Report 142705 H, 11

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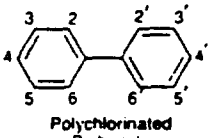
PCBs | Regulatory History and Analytical Problems

Synthesis of polychlorinated biphenyls (PCBs) was first described by Schmidt and Schultz (*1*) in 1881, but the industrial application of that discovery was not fully developed until 50 years later by the Swan Corporation. Commercial introduction of PCBs as industrial chemicals for use as nonflammable oils, notably in connection with electrical transformers, condensers, and paint, was begun in 1930. Chlorination of the parent moiety, biphenyl, results in a series of chlorinated products which exhibit unique characteristics of both thermal and chemical stability. These mixtures of PCBs (rather than individual isomers) quickly gained wide acceptance as industrial products where nonflammability and heat-resistance properties were highly desired. PCBs were marketed and used by manufacturers in products under various trade names: Aroclor, Clophen, Phenoclor, Kaneclor, and Phyralene, to name just a few of the major product lines.

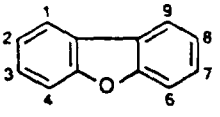
In theory, the total number of possible products resulting from chlorination of biphenyl is 209 (Figure 1), compared to 135 isomers for the closely related polychlorinated dibenzofurans (PCDFs) present as impurities in commercial PCBs. The physical properties of such mixtures are quite different from those of individual species. Aroclors will not react with acids, alkali, or water under normal or even vigorous conditions. Boiling points for Aroclors range from 278 °C for 21% chlorinated product to about 420 °C for 60% chlorinated product. PCBs can be distilled at atmospheric pressure without carbonization or decomposition. They are insoluble in aqueous media, but soluble in hydrocarbon solvents.

Regulatory History

From the initial introduction of PCBs as industrial chemicals in 1930, their widespread popularity in uncontrolled applications (hundreds of millions of pounds) over the ensuing 40 years finally resulted in their becoming a persistent and ubiquitous environmental contaminant. In 1966, Jensen (*2*), a Swedish scientist, was the first to draw attention to the fact that PCBs were found in fish and birds. By late 1971, it had become glaringly obvious that contamination of the environment had led to global contamination of wildlife (*3*). In essence, PCBs along with DDE had become the most abundant of the chlorinated aromatic pollutants in the ecosystem. The most serious consequence of the widespread contamination of the environment with PCBs was the indirect contamination of certain foods. PCBs were found in human adipose tissue and human milk.



Polychlorinated Biphenyls (PCBs)



Polychlorinated Dibenzofurans (PCDFs)

Chlorine Substitution	PCBs	PCDFs
Mono-	3	4
Di-	12	16
Tri-	24	28
Tetra-	42	38
Penta-	46	28
Hexa-	42	16
Hepta-	24	4
Octa-	12	1
Nona-	3	—
Deca-	1	—
Totals =	209	135

Figure 1. Number of possible isomers of polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs)

In 1968 an unfortunate accident in Japan (*4*) was a sneak preview of several similar but less serious accidents that occurred in the U.S. during the following decade. Rice oil became contaminated when PCBs leaked from a heat exchanger. The so-called "Yusho" poisoning incident resulted when victims consumed the contaminated rice oil. A whole spectrum of adverse symptoms was observed, e.g., chloracne, discoloration of the gums and nailbeds, swelling of the joints, waxy secretions of the glands in the eyelids, as well as more general effects such as lethargy and joint pain. This single incident served as the catalyst to focus much greater attention on the problem of PCB contamination of foods.

At that point, the U.S. Food and Drug Administration (FDA) conducted a national survey to determine the extent and levels to which PCBs might find their way into the food chain via indirect use of PCB-contaminated animal feed, industrial and environmental sources, and the use of paper food-packaging materials con-

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...g PCBs. In 1972, this survey culminated in a "Notice of Proposed Rule Making" (5) to limit the level of PCBs in foods containing unavoidable PCB residues from environmental or industrial sources. Analysis of animal feed indicated less than 5% of the samples contained PCBs (0–0.6 ppm). Isolated accidents, however, had occurred in pasteurization equipment, where PCBs from heat exchanger fluids had directly contaminated animal feed and subsequently poultry and eggs intended for human consumption. The use of PCB-containing coatings on the inner walls of silos had also resulted in the contamination of silage, which had in turn caused PCB residues to appear in the milk of dairy cows (6).

In a survey on food packaging, 67% of the packaging samples contained PCBs, with the highest level observed being 338 ppm. Of these samples, only 19% of the actual food contained in the packaging contained PCBs, with a maximum level of 0.1 ppm. In the case of packaged infant food cereals, 75% of the samples contained PCBs, with an average concentration of 0.3 ppm; the maximum found was 1 ppm. At the time of this survey, knowledge of the toxicological effects of PCBs was somewhat limited. The FDA concluded that it would be prudent to reduce human low-level exposure to PCBs by limiting the ways in which PCBs might enter the food chain, and to limit the levels of PCBs in food containing unavoidable PCB residues from environmental or industrial sources by establishing temporary tolerances (Table I) until such time as additional information warranted change (7).

The temporary tolerances established in 1973 were a result of a vigorous evaluation of the data base on PCBs prevailing at that time. Concern about the ubiquitous distribution of PCBs in the ecosystem and the "Yusho" accident led to a massive scientific probe into the toxicity of PCBs, as well as their reported presence in most wildlife (8). In 1975, the Environmental Protection Agency (EPA) sponsored a national conference on PCBs, at which the FDA announced it had initiated a review of the appropriateness of the 1973 temporary tolerances. After reviewing the wealth of data generated by the scientific community and government on PCBs, FDA proposed to reduce the temporary tolerances (9) for unavoidable residues of PCBs in several classes of food. More than 100 comments on this proposal were received from interested parties. In considering these comments, the FDA commissioner issued a final order reducing PCB tolerances (Table I) (10).

Table I. FDA Tolerances for Polychlorinated Biphenyls in Several Classes of Food

Product	Concentration (ppm) 1973	Concentration (ppm) 1979–present
Milk and dairy products (fat basis)	2.5	1.5
Poultry (fat basis)	5.0	3.0
Eggs	0.5	0.3
Fish and shellfish (edible portion)	5.0	5.0*
Infant and junior foods	0.2	0.2
Feed for food-producing animals	0.5	0.2
Animal feed components including fish meal and other byproducts of marine origin	5.0	2.0
Paper food-packaging materials intended for or used with human food	10.0	10.0

* A regulation establishing a new level of 2 ppm was promulgated but was stayed on Oct. 5, 1979, until further notice.

Since the food and feed industries were particularly vulnerable to accidental contamination from PCBs, EPA, which has regulatory control of PCBs and other toxic substances under the Toxic Substance Control Act of 1976, has issued rules governing their continued use. As of Nov. 1, 1979, the use of PCBs in new heat transfer systems in plants manufacturing or processing food, drugs, and cosmetics was no longer authorized. The EPA rule, however, did permit the continued use of PCBs in electromagnets, transformers, heat transfer and hydraulic systems until July 1, 1984. Issuance of an interagency alert notice was prepared under the sponsorship of EPA to urge voluntary removal of equipment and its replacement with non-PCB units to prevent food contamination (11).

Toxicity

Evaluation of the toxicity of PCBs has been complicated by the heterogeneity of the commercial preparations and the analytical difficulties in separation, identification and quantitation. Acute oral LD_{50} values of PCBs in mammals vary from 2–10 g/kg, with an apparent increase in mammalian toxicity with a decrease in chlorine content. In humans, chronic ingestion of small amounts (10 mg/kg) for more than 50 days causes chloracne. The fear that prolonged, continuous exposure to low doses might well result in serious human health problems has promoted a massive scientific inquiry into the toxicological and biological effects of PCBs. A recent study, involving the feeding of male broiler chickens (12) low levels of Aroclor 1254 from hatching to eight weeks of age, demonstrated marked reduction in growth with concurrent accumulation of PCBs in all tissues. Fukano et al. (13) have illustrated the accumulation of PCBs in human fat for both males and females from Tokyo within the range 1–2 ppm, while blood samples contained 1–5 ppb PCBs. Recently, the eloquent demonstration by Poland (14) that the toxicity of certain PCB congeners together with other polycyclic aromatics (PCDFs, azobenzenes, and TCDD) paralleled their ability to compete (in vitro) for specific binding sites in the cytosol carrier proteins has provided a useful tool for structure–activity and mechanistic studies.

To complicate the issues, the wealth of data concerning the metabolism of PCBs has given rise to another set of analytical problems. It is now clearly established that the major metabolic route of PCBs is hydroxylation. Metabolism in rat microsomal systems (15) of selected tri-, tetra-, and pentachlorobiphenyls resulted primarily in the formation of monohydroxylated PCBs with the number of metabolites decreasing with increasing degree of chlorination. Similar studies with PCBs in the rhesus monkey (16) have confirmed this pathway, and also demonstrated dechlorination and arene oxide formation. Four lactating Holstein cows fed single doses of Aroclors 1242 and 1254 (1.5 g) excreted in their milk 10 monohydroxy PCBs and four monohydroxy PCBs, respectively (17). Jensen et al. (18) have also reported in a study of seal blubber that methyl sulfone metabolism is clearly evident. The seal blubber contained approximately 150 ppm total PCBs, with 16 ppm of total PCB sulfones. The concentration of PCBs stored in the fat tissue increases and is then transferred at increasing concentrations to the food chain.

Analytical Considerations

It should now be clear that any attempt to extract, separate, identify, and quantify a mixture of PCBs re-

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quires knowledge of three major problem areas:

Composition. In spite of theoretical calculations establishing 209 possible congeners, Welti (19) has demonstrated experimentally via capillary chromatography that not all possible compounds are present in Aroclor 1254. Of the 69 eluting peak profiles observed, 19 represented major components while 50 represented minor contributions to the mixture. Certain positions on the biphenyl nucleus are not favored for chlorine substitution; 2-, 4-, and 6- substitutions are rare, and the occurrence of 3-, 3,5-, and 2,3-isomers is likewise infrequent. Parallel studies using packed columns have shown elution profiles with fewer than 20 discernible peaks. The complications induced by analyzing mixtures of PCBs rather than any single specific isomer have contributed a serious impediment to quantitation, since most analyses ultimately involve estimation of compounds in which the degree of chlorination can run from mono- to deca-, i.e., a range of molecular weights (188–494) with varying chemical and physical properties.

Interfering Organochlorine Pesticides. The initial confirmation of the occurrence of PCB residues in wildlife by Jensen (2) was reported after repeated and frequent encounters of similar elution patterns or profiles while analyzing for DDT and other chlorinated pesticides in general. Many earlier reported residue analyses failed to recognize this PCB in-

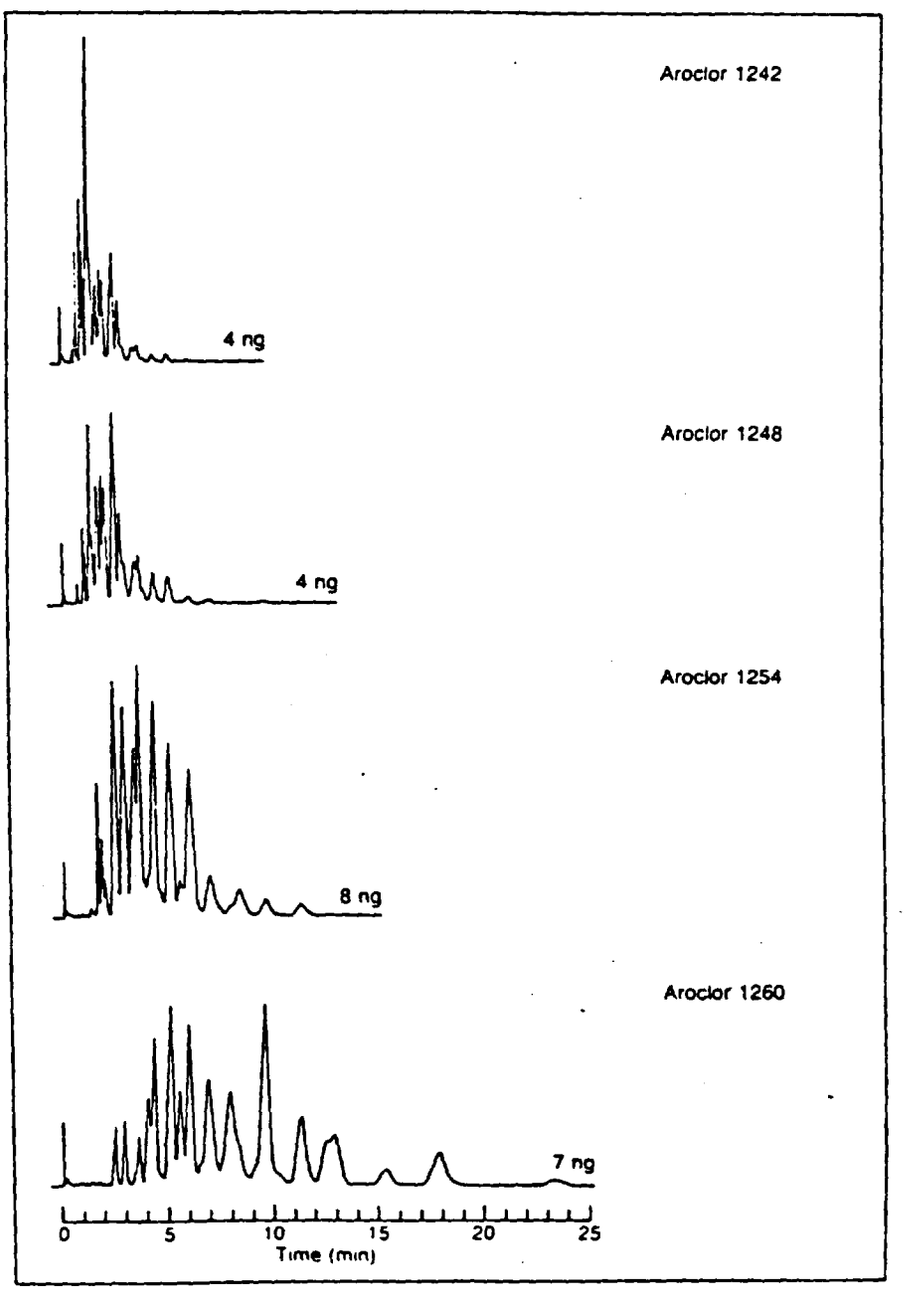


Figure 2. Electron capture responses of various Aroclor standards: 2% OV101, 6 ft × 2 mm, 200 °C

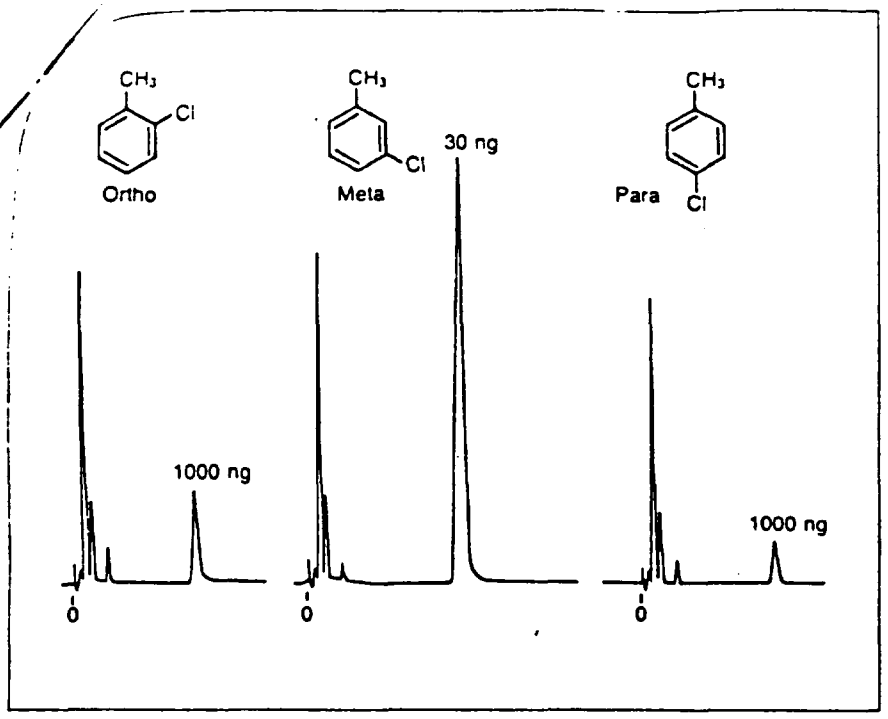


Figure 3. Electron capture responses to *ortho*-, *meta*-, and *para*-chlorotoluene: 2% OV101, 6 ft × 2 mm, 70 °C, attenuation 64 × 10^{-11} amps

terference, which must have led to an overestimation of DDT in the environment. After this historical breakthrough in 1966, the roles were suddenly reversed and the important PCB residue analyses were then deemed to have interferences by a whole host of common and ubiquitous organochlorine pesticides. Methodologies were then devised to efficiently separate PCBs from such organochlorine pesticides as DDT and Toxaphene, Heptachlorepoxide, Lindane, and Dieldrin. The stability of PCBs to treatment with acids and alkali made it possible in some procedures to either destroy or alter one or more of the interfering pesticides. On the other hand, greater attention has been paid to the use of various columns (e.g. silicic acid, celite and Florisil) and solvents (mixtures of acetonitrile, hexane, and methylene chloride) to separate PCBs from the other chlorinated pesticides (*20*). Masumoto (*21*) has pointed out, however, that such procedures can be flawed by variable column preparation and hence variable recoveries. Additionally, those congeners with the lowest chlorination were held on the silicic column and could only be eluted by a more polar solvent than petroleum ether, leading to lower recoveries for the less chlorinated Aroclors. The analyst must choose appropriate analytical procedures, depending on which interferences are present.

Metabolism. The elution patterns or profiles observed by electron capture (EC) gas chromatography for PCB residues from biological samples (*22*) very often do not resemble the profile for one of the PCB standards selected for quantitation. The presence of metabolites and degradation products is likely to be primarily responsible for this experimental observation. Dechlorination, arene oxide formation, and hydroxylation have been identified as metabolic pathways for PCBs (*23*). Dechlorination to lesser chlorinated congeners will tend to contribute to the production of a profile perhaps suggestive of a less chlorinated Aroclor and will lead to confusion in the choice of the correct Aroclor standard for quantitation by EC. More serious by far is the removal from the observed PCB residue profile of congeners that have undergone

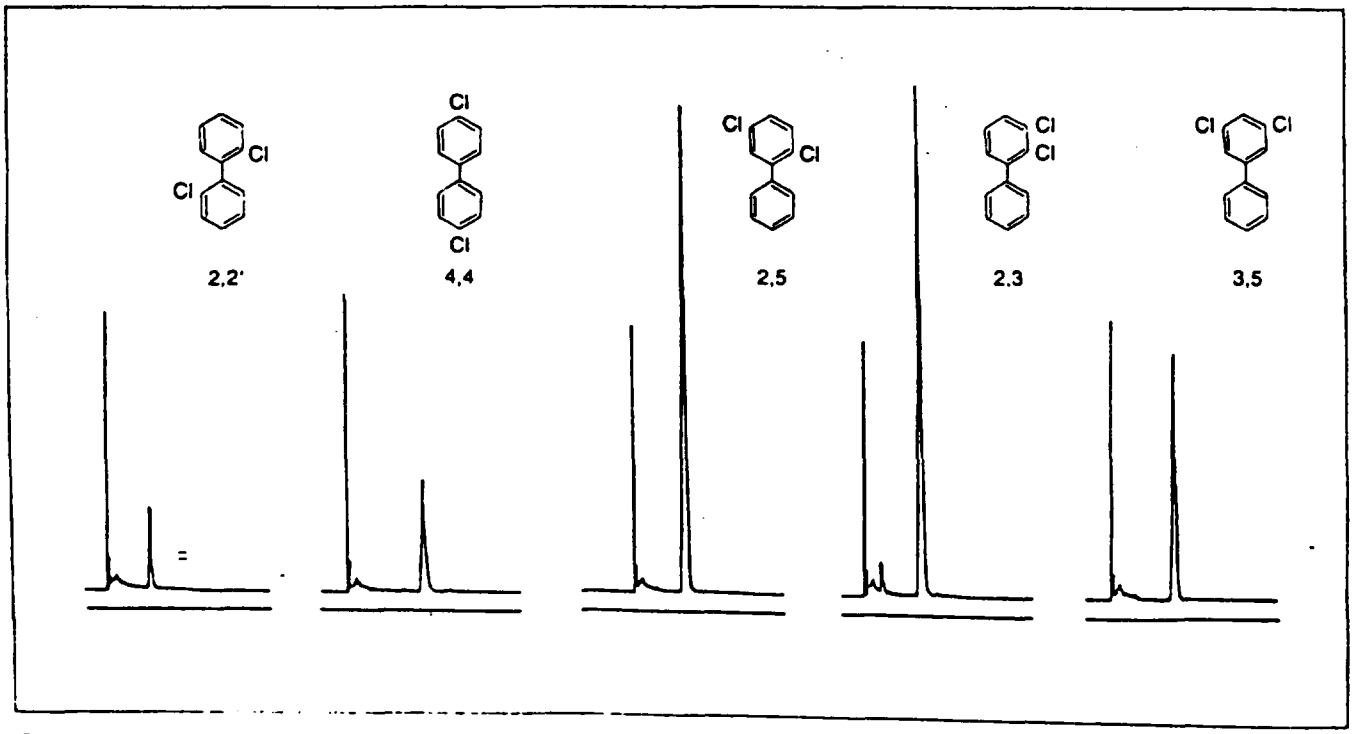
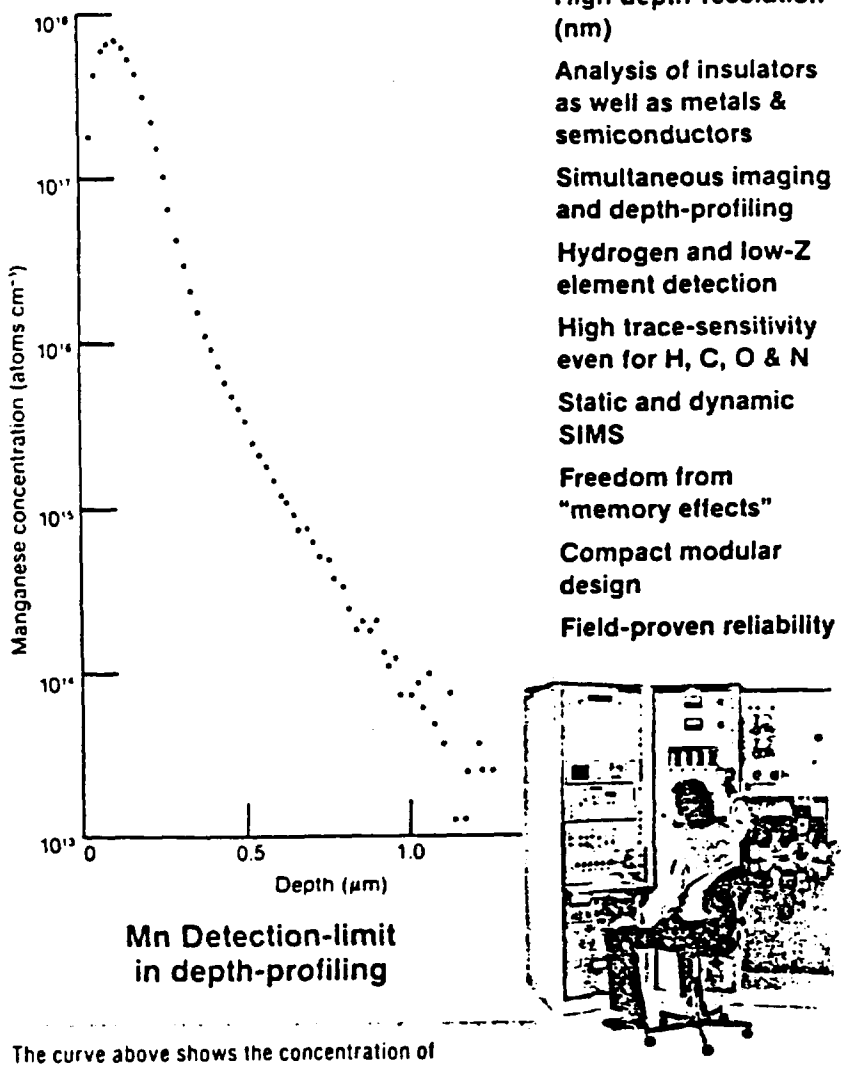


Figure 4. Electron capture responses for 1 ng injected of selected PCBs: 2% OV101, 6 ft × 2 mm, 180 °C, attenuation 16 × 10^{-11} amps



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metabolic hydroxylation. Such chlorobiphenylols do not exhibit the same elution characteristics as the parent substances, and therefore do not coelute with the solvent systems generally employed.

Metabolic processes can thus confuse the assignment of the original source of the PCB contamination and hence the correct choice of a suitable standard for quantitation. Such PCB residue profiles are further complicated by the fact that extraction and cleanup can alter the composition of mixtures because of the unique properties of these congeners.

Approaches to Quantitation

The problems encountered in the quantitation of PCB residues have focused the attention of the analytical chemist on methods by which the concentration of PCBs is related to the estimation of a single species. Such attempts have included: carbon–skeleton GC, where PCBs are reduced on-column (5% Pt at 180 °C with H_2 as carrier gas) to biphenyl (*24*); perchlorination of PCBs by antimony pentachloride to yield decachlorobiphenyl (*25*); and microcoulometric determination of the total chlorine content as HCl (*26*). These methodologies have been developed because EC-GC detector response is disproportionate, i.e., highly dependent on the degree of chlorination of the biphenyl nucleus. EC detector response has been shown to vary as much as two to four orders of magnitude between monochloro and polychloro species (*27*). The proliferation of analytical approaches to PCB analysis has resulted mainly from the existence of this disproportionality factor (*28*). This issue has overshadowed other problems, such as interferences resulting from the presence of other organochloride pesticides and PCB metabolites.

Of prime importance when using EC as the detection system for chlorinated aromatic species such as the PCBs is the problem of disproportionate responses. At the present time, Aroclor standards have been characterized by EC (Figure 2) and are employed as reference materials. The closest matching profile is used as a standard for quantitation of environmental samples. A good example of disproportionality of response by EC is that exhibited by the isomeric-monochlorotoluenes (Figure 3), in which differences of several orders of magnitude were observed. Although this case is a relatively dramatic example, the prevailing situation within the congeners of the PCB family has been found to be similar but somewhat less pronounced. The EC responses to 1 ng of five selected dichlorobiphenyls under similar conditions are illus-

trated in Figure 4. Depending on the location of the chlorine atoms within the biphenyl nucleus, varying detector responses were observed. This disturbing disproportionality has been the main criticism of PCB analysis with EC.

The analytical pitfalls created by the lack of well-defined standards when dealing with mixtures has ultimately led to the increased application of mass spectrometry in its various forms to assist in resolving difficult problems concerning identification and quantitation of these systems. A novel approach to routine quantitative analysis by chemical ionization mass spectrometry is described in the research section of this issue (29).

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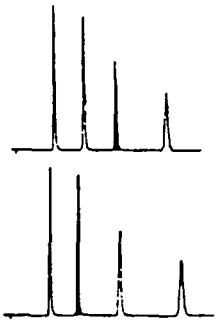
Emil G. Siegmund (seated), research chemist in mass spectrometry with the Los Angeles District Office of the FDA since 1963, received his BS degree in chemistry from the University of Massachusetts in 1959. His principal activities involve the application of GC/MS and LC/MS to regulatory analysis in the identification of suspected pesticide residues, as well as the detection of unregistered pesticides, particularly on imported fruit and vegetables. He is currently involved in developing analytical assay procedures for various glucocorticoids.

COMMON PROBLEMS IN CHROMATOGRAPHY #10



Why Has Reproducibility Been A Problem In HPLC?

Have you had to modify the composition of your mobile phase upon changing columns, even though the new column is supposedly identical to an old one?

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Table IX. Determination of Chlorine in Coal Related Samples

sample	Cl, ppm present	Cl, ppm found
NBS-1632 coal	962[a]	1137; 1217
Wyodak coal	118[b]	79; 83
coal fly ash	700[b]; 643[c]	582; 658
coal washings		
1	36.0[c]	38.2
2	15.2[c]	15.7
3	50.6[c]	50.2
4	427[c]	437

[a] Literature average of 12 values. [b] Neutron activation data. [c] Potentiometric titration with $AgNO_3$.

The normal coal samples decomposed by the Na_2CO_3 procedure and then made up to volume with pH adjustment and addition of TISAB will result in <1 μg/mL fluoride concentration in the final solution. The response of the electrode to these low fluoride levels is sluggish, and in addition the electrode potential readings slowly drift upward, making it very difficult to decide which is the true reading. This situation can be greatly improved by adding a known excess of fluoride (1 μg/mL) to both sample and blank solutions. This increases the stability of the first reading. It is essential to correct for this fluoride added before the final concentration calculations are done. Further analysis of fluorine in the solution is carried out by the standard addition method.

A Wyoming coal sample was sent to Galbraith Laboratories Inc. in Knoxsville, Tenn., for fluorine measurement. They reported a value of 72 ± 4 ppm based on triplicate determinations. The same coal sample was analyzed in our laboratory by the procedure outlined above and a value of 70 ± 6 ppm was obtained based on seven replicate samples analyzed on two separate days. Thus, our procedure seems to give satisfactory results for the fluorine determination.

Determination of Chlorine with Ion-Selective Electrode. Chlorine is a minor constituent of coal varying in concentration from 100 to 1000 ppm or more. Depending on the burning conditions in the boiler, the chlorine content of the fly ash also may vary from several hundred to <50 ppm. We use the ASTM D-2361-66 procedure for the chlorine determination. In this procedure, the final chloride measurement is done by potentiometric or Volhard titration with standard $AgNO_3$. For coals with low chlorine content, however, this method is not applicable, since the lowest concentration that can be obtained is 0.01%. Chloride ISE appears to be a viable alternative for the end measurement. The Orion 94-17 chloride ISE we used had a linear response in the range of 7 to at least 10 000 ppm chloride in solution. Below 7 ppm the response was not linear. Hydroxide ions interfere; however, this is prevented by simply making the solution acidic to pH 2 with HNO_3. Sulfide, cyanide, bromide, and iodide ions interfere in the chloride determination. The presence of sulfide or cyanide in the decomposed coal ashes is unlikely, and bromide and iodide levels in coal are far less compared with the chloride levels. Washings from coal preparation can also be analyzed by chloride ISE very rapidly. Some results by this procedure are given in Table IX. The ISE results are in good agreement with the literature, neutron activation, and potentiometric titration data where available. A coal washing solution containing 34.4 ppm chloride was analyzed six times with ISE yielding a value of 34.95 ± 0.59 ppm.

Neutron Activation Analysis of Oxygen in Coal. The analysis of oxygen is based on the nuclear reaction: $^{16}O(n,P)^{16}N$. The daughter ^{16}N has a half-life of 7.2 s and emits γ rays of 6.1 and 7.1 MeV. These high energy γ rays are far beyond the γ energies of most other elements activated by fast neutrons. The only serious interference in this determination is $^{19}F(n,\alpha)^{16}N$. However, in normal coals or rocks, fluorine concentration compared to their oxygen levels is insignificant, and this reaction can be safely ignored. The method, thus, is a rare example of an analytical technique that is virtually free from interferences and γ-ray attenuation effects, and it utilizes a short half-life, thus permitting a very rapid analysis.

Table X. Determination of Oxygen by 14-MeV Neutron Activation Analysis

sample	no. of analyses	found, %	present, %
diphenyl carbazide	1	6.75	6.60
benzophenone	6	9.42 ± 0.68	8.78
stearic acid	14	12.3 ± 0.5	11.27
hydroxylamine hydrochloride	1	22.9	23.0
benzoic acid	2	25.6 ± 0.07	26.2
salicylic acid	5	36.4 ± 1.5	34.8
citric acid	4	57.4 ± 1.5	58.3
NBS-1632a Coal	2	18.4 ± 0.7	-
NBS-1635 Coal	25	33.4 ± 1.6	-

The oxygen content of the transfer rabbit must be as low as possible to avoid a large blank correction. The usual material used is high-density polyethylene which combines the properties of light weight and low oxygen content. Olympic Plastics Co. of Los Angeles, Calif., is one source of these low oxygen polyethylene rabbits. To check the oxygen pickup from the atmosphere by these rabbits, we irradiated 10 rabbits each packed in air and in dry nitrogen, respectively. The average oxygen content of these rabbits was found to be 0.058 ± 0.008% for the rabbits packed in nitrogen and 0.062 ± 0.008% oxygen for the rabbits packed in air. Thus, no discernible difference is found between the rabbits packed either way. It should also be remembered that in the actual oxygen determination, this small oxygen content of the rabbits is blanked out.

The precision of the oxygen determination is dependent upon the counting statistics, the stability of the neutron generation rate, and the reproducibility of the irradiation, delay, and counting sequences. The irradiation, delay, and counting sequence is potentially the most serious cause of error, particularly the reproducibility of the delay time during which the sample leaves the irradiation position and the count is begun. Because of the counting statistics problems, the samples with low oxygen content (in ppm range) will have large errors while the samples with high oxygen content (in % range) should have 1–3% precision.

Some of our results on oxygen determination by 14 MeV NAA are given in Table X. The analysis of organic compounds is in good agreement with the theoretical oxygen content. No oxygen value has been reported in the literature for NBS 1632a or 1635 coals. Overall precision of the results varies from a high of 0.3%, to 5% for NBS-1635 coal. The accuracy of the determination based on organic compounds also varies between 0.5% to 4%. Thus, as normal in NAA, the accuracy and precision are about the same.

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Micromethods for Toxic Residue Screening by Negative Chemical Ionization Mass Spectrometry

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Methods were developed for the analysis of polychlorinated chemical residues found in milligram quantities of biological samples. Sample preparation by micro continuous liquid–liquid extraction steam distillation or by micro gel-permeation chromatography gave sufficiently clean residue extracts for negative chemical ionization analysis. With these techniques, chemicals such as chlorophenols and chlorobiphenyls have been confirmed in human adipose samples as small as 12.5 mg. These methods make it possible to screen <1-g samples of biological substrates for contamination with persistent toxic substances at part-per-billion levels.

Analytical procedures developed for the analysis of persistent toxic substances in environmental samples generally require between 10–500 g of substrate. However, increasing concern for the impact of environmental contamination on human health has stimulated the development of microsample methodology for residue analysis. The objective of this work was the development of an analytical procedure for the isolation and identification of persistent toxic substances at the parts-per-million to parts-per-billion level in <1-g samples of biological fluids. Our analytical strategy involves the use of efficient but simple separation techniques for sample cleanup followed by screening the cleaned up extracts with direct probe negative chemical ionization (NCI) mass spectrometry (*1*, *2*).

The two clean-up methodologies have focused on gel permeation chromatography (*3*–*7*) and steam distillation with continuous liquid–liquid extractions (*8*). Both of these techniques effectively separate persistent toxic substances from the bulk of the biomolecules, but do not involve adsorption chromatography which may reduce recoveries of the more polar chemicals.

Negative chemical ionization is uniquely suited to screening micro quantities of environmental samples for toxic chemicals, especially polychlorinated chemicals, because of high sensitivity for these chemicals and a virtual transparency to potentially interfering biomolecules. The ion forming reactions that are important for polychlorinated molecules are:

resonance capture of a thermal electron

$$M + e^-_s \rightarrow M^{-}\cdot{}^{*} \xrightarrow{N} M^{-}\cdot \quad (1)$$

disassociative capture of a thermal electron

$$A-B + e^-_s \rightarrow A^- + B \quad (2)$$

chloride attachment

$$M + X^- \rightarrow MX^- \quad (3)$$

deprotonation

$$A-H + B^- \rightarrow A^- + BH \quad (4)$$

oxygen exchange

$$A-Y^-\cdot + O_2 \rightarrow AO^- + YO \quad (5)$$

EXPERIMENTAL

Gel Permeation Chromatography. *System Description.* The GPC system is similar to that previously described (*7*) with the following modifications. A 500 mm × 10 mm glass column (Ace Glass Company) was packed with 200–400 mesh bio-beads SX-8 (Bio-Rad Inc.) and eluted with cyclohexane–methylene chloride (1:1) at 1.5 mL/min. A 254-nm ultraviolet detector (Varian Aerograph) and a strip chart recorder (Linear Inc., model 260) were used to monitor the effluent.

Recovery Studies. Arochlor (1254 (100 ng), pentachlorophenol (50 ng), and hexachlorobenzene (50 ng) were spiked into 100 mg

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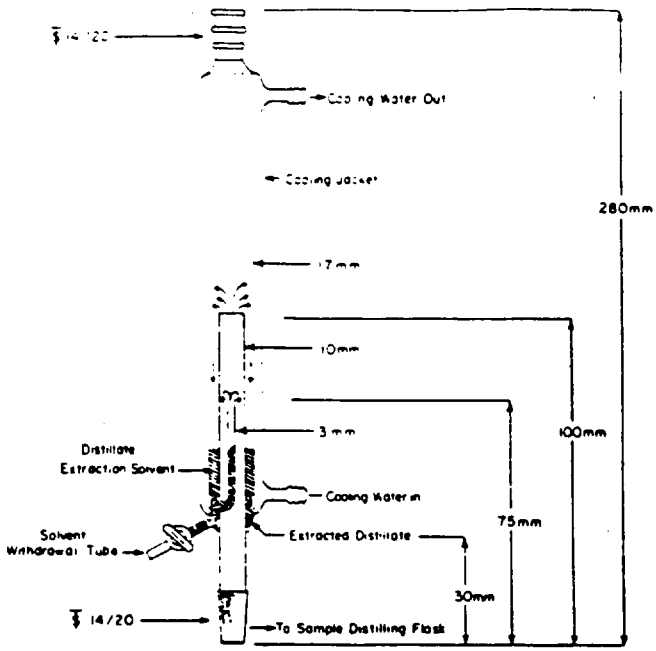

Figure 1. Microcontinuous liquid/liquid extraction steam distillation apparatus

of corn oil dissolved in 2.0 mL of methylene chloride and injected onto the gel-permeation chromatography (GPC) system. The 35–60 mL fractions were collected for analysis. Recoveries of these compounds were determined by electron capture gas chromatography (EC/GC). Pentachlorophenol was derivatized with Tri-Sil-BSA (Pierce Chemical Company) prior to GC analysis.

Sample Cleanup. In a typical experiment, 250 mg of human adipose tissue was mixed with an equal amount of anhydrous sodium sulfate. The mixture was extracted with 10 mL of hexane–acetone (1:1) in a tissue blender (Vertis). The solids were separated by centrifugation and decanting, and the solvent was reduced to 1.5 mL by concentration through a micro-Snyder column. The extract was injected into the GPC system and the 40–60 mL fraction was collected and concentrated through a micro-Snyder column for examination by NCI.

Procedural blanks were obtained by preparing a sample in the same manner without the tissue.

Steam Distillation. *System Description.* The steam distillation apparatus (Figure 1) was a modification of a similar unit previously described (*8*).

Recovery Studies. Anthracene, *trans*-chlordane, pentachlorophenol, and hexachlorobenzene in acetone were spiked into two 0.5-g samples of eastern oyster (*Crassostrea virginica*) tissue at 25 and 250-ng levels, and added to 35 mL of 10% H_2SO_4 in a 50-mL round bottom flask. The flask was placed in an ultrasonic bath for 20 min. The round bottom flask was then fitted onto the distillation head which had been loaded with 2 mL of water and 2 mL of 2,2,4-trimethylpentane. The flask was heated to 80 °C for 20 min, then to 100 °C for 40 min to distill the sample. The trimethylpentane was drained from the head and concentrated to 1.0 mL with a stream of dry nitrogen for GC determination of recoveries. Pentachlorophenol was derivatized with Tri-Sil-BSA (Pierce Chemical Company) prior to GC analysis. The chlorinated hydrocarbons were quantitated by EC/GC and the anthracene was quantitated by FID/GC.

The unspiked tissue served as a procedural blank.

Sample Cleanup. In a typical experiment 0.5 g of human adipose tissue was added to 35 mL of 10% H_2SO_4 in a 50-mL round bottom flask. The sample was then treated as described above. The drained trimethylpentane was concentrated to 100 mL with a stream of dry nitrogen for NCI analysis.

Procedural blanks were obtained by preparing a sample in the same manner without the tissue.

Reagents. Hexane, acetone, cyclohexane, methylene chloride, and 2,2,4-trimethylpentane were redistilled pesticide grade (Burdick and Jackson). Water was distilled-deionized and refluxed for 45 min on a steam distillation apparatus. Sulfuric acid (Baker Chemical Company) was diluted to 10% with the refluxed water. Standards for the recovery studies were obtained from the U.S. Environmental Protection Agency Repository at Research Triangle Park, N.C.

Gas Chromatography. A Varian Aerograph model 1400 EC/GC and a Varian Aerograph model 1700 FID/GC were equipped with identical 2 m × 2 mm glass columns packed with 3% OV-101 on gas Chrom Q. The EC/GC was operated isothermally at 185 °C and the FID/GC was temperature programmed from 100–200 °C at 4°/min. The carrier gas was N_2, 25 mL/min.

Mass Spectrometer. An AEI MS-902 mass spectrometer equipped with a chemical ionization source (Scientific Research Instruments Corporation) was operated in the negative ion mode with an electron energy of 470 eV, source temperature of 150 °C, source pressure of 0.5 Torr, and resolution 4000. A mixture of isobutane, methylene chloride, and oxygen (80:18:2) was used as the reagent gas. Direct probe sample introduction was used, and 8-s scans were recorded at 30, 60, 90, and 120 s after introduction on oscillographic paper.

The voltage of the electron multiplier (≈3.0 kV) was set such that the $(M + Cl)^-$ ion (m/z 441) for *trans*-chlordane, the $(M - 19)^-$ ion (m/z 357) for octachlorostyrene, and the $(M - 1)^-$ ion (m/z 263) for pentachlorophenol gave approximately a 50% scale reading on the oscillographic output, when spiked into a residue obtained from the steam distillation of 0.5 g of oyster.

RESULTS AND DISCUSSION

Several steam distillation units similar to the one described by Veith and Kiwus (*8*) were constructed and tested until a design was found that allowed the use of distillation volumes as small as 25 mL and also required minimal heat for steam distillation. The design that met these requirements is shown in Figure 1. Recoveries of hexachlorobenzene, pentachlorophenol, *trans*-chlordane, and anthracene spiked into 0.5 g of oyster tissue at 50 and 500 ppb ranged from 85% to 97%. These recoveries are comparable to those observed by Veith

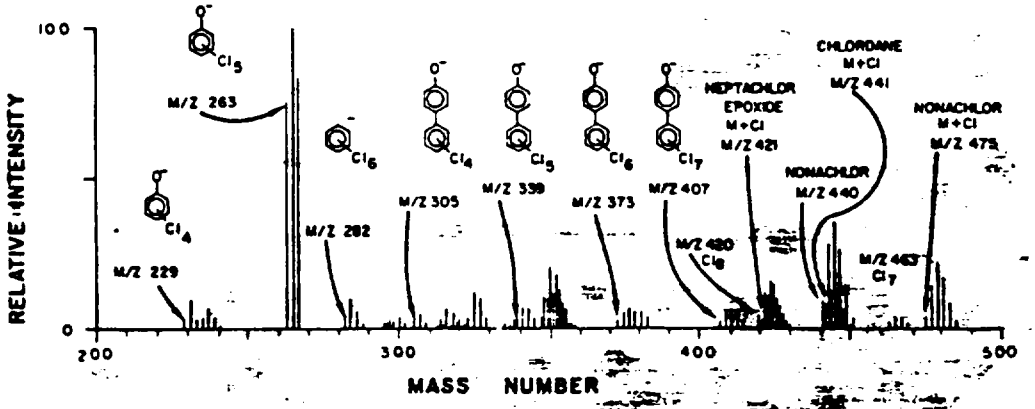

Figure 2. Typical NCI mass spectrum for steam distillation of 250 mg of human adipose tissue. Only ^{35}Cl negative mass defect ions are shown for polychlorinated chemicals.

Table I. Polychloro Ion Clusters in NCI Spectra of Steam Distillates of 250-mg Samples of Human Adipose Tissue[a]

m/z	no. of chlorine	2-20-2-MW-1	2-20-1-MW-1	2-24-8-MW-1	2-24-7-MW-1	2-24-5-MW-1	3-35-6-MW-1	2-20-3-MW-1	2-20-4-MW-1	B-1[b]	identification
217	3	*									
231	4	*									
243	3	*									
263	5	*						*			pentachlorophenol (M – 1)⁻
294	4		*					*			
305	5	*	*	*	*	*	*	*	*		pentachlorobiphenyl (M – 19)⁻
307	4							*			
314	5		*								
317	4	*	*	*	*	*		*	*		p,p'-DDMU (M + 35)⁻
323	4							*			
339	5	*						*	*	*	hexachlorobiphenyl (M – 19)⁻
341	4		*		*	*	*	*	*		
350	4		*								
351	5							*			p,p'-DDE (M + 35)⁻
373	6	*	*	*	*	*	*	*	*	*	heptachlorobiphenyl (M – 19)⁻
375	5	*	*		*		*	*	*		
387	6							*			p,p'-DDT (M – 35)⁻
407	7	*	*	*	*	*	*	*	*		octachlorobiphenyl (M – 19)
409	6		*		*		*	*			
420	8		*	*	*	*	*	*			
421	7		*	*	*	*	*	*			heptachlor epoxide (M– Cl)⁻
440	9		*	*	*	*	*	*			nonachlor M⁻·
441	9		*	*	*	*	*	*	*		chlordane (M – CL)⁻
463	7	*	*	*	*	*	*	*	*		
475	10		*	*	*	*	*	*	*		nonachlor (M + CL)⁻

[a] An asterisk indicates the presence of the ion cluster at five times the noise or higher intensity. [b] Column labelled B-1 was the procedural blank. The ion clusters appearing in these spectra were at least an order of magnitude lower in intensity than in the sample spectra.

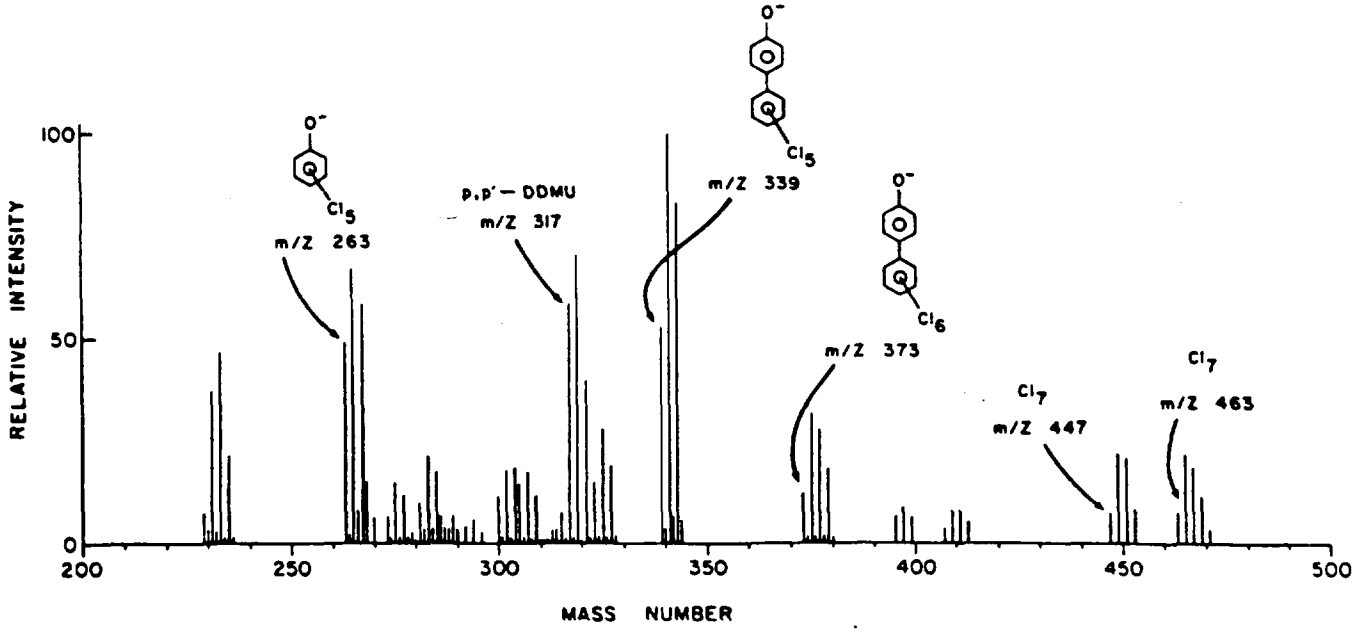

Figure 3. Typical NCI mass spectrum for steam distillation of 2.0 mL of human seminal plasma. Only ^{12}C negative mass defect ions are shown for polychlorinated chemicals

and Kiwus (*8*) for polychlorinated biphenyls (PCBs) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE) from fish tissue.

Two experiments were conducted to evaluate the utility of the microsteam distillation as a clean-up procedure for NCI screening for persistent toxic residues. First, we examined eight 250-mg samples for human adipose tissue and fourteen 2.0-mL samples of human seminal plasma to determine the different types of chemicals that may be isolated and identified in real samples by these techniques. Second, we analyzed smaller and smaller quantities of the same adipose tissue to determine the minimum quantity of tissue required for detection of the major toxic chemicals in these samples. The predominant contaminants identified in the adipose tissue survey were components of *tech*-chlordane, PCB isomers, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT) and 1-chloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDMU) (Table I). The predominant contaminants identified in human seminal plasma were PCB isomers and pentachlorophenol (Table II). The mass assignments in Tables I and II are pseudomolecular ions that were formed by reactions 1–5 above. Compound identifications are based upon a comparison with NCI spectra of standards. In each sample an ion at *m/z* 463 (CL_7) was observed; however, this compound remains unidentified. Typical NCI mass spectra for steam distillation of 250 mg of adipose tissue and 2.0 mL of human seminal

Table II. Polychloro Ion Clusters in NCI Spectra of Steam Distillates of 2.0-mL Samples of Human Seminal Plasma[a,b]

sample no.		D-7	N-2	N-5	D-12	D-2	N-21	N-14	N-10	D-5	N-1	S-7	N-22	S-10	N-29	B-1	B-9	B-11	
sperm density (10^6/mL)		8	11	12	19	20	20	20	29	106	129	146	198	227	229	X	X	X	
m/z	no. of chlorine																		compound identification
212	3	*	*	*			*	*		*	*	*	*	*	*				
229	4			*	*		*	*					*	*					tetrachlorophenol (M – 1)⁻
231	4	*		*		*	*	*		*	*	*	*	*	*				
263	5	*		*	*	*	*	*		*	*	*	*	*	*				pentachlorophenol (M – 1)⁻
285	3				*														
287	5						*												
301	4									*									
305	4		*	*				*	*			*		*					pentachlorobiphenyl (M – 19)⁻
313	5			*								*			*				
317	4	*	*	*			*	*	*	*		*	*	*	*				*p,p'*-DDMU (M + CL)⁻
321	4	*			*														
323	6						*												
339	5		*	*	*		*	*	*	*	*	*	*	*			*	*	hexachlorobiphenyl (M – 19)⁻
343	3		*	*			*	*	*			*	*	*					
345	3																		
373	6	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			heptachlorobiphenyl (M – 19)⁻
407	7		*								*								octachlorobiphenyl (M – 19)⁻
427	7	*									*								
429	5										*	*							
447	5											*							
463	7	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	

[a] An asterisk indicates observation of the ion cluster at five times the raise or higher intensity. [b] Columns labeled B-1, 9, 11 were procedural blanks. The ion clusters appearing in these spectra were at least an order of magnitude lower in intensity than in the sample spectra.

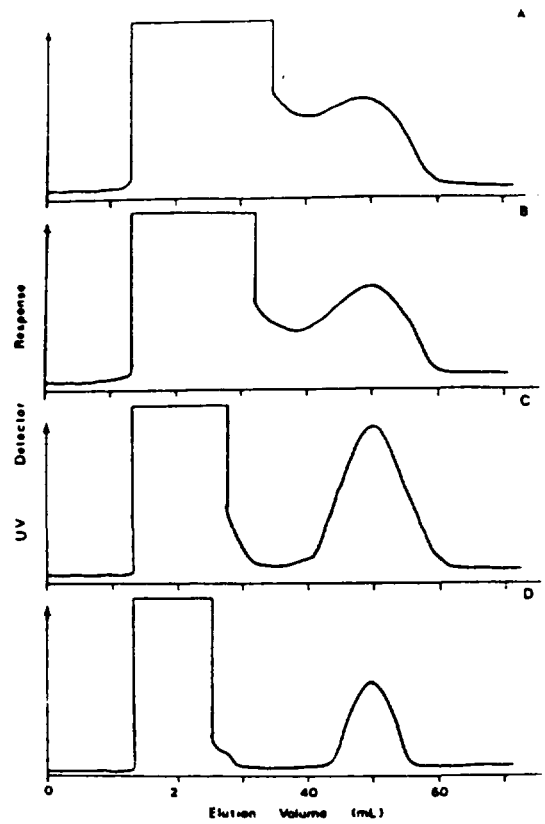

Figure 4. Gel-permeation chromatographic traces of UV detector response vs. elution volume for human adipose tissue samples of (A) 10 mg, (B) 50 mg, (C) 25 mg, and (D) 12.5 mg

plasma are shown in Figures 2 and 3, respectively. In addition to the toxic substances listed in Tables I and II, low molecular weight free fatty acids readily steam distill. Since free fatty acids have a high sensitivity for chloride attachment (*9*), they are the most consistent interference in NCI screening of environmental samples. Polychlorinated chemicals are therefore identified in the mass spectra by their negative mass defects as well as the characteristic isotopic ratios of the chlorine isotope clusters. In both the steam distillation and GPC procedures, a pentachlorobiphenyl was observed in the blanks at a level approximately an order of magnitude lower than that observed in the smallest samples. Pentachlorobiphenyl was therefore reported to be present only if it was 10 times greater than that observed in the blank.

Additional samples of 100, 50, and 25 mg of a human adipose tissue (2-20-2-MW-I, Table I) were analyzed. Although absolute peak intensity for identification decreased with decreasing sample size, all identifications made on the 250-mg sample were also possible on the 25-mg sample. An estimate of detectability of polychlorinated chemicals by NCI was established by observing the pseudomolecular ion of *trans*-chlordane, pentachlorophenol, and octachlorostyrene, which had been spiked into the residue of 500 mg of steam distilled cleaned up oyster tissue at 10 ppb. In each case the pseudomolecular ions were readily identifiable.

We have examined GPC as an alternate clean-up procedure. The system described by Kuehl and Leonard (*7*) was capable of isolating both polar and nonpolar toxic chemicals from fatty tissue; however, the system described was used for chromatography of 0.5–1.0 g of oil, which represented 5–10 g of a fish of 10% fat content. A new column 500 mm × 10 mm packed with Bio-Rad SX-8 beads and eluted at 1.5 mL/min with cyclohexane/methylenechloride provided separation of corn oil, PCBs, and pentachlorophenol comparable to that of the large columns (*7*). In addition, Arochlor 1254 (1 ppm), hexachlorobenzene (500 ppb), and pentachlorophenol (500 ppb) spiked into corn oil and eluted on this system were recovered at greater than 95%.

A series of 100-, 50-, 25-, and 12.5-mg samples of the same adipose tissue (2-12-2-MW-I, Table I) was then used to evaluate the GPC performance to determine if the same toxic chemicals could be found by GPC/NCI as were found by steam distillation/NCI. Figure 4 shows UV detector traces of the four tissue samples. As can be seen, maximum resolution and sensitivity occur with the 25-mg sample. Apparently a maximum optimum loading for this column was about 35 mg of oil. Negative chemical ionization analysis of each sample showed the same series of toxic compounds as was observed in the steam distillation/NCI experiment. In addition all compounds observed in the 100-mg sample were also observed in the 12.5-mg sample.

We applied the micro-GPC/NCI screening system to the analysis of a sample of Lake Ontario lake trout. A Soxhlet extract of 50 mg of trout tissue was cleaned up by GPC and analyzed by NCI. Polychlorinated biphenyls, hexachloro-

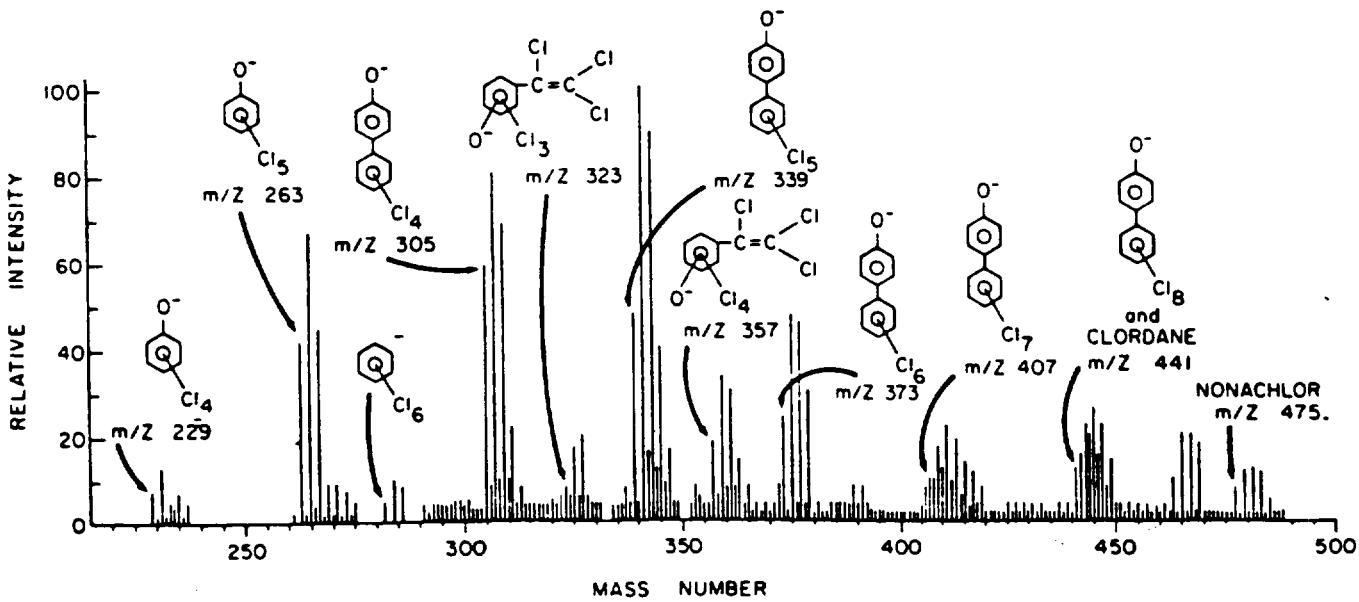

Figure 5. Negative chemical ionization mass spectrum of 50 mg of Lake Ontario lake trout cleaned up by GPC. Only ^{12}C negative mass defect ions are shown for polychlorinated chemicals

benzene, polychlorinated styrenes (*10*), chlordane, and nonachlor are readily identified in this sample (Figure 5).

Although the use of micromethods limits the possibilities for contamination of the sample, the amount of adventitious material that will appear as a positive is also smaller for microtechniques. Adequate procedural blanks must be carried through the entire procedure. We have used both water and corn oil for procedural blanks. Realistic tissue sample blanks would be desirable, but we are not aware of sources of sufficiently contaminant-free tissue. The steam distillation system may be the best for micromethods because smaller amounts of solvent and fewer pieces of glassware are required, so it is generally easier to obtain clean blanks with this system.

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Simultaneous Titrimetric Determination of Bismuth Ion and Free Nitric Acid Concentrations

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A tandem titrimetric system has been developed in which a complexometric assay for bismuth(III) ion is coupled with a simultaneous alkalimetric determination. The metallochromic indicator pyrocatecholsulfonephthalein is used to determine the end points of both titrations. The procedure has been developed in such a way as to produce four distinct end points from the indicator such that proper pH control and avoidance of bismuth(III) nitrate hydrolysis have been accomplished.

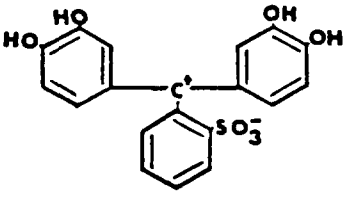
Complexometric titration of bismuth ion has remained the assay of choice even in a time when titrimetric analysis has been supplanted by newer methods. A need exists to assay for bismuth(III) ion in the presence of nitric acid and to determine the nitric acid concentration in some systems. During the manufacturing of several antidiarrheal formulations (*1*), for example, bismuth(III) appears concomitantly with nitric acid. The importance of the following titrimetric system is that it allows the tandem determination of both species.

The stability of the bismuth–EDTA complex (the log of the stability constant is 28.0 ± 0.3 (*2*, *3*)) dictates that only metal ions that form very stable complexes can successfully compete with bismuth for EDTA chelation. Thus, Th^{4+} (*4*), Fe^{3+}, Hg^{2+}, In^{3+}, Zr^{4+}, and Hf^{4+} interfere in any bismuth complexometric titration and must be absent, removed, or reduced to ensure the selectivity of the assay (*5*). Trivalent iron and mercury(II) ion, for example, are reduced by the addition of ascorbic acid.

Complexometric titrations of bismuth with EDTA have employed a host of indicators. Alizarin chrome black R, stilbazo, thorin (*6*, *7*); picramineazo compounds (*8*, *9*); the yellow BiI_4^- complex (*10*), the bismuth–thiourea complex (*5*), azoimidazole (*11*), 2-2(2-pyridylazo)-4-methylphenol (PAC) (*12*), thiazolyazonaphthol (*13*), 4-(4-nitro-2-thiazolyazo) resorcinol and 2-methyl-4-(4-nitro-2-thiazolyazo) resorcinol (*14*), semixylenol orange and xylenol orange (*15*), 1-2-naphthoquinone-4-sulfonic acid-2-thiosemicarbazone (*16*), and pyrocatecholsulfonephthalein (*17*) have been employed as indicators. For reasons that will become evident later, pyrocatecholsulfonephthalein was chosen as the indicator for this investigation.

Pyrocatechol violet, catechol violet, and brenzcatechin violet are all trivial names for pyrocatecholsulfonephthalein (*18*). Although pyrocatecholsulfonephthalein undergoes air oxidation in alkaline solutions, it is stable in neutral solution for several months and is suitable for use as a bench reagent (*19*). Analyses of bismuth in the presence of other metal ions, including pharmaceutical formulations, have been performed (*20*).

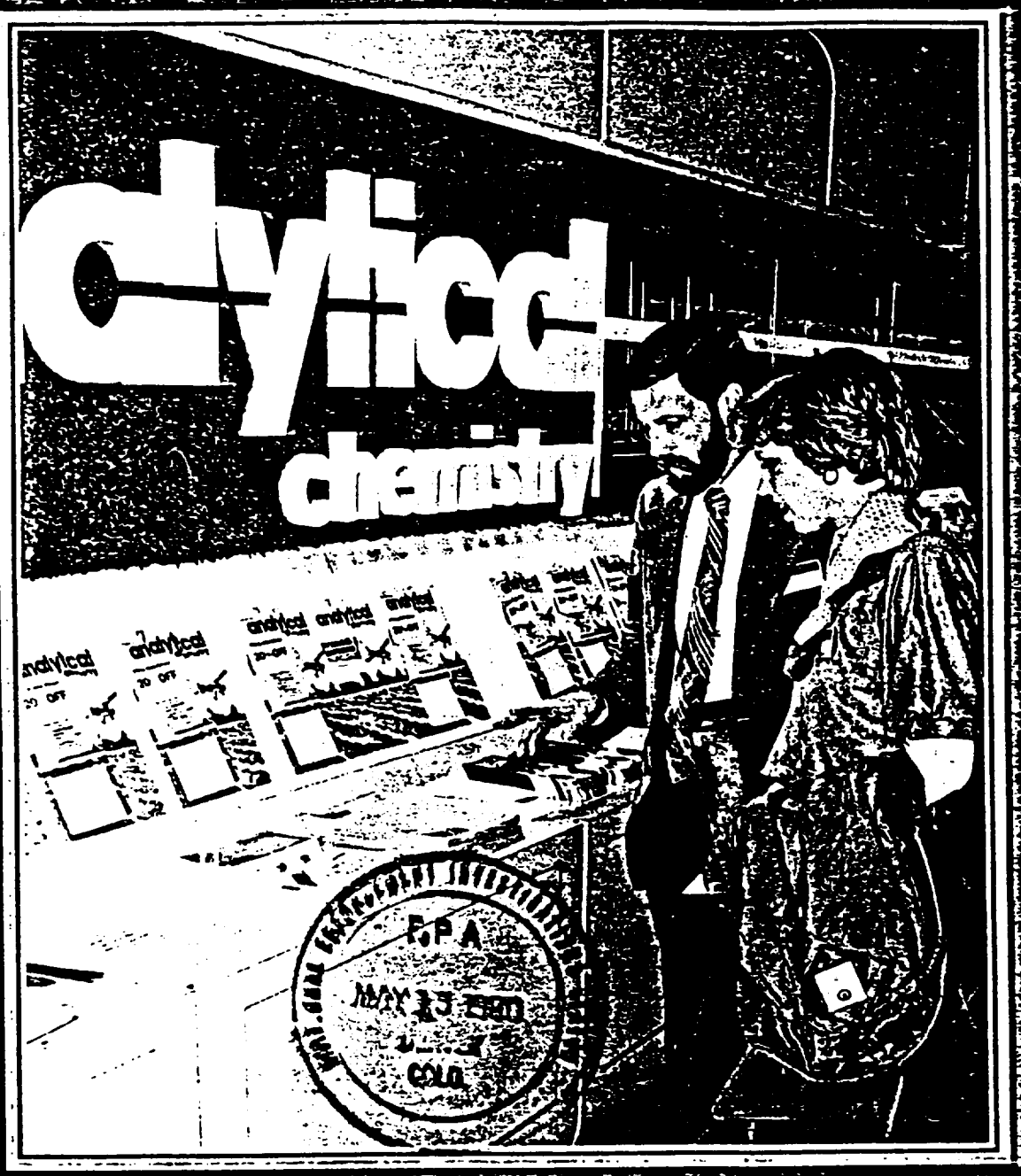
The structure of pyrocatecholsulfonephthalein reveals that



the indicator has two hydroxy groups meta to the central carbonium ion which are available for complexation. The protons on the hydroxy groups para to the central carbon atom are acidic and auxochromic; successive deprotonation results in a transition from a red aqueous solution (pH <1) to yellow (pH 1–2) to blue (pH ~8). The sulfonate group serves the function that it does in other benzaurin-like dyestuffs, namely, to sterically hinder the triphenylmethane structure from becoming a colorless carbinol by the addition of a hydroxyl group to the central carbon atom. The sulfonate group does not protonate even in very (pH <1) acidic media.

Although metallochromic indicators are always suitable as indicators in acid–base titrimetry, pyrocatecholsulphonephthalein is particularly well-suited since the end point is a sharp yellow to blue transition. The inference is that one could

analytical chemistry



ANALYTICAL CHEMISTRY at the Pittsburgh Conference in Atlantic City

Temperature-Programmed Gas Chromatographic Determination of Polychlorinated and Polybrominated Biphenyls in Serum

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U.S. Department of Health and Human Services, Public Health Service, Center for Disease Control, Clinical Chemistry Division, Toxicology Branch, Atlanta, GA 30333

An analytical method was developed to quantitate polychlorinated and polybrominated biphenyls (PCBs and PBBs, respectively) in human serum. The method includes denaturation of the proteins in serum, extraction, adsorption chromatography, and gas chromatography with electron capture detection. The coefficients of variation for determining the in vivo bound PCBs and PBBs ranged from 11.7 to 29.8% and 7.1 to 14.0%, respectively. The method is capable of measuring 10 ng PCBs and PBBs/mL in 4 mL serum.

As recognition of the extent of environmental contamination from chemicals increases, so will the need to analyze both environmental and human specimens for these contaminants. Because these compounds occur as mixtures, methods for analyzing the entire group are needed. The method reported here analyzes human serum for a series of compounds, polychlorinated biphenyls (PCBs), that have been released into the environment over a period of decades and for a smaller series of compounds, polybrominated biphenyls (PBBs), that have presumably contaminated a much more limited area. The Michigan Department of Public Health and the Center for Disease Control are using this method to monitor PCB and PBB levels in Michigan residents.

Commercial PCB mixtures vary in percentage by weight of chlorination, depending on the properties needed for a particular use; however, higher forms of mammalian life that have been exposed to all these mixtures selectively accumulate the more highly chlorinated components (1). In this study, PCBs were quantitated as Aroclor 1254, of which 54% of its weight is chlorine.

The decision to use Aroclor 1254 for quantitation was made on the following basis. Milk and fish products are reportedly the major sources of PCBs in humans (2), and market basket surveys conducted by the Food and Drug Administration showed that the gas chromatographic (GC) patterns of PCBs found in dairy and fish products most closely resembled Aroclors 1254 and 1260 (3). In a study conducted in the State of Michigan concerning PCB levels of residents who consumed fish from Lake Michigan, the PCB patterns in adipose tissue resembled Aroclors 1254 and 1260 (4). A source of the PCB contamination of dairy cattle was found to be silage that had been stored in PCB-coated silos; this PCB coating contained Aroclor 1254 (5). Another reason Aroclor 1254 was used in this study was that a preliminary investigation of a select number of serum samples from Michigan residents gave a GC pattern essentially similar to that found in goats that had been dosed with Aroclor 1254.

We were interested in developing a method that would analyze serum extracts for PCBs and PBBs so that the PBB results would reproduce those obtained from the same human serum that had previously been analyzed by isothermal GC with electron capture detection (ECD) (6). Preliminary attempts to separate the analytes by using adsorption chromatographic techniques and the more readily available adsorbents, Florisil, silica gel, silicic acid, and alumina, were unsuccessful.

We then considered approaches that would take advantage of differences in the ionic properties and molecular size of chlorine- and bromine-substituted biphenyls (7). Ion exchange resins utilize the slight difference in ionic character, but were not used because of the unpredictable character of these resins (8). Exclusion chromatography (9) utilizes the difference in molecular size and was evaluated by using 100 Å μ-Styragel columns in a high performance liquid chromatographic system; however, this system did not completely separate PCBs from PBBs.

Following these preliminary studies, we tentatively decided to follow an analytical approach that would provide simultaneous determination of both analytes. The objective then became the development of a method that would extract PCBs and PBBs from serum, provide an extract compatible with GC/ECD analysis and subsequent quantitation, and reproduce the previous

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PBB results. Previous data (4, 6) had established the efficiency of a hexane–ethyl ether extraction of methanol-denatured serum for PCBs and PBBs. Evaluation of the use of silica gel in various forms and stages of activation and deactivation (10–13) produced varying degrees of success in separating the commonly occurring chlorinated hydrocarbon insecticides from the halogenated biphenyls. On the basis of the evaluation, it appeared that procedures using deactivated silica gel provided a separation of most chlorinated hydrocarbons from halogenated biphenyls, good transferability, and processed a high volume of samples.

Evaluation of GC column packings to determine suitability for simultaneous analysis of PCBs and PBBs was done in the order of ascending value for McReynolds' constants of each liquid phase. The evaluation criteria consisted of: (1) peak-to-valley resolution of analytes; (2) total retention time; and (3) ability to withstand rapid programming and high temperatures with little column bleed. We noted that those liquid phases with the lowest McReynolds' constants, the methyl silicones, gave the best overall performance on the basis of our criteria.

A major problem in determining PCBs is the quantitation step, which is made increasingly difficult in biological samples by metabolism and/or elimination of some of the PCBs, so that the extracted product does not replicate any of the commercial PCB mixtures. Other studies (14–16) have shown that when gas chromatography is used with an electron capture detector, the method of Webb and McCall (17) gives improved interlaboratory precision and/or accuracy in quantitating PCBs as a particular Aroclor over methods that measure total peak height (or area) of all peaks or selected peaks.

On the other hand, PBB mixtures show fewer peaks than do PCBs when determined by packed column GC/ECD. Moreover, since the commercial product (Firemaster FF-1) consists largely of 2,2',4,4',5,5'-hexabromobiphenyl (18), quantitation is based on comparing the size of the hexabromobiphenyl peak in the sample to the size of this peak in a known weight of the commercial material (6).

For the quantitation of PCBs, we adapted the method to a microprocessor-controlled data system. The quantitation of PBBs is based on the largest peak in the Firemaster standard and is also accomplished by the microprocessor-controlled data system. This system has been described elsewhere (19).

METHOD

Apparatus

(a) *GLC/data system.*—Varian 3700 gas chromatograph (Walnut Creek, CA) equipped with Hewlett-Packard Model 7671A automatic injector (Avondale, PA), constant current ^{63}Ni electron capture detector, and Varian Model A-25 strip chart recorder. Chromatograph was controlled and data were calculated by Varian CDS 111(c) microprocessor.[2] Operating conditions: temperatures—column held at 190°C and programmed after PCBs elute (ca 21 min) to 230°C at 10°/min; detector 330°C, injection port 250°C; nitrogen flow 20 mL/min.

(b) *GLC column.*—Glass, 6 ft × 1/8 in. id, packed with performance-tested 3% SE-30 on 80–100 mesh Gas-Chrom Q (Applied Science Laboratories, Inc., State College, PA).

(c) *Chromatographic column.*—Glass, 18 cm × 9 mm, packed with 3.0 g 70–150 mesh silica gel (Woelm) containing 3% water and sandwiched between 1 cm layers of anhydrous Na_2SO_4.

Reagents

(a) *Solvents.*—*n*-Hexane, methanol, and ethyl ether (Distilled-in-Glass, Burdick and Jackson, Muskegon, MI).

(b) *Sodium sulfate.*—Anhydrous, reagent grade, washed with hexane and continually oven-dried at 130°C. Remove from oven and let cool in desiccator before use.

(c) *Silica gel.*—Weigh silica gel into beaker, cover with aluminum foil (punch several holes in foil) and let stand in 130°C oven for at least 24 h. Let silica gel cool in vacuum desiccator. Weigh dried gel into flask with Teflon-lined screw cap. Add water down sides of flask in amount to constitute 3% of total weight. Extract water beforehand 3 times with hexane. Shake wetted gel until there is no evidence of clumping. Rotate 3 h on mechanical rotator and let stand tightly capped overnight before use. Silica gel prepared in this manner maintains its elution characteristics for at least 7 days.

(d) *Standards.*—PCB (AR 1254, EPA STD 5700E) was provided by Environmental Protection Agency. PBB (Firemaster FF-1, Lot 7042) was provided by Farm Bureau Services, Inc. (Lansing, MI) via the Food and Drug Administration. Standard solutions, from which cali-

Use of a trade name is for identification only and does not constitute an endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

[2] A complete description of programming the Varian CDS 111(c) for this determination can be obtained from the authors.

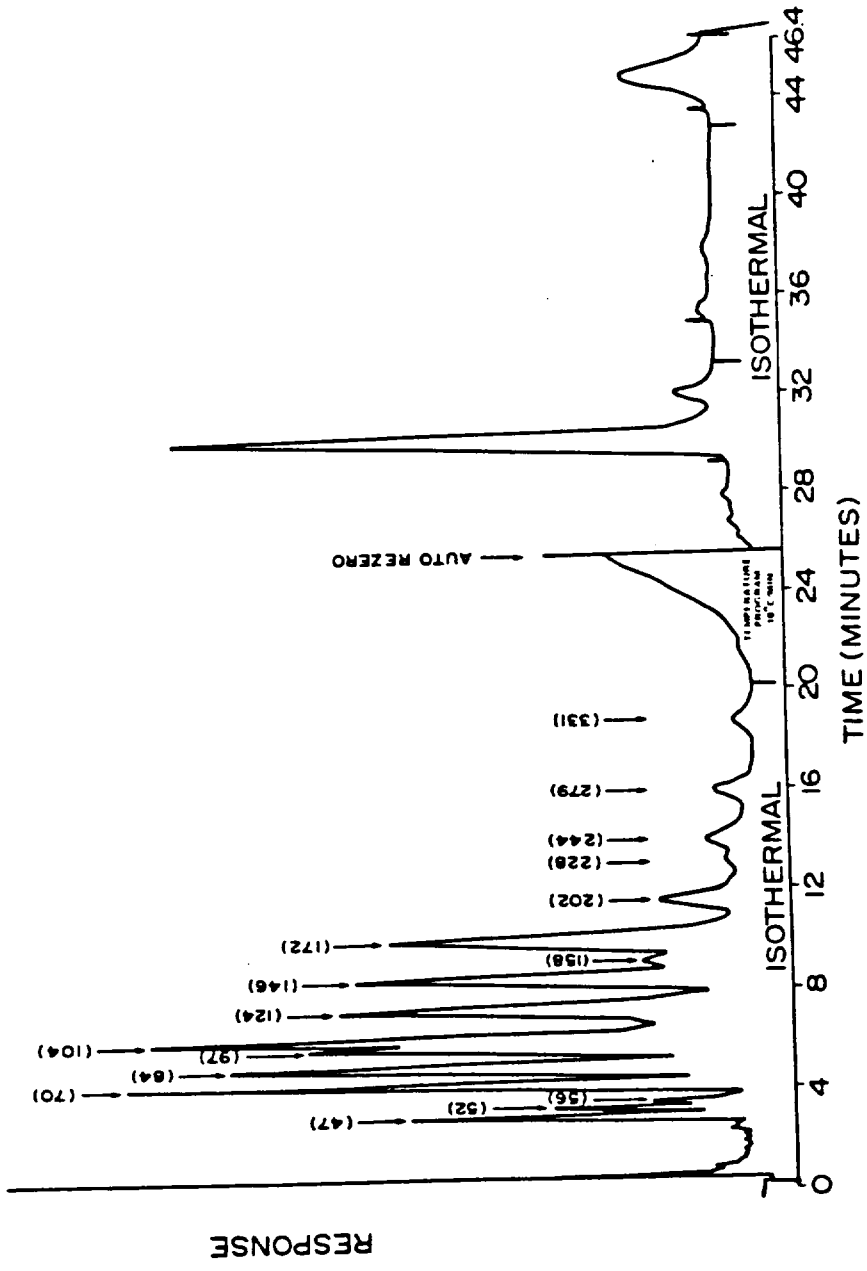

Figure 1. GC chromatogram of 3 μL of a mixed PCB/PBB standard containing 143.5 pg PCB/μL and 23.8 pg PBB/μL. Conditions as described in text.

Table 1. Estimates of precision on analyses [a] of in vivo pools containing PCBs and PBBs

Pool	N	PCB Target, [b] ppb	PCB Obtained (mean), ppb	PCB CV, %	PBB Target, [b] ppb	PBB Obtained (mean), ppb	PBB CV, %
A	49	10	10.5	13.1	10	8.6	8.4
B	48	42	41.2	15.2	164	138.6	8.7
C	46	10	12.7	23.7	41	34.8	14.0
D	47	100	95.3	11.7	10	8.9	13.8
E	44	10	12.5	29.8	484	426.9	7.1

[a] See text for pool description. Data analyzed by one-way analysis of variance.
[b] Target value is defined as the projected concentration resulting from the dilution of serum taken from cattle exposed to Aroclor 1254 and Firemaster FF-1 with PCB/PBB-free bovine serum.

bration factors are obtained, were prepared in hexane. These 4 mixed standards ranged from 430.5 pg AR 1254/μL and 95.1 pg PBB/μL to 41.0 pg AR 1254/μL and 9.5 pg PBB/μL.

Extraction

Accurately pipet 4 mL serum into 16 × 125 mm culture tube, add 2 mL methanol, and swirl briefly by vortex. Add 5 mL hexane–ethyl ether

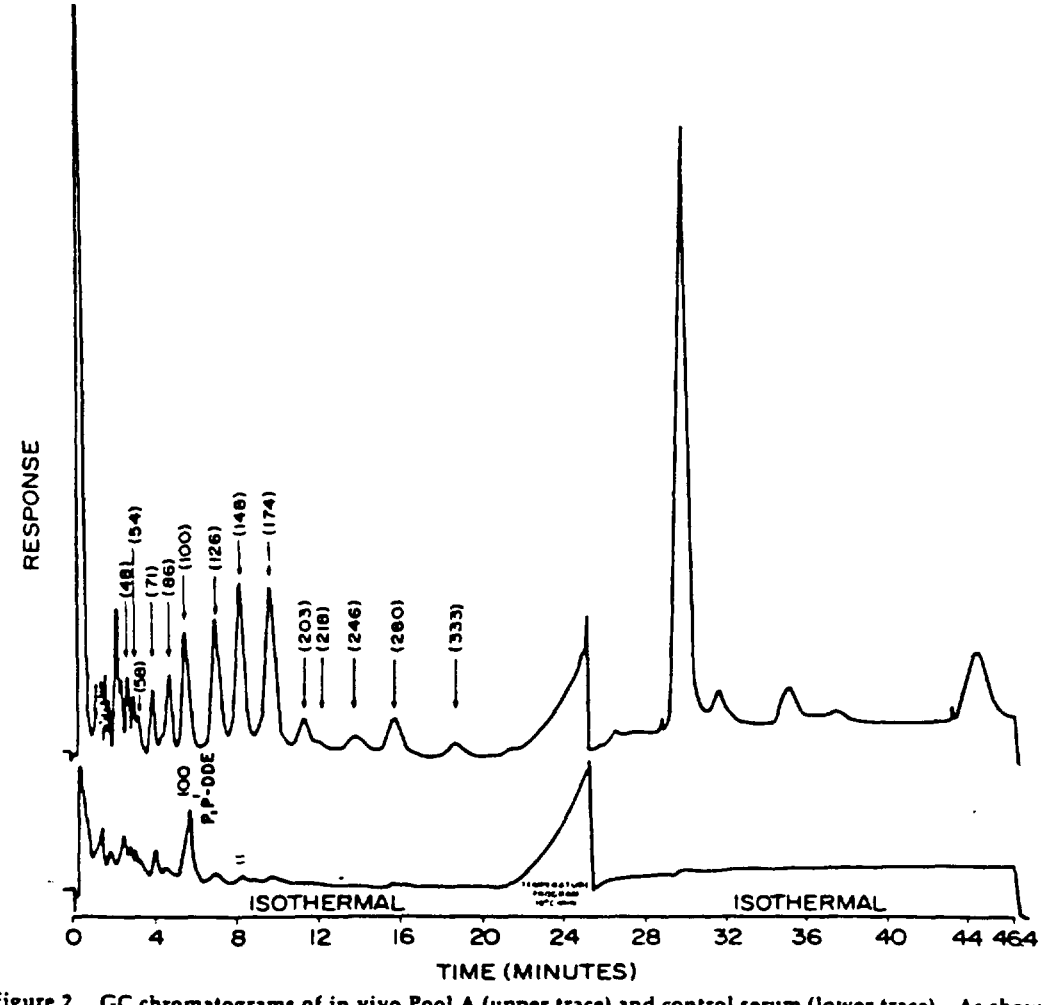

Figure 2. GC chromatograms of in vivo Pool A (upper trace) and control serum (lower trace). As shown in Table 1, the concentrations of PCBs and PBBs were approximately 10 ppb in this serum pool. Injection volume was equivalent to 12 mg of sample. Conditions as described in text.

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:Bs and PBBs

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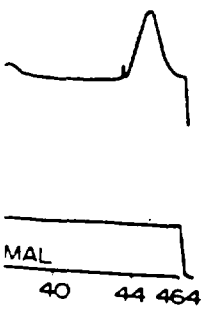
8.6	8.4
138.6	8.7
34.8	14.0
8.9	13.8
426.9	7.1

n taken from cattle exposed

n into 16 × 125 mm
methanol, and swirl
mL hexane–ethyl ether



ver trace). As shown
erum pool. Injection
in text.

(1 + 1), agitate 15 min at 50–55 rpm on rotary mixer, centrifuge 6 min at 1800 rpm, and transfer supernatant to 20 × 150 mm culture tube. Repeat this extraction step twice and combine extracts. Reduce solvent volume, at room temperature, to ca 0.5 mL under gentle stream of nitrogen.

Adsorption Chromatography

Prewash column containing silica gel with 20 mL hexane. Transfer concentrated extract to top of column. Rinse sample tube with three 0.5 mL portions of hexane and transfer each wash to head of column. Elute column with 5 mL hexane; discard first 7 mL; collect 15 mL in conical tube which has been calibrated to 1 mL. Concentrate eluate to 1 mL by blowing gentle stream of nitrogen into eluate which is heated to 40°C in a water bath. Analyze by GC/ECD.

Gas Chromatography

Inject 3 μL of PCB and PBB mixed standards. Having calculated various absolute calibration factors by external standard method (19), inject 3 μL of an appropriate aliquot of extract. Derive PCB and PBB contents of unknowns by comparing their peak areas to that of the mixed standards.

Results and Discussion

Our chromatogram of Aroclor 1254 (Figure 1) compares favorably with that of Webb and McCall (17), to which they assigned their factors. The main difference is that our chromatogram shows 3 additional peaks at relative retention times of 244, 279, and 331. Also, our chromatogram includes the response for PBB. However, the peaks 244, 279, and 331 are not characteristic of PBBs, because our GC recordings of PCB standards without added PBB give these 3 peaks. We believe the differences may be attributed to any one or combination of the following: (1) differences in the lots of AR 1254 employed in the 2 studies; (2) differences in resolution of the GC columns used; (3) differences in the response characteristics of the different electron capture detectors to certain PCB isomers.

Certain characteristics of the GC tracing led to difficulty in obtaining reproducible calibration factors (or response factors) for certain peaks, i.e., the CDC 111(c) microprocessor did not consistently detect baseline at the same point within concentrations and especially among concentrations of the Aroclor 1254 standards. This resulted in considerable variation by area counts for the same peak. This problem was especially

Table 2. Statistics[a] associated with calibration factors at 4 concentration levels

	Within concentration[b]												Among concentrations[b]		
	Std A			Std B			Std C			Std D			Stds A,B,C,D		
Webb-McCall ID No.	$\bar{X}$	CV (%)	N	$\bar{X}$	CV (%)	N	$\bar{X}$	CV (%)	N	$\bar{X}$	CV (%)	N	$\bar{X}$	CV (%)	N
47, 54, 58 group	4 812	6.1	14	4.701	6.4	13	4 506	7.0	15	4 319	8 2	13	4 182	12 1	55
70	3 988	5.4	14	3 882	5.5	13	3.736	6.2	15	3 684	8 9	13	3 822	7 2	55
84	3.752	5.5	14	3 632	6.3	13	3 483	6.8	15	3 435	9 9	13	3 576	7 6	55
98	2.378	6 0	14	2.334	5.4	13	2.246	6.2	15	2 069	17 4	13	2 258	10 4	55
104	3 055	4 8	14	2.946	6 2	13	2.838	7.1	15	2.759	11 0	12[c]	2 903	8 1	54
125 group	2 462	6.3	14	2.418	6 8	13	2.366	6 2	15	2.550	10 2	13	2 446	7 8	55
146, 160 group	1 836	5 8	14	1.802	7.0	13	1.763	6.8	15	1 888	9.8	13	1 816	8 2	55
174 group	1.318	6 3	12[c]	1.258	11.7	13	1.404	16.9	13[c]	1 490	21 0	8[c]	1 355	15 6	46
203, 232 group	0 839	9 8	14	0 874	8 8	13	0.880	8.7	15	1.045	17.2	12[c]	0 904	14 5	54
PBB (major peak)	2.523	4.2	14	2.442	5.2	13	2.255	8.2	15	2.144	13 8	13	2.341	10 2	55

[a] Among run statistics include: $\bar{X}$ = mean of calibration factors; CV (%) = coefficient of variation; N = number of determinations.
[b] Concentration (pg/μL hexane) of standards A, B, C, and D were: (A) PCB = 430 5, PBB = 95.1; (B) PCB = 287, PBB = 47.6; (C) PCB = 143.5, PBB = 23.8; and (D) PCB = 41. PBB = 9.5.
[c] Integration malfunction resulted in a smaller N.

Table 3. Potential environmental interferences calculated as PCBs and/or PBBs

Compound	Level of in vitro spike (ppb)	Total PCB level (ppb)[a]	PBB level (ppb)[a]	PCB interference factor	PBB interference factor
Halowax 1001	48.8	11.06	0.07	0.2266	0.0014
Decachlorobiphenyl	50.0	1.77	11.24	0.0354	0.2248
Halowax 1051	51.2	6.95	N.P.D.	0.1357	0
AR 5432	47.8	1.86	N.P.D.	0.0390	0
AR 5442	51.0	1.16	0.015	0.0242	0.00029
AR 5460	60.0	1.34	0.035	0.0223	0.0006
Butylphthalyl butylglycolate	150 000	1.10	0.09	7.3×10^{-6}	6×10^{-7}
Di-2-ethyl hexyl phthalate	138 800	1.54	0.02	11×10^{-6}	1×10^{-7}
Di-2-ethyl hexyl adipate	140 000	1.16	0.035	8.2×10^{-6}	2×10^{-7}
Mixed chlorinated hydrocarbon standard[b]	10 (each component)	35.06[c]	0[d]	0.3506[d]; 3.506[e]	0
trans-Nonachlor	6.0	0[f]	0	0	0
Oxychlordane	2.5	0	0	0	0
Toxaphene	100	3.40	0	0.034	0
Mirex	12.2	4.2	0	0.34	0

[a] Average of duplicates.
[b] Hexachlorobenzene, γ-hexachlorocyclohexane, β-hexachlorocyclohexane, heptachlor epoxide, *o,p'*-DDE, *p,p'*-DDE, dieldrin, *o,p'*-DDT, *p,p'*-DDD and *p,p'*-DDT.
[c] Single value. *p,p'*-DDE accounted for 94.8% of the PCB concentration.
[d] Considering concentration of all 10 components.
[e] Considering concentration of only *p,p'*-DDE.
[f] Elutes from adsorption column with PCBs but GC retention time precludes calculation as a PCB.

evident for PCB peaks 56, 124, 146, 158, and 172. However, this problem was overcome through grouping. Grouping is a process whereby areas of several peaks or a broad peak are combined and treated as a single peak; the retention time assigned is that of the midpoint of that group. Peaks 244, 279, and 331, for which there were no assigned Webb and McCall factors, were combined with peaks 202 and 228. Since Webb and McCall's mean weight percent factor for Aroclor 1254 accounted for 100% of the PCBs, this group was assigned a mean weight percent value of 2.8, which is the sum of values (1.8 + 1.0) originally assigned by Webb and McCall for peaks 203 and 232, respectively.

To better estimate the precision of this method, pooled material containing in vivo bound PCBs (as AR 1254) and PBBs was repeatedly evaluated before and during the analysis of unknowns. In the data shown in Table 1, each of the pools was analyzed in duplicate on 11 different days before the analyses of unknown samples, and 3 pools were included with each run of unknowns; this resulted in a minimum of 44 analyses for each pool. In general, the coefficients of variation (CV) were lower for the PBBs. Figure 2 is a typical gas chromatogram of Pool A (see Table 1) and the bovine control.

The variation in any procedure is the sum of all variations from various aspects of the method. We have evaluated the contribution to the total variation made by the automated instrumentation through the statistical examination of calibration factors for standards at 4 concentration levels. These calibration factors were generated over a series of analytical runs and are displayed in Table 2. The CV determined from the among-run "within-concentration" statistics increased for all analytes as the concentration of the standard decreased. The among-run "among-concentration" statistics indicate a CV of ≥10% for grouped peaks 47–58, 174, and 203–232; for the PCB peak 98; and for the major peak in PBB. Nonetheless, this agreement among the calibration factors demonstrates the linearity of the system over the concentration range studied.

We also evaluated the contribution of the adsorption chromatographic step to the total variation in the PCB and PBB data. This was done by monitoring the recoveries from the elution step of an extract of serum that originally had been PCB/PBB-free, but after extraction was fortified with PCBs and PBBs at 26 and 119 ppb, respectively. The PCB and PBB recoveries were measured daily for 7 days following silica gel deactivation; the analyses of both PCBs and PBBs had CVs of 5.9%.

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We acknowledg
Deborah Blanton

To determine if the method gave false-positive results for PCBs and PBBs, we analyzed sera from 25 employees of the Center for Disease Control. The PBB values ranged from undetectable to <1 ppb, and the PCB values ranged from 2 to 18 ppb. These data confirm the ubiquitous character of PCBs and indicate that exposure to PBBs is much more limited geographically.

Table 3 shows the results of an experiment designed to evaluate the quantitative effect on the PCB and PBB values by potential interferants by means of an "interference factor," i.e., the ratio of the apparent PCB or PBB concentration measured to the concentration of the interfering substance. For example, a value of 1.0 for the PCB interference factor for a contaminant indicates that the method gives the same response for that analyte when quantitated as Ar 1254 as does an equal amount of AR 1254. Decachlorobiphenyl gave the greatest interference for PBBs, while Halowax 1001, Halowax 1051, the mixed chlorinated hydrocarbon standard (particularly *p,p'*-DDE), and mirex significantly interfered with the quantitation of AR 1254.

In our use of this method, we routinely analyze control bovine serum that has been fortified with chlorinated hydrocarbons at levels comparable with the general population (20) so that we can detect any undesirable elution characteristics exhibited by the silica gel. This method is capable of determining 10 ng PCBs and PBBs/mL in 4 mL serum; however, based on the response of in vivo Pool A (Figure 2), lower detection limits of 5 and 1 ng/mL are achievable for PCBs and PBBs, respectively.

Acknowledgment

We acknowledge the technical assistance of Deborah Blanton and David Bagley.

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Long-term Studies of the Excretion of Polychlorinated Biphenyls (PCBs) Through the Mother's Milk of an Occupationally Exposed Worker

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Abstract. Long-term studies of the excretion of polychlorinated biphenyls (PCBs) through the mother's milk were conducted on an occupationally exposed worker, who worked with PCBs in a capacitor factory.

PCBs level in the mother's blood was 57 ppb, a level 10 to 20 times higher than that of non-exposed persons. The other tissues and fluids obtained at time of delivery were also assayed. PCB contents in umbilicus tissues, umbilicus blood and amnion fluid were 1/5, 1/4 and 1/6, respectively, the amount found in the mother's blood.

An approximate 76% decrease in PCB levels in the milk were noted 16 months after delivery; 200 mg of PCBs were excreted in about 818 liters of milk for this 16-month period.

A detailed analysis of the individual components in PCB residues clarified the remarkable changes of the PCB pattern in the blood.

Since PCBs in mother's milk were detected in the high concentration range of 0.01 to 0.7 ppm on the whole milk basis in Japan in 1972 (Isono 1972), many research institutes have been investigating the environmental behavior of PCBs. Therefore, the residue levels to be expected and related new information became available from these studies (Takeshita *et al.* 1974, Hashimoto *et al.* 1976, Shimada 1976, Nakayama and Aoki 1977, Nishimura *et al.* 1977).

In our investigation of PCBs in blood and mother's milk of non-exposed women for 6 years, we have been studying the problem of breast-feeding through the su-vey of PCBs. The levels of PCB in mother's milk and blood were almost stable from 1972 to 1977; in the range of 0.03 to 0.04 ppm on the whole milk basis (Yakushiji *et al.* 1977a, b). An average intake of 150 ml/kg of milk per day per baby would mean that each baby would consume approximately 4.5 to 6 μg/kg of PCB's per day. Then one-half the number of Japanese babies would ingest larger

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amount of PCBs than that of the allowable temporary tolerance of Japan (5 μg/kg/day). In a recent study, it is clear that remarkable amounts of PCBs transfer to suckling babies through mother's milk (Kuwabara *et al.* 1978). Actually, the PCB levels in milk after the first-delivery are higher than the levels observed after the second-delivery (Yakushiji *et al.* 1977a, b).

We had the opportunity of examining PCBs in the mother's milk of a 36-year-old housewife who was exposed to PCBs (Kanechlor® 300 and 500; chlorine: 43% and 55% respectively) in a capacitor factory from 1959 to 1966. The exposed worker, diagnosed as a chronic case of PCB intoxication, delivered her first child 13 years after marriage and two years after fasting treatment for the intoxication. The details were reported by Sagami (Sagami *et al.* 1973, Sagami 1977).

In this report, we describe a long-term investigation of the excretion of PCBs through the mother's milk, the PCB levels in the tissues obtained at time of delivery, and the changes of the PCB pattern in subsequent samples over a period of time.

Materials and Methods

Samples

Placenta, umbilicus tissues, umbilicus blood, amnion fluid and omentum adipose tissue were obtained at delivery on July 29, 1975. The blood PCBs level of the subject was high enough to recommend abandonment of breast-feeding. However, milk was collected every day from the mother for analysis. The milk volumes collected were recorded. Forty-one milk samples were analyzed for PCBs at regular intervals and 16 samples were also analyzed for *p,p'*-DDE after delivery. Nine blood samples were taken, as indicated in Figure 3, and subcutaneous adipose tissue was also taken for PCB analysis in November 1976.

Analytical Methods

The fat was extracted from the milk, omentum adipose tissue and subcutaneous adipose tissue with ethanol, ether and n-hexane. The fats, tissues and fluids were saponified with 2N KOH-ethanol solution on a water bath for 1 hr (Wakimoto and Tatsukawa 1972). The PCBs were extracted with n-hexane followed by Florisil column chromatography (Yakushiji *et al.* 1978). The eluates were concentrated and injected into a gas chromatograph.

Identification and Quantitation of PCBs

Individual components of the PCB residues were identified by mass fragmentography and mass chromatography (Yakushiji *et al.* 1978). Figure 1 shows the gas chromatographic separation on a 2% OV-1 column of PCBs in the mother's milk. It was confirmed by GC-MS analysis that the peaks 4, 9, 11, 15, 16, 18, 20, 21 and 22 numbered by the OV-1 system (Ugawa 1973) were PCBs and that no other contaminants had interfered at the same retention times on the GC. Therefore, calculation of the PCBs were made for these peaks.

The quantitation of PCBs in mother's milk as Kanechlor 500 is not accurate, since the pattern of PCB extracted from the milk or the blood are quite different from that of Kanechlor 500. Therefore, the 2% OV-1 column analysis proposed by Ugawa (1973) was used for the calculation of the PCB content of the samples. However, for the detailed analysis of the individual components of PCB residues, the 2% Apiezon L column analysis proposed by Jensen and Sundström (1974a) and applied by Nakamura (1977) was used. Two different GC numbering systems were used and they conform to those used in these references.

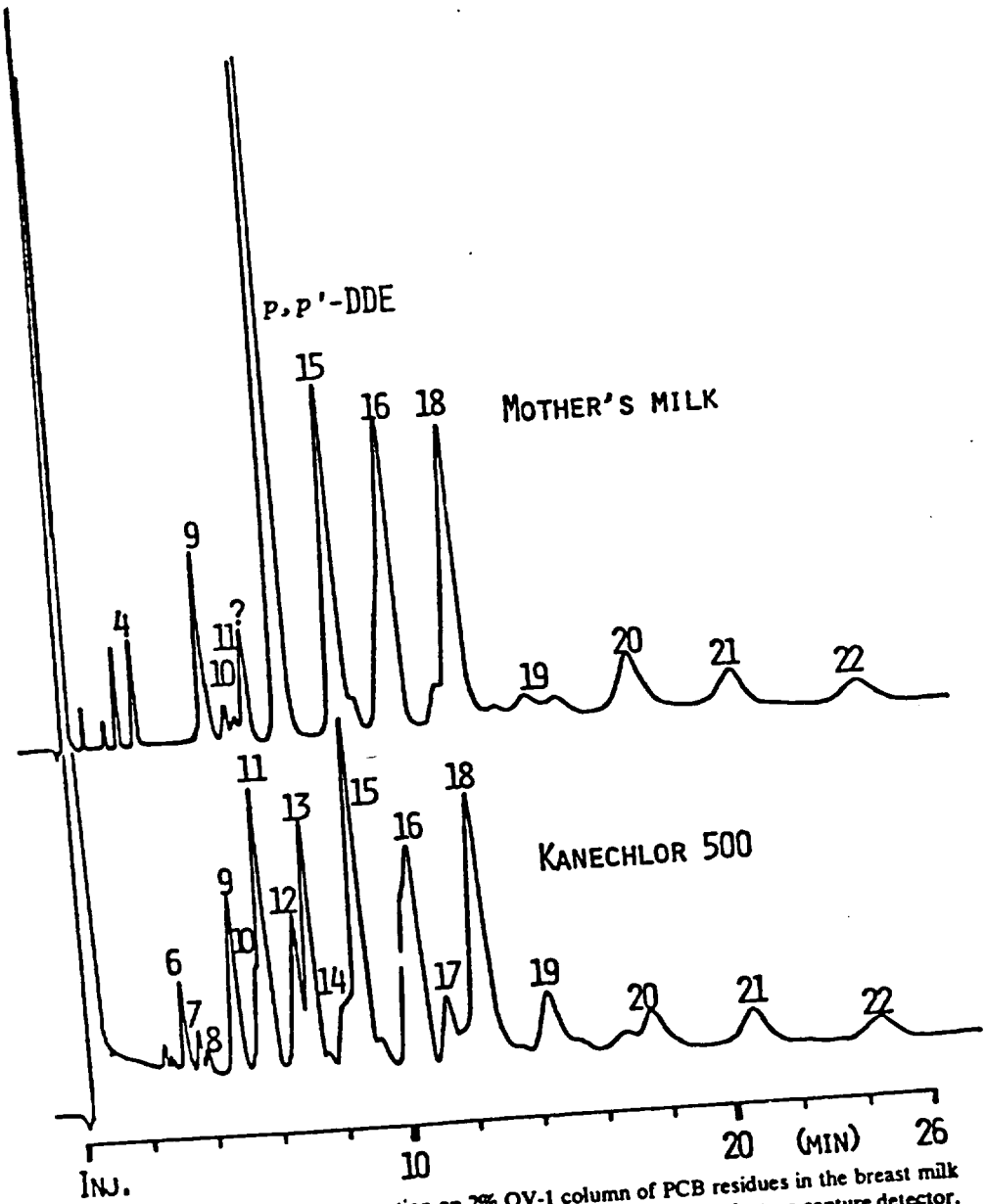

Fig. 1. Gas chromatographic separation on 2% OV-1 column of PCB residues in the breast milk and Kanechlor 500(Aroclor 1254) Varian 2100 Gas chromatograph, with electron capture detector, fitted with a glass column (1.8 m × 2 mm) packed with 2% OV-1 on Gas Chrom Q (100/120 mesh). Temperature: Detect. 210°C; Inject. 210°C; Column 180°C-Carrier gas: N_2 at 30 ml/min. Kanechlor 500: Chlorine = 55%.

Table 1. Levels of PCB in the tissues and fluids.

Sample	Sampling date	PCB concentrations	
		Fat basis ppm	Whole basis ppm
Placenta	July '75	—	0.056
Umbilicus tissues	July "	—	0.011
Umbilicus blood	July "	—	0.016
Mother's blood	July "	—	0.057
Amnion fluid	July "	—	0.010
Omentum adipose tissue	July "	16.1	12.2
Subcutaneous adipose tissue (taken from the mother)	Nov. '76	4.1	4.0
Baby's blood	Nov. '75	—	0.003
Baby's blood	Nov. '76	—	0.004

Table 2. Estimation of the excretion of PCBs through the breast milk

Month and year	N[a]	Fat[b] %	PCBs concentration		Milk[e] ml	PCBs excreted[f] mg
			Fat basis[c] ppm	Whole basis[d] ppm		
8 '75	9	2.71	13.6	0.37	49380	18.3
9	3	2.98	15.8	0.47	63445	29.8
10	3	1.87	15.2	0.28	75500	21.1
11	2	1.80	13.2	0.24	77700	18.6
12	1	2.01	11.2	0.23	74420	17.1
1 '76	2	2.22	10.1	0.22	62700	13.8
2	2	2.65	8.5	0.23	60990	14.0
3	2	2.55	9.6	0.24	66850	16.0
4	2	3.59	8.3	0.30	65100	19.5
5	2	2.22	5.0	0.11	57290	6.3
6	2	3.18	5.0	0.16	45240	7.2
7	2	3.47	5.3	0.18	38320	7.0
8	2	3.72	4.3	0.16	35930	5.7
9	2	3.42	3.5	0.12	27720	3.3
10	2	2.37	3.8	0.09	15030	1.4
11	2	4.37	3.8	0.17	2715	0.5
		Total			818320	199.6

[a] Number of samples
[b] Monthly averages
[c] $b \times c \times 10^{-2}$
[e] Monthly milk volumes collected
[f] $d \times e \times 10^{-3}$

PCBs in Mother

Results

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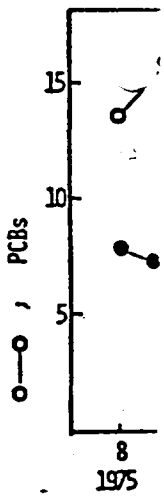

Fig. 2. Decline

Results

Excretion of PCBs

Table 1 shows the PCB levels in the tissues and fluids obtained at time of delivery and in the blood of the baby. The concentrations of PCB in umbilicus tissues, umbilicus blood and amnion fluid were 1/5, 1/4 and 1/6, respectively, the amount found in the mother's blood. However, the level of PCBs in the placenta was almost equal to that of the mother's blood. These results indicate that PCBs were transferred from the mother to her foetus. The PCBs level in the mother's blood was 10 to 20 times higher than that of non-exposed women (Watanabe *et al.* 1976). Consequently, the new-born was not given breast milk. Therefore, the blood PCBs level of the infant was fairly low, at 0.003 ppm 4 months after delivery, which was 1/19 of the level of the mother's blood.

Long-term investigations of PCB contents in the milk and blood were continued for 16 months after delivery. As previously described, 41 samples of milk were analyzed for PCBs at regular intervals. The data presented in Figure 2, which indicate monthly averages, show an approximately 76% decrease in PCB levels from August 1975 to November 1976. The half-life periods of PCBs and *p,p'*-DDE were both about 8 months. After 8 months, the rates of decline in concentration were much less.

After the recommendation to abandon breast-feeding, milk was collected every day and the volumes were checked until November 1976, when the secretion of milk stopped. In Table 2, the PCB content on the whole milk basis was conveniently calculated by the multiplication of the mean value of fat-% by that of the PCB content in the milk fat. In 9 months, approximately 170 mg of PCBs were excreted, and a total of 199.6 mg of PCBs were excreted for the 16-month period ending November 1976. Table 1 shows that the PCB content in subcutaneous adipose tissue of the mother at this time was 4.1 ppm and was 1/4

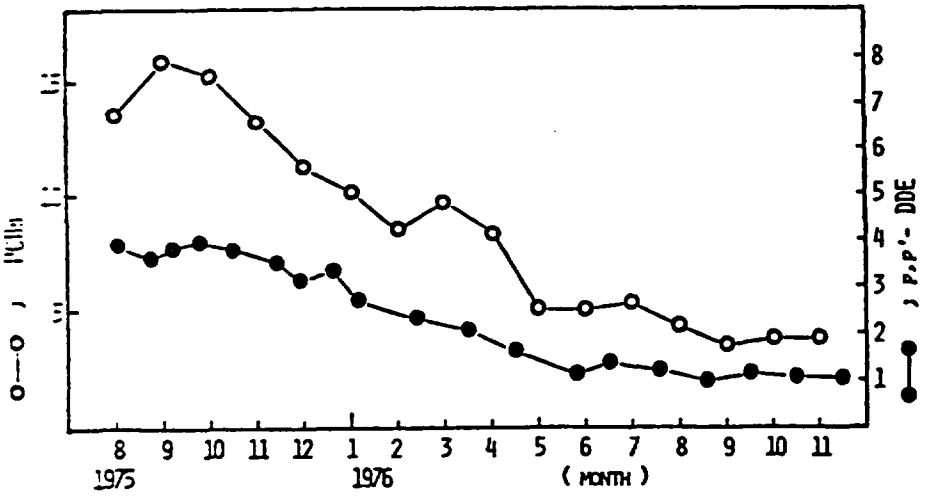

Fig. 2. Decline in concentration of PCBs and *p,p'*-DDE in the breast milk, ppm on fat basis.

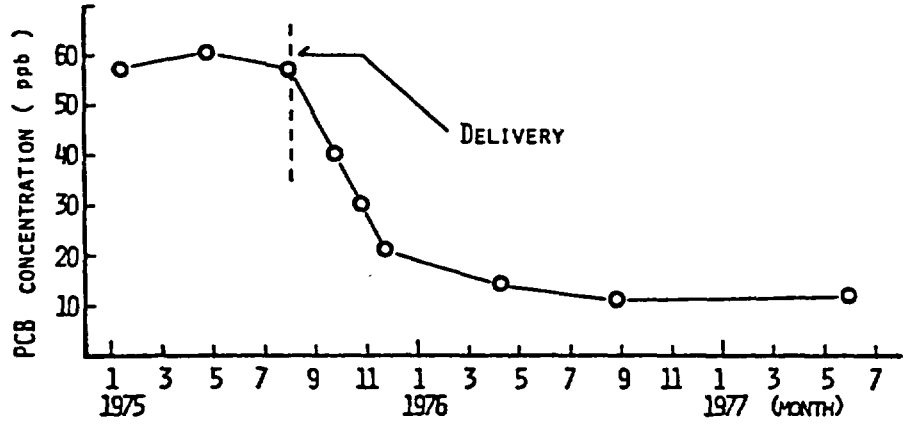

Fig. 3. Decline in concentration of PCBs in the mother's blood

the amount found in omentum adipose tissue obtained at delivery. This percentage decrease in concentration was similar in the milk fat.

Figure 3 shows the time course of PCBs concentration in the blood. Blood PCB levels were almost constant before delivery. Whereas, the sudden decrease in blood PCB concentration was roughly parallel to the decrease in breast milk. The final blood PCB level was 1/5 the amount found before delivery.

Pattern Analysis

Additional studies were made using the Apiezon L gas chromatograph column to clarify the changes of individual components of PCBs in the samples. The second GC numbering system was used. Table 3 indicates the relative percentage of each PCB component in the tissues and fluids. Peak 32 was defined as 100% for the calculation of the relative chlorobiphenyl percentage of each component. PCB patterns of low-chlorinated biphenyls of the placenta was rather similar to

Table 3. PCB patterns in the tissues and blood

Samples	Sampling date	Relative percentages of chlorobiphenyls[a]									
		k 3	9 4	11 4	25 5	28 5	29 6	32 6	47 6	49 7	50[b] 7[c]
Omentum adipose tissue	July '75	31	92	14	97	25	84	100	17	14	8
Mother's blood	July "	40	109	20	122	28	86	100	19	14	7
Placenta	July "	43	100	20	93	27	81	100	17	21	11
Baby's blood	Nov. "	81	141	37	75	23	90	100	13	41	18

[a] Peak 32 was defined as 100%
[b] Peak number by Apiezon L column
[c] The numbers of chlorines

Table 4. Changes of PCB patterns in the mother's blood

	Relative percentages of chlorobiphenyls[a]									
Sampling date	*k*[b] 3	9 4	11 4	25 5	28 5	29 6	32 6	47 6	49 7	50[b] 7[c]
Jan. '75	50	104	23	116	28	79	100	17	13	7
Sep.	40	109	20	122	28	86	100	19	14	7
Apr. '76	34	88	16	97	24	88	100	20	16	9
Aug.	20	72	14	79	21	87	100	21	20	11
May '77	16	64	10	73	17	92	100	23	24	15

[a] Peak 32 was defined as 100%
[b] Peak number by Apiezon L column
[c] The numbers of chlorines

that of the mother's blood. However, in the omentum adipose tissue, a slight difference was observed. Remarkable differences in the PCB pattern were recognized between the mother's and the infant's blood. A significant decrease in the relative percentage of some chlorobiphenyls occurred between the first and last sampling of the mother's blood, *i.e.*, peak *k* = 50% to 16%, 9 = 104% to 64%, 25 = 116% to 73% and 28 = 28% to 17% (Table 4, Figure 4). These components were relatively low-chlorinated biphenyls. In contrast, the relative percentages of peaks 29, 47, 49 and 50 increased considerably compared with the peak 32. The same changes in the PCB pattern were observed in the breast milk.

Discussion

Excretion of PCBs

The excretory route of organochlorine compounds from mammals have been previously evaluated (Morgan and Roan 1972, Fries *et al.* 1973). There are several reports of animal experiments with high level exposure to PCBs (Grant *et al.* 1971, Hashimoto *et al.* 1976, Hayes *et al.* 1971), but only a few report any low-level exposure data. Price *et al.* (1972) reported pesticide residues and PCB levels in diets, urine, and fecal matter of preadolescent girls. In their experiments, 88% of the ingested PCBs were not excreted and, presumably, they were retained in the body. In our previous study, the time course of blood PCB levels of exposed workers, non-exposed persons and Yusho patients did not show any tangible decrease (Watanabe *et al.* 1977). From these data, urine and feces seem to be the minor excretory route of PCBs from the human body.

It was recognized that large amounts of PCBs were excreted through the mother's milk, and it may be assumed that this was the major excretory route. Our studies with occupationally exposed workers, non-exposed persons and their children showed that PCB levels in infants' blood were highly influenced by

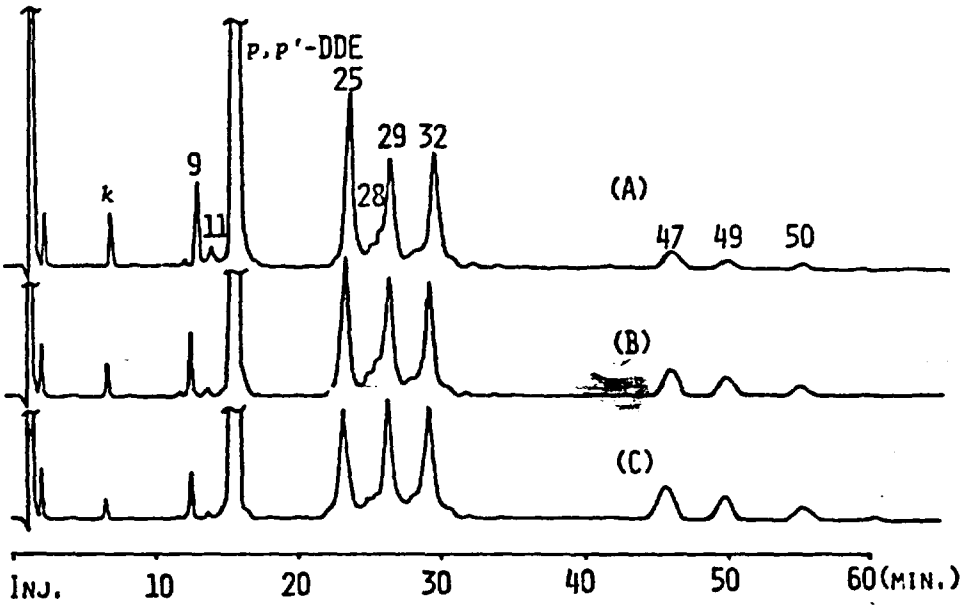

Fig. 4. Changes of the PCB pattern in the mother's blood.—Gas chromatographic separation of PCBs in the mother's blood—Shimadzu GC-6A type, with electron capture detector, fitted with a glass column (5 m × 2 mm) packed with 2% Apiezon L Grease on Gas Chrom Q (100/120 mesh). Temperature: Detect, Inject, 270°C; Column 250° C. Carrier gas N_2, 30 ml/min. Blood samples were obtained in Jan. 1975(A), Apr. 1976(B) and May 1977(C). Molecular compositions; *k* = 2,4,4', 9 = 2,4,5,4', 25 = 2,4,5,3',4', 28 = 2,3,4,3',4', 29 = 2,4,5,2',4',5', 32 = 2,3,4,2',4',5', 47 = 2,3,4,5,3' 4', 49 = 2,3,4,5,2',4',5', 50 = 2,3,4,2',3',4',5'.

the feeding period of breast milk. In many cases, PCB levels in the infants blood were the same or higher than that of the mothers with only a 3-month feeding period of the infant. The highest PCB level in the blood of workers' children was 93.2 ppb, while that of the mother was 18.3 ppb (Kuwabara *et al.* 1978).

Approximately 200 mg of PCBs were excreted from August 1975 to November 1976, in a total of 818 liters of breast milk. The data in Table 2 indicates that the average daily excretion of PCBs for the first 6 months was approximately 660 μg. The average PCB concentration of the first 6 months was estimated to be 0.30 ppm on the whole milk basis. A calculation of an average daily intake of 150 ml/kg of milk by the babies would mean that new-borns would consume 45 μg/kg of PCBs per day. Other data show that Yusho patients[8] had consumed approximately 70 μg/kg of PCBs per day (Kuratsune *et al.* 1972). As a comparison, Allen *et al.* (1973, 1974, 1976) showed that where adult rhesus monkeys exposed to 2.5 to 5.0 ppm PCBs on a continuous basis for 18 months, the infants consumed 30 to 80 μg/kg of PCBs per day. Within 2 months following birth, the infants had facial

[8] In March of 1968, PCB poisoning occurred in the northern and western parts of the Japanese island of Kushu. The disease was named "Kanemi Yusho." Yusho patients each consumed Kanemi Rice Oil containing 0.5 to 2 g of PCBs during a 3-mo. period (Umeda 1972).

acne. edema and other problems caused by the consumption of milk contaminated with PCBs. Based on these data, if the new-born in our study had consumed the mother's milk at least for 6 months, it might have ingested the same amount of PCBs as the Yusho patients or the infant monkeys of Allen's experiment.

Masuda *et al.* (1974) found 0.03 to 0.06 ppm of PCBs in 5 samples of breast milk collected from a woman with Yusho within 5 days after delivery. These levels were much lower than that of the worker reported in this study. Yoshimura (1974) also reported a case of infants with Yusho caused by the consumption of PCB-contaminated milk.

Pattern Analysis

The distribution and metabolism of PCBs in mammals and treated animals have appeared in many reports, and it was recognized that highly-chlorinated biphenyls accumulated in adipose tissues; however, low-chlorinated biphenyls were rapidly excreted from the body (Bush *et al.* 1974, Jensen and Sundström 1974 a.b, Gage and Holm 1976). From their data, this tendency has been considered to be a common one. Among the residual components of PCBs in the human body, highly-chlorinated biphenyls which have 5 to 7 chlorine atoms have already been identified by GC-MS. However, low-chlorinated biphenyls such as tri- or tetrachlorobiphenyls have not been clearly identified, because of the interference of contaminants.

In our experiments large amounts of tri- and tetrachlorobiphenyls were identified in the mother's milk, which were approximately 25% of the total PCB amounts accumulated in the milk. In another investigation on non-exposed women, approximately 30% of low-chlorinated biphenyls were identified, *i.e.*, trichlorobiphenyl 8.4 ± 2.2%, tetrachlorobiphenyls 19.1 ± 2.6%. The substituted position of the chlorine atoms of tri- and tetrachlorobiphenyls were also identified as 2,4,4', 2,4,3',4' and 2,4,5,4', respectively (Yakushiji *et al.* 1978). These results provide significant information on the studies of the mechanism of biological degradation of PCBs.

PCB patterns (Table 3) in the maternal samples were quite different from those observed in the infant. The infant never consumed the breast milk; therefore, PCB levels in the infant's blood were fairly low. It can be assumed that these differences were due to artificial feeding and the low amounts of PCBs transferred through the placenta.

Welti and Sissons (1971, 1972) have determined the composition of the isomers of Aroclor 1254 and the retention indices of the PCB compounds. Jensen and Sundström (1974a) reported the structures and the levels of most of the chlorobiphenyls found in human adipose tissue. From their work and the pattern analysis in this report, it was confirmed that all of the residual components in the breast milk of the exposed worker had specific structures, *i.e.*, peak k = 2,4,4', 9 = 2,4,5,4', 25 = 2,4,5,3',4', 28 = 2,3,4,3',4', 29 = 2,4,5,2',4',5', 32 = 2,3,4,2',4'-.5', 47 = 2,3,4,5,3',4', 49 = 2,3,4,5,2',4',5' and 50 = 2,3,4,2',3',4',5' (by the numbering system of Apiezon L column). These components all have a 4,4' substitution pattern. Bush *et al.* (1974) also mentioned this substitution pattern

found in the avian system. Our experiment was a specific case of the excretion of large amounts of PCBs through mother's milk, and it was shown that among these residual components, the low-chlorinated biphenyls, and peaks 25 and 28, which have 3,4-dichloro substitution in one of the phenyl rings, tend to be slowly excreted from the body. On the other hand, the high-chlorinated biphenyls, which have the 2,4,5-trichloro substitution in one ring and the 4-substitution in the other ring, are assumed to be one of the most stable structures in the human body.

In our previous report, the character of residual PCB patterns in the blood of non-exposed persons and workers exposed to some commercial brands of PCBs were highly influenced by the original PCBs ingested or handled by the workers. The residual PCB components satisfied the specific structures mentioned above. However, in some cases, and particularly in the Yusho patients, some accumulative components, *i.e.*, peaks *k*, 9, 25 and 28 were specifically metabolized (Watanabe *et al.* 1977). The rapid decrease of the specific components were also recognized in experimental animals with high level exposure to PCBs (Hori *et al.* 1977, Yoshida *et al.* 1977). Also, the rapid decrease of these components were roughly parallel to the activation of liver microsomal drug-metabolizing enzymes enhanced by PCBs (Shimada *et al.* 1976). These components, particularly 3,4-dichloro substituted compounds, might have a significant biological effect and be closely related to the toxicity of PCB's.

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Pesticide and PCB Residues in the Upper Snake River Ecosystem, Southeastern Idaho, Following the Collapse of the Teton Dam 1976

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Abstract. The Teton Dam in Southeastern Idaho collapsed on June 5, 1976. The resulting flood damaged a large area and caused the release of toxicants into the Snake River. A pesticide recovery team in a helicopter worked the flooded area for three weeks and collected 1,104 containers, about 35% of which contained toxicants. It was estimated that less than 60% of the lost pesticide containers were recovered.

This paper addresses the results of a one-time sampling effort designed to determine the magnitude of the chemical contamination. Over 300 samples of fish, plankton, waterfowl, sediments, water, stream drift, aquatic plants, and soil were taken. Pesticide residues were measured as μg/kg (ppb) wet weight, whole animal basis. Rainbow trout had as much as 1432 μg/kg total DDT plus analogs, 66 μg/kg dieldrin, and 1010 μg/kg PCBs. Utah suckers had up to 1420 μg/kg total DDT plus analogs, 32 μg/kg dieldrin, and 1800 μg/kg PCB. Rocky Mountain whitefish had as much as 2650 μg/kg total DDT and analogs, 30 μg/kg dieldrin and 1400 μg/kg PCBs. These PCB and DDT levels were high, approaching the 2,000 μg/kg FDA proposed tolerance, but were below the 5,000 μg/kg present tolerance. Dieldrin levels were low and organophosphates were undetectable.

An undeveloped area (the Fort Hall Bottoms) showed higher levels of contaminants than did an industrialized area (the lower Portneuf River). This apparent discrepancy remains unexplained.

Very little pre-flood data on a whole fish basis were available for comparison (Johnson *et al* 1977). However, it does not appear that any human health hazard due to pesticide levels exists in this portion of the Snake River.

The Teton Dam was located in southeastern Idaho, on the Teton River, a tributary of the Upper Snake River. The Bureau of Reclamation began construction of the dam in 1972 and it was completed in the spring of 1976. On June 5, 1976, while the Bureau was filling the Teton Reservoir, the dam broke and released 29.6 billion cubic meters of water.

The flooded portion of the Upper Snake River Valley is an agricultural area, primarily producing potatoes, small grains, beef, dairy cattle, and sugar beets (Merrell and Onstott 1965). Numerous pesticides, fertilizers, and other chemicals are used in the production of these crops. At least, three commercial facilities

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containing pesticides were damaged by the flood, and many farm storehouses were also affected. No reliable estimate is available concerning the amount of materials lost from private storage. Nevertheless, the minimum quantities of some of the lost commercial materials is known. For example, 2,000 pounds of granular Di-Syston[1] and 200 gallons of liquid Furadan[2] were known to be lost. Unknown quantities of DDT, PCBs, Guthion[3], Dinitro[4], 2,4-D, Thimet[5], Systox[6] and Malathion[7] were also lost.

The flood transported much of this material, broke the containers, and dispersed the pesticides over 240 km of the Snake River between St. Anthony and American Falls. The flood also deposited containers of toxicants over the 777 square kilometer flood plain. With time, the containers will break or rust out and more pesticides are likely to be released into the Snake River ecosystem.

A committee was formed in August, 1976 to evaluate the pesticide problems resulting from the Teton Flood. They recommended a survey to locate, recover, and dispose of hazardous material deposited by the flood. The initial survey in August, 1976 located 113 containers. Follow-up work from September 28th through October 13th located an additional 1104 containers. The area covered extended from St. Anthony to Menan Buttes, which was the area where the greatest deposition of the flood occurred. This is an area of 205 km^2 (64 km by 3.2 km).

It was estimated that less than 60% of the containers were found. The rest are buried in debris piles or under the river bottom. They will break and release their toxic contents whenever they rust through or are disturbed.

The second function of the committee was to monitor the Snake River in the flood affected area. The purpose was to determine the possible existence and extent of a human health hazard resulting from the pesticides released to the environment. The study was designed as a short-term, largescale monitoring effort. The results of this initial sampling were to show whether or not future monitoring was needed.

Methods and Materials

All samples were collected during September and October, 1976 from 13 stations along the river (Table 1). A map of the Upper Snake River Basin showing the location of the sample stations is presented in Figure 1. Sediments were collected from the top 3–4 cm of soil at each site. Plankton and drift were collected with a 13 cm diameter plankton net which has a mesh of 153 μ. Plankton samples were taken with a plankton tow representing 1000 L. of water. Stream drift samples represent 55 L. of water. All samples were placed in hexane-rinsed jars, covered with a aluminum foil and capped. Fish and birds were collected by the Idaho Department of Fish and Game. Birds were shot at or near each

[1] Di-Syston®: 0,0-Diethyl S-[2-(ethylthio)ethyl] phosphorodithioate
[2] Furadan®: 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
[3] Guthion®: 0,0-Dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl) methyl] phosphorodithioate
[4] Dinitro®: 2-sec-butyl-4,6-dinitrophenol
[5] Thimet®: 0,0-Diethyl S-[(ethylthio)methyl] phosphorodithioate
[6] Systox®: mixture of 0,0-Diethyl O-[2-(ethylthio)ethyl] phosphorothioate and 0,0-Diethyl S-[2-(ethylthio)ethyl] phosphorothioate
[7] Malathion: 0,0-Dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate

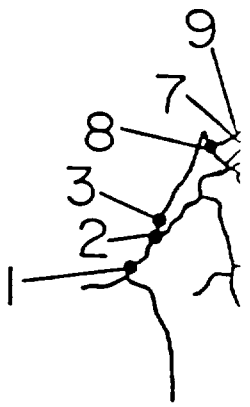

Fig. 1. Map of Upper
sampling stations, Augu

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Water samples fron
pesticides. These wells
were taken by running t
glass container. A list o

Laboratory Methods

All fish samples were an
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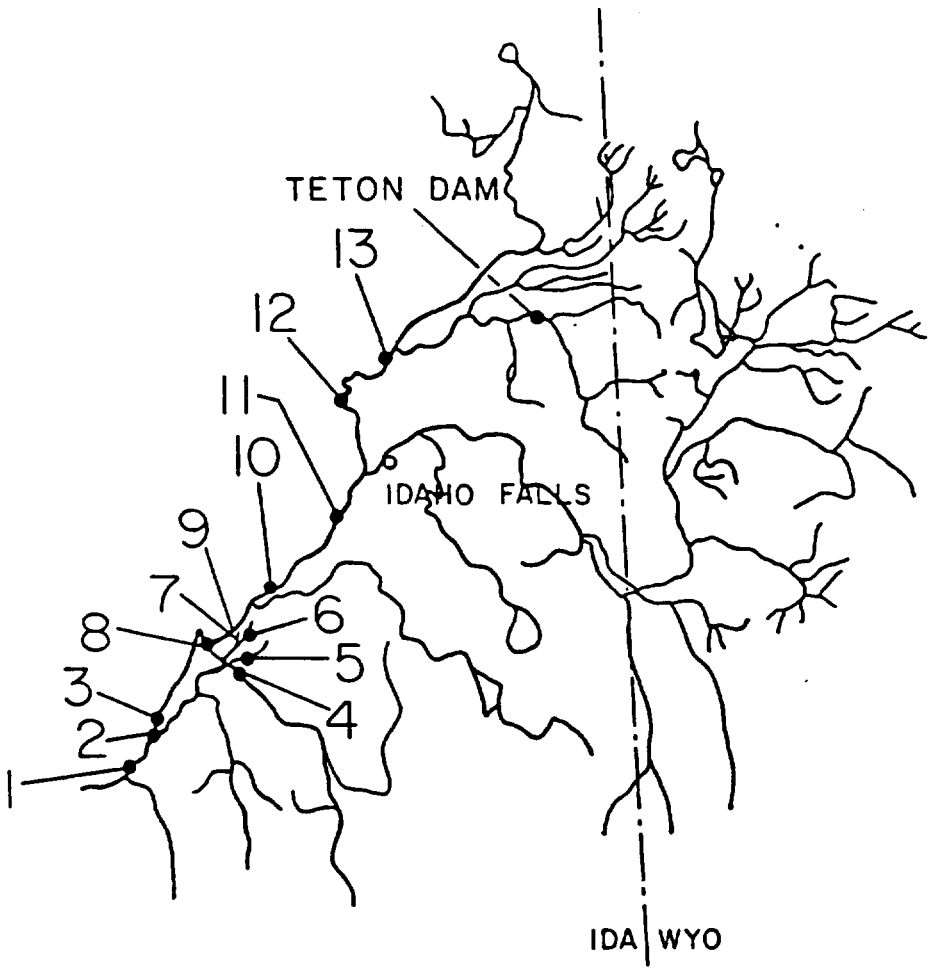

Fig. 1. Map of Upper Snake River basin showing the Teton Dam and the location of pesticide sampling stations. August–September, 1976

designated station. Fish were collected with dynamite, hook and line, gill net, seine, or electro-fishing gear. All fish and bird samples were immediately wrapped in hexane-washed aluminum foil and placed on ice. The frozen samples were then shipped to the laboratory.

Water samples from thirty-two domestic wells near Rexburg and Sugar City were analyzed for pesticides. These wells were close to areas where pesticides were known to be lost. All water samples were taken by running the water for five minutes to clear the line, then filling a 3.7 L. hexane-washed glass container. A list of the specific wells tested is available from the author.

Laboratory Methods

All fish samples were analyzed on the basis of whole animal, wet weight. Chlorinated hydrocarbons were extracted by the Mills, Onley, Gaither technique (Thompson 1976). The pesticides were

Table 1. Locations of sampling stations from the Snake River Pesticide Monitoring Program. August–September, 1976

Station no.	Station location
1	Snake River at Neeley, River Kilometer 1136.
2	Forebay of American Falls Reservoir, River Kilometer 1142.4.
3	Lower American Falls Reservoir near Aberdeen and near the "Big Hole." River Kilometer 1158.4.
4	Lower Portneuf River; joins Snake River at River Kilometer 1184. Station at River Kilometer 11.2 on Portneuf.
5	Clear Creek, enters Portneuf River at River Kilometer 3.2. Station at River Kilometer 3.2 on Clear Creek.
6	Spring Creek—River Kilometer 1.6. Spring Creek enters the Snake at River Kilometer 1187.2.
7	Big Jimmy Creek, River Kilometer 0.8; joins Spring Creek at River Kilometer 2.4.
8	Confluence of Snake River and American Falls Reservoir. River Kilometer 1196.8.
8 (a–g)	Special stations at the head of American Falls Reservoir. Seven stations, each 100 m. apart, in an arc from NW to SE.
9	Snake River at Tilden Bridge, River Kilometer 1201.6.
10	Snake River at Blackfoot, River Kilometer 1216.
11	Snake River at Shelley, River Kilometer 1254.4.
12	Snake River at Roberts, River Kilometer 1308.8.
13	Snake River near Rexburg. 3.2 km above Bannock Jim Slough. River Kilometer 1337.6.

analyzed by electron capture gas chromatography with two column confirmation. Organophosphates were analyzed by gas chromatography with a flame detector in both the sulphur and phosphorus modes. This served for both confirmation and quantification. Further confirmation was done with partition coefficients. Descriptions of all methods used may be found in the EPA Manual of Analytical Methods (Thompson 1976).

Laboratory Quality Control

Three samples of tissue with known pesticide contents were sent to each lab as "check samples." These samples were part of a group supplied by EPA Laboratories, Research Triangle Park, North Carolina. Laboratories which routinely participate in the check sample program should achieve an

Table 2. Performance of contract laboratories on quality control samples. Laboratories which use this service routinely perform in the range of ±25%

Laboratory			
W.A.R.F.		Intermountain	
Mean Error ± 18%	Range ± 0 to ± 50%	Mean Error ± 10%	Range ± 0 to ± 21.5%

accuracy of ± 25% (Jack Thompson, EPA Laboratories Division: personal communication of Joe Garcia, Idaho Department of Health and Welfare Laboratories Division, 1976). The laboratories which participated in this study performed within that range (Table 2).

Results

The following 13 toxicants were identified from the Snake River system samples:

pp'DDT	polychlorobiphenyls (as Aroclor® 1254)	dieldrin
op'DDT	benzenehexachloride (BHC)	lindane
pp'DDD	heptachlor epoxide	carbaryl
pp'DDE	hexachlorobenzene (HCB)	aldrin
		2,4-D

Total DDT[1] and analogs, dieldrin[2], and PCBs[3] are tabulated in Table 3. Other contaminants were present in very low levels (Table 4).

Well Waters

Thirty-two wells ranging from 4.2 meters to 91 meters deep were tested. Some were old wells and some were drilled after the flood. Some showed no fecal coliform bacterial contamination after the flood, and some were contaminated up to three months after the flood. Fecal coliform concentration should have indicated contamination by flood water.

Concentrations of chlorinated hydrocarbon pesticides and PCBs were less than ten ng/L in all well waters.

Discussion

DDT and Analogs

Waters from the Teton flood were sediment laden and highly turbid. DDT readily absorbs onto the surface of these sediment particles, and under these conditions, any DDT-type materials would be rapidly dispersed throughout the system (Hamelink and Waybrant 1976), while DDT levels in the waters would have been nearly undetectable. Pesticide levels in sediments would also have been low or undetectable. However, fishes may have concentrated the pesticides from the

---

[1] Total DDT Σ[1,1-dichloro-2,2-bis(p-chlorophenyl)ethane] + [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene] + [1,1-dichloro-2-(p-chlorophenyl)-2-(o-chlorophenyl)ethylene] + [1,1,1-trichloro-2,2-bis(p-chorophenyl)ethane] + [1,1,1-trichloro-2-(p-chlorophenyl)-2-(0-chlorophenyl)ethane]

[2] Dieldrin 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a, octahydro-*endo*-1,4-*exo*-5,8-dimethanonaphthalene

[3] PCB polychlorobiphenyls

Table 3. Statistical summary of pesticide residue levels in the Snake River ecosystem. Samples were taken following the collapse of the Teton Dam

		All values as μg/kg wet weight (parts per billion)											
		Total DDT and analogs				Dieldrin				Aroclor® 1254 (PCB)			
Station	Sample type	Mean	N	SD	Range	Mean	N	SD	Range	Mean	N	SD	Range
1. Snake River at Neely	Utah Sucker *C. ardens*	139	6	97	25–255	<5	6	0		213	6	172	<10–410
2. Forebay of Am. Falls Res.	Rainbow trout *S. gairdneri*	205	6	105	108–370	10	13	4	4–17	150	13	150	7–590
3. Lower American Falls Res.	Rainbow trout	179	6	124	96–412	10	6	10	2–12	140	6	190	7–510
	Utah suckers	370	12	274	55–825	10	12	10	3–32	180	12	180	<10–510
	Plankton	19	3	8	14–28	<1	3	0		5	3	3	4–8
	Mallard duck *A. platyrhynchos*	122	2	35	98–147	<1	2	0		91	2	64	45–135
	Gadwell *A. strepera*	7	1			<1	1			<10	1		
	Canada goose *B. canadensis*	6	1			<1	1			<10	1		
	White pelican *P. erythrorhynchus*	1672	2	493	1324–2021	150	2	20	137–167	280	2	60	240–320
	Sediments	<20	3	0		<10	3	0		<10	3	0	
4. Lower Portneuf River	Rainbow trout	206	6	163	35–530	5	6	1	5–7	360	6	350	69–1010
	Utah suckers	274	6	232	62–606	<5	6	0		530	6	370	100–930

	Sediments	<20	3	0		<10	3	0		<10	3	0	
4. Lower Portneuf River	Rainbow trout	206	6	163	35–530	5	6	1	5–7	360	6	350	69–1010
	Utah suckers	274	6	232	62–606	<5	6	0		520	6	370	100–930
	Stream drift	9	3	2	7–10	<1	3	0		4	3	0	
	Sediments	<20	3	0		<5	3	0		<10	3	0	
5. Clear Creek	Utah suckers	578	6	206	239–800	<5	6	0		640	6	320	340–1030
	Whitefish *P. williamsoni*	221	6	112	113–395	<5	6	0		230	6	120	91–360
	Sediments	<20	3	0		<5	3	0		<10	3	0	
6. Spring Creek	Utah suckers	216	6	276	40–770	<5	6	0		220	6	360	40–940
	Whitefish	286	6	493	50–1290	<5	6	0		40	6	40	15–110
	Sediments	<20	6	0		<5	3	0		<10	3	0	
7. Big Jimmy Creek	Rainbow trout hatchery	243	3	47	204–295	<5	3	0		75	3	23	64–94
	wild	187	3	117	81–313	<5	3	0		138	3	237	45–310
	Utah suckers	478	6	485	93–1420	<5	6	0		231	6	487	52–640
	Sediments	<20	3	0		<5	3	0		<5	3	0	

Table 3. Statistical summary of pesticide residue levels in the Snake River ecosystem. Samples were taken following the collapse of the Teton Dam (cont'd)

		All values as μg/kg wet weight (parts per billion)											
		Total DDT and analogs				Dieldrin				Aroclor® 1254 (PCB)			
Station	Sample type	Mean	N	SD	Range	Mean	N	SD	Range	Mean	N	SD	Range
8. Confluence of American Falls and Snake River	Plankton	8	1			<1	1			<4	1		
	Mallard duck	13	3	7	7–21	<1	3	0		15	3	8	<10–24
	Gadwall	<6	1			1	1			<10	1		
	Baldpate *M. americana*	<10	1			<1	1			<10	1		
	Teal *A. discors*	<6	1			1	1			<10	1		
	Sediments	<20	3	0		<5	3	0		<10	3	0	
8a–g. Sediment samples in an arc across head of Reservoir	Sediments	9	21	4	5–27	<1	21	0		4	21		0
9. Snake River below Blackfoot	Rainbow trout	107	7	36	69–198	<5	7	0		48	7	27	14–84
	Utah suckers	306	6	184	131–551	4	6	2	<1–7	255	6	219	71–490
	Whitefish	823	6	392	334–1332	10	6	5	<5–19	519	6	513	93–1400
	Sediments	<20	3	0		<5	3	0		<5	3	0	
10. Snake River at Blackfoot	Utah suckers	578	6	324	269–1128	10	6	10	<5–21	410	6	280	14–870

	Whitefish	823	6	392	334–1332	10	6	5	5–19	519	6	513	93–1400
	Sediments	<20	3	0		<5	3	0		<5	3	0	
10. Snake River at Blackfoot	Utah suckers	578	6	324	269–1128	10	6	10	<5–21	410	6	280	14–870
	Whitefish	652	4	221	450–924	10	4	10	7–20	730	4	410	150–1100
	Sediments	24	2	0		<5	2	0		<5	2	0	
11. Snake River at Shelley	Utah suckers	601	6	121	406–720	10	6	3	<5–13	480	6	650	120–1800
	Whitefish	1224	5	969	193–1650	12	5	21	<5–30	610	5	410	110–1200
	Redside shiner *R. balteatus*	1284	6	329	1063–1897	10	6	10	6–35	770	6	230	610–1200
	Sediments	12	6	9	4–22	3	6	1	1–5	20	6	20	4–54
12. Snake River at Roberts	Rainbow trout	386	9	403	145–1432	13	9	20	1–66	222	9	298	14–960
	Cutthroat trout *S. clarkii*	1531	1			16	1			50	1		
	Utah suckers	284	3	101	215–400	7	3	0.6	6–7	50	3	20	35–68
	Whitefish	988	1			9	1			100	1		
	Yellow perch *P. flavescens*	591	6	299	371–1174	30	6	20	11–69	140	6	140	44–420

Table 3. Statistical summary of pesticide residue levels in the Snake River ecosystem. Samples were taken following the collapse of the Teton Dam (cont'd)

		All values as μg/kg wet weight (parts per billion)											
		Total DDT and analogs				Dieldrin				Aroclor® 1254 (PCB)			
Station	Sample type	Mean	N	SD	Range	Mean	N	SD	Range	Mean	N	SD	Range
	Utah chubs *G. atraria*	424	6	132	263–555	30	6	10	9–43	140	6	140	23–150
	Macrophytes *Ceratophyllum sp.*	25	3	4	21–28	10	3	10	5–19	<10	3	0	
	Mallard duck	78	4	17	52–89	10	4	10	5–25	<10	3	0	
	Baldpate	20	1			<5	1			<10	1		
	Ruddy duck *O. jamaicensis*	50	1			<5	1			<10	1		
	Redhead duck *A. americana*	25	1			<5	1			<10	1		
	Avocet *R. americana*	307	1			31	1			<10	1		
	Sediments	12	3	9	6–22	<1	3	0		<1	3	0	
13. Snake River near Rexburg	Cutthroat trout	613	6	279	295–918	9	5	2	6–11	368	5	135	230–410
	Whitefish	405	8	96	241–510	10	8	10	<5–20	210	8	50	130–290
	Macrophytes	22	3	3	20–26	<5	3	0		<10	3	0	
	Sediments	48	3	22	24–68	<1	3	0		<4	3	0	

Table 4. Concen

Pesticide and

Pesticide	HCB[1]																								Heptachlor Epoxid

Table 4. Concentrations of pesticides other than DDT and Dieldrin in the Snake River ecosystem

Pesticide	Species of group	Number tested	Percentage of samples over detectable limits	Pesticide concentration (μg/kg)		
				Mean	S.D.	Range
HCB	Rainbow trout	17	0%	<5		
	Cutthroat trout	5	0%	<5		
	Utah suckers	32	0%	<5		
	Mountain whitefish	18	0%	<5		
	Yellow perch	6	0%	<5		
	Utah chubs	6	0%	<5		
	Redside shiner					
	Plankton					
	Stream drift					
	Macrophytes (*Ceratophyllum*)					
	White pelicans					
	Other waterfowl[a]	8	0%	<5		
	Sediments					
Heptachlor Epoxide[a]	Rainbow trout	11	100%	7.4	14.6	1–51
	Cutthroat trout	1	100%	32		
	Utah suckers	6	33%	3.0	3.3	<1–9
	Mountain whitefish	3	100%	19.3	18.9	6–41
	Yellow perch					
	Utah chubs					
	Redside shiner					
	Plankton					
	Stream drift					

Table 4. Concentrations of pesticides other than DDT and Dieldrin in the Snake River ecosystem (cont'd)

Pesticide	Species of group	Number tested	Percentage of samples over detectable limits	Pesticide concentration (μg/kg)		
				Mean	S.D.	Range
	Macrophytes *(Ceratophyllum)*					
	White pelicans	2	100%	51.5	54.4	13–90
	Other waterfowls[a]	10	60%	1.4	0.5	<1–2
	Sediments					
BHC[3]	Rainbow trout	26	58%	10.9	7.6	<5–36
	Cutthroat trout	5	80%	8.2	2.0	<5–10
	Utah suckers	36	44%	9.3	8.7	<5–47
	Mountain whitefish	28	64%	30.8	47.7	<5–170
BHC	Yellow perch	6	50%	5.8	1.0	<5–7
	Utah chubs	6	50%	11.7	10.1	<5–22
	Redside shiner	5	100%	17.4	4.9	12–25
	Plankton					
	Stream drift					
	Macrophytes *(Ceratophyllum)*					
	White pelicans					
	Other waterfowl[a]	8	0%	<5		
	Sediments					
Lindane[4]	Rainbow trout	25	0%	<5		
	Cutthroat trout	5	0%	<5		
	Utah suckers	33	0%	<5		
	Mountain whitefish	19	16%	5.5	1.2	<5–9
	Yellow perch	6	0%	<5		

Table 4. Concentrations of pesticides other than DDT and Dieldrin in the Snake River ecosystem (cont'd)

Pesticide	Species of group	Number tested	Percentage of samples over detectable limits	Pesticide concentration (μg kg) Mean	S.D.	Range
	Utah chubs	6	0%	<5		
	Redside shiner					
	Plankton					
	Stream drift					
	Macrophytes (*Ceratophyllum*)					
	White pelicans					
	Other waterfowl[a]	8	0%	<5		
	Sediments					
2,4-D	Rainbow trout	11	9%	1.4	1.5	<1–6
	Cutthroat trout	1	100%	1		
	Utah suckers	9	0%	<1		
	Mountain whitefish	3	0%	<1		
	Yellow perch					
	Utah chubs					
	Redside shiner					
	Plankton					
	Stream drift					
	Macrophytes (*Ceratophyllum*)					
	White pelicans					
	Other waterfowl[a]	3	33%	6	1.7	<5–8
	Sediments					
Carbaryl[c]	Rainbow trout	11	9%	50		<50–50

Table 4. Concentrations of pesticides other than DDT and Dieldrin in the Snake River ecosystem (cont'd)

Pesticide	Species of group	Number tested	Percentage of samples over detectable limits	Pesticide concentration (μg kg)		
				Mean	S.D.	Range
	Cutthroat trout	1	100%	110		
	Utah suckers	9	11%	35	22	<5–50
	Mountain whitefish	3	0%	<5		
	Yellow perch					
	Utah chubs					
	Redside shiner					
	Plankton					
	Stream drift					
	Macrophytes (*Ceratophyllum*)					
	White pelicans					
	Other waterfowl[a]	4	100%	225	96	100–300
	Sediments					
Organophosphates	Rainbow trout	11	0%			
	Cutthroat trout	1	0%			
	Utah suckers	9	0%			
	Mountain whitefish	3	0%			
	Yellow perch					
	Utah chubs					
	Redside shiner					
	Plankton					
	Stream drift					
	Macrophytes (*Ceraphyllum*)					

Table 4. Concentrations of pesticides other than DDT and Dieldrin in the Snake River ecosystem (cont'd)

Pesticide	Species of group	Number tested	Percentage of samples over detectable limits	Pesticide concentration (μg/kg) Mean	S.D.	Range
	White pelicans					
	Other waterfowl[a]	4	0%			
	Sediments					

HCB: hexachlorobenzene.
Heptachlor epoxide: 1,4,5,6,7,8 heptachlor,2,3,-epoxy,3a,4,7,7a, tetrahydro,4,7 methanoindan.
BHC: 1,2,3,4,5,6-hexachlorocyclohexane.
Lindane: gamma isomer of hexachlorocyclohexane.
2,4-D: 2,4-dichlorophenoxyacetic acid.
Carbaryl: 1-naphthyl methyl carbamate.
[a] "Other Waterfowl" includes 9 Mallard ducks, 2 Gadwalls, 2 Baldpates, 1 Ruddy duck, 1 Redhead duck, 1 Blue-Winged teal, 1 Canada goose, and 1 Avocet.

system (Warlen 1974; Warlen and Wolfe 1976). DDE[1] has been shown to be concentrated 110,000 times from water into fish tissues (Bluegills) (Hamelink and Waybrant 1976).

Levels of DDT and analogs in Snake River sediments were too low to be detected in most samples. Fishes from the Snake River have concentrated DDT into their tissues (Figure 2). Concentrations were high in rainbow trout; about 200 μg/kg. Theoretically, these levels were high enough to be detrimental to the fish population; 90 μg/kg in trout eggs resulted in 75% mortality over 75 days (Hogan and Brauhn 1975). DDT levels in suckers and whitefish were still higher and increased upstream; i.e., toward the source of most flood-related contaminants. Trout were planted in the streams on the Fort Hall bottoms after the flood. These fish would not have been exposed to the contaminated flood waters. Hamelink and Waybrant (1976) reported on a similar situation. They introduced rainbow trout into their system 134 days after introduction of the pesticide. Within 108 days, the trout had flesh levels of DDT of 180 μg/kg, even though the concentration of DDT in the water was only 0.001 μ/L. These Snake River samples were taken 115 days after the flood and mean DDT levels in wild rainbow trout were 187 μg/kg. Mean concentrations in hatchery fish were 243 μg/kg. No data are available on the pesticide concentration of the hatchery fish before they were planted. However, hatchery-raised fish had significantly ($P<0.05$) higher DDT and PCB levels in their flesh than did wild trout from the same area (Table 3, Station 7).

Two adjacent streams at the head of American Falls Reservoir show puzzling differences in DDE/DDT concentrations. Spring Creek and its tributary (Big Jimmy Creek, Stations 6 and 7) drain part of the Fort Hall bottoms. Clear Creek

[1] DDE: 1,1-dichloroethenylidene bis (4-chlorobenzene)

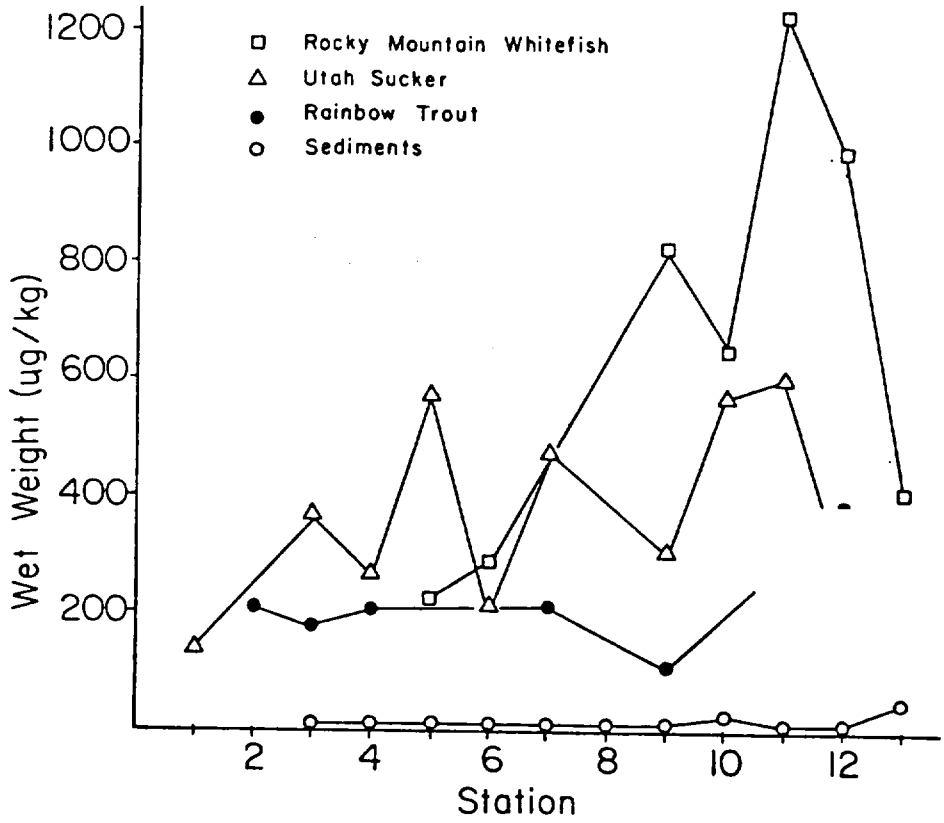

Fig. 2. Mean total DDT concentrations in fish and sediments from the Snake River three months after the Teton Dam collapse, 1976

(Station 5), a tributary of the Portneuf River, also drains part of the bottoms. Suckers and whitefish from the Spring Creek drainage are low in DDE/DDT proportions (24–34% DDE). Suckers and whitefish from the lower Portneuf drainage are higher in DDE/DDT concentrations (54–63% DDE). This indicates a difference in the source of contamination or in the food habits of the fishes in the two adjacent watersheds and it is not known where the differences arise.

Two groups of samples showed DDT levels where the DDE portion was abnormally high: redside shiners had DDE levels which averaged 92% of the total DDT. Similarly, the one avocet that was collected had DDE levels which represented 94% of the total DDT. These levels probably reflect specific food habits or metabolic differences.

PCBs (as Aroclor® 1254)

PCB is quite insoluble in water (Schoor 1974) and any of this material encountered by the flood would have been adsorbed onto sediment particles or would have been deposited in the sediments immediately (Nisbet & Sarofim 1972: as

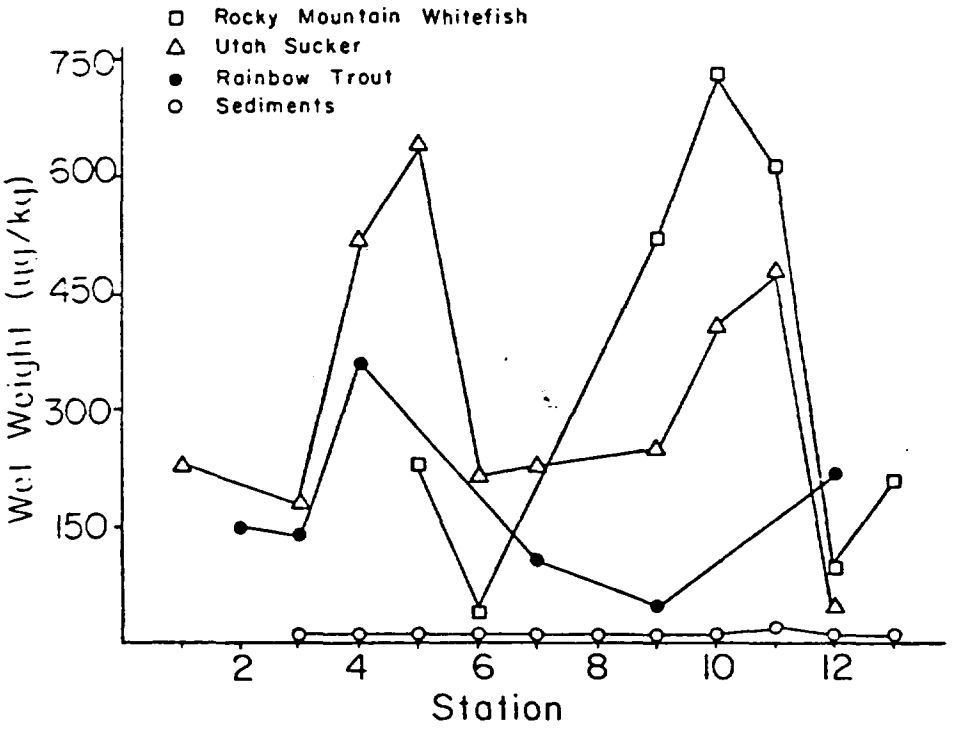

Fig. 3. Mean PCB concentrations as Aroclor® 1254 in fish and sediments from the Snake River three months after the Teton Dam collapse, 1976.

quoted by Dennis (1976) p. 193). Therefore, the distribution of PCBs in the Snake River should be highly localized.

Nebeker (1976) reports that 5 μg/L of PCBs in water are toxic to small fish, insects, and crustaceans. PCBs were generally undetectable in the Snake River water column, but PCBs in fishes of the Snake River were high; up to 1800 μg/kg (Table 3, Figure 3). Whitefish and suckers have concentrated the contaminant most efficiently.

In Lake Ontario, PCB levels were high enough to be of concern (Haile *et al.* 1975). The mean level of PCBs in the water was 0.055 μg/L. The ratio of PCB to Total DDT was 7.0 for sediments, 5.3 for Benthos, and 2.6 for all other samples (Haile *et al.* 1975). In the Snake River, PCB to DDT ratios were between 0.00 and 2.00, with most samples having ratios approaching 1.00. With one exception, suckers, whitefish, and trout, had similar ratios at a given station. Spring Creek on the Fort Hall Bottoms was unlike other stations in this regard; its sucker PCB/DDT ratios averaged 1.02, while the whitefish average ratio was 0.14. This indicates that, in this case, the whitefish concentrated the DDT to a greater degree, or the PCBs to a lesser degree than did the suckers. Nearly all other PCB/DDT ratios from this study were less than 1.00; however, the lower Portneuf River had ratios of 1.11 for suckers, and 1.04 for whitefish. The increased PCB levels at this station were probably due to the industrialization of the lower Portneuf River flood plain.

Other Pesticides

Hexachlorobenzene (HCB) was undetectable in all samples. Lindane (the gamma isomer of HCB) was detectable in only one of 102 samples. Benzene hexachloride (BHC) was usually just over the detectable limit: 2,4-D was nearly always undetectable, and the organophosphates were never detectable. Heptachlor epoxide was nearly always over the detectable limit, being most concentrated in pelicans, whitefish, and the one cutthroat trout; i.e., organisms higher in the food web. Heptachlor epoxide never approached the FDA tolerance limit in the samples from the Snake River system.

Carbaryl was below the detection limit in all but the waterfowl samples and the one cutthroat trout. The four birds containing carbaryl were three mallard ducks and a canada goose. The duck levels were all near the FDA tolerance limit of 300 μg/kg.

Distribution of Pesticides along the Snake River

Several areas of the Snake River have pesticide residue levels unlike the rest of the river. The head of American Falls Reservoir would be expected to have had high residue immediately after the flood due to the deposition of sediment from the Snake River. The Snake River near Rexburg would be expected to have high levels of pesticides stemming from materials lost in the flood. The Fort Hall Bottoms, an isolated area of little development, but extensive post-Teton flooding, would be expected to have lower levels of pesticide residues.

The results were not as expected. Sediment samples from the head of American Falls Reservoir (Station 8) were uniformly low in pesticides. Pesticide residue levels were no higher near Rexburg than they were in the rest of the river. The Fort Hall Bottoms generally had higher residue levels than the industrialized Portneuf River. This phenomenon is probably due to the Teton Flood or to agricultural impacts in the bench land above the Fort Hall Bottoms. The area near Blackfoot (Stations 9–11) showed the highest residue levels, probably due to irrigation return flows entering the river in this area.

Comparisons with previous American Falls Reservoir data

Johnson *et al.* (1977) reported on pesticide residue levels in American Falls Reservoir. Their data were collected before the Teton flood, and were collected on whole fish of 1 to 10 grams. Some data were also collected on flesh levels of pesticides in older fish. Post-flood data from this study were collected on the basis of whole fish, for fish over four years old. Johnson *et al.* (1977) discuss the differences in DDD/DDE levels attributable to steroid metabolism in these fishes. These differences in metabolism and sample type make it impossible to compare the pre-flood and post-flood data.

Human Health Implications of Findings

Proposed tolerances for several contaminants and levels detected in this study are presented in Table 6.

Pesticide and PCB

Table 5. Organoch
Johnson et. al. (19

Pesticide residues
Species
Utah sucker
Yellow perch
Utah chub

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(Table 5). Flesh
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humans.

Table 6. Proposed
Snake River. Note
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| Proposed human he
(Wet weight in flesh |
|---|
| Utah sucker |
| Rainbow trout |
| Cutthroat trout |
| Whitefish |
| Yellow perch |
| Utah chubs |
| Redside shiner |
| Mallard duck |
| Gadwall |
| Baldpate |
| Teal |
| Ruddy duck |
| Redhead |
| Canada goose |

Table 5. Organochlorine residues from American Falls Reservoir fish, 1975. Table summarized from Johnson et. al. (1977)

Pesticide residues (μg/kg wet weight)					
Species		DDD	DDE	Dieldrin	PCB
Utah sucker	—whole fish	51.6	68.7		
	—flesh	187.4	28.4	ND	671.7
Yellow perch	—whole fish	42.9	50.7		
	—flesh	5.7	14.7	34.4	ND
Utah chub	—whole fish	29.6	51.0		
	—flesh	37.6	65.4	ND	ND

There is apparently no meaningful way to compare flesh levels of contaminants with whole fish levels. Johnson *et al.* (1977) presented data on both bases (Table 5). Flesh residue levels compared to whole fish levels varied with species as well as contaminant. However, there does not appear to be a hazard to human health based on these data. The PCB and DDT levels do approach the FDA tolerances, however, and increases above the tolerances would imply a hazard to humans.

Table 6. Proposed human health tolerance for several contaminants and residue levels from the Snake River. Note that tolerances are flesh basis and Snake River data are whole fish basis (see Table 5)

	Total DDT (μg/kg)	PCB (μg/kg)	Dieldrin (μg/kg)
Proposed human health tolerance (Wet weight in flesh)	2,000	2,000	300
	Range of mean levels from each station (in wet weight of whole animal)		
Utah sucker	139–601	50–640	4–10
Rainbow trout	107–386	78–360	<5–13
Cutthroat trout	613–1531	50–368	9–16
Whitefish	221–1224	40–730	<5–12
Yellow perch	591	140	30
Utah chubs	424	140	30
Redside shiner	1284	770	10
Mallard duck	13–122	<10–91	<1
Gadwall	6–7	<10	<1
Baldpate	<10–20	<10	<1–<5
Teal	<6	<10	1
Ruddy duck	50	<10	<5
Redhead	25	<10	<5
Canada goose	6	<10	<1

Fish and Wildlife Implications of Findings

Fish and wildlife toxicity levels of pesticides are commonly expressed as μg/L in the water column. Pesticides were generally undetectable in Snake River waters. However, pesticide concentrations in the flesh of fishes, birds, or mammals often indicate contamination of the system and may show that biomagnification is occurring. Organochlorine concentrations over 500 μg/kg in any species of wildlife clearly pose a threat to specific species as well as the entire ecosystem (Dr. C. Trost, Department of Biology, Idaho State University, personal communication, August 1977). On that basis, all seven species of fishes from the Upper Snake River are in danger. The elevated organochlorine levels indicate that sublethal effects are being felt by the fauna. These effects will progressively increase and may endanger the entire ecosystem.

Summary and Conclusions

The data from this study show that fishes from the Upper Snake River have concentrated DDT and its analogs into their bodies. The mean total DDT levels in rainbow trout were about 200 μg/kg. Total DDT levels for suckers and whitefish at the various stations ranged from 25 to 1420 μg/kg wet weight for suckers and 50 to 2650 μg/kg for whitefish. The FDA proposed human tolerance level of total DDT in fish flesh is 2,000 μg/kg.

PCB levels in trout, suckers, and whitefish from the Snake River came very close to the proposed FDA tolerance of 2,000 μ/kg wet weight in fish flesh for human consumption.

Samples from the upper river, i.e., near the Teton Dam, generally had higher pesticide residues than the lower river. River fauna from the Snake River near Blackfoot and Shelley had the highest pesticide residues. No marked increases were noted in areas of high sediment deposition, such as the head of American Falls Reservoir. These areas were expected to show high levels because of the sediment adsorption property of most pesticides. The undeveloped Fort Hall Bottoms had higher organochlorine residues than did the lower Portneuf River, an undustrialized area.

Due to differences in the sample type sent for analysis, i.e., old vs. young fish, and whole fish vs. edible flesh, comparisons with previous data from the same area are not available. No apparent hazard to human health was detected based on this sampling program. PCB levels in the fishes may be hazardous to humans if they show significant increases above the present levels. Snake River fishes had PCB levels as high as 1800 μg/kg and the FDA tolerance limit is 2,000 μg/kg. Therefore, the fishes of the system should be monitored in order to guard against undetected hazard. A potential does exist for diminishing the fauna and/or biomagnification of pesticides. A monitoring program should also address this aspect.

Acknowledgments. The following people represent the Committee which was formed to monitor pesticide contamination in the Upper Snake River after the Teton flood. All members have contributed to this effort and their assistance is gratefully acknowledged.

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John Heimer, Idaho Fish and Game Department;

David Lenhart and Rex Van Wormer, U.S. Fish and Wildlife Service;

Bruce Chelikowsky, U.S. Public Health Service;

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PCB Episode Could Happen Again, NRC Committee Warns EPA

The Environmental Protection Agency has toxic substance control regulations that are inadequate to prevent certain potentially hazardous chemicals from being introduced to the marketplace, the National Research Council reported this summer. The Committee on the Assessment of Polychlorinated Biphenyls (PCBs) in the Environment, chaired by Alfred M. Beeton of the University of Michigan, concluded that the agency's two principal testing schemes—designed in accordance with the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), respectively—would not detect most of the long-term, chronic ...h hazards now known to be associated with nonoccupational exposure to PCBs. This confirmed to them what many have already feared: that a host of chemical time bombs may have been allowed on the market in accordance with the regulatory standards of the day, only to be revealed 20 or 25 years later to cause serious diseases in people subjected to low-dose, long-term exposure.

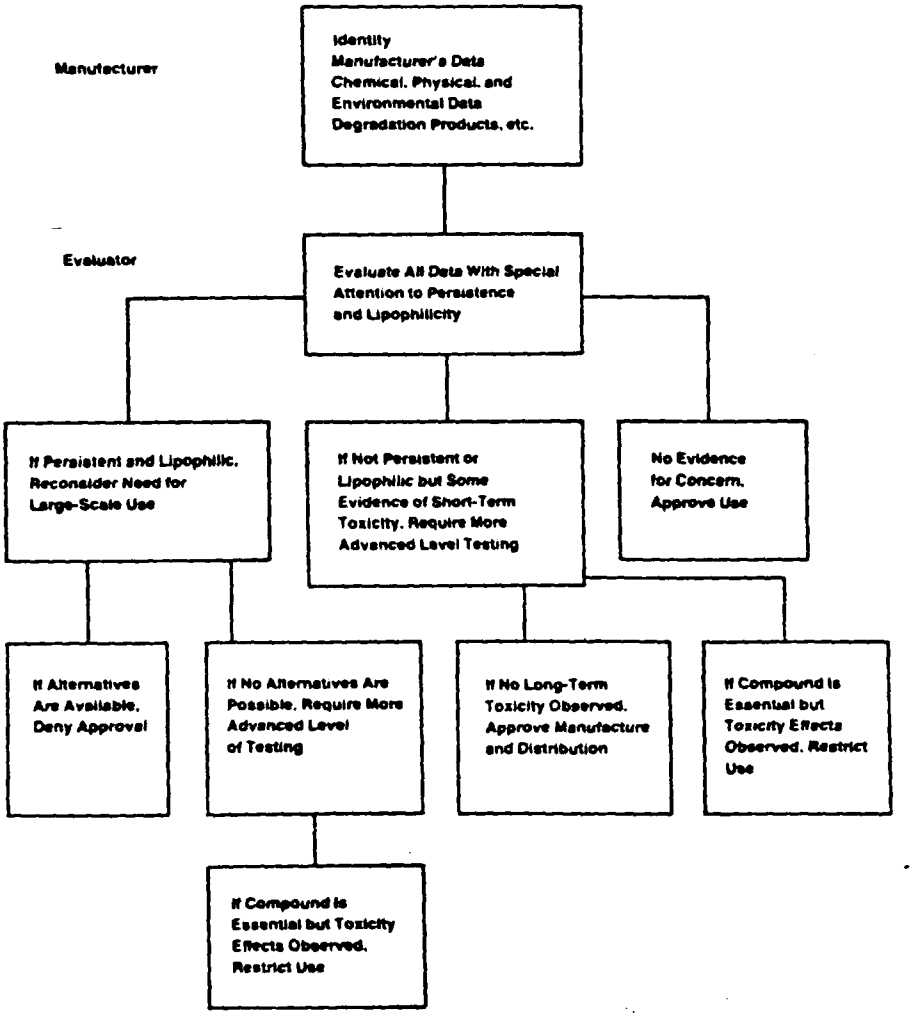
The NRC committee evaluated controls for PCBs, even though their sale and distribution have been banned as of 1 July, as a model for the mechanism now used to evaluate potential health hazards of other chemicals. NRC was interested in recommending ways EPA ...ould avert in the future the unanticied persistence of hazardous chemicals in the environment. They chose this broad course rather than the easier way of writing "yet another extensive review of the fate of PCBs and the risks to health associated with them."

The Beeton committee recommended that EPA pay especially close attention to new chemicals with two characteristics that make PCBs and chemicals like them especially troublesome: a demonstrated tendency to accumulate in fatty tissue (lipophilia) and a tendency to persist in the environment. These two indicators, the committee said, are "perhaps the most important properties of a chemical that relate to potential hazards to health and the environment." They signal the "inevitability of environmental contamination and health problems" that would result from the approval, marketing, and widespread use of the compound under review. When they appear, the committee said, a red flag should spring up in the regulators' minds, and more intensive toxicity tests should be conducted. The accompanying chart, reproduced from the report, shows the "evaluation scheme" proposed by the NRC.

The correct choice of animal species for such tests was another matter addressed by the Beeton committee. The TSCA and FIFRA regulations currently proposed require one rodent plus one other mammal to be used in toxicity testing, but these animals' responses, said the committee, are rarely "typical" of the responses of humans. The group pointed out that the common laboratory animals (rats and dogs) show only minimal liver and reproductive changes after PCB exposure. The other possible species that could be used (mouse, monkey, chicken, mink, pelican, wildfowl, and aquatic species) also differ from humans in their response to PCBs and probably to other toxic chemicals as well. In addition, these animals usually are used to detect "gross intoxication" only. "Subtle effects," the committee said, "are not considered at all in the scheme proposed for TSCA and only peripherally in those drafted by FIFRA."

To supplement the evaluation scheme proposed in the chart, the committee also urged EPA to assess a new chemical's movement through the environment as another measure of its "hazard potential." The group said the agency should insist on "identification of 'problem areas' or sites of potential chemical accumulation (e.g., agricultural lands, urban areas, marine or freshwater sources, or combinations of these); [and] specification of tests to assess toxic effects within the 'problem areas,' especially measures of reproductive alterations, pathological effects, and behavioral and learning impairments."

—R.M.H.



Evaluation scheme for assessing potential hazards to health and the environment. (Source: *Polychlorinated Biphenyls*, National Research Council.)

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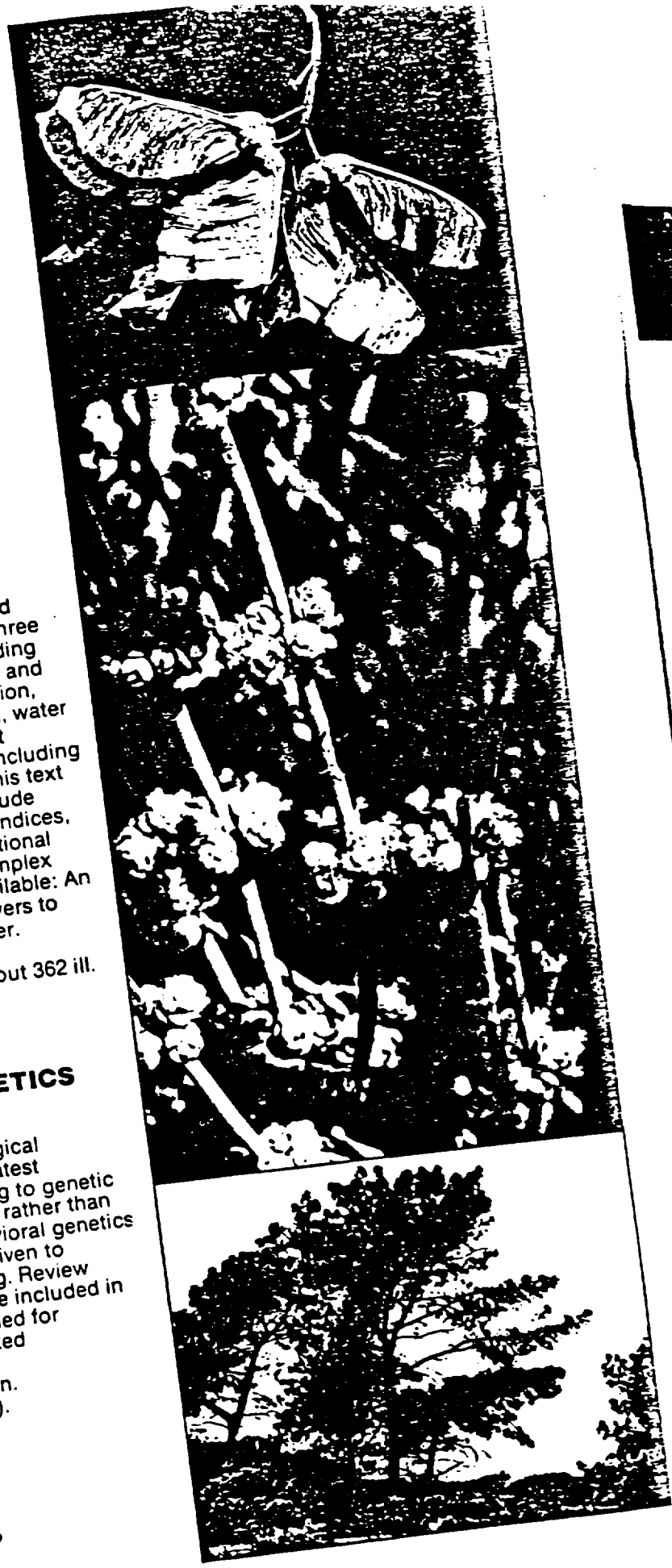

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Polychlorinated Terphenyls in the Environment

by
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The presence of polychlorinated biphenyls (PCB's) in the environment has frequently been reported in the literature. Good review papers on this subject are available (see e.g. 1, 2).

Besides the PCB's a group of analogous substances is being produced for tens of years: the polychlorinated terphenyls (PCT's). The PCT's are in use for similar applications as the PCB's. Because of the analogy between the two groups of substances one might expect that the PCT's also accumulate in biological systems. In two recent publications the presence of PCT in eggs, fatty tissue of herring gulls (Larus argentatus) and tissues of cod (Gadus morhua) has been reported (3, 4). These investigations were carried out with gas chromatographic techniques.

The objective of the present research is to investigate several different samples on the presence of PCT. The number of samples was not such that the figures are representative from a statistical point of view.

The samples were: water of the Rhine river (mixture of samples of the water), oysters, eel and human fat. In the samples the amounts of PCB's were also determined for comparison purposes.

Experimental

Extraction and clean-up

The samples were extracted with n.hexane. The extracts were cleaned by column chromatography, either on alumina (5) or Florisil (6). The eel extracts contained much oily residue and were cleaned over both Florisil and alumina.

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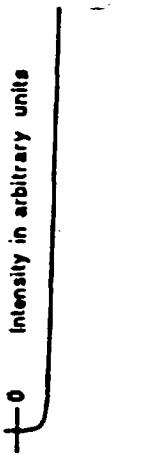

Mass fr

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Method of analysis

The analysis of PCT was carried out with a combination of a mass spectrometer (Varian Mat CH 5) and a gas chromatograph (Varian 1740) used in the mass fragmentography mode. Two m/e-values were selected (m/e = 436 and m/e = 470) and from all samples mass fragmentograms were taken on these m/e-values (see Fig. 1 and 2).

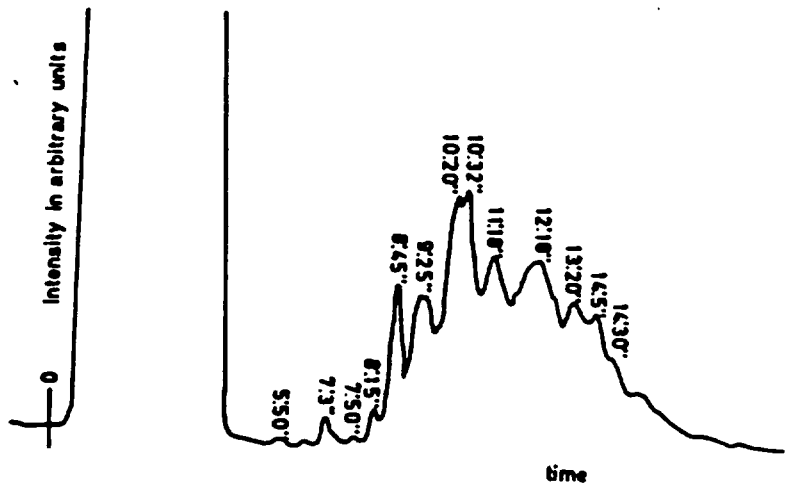

FIGURE 1
Mass fragmentogram on m/e = 436 of a standard of the polychlorinated terphenyl Clophen Harz (W)

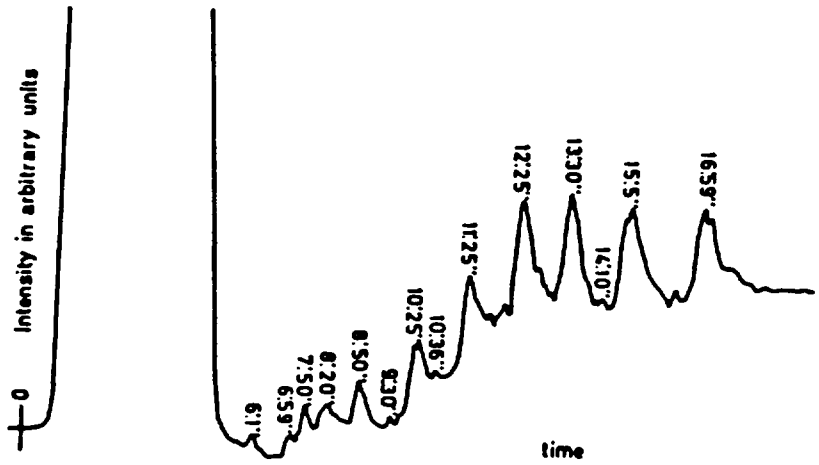

FIGURE 2
Mass fragmentogram on m/e = 436 of a sample of oyster

The gas chromatographic conditions were: column length 3', 1/8" O.D. (Pyrex), filled with 3% OV-17 on Varaport 30. Temperature programming was used: 6°C/min., 200° —> 285°C for m/e = 436 and 8°C/min., 200° —> 285°C for m/e = 470. The gas chromatographic conditions for the PCB's were: same column, temperature programming 4°C/min., 125° —> 200°C for m/e = 256; 6°C/min., 125° —> 200°C for m/e = 290, and 6°C/min., 150° —> 250°C for m/e = 324.

Results

The content of PCT was compared with the Clophen Harz (W) standards. Since two fragmentograms were made, we will give the separate results of each mass fragmentogram (the figures of the mass fragmentograms are of course averages too). The results are represented in Table 1.

In all recent samples PCT was found. Two of the older samples of human fat (1969) did not show PCT, while a third one did contain PCT. The amounts are slightly lower than those of PCB but of the same order of magnitude. The investigation on the occurrence of PCT will be continued.

TABLE 1

sample	PCT m/e = 436	PCT m/e = 470	PCT average	PCB comparison	remarks
water of Rhine river (1972)	0,08 ppb	0,05 ppb	0,07 ppb	0,08 ppb	water basis
oysters from Oosterschelde near Ierseke (1971)	0,15 ppm	0,08 ppm	0,12 ppm	0,2 ppm	tissue basis
eel from IJssel-meer (1971)	0,5 ppm	0,2 ppm	0,4 ppm	4,7 ppm	fat basis
human fat (1969)	not observed	not observed	-	0,5 ppm	fat basis
human fat (1969)	0,5 ppm	0,5 ppm	0,5 ppm	0,4 ppm	fat basis
human fat (1969)	not observed	not observed	-	1,0 ppm	fat basis
human fat (1972)	1 ppm	0,5 ppm	0,8 ppm	0,8 ppm	fat basis

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Presence of Organochlorine Pesticides, Polychlorinated Biphenyls, and Mercury in Spanish Human Milk Samples

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Organochlorine pesticide residues have been detected in human milk in various countries, among these are : England (EGAN et al. 1965), Belgium (HEYNDRICKX & MAES 1969), Germany (ACKER & SCHULTE 1970), Russia (GRACHEVA 1970), U.S.A. (DYMENT et al. 1971), Holland (TUINSTRA 1971), Poland (KONTEK et al. 1971), Sweden (WESTOO & NOREN 1972), Guatemala (WINTER et al. 1976), and Canada (MES & DAVIES 1979). Polychlorinated biphenyls have also detected in Germany (ACKER & SCHULTE 1970), Sweden (WESTOO & NOREN 1972), U.S.A. (SAVAGE et al. 1977) and Canada (MES & DAVIES 1979). Mercury was also detected in Sweden (WESTOO 1973, PITKIN 1976) and Japan (HARADA 1968, WAKATSUKI 1973, FUJITA & TAKABAKATE 1977). The objective of the present study has been to investigate the levels of organochlorine pesticides, polychlorinated biphenyls and mercury in whole breast milk of Spanish mothers.

MATERIALS AND METHODS

A total of twenty milk samples was collected during the period of April to July 1981 from lactating mothers from Madrid (Spain) by manual extraction and stored at -20°C until time of analysis.

For the analysis of organochlorine compounds, samples of whole breast milk (5g) were dispersed in anhydrous sodium sulfate and the mixture extracted with hexane:acetone (40:60) in a Soxhlet apparatus for six h.

The REYNOLDS (1969) procedure was used to cleanup the extract and to separate polychlorinated biphenyls from most of the organochlorine insecticides.

The organochlorine residues were identified by the conventional methods of EC-GLC. For quantitation a mixture of 5% DC-200 and 7.5% QF-1 on 80-100 mesh Chromosorb W HP was used; temperature parameters were: column 200°C, detector 210°C, injector 215°C; nitrogen flow rate: 67 mL/min.

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Simultaneously, 10 g of whole breast milk were digested with nitric and sulfuric acids and the mercury quantitated by the method of reductive aeration with stannous chloride and flameless atomic absorption spectrophotometry (UTHE 1972).

RESULTS AND DISCUSSION

The data in Table 1, expressed in parts per million, show the average residue levels, ranges, standard deviation and percentage of samples with residues ≥ 0.001 µg/g in whole breast milk samples collected in the period of April to July of 1981.

Table 1. Organochlorine compounds and total mercury (ppm) in the whole breast milk of Spanish mothers residing in Madrid.

	a) Average	Maximum observed	Minimum observed	S.D.	% of samples with residues ≥ 0.001 µg/g
γ HCH	0.019	0.043	0.001	0.012	100
H. epox.	0.004	0.014	0.000	0.004	77
Dieldrin	0.003	0.014	0.000	0.002	46
pp'-DDE	0.17	0.25	0.052	0.062	100
pp'-TDE	0.003	0.008	0.000	0.002	54
pp'-DDT	0.083	0.15	0.027	0.038	100
PCBs	0.25	0.32	0.19	0.038	100
Hg	0.0095	0.019	0.0009	0.0055	100

a) Averages are based on the number of samples with residues ≥ 0.001 µg/g, except for mercury levels;
H. epox. = Heptachlor epoxide

All of the twenty samples analyzed showed measurable quantities of γ-HCH, pp'-DDE, pp'-DDT, polychlorinated biphenyls and mercury. In addition, a large majority of the samples showed detectable heptachlor epoxide, dieldrin and pp'-TDE residues.

Although laboratory differences in methodology, sample origin and collection time make direct comparisons difficult, it can be noteworthy, however, to relate these residue concentrations with others of the same nature stated in a previous publication (POZO et al. 1979). According to those results, it may be assumed that in a period of two years a decreasing concentration is observed in the residue levels of α- and γ-HCH, chlordane and heptachlor, but an increase is noted in the accumulation of heptachlor epoxide, dieldrin, pp'-DDE, pp'-TDE and pp'-DDT residues (Table 2).

Therefore, the pesticide levels in whole human milk found in this study ranged from one fourth (γ-HCH) up to eight times more (total DDT) than the legal limits for organochlorine compounds established by FAO/WHO (1972) for the acceptable daily intake in human beings.

Table 2. Trends of organochlorine pesticides residues in Spanish human milk.

	Average µg/g whole milk	
	Year of publication	
Compound	1979	1981
α-HCH	0.001	0.001
γ-HCH	0.040	0.019
Chlordane	0.0003	ND
Heptachlor	0.039	0.001
Heptachlor epoxide	0.0003	0.003
Dieldrin	0.0005	0.003
pp'-DDE	0.11	0.17
pp'-TDE	0.001	0.003
pp'-DDT	0.065	0.083

Studies on DDT levels in the newborn's blood suggest that there may exit a correlation between high levels of DDT in their blood and infant's health, and the mother's milk is an important source of infant nutrition.

In Table 3, pp'-DDE, pp'-DDT (pesticides very frequent in different studies), total DDT and PCB levels in whole human milk of various countries are given. The average content of total DDT in Spanish milk (0.25) is about the middle of the DDT range typically found in many European and American countries. Data on polychlorinated biphenyls are not so abundant as DDT, but those values shown in Table 3 indicate that PCBs in Spanish milk are higher than individual organochlorine pesticides and also higher than PCB levels reported by other countries.

With regard to the mean of total mercury content in Spanish breast milk (0.0095) it appears to be higher than in Sweden, were WESTOO (1973) reported 0.00093 ppm and PITKIN (1976) a range of 0.0008-0.0016 ppm. On the other hand, mercury concentration in milk of healthy mothers from the Minamata area of Japan was reported by a mean of 0.063 ppm (HARADA 1968). However, WAKATSUKI (1973) reported a range of 0.50-0.54 ppm in breast milk of mothers from an agricultural district of Japan but FUJITA and TAKABAKATE (1977) have found a mean of 0.0036 ($\pm$ 0.0022) ppm in the mother's milk sampled in two hospitals of Tokyo.

Table 3. Comparison of pp'-DDE,pp'-DDT, total DDT and PCBs mean levels in whole human milk of various countries (ppm).

Country	Year	PCBs	pp'-DDE	pp'-DDT	DDT_{total}	Ref.
Guatemala	1974				0.38	a
Poland	1971*		0.20	0.08	0.28	b
Spain	1981	0.25	0.17	0.08	0.25	c
Russia	1970*		0.10	0.10	0.20	d
Belgium	1969*		0.07	0.05	0.12	e
England	1964		0.07	0.04	0.12	f
Germany	1970*	0.10	0.08	0.03	0.11	g
USA	1971*		0.08	0.02	0.11	h
USA	1977*	0.04				i
Sweden	1972	0.02	0.06	0.02	0.08	j
Holland	1969		0.03	0.02	0.05	k
Canada	1975	0.01	0.03	0.01	0.04	l

* Year published; a = WINTER et al. (1976); b = KONTEK et al. (1971); c = This study; d = GRACHEVA (1970); e = HEYNDRICKX & MAES (1969); f = EGAN et al. (1965); g = ACKER & SCHULTE (1970); h = DYMENT et al. (1971); i = SAVAGE (1977); j = WESTOO (1972); k = TUINSTRA (1971); l = MES & DAVIES (1979).

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Polychlorinated Biphenyl and Other Chlorinated Hydrocarbon Residues in Adipose Tissue of Canadians

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The presence of organochlorine (OC) pesticides in adipose tissue of Canadians have earlier been reported by RITCEY et al. (1973) in the first nation-wide survey. At approximately the same time different chlorinated environmental pollutants were reported in human adipose tissue, such as hexachlorobenzene (HCB) (BRADY 1972), oxychlordane (BIROS 1973) and polychlorinated biphenyls (PCBs) (PESENDORFER 1973). This led to a national survey by MES et al. (1977) on chlorinated hydrocarbon residues in adipose tissue of Canadians, which incorporated these newly reported residues.

The data reported in this paper are part of a continuing monitoring program of chlorinated hydrocarbons in adipose tissue of Canadians in order to determine a possible trend in both the disappearance of restricted OC pesticides, such as p,p,-DDT, as well as the appearance of new environmental contaminants, such as hexachloro-1,3-butadiene (HCBD) (FISHBEIN 1979).

MATERIALS AND METHODS

Sampling

Human adipose tissues were obtained during autopsies on accident victims. The fat samples were collected in glass jars, previously washed, heated at 350°C and rinsed with residue free acetone and hexane and supplied with teflon lined screw caps. Samples were immediately frozen and kept frozen until analysed. A total of 99 samples was collected from the following provinces: Prince Edward Island (4), Nova Scotia (6), Quebec (39), Ontario (6), Manitoba (8), Saskatchewan (7), Alberta (8), British Columbia (19) and Yukon (2).

Analytical Methods

All solvents were glass distilled and free of interfering residues. Standards were gifts from the Environmental Protection Agency (U.S.A.) and 98-100% pure. Florisil, silicic acid, Celite, anhydrous Na_2SO_4 and glass-wool were treated as previously reported (MES and DAVIES 1978).

Extraction and Cleanup. Two gram samples were extracted with 100 ml of benzene:acetone (1:19 v/v) for 3 min. with a Silverson Homogenizer (Canlab Ltd). Extracts were filtered through glass wool and evaporated on an all-glass rotatory evaporator (< 30°C). Traces of benzene were removed by repeated evaporation with a few milliliters of hexane. The solvent free oily residue was redissolved in hexane and filtered through anhydrous Na_2SO_4 and the hexane evaporated to dryness. The residue was

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then transferred to a 15 mL graduated centrifuge tube, using acetone and the volume adjusted to 10 mL. A 0.5 ml aliquot was evaporated in a preweighed aluminum dish to determine benzene:acetone soluble lipids.
Fat was removed from the remaining extract by low temperature precipitation (McLEOD and WALES 1972).
Separation. The fat free extract in ~ 1 mL hexane was chromatographed on a combined Florisil-silicic acid column as previously described (MES and DAVIES 1978) and the following fractions collected:

Fraction	Eluting solvent	Eluted compounds
I	35 mL hexane	HCBD, penta chlorobenzene (PCBz), HCB, p,p'-DDE, PCB in Arochlor 1260. Some PCBs in Aroclor 1242, Mirex.
II	40 mL of 20% CH_2Cl_2 in hexane	some PCBs in Aroclor 1242, α, β and γHCH, oxychlordane, trans-nonachlor, p,p'-DDE, o,p'-DDT, p,p'-DDT
III	40 mL of 60% CH_2Cl_2 in hexane	βHCH, heptachlor epoxide, dieldrin.

The fractions were concentrated to ~ 1 mL on a rotatory evaporator as above and transferred to 15 mL stoppered graduated centrifuge tubes, using hexane. The final volume was adjusted to 4 mL in most cases.
Identification and quantification. The fractions were chromatographed on a Varian 2100 series gas chromatograph with electron capture detector (Tritium foil).
Identification of individual residues was carried out on a 0.6 x 183 cm glass column, packed with 6% OV-210 + 4% SE-30 on chromosorb W (AW) 100/120 and a flow rate of 40-50 mL/min. Injection, column, and detector temperatures for the PCB determinations were 212, 199 and 250°C respectively, while for OC pesticides these temperatures were held at 220, 195 and 258°C. The β and γ HCH isomers were quantitated from chromatograms obtained on a column similar to the one above and under exactly the same GC conditions, but with 5% OV-210 as stationary phase.
In order to separate Mirex from PCBs fraction I was also chromatographed on a 0.5 mm x 36 m SCOT column, coated with SP 2100, using a Varian 1400 gas chromatograph with electron capture detector (Scandium Tritide). Injection, column and detector temperatures were 249, 225, and 241°C respectively. A linear flow rate of nitrogen of 21 cm/sec was maintained.
Aliquots (5 µL) of standard solutions of Aroclor 1260 (50×10^{-5} µg/µL), Aroclor 1242 (100×10^{-5} µg/µL) and other halogenated hydrocarbons ($0.25 - 10 \times 10^{-5}$ µg/µL, depending on individual response) were injected before and after every two sample injections.
Individual compounds were quantitated by measuring peak heights. PCBs were estimated by summation of peak heights of peaks 8 and 10-15 in Aroclor 1260. In Aroclor 1242, however, different peaks were used to estimate PCBs in fractions I and II. Peaks C to J of Aroclor 1242 in fraction I and peaks E and G-J in fraction II were used. (Fig. 1)

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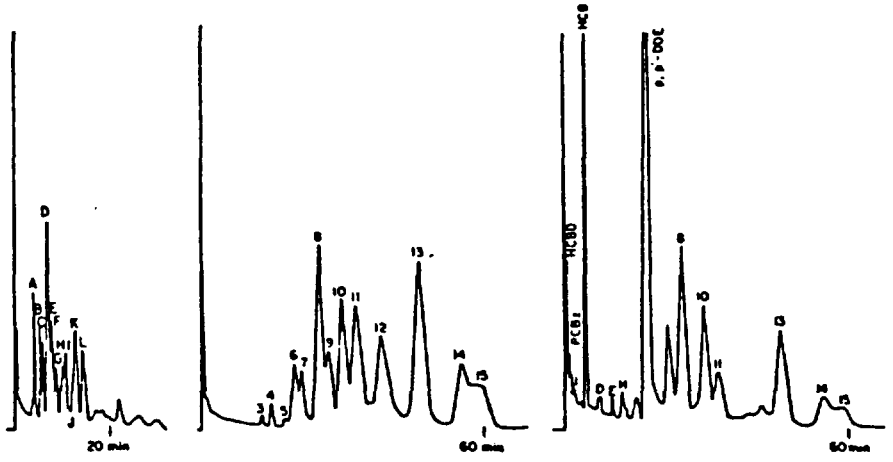

FIG. 1. Typical GC elution patterns of Aroclor 1242, 1260 and PCBs in human adipose tissue (from left to right respectively) on the mixed stationary phase GC column.

Confirmation of compounds in fraction I. The presence of HCB, HCBD, PCBz, p,p'-DDE and PCBs in fraction I were confirmed by further gas liquid chromatographic (GLC) in 5% OV-210.

Thin layer chromatography (TLC) separation of fraction I was carried out as described by MES et al., (1977), using 60% CH_2Cl_2 in hexane to elute the compounds from the adsorbent.

Appropriate fractions were re-chromatographed on a GLC column of mixed stationary phase as above and single ion monitored by mass-spectrometry (MS) for HCB (m/z = 284), HCBD (m/z = 260), PCBz (m/z = 250) and Mirex (m/z = 546), using a Varian Mat 311A coupled to a Varian 1400 series gas chromatograph by a two-stage Watson-Bieman separator at a resolution of 1000. A full scan probe analysis was performed on the same instrument for pentachlorobiphenyl (m/z cluster 224, 326, 328, 330), hexachlorobiphenyl (m/z cluster 358, 360, 362, 364) and heptachlorobiphenyl (m/z cluster 392, 394, 396, 398).

Confirmation of compounds in fraction II and III. Pesticides in fractions II and III were confirmed on 5% OV-210 as above.

TLC confirmation was carried out as above, except β and γ HCH, heptachlorepoxide, dieldrin and o,p'-DDT, which were re-chromatographed on 5% OV-210.

Single ion monitoring MS was carried out on appropriate fractions for β HCH (m/z = 290), γ HCH (m/z = 219), o,p'-DDT (m/z = 354 and 235), dieldrin (m/z = 380 and 345) and heptachlorepoxide (m/z = 390 and 353) as above. A full scan probe MS analysis was also carried out for oxychlordane (m/z clusters (420, 422), (424, 426), (385, 387, 389)), trans-nonachlor (m/z cluster 405, 407, 409), p,p'-DDE (m/z clusters (316, 318, 320), (246, 248), 210) and p,p'-DDT (m/z clusters (352, 354, 356), (317, 319), (235, 237)).

Controls. Samples were fortified with all compounds analysed for, except α HCH, Aroclor 1242, o,p'-DDT and Mirex. Fortification levels were 1.25 ppm for Aroclor 1260 and p,p'-DDE, 0.313 ppm for p,p'-DDT, 0.015 ppm for γ HCH and 0.05 ppm for all others. These fortifications were carried out in different combinations of pesticides in order to avoid overlapping of gas chromatographic retention times. At regular intervals during the survey solvent blanks were run through the entire analytical procedure.

RESULTS AND DISCUSSION

The results in Table 1 show the occurrence and very high incidence of environmental chemicals in adipose tissue of Canadians, as collected in 1976. All residues in Table 1 were confirmed by gas chromatography

TABLE 1

Chlorinated hydrocarbon residues in lipid extracts of adipose tissues of Canadians

Compound	μg/g wet weight		% of samples Containing residues
	Average[a] ± S.D.[b]	Range	
PCB, as Aroclor 1260	0.944 ± 0.902	0.040 - 6.801	100
PCB, as Aroclor 1242	0.307 ± 0.272	0.054 - 2.300	100
HCBD	0.004 ± 0.000	0.001 - 0.008	93
PCBz	0.002 ± 0.000	0.001 - 0.020	93
HCB	0.095 ± 0.103	0.010 - 0.667	100
α HCH	0.004 ± 0.000	0.001 - 0.043	97
β HCH	0.151 ± 0.459	0.016 - 4.413	100
γ HCH	0.003 ± 0.000	0.001 - 0.030	90
Oxychlordane	0.055 ± 0.026	0.005 - 0.186	100
t-Nonachlor	0.056 ± 0.032	0.015 - 0.244	100
Heptachlor epoxide	0.037 ± 0.045	0.004 - 0.404	100
Dieldrin	0.049 ± 0.030	0.003 - 0.211	100
p,p'-DDE	1.721 ± 1.390	0.034 - 7.819	100
o,p'-DDT	0.032 ± 0.058	0.005 - 0.578	96
p,p'-DDT	0.311 ± 0.493	0.016 - 3.998	100

[a] Average of 99 samples [b] Standard deviation.

(GC), TLC and MS in individual or pooled samples. PCBs in fractions I and II were confirmed by the presence of penta-, hexa-, and heptachlorobiphenyl. HCBD and PCBz residue determinations cannot be considered quantitative with eratic recoveries of 0-56 and 3-84 % respectively, causing the average recovery to be < 30%. Recoveries of other chlorinated hydrocarbon residues ranged from 70-100%. Figure 1 shows a typical GC elution pattern of HCBD, PCBz, HCB, PCBs and p,p'-DDE in fraction I of human adipose tissue extract.

Table 2 compares the present data with those obtained by RITCEY et al. (1973) in 1969 and MES et al. (1977) in 1972. In general it can be observed, that the average levels of PCBs, γ HCH and oxychlordane

TABLE 2

Trends in chlorinated hydrocarbon residues in adipose tissues of Canadians

Compound	µg/g wet weight		
	RITCEY et al.(1973)	MES et al.(1977)	THIS SURVEY
PCB, as Aroclor 1260		0.907	0.944
HCB		0.062	0.095
α HCH		0.004	0.004
β HCH		0.054	0.151
γ HCH	0.015	0.007	0.003
Oxychlordane		0.055	0.055
t-Nonachlor		0.065	0.056
Heptachlor epoxide	0.040	0.043	0.037
Dieldrin	0.122	0.069	0.049
p,p'-DDE	3.430	2.095	1.721
o,p'-DDT		0.031	0.032
p,p'-DDT	1.017	0.439	0.311
Total DDTs	4.543	2.571	2.064

appear to have remained constant; the average levels of γ HCH, t-nonachlor, heptachlor epoxide, dieldrin and total DDT appear to have decreased, and the average levels of HCB and β HCH appear to have increased.

The increase in HCB residue level probably reflects more the improved recovery in this study compared to the 1972 survey (MES et al. 1977), than an actual increase in the HCB body burden. The decrease in p,p'-DDT level between 1972 and 1976 was less than between 1969 and 1972. This could possibly be attributed to the fact that PCBs were not determined in 1969 and if present at that time could have contributed to the DDT level.

The geographic distribution of environmental contaminants is shown in Table 3 (MES et al., 1977). The results indicate higher residue levels of Aroclor 1242, HCB, γ HCH, oxychlordane, t-nonachlor, heptachlor epoxide, dieldrin and p,p'-DDE in the central region than in the Eastern and Western regions and Quebec. This is in contrast with reported values in the 1972 survey, where the residue levels of the Central region were consistently lower than in other regions, except for heptachlor epoxide. Although the levels of these residues were also higher than those found in Ontario and the Yukon, the latter two regions only represented a limited number of samples, especially in the case of populated Ontario. Only HCB, oxychlordane and heptachlor epoxide showed a statistically significant difference ($p < 0.05$) between the regions (Yukon not included).

There appears to be little difference in residue levels of males and females (Table 4), except for HCB, which is 2 x higher in females than males and β HCH which is higher in males. The difference in HCB was statistically significant.

Table 5 shows the residues in artificially grouped ages. Although the results indicate an increase in residue levels with age, except for β HCH and p,p'-DDT, the effect was only statistically significant ($p < 0.05$) for

TABLE 5

Regional distribution of chlorinated hydrocarbon residues in adipose tissue of Canadians.

Compound	Average µg/g wet weight ± S.D.					
	Region					
	Eastern[a]	Quebec	Ontario	Central[b]	Western[c]	Yukon
PCB, as Aroclor 1260	0.803 ± 1.094	0.890 ± 0.409	1.791 ± 1.468	0.779 ± 0.660	0.947 ± 1.233	1.398 ± 0.819
PCB, as Aroclor 1242	0.219 ± 0.304	0.293 ± 0.156	0.253 ± 0.073	0.416 ± 0.553	0.306 ± 0.181	0.367 ± 0.256
HCBD	0.002 ± 0.000	0.004 ± 0.000	0.004 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.004 ± 0.000
PCBz	0.001 ± 0.000	0.002 ± 0.000	0.003 ± 0.000	0.002 ± 0.000	0.003 ± 0.000	0.001 ± 0.000
HCB	0.041 ± 0.020	0.072 ± 0.033	0.082 ± 0.048	0.149 ± 0.189	0.119 ± 0.121	0.126 ± 0.046
α HCH	0.002 ± 0.000	0.003 ± 0.000	0.004 ± 0.000	0.004 ± 0.000	0.006 ± 0.000	0.005 ± 0.000
β HCH	0.078 ± 0.087	0.074 ± 0.037	0.179 ± 0.318	0.126 ± 0.254	0.308 ± 0.848	0.089 ± 0.033
γ HCH	0.002 ± 0.000	0.002 ± 0.000	0.001 ± 0.000	0.004 ± 0.000	0.003 ± 0.000	0.003 ± 0.000
Oxychlordane	0.043 ± 0.017	0.047 ± 0.014	0.054 ± 0.017	0.074 ± 0.046	0.059 ± 0.028	0.076 ± 0.022
t-Nonachlor	0.047 ± 0.022	0.051 ± 0.020	0.048 ± 0.022	0.071 ± 0.057	0.059 ± 0.030	0.081 ± 0.025
Heptachlor epoxide	0.014 ± 0.000	0.029 ± 0.010	0.052 ± 0.069	0.076 ± 0.097	0.030 ± 0.014	0.042 ± 0.026
Dieldrin	0.036 ± 0.017	0.053 ± 0.028	0.049 ± 0.026	0.056 ± 0.048	0.045 ± 0.022	0.036 ± 0.010
p,p'-DDE	0.965 ± 0.697	1.764 ± 1.248	1.531 ± 1.215	2.268 ± 1.704	1.663 ± 1.590	1.915 ± 1.167
o,p'-DDT	0.017 ± 0.010	0.028 ± 0.020	0.034 ± 0.041	0.031 ± 0.014	0.051 ± 0.113	0.032 ± 0.000
p,p'-DDT	0.118 ± 0.030	0.308 ± 0.328	0.225 ± 0.146	0.332 ± 0.323	0.404 ± 0.819	0.261 ± 0.064

[a] Prince Edward Island and Nova Scotia

[b] Manitoba and Saskatchewan

[c] Alberta and British Columbia.

TABLE 4

Chlorinated hydrocarbon residues in adipose tissue of Canadians as related to sex

Compound	Average µg/g wet weight ± S.D.	
	Male (N[a] = 53)	Female (N = 45)
PCB, as Aroclor 1260	0.887 ± 0.689	1.007 ± 1.114
PCB, as Aroclor 1242	0.321 ± 0.335	0.291 ± 0.178
HCBD	0.004 ± 0.000	0.003 ± 0.000
PCBz	0.002 ± 0.000	0.002 ± 0.000
HCB	0.066 ± 0.037	0.130 ± 0.141
α HCH	0.005 ± 0.000	0.004 ± 0.000
β HCH	0.183 ± 0.612	0.116 ± 0.166
γ HCH	0.003 ± 0.000	0.003 ± 0.000
Oxychlordane	0.053 ± 0.020	0.057 ± 0.033
t-Nonachlor	0.058 ± 0.033	0.054 ± 0.030
Heptachlor epoxide	0.035 ± 0.028	0.040 ± 0.059
Dieldrin	0.050 ± 0.024	0.047 ± 0.035
p,p'-DDE	1.726 ± 1.497	1.726 ± 1.284
o,p'-DDT	0.039 ± 0.078	0.025 ± 0.017
p,p'-DDT	0.355 ± 0.614	0.264 ± 0.300

[a] Number of samples

TABLE 5

Chlorinated hydrocarbon residues in adipose tissue of Canadians as related to age

Compound	Average µg/g wet weight ± S.D.		
	0-25 years (N = 33)	26-50 yrs (N = 41)	51 + yrs (N = 24)
PCB, as Aroclor 1260	0.697 ± 0.640	0.926 ± 0.567	1.233 ± 1.426
PCB, as Aroclor 1242	0.237 ± 0.215	0.319 ± 0.178	0.384 ± 0.428
HCBD	0.003 ± 0.000	0.004 ± 0.000	0.004 ± 0.000
PCBz	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000
HCB	0.051 ± 0.026	0.094 ± 0.079	0.160 ± 0.164
α HCH	0.005 ± 0.000	0.004 ± 0.000	0.004 ± 0.000
β HCH	0.067 ± 0.051	0.260 ± 0.698	0.082 ± 0.037
γ HCH	0.002 ± 0.000	0.003 ± 0.000	0.004 ± 0.000
Oxychlordane	0.045 ± 0.020	0.056 ± 0.024	0.067 ± 0.037
t-Nonachlor	0.049 ± 0.039	0.059 ± 0.024	0.063 ± 0.030
Heptachlor epoxide	0.021 ± 0.000	0.047 ± 0.066	0.042 ± 0.024
Dieldrin	0.033 ± 0.010	0.056 ± 0.028	0.059 ± 0.039
p,p'-DDE	1.052 ± 1.292	2.024 ± 1.318	2.139 ± 1.377
o,p'-DDT	0.036 ± 0.098	0.029 ± 0.017	0.034 ± 0.024
p,p'-DDT	0.195 ± 0.339	0.412 ± 0.661	0.301 ± 0.276

HCB, oxychlordane, heptachlorepoxide and p,p'-DDE and for dieldrin in the 0-25 year age group, which was significantly lower than in the other two groups.

Sixteen samples from the Eastern region and Quebec were analysed for Mirex. An average Mirex residue level of 6 ppb was found. The range was 2-19 ppb. Figure 2 shows a typical GC elution pattern of PCBs and Mirex on a capillary column. The presence of Mirex in the total pooled sample was confirmed by MS.

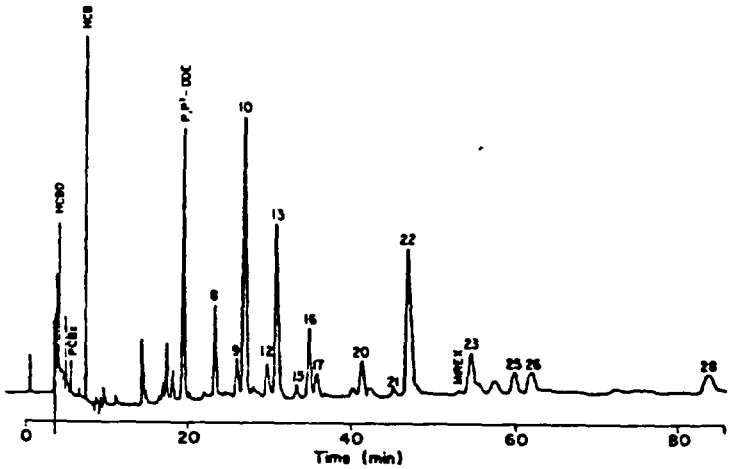

FIG. 2. A typical GC elution pattern of PCBs in human adipose tissue by capillary gas liquid chromatography. The numbers are not related to those in fig. 1, but are from an arbitrary numbering system for capillary GC, used in our laboratory.

ACKNOWLEDGEMENTS

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An Assessment of the Reproductive Toxic Potential of Aroclor 1254 in Female Sprague-Dawley Rats

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Polychlorinated biphenyls (PCBs) and their derivatives are regarded as major global pollutants (RISEBROUGH et al. 1968). In the United States, KUNTZ & STRASSMAN (1976) have found that residual amounts of PCBs accumulate in the tissues of many human subjects. From a toxicological viewpoint, PCB compounds have caused atrophy in reproductive organs, such as the uterus of rabbits, following oral administration of 300 mg of Aroclor 1254 (KOLLER & ZINKL 1973). However, this PCB compound did not produce any trophic response in the uterus of the weanling rats at doses up to 1000 µg/kg, nor did the PCB induce persistant vaginal estrus syndrome when given to these neonates (GELLERT 1978). Furthermore, VILLENEUVE et al. (1971) found no effects on the number of litters, litter size and litter weights of fetuses from rats fed dosages of 100 mg/kg/day Aroclor 1254. Oral administration of 12.5-50.0 mg/kg/day of Aroclor 1254 to rabbits for 28 days of gestation was embryotoxic (VILLENEUVE et al. 1971).

MATERIALS AND METHODS

Female albino rats (Holtzman strain) ranging from 200-300 g were maintained under standard conditions and subjected to decidualized pseudopregnancy and pregnancy inductions in accordance with previously described procedures (TAT SING & SPENCER 1981). Biochemical and physiological assays and pesticide feeding schedules were conducted in conformity with those in the fore-cited study. Similar statistical measures were employed.

RESULTS

Uterine protein (Fig. 1), uterine glycogen (Fig. 2), and ovarian protein (Fig. 3) of PCB-treated decidualized pseudopregnant rats were reduced. Placental protein (Fig. 4) and placental glycogen (Fig. 5) concentrations were decreased in pregnant rats after 10 days of PCB treatments. No embryonic resorption was observed in Day 12 dams fed PCB up to 900 ppm (Table 1A). Fetotoxicity of the PCB was demonstrated by a decrease in fetal survival rate per litter, and also by a loss in fetal weight

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loss recorded at birth (Table 1A). Reductions in chow consumption were observed at 300, 600, and 900 ppm test groups (Table 1A). Maternal toxicity as manifested by the loss in body weight appeared in test groups of 600 and 900 ppm (Table 1B).

DISCUSSION

The data described here demonstrate that dietary administration of the PCB compound (Aroclor 1254) during decidualized pseudogestation produced acute alterations in uterine and ovarian biochemistry. Uterine reductions in protein and glycogen and decreases in ovarian protein occurred in dose-related manners. Moreover, uterine weights were significantly lowered at 750 ppm and above. Hence, there exists a definite and robust uterotoxic influence for Aroclor 1254 in decidualized pseudopregnant rats. Taken altogether, this induced incompetence in uterine biochemistry (endocrine target organ) is paralleled by a significant decrease in a prominent parameter in the biochemistry of the ovary (the juxtaposed endocrine support organ). These results suggest that the lytic action of Aroclor 1254 on the uterus, especially with regards to glycogen, involves a loss of anabolic stimulation from the ovary. Since uterine glycogen is an extremely sensitive criterion for estrogen action (BITMAN et al. 1965), this response here indicates an anti-steroidal role for Aroclor 1254. In harmony with this mechanism, are studies citing the occurrence of metabolic changes in sex steroids in normal cyclic rats following PCB treatment (OBERG & INGVAST 1977; DERR 1978).

In pregnant rats, Aroclor 1254 also affected placental glycogen. Albeit, protein concentrations declined steadily in groups fed low levels of Aroclor 1254, and became progressively reduced with increasing dosages.

VILLENEUVE et al. (1971) reported that no fetal resorption was produced in pregnant rats given daily doses of Aroclor 1254 by oral gavage. In our study, wherein the toxicant was administered via the diet, the fetal survival potential was drastically affected at 300 ppm and greater (Table 1A). Moreover, at dietary exposures of 300, 600 and 900 ppm, the food intakes were diminished. By corroboration, it has been documented that pregnant rats are more susceptible to the toxicological effects of Aroclor 1254 during dietary deprivation (BYRE 1974). Therefore, reductions in the fetal survival rates and neonatal weights may be manifested through a joint function on the part of Aroclor 1254 toxicity and by means of a decrease in nutritional intake by maternal rats (Table 1A).

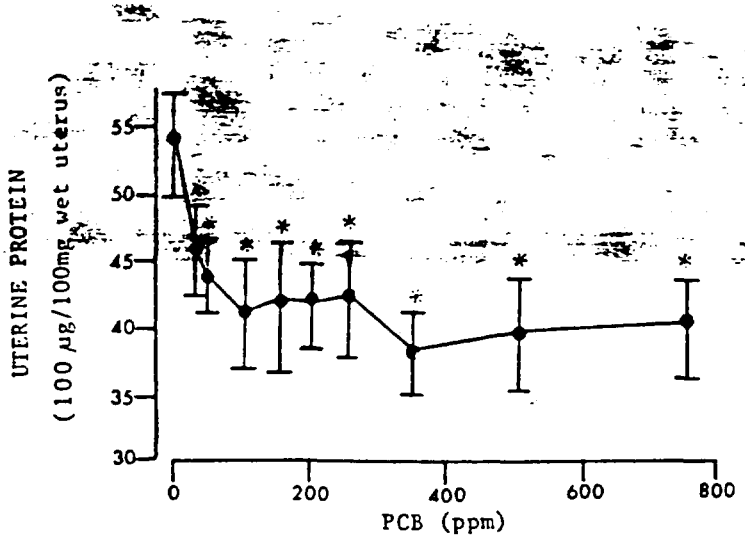


Fig. 1. Uterine protein content of Day 10 decidualized pseudopregnant rats fed different concentrations (ppm) of Aroclor-1254 containing diet. Each point represents the mean of 10 rats and the bar indicates the SE. An asterisk shows values that are significantly different from the control, using Student's t-test ($p < 0.05$).

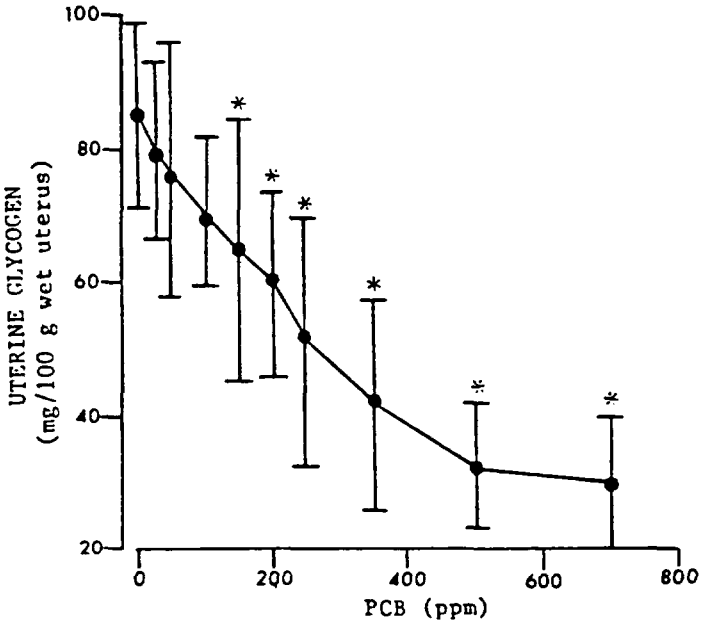
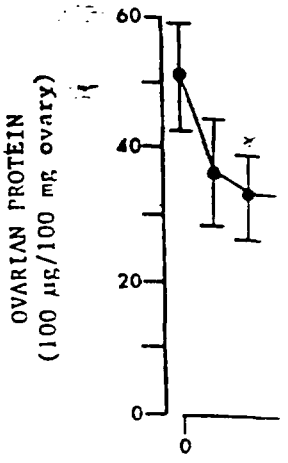


Fig. 2. Uterine glycogen content of Day 10 decidualized pseudopregnant rats fed different concentrations (ppm) of Aroclor-1254 containing diet. Each point represents the mean of 10 rats and the bar indicates the SE. An asterisk shows values that are significantly different from the control, using Student's t-test ($p < 0.05$).



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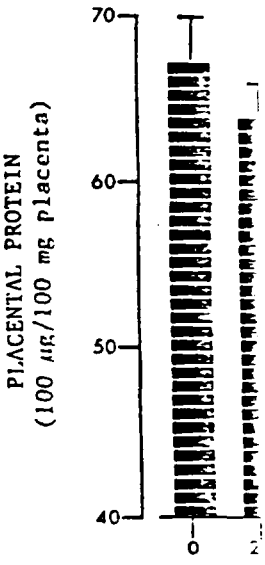

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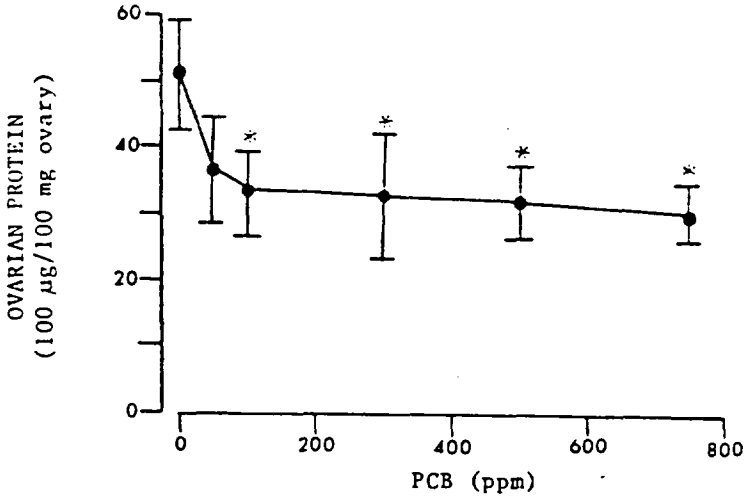

Fig. 3. Ovarian protein content of Day 10 decidualized pseudopregnant rats fed different concentrations (ppm) of Aroclor-1254 containing diet. Each point represents the mean of 5 rats and the bar indicates the SE. An asterisk shows values that are significantly different from the control, using Student's t-test ($p < 0.05$).

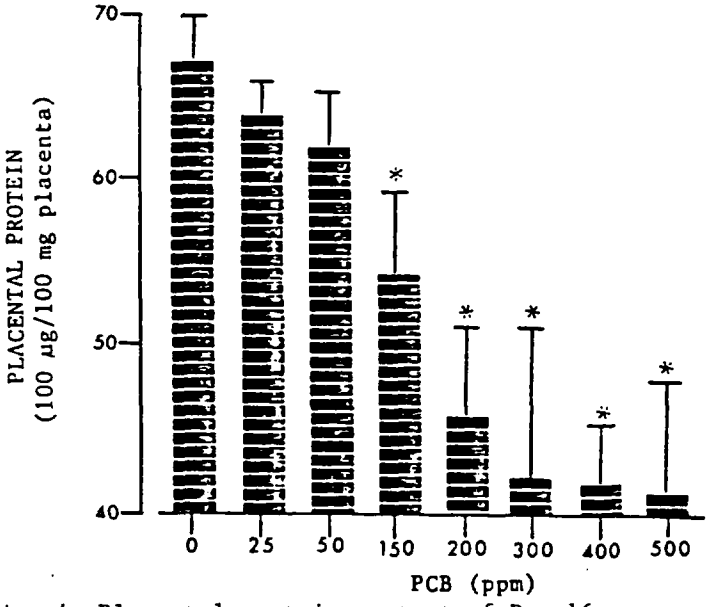

Fig. 4. Placental protein content of Day 16 pregnant rats fed different concentrations (ppm) of Aroclor-1254 containing diet. Each bar represents the mean of 7 rats. The standard errors of the means are plotted above the bars. An asterisk shows values that are significantly different from the control, using Student's t-test ($p < 0.05$).

TABLE 1A
EFFECT OF PCB (AROCLOR 1254) ON PREGNANCY

Dietary[a] treatment with PCB (ppm)	Daily intake of PCB+chow (g/day/rat) $\bar{X}\pm SE$	Number of litter	Implantations at Day 6 per dam $\bar{X}\pm SE$	Number of conceptuses at Day 12 $\bar{X}\pm SE$	Percentage of fetal survival rate per litter at birth $\bar{X}\pm SE$	Average fetal weight per litter at birth (g) $\bar{X}\pm SE$
0	20.8±0.6	7	12.29±1.17	12.29±1.17	79.54± 3.84	7.02±0.12
25	18.6±0.4	7	12.40±0.55	12.40±0.55	66.21± 9.42	6.84±0.08
50	18.0±0.6	7	12.60±0.68	12.60±0.68	65.87±10.33	6.67±0.06
100	19.3±0.1	7	12.80±0.37	12.80±0.37	65.38±12.20	6.19±0.15[b]
200	17.7±0.9	7	10.60±0.51	10.60±0.51	63.05± 8.44	5.61±0.10[b]
300	15.8±0.6[b]	7	12.40±0.40	12.40±0.40	28.03±12.29[b]	5.58±0.19[b]
600	9.6±0.7[b]	7	12.43±1.37	12.43±1.37	17.14±11.28[b]	---
900	7.0±0.6[b]	7	12.14±0.75	12.14±0.75	00.00± 0.00	---
ANOVA	< 0.05				< 0.05	< 0.05

[a]Rats were given Aroclor 1254 at respective concentrations in a diet from Days 6 through 15 of pregnancy.

[b]Significantly different from the control, using Student's t-test ($p < 0.05$).

TABLE 1B

BODY WEIGHT OF PREGNANT RATS GIVEN PCB (AROCLOR 1254)

Dietary[a]	Maternal body weight	Δ body weight

Rats ... Aroclor 1254 at respective concentrations in a diet from Days 6 through 15 of pregnancy.

[b]Significantly di ... rent from the control, using Stude... t-test ($p < 0.05$).

TABLE 1B

BODY WEIGHT OF PREGNANT RATS GIVEN PCB (AROCLOR 1254)

Dietary[a] treatment with PCB (ppm)	Maternal body weight (g) $\bar{X} \pm SE$		Δ Body weight (g)
	Day 6 of gestation	Day 16 of gestation	Day 16- Day 6
0	273± 8	302± 4	+ 29
25	232± 8	265±12	+ 33
50	226±10	255± 8	+ 29
100	243± 7	274±10	+ 31
200	220± 6	245± 7	+ 25
300	268±12	288±10	+ 20
600	233±10	215± 5	- 18
900	223± 6	174± 5	- 49
ANOVA		< 0.05	

[a]Rats were given Aroclor 1254 at respective concentrations in a diet from Days 6-15 of pregnancy.

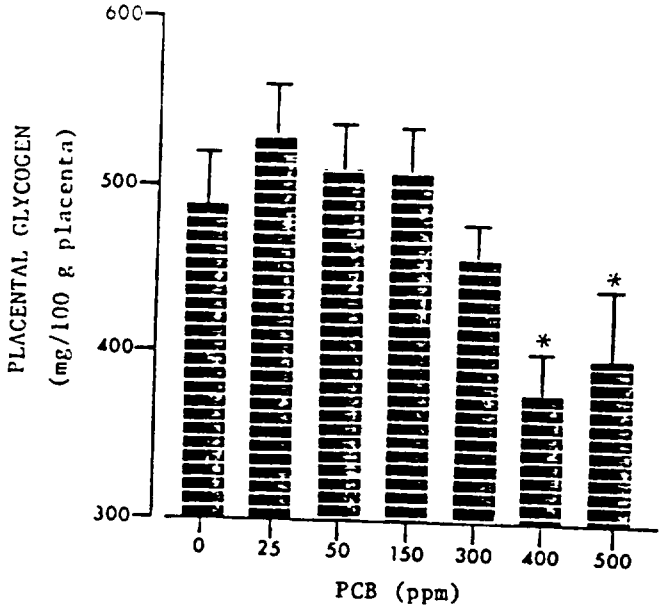

Fig. 5. Placental glycogen of Day 16 pregnant rats fed different concentrations (ppm) of Aroclor-1254 containing diet. Each bar represents the mean of 7 rats. The standard errors of the means are plotted above the bars. The asterisk shows values that are significantly different from the control, using Student's t-test ($p < 0.05$).

The study indicates that Aroclor 1254 caused imbalances in hormonally-mediated biochemical effects in pseudopregnant and pregnant rats. Furthermore, the compound has both indirect or systemic and direct action (reduced fetal survival rates and neonatal weights) on fetal growth and development.

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Toxicity of the PCBs Aroclor 1254 and 1242 to Embryos and Larvae of the Mummichog, *Fundulus heteroclitus*

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PCBs as contaminants of the aquatic environment have aroused concern because of their toxicity, persistence, and tendency to bioaccumulate. Numerous studies of their effects on aquatic biota have been published. HALTER & JOHNSON (1974) found premature hatching of coho salmon (Oncorynchus kisutch) in 15 ug/L of Aroclor 1254. HANSEN et al. (1975) found that hatching time of Cyprinodon variegatus was unaffected by Aroclor 1016, and that eggs and larvae had comparable tolerances. However, SCHIMMEL et al. (1974) reported C. variegatus larvae to be more sensitive than embryos to Aroclor 1254, and MAUCK et al. (1978) reported that larvae of the brook trout (Salvelinus fontinalis) were also more sensitive than embryos. NEBEKER et al. (1974) found that embryos of the fathead minnow and flagfish were also more resistant to Aroclor 1242 than larvae of these species.

mummichog, Fundulus heteroclitus, is an abundant estuarine species. t is easily maintained and its embryos have been staged. In previous studies e have described teratological effects of insecticides (WEIS & WEIS 1974), nd of heavy metals (WEIS & WEIS 1977 a,b) on this species, and have ted that different females produced eggs with differing degrees of sensitiy. When subjected to the same concentration of methylmercury (MeHg) ne batches were severely affected while others were only mildly affected EIS et al., in press).

In the present study we wished to learn the effects of the PCBs Aroclor 2 and 1254 on mummichog embryos, and the degree of variation in eptibility among eggs of different females. We also wished to see if embryonic tolerance of a batch of embryos would be paralleled by larval ance, or by the embryonic MeHg tolerance, and whether prior exposure CB as embryos could alter the tolerance of the larvae.

MATERIALS AND METHODS

lt fish were collected from Southampton, NY by seining. Eggs and ere stripped from fish into individual fingerbowls of filtered seawater o salinity). Toxicants were added after cleavage began, at which s which failed to cleave were removed. The standard length of each as recorded. Each batch of eggs was divided into a control group, reated with 0.05 mg/L MeHg (I.C.N. Pharmaceuticals, Plainview olved first in 0.2% $NaHCO_3$) and groups treated with Aroclor 1254 or 1242 (Analabs Inc. N stock solution of 10 mg/ water for an intermediate daily. Previous work hav embryos (WEIS & WEIS 1 groups contained 20 or m four days of development

After one week of inc stereomicroscope for mal evaluated by indices pre in press) which rank the skeletal system. Mean in

At the time of hatchi noted. To stimulate hatc water changed daily start used in all cases. PCB-tr (HR) which is %hatch in the first day in which at generally below 1.0, ref

Larval susceptibility 5.0 mg/L PCB. Newly h groups were separated int water. At least 10 experi changed and re-dosed da recorded. All larvae we

Spearman's Rank corr to correlate the HR respo indices of MeHg suscepti larval susceptibility of c which had previously bee susceptibility of larvae w

The first group of exp of eggs were exposed to c on embryonic developmer and some groups showed c on hatching. Larval tests of former control eggs in four batches in which we mg/L as embryos, larval mg/L.

The second group of e seven batches of eggs v malformations, but d

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or 1242 (Analabs Inc. North Haven CT) dissolved first in acetone for a stock solution of 10 mg/mL. This stock solution was dispersed in filtered sea water for an intermediate dilution of 0.1 mg/mL which was made up fresh daily. Previous work having shown that acetone had no gross effects on these embryos (WEIS & WEIS 1974); controls were untreated. All experimental groups contained 20 or more eggs. Solutions were changed daily for the first four days of development.

After one week of incubation at 24° C embryos were examined under a stereomicroscope for malformations. The MeHg response of each egg was evaluated by indices previously described (WEIS & WEIS 1977b, WEIS et al. in press) which rank the relative severity of defects in the head, heart, and skeletal system. Mean indices for each batch were calculated.

At the time of hatching, a retardation of hatch due to PCB was often noted. To stimulate hatching, both control and PCB-treated batches had water changed daily starting on day 12 of development. Clean water was used in all cases. PCB-treated batches were evaluated using a hatch ratio (HR) which is %hatch in PCB/ % hatch in controls. This was calculated on the first day in which at least 50 % of the controls had hatched. The HR was generally below 1.0, reflecting retardation of hatching in PCB.

Larval susceptibility was determined by a short-term toxicity test with 5.0 mg/L PCB. Newly hatched fry from control groups and PCB-treated groups were separated into control and experimental dishes in 50 mL seawater. At least 10 experimental larvae were used in each dish. Water was changed and re-dosed daily for a week. The percent dead every 24 h was recorded. All larvae were unfed.

Spearman's Rank correlation coefficient (SIEGEL 1956) was performed to correlate the HR response of each batch of embryos to PCB with the indices of MeHg susceptibility, the standard length of the female, and the larval susceptibility of controls from that batch. The susceptibility of larvae which had previously been exposed as embryos was also compared with the susceptibility of larvae which had not received prior exposure.

RESULTS

The first group of experiments was done with Aroclor 1254. Eight batches of eggs were exposed to concentrations of 0.01 - 10.0 mg/L with no effect on embryonic development or hatching. Embryonic mortality was negligible, and some groups showed acceleration, some retardation, and some no effect on hatching. Larval tests for seven days likewise showed no larval mortality of former control eggs in concentrations up to 10.0 mg/L. However, in the four batches in which we tested larvae which had been pre-exposed to 10.0 mg/L as embryos, larval mortality (about 20 %) was noted after 72 h in 5.0 mg/L.

The second group of experiments was done with Aroclor 1242. Twenty-seven batches of eggs were exposed to 10.0 mg/L which also did not cause any malformations, but did cause a consistent retardation of hatching. The HR

varied from 0 (none hatched by the day 50% of controls had hatched) to 1.0 (equal percentages of controls and experimentals hatched). There was no correlation (by Spearman's test) of this PCB response with the indices of MeHg response. However, the HR was positively correlated with female standard length (Fig. 1). It can be seen that longer (and presumably older) females produced eggs that were less affected by the PCB. There was also a correlation of HR with fecundity (number of eggs in the batch) ($P < 0.01$) although this is probably a secondary relationship, since larger fish tend to produce more eggs.

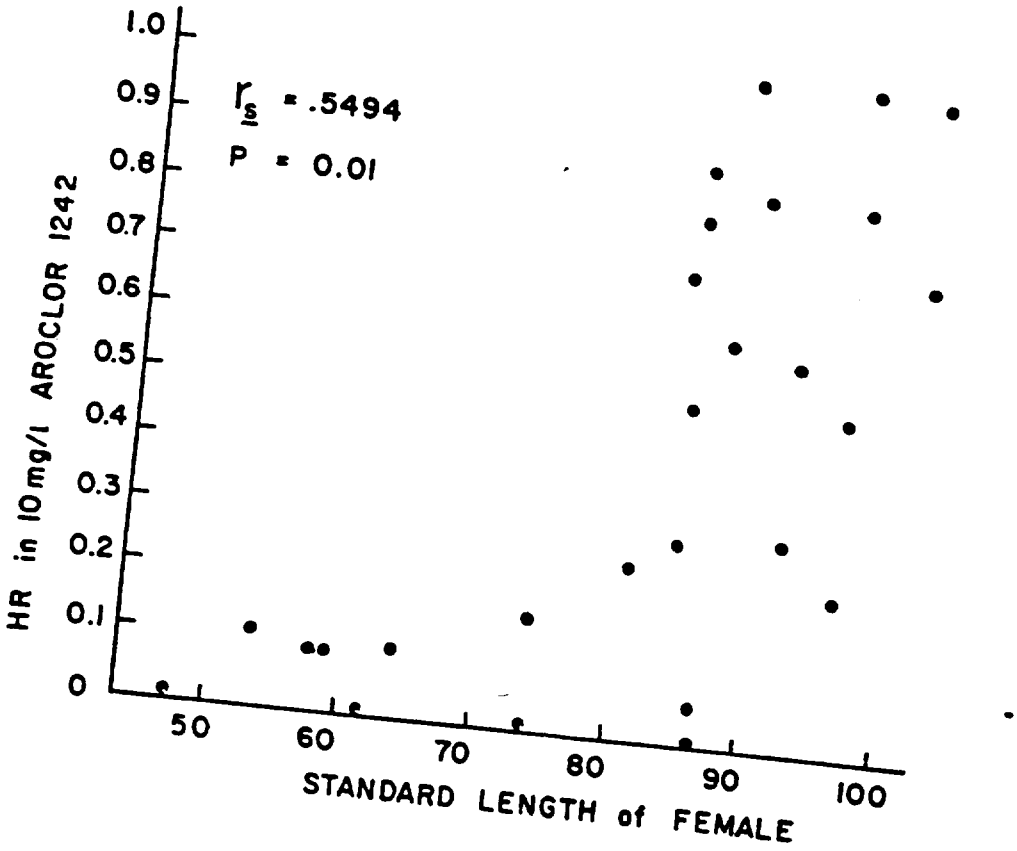

Figure 1. Relationship of Hatch Ratio (HR) of a batch of eggs exposed to 10 mg/L Aroclor 1242 to maternal length.

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For the short-term larval toxicity test 5.0 mg/L was chosen as the standard dose, since this concentration did not kill all larvae immediately, nor did they all survive the 7-day period. The percentage dead at 72 h varied from 0 - 100%, and averaged 45 ± 11 percent among 17 batches of larvae that hatched from control groups of embryos. This larval tolerance was not correlated with the HR of the embryos, however. Larval mortality in 5.0mg/L was considerably higher among the 9 groups which had previously been exposed to 10.0 mg/L as embryos. In these groups, the percentage dead at 72 h was 11 % in one group and 100 % in all the others. Nine batches exposed to 1.0 mg/L as embryos showed an intermediate response. Fig. 2 shows the comparison of mortality among groups of larvae with no pre-exposure, pre-exposure to 1.0 mg/L, and pre-exposure to 10.0 mg/L as embryos. Larval deaths in pre-exposed groups tended to be precipitous, going from 10-20% dead at 48 h to 100% dead at 72 h. No mortality occurred in any control larvae, including in groups which had had prior exposure to PCB as embryos.

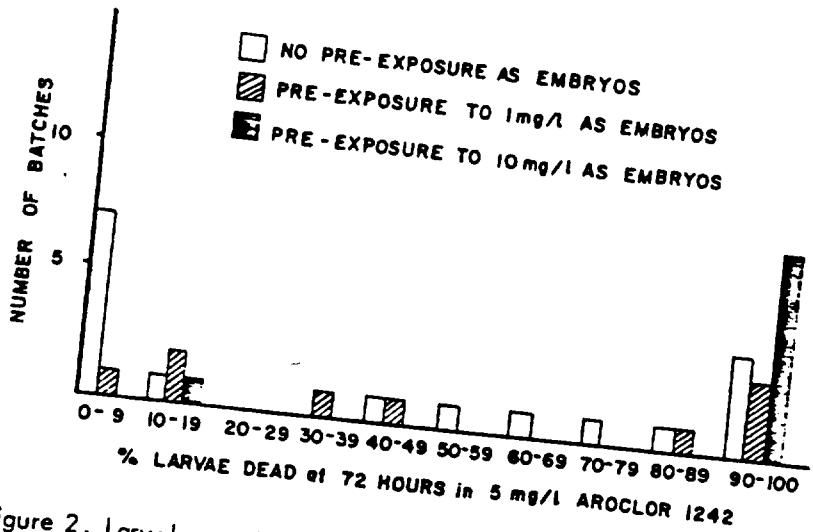

Figure 2. Larval mortality in 5.0 mg/L Aroclor 1242 in groups with no embryonic pre-exposure, pre-exposure to 1.0 mg/L, and pre-exposure to 10.0 mg/L.

DISCUSSION

Mummichog embryos proved to be highly resistant to the PCBs in these short-term tests. No teratological effects were noted in embryos in

up to 10 mg/L. It is likely that the actual concentrations were less than the nominal concentrations introduced. LAUGHLIN et al. (1977) and ROESIJADI et al. (1976) have shown actual concentrations to be considerably less than the nominal concentrations, and that a decrease of 30-60% took place after 24 h. ROESIJADI et al. (1976) attributed the rapid loss of PCB to volatilization, adsorption onto the glass, and uptake by organisms. The daily changing of our solutions helped to bring the level up, but we did not measure actual concentrations.

We found the larvae to be more sensitive to Aroclor 1242 than embryos. This is similar to the findings of SCHIMMEL et al. (1974) with Aroclor 1254 on Cyprinodon variegatus. The concentrations used by those investigators were considerably lower than ours, reflecting the long-term nature of their study, as compared to the short-term nature of ours, but also, probably, a greater tolerance on the part of F. heteroclitus than other species tested. HALTER & JOHNSON (1974) noted embryo mortality of coho salmon (Oncorynchus kisutch) in 15 ug/L of Aroclor 1254, and FREEMAN & IDLER (1975) noted great reduction in hatchability of brook trout (Salvelinus fontinalis) eggs in 0.2 mg/L Aroclor 1254. SCHIMMEL et al. (1974) noted decreased embryo survival of C. variegatus in 10 ug/L Aroclor 1254. These are all much lower concentrations than used in the present study.

Aroclor 1254 had an extremely low toxicity to F. heteroclitus in that (nominal) levels up to 10 mg/L produced no noticeable effects on embryonic development, hatching, or larval survival during a 7-day period (except for pre-exposed larvae). Other investigators have also noted differential toxicities of different PCBs. NEBEKER et al. (1974) noted that Aroclor 1254 was more toxic than Aroclor 1242 to the fathead minnow, Pimephales promelas (this being the reverse of our findings), but MAYER et al. (1977) found Aroclor 1242 to be more toxic than 1254 to cutthroat trout. DEFOE et al. (1978) found Aroclor 1260 to be more toxic than 1248 to P. promelas, and HANSEN et al. (1975) found Aroclor 1016 only 0.01 times as toxic as 1254 to C. variegatus.

The degree to which hatching was retarded by Aroclor 1242 was inversely correlated with the length of the female that produced the eggs. This is probably a reflection of the general phenomenon that older fish produce more successful eggs with greater hatchability (PITTMAN 1979). We have previously found embryonic Pb tolerance to be correlated with female length in F. heteroclitus (WEIS & WEIS, in press).

No correlation of PCB tolerance with MeHg tolerance was observed, showing that tolerance to one toxicant does not imply tolerance to others, as was previously noted with MeHg and Pb tolerance (WEIS & WEIS, in press).

The effects of PCB on larval survival indicate, as mentioned previously, greater susceptibility than the embryos to Aroclor 1242. Larval tolerance

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was not correlated with embryonic tolerance, indicating that different mechanisms of toxicity are involved. Since embryonic tolerance was correlated with maternal length, it is likely that some maternally derived trait, probably chorionic permeability, is involved in conveying tolerance to the embryos. It is likely that the chorion protects embryos to differing degrees, but in all cases, the embryos would be exposed to less toxicant than the larvae. The differential susceptibility of different groups of larvae, on the other hand, must be due to differences among the larvae themselves.

Larvae which were pre-exposed as embryos were much more susceptible, indicating that the pre-exposure does not produce acclimation and then increased tolerance, as has been reported for a number of pollutants including heavy metals (DIXON & SPRAGUE 1981 a,b; LLOYD 1960) and pesticides (ORCIARI 1979). On the contrary, the pre-exposure to PCB served to weaken the larvae and make them more susceptible to further exposure. A similar increase in sensitivity was seen by DIXON & SPRAGUE (1981a) in cyanide-exposed rainbow trout (S. gairdneri), and by SWARTS et al. (1978) in sulfuric acid-exposed brook trout (S. fontinalis).

There is evidence that PCBs have cumulative, slow acting effects. MAUCK et al. (1978) have noted long-term effects on brook trout (S. fontinalis) larvae, showing that levels of Aroclor 1254 which had no effect on hatchability, hatching time and sac fry survival, did finally cause fry mortality after 48 days. Similar slow cumulative effects of Aroclor 1016 on pinfish (Lagodon rhomboides) were noted by HANSEN et al. (1974) and on C. variegatus by HANSEN et al. (1975). NIMMO et al. (1975), after two-week tests of Aroclor 1254 on several species of shrimp, long nose killifish (F. similis), and spot (Leiostomus xanthurus), concluded that acute tests did not show the true sensitivity of these species. In comparison to 48 h tests, those lasting two weeks showed a 100-fold increase in toxicity.

Although we have not done long-term, low dose experiments, our data showing increased larval susceptibility after embryonic pre-exposure, and the precipitous nature of the larval deaths indicate that a similar cumulative process is occurring in this species. It is likely that longer term larval tests would have revealed significant mortality at much lower dose levels.

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Depuration and Biological Half-life of ^{14}C-PCB in Aquatic Organisms

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Polychlorinated biphenyls (PCBs) are a class of man-made halogenated aromatic hydrocarbons that are distributed throughout the environment (SCHNEIDER 1979). Because PCBs are hydrophobic and resistant to environmental degradation, aquatic plants and animals tend to accumulate them from the surrounding water environment (KALMAZ and KALMAZ 1979). Results of several investigators indicated that aquatic organisms accumulate total body concentrations of PCBs thousands of times greater than that of the surrounding water. SANDERS and CHANDLER (1972) showed that fresh water crustaceans and insects accumulated PCB (Aroclor 1254) up to 48,000 times greater than the concentration in the water. Similar exposure experiments conducted with estuarine animals showed oysters concentrating 101,000 (LOWE et al. 1972), and fish 37,000 (HANSEN et al. 1971) times the amount of PCB in the water.

The biomagnification of PCBs by organisms is affected by several factors, such as ambient concentration, species, trophic level variations, adsorption, and lipid content (KALMAZ and KALMAZ 1979). SKEA et al. (1979) studied bioaccumulation of Aroclor 1016 in Hudson River fish and found that the total quantity of PCBs accumulated by the different species of fish was closely related to the fat content and weight of the fish. Fish tissues analyzed from the River Avon showed that those fish species containing a higher percent lipid tended to accumulate more PCB (KPEKATA 1975). DEFOE et al. (1978) reported that bioconcentration in minnows was independent of the PCB concentration in the water. However, CAMP et al. (1974) found that there was a direct relationship between biological magnification and concentration of Aroclor 1254 in aquaria water for catfish.

Contaminated aquatic organisms, if transported to clean water after the exposure period, will depurate or eliminate a xenobiotic chemical from their bodies over a time span that is dependent upon differences in the species' metabolism and excretion (HAMDY and PRABHU 1978). HATTULA and KARLOG (1973) stated that the cytochrome P-450 enzyme complex, absent in fish but present in mammals and birds, dramatically affects the metabolism of PCB.

NIMMO et al. (1974) examined the accumulation and depuration of Aroclor 1254 in grass shrimp and reported that 60 to 90% of the accumulated PCB was lost from the shrimp within 4 weeks after exposure was stopped. HANSEN et al. (1971) found that after 84

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days of flushing in PCB-free water, the relative amount of Aroclor 1254 in spot fish had decreased 73%.

Therefore, this study was conducted to follow the absorption, elimination, and biological half-life (BHL) of ^{14}C-PCB (Aroclor 1254) in selected organisms representing a simple aquatic ecosystem. Factors affecting the loss of ^{14}C-PCB from labeled bacterial cells following washing were also examined.

MATERIALS AND METHODS

Trophic Systems. The following 4 organisms were used: cells of a PCB-resistant Bacillus sp. previously isolated in our laboratory representing the lowest level (NUNN 1979), mosquito larvae (Aedes aegypti), guppies (Lebistes reticulatus), and Cichlids (Cichlasoma facetum); each representing the next higher food chain system, respectively. Cichlids are a species of medium-sized tropical fish whose common name is Jack Dempsey.

Labeling of Test Organisms. Uniformly labeled carbon 14-PCB (approximately 50% chlorine by weight and with a gas chromatographic pattern closely resembling that of Aroclor 1254), purchased from Amersham Corp., was used for all isotopic labeling. An 18 h actively growing Bacillus spp. culture was inoculated into flasks containing 250 ml glucose basal salt broth (HAMDY and NOYES 1975) plus 0.1% Tween 80 (a surface active agent for PCB emulsification), 200 ppm non-radioactive Aroclor 1254, and 2 µCi ^{14}C-PCB. The culture was allowed to grow for 48 h at 37°C, and then the labeled cells were harvested by centrifugation (17,000 x g) and washed once with sterile saline.

Approximately 200 eggs of A. aegypti were sterilized by immersion in 75% ethanol for 5 min (LEA et al. 1956). The eggs were allowed to hatch in a beaker containing 400 ml distilled water (27°C), 0.1% Tween 80, and sterile diet (Purina guinea pig chow) to yield sterile larvae. When the larvae were 1 d old, 0.8 µCi of ^{14}C-PCB was added to the beaker; the larvae were then allowed to grow for 48 h. The labeled larvae were collected and washed once with distilled water.

Guppies (L. reticulatus) grown in aquarium tanks (25°C) were labeled by placing 12 fish in beakers containing 1 L distilled water, 0.1% Tween 80, 1.6 µCi ^{14}C-PCB, and incubated for 48 h. Cichlids were labeled by the same procedure used for guppies.

Counting System. A known weight of water, saline, or sample (bacterial cells or nitric acid digest of the larvae, guppies, or Cichlids) was each placed in a standard scintillation vial containing 10 ml of toluene-based scintillation fluor and counted in a Beckman LS 7500 liquid scintillation system. The instrument was set to count all samples to ± 2% error or 10 min, whichever occurred first; all samples were corrected for background.

Biological Depuration. Each labeled system was transferred to isotope-free saline (bacteria) or distilled water (larvae, guppies, and Cichlids) and incubated. Depuration rate of ^{14}C-PCB by bacterial cells was studied at 37°C. Temperatures of 27 and 25°C were used for larvae and fish, respectively. During incubation, samples of labeled bacterial cells, aliquots of the

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Data, reported as log of initial activities (1.0), were plotted as a function of incubation (d); and the rate of depuration (k, slope of line) was calculated for each system. The 50% biological retention time (biological half-life, BHL) of ^{14}C-PCB was also determined and reported as the time, in d, required for half of the radioactivities of ^{14}C-PCB (cpm/g) to be eliminated from each trophic system.

Confirmation of the Presence of PCB during Bacterial Depuration. This was conducted by extracting 9 ml saline from the depuration experiments with 5 ml pesticide-grade hexane. The top layer containing the PCB and hexane mixture was removed and subjected to chromatographic analyses using a Tracor Model 560 Gas Chromatograph equipped with a Pyrex glass column 6' x ¼" packed with 3% OV-1 on 80/100 Chromosorb W Hp. The carrier gas was 95% argon and 5% methane at a flow rate of 60 ml/min. The operating temperatures for the injector, column, and electron capture detector (^{63}Ni) were 150, 200, and 350°C, respectively. Chromatograms of the hexane extract were compared with those of a control system containing Aroclor 1254 PCB, but no bacteria.

Loss of ^{14}C-PCB from Bacterial Cells by Washing. Fresh and frozen Bacillus sp. cells, labeled with ^{14}C-PCB (same as used in depuration experiments) were used. The frozen cells were allowed to thaw and both types of labeled cells were then washed 5 times with pesticide-grade hexane. In another experiment, the labeled cells were washed 5 times with sterile 0.05 M sodium phosphate buffer (pH 7.0), immediately followed by 5 washes with pesticide-grade hexane. After each wash the cells were centrifuged (17,000 x g) and aliquots of supernatant counted to determine the % loss of ^{14}C-activities by cells as a function of wash numbers and reported as % of initial counts.

RESULTS AND DISCUSSION

Data on the depuration of ^{14}C-PCB by all 4 systems during incubation is presented in Fig. 1. Three phases of elimination were noted for the Bacillus culture. Rapid elimination was seen through the 7th d ($k = -0.0305$ d^{-1} for 0-3 d, and -0.0663 d^{-1} between 3-7 d) followed by a very slow rate ($k = -0.0009$ d^{-1}) to the 17th d. A BHL of 6.4 d was evident from the graph based on the 1st phase only. In larvae, 3 depuration phases were also present. A slope (k) of -0.0229 d^{-1} occurred after the 1st d, -0.0859 d^{-1} between 1-2 d, and -0.0899 d^{-1} between 2-5 d with a BHL of 4.4 d. Guppies showed 2 distinct depuration phases; the 1st exhibited a rate of -0.1308 d^{-1} and -0.1373 d^{-1} for the 2nd with a BHL of 3.3 d. The Cichlids also showed 2 phases of elimination; a rapid rate ($k = -0.1024$ d^{-1}) occurring during the 1st d followed by a slower one thereafter ($k = -0.0574$ d^{-1}) with a BHL of 5.1 d.

Table 1 summarizes all data including BHL for each system

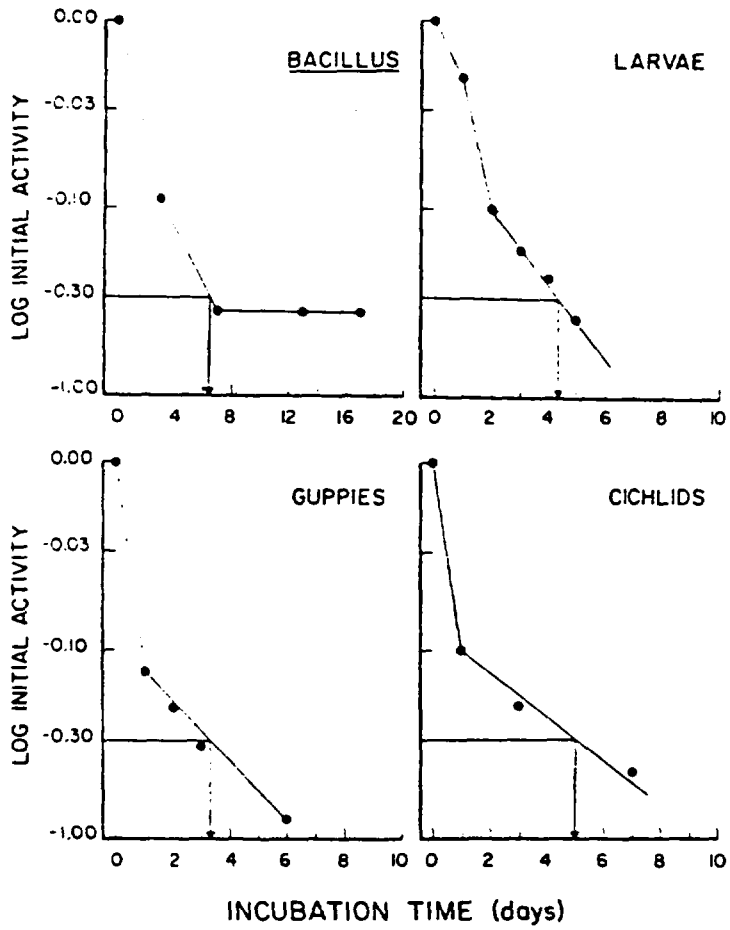

Fig. 1. Depuration and biological half-life of ^{14}C-PCB in four trophic systems. Initial activity = 1.0.

calculated from log values using regression analyses. In bacteria, using all data between 0-17 d, the BHL was 10.46 d. Whereas, using values for d 7-17 (the 2nd depuration phase), the BHL was 55.94 d. Note that all subsequent half-lives for larvae and fish based on log values using regression analyses very nearly approximate those calculated from the graphs of log values.

Several earlier studies conducted on depuration of PCBs in fish indicated that the BHL ranged from 1 to 3 months and that certain factors affected the BHL. Among these were: species differences; differences in metabolic behavior or PCB components of different chlorine content; differential solubility of PCB components, and lastly, that clearance is a combination of movement back out through the gills plus excretion in the urine and

TABLE 1.. Depuration rates (k, slope of line) in various trophic levels labeled with ^{14}C-PCB.

Incubation	k value[a]	Correlation	Biological Half-Life (Days)

TABLE 1. Depuration rates (k, slope of line) in various trophic levels labeled with ^{14}C-PCB.

Trophic System	Incubation Time (Days)	k value[a] Days^{-1}	Correlation Coefficient	Biological Half-Life (Days)	
				Graph[b]	Calculated[c]
Bacteria	0-3	-0.0305	-1.0		10.46 (0-17d)
(PCB-resistant	3-7	-0.0663	-1.0	6.4 d	
Bacillus spp.)	7-17	-0.0009	-0.8030		55.94
Mosquito Larvae	0-1	-0.0229	-1.0		4.47 (0-5d)
(Aedes aegypti)	1-2	-0.0859	-1.0	4.4 d	
	2-5	-0.0899	-0.9707		4.35
Guppies	0-1	-0.1308	-1.0		2.47 (0-6d)
(Lebistes reticulatus)	1-6	-0.1373	-0.9927	3.3 d	2.54
Cichlids	0-1	-0.1024	-1.0		4.62 (0-7d)
(Cichlasoma facetum)	1-7	-0.0574	-0.9988	5.1 d	4.57

[a]Slopes were calculated from graphs of initial activities vs. incubation time.
[b]From graph of log values. [c]Regression analyses.

feces (HUTZINGER et al. 1972; HANSEN et al. 1974; and BRANSON et al. 1975). CALAMBOKIDIS et al. (1979) found that the concentration of PCB in mussels decayed logarithmically with calculated half-lives ranging from 3 d for a PCB component with 2 and 3 chlorines to 50 d for a PCB component with 6 and 7 chlorines. These researchers established that Aroclor 1242, 1254, and 1260 had half-lives of 8, 23, and 30 d, respectively.

Our results showed a much shorter BHL for ^{14}C-PCB than others. It is possible that the size of the trophic system used, as well as differences in species and metabolic rates, may affect the data. NARBONNE (1979) reported that PCB concentration in mullets was dependent on food intake, so size of the organism is another important factor to be considered when evaluating the data.

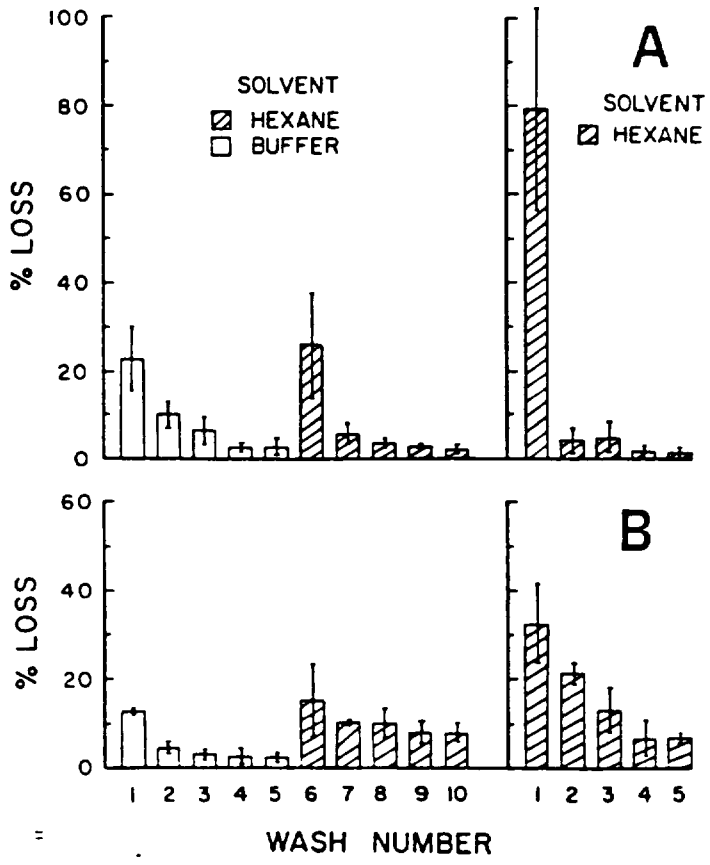

Fig. 2. Loss of ^{14}C-PCB from bacterial cells by washing. A represents data for fresh cells and B, frozen (thawed) cells.

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Comparison of the chromatograms between the hexane extracts of saline during depuration by bacterial cells showed that PCB (1254) was being eliminated from the cells.

Experiments were also conducted to ascertain if depuration was due to excretion of PCB or by merely washing of PCB absorbed onto the cell wall. Data obtained (Fig. 2A & B) revealed that after 5 washes in pesticide-grade hexane, a total of 90.6 and 79.5% of the ^{14}C-PCB (based on initial activites as 100%) radioactivities were lost from the fresh and frozen cells, respectively. When the labeled cells were first washed (5 times) with 0.05 M sodium phosphate buffer, a total of 44.3 and 25.4% of the ^{14}C-PCB radioactivities were lost from the fresh and frozen cells, respectively. After the remaining 5 washes with pesticide-grade hexane, an additional 39.5% was lost from the fresh cells and 51.9% from the frozen ones. This indicated that the percent of the ^{14}C-PCB lost by washing is dependent upon the condition of the cells, the solvent and the number of washes.

This study established the depuration of PCB (Aroclor 1254) in all trophic systems used, but the rates differed in magnitude. All systems, except bacteria, followed exponential elimination with BHLs ranging from 3 to 5 d. Washing may also play a significant role during the depuration phases.

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Biodegradation of Aroclor 1221 Type PCBs in Sewage Wastewater

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Environmental Contaminants Division, National Water Research Institute, Burlington, Ontario, Canada L7R 4A6

Highly valued for their electric insulating and other properties, polychlorinated biphenyls (PCBs) have, until recently, found extensive use in industry as dielectric fluids in transformers, hydraulic fluids in heavy machinery, and in formulations for paint and ink (BROADHURST 1972). It has been estimated that the total world production of PCBs during the past 45 years has exceeded one million tons, of which about 40% has been released to the environment (Interdepartmental Task Force 1972). Unfortunately, their stability also increases their persistence, and indeed they do not break down easily in the natural environment including the lower chlorinated ones (DUINKER et al. 1980). As a result, high concentrations of PCBs have accumulated at various levels in the food-chains in our ecosystem. Thus PCBs have been detected in both fresh water and sea water (PEAKALL 1975), in sediments (FRANK et al. 1977), plankton (WARE & ADDISON 1973), fishes (VEITH et al. 1979), birds (BRAESTRUP et al. 1974), and in human tissues (MUSIAL et al. 1974) indicating that they are ubiquitous contaminants in the total environment.

The literature now abounds with reports concerning the metabolism of PCBs in living organisms and several excellent works regarding microbial degrdation of PCBs have been published (AHMED & FOCHT 1973; HUTZINGER et al. 1974; WONG & KAISER 1975; FURUKAWA & MATSUMURA 1976). Studies on microbial degradation of PCBs are essential to understand the behaviour of PCBs in the environment. One difficulty encountered in the study of the interaction between PCBs and biota is that PCBs are not single compounds, but complex mixture of different isomers (TUCKER et al. 1975), which greatly complicates the assessment of the processes of interaction with microorganism such as biodegradation and biotransformation. For example, PCBs mixtures were reported to biodegrade much faster than the pure components (BAXTER et al. 1975) implying that different mechanisms could be involved in the degradation of commercial PCBs formulations and their correponding single isomer. As significant amounts of PCBs enter the aquatic environment via the route of the wastewater effluents (BERGH & PEOPLES 1977), there is a need for information concerning the fate and behaviour of PCBs in the sewage effluent. The objective of this study was to assess the biodegradation of commercial PCBs formulations in municipal sewage including the factors affecting the degradation.

MATERIALS AND METHODS

Chemicals: The commercial PCBs mixture (Aroclor 1221, lot no. KD-07-101) was courteously provided by the Monsanto Co., St. Louis,

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MO. All pure PCB isomers were obtained from Analab, North Haven, CT. Sodium ligninsulfonate was acquired from the Matheson Coleman and Bell, Cincinnati, OH. Organic solvents were glass distilled as supplied by Caledon Laboratories Ltd., Georgetown, ON.

PCBs Emulsion: A small stainless steel sonic cup containing 1000 mg of PCBs, 1 mL of sodium ligninsulfonate solution (50 mg mL^{-1}) and 10 mL of distilled water was placed in a model 350 ultrasonic apparatus (Heat Systems Ultrasonics Inc., Plainsview, N.Y.) and the contents were subjected to a pulse sonification (50%) for 2 min. The resultant emulsion was very uniform and stable with an average PCBs droplet size of 0.5-1 um.

Bacterial Culture and Media: The bacterial culture used in the present study was a PCB-degrading Pseudomonas sp. 7509 originally isolated from the activated sludge (Liu 1980). The culture was maintained on the following basal medium with Aroclor 1221 as the sole carbon and energy source (g L^{-1}): K_2HPO_4, 1.3; KH_2PO_4, 0.82; $(NH_4)_2SO_4$, 1.0; $MgSO_4.7H_2O$, 0.05; $FeSO_4.7H_2O$, 0.01; $CuSO_4.5H_2O$, 0.01; $CoCl_2.6H_2O$, 0.01; $MnCl_2.4H_2O$, 0.01; NaCl, 0.05. The final pH of the medium was 6.9 and was sterilized at 121°C for 15 min. The PCBs emulsion and ligninsulfonate were subsequently added to the medium at concentrations of 100 and 50 ppm, respectively. For the bench scale biodegradation test, fresh municipal raw sewage fortified with nitrogen (as NH_4Cl) and phosphorus (as KH_2PO_4 + K_2HPO_4) at 20 ppm each was employed as the growth medium. The raw sewage was not sterilized with a typical BOD concentrations of 140-170 ppm.

Determination of Degradation: In all experiments, only the primary biodegradation of PCBs was followed, i.e., the disappearance of PCBs from the growth media or by measurement of the oxygen uptake rate. Upon acidification with 2 drops of 12 N H_2SO_4, 5 mL of the cultural broth and 1 mL of n-hexane were vigorously mixed in a 15-mL conical glass centrifuge tube for 1 min on a vortex mixer. The emulsion was broken down by centrifugation at 2,000 x g for 5 min and the clear hexane extract was used for g.c. analysis. Gas chromatographic analyses were carried out on a Hewlett-Packard 5730A gas chromatograph equipped with dual FID and interfaced with a Spectra Physics SP 4000 chromatographic data system. The 1.8 m x 2 mm i.d. stainless steel columns containing 10% OV-1 on Chromosorb W(AW-DMCS) were temperature programmed from 150-250°C at 8°C min^{-1}, while the injector and detector were kept at 250 and 280°C, respectively. The carried gas was nitrogen at a flow rate of 30 mL min^{-1}.

Manometry: Oxygen uptake was determined at 20°C using a Gilson differential respirometer. Resting cell suspensions were prepared from culture grown in the basal medium with Aroclor 1221 as the carbon and energy source as reported above. The cells were harvested by centrifuging at 15,000 g for 20 min. The cells were washed three times in cold 0.05 M (pH 7.0) phosphate buffer and resuspended in the same buffer. The cell suspensions were so adjusted that 1 mL of suspension contained 3-4 mg cell (dry wt.). The

standard incubation mixtures for the assay were: 1 mL of cell suspension, 1 mL of 0.05 M phosphate buffer (pH 7.0), 0.8 mL of distilled water, 0.2 mL of substrate (in the side arm) and 0.15 mL of 20% KOH in the center well for CO_2 absorption.

Bench Scale Test: The bench scale test of PCBs biodegradation was carried out in a 14-L microferm fermentor (New Brunswick Scientific Co., Edison, N.J.) equipped with automatic temperature, pH, dissolved oxygen and agitation speed controls. In a typical batch operation, the fermentor was charged with 10 L of fresh raw sewage which was fortified with 20 ppm of phosphorus and nitrogen each. The temperature was automatically controlled at 20°C at 400 rpm and dissolved oxygen (D.O.) in the fermentor broth at 2 ppm.

RESULTS AND DISCUSSIONS

The results presented in this and a previous paper (LIU 1980) suggested that P. sp. 7509 could oxidize commercial PCBs formulation (Aroclor 1221) at a rapid rate. If this bacterium is to be considered as a seeding culture for wastewater treatment, it is essential that the bacterium be able to degrade PCBs in the presence of other rich organic nutrients normally found in wastewater. Investigations in this area could also provide useful information on the possible interference from other organic compounds in the removal of PCBs by wastewater treatment process. As shown in Figure 1, P. sp. 7509. oxidized the Aroclor 1221 at a rate approximately 10 times faster than sewage. There are at least two possible explanations for this observation. First, the sewage contained some toxic materials that inhibited the bacterial respiration. This was unlikely, because addition of Aroclor 1221 (emulsion) to the sewage after 80 min resulted in an immediate increase in oxygen consumption. The second possibility was that P. sp. 7509 preferred Aroclor 1221 to the other organic substrates. This preference was demonstrated in the same Figure 1. Glucose, a substrate used readily by most bacteria, was poorly oxidized by this bacterium.

Since the temperature of sewage treatment plants in Canada vary considerably with seasons, it was of interest to test the ability of P. sp. 7509 to oxidize PCBs at different temperatures. Figure 2 shows the effect of temperature on the oxidation rate of Aroclor 1221 emulsion by this bacterium. From 15 to 35°C, the bacterium could rapidly oxidize Aroclor 1221 with the corresponding Qo_2 of 81 (15°C), 182 (25°C) and 338 (35°C). Decreasing the temperature to 4-10°C drastically retarded the microbial oxidation rate of Aroclor 1221 (Qo_2 = 15 at 4°C, 32 at 10°C). Similarly, a raise of temperature from 10 to 15°C resulted in an increase of Aroclor 1221 oxidation rate by 2.5 times. Nevertheless, even at such a low temperature of 4°C, this bacterium was still able to oxidize the Aroclor 1221. To provide a more realistic and optimal degradation temperature, 20°C was therefore used for all experiments including the bench scale biodegradation test.

Nitrogen and phosphorous which are required for bacterial growth are present in limited amounts in certain wastewater. Bench scale

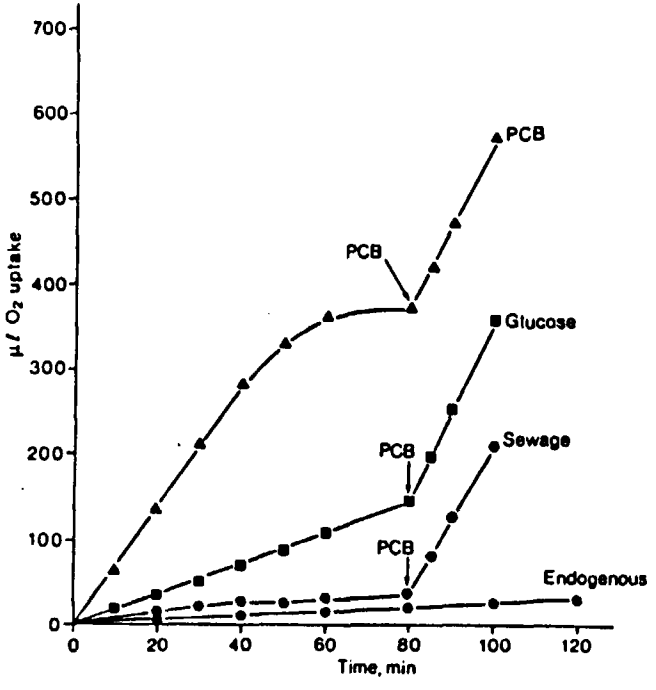

Figure 1. Comparison of raw sewage, glucose and Aroclor 1221 oxidation by P. sp. 7509. Additional 200 ul of Aroclor emulsion (2 mg) were added after 80 min, as indicated in the figure.

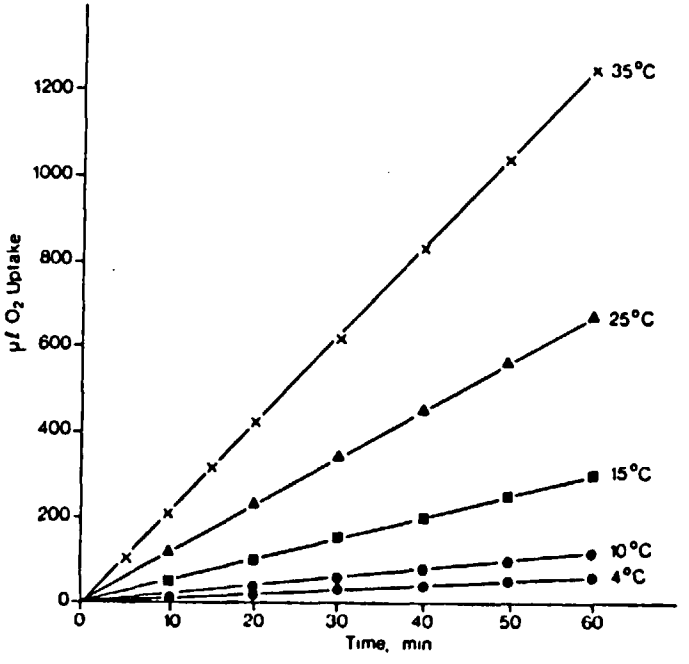

Figure 2. Effect of temperature on the oxidation of Aroclor 1221 by P. sp. 7509. Data have been corrected for endogenous respiration.

experiments were conducted to study the effect of nitrogen and phosphorous concentrations on the degradation rate of Aroclor 1221 by P. sp. 7509 in raw sewage. Addition of nitrogen and phosphorous concentrations ranging from 2 mg to 20 mg L^{-1} to sewage (the minimal value roughly equivalent to that found in municipal raw sewage) did not significantly affect the biodegradation rate of Aroclor 1221. However, raw sewage with N and P fortifications constantly yielded more reproducible results implying that the concentrations of N and P in raw sewage were on the border of rate limiting. Consequently, fortified raw sewage (N and P at 20 mg L^{-1} each) was used in all bench scale tests.

Although several studies on the relative rate of PCBs biodegradation have been reported (AHMED & FOCHT 1973; WONG & KAISER 1975; REICHARDT et al. 1981), the effect of dissolved oxygen on the degradation rate of PCBs by bacteria has not received any attention. Bench scale tests were thus initiated to study this effect. In these experiments, the fermentor was charged with 10 L of fortified raw sewage seeded with P. sp. 7509. The fermentor's impeller was kept at 400 rpm with the dissolved oxygen concentrations in the fermentor broth varying from 0 to 4 ppm level depended on the experimental conditions desired. Data presented in Figure 3 indicates that the rate of Aroclor 1221 biodegradation occurred without limitation at dissolved oxygen concentrations of 1 ppm and above. At dissolved oxygen concentrations less than 1 ppm, a strong reduction of the PCBs degradation rate resulted. These data indicated that P. sp. 7509 could degrade Aroclor 1221 under the operational conditions of the sewage treatment plant where the dissolved oxygen in the activated sludge reactor is generally maintained about 2-3 ppm level (ECKENFELDER & O'CONNOR 1961). Thus, the level of dissolved oxygen in the growth medium does not appear to be the rate-limiting factor for microbial degradation of PCBs. This is consistent with results by MACLENNAN & PIRT (1970) who investigated the effect of dissolved oxygen on the rate of hydrocarbon degradation. They reported that the biodegradation rate was not affected until the dissolved oxygen in the growth medium fell below 0.3 ppm level.

Perhaps the most interesting observation noted in the present study was that the rate of Aroclor 1221 degradation was intimately depended on the agitation speed of the fermentor's impeller. In these batch exerpiments, the dissolved oxygen in the fermentor broth was maintained at 2 ppm, while the impeller speed was varied from 0 to 800 rpm. Data in Figure 4 indicated that the rate of Aroclor 1221 degradation (expressed in terms of half-life) was a logarithmic function of the impeller speed between zero and 800 rpm. This excellent correlation ($r = 0.96$; $n = 5$) implies that good mixing is essential in the study of PCBs biodegradation.

The effect of impeller speed on Aroclor 1221 degradation is further demonstrated by the gas chromatograms of Figure 5. After 66 h incubation, 86.3% of the added PCBs was degraded by P. sp. 7509 at 800 rpm, while only 16.9% PCBs was reduced at 100 rpm. The results

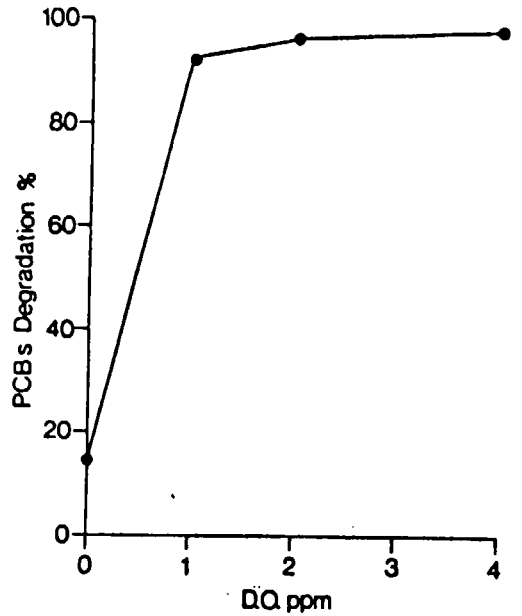

Figure 3. Effect of dissolved oxygen on the rate of Aroclor 1221 degradation in the fermentor at 20°C.

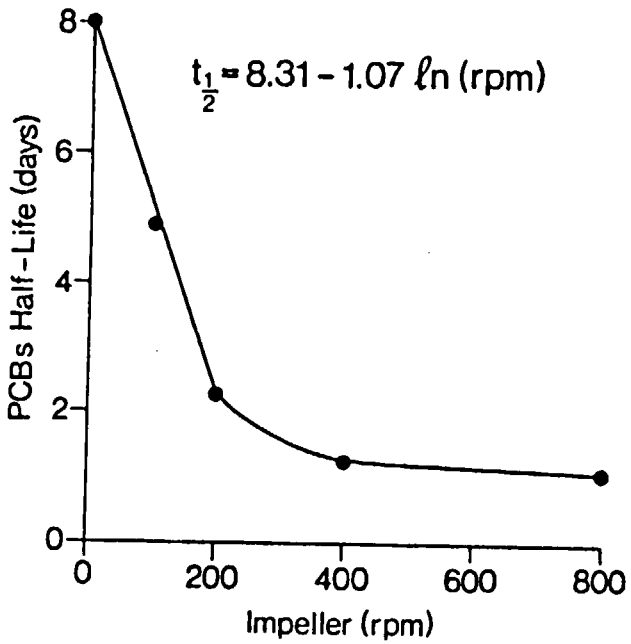

Figure 4. Effect of impeller speed on the rate of Aroclor 1221 degradation. The initial concentration of Aroclor 1221 was 100 ppm with dissolved oxygen at 2 ppm.

ppm.

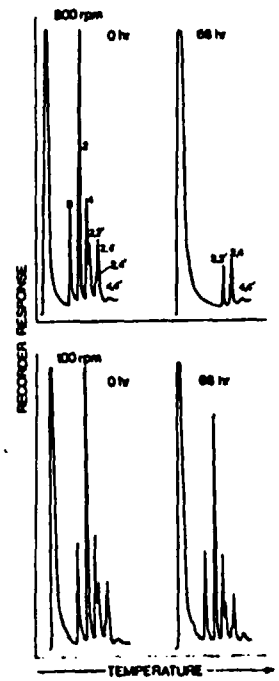

Figure 5. FID gas chromatograms of hexane extracts of Aroclor 1221 at 0 h and after 66 h incubation with P. sp. 7509 in fortified raw sewage.

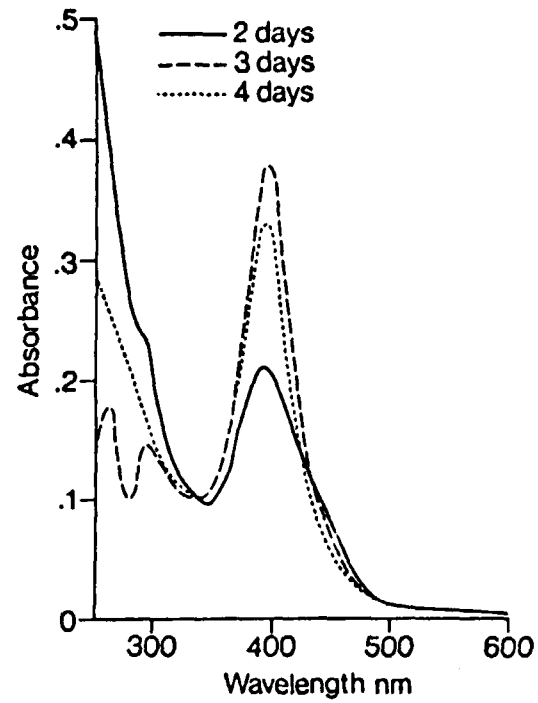

Figure 6. Production of yellow color from fermentor broth.

in the present investigation and the earlier study (LIU 1980) suggested that the chance or frequency of contact between bacteria and PCBs droplet is one of the major factors in determining the rate of microbial degradation of PCBs. To verify the observation that the rapid loss of Aroclor 1221 from the fermentor broth at high impeller speed was not due to volatilization, microbial inhibitors ($HgCl_2$ at 100 mg L^{-1}, KCN and NaN_3 at 5 m moles L^{-1} each) were added to the fermentor broth in a separate experiment. With dissolved oxygen at 2 ppm level and impeller speed at 400 rpm, the loss of Aroclor 1221 from the fermentor due to physical processes amounted to only 8% after 10 days incubation. The inclusion of sodium ligninsulfonate in the fermentor broth was probably responsible for preventing the rapid loss of Aroclor 1221 from the fermentor by lowering the PCBs vapor pressure. Other evidence for microbial degradation of PCBs in fermentor was also noted by following the bright yellow color production in the supernatant of the fermentor broth (Figure 6). The yellow color product had an absorption maximum at 395 nm during the early stage of incubation and rapidly disappeared from the broth. The yellow color compound appeared to be the chlorinated derivatives of α-hydroxymuconic acid as suggested by FURUKAWA and MATSUMURA (1976). The results in the present study clearly indicated that P. sp. 7509 was capable of degrading the lower chlorinated Aroclor 1221 in municipal wastewater under the batch operational conditions. Experiments are being carried out to assess the feasibility of degrading higher chlorinated PCBs in wastewater under the continuously operational conditions.

ACKNOWLEDGMENTS

The author wishes to thank K. Thomson for assistance and expertise in the analytical determinations of PCBs.

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855 Zitko, V. "Uptake of Chlorinated Paraffins and PCB from Suspended Solids and Food by Juvenile Atlantic Salmon." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 12 (1974): 406-12.

Chlorinated paraffins are suggested as replacements for PCBs. They are much less accumulated than PCB, if at all. Possibly toxic to juvenile salmon.

POLYCHLORINATED BIPHENYL (PCB)

856 Ahnoff, M., and Josefsson, B. "Polychlorinated Biphenyl (PCB) in Gota River Water." AMBIO 4 (1975): 172-74.

Little is known about waters with low levels PCB. Describes new analytical method when PCB is present only in trace amounts. In this instance it was shown that river played little role in dispensing PCB.

857 Allen, J.R., and Abrahamson, L.J. "Morphological and Biochemical Changes in the Liver of Rats Fed Polychlorinated Biphenyls." ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 1 (1973): 265-80.

Following administration in diet for six weeks of 0.1 percent PCB in three Arochlor types, liver became enlarged and liver microsomal enzymes increased activity. Three-fold enlargement of liver in animals killed after six weeks on above diet.

858 Ayres, David C., and Denton, Gary R.W. "On the Diversity of Products Obtained during Synthesis of Polychlorbiphenyls by the Van Roosmalen Procedure." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 14 (1975): 361-69.

Many peaks on chromatograms, as many as fourteen. Illustrates compound nature of PCB and difficulty in analysis.

859 Babish, John G., et al. "Polybrominated Biphenyls: Tissue Distribution and Effect on Hepatic Microsomal Enzymes in Japanese Quail." JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 23 (1975): 879-82.

Maximum enzyme induction occurred at 20 ppm intake in males, at 100 ppm in females. Potential toxicity of PBBs as great as PCBs.

860 Bagley, G.E., et al. "Identification of Polychlorinated Biphenyls in Two Bald Eagles by Combined Gas-Liquid Chromatography--Mass Spectrometry." JOURNAL OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS 53 (1970): 251-61.

PCB found in eagle tissues; article concerns technique of separating "interfering" chlorinated hydrocarbons for individual chemical determinations.

861 Bush, Brian, et al. "Toxicity and Persistence of PCB Homologs and Isomers in the Avian System." ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 2 (1974): 195-212.

Detailed comprehensive experimental review of PCBs in birds. Good bibliography.

862 Cecil, Helene C., et al. "Effects of Polychlorinated Biphenyls and Terphenyls and Polybrominated Biphenyls on Pentobarbitol Sleeping Times of Japanese Quail." ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 3 (1975): 183-92.

DDT and methoxychlor lengthen sleeping time, but it is shortened with the several Arochlors used in this study. Presumably means increased metabolism of pentobarbitol by induced liver microsomal enzymes. Degree of expression correlated with chlorine content.

863 Clausen, J.; et al. "The Content of Polychlorinated Hydrocarbons in Arctic Mammals." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 12 (1974): 529-34.

Mammals studied were four seal species, polar bear, polar fox, and sheep. DDE and PCB were found in all. Highest level was DDE (1.25 ppm, lipid) in bear which also contained 21.0 ppm PCB; highest PCB in porpoise was 11.4 ppm. Aldrin was present in most samples. Sources are probably algae and fish which concentrate the compounds and which are distributed northward by the Gulf Stream.

864 Craigie, J.S., and Hutzinger, Otto. "Effects of Commercial Chlorinated Hydrocarbons and Specific Chlorobiphenyls on the Growth of Seven Species of Marine Phytoplankton." CHEMOSPHERE 3 (1975): 139-44.

Many PCB formulations used to determine effects on growth. Some PCB had no effect; some species depressed, others not depressed at lower ranges. But no species grew at 100 ppm of any PCB. Highly chlorinated biphenyls seem less toxic.

865 Crosby, Donald G., and Moilanen, Kenneth W. "Photodecomposition of Chlorinated Biphenyls and Dibenzofurans." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 10 (1973): 372-77.

DDT and DDE are converted in part to di-, tri-, and tetrachlorobiphenyls under simulated atmospheric irradiation.

Would be expected to precipitate in rainfall if formed in atmosphere. Possibly degradative effect from ultraviolet component of sunlight. For a review of this work, see T.H. Maugh II in section 5, DDT and Its Derived Relatives, DDD and DDE.

866 Crump-Wiesner, Hans J., et al. "A Study of the Distribution of Polychlorinated Biphenyls in the Aquatic Environment." PESTICIDES MONITORING JOURNAL 8 (1974): 157-61.

Residues ubiquitous in aquatic environment. Range of sampling in nineteen states showed 0.1 microg/kg in water, and from 0.5 microg/kg to 3200 microg/kg in bottom sediments. PCB also in plants and fish. Generally, monitoring plan described.

867 Dahlgren, Robert B., and Linder, Raymond L. "Effects of Polychlorinated Biphenyls on Pheasant Reproduction, Behavior, and Survival." JOURNAL OF WILDLIFE MANAGEMENT 35 (1971): 315-19.

Experiment with pheasant chicks from hens receiving 50 mg PCB weekly. Chicks placed on a "visual cliff" for up to five minutes either jumped to the visually deep side or made no choice in significantly larger proportion than did those from untreated hens. Effects are similar to those of dieldrin on behavior.

868 Dahlgren, Robert B., et al. "Effects of Stress on Pheasants Previously Given Polychlorinated Biphenyls." JOURNAL OF WILDLIFE MANAGEMENT 36 (1972): 974-78.

Birds receiving 50 mg PCB twice a week and then alternatively starved and fed for two- to three-day intervals didn't live as long as controls, lost weight, and accumulated brain tissue residues as high as 240 ppm. Conclusion: stress causing loss of weight in birds with PCB body burden can be dangerous.

869 _____. "Storage and Excretion of Polychlorinated Biphenyls in the Pheasant." JOURNAL OF WILDLIFE MANAGEMENT 35 (1971): 823-28.

Average recovery of PCB in pheasant tissues (up to 200 ppm) was 95 percent. Absorption and storage rapid and high. Eggs important source of excretion of residue, but tissue retention very high nonetheless.

870 _____. "Residue Levels and Histopathology in Pheasants Given Polychlorinated Biphenyls." JOURNAL OF WILDLIFE MANAGEMENT 36 (1972): 524-33.

Birds dead from 210 mg PCB daily had brain residue levels of 2,500 ppm, and muscle levels of 140 ppm (averages). Brain levels of 300-400 ppm indicate PCB toxicosis. Birds that died from PCB had smaller hearts and very small spleens (due to lymphocyte depletion). Pheasants are more sensitive to PCB than to DDT.

871 Doguchi, M., and Fukano, S. "Residue Levels of Polychlorinated Terphenyls, Polychlorinated Biphenyls and DDT in Human Blood." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 13 (1975): 57-63.

In Japan average levels in blood of PCTs, PCBs, and DDE were 5.0 ppb, 3.2 ppb, and 11.2 ppb respectively. Interestingly, PCTs have higher levels than PCBs which are produced industrially in much greater volume and which have received a good deal more attention as environmental pollutants. See references in this paper for more on PCTs.

872 Dustman, Eugene H., et al. "The Occurrence and Significance of Polychlorinated Biphenyls in the Environment." TRANSACTIONS OF THE THIRTY-SIXTH NORTH AMERICAN WILDLIFE AND NATURAL RESOURCES CONFERENCE, 1971, pp. 118-33.

Good general coverage and reference list with regard to fish and game species.

873 Fujiwara, Kunisato. "Environmental and Food Contamination with PCB's in Japan." SCIENCE OF THE TOTAL ENVIRONMENT 4 (1975): 219-47.

Excellent summary paper that should be a model for other industrial nations. Japan has had the only large scale PCB poisoning in man (1968) although at first it was called food poisoning. In 1971 Japan abruptly stopped the production, use, and importation of PCBs. No other nation has.

874 Greichus, Y.A., et al. "Effects of Polychlorinated Biphenyls in Bird Tissues and Arochlor Standards with Gas Chromatography and Mass Spectrometry." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 11 (1974): 113-20.

All major PCBs occur in cormorant tissues and eggs; Arochlor 1254 and 1260 (commercial mixtures) also occurred in pelican fat.

875 _____. "Physiological Effects of Polychlorinated Biphenyls or a Combination of DDT, DDD, and DDE in Penned White Pelicans." ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 3 (1975): 330-43.

Detailed accounting of various chemical effects on organs or blood chemistry. There seem to be no particular additive effects of the several types of chlorinated hydrocarbons.

876 Hansen, D.J., et al. "Arochlor 1016: Toxicity to and Uptake by Estuarine Animals." ENVIRONMENTAL RESEARCH 7 (1974): 363-73.

This PCB was tested with oysters (Crassostrea), brown shrimp (Penaeus), grass shrimp (Palaemonetas), and pinfish (Lagodon). It quickly became quite toxic to invertebrates, but prolonged exposure was required in pinfish. Tissue effects were exhibited in pinfish.

877 Haque, Rizwanul, et al. "Aqueous Solubility, Adsorption and Vapor Behavior of Polychlorinated Biphenyl Arochlor 1254." ENVIRONMENTAL SCIENCE AND TECHNOLOGY 8 (1974): 139-42.

Aqueous solubility about 56 ppm. Adsorption depends on substrate; sand adsorbs little, loams much more. Vapor loss is significantly higher from sand than from soil.

878 Harvey, G[eorge]. R., et al. "Polychlorobiphenyls in North Atlantic Ocean Water." SCIENCE 180 (1973): 643-44.

Average concentration of PCBs in North Atlantic is 20 ppt; estimated 2×10^4 metric tons of PCBs in upper 200 meters of water. Less in Sargasso Sea. Environmental half-life of PCBs much longer than DDT metabolites.

879 Hesselberg, Robert J., and Scherr, David D. "PCB's and p,p'DDE in the Blood of Cachetic Patients." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 11 (1974): 202-5.

Patients had severe stresses, hence high metabolic fat withdrawal, as do wild species under stress. Average DDE (21 ppb) in patients' blood was higher than in normal blood. Still, no clear evidence of nervous system effects.

880 Hill, Elwood F., et al. "Polychlorinated Biphenyl Toxicity to Japanese Quail as Related to Chlorination." POULTRY SCIENCE 53 (1974): 597-604.

Chlorine percentage (in the different PCBs) correlates positively with avian toxicity, but apparently PCB toxicity is not simply a function of chlorination. Positioning of chlorine atoms on benzene rings may affect toxicity.

881 Holmes, D.C., et al. "Chlorinated Hydrocarbons in British Wildlife." NATURE 216 (1967): 227-29.

PCBs detected in wildlife. In bird livers and eggs there is often more PCB than organochlorine pesticide residues. PCBs toxic but adverse effects not known. Highest levels in terrestrial predators and in marine fish-eating birds. This paper was an early mention of PCB residue contamination, following the Swedish announcement in prior year.

882 Hutzinger, Otto, et al. THE CHEMISTRY OF PCB'S. Cleveland, Ohio: CRC Press, 1974. 269 p.

Comprehensive summary of the chemistry of the chlorobiphenyls, but does not include much on toxicology and other biological aspects. Does have section on occurrence and accumulation in the environment.

883 Hutzinger, O[tto]., et al. "Polychlorinated Biphenyls: Metabolic Behavior of Pure Isomers in Pigeons, Rats and Brook Trout." SCIENCE 178 (1972): 312-13.

Excreta examined showed alterations of some isomers in rats and pigeons; but no hydroxymetabolites were detected in excreta of brook trout.

884 Jensen, S. "Report of a New Chemical Hazard." NEW SCIENTIST (England) 36 (1966): 612.

First report on PCB residues in wild species in Sweden.

885 Kaiser, Klaus L.E., and Wong, Paul T.S. "Bacterial Degradation of Polychlorinated Biphenyls. I. Biodegradation of Some Metabolic Products from Arochlor 1242." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 11 (1974): 291-96.

A bacterial culture, isolated from lake water, degrades fairly high concentration of commercial PCB. Some metabolites identified.

886 Koeman, J.H., et al. "Chlorinated Biphenyls in Fish, Mussels and Birds from the River Rhine and the Netherlands Coastal Area." NATURE 221 (1969): 1126-27.

PCBs are widely occurring in these regions. PCBs with fewest chlorine atoms were found in fishes but not in birds. This implies that lesser chlorinated PCBs persist less and perhaps have lesser toxicity. PCBs make detection of other chlorinated hydrocarbons difficult. DDT, DDE, and teladrin were also found.

887 Lincer, Jeffrey L., and Peakall, David B. "Metabolic Effects of Polychlorinated Biphenyls in the American Kestrel." NATURE 228 (1970): 783-84.

Estrodiol breaks down to a more polar metabolite in livers of Arochlor-fed kestrels. The rate of breakdown is dose-dependent. PCBs induce liver enzymes. Action is similar to DDT and metabolites.

888 _____. "PCB Pharmacodynamics in the Ring Dove and Early Gas Chromatographic Peak Diminution." ENVIRONMENTAL POLLUTION 4 (1973): 59-68.

Ring doves were given daily dose of 10 ppm Arochlor 1254. There was linear increase of PCB in tissue until 105 days with asymptote at 80 ppm. Total analysis: in fat 797 ppm; in feces 2.27 ppm. Authors suggest that comparable levels found in starvation-stressed birds in Irish Sea in 1969 indicate that PCB was probably not the cause of death in these birds.

889 Linko, R.R., et al. "PCB Residues in Plankton and Sediment in the Southwestern Coast of Finland." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 12 (1974): 733-38.

Plankton has relatively high amounts--0.04 to 0.75 ppm wet weight, and 4 to 77 ppm lipid weight. About the same as in fish. Amounts in sediments very low compared to plankton. PCBs do not sink to sea bottom. The source of animal contamination is the immediate surface of the sea.

890 Litterst, Charles L., and van Loon, Edward J. "Time-Course of Induction of Microsomal Enzymes Following Treatment with Polychlorinated Biphenyl." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 11 (1974): 206-12.

Significant levels of enzyme induction within seven days. Occurs even with single oral doses. Discontinuation of dosage slowly reduces induction to normal in about ten days.

891 Masuda, Yoshita, et al. "Comparisons of Polychlorinated Biphenyls in Yusho Patients and Ordinary Persons." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 11 (1974): 213-16.

PCB hazard known since 1966 (Sweden). There was a mass outbreak of human poisoning (called "Yusho") by PCB-contaminated rice oil in Japan in 1968. Average value of PCBs slightly higher in Yusho patients. Milk in one Yusho patient contained 0.06 ppm.

892 Maugh, Thomas H. II. "DDT: An Unrecognized Source of PCB." SCIENCE 180 (1973): 578-79.

Reported that DDT vapor can be converted to PCBs by irradiation with ultraviolet light of the same wavelengths present in

sunlight in the lower atmosphere. DDT and DDE are generally inert in sunlight, but not true of their vapors. Effect is substitution of one environmental contaminant with another.

893 Metcalf, Robert L., et al. "Laboratory Model Ecosystem Studies of the Degradation and Fate of Radiolabelled Tri-, Tetra-, Pentachlorobiphenyl Compared with DDE." ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 3 (1975): 151-65.

"Tri-" degraded much more rapidly than others. "Penta-" about the same as DDE. Linear relationship between lipid/water partition water solubility, and ecological magnification. No evidence of conversion of DDE to PCB. Good reference list.

894 Morgan, Janet R. "Effects of Arochlor 1242 (a Polychlorinated Biphenyl) and DDT on Cultures of an Alga, Protozoan, Daphnid, Ostracod, and Guppy." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 8 (1972): 129-37.

Arochlor (but not DDT) inhibited growth of Chlamydomonas (alga) and concentrated over medium in the alga. Daphnia and the Cladoceran (Cypridopsis) highly susceptible to both Arochlor and DDT. Young guppies died in medium of 2 ppm Arochlor, a lower toxicity than p,p'-DDT. Clearly, Arochlor can concentrate in basal members of food chain.

895 Mosser, Jerry L., et al. "Interactions of PCBs, DDT and DDE in a Marine Diatom." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 12 (1974): 665-68.

Authors suggest that organochlorines and PCBs may interact for greater toxicity than shown singly.

896 _____. "Polychlorinated Biphenyls and DDT Alter Species Composition in Mixed Cultures of Algae." SCIENCE 176 (1972): 533-35.

Each chemical was added to mixed cultures of a marine diatom and a marine green alga and produced similar effects. Diatoms grew faster in controls; dominance diminished in treated cultures, even at concentrations of chlorinated hydrocarbons that had no effect in pure cultures. Stable pollutants could disrupt species composition of phytoplankton communities, thereby affecting whole ecosystems. (There is no field evidence of this, however.)

897 Nimmo, D.R., et al. "Accumulation of Arochlor ®1254 in Grass Shrimp (Palaemonetes pugio) in Laboratory and Field Exposures." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 11 (1974): 303-8.

Study of a shrimp that is estuarine throughout its life cycle; shown to be bioaccumulator of residues. But lesser exposure than species with pelagic phase in life cycle. Shrimp exposed to PCB-contaminated sediments accumulated 0.42 mg/kg after three months without significant mortality. Higher accumulation and mortality in laboratory tests.

898 _____. "Toxicity of Arochlor 1254 and Its Physiological Activity in Several Estuarine Organisms." ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 3 (1975): 22-39.

High concentrations in Florida estuary; present in all components. Toxicity tests indicate organisms sensitive at ppb levels; a ciliate protozoan, two genera of shrimp, and killifish effected at or near 1.0 ppb. Shrimp concentrated PCB in tissue from as low as 0.04 ppb in water. Pathological changes in oysters, in spot (fish), and in shrimp. Paper combines field and laboratory data well.

899 Nisbet, Ian C.T., and Sarofim, Adel F. "Rates and Routes of Transport of PCB's in the Environment." ENVIRONMENTAL HEALTH PERSPECTIVES 1 (1972): 21-38.

Identifies sources, amounts, and problems connected with PCBs in all parts of the environment. Note particularly bioconcentration model and DDT/PCB residue ratios.

900 Panel on Hazardous Trace Substances. "PCBs-- Environmental Impact." ENVIRONMENTAL RESEARCH 5 (1972): 249-362.

Excellent detailed review of PCBs. Much original data. Tables in text and extensive bibliography.

901 Peakall, David B. "PCB's and Their Environmental Effects." CRITICAL REVIEWS IN ENVIRONMENTAL CONTROLS 6 (1975): 469-508.

The most important review of the subject. Two hundred fifty-one references. All aspects of PCBs discussed.

902 _____. "Polychlorinated Biphenyls: Occurrence and Biological Effects." RESIDUE REVIEWS 44 (1972): 1-21.

PCBs now widespread, paralleling DDT in concentration. Very stable; will persist for many years. Chief manufacturer has restricted production. PCBs embryotoxic in 10 ppm range.

903 Risebrough, Robert W., et al. "PCB Residues in Atlantic Zooplankton." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 8 (1972): 345-55.

In many areas of the Atlantic Ocean PCB residues exceed those of DDT. PCB in lipids of zooplankters from the continental shelf ranged from 2.4 to 260 ppm. From shelf and slope (wet weight) levels were about 1.5 ppm and 0.15 ppm respectively.

904 _____. "Polychlorinated Biphenyls in the Global Ecosystem." NATURE 220 (1968): 1098-1102.

This was the first announcement that these persistent waste products are distributed worldwide, survive in environment and tissues for long periods, and accumulate in food chains.

905 Rote, James W., and Morris, William J. "Use of Isotopic Abundance Ratios in Identification of Polychlorinated Biphenyls by Mass Spectrometry." JOURNAL OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS 56 (1973): 188-99.

This method provides unambiguous identification of PCBs in the presence of other substances.

906 Rote, J[ames]. W., and Murphy, Philip G. "A Method for the Quantitation of Polychlorinated Biphenyl (PCB) Isomers." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 6 (1971): 377-84.

PCBs are complex mixtures of isomers having different degrees of chlorination. Peaks on chromatograms depend on chlorination in varieties of Arochlors.

907 Stickel, Lucille F. "Biological Data on PCBs in Animals Other Than Man." In POLYCHLORINATED BIPHENYLS AND THE ENVIRONMENT, pp. 158-72. Interdepartmental Task Force on PCBs. Springfield, Va.: National Technical Information Service, for U.S. Department of Commerce, 1972.

A summary review of PCB effects on wild species of animals. Comparisons made with chlorinated hydrocarbon pesticides. Author is one of several representatives of federal agencies contributing to this publication.

908 Sugiura, K., et al. "Accumulation and Excretion of PCBs in the Mouse." CHEMOSPHERE 3 (1975): 181-87.

Detailed chemistry indicating that residues of PCB depend on an adsorption-diffusion mechanism in adipose tissue, as do other chlorinated hydrocarbons. Seemingly, PCBs with fewer chlorine atoms are more rapidly metabolized and excreted.

909 Sundstrom, Gonar. "Polychlorinated Biphenyls. V. Synthesis of ^{14}C-Labelled 2,2',4,4',5,5'--Hexachlorobiphenyl, a Major Component in PCB-Mixtures." BULLETIN OF ENVIRONMENTAL CONTAMINATION

AND TOXICOLOGY 11 (1974): 39-44.

Report of synthesis of PCB component material.

910 Thompson, Neal P., et al. "Polychlorinated Biphenyls and p,p'-DDE In Green Turtle Eggs from Ascension Island, South Atlantic Ocean." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 11 (1974): 399-406.

Multiple PCBs found. All residues low. Island remote. No effects shown including in eggs brought to Florida for hatching.

911 Tucker, E.S., et al. "Migration of Polychlorinated Biphenyls in Soil Induced by Percolating Water." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 13 (1975): 86-93.

PCBs are not readily leached from soil by percolating water, and only the less chlorinated, more degradable, homologs are leached from soil. Problem is disposal of Arochlor-impregnated capacitors. Authors conclude that landfills are only a minor source of PCB environmental contamination.

912 Tumasonis, Casimir Franco, et al. "PCB Levels in Egg Yolks Associated with Embryonic Mortality and Deformity of Hatched Chicks." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 1 (1973): 312-24.

Hens were exposed for six weeks to Arochlor 1254. Decreased egg production but no effects on egg weights or fertility. Leg, toe, and neck deformities occurred in chicks hatched from eggs in which PCB levels were 10-15 ppm or more. Liver is a target for PCB action. Good bibliography.

913 U.S. Department of Health, Education, and Welfare. "Perspective on PCBs." ENVIRONMENTAL HEALTH PERSPECTIVES, Experimental Issue no. 1 (1972): entire issue (185 p.).

Thirty contributions on a wide range of subjects. Published results of a conference held in 1971. Topics include toxicity to vertebrates, transport means in the environment, and effects in aquatic systems.

914 Yoshida, T., et al. "Distribution of [^{14}C] PCB's in Carp." AMBIO 2 (1973): 111-13.

Study relative to known bioconcentration in fishes and fish-eating birds. Which organs in fish concentrate? Autoradiography shows localization mainly in gall bladder, fat, and hepatopancreas. Highest in gall bladder and in fatty tissue of skulls.

915 Zepp, R.L., Jr., et al. "Reduction of Pentobarbital-induced Sleeping Times In PCB-treated Cottontail Rabbits." BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 12 (1974): 518-21.

Ten ppm Arochlor 1254 significantly reduced sleeping times of both sexes. Relates to hepatic microsomal enzyme activity.

916 Zinck, M.E., and Addison, R.F. "The Fate of 2-, 3-, and 4-, Chlorobiphenyl Following Intravenous Administration to the Thorny Skate (Raja radiata) and the Winter Skate (Raja ocellata)." ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 2 (1974): 52-61.

Isomers of PCB with low chlorine content. All accumulated in tissues. The 3- isomer cleared most rapidly in plasma and from tissue. Considerable variance in residue levels from fish to fish.

917 Zitko, V., et al. "Distribution of PCB and p,p'-DDE Residues in Atlantic Herring (Clupea harengus harengus) and Yellow Perch (Perca flavescens) in Eastern Canada--1972." PESTICIDES MONITORING JOURNAL 8 (1974): 105-9.

Detailed study intended to determine temporal trends in contaminants. Concluded impossible on year-to-year basis because of extreme variability.

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Occurrence and Methods of Control of Chemical Contaminants in Foods

by C. Jelinek*

Contamination of food by chemicals can result from their use on agricultural commodities; accidents or misuse during food handling and processing; nuclear weapon testing and operation of nuclear power plants; and disposal of industrial chemicals or by-products with subsequent dispersal into the environment. The Food and Drug Administration (FDA), as the Federal agency mainly responsible for evaluating the hazards of chemical contaminants and enforcing any established tolerance levels for them in foods, has been monitoring pesticides, industrial chemicals, metals, and radionuclides in foods in its nationwide programs for many years. In addition, FDA searches for potential contaminants among the approximately 50,000 industrial chemicals manufactured in the United States and coordinates its efforts with those of other Federal and state agencies in these investigations. The overall results of the FDA surveillance and compliance programs for chemical contaminants in foods, as well as specific examples illustrating the wide range of incidents and types of occurrences, are presented.

Introduction

Incidents that release chemicals into the environment often occur as a result of human activities. Such chemicals, which include pesticides, other industrial chemical contaminants, metals, and radionuclides, can also enter food by accident or misuse during food handling or processing. The Food and Drug Administration (FDA) programs for monitoring and controlling levels of contaminants in foods, the results the agency has obtained, and the steps being taken to improve these monitoring programs are presented.

The overall planning, coordinating, and evaluating required to make these programs productive is carried out at the Bureau of Foods. The samples are collected and analyzed in most cases, however, by 16 of the 21 FDA Districts in various Regions throughout the United States. These Districts have laboratories equipped for carrying out chemical analyses; this arrangement provides considerable flexibility in dealing with local problems when they arise.

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The purpose of the monitoring activities can vary, depending on the nature of the contaminant involved and FDA's knowledge of the extent of its occurrence in the food supply. Consequently, some continuing field programs such as the Total Diet Studies, the Heavy Metals Program, and the Radionuclides Program are primarily surveillance-oriented, whereas FDA field programs for pesticides and polychlorinated biphenyls (PCBs) in agricultural products and for pesticides, PCBs, and metals in fish are compliance-oriented. These latter programs are carried out primarily to determine whether the contaminants in question are present in excessive levels in foods and to remove the foods from the market if they contain violative levels. The programs dealing with special contaminants, however, are exploratory in nature and, depending on the particular situation, may involve either surveillance or compliance.

Pesticides

Residues of pesticides occur in foods or animal feed as a result of their use (either permitted or unauthorized) on the commodities concerned and through their entry into the general environment.

As the first step in the control of pesticide occurrence in foods, the Environmental Protection Agency (EPA) permits the commercial use of a pesticide only after the petitioner has submitted sufficient information to demonstrate that the pesticide can be used safely and efficaciously on the crops concerned. EPA issues regulations concerning labeling , use on specified crops, and levels or tolerances that will be allowed.

Surveillance for pesticides on various commodities is carried out by the states on products moving in intrastate commerce and by FDA and the United States Department of Agriculture *USDA) on those moving in interstate commerce. Finally, EPA can and does change the registration of a pesticide to reduce or prohibit its use if new information indicates this is necessary.

There are about 280 pesticides registered by EPA for current use on food or animal feed commodities. Although monitoring for these pesticides is complicated by the number of different crops on which each may be used, the acreage involved in food and feed production, and the sophistication of many of the residue analytical methods required, FDA has, nonetheless, carried out nationwide programs for many years.

At present, FDA collects and analyzes about 8500 samples of various domestic and imported agricultural commodities for pesticides and PCBs; determines pesticides, PCBs, mercury, and other contaminants in about 1200 samples of domestic and imported fish; and also examines about 750 samples of animal feeds. Other government agencies (Federal and state) also monitor various foods for chemical residues.

As might be expected, the occurrence of the organochlorine pesticides has decreased significantly, as a result of the complete or partial bans on their use on crops. This downtrend, however, is not so noticeable in fish, milk, and eggs because of the stability of these pesticides in the environment and their propensity to bioaccumulate in fatty tissues. The overall level and rate of occurrence of the organophosphorus pesticides has increased to some extent, but not so much as might be expected from their greatly increased usage. This difference reflects their relative instability in the environment. The rate of overtolerance findings for domestic commodities in these programs is about 3%; that for imports is slightly higher.

Since it is much more efficient to determine a group of chemicals in one analysis, rather than one at a time, FDA relies mainly on the use of multiresidue methods and can determine about 230 pesticides and their alteration products by these means.

In the past year or two, FDA has instituted several changes to make these field programs even more effective. Each pesticide is presently being evaluated to determine the relative hazard it may present as a food contaminant. Such factors as production volume, crop usage, environmental stability, and toxicity are used in this assessment. For those pesticides having higher hazard classifications, monitoring will be carried out on crops on which they are used. This evaluation will also be valuable in guiding analytical methods research for pesticides, including those not amenable to analysis by multiresidue methods.

Until recently, FDA's Pesticide Program was planned and directed in detail from headquarters in Washington. In order to provide more flexibility, each District now plans its own monitoring program for chemical contaminants in agricultural products and fish, with general guidance from headquarters, and also develops information on pesticide usage and on disposal of industrial chemicals in its own area. More resources have been made available to each District to carry out these activities. General crop and usage information will still be developed and be made available to the field, and results from the field will be followed on a continuing basis by headquarters so that significant information can be exchanged between Districts.

Industrial Chemicals

In the late 1960s, it became evident that industrial chemicals can contaminate foods, even though they are not intentionally used on agricultural products or in processed foods. Contamination by industrial chemicals may be due to various causes, the chief of which are: their use as components in food processing equipment, cross-contamination of food and nonfood formulations, use as components in chemical processes or equipment, disposal of unwanted by-products, and ultimate dispersal into the environment.

Contamination by Components of Food Processing Equipment

A prime example of widespread food contamination by a component of food processing equipment was the leakage of a PCB-containing heat exchange fluid in 1972 into fish meal which was being pasteurized in a North Carolina plant. This meal was incorporated into poultry feed and resulted in the contamination of poultry and eggs. As a consequence of this and similar instances, FDA established regulations in 1973 which prohibit the use of

PCBs in "unsealed" equipment in plants processing food, feeds, and food packaging materials (*1*). Since then, some leaks of PCB fluids have occurred from "sealed" electrical equipment such as transformers and capacitors. A leaking transformer in a Montana packing plant in 1979 led to the widespread PCB contamination of animal feed, poultry, hogs, eggs, and foods containing the latter (*2*). As a result FDA, EPA, and USDA have proposed regulations which would ban PCB-containing transformers and capacitors in food, feed, food packaging material, and agricultural chemical plants (*2*). In the meantime, all three agencies have sent a technical brochure (*3*) to all establishments under their jurisdictions describing what can be done on a voluntary basis to prevent such incidents.

Cross-Contamination of Food and Nonfood Formulations

Sometimes industrial chemicals enter the food supply by a mistaken use of a product in a formulation. The accidental substitution of a polybrominated biphenyl (PBB), a flame retardant, for magnesium oxide in an animal feed product in 1974 (*4*) is a good example. Foods such as milk and meat were extensively contaminated. After an incident of this type, FDA uses as much of its resources as possible to aid the state affected and to prevent contaminated foods from entering widespread interstate commerce. In the PBB incident, FDA's Detroit District maintained constant contact with the State of Michigan and provided extensive assistance, especially laboratory support. In addition, FDA carried out a special nationwide survey for PBBs in products such as milk and eggs (*5*, *6*). The results showed that the contamination of food did not extend to other states to any significant degree.

Component of Chemical Processing Equipment

An example of the contamination of food by a component of chemical processing equipment was the extensive contamination of fish by the mercury used in the electrolytic cell for production of chlorine and sodium hydroxide (*7*). After it was discovered in 1969 that mercury entering the waterways could be converted to methylmercury by bacteria in sediments and could accumulate in fish (*8*), strict effluent guidelines for mercury were established. As a result, the amount of mercury introduced into water from those plants was reduced from about 1,200,000 lb to less than 20,000 lb per year by 1973. This in turn has led to a decrease in mercury levels in estuarine and freshwater fish. At the same time, FDA has enforced its action level for mercury in fish.

Disposal of Unwanted By-Products

Contamination of the food supply by an unwanted by-product occurred several years ago when the still-bottoms from a perchloroethylene plant were routinely disposed of while hot (*5*). This material contained various by-products including hexachlorobenzene (HCB), which subsequently escaped into the atmosphere and settled on crops and forage. Thousands of cattle feeding nearby became contaminated with HCB. Subsequently, the company effected better control of its effluents, and there have been no more known instances of HCB contamination of food animals near this plant. Another producer has installed well-designed incinerators to burn the tar; this method is probably the best way to dispose of such by-product materials.

The common presence of the DDT family and of PCBs in fish is a graphic illustration of the escape of these products into the aqueous environment at various steps of their processing, use, and disposal.

PCBs

For years FDA has been monitoring agricultural products and fish for PCBs in its field programs. The multiresidue analytical method employed for organochlorine pesticides also determines PCBs. Generally, PCBs have been found only in animal-derived products—fish, milk, eggs, cheese—and also in animal feed components such as fish meal or other animal-derived products.

PCBs still continue to be found in animal feeds and their components, but to a lesser degree than in fish. The occurrence in feeds is generally not reflected in milk, eggs, cheese, or meat, where findings each year vary from zero to several percent of the samples examined. Minor increases in findings in certain years usually reflect isolated incidents of PCB contamination. Such incidents can be expected to continue, and FDA undoubtedly will need to monitor for PCBs on a nationwide basis for many years.

The PCB findings in individual foods are confirmed by the results obtained in FDA's Total Diet Studies (*9*). The Composition of the Total Diet is based on the USDA survey of 1966, and is typical of the intake of the teenage male, the heartiest eater in the United States. (A new Total Diet, reflecting more recent surveys, is now being developed.) The foods in the market baskets are made table-ready and combined into composites of 12 food categories for subsequent analysis.

Table 1. Percentages of Total Diet composites containing PCBs.

Fiscal year	Food class composites								
	Dairy products	Meat, fish, poultry	Grains, cereals	Potatoes	Legume vegs	Root vegs	Garden fruits	Oils, fats, shortening	Sugar and adjuncts
1972	6	46	6	—	6	3	3	.17	6
1973	10	33	17	3	—	—	—	3	—
1974	—	43	—	—	—	—	—	—	3
1975	—	40	—	—	—	—	—	—	—
1976	5	5	—	—	—	—	—	—	—
1977	—	35	5	—	—	—	—	20	—
1978	—	30	—	—	10	—	—	5	—
1979	5	10	—	—	—	—	—	5	—

In FDA's Total Diet Studies, PCBs have been found consistently in only the meat, fish, and poultry composite (Table 1). In most cases, the source is the fish in this composite. Significantly, PCBs have been much less prevalent in the diet since 1973, when FDA promulgated tolerances for PCBs in various foods, prohibited their use in most industrial fluids in food and feed plants, and established an action level in paper food packaging materials (*1*). Some of these tolerance levels were subsequently lowered in 1979, when new information indicated PCBs are more toxic and that they are more persistent in the environment than had previously been thought (*10*).

Metals

FDA has accorded highest priority to mercury, lead, cadmium, arsenic, selenium, and zinc in its investigations of contamination of foods by toxic elements (*11*). All of these elements occur in the earth's crust and, therefore, are present naturally in all foods to some extent. In addition, they have a variety of uses. It is important that their disposal does not lead to a substantial increase in their levels in the aquatic environment or in the soil.

Because of their toxic behaviors and their levels in foods, FDA has concentrated most of its attention on mercury, lead, and cadmium and, since 1972, has devoted more of its resources to lead and cadmium than to the other metals.

Infants are particularly susceptible to the hazards of lead contamination (*12*) for a variety of reasons: (1) toxic effects occur at lower threshold levels in children; (2) they absorb a higher proportion of the lead ingested than do adults; (3) they ingest more food per pound of body weight; (4) their central nervous systems are more sensitive to lead than those of adults; and (5) infants and children frequently ingest high levels of lead from nonfood sources such old paint, dirt, or house dust. For cadmium, damage to the kidneys in middle age after cumulative ingestion of low concentrations of this metal is the first effect to be noted from the low level intake of this metal (*13*).

In the early 1970s, FDA carried out extensive surveys on individual foods and on composites in the Total Diet Studies to relate levels of lead and cadmium found in food to daily intakes. The average daily intakes can be calculated from the Total Diet Studies because both the level of lead and cadmium in each composite and the average amount of each composite consumed per day are known. The daily intakes can also be estimated if the levels of the metals in enough foods of dietary importance have been measured because the average amount consumed per day of each food item will be known. The intake estimated by the latter approach is always higher (and probably more accurate) than that calculated from the Total Diet Studies because food items which do not contain measurable amounts of a contaminant may have a diluting effect and prevent its detection in the same Total Diet Study composite.

For a teenage male, we have estimated the average lead intake derived from the individual foods to be about 220 μg/day, compared with the tolerable daily intake of about 430 μg/day (*13*), proposed by the Food and Agriculture Organization/World Health Organization (FAO/WHO). For cadmium, however, the estimated dietary intake of 57 μg/day (*14*) approximates the suggested FAO/WHO tolerable intake of 57-71 μg/day (*13*).

One controllable source of lead in the food supply is the lead-containing solder in tin cans. In 1972, FDA encouraged the can-making and the canned food industries to reduce the lead levels in their products. Since then, FDA has followed their programs to decrease lead in foods, especially for infants (*15*). From FDA and industry data, it was estimated that the daily dietary intake for lead in

1974 was about 65-115 μg/day for infants in the 2-month to 2-year age group (*16*). In comparison, it has been recommended that for infants under 6 months of age and for children in the 6-month to 2-year age group, total lead intake from all sources should not exceed 100 and 150 μg/day, respectively (*17*).

The average lead levels in various infant foods are now only 10-20% of those originally found several years ago (Table 2). Industry has achieved this significant improvement by a variety of means, including better control of raw products, technical improvements in can-making and canning, and changing from lead-soldered cans to other containers. Another factor contributing to this accomplishment was the fact that FDA informed industry of its concern, convinced them of the need to improve, and stayed in close touch with them while their programs were implemented.

In July, 1979, FDA published a Notice of Intent (*15*) in which the achievement of a 50% reduction in the amount of lead contributed to food by lead-soldered cans was stated as a goal to be attained within 5 years, and certain information and data were requested to guide subsequent Agency actions. The Notice of Intent stated that, after the receipt and evaluation of this information, action levels for lead in infant juices, glass-packed infant foods, infant formula, and evaporated milk would be set by FDA, followed by action levels for lead in "adult foods" eaten by the young.

With respect to cadmium, the data developed by FDA do not indicate that the handling, processing, and shipping of food cause a noticeable increase in cadmium content in foods (*14*). FDA estimates for cadmium intake do suggest, however, that no new practice leading to a significant increase in cadmium in the diet should be instituted. The increased application of municipal sewage sludge to crop land several years ago with little or no control is an example of such an action (*18*). The appropriate utilization of municipal sludge on lands (*19*) with specific controls for cadmium has been developed by the combined efforts of FDA, EPA, and USDA. EPA's latest criteria for the application of solid waste to land will unquestionably reinforce other efforts being made to reach this goal.

Table 2. Decrease in lead levels in foods for infants.

Type of food	Average lead levels, ppm	
	Start of program	Current
Glass-packed	0.15	0.25
Infant juices	0.30[a]	0.15[b]
Infant formula[c]	0.10[a]	0.015[d]
Evaporated milk	0.52[a]	0.09[a]

[a]Lead-soldered cans.
[b]Glass-packed.
[c]Ready-to-feed basis.
[d]Some changes from lead-soldered cans.

Radionuclides

The presence of radionuclides in foods may result from natural occurrence (such as potassium-40), atmospheric testing of nuclear weapons, operations of nuclear power plants, and accidents.

FDA's monitoring program for radionuclides consists of three main segments: radionuclides in imported foods, the Total Diet, and foods collected near nuclear plants (*20*).

FDA has monitored imports of various foods for strontium-90, iodine-131, ruthenium-106, cesium-137, and potassium-40. The first four are representative of fallout products, and potassium-40 is a naturally occurring radionuclide. Strontium-90 is the only man-made nuclide found regularly in all imported food types, but at levels too low to present a hazard to the consumer. Cesium-137 is found less regularly, and iodine-131 and ruthenium-106 have almost never been found in any samples analyzed by FDA.

Only strontium-90 (and, of course, potassium-40) are detected with any consistency in the Total Diet samples. The average daily intake calculated from these findings is around 11 picocuries/day of strontium-90, well below any level of concern. No particular trend has been noted in the levels of strontium-90 intake in the Total Diet samples since this progam was instituted in 1973.

In the FDA studies of foods grown near nuclear plants, samples of milk, fish, vegetables, fruits, and grains harvested near eight nuclear plants are regularly examined. Four different plants per year are now substituted for a like number on the list. Originally, tritium was the only radionuclide measured, and an increase in tritium levels is an indication of reactor leakage. More recently, scanning for beta- and gamma-emitters was added to detect radioactive corrosion products and fallout products that would result from an incident or from faulty operations.

The levels of tritium found in the food samples have been extremely low—almost down to the limit of detection. No upward trends have been noted since this part of the program was instituted in 1975. Generally, no findings have resulted from the beta- and gamma scans of these food products.

Surveillance of foods collected near nuclear plants was of special significance during the Three Mile Island accident, which occurred in 1979. FDA was

in a position to measure and evaluate the resulting contamination of food by radionuclides because broad monitoring programs were already in operation. The FDA regular program called for the monitoring of foods produced near the Peach Bottom, Pennsylvania plant, 40 miles below Three Mile Island, and, as part of the Total Diet Studies for fiscal year 1979 (*21*), a market basket was due to be collected at Harrisburg shortly before the incident happened. Elevated radionuclide levels were not found in either group of food samples.

Additionally, FDA collected and analyzed many samples of food, primarily milk and water, from the Three Mile Island area during and after this incident. Generally, the radionuclide levels were no higher than those observed previously in the regularly screened samples.

The background information collected in the FDA surveillance program is similarly used to assess radionuclide contamination of food caused by atmospheric testing of nuclear weapons, such as the Chinese bomb blast which produced fallout over the Northeast several years ago.

FDA has also analyzed food samples from a phosphate mine tailings area of Florida for radium-226 (*21*). Levels in certain locally grown foods such as citrus fruits are slightly higher there than those found in fruit grown elsewhere; however, there appears to be no potential danger to the consumer. FDA is still conducting some surveillance on food grown in the area to identify any uptrends in radionuclide levels.

Although the FDA surveillance program for radionuclides has never found levels believed to be truly hazardous in any of the monitored food samples, FDA plans to conduct these broad monitoring programs on a continuing basis so as to detect and evaluate both gradual uptrends and sharp increases in radionuclide levels.

Suspected Industrial Chemical Contaminants

It was discovered in 1969 and the years immediately following, as described earlier in this report, that industrial chemicals, such as PCBs, can contaminate food.

In 1971 FDA launched a formal, continuing program to search for as-yet-unrecognized industrial chemical contaminants in foods. The chief objectives of this program were: to anticipate and search for these potential contaminants; to evaluate their hazards if found in foods; and to take actions to eliminate or minimize the problem (*22*).

According to information developed by EPA's Office of Toxic Substances, approximately 50,000 industrial chemicals, each containing impurities and by-products, are manufactured in the United States. It is possible, therefore, to investigate only a very small fraction of these chemicals or chemical families.

The criteria used by FDA since 1972 for selection of potential industrial chemical food contaminants are: production volume (the greater the amount of a product produced and used, the more likely it is to contaminate foods); toxic by-products; toxicity; environmental stability; solubility behavior or partition coefficient (a fat-soluble, water-insoluble material is more likely to concentrate in the fatty portion of fish, meat, milk, and eggs); end-use pattern (the less confined the uses of a product, the more likely it is to cause environmental and contamination problems); and means of disposal. These criteria are not listed in any order of priority and are similar to the factors now used by other workers in the environmental field including FDA's appraisal of pesticides as potential food contaminants. FDA assesses information from all sources—industry, government agencies, universities, and the literature—in making these selections. Among the chief agencies with which FDA exchanges industrial chemical information are EPA and USDA.

Classes of materials that have been evaluated for investigation include flame retardants, plasticizers, electrical fluids, halogenated solvents, various chemical intermediates, functional fluids, lubricating oil additives, replacements for PCBs, and chemicals present in sludges or wastewater.

Investigation of an industrial chemical involves several steps (*23*). First, a satisfactory analytical approach to detect and measure low levels of the compound in the presence of many other chemicals in food such as fish has to be developed. FDA has established a collection of over 600 different industrial chemicals for use as reference materials by FDA district laboratories, other Federal and state agencies, and selected researchers.

Next, information about location of manufacture and quantity of production is needed to obtain food samples from specified locations, often with the active assistance of individual states. If the suspected contaminants are found, the appropriate state or Federal agencies are notified. FDA's monitoring activities may be expanded, up to and including nationwide surveys of various foods by the field laboratories. On the basis of these results, FDA toxicologists may then judge whether the levels of contaminant in foods constitute a hazard to the consuming public.

Many of the less commonly encountered industrial chemical contaminants have been detected in

these investigations. These are summarized in Table 3. The freshwater fish has been a prime indicator food, because most contaminants eventually enter the waterways. Since almost all of the contaminated food samples were fish from inland waters near manufacturer or user sites, the results do not necessarily reflect general public exposure but may indicate that some chemicals are in the effluents from nearby plants or are leaching from treatment operations or disposal sites.

Some of the main chemical families found as contaminants in these investigations are: chlorinated aromatics, chlorinated aliphatics and cyclic aliphatics, brominated aromatics, phosphate esters, and aromatic amines.

Chlorinated Benzenes

Residues of these compounds have been found in fish from many different geographic locations and are apparently ubiquitous. Levels up to about 7 ppm have been found in the edible portion.

Table 3. Industrial chemicals found in foods: FDA exploratory program.

Type	Principal areas
Chlorinated benzenes	Many lakes and rivers
Chlorinated toluenes	Niagara River, N. Y. Bald Eagle Creek, Pennsylvania Raritan River, N. J. Newark Bay, N. J.
Chlorinated benzotrifluorides	Niagara River, N. Y. Raritan River, N. J. Newark Bay, N. J.
Other chlorinated aromatics	
Nitrobenzenes	Mississippi River; Delaware River
Anilines, phenylmethyl sulfides	Mississippi River
Styrenes	Lake Ontario, N. Y. White Lake, Michigan
Phenols and anisoles	Many lakes and rivers
Chlorinated cyclics	
Norbornenes	Mississippi River; Wolf Lake, Illinois
Cyclopentenes	White Lake, Michigan
Chlorinated aliphatics	
Butadienes	Mississippi River; Ohio River; White Lake, Michigan
Haloforms, ethylenes, and ethanes	Many lakes and rivers
Brominated aromatics	Pine River, Michigan; Ohio River, Ohio Ohio River, West Virginia Mississippi River, Iowa
Aromatic amines	Buffalo River, N. Y. Delaware River, Delaware
Aryl phosphates	Many lakes and rivers

Chlorinated Benzyl Chlorides and Benzotrifluorides

These chemical intermediates have been found mainly in fish from the lower Niagara River, New York, in levels up to about 1 ppm in the edible portion (*24*).

Other Chlorinated Aromatics

Pentachloroaniline and pentachlorophenyl methyl sulfide are associated with the pesticide, pentachloronitrobenzene (PCNB). Pentachloroanisole is derived from the pesticide, pentachlorophenol, and is frequently found as a residue in fish in many areas.

Chlorinated Cycloaliphatics

The chlorinated norbornene derivatives are precursors of the pesticide endrin and have been found in Mississippi River fish below a manufacturing plant in Memphis, Tennessee, in levels up to about 16 ppm in the edible portion (*25*). The chlorocyclopentenes were found in fish near a plant manufacturing hexachlorocyclopentadiene (HCP) near White Lake, Michigan, and are probably by-products.

Chlorinated Aliphatics

The chlorinated butadienes are by-products of chlorinated hydrocarbon manufacture. FDA has found hexachlorobutadiene (HCBD) frequently as a contaminant of fish, but not to any extent in other foods (*26*). The findings have varied from about 0.03 ppm to 4.6 ppm in the edible portion.

Brominated Aromatics

In addition to the PBBs, low levels of several bromobenzenes have been detected in fish collected near a bromocarbon producer in Michigan and in other waterways.

Phosphate Esters

FDA interest in these orthophosphate esters results from the fact that they have replaced PCBs in some flame retardant, plasticizer, and functional fluid applications. Aryl phosphate residues have been found in fish obtained near various user sites (*27*) and are believed to be ubiquitous contami-

nants, albeit at low levels (up to around 1 ppm in the edible portion).

Aromatic Amines

These chemical intermediates have been found in fish near dyestuff producers (*28*). Although the concentrations found were low, FDA has maintained interest in this class of compounds because of their carcinogenic potential.

FDA regards assistance furnished to states and municipalities as part of a policy of mutual cooperation. Investigations of potential chemical contaminants are typically combined FDA-state efforts. The following instances illustrate the wide range of incidents and chemical types that have been involved in these investigations:

FDA found isopropylphenyl diphenyl phosphate in fish furnished by the State of Pennsylvania. The samples were obtained in a creek near a steel plant known to discharge aryl phosphates (*27*).

At the request of the State of Maine, FDA analyzed samples of sediment and fish obtained in rivers containing floating drums of tris(2,3-dibromopropyl) phosphate (TRIS). No residues of TRIS were found.

FDA analyzed for residues of methylene bis(o-chloroaniline) (MOCA) in various samples from Adrian, Michigan, furnished by the State of Michigan. MOCA was present in the water and sludge samples from an industrial lagoon, but none was detected in fish from the nearby Raisin River (*29*).

Benzidine and dichlorobenzidine were found in water but not in fish in samples furnished by the State of Michigan. Samples were obtained near a plant discharge point in Muskegon County.

Bis(β-chloroethyl) ether was found in water and fish from a lake near Pitman, New Jersey, near a dump site. Samples were furnished by the State of New Jersey.

In Louisville, Kentucky, still-bottoms from HCP production were dumped into the sewer system. FDA provided assistance to Louisville and other municipalities and supplied analytical reference samples. High concentrations of HCP and octachlorocyclopentene, as well as many other organochlorine compounds, were found in the sewage sludge.

Methylchloroform was found in samples of ice cream and process water furnished to FDA by the State of Massachusetts.

Conclusion

When significant levels of a recognized chemical contaminant such as PCBs are reported, the problem presented is one requiring evaluation and control. The search for new chemical contaminants, however, poses a greater challenge because the number of potential contaminants is always much larger than the actual total detected. Neither FDA nor any other single agency will ever have sufficient personnel and resources to investigate all the possibilities.

With the passage of the Toxic Substances Control Act, the Resources Recovery and Conservation Act, and the Clean Water Amendments in recent years, more and better information about the chemicals produced, the composition of effluents and wastewaters, the levels of chemicals in drinking water, and the number and character of dump sites is now available. In spite of this progress, increased and unexpected demands on present resources will make cooperation among Federal and state agencies even more necessary than ever before.

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Polychlorinated Biphenyls in Indoor Air

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■ Polychlorinated biphenyls (PCBs) have been recognized as environmental contaminants since the mid-1960s. Until recently, however, little work has been done on identifying levels of PCBs in the indoor air. This paper describes a method for the analysis of PCBs utilizing low-volume indoor air sampling. The method uses polyurethane foam as a collector and has a limit of detection of ~0.01 $\mu g/m^3$. This study shows that indoor air, whether in commercial, industrial, or residential buildings, contains levels of PCBs at least 1 order of magnitude higher than outdoor levels. Defective fluorescent light ballasts are also shown to emit PCBs and to be an important source of indoor atmospheric contamination.

Polychorinated biphenyls (PCBs), which were commercially available for almost 50 yr in the United States, were in great demand because of their nonflammability, electrical-insulating properties and their chemical and thermal stabilities. From 1957 to 1974 over 300 million kg were manufactured by Monsanto Corp. and used in this country (*1*).

Starting in 1966, PCBs were discovered to be environmental contaminants present in such diverse media as Antarctic snow (*2*), human mothers milk (*3*), and Icelandic plants and animals (*4*). In 1971, Monsanto voluntarily cut back production and ceased making all PCBs in October 1977, but the problem of universal contamination is still present. For example, atomospheric concentrations in the ng/m^3 range have been reported recently from such widely distributed places as Rhode Island (*5*), Bermuda (*6*), and Southern California (*7*).

Even with the present interest in documenating PCB levels in the atmosphere, most of the work has been done on ambient air, and very little is known about PCB concentrations found indoors. However, many of the products containing PCBs were for indoor use: textile dyes, printing inks, paints, carbonless copy paper, fireproofing agents, small electrical capacitors, and fluorescent light ballasts. Previous researchers have reported on PCB emissions for fluorescent lighting fixtures in offices (*8*), but there have been no surveys published of PCB levels in private homes.

Until recently, the method most often used for the collection of PCBs from air was to draw the air through ethylene glycol-filled impingers (*9*). The hygroscopic nature of the ethylene glycol made sampling difficult and limited sampling time under all but the most arid conditions (*10*). These difficulties were eliminated with the introduction of polyurethane foam as a collection medium (*5*). Foam plugs are light weight and much easier to transport than the liquid ethylene glycol in impingers. Background levels of PCB using precleaned foam plugs give much cleaner gas–liquid chromatography (GLC) chromatograms than that associated with ethylene glycol reagent blanks. The PCB detection limits when using ethylene glycol in midget impingers are in the range of 0.5 $\mu g/m^3$ for a 5-h sample (*8*). When foam plugs are used as described in this method, the detection limit for a 4-h sample is ~0.01 $\mu g/m^3$. This higher sensitivity permits a more accurate assessment of air levels, which is especially important in light of the proposed National Institute for Occupational Safety and Health (NIOSH) criterion for a safe workplace atmosphere of 1.0 $\mu g/m^3$ over an 8–10-h workday and a 40-h workweek (*11*).

In this study, we took air samples in laboratories, offices, and homes. All of the laboratories and offices had fluorescent light and forced air heating and air conditioning. Of the nine houses, four contained fluorescent lighting in their kitchens, all but two of the homes had forced air heating, and all but one had air conditioning. There was also an opportunity to monitor contamination resulting from the burnout of a PCB-containing fluorescent light ballast.

Experimental Section

Apparatus. Two types of air samplers were used in this study. The first type, which utilized a Bendix Hurricane pump, collected large amounts of air (100–500 L/min) but was unsuited for indoor sampling. This sampler was used for the outdoor comparisons and to collect samples in the laboratories and offices when they were unoccupied. The smaller, low-volume pumps analyzed were the Mine Safety Appliance (MSA) portable pump Model S and the Du Pont constant-flow sampling pump Model P4000A. These personal samplers were much better suited to indoor monitoring because they were quiet and unobtrusive, although they sampled at only 2.5–4.0 L/min.

The sampling cartridges used with the low-volume samplers were made from glass tubes 2 cm in diameter and 7.5 cm long with 8-mm diameter × 3-cm long connectors. They were attached to the pumps with PVC tubing. The cartridge used with the Bendix pumps consisted of the 10-cm round filter holder that is standard equipment modified by epoxying a cylindrical metal chamber 5-cm i.d. × 25 cm long behind the filter holder. This was then attached to the pump by 7.6 m of flexible hose.

The sorbent used in both sampling cartridges was polyether-type polyurethane foam (density 0.022 g/cm^3). The plugs (5.5-cm diameter × 8 cm long for the large samplers and 2-cm diameter × 4 cm long for the small ones) were cut from sheets of polyurethane upholstery material and shaped to fit into the cartridges under slight compression to assure that all of the air sampled would be pulled through the foam.

All analyses were carried out with a Tracor 222 gas chromatograph equipped with ^{63}Ni electron capture detectors. The columns used included 3% OV-225 on Supelcoport 80/100 mesh, 3% OV-1 on Chromosorb WHP 80/100 mesh, and 4% SE-30/6% OV-210 on Gas-Chrom Q 100/120 mesh. All columns were 6-mm i.d. and 1.8 m long.

Reagents. Aroclor standards were obtained from the Pesticide Repository, U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, N.C. The standard solutions were made by dissolving the Aroclor in pesticide-grade benzene and diluting with hexane. Stock solutions were stored at −10 °C, and working standards were remade periodically from these.

The benzene and hexane used throughout were from Burdick and Jackson Laboratories. Analytical reagent-grade diethyl ether containing 2% ethanol was obtained from Mallinckrodt.

Basic alumina (60 mesh) was from Alfa Products, and the silicic acid used was Mallinckrodt AR, 100 mesh.

Procedure. Before use the foam plugs were extracted twice in Soxhlet extractors for at least 7 h each time with 5% diethyl ether in hexane. The solvent from the second extraction was concentrated, passed through a deactivated alumina column (see below), and analyzed by GLC to determine background

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Table I. Airborne PCB Levels in Workplaces

site	period of sampling	no. samples	av concn,[a] ng/m³	% RSD
	Industrial Research Facility			
laboratory A	6 months	11	200	27
laboratory B	2.5 months	6	240	36
office A	1 week	2	110	20
office B	2 days	2	80	24
outdoors[b]	1 week		18	
	Academic Facility			
laboratory	2 days	2	210	10
outdoors	2 days	2	4	11
	Shopping Complex			
office	2 days	2	44[c]	35

[a] Concentrations are calculated as Aroclor 1242 and Aroclor 1254. [b] Unpublished data from A. Brown and M. Jackson, Analytical Chemistry Branch, Health Effects Research Laboratory, U.S. Environmental Protection Agency. [c] No Aroclor 1254 was present.

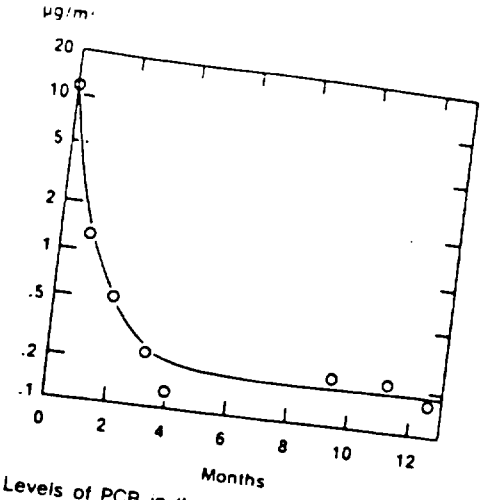

Figure 1. Levels of PCB in the air after a fluorescent light ballast burnout.

levels of of PCBs present. The plugs were then wrapped in hexane-rinsed aluminum foil for storage. When needed for sampling, the plugs were loaded into the cartridges by using forceps and rewrapped for transport to and from the sampling site.

The efficiency of the extraction and cleanup process was tested by carrying plugs spiked with 100 ng each of Archlor 1242 and Aroclor 1254 through the same procedure as that for the exposed plugs. The retention efficiency of the foams was tested by spiking a plug with a standard solution and then drawing air through both the fortified plug and then a second plug at 4 L/min for 4 h. The collection efficiency of the low-volume samplers was tested by placing a hexane solution containing 500 ng each of Aroclor 1242 and Aroclor 1254 into the bottom of midget impingers and drawing air through the impingers and then through foam plugs. Four samplers were run side by side to determine the actual variation of the method in a sampling situation.

The facilities sampled were placed into four categories: (1) an industrial research facility, (2) an academic laboratory building, (3) a shopping complex, and (4) private residences. The focus of the study was to determine "normal" levels of PCBs that people come in contact with during everyday activities (categories 3 and 4) and compare those to laboratory situations (categories 1 and 2). The ambient air outside two of the buildings and one home was also sampled for an indoor/outdoor comparison. The sampling was carried out at various times of the year, but the heating and air conditioning systems assured that all indoor samples were collected at temperatures of 20 ± 6 °C. The outdoor samples were collected in March (house) and September (industrial research facility and academic building) when the ambient temperatures approximated those found indoors.

The sampling periods were usually either 8 or 16 h so that a total sample equivalent to 1–4 m^3 of air was collected. The air intake during sampling was positioned 1–2 m above the floor and oriented downward or horizontally.

A fluorescent light ballast burnout which occurred in an office during this study presented the opportunity to examine the persistence of PCB in the air. The ballast was replaced almost immediately after burnout and was placed in another room overnight while the first air sample was collected. The ballast was removed and disposed of the next morning, and sampling was continued in the affected room intermittently for over a period of ~1 yr.

After sampling, the plugs were Soxhlet extracted with hexane for at least 40 cycles. The extracts were concentrated to ~5 mL with a rotary evaporator and then to less than 1 mL under a gentle stream of nitrogen at ambient temperature.

Table II. Airborne PCB Levels in Homes

site	month sampled	no. samples	av concn,[a] ng/m³	% RSD	fluorescent lighting	forced air heat, air conditioning[b]
kitchen A	June	1	480			1, 2
kitchen B	June, Nov	1	180			1, 2
kitchen C	Apr	2	250	28		1, 2
kitchen D	July, Dec	2	210	21	yes	1, 2
kitchen E	June, July, Oct, Nov	3	240	32	yes	2
kitchen F	July, Sept	5	150	27		
kitchen G	Mar, Apr, Nov	2	580	36		1, 2
kitchen H	Mar, Apr	3	260	24	yes	1, 2
kitchen I	Apr	5	500	29		1, 2
living room D	July, Dec	1	39		yes	1, 2
bedroom E	July, Dec	3	170	23		1, 2
basement E	June, July, Oct	3	120	28		2
garage F	Mar, Apr	4	64	30		
library I	Mar	3	400	26		
outdoors I		1	4			

[a] Concentrations are calculated as Aroclor 1252 and Aroclor 1254. [b] Forced air heat = 1, forced air conditioning = 2.

Column Chromatography. The alumina was weighed into a flask, distilled water equal to 6% by weight of the alumina was added, and the flask was stoppered and shaken well. The adsorbent was then allowed to stand for at least 15 h before use. A plug of preextracted glass wool was placed in the bottom of a 7-mm i.d. × 22-cm long glass column and washed with 10 mL of hexane. The column was then packed to a depth of 17 cm with the deactivated alumina. The sample was added to the column and eluted with 15 mL of hexane, and the volume of eluate was adjusted for GLC analysis.

The method used to characterize the PCBs found in the sample has been previously described (*12*).

When it was necessary to separate the PCBs from interfering chlorinated pesticides, especially residues of technical chlordane, the sample extracts were fractionated by silicic acid column chromatography (*13*).

Results and Discussion

The results indicate that the foam plugs collect PCBs nearly quantitatively compared to a collection efficiency of ~80% for ethylene glycol (*6*). The extraction and cleanup of four fortified plugs gave 100 ± 3% recovery for Aroclor 1242 and 99 ± 5% for Aroclor 1254. The retention of PCBs by foam was at least 97% when two plugs were used in series. In the worst case (Aroclor 1242 on only the first plug) the retention was 87 ± 7%. The collection efficiency was 96 ± 15% for Aroclor 1242 and 95 ± 7% for Aroclor 1254. The efficiency of the foam plug sampling system was studied by using a minimum of five replicates for each experiment.

When four samplers were run side by side in the same laboratory and the samples were taken through the analytical procedure at the same time, the results showed that the overall method had a relative standard deviation of 17%.

The percent relative standard deviations for the actual sampling in the field varied from 10 to 36%. This larger variation is due, at least in part, to the fact that sampling at a given location was spread over as much as several months with some of the samples taken when the forced air heating or cooling systems were in use and others taken when the house air was more or less stagnant.

The average level of PCBs, calculated as Aroclor 1242 plus Aroclor 1254, found outside the industrial research building was less than 0.02 $\mu g/m^3$ while the level inside the offices was 5 times higher, 0.10 $\mu g/m^3$. Inside the laboratories the level was 10 times higher than ambient, averaging 0.21 $\mu g/m^3$ (Table I). The air outside one home contained 0.004 $\mu g/m^3$ of PCB while the average level inside that house on the same day was 0.31 $\mu g/m^3$. While these data indicate that indoor air contains much higher levels of PCBs than the surrounding outdoor air contains, the amounts found are still well below the NIOSH proposed criterion.

In the room containing the burned-out light ballast, air levels of PCBs on the day of burnout were found to be over 50 times higher than normal (11.6 vs. 0.2 $\mu g/m^3$) for that room, and levels remained elevated for 3–4 months afterward (Figure 1). In another study of a ballast burnout where ethylene glycol in impingers or on nylon screens was used as the collection medium, the levels had reached the detection limit of the method (0.5 $\mu g/m^3$) "within weeks" (*8*). Comparison of these studies illustrates the benefits of the foam collection system's lower limit of detection when following trends caused by PCB spills or contamination.

Of the nine houses tested, four had pre-1972 fluorescent lighting fixtures in their kitchens. Besides the nine kitchens, a total of six other rooms also were tested. As can be seen in Table II, in every case the levels of PCB found were higher in the kitchen than the other rooms tested, but there was little or no correlation between PCB concentration and the type of lighting used. However, other electrical appliances typically found in kitchens may emit PCBs to account for the observed effect. It is interesting to note that the levels found in laboratories were no higher than those found in the houses.

It is apparent from this work that the "normal" levels of PCBs found in indoor air are at least 1 order of magnitude higher than those in the surrounding outdoor atmosphere. The relatively high levels of PCBs that have been reported in urban air may be the result of PCBs leaking from the many buildings present rather than from one or two sources of high PCB contamination. More work needs to be carried out to pinpoint the sources of indoor PCB emissions and to determine to what degree the levels are dependent on air circulation in a given residence.

Acknowledgment

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Uptake of Polychlorobiphenyls Present in Trace Amounts from Dried Municipal Sewage Sludge Through an Old Field Ecosystem

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Municipal sewage wastes are increasingly being used as fertilizers and soil conditioners. FURR et al. (1976) report that several major municipalities are already using sludge in this capacity. The cities of Chicago, Milwaukee, and Schenectady commercially market sludge under the tradenames Vertigreen, Milorganite, and Orgro, respectively.

Although the use of sludge as a fertilizer or conditioner is attractive in terms of cost, nutrient recycling, and waste disposal, a question has arisen regarding pathogens and trace levels of organic and inorganic toxic materials contained in the sludge, that may affect human health or the ecosystem where the sludge is eventually disposed (FURR et al. 1976; TORREY 1979). In particular, dried sewage sludge has been found to contain polychlorinated biphenyls (PCBs) at the ppm level.

This study examined PCB concentrations in a sewage sludge used as a fertilizer, in the treated soil, in the plants growing in that soil, and in arthropods in contact with the soil and feeding upon the plants. By repeated sampling of each level, the uptake of PCBs through a terrestrial food chain could be proposed.

MATERIALS AND METHODS

The research was performed at the Miami University Ecology Research Center, two miles north of Oxford, OH. Four acres (1.6 ha) of grassland, previously enclosed by 20 gauge galvanized steel walls extending 61 cm above the ground and 46 cm below the ground, were subdivided into 16 0.1 ha enclosures. In previous experiments at this site, cross contamination of pesticides and fertilizers had been negligible.

Five replicate plots were randomly selected for treatment, of which three were sludge-treated and two, serving as controls, received no treatment. Soil particle size analysis by the method of FOTH & TURK (1972) averaged 30.0% sand, 48.2% clay, and 21.8% silt. The soil samples averaged 5.9% organic matter. The plots were last planted in 1974 with a mixture of Kentucky Bluegrass (Poa pratensis), Rye grass (Lolium perenne), and Fescue (Festuca elatior). In 1979, Goldenrod (Solidago rigida) and the fescue comprised over 95% of the plant biomass present.

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The sludge used was "Milorganite," a dried, granular, processed sludge marketed by the Sewerage Commission of Milwaukee, WI, of nutrient composition 6-2-0 (N-P-K). Four hundred pounds were applied monthly by cyclone seeder on the 22nd of each month from May to September in both 1978 and 1979.

Specimens of sludge, soil, plants, and above-ground arthropods were collected monthly between the 18th and 21st of each month in 1979, starting immediately before the initial May sludge spreading and continuing through October. All samples were collected from three randomly selected locations within each plot and combined to make a monthly composite plot sample. Arthropods were collected by a vacuum collector process as described by BARRETT (1968) and were manually sorted from the litter. Plants were collected through the random location of a one-quarter square meter quadrat and the collection of all material within. Soil was collected by the removal of the upper 2 cm of soil from around the roots of the plants. All samples were preserved by freezing in glass containers until analysis.

Extraction was performed on 50 g aliquots of soil, 25 g aliquots of plants, and on the entire biomass of the arthropods collected. The aliquot was blended with a 1:1 hexane:acetone solution in a proportion of 2 mL solvent per gram of aliquot. After vacuum filtration, the soil and plant extracts were washed with 30 mL 2% saline and partitioned three times with 30 mL acetonitrile as described by THOMPSON (1977). Florisil and silicic acid fractionation were also done as described by Thompson. Fortified specimens of potting soil and greenhouse plants averaged 92% recovery from soil and 85% from plants.

To insure that arthropods were not contaminated by soil or detritus, individuals were cleaned and inspected manually under a 20 power microscope. Twelve of the arthropod samples were macerated and extracted as described by PETERSON et al. (1976). The remainder were suspended in 25 mL of the hexane-acetone solution and extracted in blenders before washing with saline and Florisil fractionation. Fortified specimens of a commercial hamburger-soybean mixture averaged 76% recovery of PCB.

Gas chromatography was performed using a tritium electron capture detector. Injector, column oven, and detector temperatures were all 200° C. All samples were injected into a 1/8 inch o.d., 3 m nickel column of 1.5% OV-17/1.95% OV-210 on 100-120 mesh Chromosorb W at a flow rate of 60 mL/min nitrogen. In addition, an auxiliary column of 5% OV-210 on Chromosorb W was used at 45-55 mL/min nitrogen for the separation and detection of PCBs. Retention times measured from chromatograms obtained on both columns were compared to Arochlor 1254 and 1260 standards obtained from the USEPA Environmental Toxicology Division, Research Triangle Park NC. Quantitation of the peaks was carried out on the OV-17/OV-210 column chromatograms.

Results were expressed numerically where they were above 20 ppb, while lesser detectable levels were called "trace" and were not used in the statistical analysis. Raw values were corrected for the percent recovery based on fortified specimens. The limit of detection was estimated to be approximately 8 ppb. The data were tested for significance using an analysis of variance (ANOVA) and the soil analyses were fitted to a line using least squares regression.

RESULTS

The Milorganite sludge was found to contain an average of 3.3 ppm (dry weight) PCB in the retention time range of peaks derived from a standard mixture of Aroclor 1254 and 1260. The range of values for six randomly chosen 50 lb bags was 3.1 to 3.4 ppm.

Soil samples collected from the treated plots showed trace amounts (i.e., less than 20 ppb) of PCB residues from the previous year's sludge treatment in the May 15, 1979, samples (Table 1). With additional sludge applications, the amount of detectable PCB in the soil increased to a quantifiable level in the July samples, and further to a mean of 34 ppb by October, three weeks after the final sludge application. From the control plots, one out of twelve samples showed trace residue levels.

Table 1. PCB residues in soil (ppb dry weight)

Treatment and Plot	May 15	June 15	July 19	Aug. 20	Sept. 18	Oct. 20
Control #1	N	N	N	N	N	N
Control #2	N	N	N	N	T	N
Sludge #1	T	20	21	22	36	39
Sludge #2	T	T	26	29	31	29
Sludge #3	T	T	19	27	29	35

"N" are no detectable PCB residues.
"T" or trace residues are detectable PCB residues, but at less than 20 ppb.

These soil concentrations are significant ($p < 0.05$) using ANOVA when compared to the controls. Least square regression of the PCB concentration vs. time in the treated soil samples yields an intercept of 14 ppb (with a 95% confidence interval of 6.4 to 21 ppb) as the May 22 PCB soil residue, or as the residue from the previous year's treatment. The slope of the regression is 0.14 ppb/day, or 4.0 ppb/treatment, and is significant ($p < 0.05$). The Pearson Correlation Coefficient between PCB concentration and time is 0.82.

Of the 72 plant samples collected, 67 were extracted and analyzed. Five received contamination in the laboratory during extraction and cleanup. The remaining 62 specimens had no detectable PCBs. In a separate experiment, three specimens of the Goldenrod, one from each treated plot, collected on September 18 were sorted and analyzed by leaves, stems, and flowers, and each subsample yielded no detectable PCBs.

For the arthropod analyses, none from the control plots showed detectable PCBs, nor did any animals collected in May and June. Among primary consumers, four samples from the treated plots from July and August showed a mean of 31 ppb PCB. In September, no residues were detected, and October arthropod samples were of insufficient biomass to be analyzed.

Arthropods in the May and June collections were primarily leafhoppers (Family Cicadellidae) and aphids (Family Aphidae) of the order Homoptera. In July and August, the greatest contribution to the biomass were crickets (Gryllus assimilus), Bean Beetles (Epilachna borealis), and grasshoppers (Dissostera carolina).

The secondary consumers were predominantly large spiders (Family Arachnida), Daddy Longlegs (Family Phalangida), Ladybugs (Adalia bipunctata), and Mantids (Mantis religiosa). Samples from the control plots yielded no detectable PCB residues, nor did samples from the treated plots in May, June, and September. Three samples from July and August did yield an average of 24 ppb. Differences between the primary and secondary consumers were not significant by ANOVA ($p < 0.10$).

DISCUSSION

The retention and increase of PCB residues in the soil confirms expectations that PCBs are relatively immobile and persistent in the A soil horizon. The lack of detectable PCBs above ground in the fescue grasses, Goldenrod, and miscellaneous plants confirms previous work with DDT. BROOKS (1974) found that DDT is taken up into those plant tissues with which it is in direct contact, in small amounts, but it is unlikely that the compound would be degraded or translocated unless the plant has a high oil or lipid content. This has been confirmed with PCBs in carrots (IWATA & GUNTHER 1976) and linseed (FINLAYSON & McCARTHY 1973; NASH 1974).

The largest contributors to the May and June insect biomass are aphids and leafhoppers, animals which rarely contact the ground and which showed no detectable PCBs. Whatever translocation of PCBs in plants may occur does not lead to biomagnification such that the limits of detection are reached. In July and August, the largest contributors are grasshoppers, bean beetles, and crickets, all larger animals that contact the ground frequently. The latter of these is a direct detritus consumer. The secondary consumers reflect uptake of the PCBs into these animals during these months. The bioconcentration values (1.3 times the soil for

primary consumers, 0.8 for secondary consumers for July and August) are considerably lower than those reported for aquatic insects (THOMPSON 1973) and for PCB-like compounds in beetles (GREICHUS et al. 1977).

Many factors are responsible for the movement of pesticides from soil to plants (NASH 1974). Other factors not presently considered, e.g., the route of application to both the ecosystem and to the organism (cutaneous contact vs. ingestion), climate, and life histories may also need to be included. One factor implied from this study is that PCBs can enter the food chain by direct consumption of the contaminated material (sludge or soil) rather than through transfer from plant to herbivore to carnivore.

Another point is that, although the insect residues are small, they can be significant when biomagnified. For example, an insect biomass of 1300 mg/m^2 at a PCB residue of 30 ppb means that there are 390 mg PCB/ha in the insects of a hypothetical field. If a half kilogram bird of prey consumes 10% of the field's insects in a season, then the bird accumulates a body burden of 39 mg, or a residue of 78 ppm. This does can be injurious to birds of prey and has been shown to cause eggshell thinning (MOORE 1974; HILL et al. 1976).

A review by TORREY (1979) recognizes this problem and proposes several criteria for the site selection and for the spreading procedures to be used for sludge disposal. These criteria include soil pH and composition, ground water and bedrock considerations, and drainage, moisture, and slope criteria which can reduce environmental hazards from the use of sludge as a fertilizer or soil conditioner.

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Accumulation of Polychlorinated Biphenyls in Atlantic Tomcod (*Microgadus tomcod*) Collected from the Hudson River Estuary, New York

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The Hudson River estuary contains elevated levels of polychlorinated biphenyls (PCBs) throughout its course. HORN et al. (1979) estimated that almost 300 metric tons of residual PCBs are present in the sediments, a level of contamination exceeding other aquatic ecosystems in the United States (US EPA 1976). General Electric's capacitor manufacturing facilities in the upper river at Fort Edward and Hudson Falls, New York, were the major sources of PCBs (HETLING et al. 1979). Discharges of PCBs from these facilities were sharply curtailed in 1976 and terminated in June 1977. PCB concentrations in the Hudson estuary recently ranged from 4 to 6 µg/g in the sediments and 0.0001 to 0.0011 mg/l in the water column.

PCB contamination in the estuary resulted in the closure of the striped bass and American eel fisheries in early 1976. PCB levels in the edible flesh of several fish species are now regularly monitored (HETLING et al. 1979, ARMSTRONG & SLOAN 1980). These surveys are concerned with human health protection. PCB effects on the health of the exposed fish populations have not been investigated.

A recent study of adult Atlantic tomcod (Microgadus tomcod) collected during the winter spawning season observed grossly visible abnormalities in livers characterized by enlargement and discoloration (SMITH et al. 1979). Histopathological examinations suggested a 25 percent frequency of hepatocellular carcinoma in the 1977-78 spawning population. The cause(s) of the hepatomas was unknown; but based on preliminary tests which revealed PCB residues in liver tissues, SMITH et al. (1979) speculated that PCBs may play a role.

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We present here the results of PCB residue measurements in liver, gonadal and body tissues of adult Atlantic tomcod collected from the same spawning population studied by SMITH et al. (1979). SPAGNOLI & SKINNER (1977) and HORN et al. (1979) presented data on PCB levels in edible body tissues of Atlantic tomcod collected in the Hudson estuary, but PCB levels in liver and gonadal tissues have not been reported. We also examined the available data for evidence that PCBs were involved in hepatoma induction.

MATERIALS AND METHODS

Test Animals

The histopathological studies of SMITH et al. (1979) and our tissue contaminant studies were incidental to a larger project investigating the impact of power plant operations on Atlantic tomcod and other fish populations. Adults were collected during the winter spawning season (mid-January through mid-February 1978) on the spawning grounds in box traps (0.9m x 0.9m x 1.8m) set along the shore of the estuary. Random samples of fresh specimens were regularly returned to the laboratory for routine age, growth, maturity and fecundity measurements.

Three adults from each of the three categories of gross abnormal liver appearance defined by SMITH et al. (1979) as hemorrhagic, small pustules, tumors, were randomly selected for PCB analysis from samples collected for routine age and growth measurements. Three adults whose livers were normal in gross appearance were also randomly selected for PCB analysis. The liver, gonadal and body (remaining) tissues from eight males and four females ranging from 142 to 213 mm (total length) were dissected, separated, individually labelled and frozen at -5°C in containers washed with methanol and rinsed with hexane.

Chemical Analyses

Tissues were analyzed in accordance with THOMPSON (1977). Frozen tissues were thawed, macerated with a glass rod, and the fatty portions separated by repetitive extractions with petroleum ether. Halogenated organics were then separated by acetonitrile partitioning. The resulting extracts were concentrated by evaporation in a Kuderna-Danish (K.D.) apparatus, eluted through a Florisil column with 6% ethyl ether in petroleum ether, and concentrated for analysis by another K.D. evaporation.

A portion of the extract was perchlorinated using the method described by ARMOUR (1973). Analysis of this extract for decachlorobiphenyl was used to quantify total PCBs in the sample. Analyses of all extracts were performed on a Perkin-Elmer Model 910 gas chromatograph equipped with electron capture detectors. A packed column of 5% OV-210 on Supercoport was used for initial identification and for quantification of the decachlorobiphenyl. Another packed column consisting of 1.5% OV-17 and 1.95% OV-210 on Supercoport was used to aid

identification of different Aroclors. Recoveries were determined from Aroclor standards which were added as spikes and taken through all of the separation and concentration steps. Results were corrected for both solvent blanks and for recoveries. Concentrations are reported in µg/g wet weight of tissue.

RESULTS AND DISCUSSION

PCBs, identified as a mixture of Aroclors 1016 and 1254, were detected in all tissues (Table 1). All of the major gas chromatograph peaks could be attributed to these Aroclors; therefore, there was no evidence of other halogenated organic compounds in the 6% ethyl ether Florosil fraction. The small sample size (n = 12) and wide variation in the data limited statistical analysis, however, some potentially important trends were apparent.

Concentrations of PCBs in body tissues measured in our study (mean 0.17 µg/g, range 0.01 to 0.67 µg/g) are lower than other reports on Atlantic tomcod collected in the Hudson estuary. HORN et al. (1979) reported a mean concentration of 0.96 µg/g total PCBs (range 0.44 to 4.48 µg/g) from edible flesh. SPAGNOLI & SKINNER (1977) reported mean concentrations of 6.5 µg/g and 7.7 µg/g, respectively, for Aroclors 1242/1016 and 1254 in whole carcasses, with concentrations of 1.5 µg/g and 1.3 µg/g respectively in edible flesh.

Mean concentrations of total PCBs in gonads and livers were 1.17 µg/g (range 0.08 to 7.35 µg/g) and 37.52 µg/g (range 10.94 to 98.22 µg/g) respectively (Table 1). PCB residues in ovarian tissues were generally lower than in testes. We are unaware of previous measurements of PCB residues in Atlantic tomcod gonads and livers. UTHE et al. (1980) reported concentrations of PCBs in testes and livers of a related species, Atlantic cod (Gadus morhua), ranging from 0.05 to 5.3 µg/g and from 3.5 to 374 µg/g wet weight respectively. These concentrations were observed in groups of sexually maturing males fed 1, 5, 10, 25 or 50 µg of Aroclor 1254 per g of diet three times per week for 92 days.

We noted a trend toward lower levels of PCBs in the tissues of larger Atlantic tomcod (Table 1). However, the only statistically significant ($p < 0.05$) association was between body length and PCB concentration in livers of males and females combined ($r = -0.602$). This association was not significant for either males or females analyzed separately. We did not determine the percent lipid in any of the tissue samples.

Levels of PCB residues among body, liver, and gonadal tissues were generally not significantly associated. The single significant ($p < 0.05$) direct association ($r = +0.811$) between the concentration of PCBs in gonads and livers may have been spurious because it was dependent upon the relatively high concentration in the testes of one male (Table 1). When this

TABLE 1

Age, length, liver appearance category, and total PCB concentration in tissues of twelve Atlantic tomcod collected in the Hudson River estuary during January and February 1978

Liver Category[b] (gross external appearance)	Age (yrs)	Sex	Length (mm TL)	Total PCBs (µg/g)[a]			
				Body[c]	Testis	Ovary	Liver
Normal	1	M	163	0.10	1.64		29.43
Normal	1	M	179	0.02	0.86		20.32
Normal	1	M	150	0.08	0.64		49.18
Hemorrhagic	1	F	165	0.04		0.10	15.09
Hemorrhagic	1	M	150	0.10	0.19		31.50
Hemorrhagic	1	M	152	0.09	0.83		59.07
Small Pustules[d]	1	F	187	0.08		0.08	10.94
Small Pustules	1	M	142	0.67	7.35		98.22
Small Pustules	1	M	168	0.04	0.89		56.35
Tumors[d]	1	F	146	0.05		0.83	36.96
Tumors	1	F	190	0.66		0.12	16.02
Tumors	2	M	213	0.11	0.46		27.12
Mean				0.17	1.61	0.28	37.52

[a] Based on wet weight
[b] After categories described by SMITH et al. (1979)
[c] Homogenate of entire fish after liver and gonad were excised
[d] Categories of livers which contained histological evidence of neoplastic nodules of hepatocytes and hepatocellular carcinoma (SMITH et al. 1979)

M = male
F = female
TL = total length

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a Based on wet weight
b After categories described by SMITH et al 979)
c Homogenate of entire fish after liver and gonad were excised
d Categories of livers which contained histological evidence of neoplastic nodules of hepatocytes and hepatocellular carcinoma (SMITH et al. 1979)

individual was removed from the analysis, the association was no longer significant.

PCB residues in livers whose external appearance was normal varied by a factor of 2.4 and the mean concentration was not discernably different from mean PCB concentrations in abnormal livers (Table 1). Hence, no association between PCB concentration and type of liver abnormality was discernable. More recent histopathological studies revealed that several Atlantic tomcod livers, collected from the 1980-81 spawning population, which were normal and hemorrhagic in external appearance contained neoplastic nodules (personal communication with C. E. Smith, USFWS Fish Cultural Development Center, Bozeman, Montana). Thus, some or all livers classified as normal and hemorrhagic by SMITH et al. (1976) and analyzed in our study for PCBs (Table 1) may have eventually developed neoplastic nodules and hepatocellular carcinoma. Normal livers may be rare in the Hudson River population of Atlantic tomcod.

In conclusion, this study shows that the tissues of adult Atlantic tomcod collected in the Hudson River estuary contain elevated levels of PCBs, especially in livers. However, any role of PCBs in the induction of hepatomas observed by SMITH et al. (1979) is unclear. The PCB-hepatoma induction hypothesis is plausible and deserves further study because: 1) Hudson River sediments are heavily contaminated with PCBs in areas inhabited by Atlantic tomcod (HORN et al. 1979), 2) Atlantic tomcod are bottom-feeding fishes (SCOTT & CROSSMAN 1973) and therefore probably continually exposed to PCB contaminated sediments, 3) PCBs concentrate in fish livers (HANSEN et al. 1971, 1976; CAMP et al. 1974; ZITKO & HUTZINGER 1976; NARBONNE 1979), 4) PCBs have been associated with hepatocellular carcinoma and other liver abnormalities in wild fish populations (FALKMER et al. 1977; McCAIN et al. 1977; PIERCE et al. 1978, 1980), 5) Liver anomalies have been induced in fish exposed to PCBs under laboratory conditions (JENSEN et al. 1970; JOHANNSON et al. 1972; HANSEN et al. 1974; COUCH 1975; LIPSKY et al. 1978; KLAUNIG et al. 1979; UTHE et al. 1980), and 6) Even though there is no evidence that PCBs are hepatocarcinogenic in teleosts (PIERCE et al. 1978), they are hepatocarcinogenic in some mammals (FISHBEIN 1974). Further studies will be required to identify the causal relationship, if any, between PCBs and hepatocellular carcinoma observed in Atlantic tomcod from the Hudson River estuary.

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Macro-analytical Methods Used to Analyze Tissues of the Hawaiian Monk Seal, *Monachus schauinslandi*, for Organochlorine Pesticides, Polychlorobiphenyls, and Pentachlorophenol

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Among the specimens which were processed by the Hawaii Epidemiologic Studies Program (HESP) laboratory as poisoning incidents, were tissues of an adult male Hawaiian Monk Seal, Monachus schauinslandi, which had died while in captivity without known cause. Various tissues of the animal were brought to the laboratory for analyses by the veterinary pathologist, State of Hawaii, Department of Agriculture, to determine whether the death of the monk seal had any connection with exposures to environmental pesticides or pollutants, particularly to organochlorine compounds. The specimens included urine, parts of liver, lung and blubber, an entire testis, and an entire kidney.

The monk seal, an endangered species in Hawaii, is a relatively large animal, the adult male averaging 175 kg (380 lb), TOMICH (1969), and consequently solid tissues from the animal were from correspondingly large organs. Macro methods for representative analyses of these tissues for multiresidues of organochlorine pesticides, polychlorobiphenyls (PCBs) and pentachlorophenol (PCP) were devised. The methods utilize portions of reported analytical protocols for the determination of organochlorines in human and animal tissues and combines them with other techniques associated with pesticide residue analyses. Generally, the tissues with the aid of distilled water and drops of concentrated sulfuric acid are first extracted with hexane or benzene, the separated extractants are then cleaned and fractionated by passage through Florisil columns, and the resulting eluates are analyzed by gas-liquid chromatography (GLC) with electron capture (EC) detection.

METHODS AND MATERIALS

Organochlorine Pesticides and PCBs

Extractions in the devised methods are similar to the extraction of organochlorines from blood serum with hexane according to

[1]Presently, Pesticide Hazard Assessment Project

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the method by DALE et al. (1966), which is the multiresidue method outlined in the EPA, Manual of Analytical Methods (THOMPSON, ed. 1977). The solid tissues, however, are minced well before extracting them, and larger sample sizes, 5 - 12 g, are used with correspondingly larger amounts of hexane. An extraction is also conducted in a larger culture tube, 25 OD x 180 mm, with teflon-lined screw-cap, and with ca. 5 mL, distilled water, and ca. 10 drops of concentrated sulfuric acid. The minced sample is weighed and placed into the culture tube. Distilled water is added while rinsing the tissue down the culture tube. Acid is then added, followed by hexane. Both water and acid facilitate the extraction with hexane and assist in separating a relatively clean extract. About 15-20 mL hexane are suitable for sample sizes ranging from 5 to 7 g. The amount of hexane used for an extraction may vary and depends on the quantity and consistency of the tissue sample being analyzed, but the total amount used per sample must be accurately measured. Larger quantities of both water and hexane may be used to ensure efficient mixing of the extractant and tissues during slow rotation (ca. 50 rpm) on a Roto Rack^{R}. The extraction mixture is rotated for a period of about 4 h and is allowed to stand, usually overnight. The tube is then spun in a large capacity centrifuge at 2,000 rpm, and the upper hexane layer is collected by means of a disposable pipet. As in the analysis of blood serum, an accurately measured amount of most of the total volume of hexane is collected but not necessarily the entire amount. The extractant is then concentrated to ca. 0.5 mL with a gentle stream of nitrogen gas.

A Chromaflex column, 9 OD x 200 mm long, with a 50-mL reservoir (Kontes 420100, size 22) is packed with Florisil of chromatographically reproducible quality. Florisil, which is purged overnight in an oven at 135°C, allowed to cool while exposed to ambient laboratory temperature, and stored in reagent bottles with screw-caps lined with aluminum foil, is suitable. The column is filled with Florisil, which is retained in the column with a small wad of glass wool to a height of 100 mm after gentle tapping, and the Florisil is topped with 20 mm of anhydrous granular sodium sulfate. Adhering to chromatographic techniques, the column is first washed with 10 mL hexane, the concentrated extract is then placed on the column with washes kept to a minimum 2 mL, and the sample is eluted off the column by the following protocol with each fraction being collected separately: Fraction 1 ..... 10 mL, hexane; Fraction 2 ..... 20 mL, 20% benzene in hexane; Fraction 3 ..... 20 mL, 40% benzene in hexane; Fraction 4 ..... 10 mL, 80% benzene in hexane.

The concentrations of the fractions are adjusted for GLC analysis, EC detection. The quantities of residues are determined by including in the calculations peak heights, sample weights, dilution or concentration factors, and the fractions of total extractants analyzed, as in routine residue analyses of biological extracts by GLC.

PCBs are collected in fraction 1 with the organochlorine pesticides, HCB, aldrin, oxychlordane, trans-nonachlor, p,p'-DDE,

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For Aroclor 1260, the averages of the recoveries of each of the components corresponding to 7 GLC peaks quantitated, ranged from 80 to 99%.

Pentachlorophenol

The analysis of PCP in solid tissues was conducted by the macro method described for the organochlorines except that benzene was used as the extractant in place of hexane. Also, derivatization (alkylation) of the chlorinated phenol in the benzene extract, an additional step, was conducted with diazomethane as in the analysis of PCP in urine and blood, CRANMER & FREAL (1970), RIVERS (1972), and the EPA Manual of Analytical Methods (THOMPSON, ed. 1977). The derivatization is also outlined in a modification of the analysis of PCP in urine and blood by the HESP laboratory, HESP Annual Report No. 8 (1975). Briefly, benzene extracts are first adjusted with a stream of nitrogen gas (or concentrated by other means without loss of PCP) to ca. 5 mL. Derivatization is then conducted in a high draft-fume hood by adding diazomethane reagent prepared in basic hexane from N-methyl-N'nitro-N- nitrosoguanidine, the reaction mixture is cleaned by passage through a column of Florisil, and the methyl ether of PCP is analyzed by GLC. The derivative is eluted off the Florisil column with 20 mL of 20% benzene in hexane, followed by an additional 20 mL of 40% benzene in hexane to ensure complete elution. Monk seal urine was analyzed using 2.00 mL samples extracted with 6.00 mL benzene and 2 drops of concentrated sulfuric acid, as in routine urine analyses, HESP Annual Report, No. 8 (1975).

The PCP determinations were monitored by concurrent recovery runs with 5-12 g, commercial liver samples fortified with solutions of the sodium salt of PCP (prepared in 0.01 N NaOH). The level of fortification was 100 ng for the solid tissues. For urine, recoveries of 50 and 100 ng levels of PCP (Na salt) from 2.00 mL control samples were conducted and analytical quality control requirements for the laboratory's routine urine analyses were maintained. The recoveries of PCP from these tissues after blank and sample corrections were 70 to 96%.

Blubber extracts in the PCP analyses also required additional cleanup processes before analyzing by GLC. With an original 5 g sample, a benzene fraction readily separated after extraction by slow rotation. Methylation of the separated benzene extract with diazomethane was conducted without diffficulty. However, when the derivatized extract was concentrated, ca. 3 mL of viscous oil, which could not be reduced in volume any further, resulted. This was too much lipid to be cleaned by passage through a microcolumn of Florisil. Therefore, the extract was first processed by liquid-liquid partitioning using large volumes of acetonitrile, hexane and 2% sodium sulfate solution, a modification of the acetonitrile partitioning by TESSARI & SAVAGE (1980), and then passed through a macro Florisil column. With ca. 100 mL, acetonitrile, and hexane washes totalling ca. 20 mL, the oily extract was transferred to a 1-L separatory funnel containing 550 mL 2% sodium sulfate solution and 100 mL hexane. The funnel was then shaken for 2 min and

allowed to stand until the aqueous and hexane layers clearly separated. The lower aqueous layer was drained into another 1-L separatory funnel containing another 100 mL hexane and the liquid-liquid extraction repeated. The aqueous layer which was extracted a second time with hexane was then discarded and the hexane fractions were combined and concentrated to 5 mL by low temperature, rotary flash evaporation. The hexane concentrate was then chromatographically passed through a 25 x 300 mm glass column which was prepared by packing gently with Florisil to a height of 100 mm, topping with 25 mm anhydrous granular sodium sulfate, and prewashing with ca. 100 mL hexane. The methylated PCP was eluted off the column with 100 mL hexane. The eluate was concentrated and adjusted without difficulty for GLC analysis and gave rise to suitable chromatograms.

The devised methods are simple, relatively fast, flexible, and may be modified to improve the analyses or to accommodate new and problem situations. Fractions eluted off the Florisil column may be concentrated again and rechromatographed through freshly prepared Florisil columns to reduce materials which cause interferences and high backgrounds in gas chromatograms. Background materials may also be reduced by using 2% sodium sulfate solution rather than distilled H_2O during extractions of tissues with hexane or benzene. Smaller amounts of original lipid samples may be used to preclude additional cleanup processes. Finally, elution patterns of organochlorines off Florisil columns should be determined for different batches of Florisil and modified accordingly during column chromatography to account for differences in batches.

RESULTS AND DISCUSSION

The results of the analyses of monk seal tissues for organochlorine pesticides and PCBs are shown in Table 1. p,p'-DDE and Aroclor 1260 were the only organochlorines detected in the tissues analyzed. Blubber had the highest levels of both residues, followed by liver and kidney, respectively. Lung and testis had approximately similar but even lower levels. There was no detection in urine. Seven components of Aroclor 1260 which gave rise to the more consistent and prominent GLC peaks were quantitated. Separation of p,p'-DDE which was in the same fraction off the Florisil column as Aroclor 1260 (fraction 1) was not necessary since its GLC peak did not interfere with the 7 Aroclor 1260 peaks, and quantitations with the two materials in the same fraction was not a problem. Figure 1 shows typical chromatograms of fraction 1 and is one of the liver samples. The relative amounts of p,p'-DDE and Aroclor 1260 present were so different that fraction 1 had to be greatly diluted for the quantitation of p,p'-DDE (see Figure 1). The same situation was experienced with the other solid tissues. The results listed in Table 1 are based on total weights of samples analyzed. The results for p,p'-DDE are from the SE-30/OV-210 column and the results for Aroclor 1260 are from the OV-17/QF-1 column. Use of larger amounts of urine in an analysis may have detected very low levels of p,p'-DDE and Aroclor 1260. However, the small quantity of original specimen limited the analyses to 2 mL sample sizes.

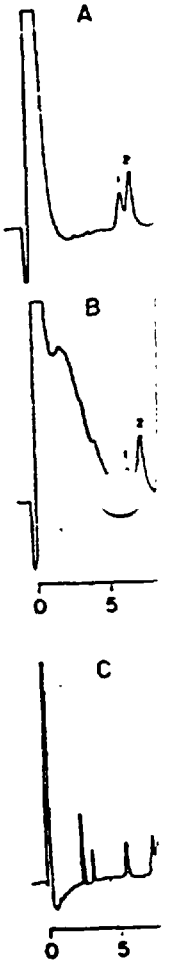
TABLE 1. Results of analyses of Hawaiian Monk Seal samples by the macro method, multiresidue screen for organochlorine pesticides and PCBs.

Sample	p,p'-DDE ppm	Aroclor 1260, ppm Peak: 4	5	6	7	8	11	12	ave.
Liver-1	0.67	0.68	1.2	0.33	1.1	0.62	0.50	0.39	0.69
Liver-2	0.94	0.89	1.4	0.41	1.4	0.82	0.72	0.56	0.88
Liver-3	0.44	0.49	0.79	0.55	0.98	0.38	0.33	---	0.59
Lung-1	0.065	0.058	0.086	0.014	0.086	0.053	0.036	0.035	0.052
Lung-2	0.061	0.084	0.13	0.027	0.13	0.080	0.056	0.048	0.079
Lung-3	0.040	0.043	0.055	0.012	0.057	0.033	0.023	0.016	0.034
Kidney-1	0.058	0.079	0.13	0.04	0.12	0.058	0.050	0.037	0.074
Kidney-2	0.14	0.19	0.28	0.094	0.28	0.11	0.12	0.089	0.17
Testis-1	0.051	0.083	0.12	0.041	0.12	0.057	0.054	0.041	0.074
Testis-2	0.063	0.076	0.11	0.035	0.11	0.055	0.051	0.035	0.068
Blubber-1	13	17	24	9.1	23	8.6	9.0	7.6	14
Blubber-2	13	15	21	7.9	20	7.9	8.5	6.3	12
Urine	N.D.[a]				N.D.				

[a] Not detected

Table 1 includes the results of repeated analyses for each of the solid specimens. Each analysis was conducted with a composite tissue sample prepared by combining samplings cut from different regions of a specimen. The samplings were minced and mixed together well, and the composite which resulted was considered to be representative of the entire specimen or organ being analyzed. For each repetition a separate composite sample was prepared in the same manner and analyzed. An analysis therefore, covered different regions of a specimen and the apparent variation in results for a particular specimen is due to the actual variation in levels of organochlorines in different regions of the specimen and not due to variation in the method of analysis. By following such techniques, an overall analysis of a large specimen was believed to have been possible without having to macerate and sacrifice the entire specimen or organ. For the larger specimens, liver and lung, a second repetition was run.

The monk seal tissues were also analyzed by an alternate micro method for organochlorine pesticides in human or animal tissue and human milk according to the procedure by ENOS et al. (1967), which is outlined in the EPA Manual of Analytical Methods (THOMPSON, ed., 1977). In this method, duplicate 0.5 g samples were analyzed. As in the macro method tissues were first sampled from different



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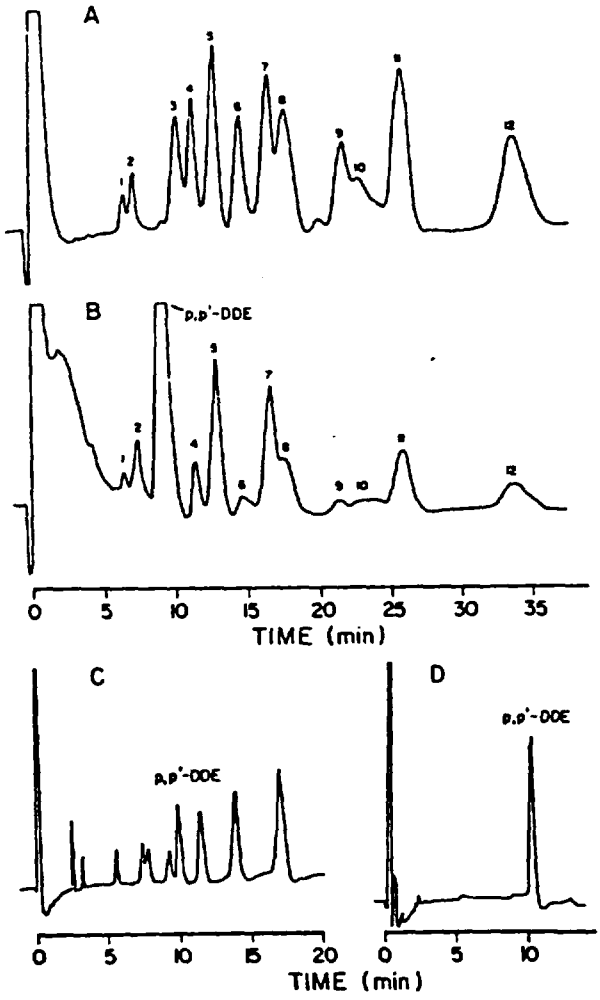

Figure. 1. Gas Chromatograms for standards and for an extract, fraction 1, off Florisil column of a Hawaiian Monk Seal liver sample with p,p'-DDE and Aroclor 1260. A - Aroclor 1260 standard showing 12 characteristic peaks, 5 ng. B - liver extract, fraction 1, concentrated to 5 mL, 5 µL injection; p,p'-DDE present with Aroclor 1260. C - mixture of 10 organochlorine pesticide standards; peaks, left to right, HCB, β-HCH, aldrin, oxychlordane, heptachlor epoxide, trans-nonachlor, p,p'-DDE, dieldrin, p,p'-DDD, and p,p'-DDT; p,p'-DDE, 100 pg. D - same liver extract as B, but diluted 10 x to 50 mL; 5 µL injection.

regions of a specimen. Then the samples were minced and mixed together. From this one composite, two 0.5 g samples were weighed and analyzed. Table 2 shows the results of the duplicate analyses for each solid specimen. The results are consistent with those obtained by the laboratory's multiresidue macro method, considering the variations between samplings of large specimens, and p,p'-DDE and Aroclor 1260 were the only organochlorines detected. As expected, the precisions of the results for a specimen by the micro method are much better than the precisions of the results by the macro method. However, the results in the micro method are for composites of relatively small amounts of tissues, only a little more than 1 g being needed for duplicate analyses, and are consequently representatives of small parts of the specimens and not of the entire specimens.

Further confirmation of the presence of p,p'-DDE and Aroclor 1260 in monk seal tissues

Table 2. Results of analyses by the micro method for organochlorine pesticides and PCBs in tissues of the Hawaiian Monk Seal

Sample	p,p'-DDE ppm	PCB 1260, ppm Peak: 4	5	6	7	8	11	12	ave.
Liver-1	1.2	1.6	2.9	1.0	2.7	1.4	1.2	1.1	1.7
Liver-2	1.3	1.6	2.9	0.98	2.8	1.4	1.3	1.1	1.7
Lung-1	0.072	0.10	0.14	0.064	0.15	0.066	0.063	0.050	0.092
Lung-2	0.075	0.091	0.12	0.048	0.12	0.055	0.051	0.046	0.075
Kidney-1	0.38	0.52	0.81	0.26	0.74	0.31	0.34	0.27	0.46
Kidney-2	0.32	0.44	0.67	0.23	0.62	0.27	0.28	0.22	0.39
Testis-1	0.20	0.35	0.51	0.17	0.48	0.22	0.24	0.18	0.31
Testis-2	0.20	0.34	0.48	0.18	0.46	0.22	0.23	0.18	0.30
Blubber-1	11	10	14	5.8	14	5.6	5.6	4.7	8.6
Blubber-2	9.9	9.2	12	4.8	13	5.9	4.9	4.3	7.8

was conducted by GC/Mass Spectrometry (MS). One liver extract and one blubber extract were selected for the confirmation. p,p'-DDE was separated from Aroclor 1260 by passing the extracts through a Silica Gel 60 column according to the protocol outlined by the Texas Epidemiologic Studies Program, EPA, (unpublished) for the separation of PCBs from organochlorine pesticides, a modification in the analysis of human milk by TESSARI & SAVAGE (1980). The Finnigan Model 3000 operating at 70 electron volts was the instrument used in the GC/MS analyses.

The results for the determination of pentachlorophenol in monk seal tissues, which were conducted separately from the analyses of the organochlorine pesticides and PCBs are shown in Table 3. PCP was detected in all specimens. Urine and blubber contained the greatest amount of PCP, while the solid tissues had very low but nearly equal amounts. Figure 2, are representative gas chromatograms for the PCP analyses.

From this study, we cannot attribute the death of the Hawaiian Monk Seal to the organochlorines and pentachlorophenol which were found in tissues of this mammal. Baseline (normal) data of residue levels for these pollutants in animal tissues in Hawaii are so limited that pathogenic levels in our fauna cannot be predicted. Moreover, it is difficult to even speculate whether the residues detected in the monk seal tissues were due to exposures of earth's background (ambient) atmosphere or to exposures of the mammal's immediate (specific) environment. The possibility still remains

Table 3. Results of the analysis of pentachlorophenol in Hawaiian Monk Seal Specimens

Specimen	PCP, ppb
Liver	1.0
Lung	1.5
Kidney	Trace (0.8)
Testis	Trace (0.9)
Blubber	77
Urine	66

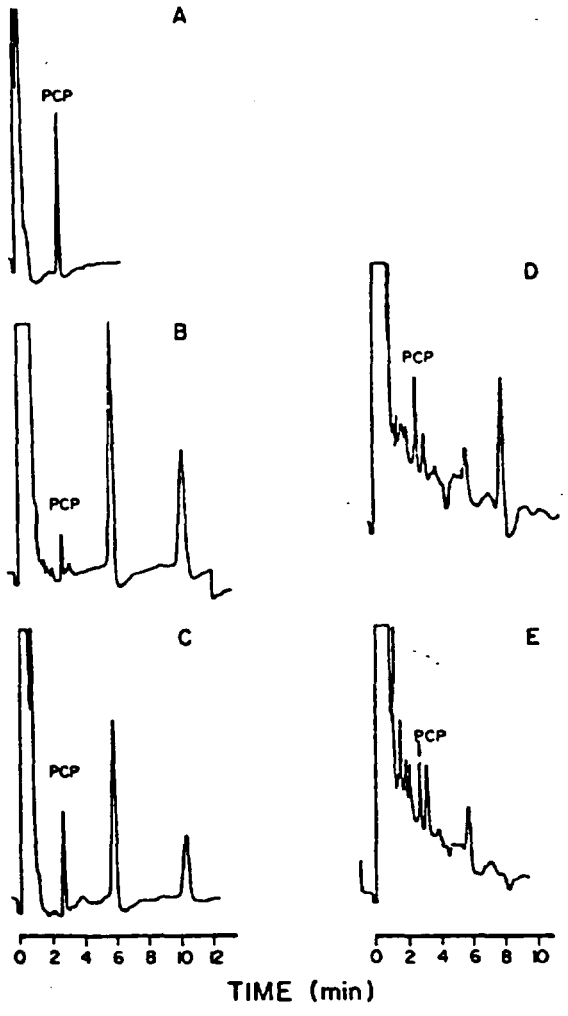

Figure 2. Typical gas chromatograms, A-E, for the analysis of pentachlorophenol in Hawaiian Monk Seal tissues. A - PCP standard, 30 pg; B - lung extract; C - same lung extract as B, but fortified with PCP standard; D - kidney extract; E - testis extract.

though that these residues or their parent forms may be implicated as contributing factors toward endangering the monk seal which is native to the Hawaiian Archipelago. With the exception of PCP, the levels of the residues found in monk seal appear to be higher than what would be expected as normal, particularly in blubber where results are also based closer to lipid content. Interestingly though, the detection of PCP in animal tissues other than in urine and blood after the review by BEVENUE & BECKMAN (1967) and the work by BARTHEL et al.(1969) is believed not to have been extensively studied, except for an isolated determination of PCP in human adipose tissue by SHAFIK (1973). Although PCP is water soluble and easily excreted from animals, it apparently is translocated throughout the system and most likely stored, over a period, in the lipids of tissues.

The macro methods followed in this study, however, are streamlined without loss of low detection limits and accuracy, and are convenient in handling great numbers of variable specimens. Large specimens which are difficult to sacrifice entirely for analysis

due to size or specimens of tissues which must be preserved, such as those from endangered species, are particularly suitable. Since the monk seal analyses, the macro methods have been applied to human tissues, mainly liver, kidney, fat, and brain of infants, with reproducible and confirmed results for multiresidues of organochlorine compounds and PCP. Also, these recent analyses have been carried out with an abbreviated elution protocol for the Florisil column and without additional cleanup.

ACKNOWLEDGMENTS

GC/MS analyses were conducted by K. H. Yanagihara, Department of Agriculture Biochemistry, College of Tropical Agriculture and Human Resources, University of Hawaii, Honolulu. T. R. Sawa, DVM, Chief, Veterinary Laboratory, Department of Agriculture, State of Hawaii.

Study was supported through a contract with the Epidemiologic Studies Program, Human Effects Monitoring Branch, Technical Services Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. 20460.

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Monitoring of DDT, PCBs, and Other Organochlorine Compounds in Marine Organisms from the North Aegean Sea

S. D. Kilikidis, J. E. Psomas, A. P. Kamarianos, and A. G. Panetsos

Department of Food Hygiene, Faculty of Veterinary Medicine, Aristotelian University, Thessaloniki, Greece

It is well established that human activities have resulted in serious environmental changes, especially in the coastal marine environment. Organochlorine insecticides and polychlorinated biphenyls are among the most persistent and toxic pollutants in aquatic and terrestrial ecosystems. The organochlorine insecticides can enter into the marine ecosystem, mainly, from the atmosphere. However, there is frequently a relationship between the presence of some specific insecticides and local pollution sources such as agricultural or municipal wastes. On the other hand, PCBs are related to industrial pollution. The distribution of these micropollutants has been extensively studied in the last decade, and considerable efforts have been made to understand the mechanisms of their transport through the biotic community. While the level of organochlorine residues in any organisms is a static index of a dynamic process, it identifies the presence and relative amounts of particular compounds (KERR & VASS 1973).

A comparison of residues level in predator and prey organisms in such an environment could establish present day background level typical of unpolluted coastal marine waters and indicate those animals most susceptible to accumulation through food chain transfer.

This paper describes the monitoring of organochlorine compounds in several biota species of coastal waters of North Aegean Sea.

MATERIALS AND METHODS

Areas studied: The Aegean Sea, especially its northern part, being a semi-enclosed formation and relatively shallow and fairly small water body, belongs to those marine ecosystems whose coastal waters offer a suitable location for investigations into the fate of organochlorine compounds.

The survey of DDT, PCBs and other organochlorine in marine organisms covered the whole of the north coast of the Aegean Sea, and sampling stations were located in the three important, industrial and agricultural, areas. The areas covered correspond to an anomalous coastline, formed by the deltas of the six main rivers namely Aliakmon, Loudias, Axios, Gallikos, Strymon and Nestos. These rivers flowing in the N.Aegean Sea, are the main sources of pesticides and PCBs contamination of the N.Greece coast. From this area three main gulfs were selected for study: The Thermaikos (40° 30'N-21° 50'E), the Strymonikos (40° 50'N-23° 60'E) and the Kavala gulf (40° 55'N - 24° 24'E) (Fig. 1).

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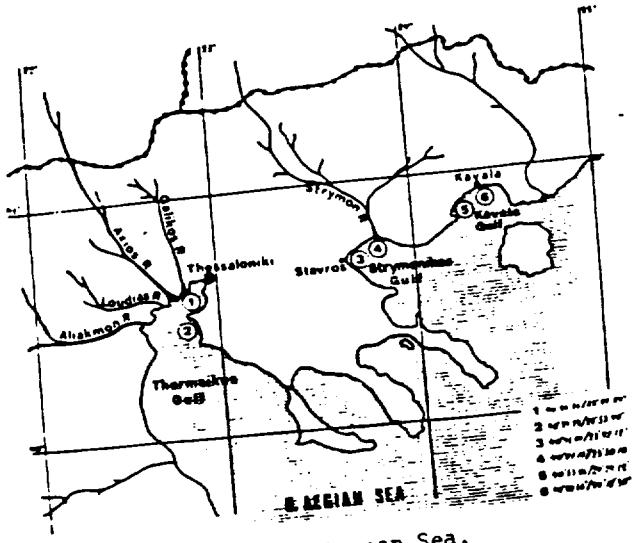

Fig. 1. Map of N.Aegean Sea.

The Thermaikos gulf receives the wastes of the city of Thessaloniki, industrial wastes (west coast) and agricultural wastes (east coast). The west coast is not suitable for swimming. The Strymonikos gulf receives mainly agricultural wastes, while the gulf of Kavala receives the wastes of the city of Kavala and agricultural wastes.

Sampling: To investigate the pollution of marine organisms a total of 142 samples (fish and shellfish) were collected from the above mentioned areas.

Sampling started in Sept. 1975 and continued through Dec. 1979. To monitor organochlorine insecticides (HCB, HCH and its metabolites, all DDT's, aldrin, dieldrin, heptachlor and heptachlor epoxide) and PCBs we started by collecting *Mytilus galloprovincialis*, *Mullus barbatus*, *Xiphias gladius*, *Thunnus thynnus* and *Merluccius merluccius*. *M.galloprovincialis* samples from six sampling stations indicated on the enclosed map (Fig. 1), while the samples of fish caught in the three above mentioned gulfs. The Kind and the number of samples are summarised in table 1.

Table 1. Sampling Stations and Number of Samples

FISH AND SHELLFISH	SAMPLING STATIONS			TOTAL (N.AEGEAN SEA)
	THERMAIKOS gulf	STRYMONIKOS gulf	KAVALA gulf	
M.galloprovincialis	39	18	39	96
M.barbatus	12	13	9	34
T.thynnus	2	1	1	4
X.gladius	2	-	-	2
M.merluccius	2	2	2	6
TOTAL	57	34	51	142

All organisms were placed in glass jars and held at -20°C until analysed. Containers were prerinsed with hexane and acetone and similarly rinsed aluminium foil was used to line caps to prevent contamination.

Handling samples and analytical techniques: Samples were treated with sodium sulfate and then Soxhlet-extracted, using petroleum ether, bp 40-60°C. The extractable organic organic matter was treated by the JOHNSON's (1965), and JENSEN et al (1973) methods.

In analyzing fish and shellfish for organochlorine insecticides and PCBs, chromatographic meyhods were used. A gas-chromatograph with a ^{63}Ni detector was used. The column was a coiled pyrex glass of 2 m (id, 2 mm), packed with 15% QF-1 and 10% DC-200 on 80-100 mesh Chromosorb W. Operation conditions were as follows:
a) Temperatures: Detector and injector 300°C; Column 210°C,
b) Flow rate of nitrogen: 14 mℓ/min.

All determinations were conducted in duplicate, and mean values were calculated.

RESULTS AND DISCUSSION

The mean analytical results for organochlorine residues in five species of fish and shellfish studied are presented in Tables 2 and 3, along with the necessary suplementary information. Those results are presented as concentration on a wet weight basis.

Table 2. Organochlorinesin *Mytilus galloprovincialis* (M.G.) and *Mullus barbatus* (M.B.) (ppb wet weight).

Compound	THERMAIKOS GULF		STRYMONIKOS GULF		KAVALA GULF	
	(M.G.)	(M.B.)	(M.G.)	(M.B.)	(M.G.)	(M.B.)
	(40 sampl.)	(12 sampl.)	(20 sampl.)	(12 sampl.)	(36 sampl.)	(10 sampl.)
DDE	6 (100)*	30 (100)*	8 (100)*	31 (100)*	14 (100)*	57 (100)*
DDD	4 (89)	9 (83)	7 (95)	11 (92)	9 (86)	26 (90)
DDT	5 (80)	12 (83)	6 (90)	12 (92)	11 (92)	33 (90)
Total DDT	14	51	22	54	33	120
Aldrin	0.5 (17)	ø	0.7 (85)	ø	3 (38)	ø
HCB	1 (47)	1 (77)	2 (20)	3 (67)	0.6 (42)	3 (80)
PCBs	340 (100)	230 (100)	261 (100)	1600 (100)	290 (100)	180 (100)

* Positiveness per cent.

Table 3. Organochlorinesin Marine Organisms from N.Aegean Sea (ppb wet weight).

Compound	*Mytilus galloprovincialis* (96 samples)	*Mullus barbatus* (34 samples)	*Merluccius merluccius* (6 samples)	*Xiphias gladius* (2 samples)	*Thunnus thynnus* (4 samples)
DDT	9	38	18	190	600
DDD	6	15	10	46	320
DDT	ø	19	16	210	320
Total DDT	23	71	43	450	1200
HCB	0.9	4	2	2	37
Aldrin	1	ø	ø	ø	ø
PCBs	310	700	510	360	2600

Three DDT related compound (pp'-DDE, pp'-DDT and pp'-DDD) were present in sufficient quantities for reliable determination. No peak corresponding to op'-DDT, dieldrin or heptachlor was observed in any samples. In the case of aldrin in fish, all samples had concentra-

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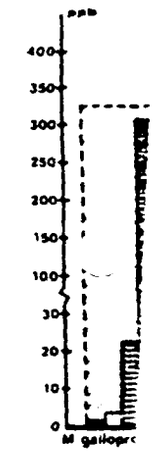

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tion lower than the detection limit (0.01 ng). Also, peaks attributable to PCBs, some of which have a column retention time similar to that of pp'-DDD and pp'-DDT (ADDISON et al. 1972), were absent.

All samples analyzed contained PCBs and DDE. PCB residues are found as ubiquitous contaminants of aquatic life (PEAKALL 1975) and DDE is, under reducing conditions, the most persistent metabolite of the previously widely used insecticide DDT. Usually DDE represents between 60 and 90% of the total DDT (DDT plus all DDT metabolites) (KAISER 1977). This is in agreement with our data from tables 2 and 3. Residues of pp'-DDE present in all organisms examined ranged in concentrations from 1 ppb, wet weight, in *M.gallocprovincialis* to over 1,000 ppb in samples from *T.thynnus*. On the other hand, residues of pp'-DDT were not found in every sample and when present, concentrations were usually equal to or less than those of pp'-DDE (Tables 2 and 3).

As can be seen from the results of Table 2, no significant difference was found in the distribution of total organochlorines in the organisms investigated (*M.galloprovincialis*, *M.barbatus*), except of PCBs of *M.barbatus* from the Strymonikos gulf. This high concentration of PCBs is attributed not only to local sources of pollution but to the inflow effect of the Strymon river.

The means of total organochlorines were higher in *T.thynnus* than in *X.gladius*, *M.merluccius* and *M.barbatus* (Fig. 2). This may be par-

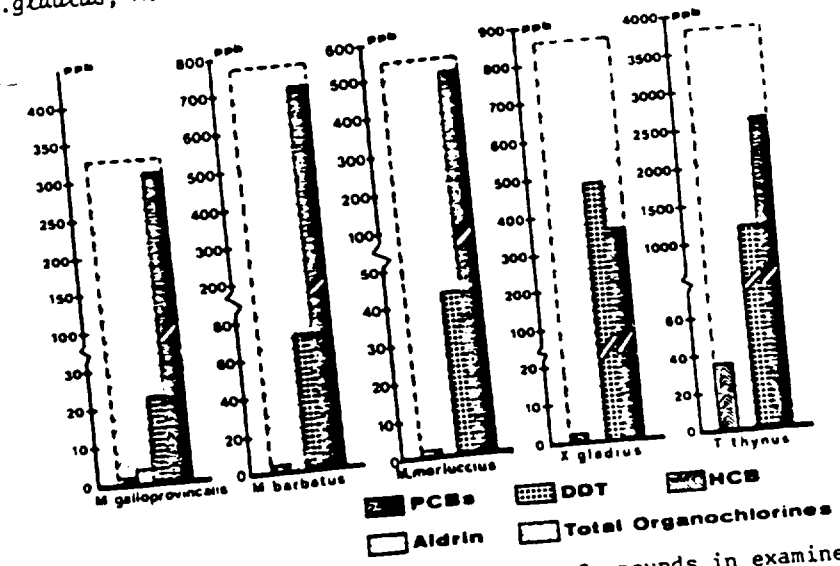

Fig. 2. Concentration of Organochlorine Compounds in examined marine organisms. (ppb wet weight).

tly due to differences in their body chemistry and location in the food chain. Data from diverse geographic locations do indicate, however, a relative uniformity in total DDT residue levels in widely separated marine habitats (FOWLER et al. 1978, BALKAS et al. 1978, NAZANSKY et al. 1978). What has been established from the data di-

scussed here, however, is that a minimum level of contamination may exist, which can be considered a background level, in all marine food chains.

From the results obtained (Table 2) it can be observed that the N.Aegean Sea is characterized by high value of PCBs (340 ppb wet weight in *M.galloprovincialis*), while the value of total DDT in the same organisms is low (14 ppb wet weight).

Generally, local urban and industrial wastes seem to be the main sources of the pollution in the areas investigated.

M.galloprovincialis samples show significant differences of organochlorine pesticides and PCBs, during the period of investigation (Table 4). The concentration of PCBs in *M.galloprovincialis* increased in the last year of investigation (1979), two times more than the first two year-period (1975-76). In Fig. 3 it is possible to see that, how the concentration of pesticides and PCB in *M.galloprovincialis* varied annually.

Table 4. Annual Variations in the Level of Organochlorine Compounds in *Mytilus galloprovincialis* (ppb wet weight).

Compound	1975-76 (40 samples)	1977 (10 samples)	1978 (23 samples)	1979 (23 samples)
DDE	9	9	11	9
DDD	3	9	11	6
DDT	8	4	8	10
Total DDT	20	22	30	25
HCB	Ø	2	2	2
Aldrin	3	Ø	Ø	0.8
PCBs	270	350	340	580

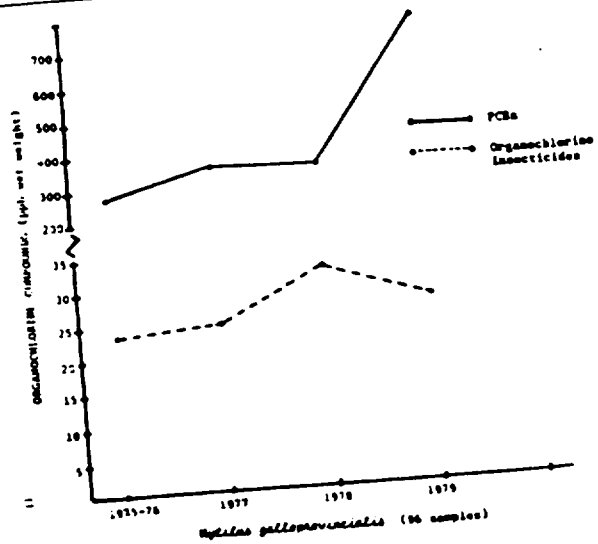

Fig. 3. Annual variation of Organochlorine Compounds in *M.galloprovincialis*.

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Organochlorine Pesticides

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There are few results reported for the organochlorine residues in *M.galloprovincialis* and *M.barbatus* from the Mediterranean. The total DDT values reported from Saronikos Bay, from France and from the Adriatic Sea vary between 3 and 120 ppb for *M.galloprovincialis* and between 8 and 140 for *M.barbatus*, while PCBs concentration varied from 300 to 9,800 ppb (SATSMADJIS & GABRIELIDES 1977, MESTRES 1978, NAZANSKY et al. 1978, DUJMOV 1978).

Finally, marine organisms from the heigher trophic level (e.g. *T.thynnus*) were found more contaminated than those from lower (e.g. *M.galloprovincialis*) levels.

It can be thus concluded that an understanding of organochlorine pollutants, as regards their types and concentrations, depends on background information on the compounds consumed, their tendency for biomagnification or biodegradation and, perhaps, seasonal variations.

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Efficiency and Rate of Elimination of Polychlorinated Biphenyls from Waste Waters by Means of Algae

Karel Véber[1], Jarka Zahradnik[1] and Ivo Breyl[2]

[1] *Biotechnological Laboratory of Institute of Microbiology CSAS, Trebon, Czechoslovakia;* [2] *State Veterinary Institute for Game, Jihlava—Horni Kosov, Czechoslovakia*

Polychlorinated biphenyls (PCBs) are being detected in an ever increasing number of components of the environment and accumulate in natural ecosystems.

The occurrence and accumulation of PCBs are the subject of many studies while the kinetics of their sorption by organisms, and possible methods related to the decontamination of the environment, are studied much less extensively.

The currently available results show that the commonly encountered concentrations of PCB residues do not give rise to dangerous acute toxicity; a higher risk, however, is represented by chronic toxicity due to accumulation of PCBs in organisms and in food chains. The high coefficient of accumulation of PCBs in microorganisms such as algae could be utilized for the decontamination of the most important environmental components such as drinking and feed water as well as the water from economically exploited reservoirs. The technological solution of the problem would require further applied research.

The data on the tolerance of algae towards PCB residues are controversial and hardly comparable owing to differences in the parameters of cultivation experiments. The values of accumulation ratios in different algae also differ.

SODERGREN (1971) found no appreciable damage to Chlorella cells even in saturated aqueous solutions of PCB; he found a 88% elimination of PCB from the medium. UREY et al. (1976) reported that PCBs do not inhibit the growth of Chlorella on short exposure. On the other hand, GLOOSCHENKO & GLOOSCHENKO (1975) found an inhibition of growth of Scenedesmus and other algae already at 1 ppb PCB. MOSSER et al. (1972) found inhibition of diatomes at 10 - 100 ppb; EWALD et al. (1976) determined the ID_{50} for the growth of the most resistant alga under study to be 4.4 ppm PCB. KEIL et al. (1971) found a 1000-fold accumulation of PCB in algae as compared to their medium.

This study is concerned with the efficiency and kinetics of elimination of PCB residues by microscopic algae. The experiments were carried out in southern Bohemia under outdoor conditions on open cultivation devices. The results point to one of the real possibilities of elimination of PCB residues from water environments.

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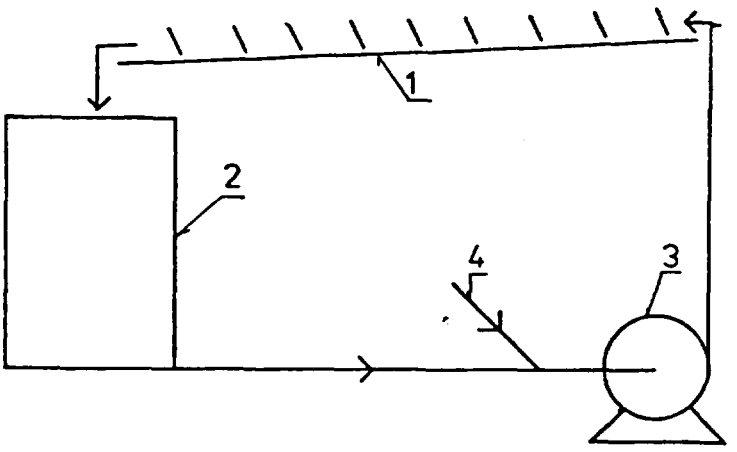

Fig. 1: Cultivation device. 1. Cultivation surface with transverse baffles (side view). 2. Retention tank. 3. Suspension pump. 4. CO_2 supply.

MATERIALS AND METHODS

Waste water used for cultivation came from a municipal water treatment plant and from large-scale piggery. It contained about 25% of biogenic and trace elements as compared with the synthetic medium for algae according to SETLÍK (1968) and, on average, 40.5 ppb PCB.

Cultivation device is shown in Fig. 1. Algal suspension was left to flow down the cultivation surface (1) equipped with a system of transversal glass baffles. The cultivation surface was tilted lengthwise by 3% and oriented towards south and its area was 2 m^2. Underneath the lower rim of the cultivation surface was a calibrated retention tank (2) which could accommodate the whole volume of algal suspension, i.e., 150 L, and served for measurement and adjustments of suspension volume. During circulation, the suspension was pumped from the bottom of the tank by means of a pump (3) and delivered on the upper lip of the cultivation surface. Carbon dioxide was introduced in front of the pump by feed line (4).

The device operated outdoors, the suspension circulated overnight, only without CO_2 supply. The thickness of algal suspension layer on the cultivation surface was 4 cm.

Once a day a volume of the suspension corresponding to the chosen dilution rate was withdrawn and replaced by the same volume of waste water. Evaporation losses were made up by tap water. The dilution rates tested were in the range 0.1 - 0.5 day^{-1}.

The algal strains under study included: Scenedesmus acutus (in 1973) and Chlamydomonas geitleri (in 1979). Before experiments, both strains were tested as to their growth characteristics in a medium according to SETLÍK (1968).

fluctuated between pH 6.3 and 8.3. No growth inhibition was observed at low PCB concentrations in the medium; the rate of elimination grew with increasing proportion of waste water in the medium. Table 1 indicates in both algal strains under study a certain trend to a decreased efficiency of PCB sorption with increasing dilution rate. The trend is not completely clear-cut and may reflect also the effect of weather in individual periods on the photosynthetic activity of the cultures. The trend cannot be also irrevocably ascribed to the inhibitory effect of increasing PCB concentration in the medium since the waste water may also contain other components inhibiting algal growth.

The PCB content in dry algal biomass at a maximum dilution rate was 213 mg kg^{-1} (S. acutus) and 157 mg kg^{-1} (C. geitleri). For comparison, we analyzed also algae of different origin from other cultivations. The biomass of the alga Scenedesmus obliquus cultivated in an inorganic medium outdoors in a pilot-plant device in southern Bohemia was found to contain 45 mg kg^{-1}, samples of laboratory cultures of Chlorella vulgaris up to 72 mg kg^{-1}, a sample of S. obliquus from a heterotrophic cultivation, 95 mg kg^{-1}; samples of S. obliquus grown in an outdoor industrial device in southern Bulgaria showed negative results of analyses in 1976 - 1979. Algae of genus Chlorella, grown experimentally in water from south-Bohemian ponds and in drain water from ameliorated plots, contained also up to 10 mg PCB per kg biomass.

The results point to a wide occurrence of PCB residues in the environment and confirm the considerable accumulating ability of algae for these substances.

The mechanism of sorption is not quite clear. UREY et al. (1976) found that the accumulating ability with regard to PCB is retained also by dead algal cells and the process may thus represent absorption in lipid cell components or simple adsorption phenomena.

The results permit the following conclusion. The presence of PCB in waste waters, at least up to the above concentration, does not preclude the semicontinuous cultivation of unicellular algae during which a major part of the substances in accumulated in the algal biomass. C. geitleri seems to be more efficient in the elimination process than S. acutus. The process can be carried out successfully under variable outdoor climatic conditions; a scale-up of the process could give rise to a promising technology of decontamination of some contaminated waters in suitable concrete cases. The liquidation of the relatively small volume of algal mass with accumulated PCB would be technologically very simple.

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Bull. Environm. Contam. Toxicol. 25, 43-48 (1980)

Toxicity of Arsenic and PCB to a Green Alga (*Chlamydomonas*)

Erik R. Christensen[1,2] and Peter A. Zielski[2]

[1]*Center for Great Lakes Studies, University of Wisconsin, Milwaukee, WS 53204*
[2]*Department of Civil Engineering, University of Wisconsin, Milwaukee, WS 53201*

High arsenic and PCB concentrations have recently been reported for Green Bay, Lake Michigan (VEITH 1972, ANDERSON et al. 1978, HOLM 1979). The source of arsenic has been localized to a chemical company producing arsenic herbicides in Marinette, Wisconsin. While the production ceased several years ago, the arsenic pollution persists because of ground water infiltration of leachates from storage piles of arsenic-containing wastes. The sources of PCBs are less well known. The sources are probably non-point in nature, and originate in part from local dumpings of PCBs previously used as dielectrics and hydraulic fluids.

The levels of these contaminants, e.g., up to 10 µg As/L and 0.45 µg PCB/L could present a hazard to the biota. The effect of arsenic and PCB on algae have been studied for each of these toxicants acting separately, but not in combination. PCB at 10-100 µg/L inhibit chlorophyll production and RNA synthesis (KEIL et al. 1971, SINCLAIR et al. 1977). On the other hand, arsenate at 1 µM behaves as an antimetabolite occupying sites for phosphate (PLANAS & HEALEY 1978). Based on the different toxic mechanisms for the two compounds, it is hypothesized that they will show independent joint action (FINNEY 1971), i.e., that they will not form a particular toxic combination. The purpose of this study was to establish toxic thresholds and possible interactive effects of arsenic and a PCB (Aroclor 1248) for the green alga Chlamydomonas isolated from Lake Michigan.

MATERIALS AND METHODS

A slightly modified version of the culture medium of GUILLARD (1975) was used as growth medium for Chlamydomonas. Major nutrients, EDTA, and vitamins were added as specified, as well as all trace elements except boron which was added as boric acid at a final concentration of 0.6 mg/L, a factor ten lower than required by the recipe. This lower boron level was chosen so as to better corespond to the levels specified in other standard media and to concentrations found in natural waters.

The test alga Chlamydomonas was obtained from the University of Michigan Great Lakes and Marine Waters Center at Ann Arbor.

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The alga was isolated from Lake Michigan by Richard Steinberger. The mean diameter was found to be about 4.9 µm using an electronic particle counter. A stock of actively growing Chlamydomonas was maintained by weekly transfers to fresh nutrient media.

Algal assays were carried out essentially according to the EPA procedure: Algal Assays Procedure Bottle Test (U.S. EPA 1978). The adopted procedure included maintenance of the cultures at 15°C in incubators with continuous 20 W "cool-white" fluorescent lighting. Aseptic techniques were applied throughout the bioassays. The algae were kept in autoclaved, cotton-plugged Erlenmeyer flasks filled with 100 mL nutrient solution. The flasks were shaken every 24 hours to maintain the cultures in homogeneous suspension.

First, two screening experiments were conducted to establish the toxicity ranges of the two toxicants using dibasic sodium arsenate and PCB (Aroclor 1248). Next, a combination experiment was set up based on these ranges. For fortifications, aqueous solutions of dibasic sodium arsenate were prepared, while the PCB was diluted with hexane, acetone, and water, successively. As an example, the 111 µg/L PCB flasks contained 711 mg acetone/L and 69 mg hexane/L, while the other PCB flasks contained proportionately lower concentrations of hexane and acetone. Control flasks with these solvents, but without PCB, were also set up.

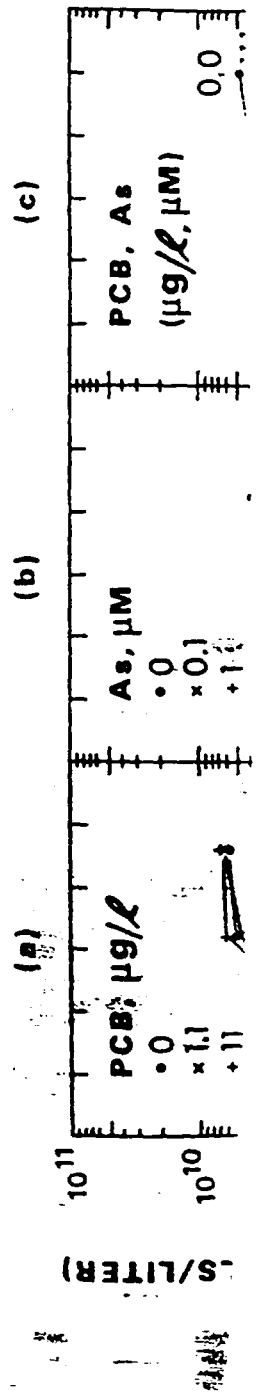
After fortification, the flasks were inoculated with 5×10^6 cells/L. Algal growth was monitored at least once a week for about four weeks. Algal population and size distribution were measured by means of a Particle Data Inc. Electrozone/Celloscope, Model 112 LTH, coupled to a PDP 8 computer and a Teletype terminal.

Data analysis, based on maximum standing crop was performed at the 95% confidence level using a two-tailed t-test for the independent joint action hypothesis (CHRISTENSEN et al. 1979).

RESULTS AND DISCUSSION

The results of the algal assays are plotted in Fig. 1. The screening tests (Figs. 1a, b) with PCB and arsenic added singly were carried out first to establish the toxic ranges of these compounds. Next, the combination experiment (Fig. 1c) was conducted based on these levels. Interactions could not be excluded outside the toxic ranges of the individual toxicants, but were considered most likely to occur there.

From Fig. 1a, PCB depresses growth at concentrations between 11 and 111 µg/L. This is consistent with the levels of 10-100 µg/L found by KEIL et al. (1971) to inhibit chlorophyll production and RNA synthesis of a marine diatom (Cylindrotheca



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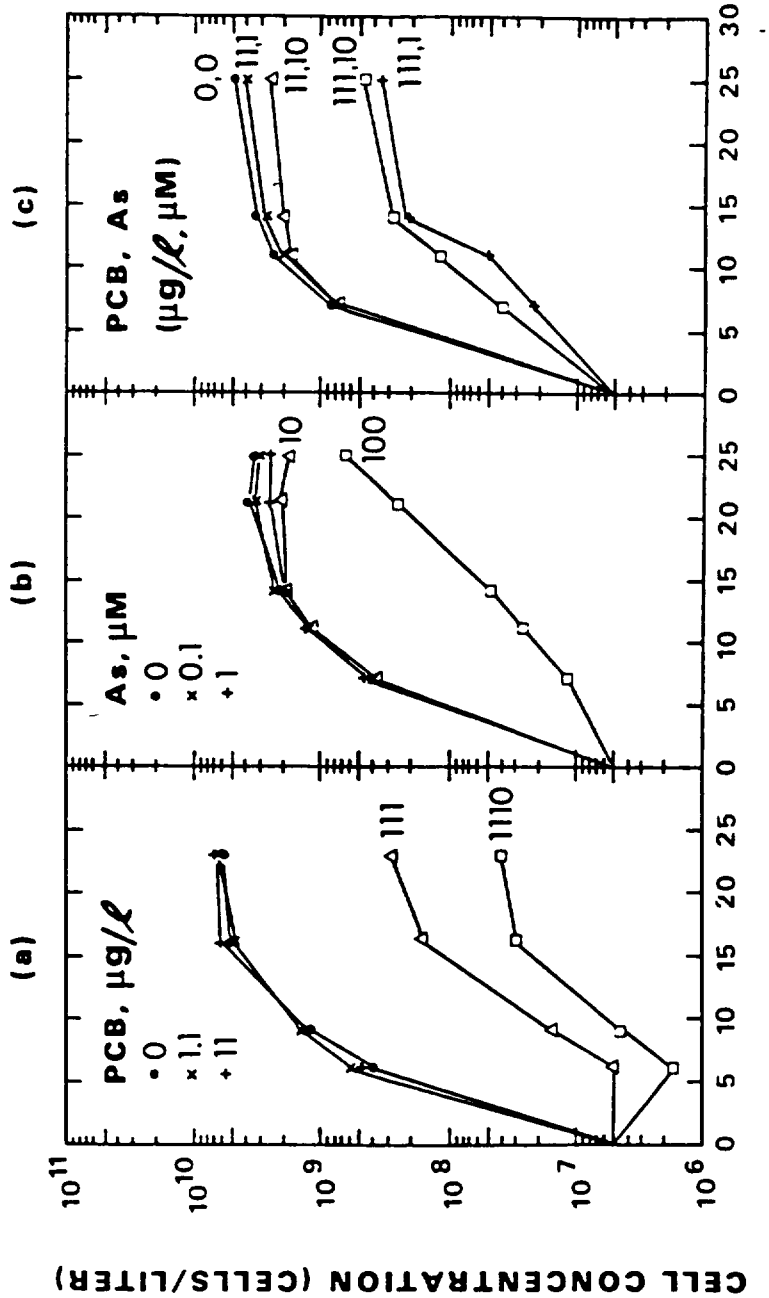

Fig. 1 Growth response of Chlamydomonas fortified with (a) PCB (Aroclor 1248), (b) dibasic sodium arsenate, and (c) these toxicants in combination.

closterium) and with similar findings of SINCLAIR et al. (1977) who measured the respiration rate of Chlorella vulgaris. None of the control flasks with hexane and acetone, but without PCB, showed any significant growth depression due to these solvents.

From Fig. 1b, arsenate is inhibitory to growth at a concentration of 1 µM. By comparison, PLANAS & HEALEY (1978) demonstrated growth inhibition for five different algae, including Chlamydomonas reinhardtii, at arsenate concentration between 1 and 100 µM.

The combination experiment conducted with fortifications of PCB and As was performed at the levels indicated in Fig. 1c. No interactive effects are immediately apparent based on this figure. Note that despite the antiseptic techniques used throughout the experiments, a contaminant organism dominated at any concentrations of or above 111 µg PCB/L and 100 µM As, added singly or in combination. The organism was unicellular, clear, flagellated, and slightly longer and narrower than Chlamydomonas. The organism may have been present in the original cultures received from Ann Arbor, Michigan.

A statistical analysis of data from the combination experiment is given in Table 1. The hypothesis tested is independent joint action, that is a product model for the combined yield. It is evident that this hypothesis can not be disproved at any of the two levels, since the associated t-values are less than 4.30 for a p equal to 0.05. Consequently, PCB and As act independently in the above sense, and do therefore not form a particularly toxic combination. Since the actual concentrations in Green Bay of these contaiminants are less than or equal to about 10 µg As/L and 0.45 µg PCB/L it would therefore appear that current levels of PCB and As present little, if any, hazard to the algae in this area. However, several factors not considered here could conceivably change this situation. These factors include: differential species sensitivity to these toxicants, geographic differences in phytoplankton sensitivity to PCB (FISHER et al. 1973), differences in toxicity depending on levels of major nutrients, and possibly greater upconcentration of PCB and As in the algal cells under long-term continuous culture conditions.

TABLE 1

Statistical analysis of cell concentration (10^6 cells/L) of Chlamydomonas on day 25 in the PCB - arsenic combination experiment.

ACKNOWLEDGEMENTS

This research was supported by a grant from the University of Wisconsin - Milwaukee Graduate School. We thank James Bowers of the University of Michigan Great Lakes and Marine Waters Center at Ann Arbor for supplying the algae.

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TABLE 1

Statistical analysis of cell concentration (10^6 cells/L) of Chlamydomonas on day 25 in the PCB - arsenic combination experiment.

Level*	Test	Fortification	Replicate 1	Replicate 2	Mean	Normalized mean	Predicted normalized mean, product model	t*
	a	None	5830	5680	5760±110	1.00±0.02		
	b		2630	4080	3360±1030	1.00±0.31		
	c		4470	5060	4770±420	1.00±0.09		
1	a	PCB	6030	6920	6480±630	1.13±0.11		
	b	As	1720	2050	1890±230	0.56±0.18		
	c	PCB,As	3200	1680	2440±1080	0.51±0.23	0.63±0.21	-0.54
2	a	PCB	290	296	293±4	0.051±0.001		
	b	As	1720	2050	1890±230	0.56±0.18		
	c	PCB,As	291	600	466±218	0.094±0.047	0.029±0.0093	1.92

*PCB: 11 µg/L (1), 111 µg/L (2)
As: 10 µM

**t(2): 4.30, p = 0.05

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Distribution Differences between Polychlorinated Terphenyls and Polychlorinated Biphenyls in Human Tissues

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Polychlorinated terphenyls (PCTs) are compounds which require further studies to investigate their effects on human health and on the environment (U.S. ENVIRONMENTAL PROTECTION AGENCY 1978). PCTs are similar to polychlorinated biphenyls (PCBs) in their chemical characteristics and have been used industrially in the same way as PCBs, which are ubiquitous pollutants. PCT residues were identified in the eggs and fatty tissue of herring gulls by ZITKO *et al.*(1972). In Japan, PCT residues were subsequently reported in various samples, including human tissues (DOGUCHI 1977, MINAGAWA 1979). It was also reported that the PCT distributions in the environment and human body were not necessarily the same as those of PCBs. For instance, the levels of PCTs in human fat and blood were almost equal to those of PCBs, although the PCT residue levels in environmental samples such as water, fish and foodstuffs were much lower than those of PCBs.

The present study was performed in order to clarify the tissue distribution of PCTs in the human body. Several differences between the PCT and PCB distributions in human tissues were found.

EXPERIMENTAL

Materials. Seventeen samples of human adipose tissues and 6 liver samples were obtained through hospitals located in the Osaka area from 1975 to 1978. Human milk and blood from 24 nursing women were collected in 1975. Blood samples from 17 children, aged from 0 to 6 years, and from 16 of the mothers were collected in 1976. Thirty fetal samples (skin, liver, and fat) were obtained through Kyoto University in 1976.

Extraction and clean-up procedures. According to the standard analytical method for PCBs (Ministry of Health and Welfare, Japan, 1972), 0.5 g of adipose or milk fat, 10 g of whole blood, or 1 g of other tissues was refluxed in 20 ml of 2N ethanolic potassium hydroxide for on hour. Each saponified solution was poured into 40 ml of water, then extracted with 30 ml of n-hexane. The extract was washed twice with 2% (w/v) sodium chloride and dried over anhydrous sodium sulfate.

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Twenty-five ml of the extract was placed in a 10 mm x 20 cm glass column containing 5 g of Florisil, 60 - 100 mesh, non-activated. The elute was concentrated to a suitable volume and assayed for PCTs and PCBs by gas chromatography. Recovery rates of PCTs and PCBs by this procedure were better than 90 %.

Gas chromatographic analysis. The sample extract was injected into a Varian 2100 gas chromatograph equipped with an electron capture detector (^{63}Ni). The glass column (150 cm x 0.2 cm, packed with 2 % OV-1 on Gas Chrom Q, 100-120 mesh) was held at 245°. The injector and detector temperature were 255° and 300°, respectively. The nitrogen flow rate was 10 ml per minute. The PCT residues in the samples were quantified by comparison of the total peak height of the 11 major peaks given by Aroclor 5460 with those given by the samples.

The quantitation of PCBs followed the method of UGAWA *et al.* (1973), using a 2 % OV-1 column at 180°.

RESULTS AND DISCUSSION

Figure 1 shows the gas chromatograms obtained with Aroclor 5460 and hexane extracts of human tissue samples. It can be seen that there is a characteristic PCT profile. Though the early peaks (peaks 1 to 5) in the PCT patterns of the sample extract profiles differed slightly, the similarity between the profiles of the sample extracts and that of standard Aroclor 5460 is apparent.

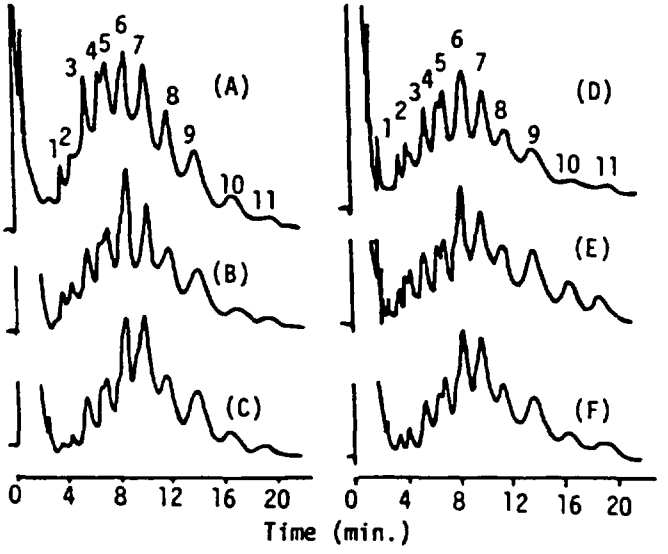

Fig.1. Gas chromatograms of PCTs in human tissues. (A) Aroclor 5460, (B) Adipose tissue (adult), (C) Liver (adult), (D) Milk (adult), (E) Blood (adult), and (F) Liver (fetus).

Adipose tissue and liver. The analytical data for the tissues of Japanese are given in Table 1. PCT residues were found in almost all samples. The average level of PCTs in adipose tissue was 0.89 ppm on a fat basis, which was about one-half that of PCBs. The results obtained in this study are similar to the data reported by FREUDENTHAL *et al.*(1973), MINAGAWA (1979) and DOGUCHI (1977).

The avrage level of PCTs in human liver was 0.05 ppm, with a range of 0.007 - 0.162 ppm on a whole liver basis. The ratio of PCT/PCB was again about one-half.

Human milk and blood. The average levels of PCTs and PCBs in milk were 0.001 and 0.023 ppm on a whole milk basis, respectively. The levels of PCTs were extremely low (4 or 5 % of the PCB content). The present results are consistent with the data reported by MINAGAWA (1979). The PCB content of body tissues is greatly influnced by their fat content. The PCB concentration

TABLE 1

PCT and PCB concentrations in the tissues of Japanese

Tissue sample	N[b]	PCTs		PCBs	
		Conc.[a]	Occurrence	Conc.[a]	Occurrence
Adult(over 20 years)					
Adipose	17	0.89 ± 1.3[c,d] (0.89 -39.3)	24/24	1.52 ± 0.47 (0.93 - 2.95)	17/17
Liver	6	0.05 ±0.058 (0.007 -0.162)	6/6	0.091 ±0.116 (0.025 -0.323)	6/6
Milk	24	0.001 ±0.001 (0.0001-0.003)	24/24	0.023 ±0.011 (0.006 -0.051)	24/24
Blood	24	0.003 ±0.003 (0.0005-0.025)	24/24	0.002 ±0.001 (0.0006-0.005)	24/24
Child(0 to 6 years)					
Blood	17	0.001 ±0.002 (ND -0.007)	6/17	0.004 ±0.004 (ND -0.013)	13/17
Mother's Blood	16	0.005 ±0.007 (0.001 -0.025)	16/16	0.003 ±0.001 (0.002 -0.005)	16/16
Fetus(4 to 9 months)					
Skin	12	0.007 ±0.014 (ND -0.050)	9/12	0.084 ±0.137 (0.004 -0.448)	12/12
Liver	14	0.009 ±0.011 (ND -0.033)	12/14	0.014 ±0.005 (0.005 -0.020	14/14
Fat	4	0.005 ±0.004 (ND -0.008)	3/4	0.006 ±0.003 (0.004 -0.009)	4/4

a) Mean±S.D. on a whole organ basis. Values in parentheses indicate the range.
b) N is the number of samples.
c) On a fat basis.
d) One sample, having 39.3 ppm PCTs, was rejected on the basis of Grubb's test.

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in milk fat was the same as that in adipose tissue (YAKUSHIJI *et al.* 1978). However, the PCT level in milk fat was about 0.03 ppm and was significantly lower than that in adipose tissue, *i.e.*, 0.89 ppm. The reason for this difference between the PCT and PCB tissue distributions in the human body is unclear.

Blood samples obtained from 24 nursing women were analyzed. The average level of PCTs in these blood samples was 0.003 ppm on a whole blood basis, and was slightly higher than that of PCBs, *i.e.*, 0.002 ppm. DOGUCHI (1977) reported a similar result. The average PCT/PCB ratio of blood was about 1.5, while the ratio in adipose tissue was about 0.5. Thus, it appears that PCT tends to accumulate preferentially in the blood. In animal experiments, SOSA-LUCERO *et al.* (1973) and MINAGAWA (1979) reported that PCTs tended to accumulate more in the liver and blood compared with PCBs. However, the reason for this is not clear.

Children's blood. The levels of PCTs and PCBs in the blood of children and their mothers are given in Table 1. The average PCB level in children's blood was 3.8 ppb on a whole blood basis, which was slightly higher than in the mother's blood, *i.e.*, 2.8 ppb. KUWABARA *et al.* (1979) reported that an increase in the feeding period of mother's milk tended to cause an increase in the PCB level in the blood of children. They suggested that most of the PCBs in the children had been transported via their mother's milk. On the other hand, the average PCT level in children's blood was very low, *i.e.*, 1.0 ppb, while that in mother's blood was 5.4 ppb. In addition, only six out of 17 samples of children's blood were contaminated by PCTs. This may be due to the low level of PCTs in milk mentioned above. It appears that the amount of PCTs transported into children via their mother's milk was less than in the case of PCBs.

Fetal organ. The placental transfer of PCBs was reported in humans by NISHIMURA *et al.* (1977). However, there is no information available on the placental transfer of PCTs. Table 1 shows the levels of PCTs and PCBs in fetal organs. The average levels of PCTs in fetal skin, liver, and fat were 0.007, 0.009, and 0.005 ppm on a whole organ basis, respectively. These PCT levels were much lower than those in adult tissue, suggesting that the extent of placental tranfer of PCTs is relatively small.

There was a difference between the PCT and PCB tissue distributions in the fetus. NISHIMURA *et al.* (1977) reported that the PCB concentration in fetal organ was highest in the skin, and suggested that this was due to adipose tissue of the skin. The same result was obtained in the present study. On the other hand, the PCT level of fetal skin was almost the same as

that of the other organs. This again indicates that the placetal transfer of PCTs is less than that of PCBs. This may be because PCTs have less affinity for fat than PCBs.

The range of PCT levels in tissues. Figure 2 showes histograms of the PCT and PCB levels in adult adipose tissue and blood. There was a significant difference between the PCT and PCB concentration ranges not only in these tissues, but in all the tissues. The levels of PCTs showed a much wider range than those of PCBs. This may possibly be a result of relatively restricted PCT pollution in the environment. In contrast, environ mental pollution by PCBs is widespread, partly because of the higher volatility and solubility of PCBs compared with PCTs.

Comment. Several tissue distribution differences between PCTs and PCBs in the human body are described in this paper. It has been observed that the toxicity of PCTs is similar to or rather less than that of PCBs (SOSA-LUCERO *et al.* 1973, ALLEN & NORBACK 1973, and CECIL *et al.* 1975), and the present results indicate that little placental transport and little transfer via mother's milk occur in the case of PCTs. In fact, none of the fetuses or children in this study showed any clinical symptoms of toxicity due to PCTs and PCBs. Therfore, the effect of PCTs on the human fetus and suckling baby may be negligible.

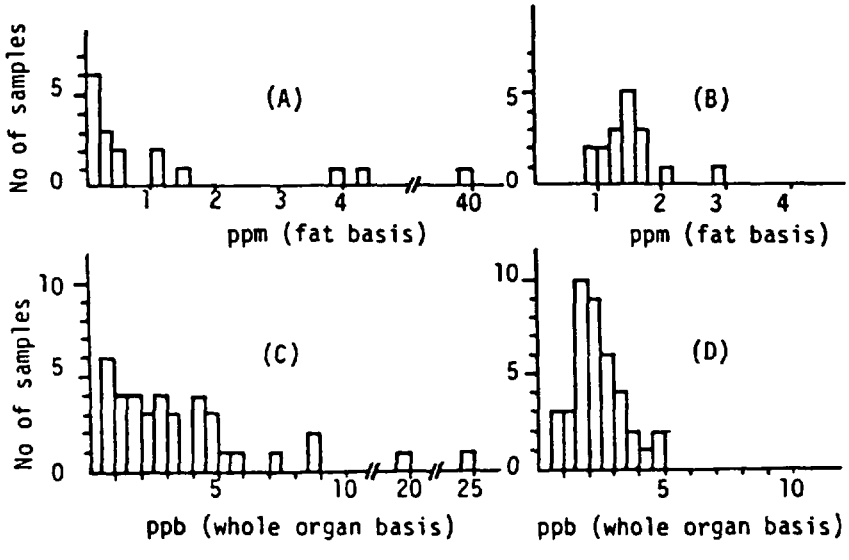

Fig. 2. Histograms of (A) PCT and (B) PCB concentrations in fat, and (C) PCT and (D) PCB concentrations in blood obtained from 24 nursing women and 16 mothers of children.

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We mentioned above that the levels of PCTs showed a much wider range than those of PCBs in case of human exposure, though a few subjects in this study showed abnormally high PCT level, *i.e.*, over than 10 ppm in fat or 10 ppb in whole blood. In cases of human PCB exposure, such high levels have seldom been observed except in occupationally exposed workers (WATANABE *et al.* 1978). According to HARA *et al.*(1974), symptoms related to PCB toxicity, especially skin lesion, have been observed in workers in a Japanese condenser factory, but these symptoms disappeared when the use of PCB ceased.

Unfortunately, there is no information available on the carcinogenicity and chronic effects of PCTs at low levels of exposure. It is not possible at present to evaluate the health hazard of PCTs to man at any level of exposure.

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Presence of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Wildlife Living near Seveso, Italy: A Preliminary Study

R. Fanelli, M. G. Castelli, G. P. Martelli, A. Noseda, and S. Garattini
Istituto di Ricerche Farmacologiche "Mario Negri" Via Eritrea, 62 — 20157 Milan, Italy

As a consequence of an explosion in a chemical plant near Seveso (Italy), a large inhabitated area was contaminated by a number of chemicals including the extremely toxic 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) (WHO 1977, GREIG et al. 1973). Specific reports on contamination of soil, vegetables and domestic animals have been already published (ABBRUZZI et al. 1978, GARATTINI et al. 1979, MARAZZA & PEZZA 1979) and a continuous monitoring plan is operating to keep the possible exposure of the people living in the polluted zones under control. However, no data on wildlife contamination have appeared in the literature so far. In this paper we present some preliminary findings on TCDD levels in wild animals captured in the contaminated areas. The main objectives of this study were:

- to gain indications about the range of TCDD levels in animals.
- to establish any correlations between environmental contamination and wildlife levels.
- to verify that available analytical methods were suitable for a monitoring program, in terms of sensitivity and specificity.

MATERIALS AND METHODS

All animals except hares were captured in zones where there was considerable environmental contamination. Hares were collected in various places inside and around the contaminated areas. So far the list of animal species analyzed includes the common field mouse (Microtus arvalis), toad, snake, earthworm and hare. All these animals were collected two years after the contaminating event.

Animal tissues were analyzed for TCDD levels after extraction and cleanup according to the method developed in our laboratory (FANELLI et al. 1979), by gas chromatography-low resolution mass fragmentography.

RESULTS AND DISCUSSION

TCDD levels found in animals captured in contaminated areas are reported in Table 1. All field mice were found positive for TCDD with whole body concentrations ranging from 0.070 to 49 ppb (mean value 4.5 ppb; median value 1.2 ppb). Most of

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these rodents were collected in a trap placed in an area (6000 m^2) where contamination of the top 7 cm of soil varied from 0.010 to 12 ppb (mean value of 23 determinations: 3.5 ppb). These data, although only preliminary, are in agreement with the conclusion of experiments carried out in a test area sprayed with 2,4,5-T herbicide (YOUNG et al. 1979), showing that rodent populations living on polluted soil accumulate TCDD in the body up to concentrations of the same order of magnitude as the soil itself, probably through pelt contamination (AIR FORCE ARMAMENT LABORATORY 1975) and subsequent ingestion of soil particles.

TCDD assays made in hares, toad, snake and earthworms are too few to permit any correlations. However the high TCDD levels found in hare livers are particularly interesting because these animals have a wide habitat radius and constitute one direct route by which humans can be exposed to the toxic substance.

From these preliminary data, it appears that high TCDD levels are accumulating in wildlife exposed to TCDD in the contaminated areas. This represents a unique opportunity to investigate the effects and behaviour of TCDD in wildlife. It should, furthermore, be borne in mind that TCDD could easily be spread to so far uncontaminated places by mobile animals which might have accumulated TCDD through the food chain.

TABLE 1

TCDD Levels in Wildlife

Animal	Number of analyzed samples	Tissue	Positive	TCDD level ng/g (ppb) average	min-max
Field Mouse	14	whole body	14/14	4.5	0.07- 49
Hare	5	liver	3/5	7.7	2.7 - 13
Toad	1	whole body	1/1	0.2	
Snake	1	liver	1/1	2.7	
		adipose tissue	-	16	
Earthworms	2^	whole body	1/2	12	

^Each sample represents a pool (5 g) of earthworms.

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Levels of Polychlorinated Biphenyls in Blood of Breast-fed Children Whose Mothers are Non-occupationally Exposed to PCBs

K. Kuwabara, T. Yakushiji, I. Watanabe, S. Yoshida, K. Koyama, and N. Kunita

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The environments is widely contaminated by polychlorinated biphenyls (PCBs). Because of their stable and lipophilic properties, they can accumulate in human tissues and breast milk (ACKER and SCHULTE 1970). There have been few reports on the transfer of PCBs from mother to child.

KODAMA and OTA (1977) demonstrated the presence of PCBs in the blood of breast-fed infants and reported that blood PCB levels of them rose gradually with ingestion of breast milk. We have previously reported similar findings for children whose mothers had been occupationally exposed to PCBs; we found extremely high levels of PCBs in the children's blood and some kinds of clinical signs (HARA *et al.* 1976, KUWABARA *et al.* 1978). In a further survey on these children, we discovered a baby with an unusually high blood PCB level of 115 ppb (μg/kg). The baby was 8-month-old and had been fed on mother's milk for approximately 6 months (KUWABARA *et al.* manuscript in preparation).

Here, we report on the blood PCB levels of children whose mothers are non-occupationally exposed, and the relationship between breast feeding and those levels.

MATERIALS AND METHODS

Blood (3 - 10 ml) was drawn from non-occupationally exposed mothers and their children in Osaka prefecture in 1976. The blood samples were analyzed by essentially the same methods as described before (KUWABARA *et al.* 1978). Recovery rate of PCBs by this procedure was more than 90 %.

RESULTS AND DISCUSSION

PCB level in the blood of mother and child: The results are listed in Table 1. The PCB levels in 9 of the 17 children are higher than those of their mothers. The maximum level is 12.8 ppb and is 6 times higher than that of the child's mother. Four samples were below the 0.2 ppb detection limit. Compared to the levels found for mothers, the levels for children have a wider variation.

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TABLE 1

PCB Levels in Blood of Mother and Child

Child				Mother	
No.	Sex	Age year, month	PCB level in blood (ppb)	No.	PCB level in blood (ppb)
C -1	M	4,7	3.3	} M -1	2.0
C -2	M	1,2	9.1		
C -3	M	0,7	ND	M -3	2.2
C -4	F	0,4	ND	M -4	3.0
C -5	M	0,4	0.8	M -5	2.4
C -6	F	0,3	ND	M -6	1.7
C -7	F	0,3	ND	M -7	3.2
C -8	M	1,8	7.3	M -8	2.9
C -9	M	6,2	4.0	M -9	2.4
C-10	F	2,5	12.8	M-10	2.1
C-11	M	3,3	2.8	M-11	2.5
C-12	M	0,1	2.8	M-12	2.9
C-13	F	3,9	8.2	M-13	2.6
C-14	F	0,7	3.4	M-14	4.2
C-15	F	1,4	3.4	M-15	2.8
C-16	F	2,1	3.6	M-16	4.6
C-17	M	4,3	3.5	M-17	3.1
Mean ± S.D.			3.8 ± 3.6 ppb		2.8 ± 0.8 ppb

M designates male and F designates female.
ND: value below the detection limit, 0.2 ppb.
Mean and S.D. of child are evaluated assuming that ND is 0.1 ppb.
Sample No. C-1 and C-2 are brotherly (No. M-1's children).

Fig.1 shows the relation between breast feeding and blood PCB level of the children. It appears that increasing feeding period of the mother's milk tends to increase the level of PCBs in blood of the children. It is especially noteworthy that the levels of 1-month-old and 7-month-old infants fed on mother's milk are much higher than babies of approximately the same age who ingest only artificial milk.

The average PCB concentration in modified milk powder for infants was 1 ppb (Osaka Prefectural Government, 1976) or 3 ppb (Science and Technology Agency, Japan, 1972). We found that the PCB daily intake by meal of non-occupationally exposed adults was about 3 or 20 μg per person (WATANABE *et al.*

manuscript in preparation), and the average for human milk PCB level was 33 ppb (YAKUSHIJI *et al.* 1977). Compared to the value of artificial milk or diet, the amount of PCBs which infants receive is considerable. Assuming that a baby ingests 1 kg of the breast milk contaminated by 30 ppb PCB, the baby would receive 30 μg of PCBs each day.

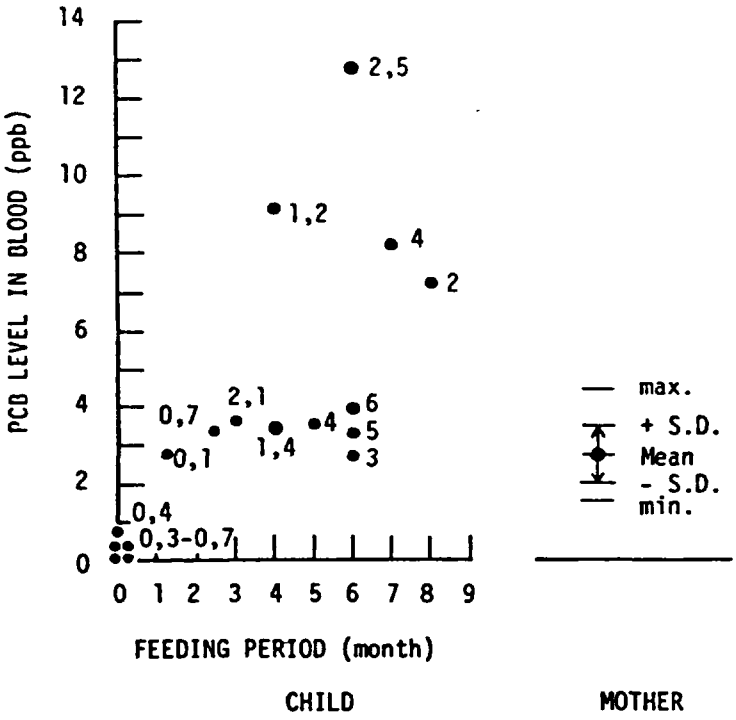

Fig.1. Relation between PCB level in blood of the children and the feeding period of mother's milk. Each number designates the age of a child (years, months). The average level in blood of mothers is also shown.

PCB pattern in the blood of mother and child: In the previous study on the child and mother occupationally exposed to PCBs, we found that the gas chromatographic patterns of blood PCB of the children fed mother's milk for a long time were quite different from those of their mothers. The pattern change of this type is remarkably observed in the blood PCBs of "Yusho" patients who suffered from PCB poisoning in Japan in 1968 (MASUDA *et al.* 1974). These patterns have been discussed in detail in a previous report (KUWABARA *et al.* 1978).

In the current study, there are no clear cases of change in blood PCB patterns between mothers and children as shown in Fig.2. However, regarding this point, more examinations will be performed.

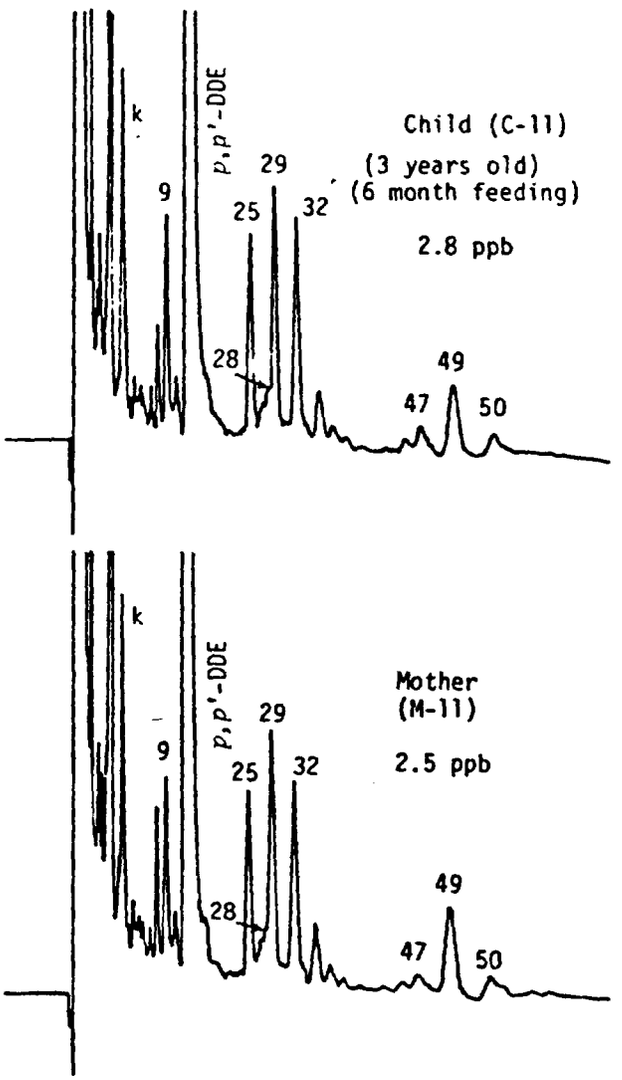

Fig.2. Gas chromatograms of PCBs in blood from a mother and her child on a 2 % Apiezon L column employing an electron capture detector. Peak No. k and 9 are identified as tri- and tetrachlorobiphenyls (YAKUSHIJI *et al.* 1978), and from No. 25 to 50 are identified as penta- to heptachlorobiphenyls, respectively (JENSEN and SUNDSTRÖM 1974, NAKAMURA and KASHIMOTO 1977).

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Presence of Polychlorinated Biphenyl and Organochlorine Pesticide Residues and the Absence of Polychlorinated Terphenyls in Canadian Human Milk Samples

Jos Mes and David J. Davies

Department of National Health and Welfare, Health Protection Branch, Food Directorate, Tunney's Pasture, Ottawa, Canada

Polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides in human milk have been reported both in Canada (RITCEY 1972, MUSIAL 1974) and elsewhere (SIYALI 1973, SAVAGE 1973, GRACA 1974, PESENDORFER 1975). Concurrently, Japanese reports indicated the presence of polychlorinated terphenyls (PCTs) in human tissues (DOGUCHI 1973) and milk (NISHIMOTO 1973). This paper reports on a nation-wide survey of PCBs, PCTs and OC pesticides in the milk of Canadian mothers as part of a continuing program to follow trends in the levels of pesticides and industrial contaminants.

MATERIALS AND METHODS

Sampling

Samples were collected as previously described (MES et al. 1978). Since manual expression of the milk could not be guaranteed in all cases, instructions were to immediately transfer the milk from the breastpump to the residue free bottle, to avoid prolonged contact with any plastic or rubber parts.
Out of a total of 100 samples, 10 came from the Eastern region (Newfoundland, Prince Edward Island, Nova Scotia, New Brunswick), 25 from Québec, 35 from Ontario, 10 from the Central (Manitoba, Saskatchewan) and 20 from the Western (Alberta, British Columbia) regions.

Analytical Methods

All solvents were glass-distilled and free of interfering residues. Adsorbents were prepared as earlier reported (MES et al. in press). Oxychlordane and α-hexachlorocyclohexane (HCH) were 98% pure; Aroclor 1260 was of a technical grade and all other standards were 99% pure.
Extraction and cleanup. Fifty gram samples were extracted and their fat content determined according to MES et al. (in press). The fat was removed from the extract by low temperature precipitation (MCLEOD and WALES 1972).

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Separation. The fat free extract was chromatographed on a semi-micro silicic acid column (MES et al. 1976), which was extended with 4.5 g Florisil and topped with some annhydrous Na_2SO_4. The following 3 fractions were collected:

Fraction	Eluting solvent	Eluted compounds
I	35 ml hexane	PCB, HCB, some PCT, and p,p'-DDE
II	40 ml of 20% CH_2Cl_2 in hexane	PCT, p,p'-DDE, DDTs and chlordanes
III	40 ml of 60% CH_2Cl_2 in hexane	β-HCH, dieldrin, heptachlor epoxide

The fractions were carefully evaporated on a glass rotatory evaporator (< 30°C), the residues transferred in hexane to 15-ml centrifuge tubes and the volume adjusted to 1 ml.

Identification and quantification. Gas chromatography (GC) and quantification of PCBs and most OC pesticides was carried out on a 6% OV-210 + 4% SE-30 column (MES and CAMPBELL 1976). The β- and γ-HCH isomers were quantitated on a 5% OV-210 column (MES et al. 1977). Fractions I and II were also chromatographed on a Varian 1400 with Scandium Tritide electron capture detector for the determination of PCTs in every 5th sample, under the following conditions:

Column: 0.3 x 183 cm glass, packed with 3% OV-210 + 3% SE-30 on 60/80 Chromosorb W(AW) (0.3 g of each phase on 10 g solid support).

Temperatures: Injection: 255°C; Column: 264°C; Detector 268°C.

Aroclor 5460 (2.5×10^{-3} µg/5 µl injection) was completely eluted after 50 min at a flow rate of 45 ml N_2/min.

Confirmation of PCB. The fractions containing PCB (I) of every other 10 samples, exclusive of every 10th, were pooled to give ~ 4.5 µg of estimated PCB/pooled sample.

Thin layer chromatography (TLC) was carried out on precoated, aluminum oxide (type E) F_{254}, 20 x 20 cm plates (Brinkman Ltd., Canada), activated at 110°C for 1 hr. Half of the pooled sample was applied per spot. Reference spots of Aroclor 1260, p,p'-DDE and HCB at 5, 2.5 and 2.5 µg/spot respectively were applied at each end of the line of origin, together with a blank spot. The plates were developed in 1% acetone in hexane (v/v) and the reference spots visualized with $AgNO_3$ (MCLEOD and RITCEY 1973). Adsorbent from the areas of the sample and blank, corresponding to those of the respective standards, were scraped, eluted with 60% CH_2Cl_2 in

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hexane and gas chromatographed as above.
The remaining fractions I, which were not pooled, were re-chromatographed on a 5% OV-210 column to confirm the identity of HCB, p,p'-DDE and PCB and then perchlorinated according to ARMOUR (1973) and the decachlorobiphenyl chromatographed on a 0.6 x 60 cm glass column packed with 4% SE-30 on 60/80 Chromosorb W(AW). The PCB fractions from TLC were analysed by mass spectrometry (MS) using a Varian Mat 311A coupled to a Varian 1400 Series gas chromatograph by a two-stage Watson-Biemann Separator and monitored for a single ion at m/e = 359.8.

Confirmation of OC pesticides. Components in fractions II and III were confirmed on 5% OV-210 (MES et al. 1976). Every other 10 samples were pooled and chromatographed (1-30 µg of estimated pesticide/spot) on TLC plates with appropriate standards (5-10 µg of individual pesticide/spot), eluted and re-chromatographed as above on two different GC columns.
Single ion monitoring MS was carried out on HCB (m/e = 283.8), β-HCH (m/e = 218.9), oxychlordane (m/e = 386.8), trans-nonachlor (m/e = 408.8), heptachlor epoxide (m/e = 272.0), dieldrin (m/e = 380.0), p,p'-DDE (m/e = 317.9), and p,p'-DDT (m/e = 235.0).

Controls. Every 20th sample was repeated by fortifying it at the 0.1 ppm level with every compound analysed for, except γ-HCH and trans-nonachlor. At different times during the survey blanks were run through the complete analytical procedure.

RESULTS AND DISCUSSION

The data in Table 1 show the average residue levels in the milk of Canadian mothers, as collected in 1975.

TABLE 1

Organochlorine residues in Canadian human milk

Compound	ng/g whole milk		% of samples with residues ≥ 1 ng/g whole milk
	Average[a]	Max. observed	
PCB, as Aroclor 1260	12	68	98
HCB	2	21	81
β-HCH	2	21	91
Heptachlor epoxide	1	3	69
Oxychlordane	1	2	77
trans-Nonachlor	1	2	68
p,p'-DDE	35	144	100
Dieldrin	2	6	84
o,p'-DDT	3	48	32
p,p'-DDT	6	21	100

[a]Averages are based on the number of samples with residues ≥ 1 ng/g whole milk.

Polychlorinated biphenyls, hexachlorobenzene (HCB), ß-HCH, p,p'-DDE, dieldrin and p,p'-DDT were present in more than 80% of the samples. All samples contained p,p'-DDE and p,p'-DDT. All samples, which did not register residues of oxychlordane and trans-nonachlor ≥ 1 ppb, did indicate what appeared to be trace (< 1 ppb) amounts of these two compounds.
Compared to previous surveys on Canadian human milk by RITCEY et al. (1972) and unpublished data from this laboratory, heptachlor epoxide, p,p'-DDE, dieldrin, o,p'-DDT and p,p'-DDT levels all decreased over an eight year period.

TABLE 2

Trends of organochlorine residues in Canadian human milk

	Average ng/g whole milk		
	Year of sample collection		
Compound	1967	1970[a]	1975
PCB, as Aroclor 1260		6	12
HCB			2
ß-HCH			2
γ-HCH	3	2	
Heptachlor epoxide	3	4	1
Oxychlordane			1
trans-Nonachlor			1
p,p'-DDE	103	56	35
Dieldrin	5	5	2
o,p'-DDT	5	3	3
p,p'-TDE	4	3	
p,p'-DDT	33	15	6

[a] Unpublished data

At the same time other compounds have been detected such as HCB, ß-HCH, oxychlordane and trans-nonachlor. Lindane (γ-HCH) was only found in 8 samples at levels ≥ 1 ppb and all in Ontario. Nevertheless 71% of the samples apparently still contained traces of lindane, which could not technically be confirmed by MS.
Whether or not the PCB increase between 1970 and 1975 was significant will be difficult to determine with the limited data. It was observed, however, that perchlorination accounted for only 38% of the PCBs as calculated on the basis of GC results. A duplicate sample fortified before the perchlorination step with Aroclor 1260 at 0.2 ppm gave 75.7% recovery with a standard deviation of ±0.5. A single perchlorination of 10 µg Aroclor 1260 gave 110% recovery. If this apparent discrepancy is indeed valid, the average 1975 PCB level

could well be lower than the 1970 one. Consequently, at the same time, this could indicate the presence of unknown contaminants in the PCB fraction of Canadian human milk. The authors earlier described (MES et al. in press) the unusual ratios of two PCB peaks in 14% of the milk samples, which could not be attributed to Mirex.

Table 3 shows the geographic distribution of the residues in the latest survey. The highest average PCB, p,p'-DDE and p,p'-DDT levels were found in Ontario

TABLE 3

Regional distribution of organochlorine residues in Canadian human milk

Compound	Regional averages[a] in ng/g whole milk				
	Eastern	Quebec	Ontario	Central	Western
PCB, as Aroclor 1260	8	10	17	8	15
HCB	1	1	2	1	4
ß-HCH	1	1	3	2	2
Heptachlor epoxide	1	1	1	1	2
Oxychlordane	1	1	1	1	1
trans-Nonachlor	1	1	1	1	1
p,p'-DDE	29	34	34	21	59
Dieldrin	2	1	2	1	2
o,p'-DDT	3	1	6	-[b]	1
p,p'-DDT	5	7	6	5	8

[a]Averages based on the number of samples with residues ≥ 1 ng/g whole milk.

[b]None detected.

and the Western region. The Central region had the lowest residue levels. The latter was also observed in data from human adipose tissue (MES et al. 1977).

Figure 1 shows two unknown peaks in fraction III, chromatographed on two different columns. It is plausible that peaks x and y in chromatogram A represents the same compounds as peaks A and B in chromatogram B, but further investigation is needed. These two unknown peaks appear in most samples and only differ in their magnitudes.

All reported compounds were confirmed by MS. Recoveries from fortified samples ranged from 84-98% for all pesticides, except HCB and PCB, which were recovered at 78 and 70%, respectively. The low PCB recovery will be further investigated, since preliminary work had indicated >95% recovery from Florisil-silicic acid column. The data in Table 1 were not corrected for these low recoveries.

The average lipid content of the human milk samples was 2.20% with a standard deviation of ± 1.35.

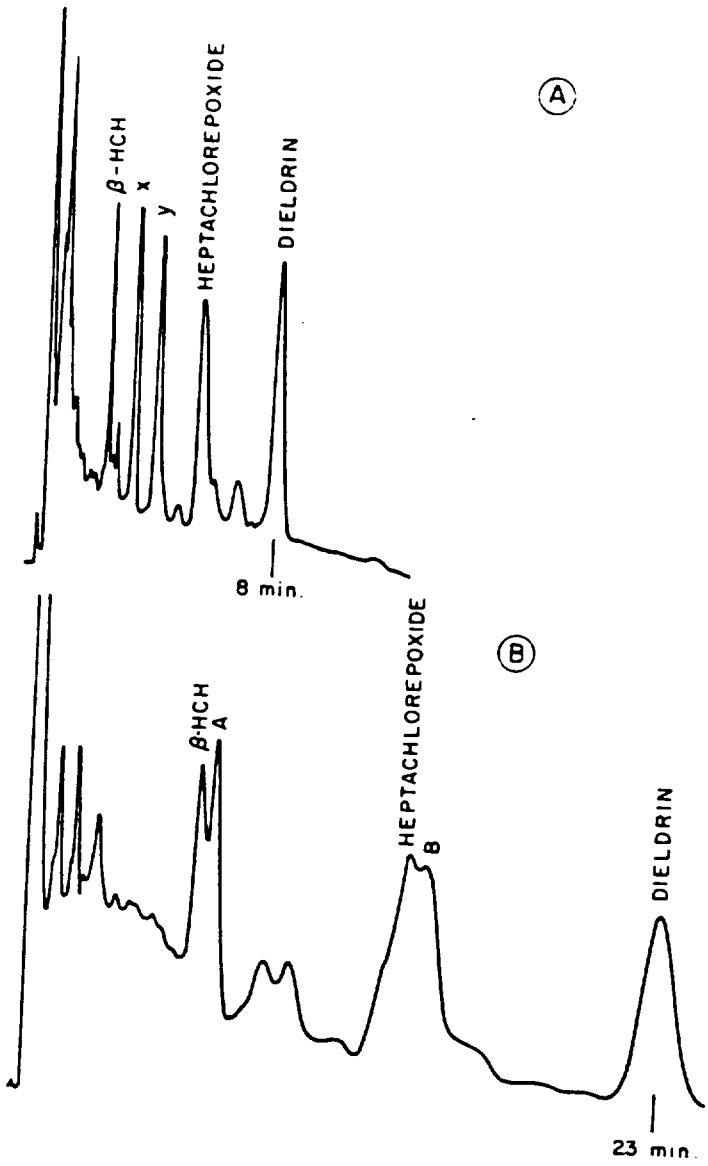

FIG. 1 Chromatogram A represents a GC elution pattern of a particular fraction III on 6% OV-210 + 4% SE-30. Chromatogram B represents the same fraction but on 5% OV-210.

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Traces of Mirex in Some Canadian Human Milk Samples

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Department of National Health and Welfare, Health Protection Branch, Food Directorate, Tunney's Pasture, Ottawa, Canada

Introduction

Mirex (1,2,3,4,5,5,6,7,8,9,10,10-dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta (c,d) pentalene) has mainly been associated with the control of the Fire ant in the Southeastern U.S.A. (ALLEY 1973), where it was subsequently found in non-target organisms (COLLINS et al. 1973, WOJCIK et al. 1975) including man (ANONYMOUS 1976). Recently Mirex has been found in Canada in fish from Lake Ontario (KAISER 1974) and in eggs of double crested cormorants (ZITKO 1976). This paper reports on a limited study of Mirex in human milk samples from across Canada, using U.V. irradiation to limit interference from polychlorinated biphenyls (PCBs).

Sampling

Fourteen human milk samples were selected from samples acquired during a recent human milk survey of polychlorinated biphenyls. Five samples came from Ontario, 2 from the Eastern (New Brunswick, Nova Scotia), 3 from the Central (Manitoba, Saskatchewan) and 4 from the Western (Alberta, British Columbia) region.
The selection was based on observed Aroclor 1260 patterns, where a proportionally large peak 13 (REYNOLDS, 1969) could indicate the presence of Mirex under the gas chromatographic (GC) conditions used. The samples were collected, during the first 4 to 5 days of lactation, in previously washed, heated (350°C) and hexane rinsed bottles with aluminum lined caps. Sample weights ranged from 18.5 to 114.0g.

Analytical Methods

Glass-distilled, residue free solvents were used. Aroclor 1260 and Mirex (99.9%, pure) were obtained from Monsanto Chemical Co. and EPA-Research Triangle Park N.C. respectively. Silicic acid (Mallinckrodt 2847), Florisil, anhydrous Na_2SO_4 and Celite (545,

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AW) were soxhlet extracted with CH_2Cl_2 until free of interfering residues, and heated at 130°, 300°, 90° and 80°C respectively. Silicic acid was deactivated with 4.4 % and Florisil with 2% H_2O.

Extraction and cleanup. The total milk sample was weighed and centrifuged at 1200 rpm for 20 min in a Squibb separatory funnel. The fat layer was saved and extracted with 100 ml of 5% benzene in acetone (v/v) for 2 min. Any precipitate was removed by a Buchner funnel and the acetone evaporated on a glass rotatory evaporator (<30°C). The wet residue was redissolved in hexane and filtered through ~ 2.5 cm of anhydrous Na_2SO_4. The hexane was evaporated as above and the residue redissolved in acetone for precipitation of the fat at - 78°C (MCLEOD et al. 1972).

Separation. A slurry of 4.5 g silicic acid and 1 g Celite in hexane was poured into a glass column (12 mm O.D. x 40 cm) with a 50 ml reservoir and teflon stopcock and packed under N_2 pressure. Excess hexane was eluted until a few mm from the top of the adsorbent. Dry Florisil (4.5 g) was added to fill the remaining column space. The fat free residue was applied in hexane to the Florisil-silicic acid column. Mirex and PCBs were collected under pressure in 35 ml of hexane at a flow rate of 2-2.5 ml/min.

Mass spectrometry (MS). A Varian Mat 311A coupled to a Varian 1440 GC by a Watson-Biemann separator was used.

The hexane fraction from the column was evaporated under N_2 and redissolved in 100 µl CH_2Cl_2. Single ion monitoring was carried out with 5 µl injections of sample and standard (2 ng/µl) at m/e = 545.6 and m/e = 271.8. Mirex was estimated using the molecular ion peak (m/e = 545.6).

U.V. irradiation. Standard solutions were prepared in 5% diethylamine (DEA) in hexane (Table 1). Two

TABLE 1

Standard solution	Concentration in ug/ul Arochlor 1260	Mirex	Ratio PCB/Mirex
A		0.96×10^{-4}	
B	9.4×10^{-4}		
C	9.4×10^{-4}	0.96×10^{-4}	10
D	9.4×10^{-4}	0.19×10^{-4}	50
E	3.1×10^{-3}	0.96×10^{-4}	32
F	6.3×10^{-3}	0.96×10^{-4}	66

ml of solutions A and B were irradiated according to LEWIS et al. (1976) in a quartz cuvette with cork stopper at 8 cm distance from the U.V. light source (General Electric germicidal lamp 615T8, 15W).

Recoveries were checked at 20, 25, 30, 45 and 60 min. A 2 ml aliquot of solutions C to F was irradiated for 30 min to determine Mirex recovery.
The hexane fraction remaining after MS analysis was concentrated, transferred to a quartz cuvette, evaporated and redissolved in 1 ml of 5% DEA in hexane. Samples were irradiated for 30 min.
Gas chromatography. After irradiation 10 µl out of 250 µl were injected into a Varian 1400 with Scandium Tritide electron capture detector, under the following conditions:
Column: 0.6 x 183 cm, glass, packed with 3% OV-210 + 3% SE-30 on chromosorb W(AW) 60/80 (0.3 g of each phase on 10 g solid support).
Temperatures: Injection : 222°C; Column : 183°C; Detector : 262°C. Under these conditions the absolute retention time of Mirex was approximately 60 min.
Ten microliter injections of solutions A and B were made before and after every two sample injections. Peak heights were used for quantification.
Controls. Two human milk samples, spiked with 0.1 and 0.01 ppm Mirex, and one blank were subjected to the entire analytical procedure including the U.V. irradiation.

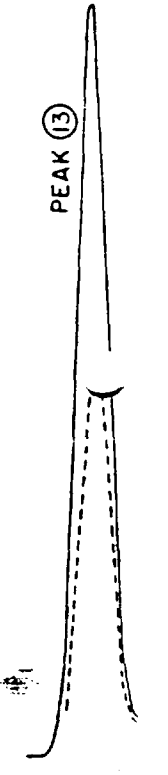
Results and Discussion

The calculated peak height ratios of peaks 13/8 in aroclor 1260 in human milk samples were 0.56 and 0.40 for a 3% OV-210 + 3% SE-30 and 5% OV-210 GC column respectively. Standard aroclor 1260 gave ratios of 1.03 and 0.65 for the same columns. The ratios of most of the samples used in this investigation were considerably > 1.00 (Fig. 1). The PCBs of these samples were chromatographed under conditions where Mirex and peak 13 would give identical retention times. The unusual ratios could have been caused by the presence of Mirex.
The length of time Mirex was irradiated did not seem to affect its recovery (Table 2), while the overall effect on PCB recovery was not considered large enough to irradiate more than 30 min.

TABLE 2

Effect of duration of U.V. irradiation on Mirex and PCB recoveries

Time (min)	% Mirex recovered	% Peak 13 of aroclor 1260 recovered
20	90	12
25	87	8
30	86	10
45	91	7
60	90	5



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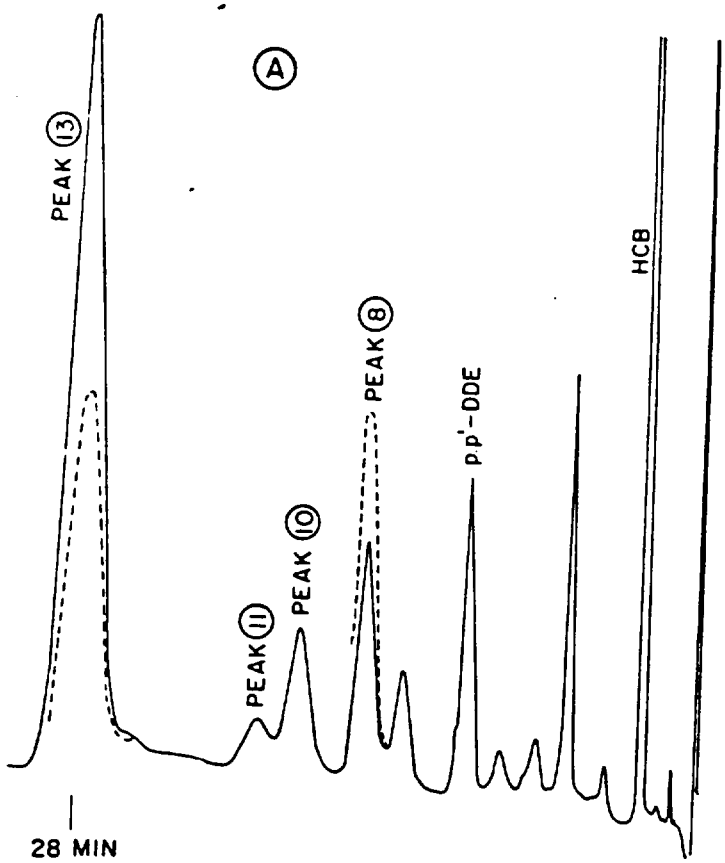

Fig. 1. Chromatogram A represents a typical PCB elution pattern of human milk used in this investigation. The broken line indicates relative response of peak 8 and 13 in standard aroclor 1260 under the same conditions.

In mixtures of Mirex and PCB, varying the mirex content did not seem to affect recovery of the latter (Table 3).

TABLE 3

The effect of varying the PCB/Mirex ratio during irradiation on Mirex recovery

Ratio PCB/Mirex	Quantity kept constant	% Mirex recovered[a]
10	PCB	90.9 ± 7.4
50	PCB	92.7 ± 5.0
32	Mirex	78.3 ± 1.5
66	Mirex	82.6 ± 3.7

[a] 3 determinations.

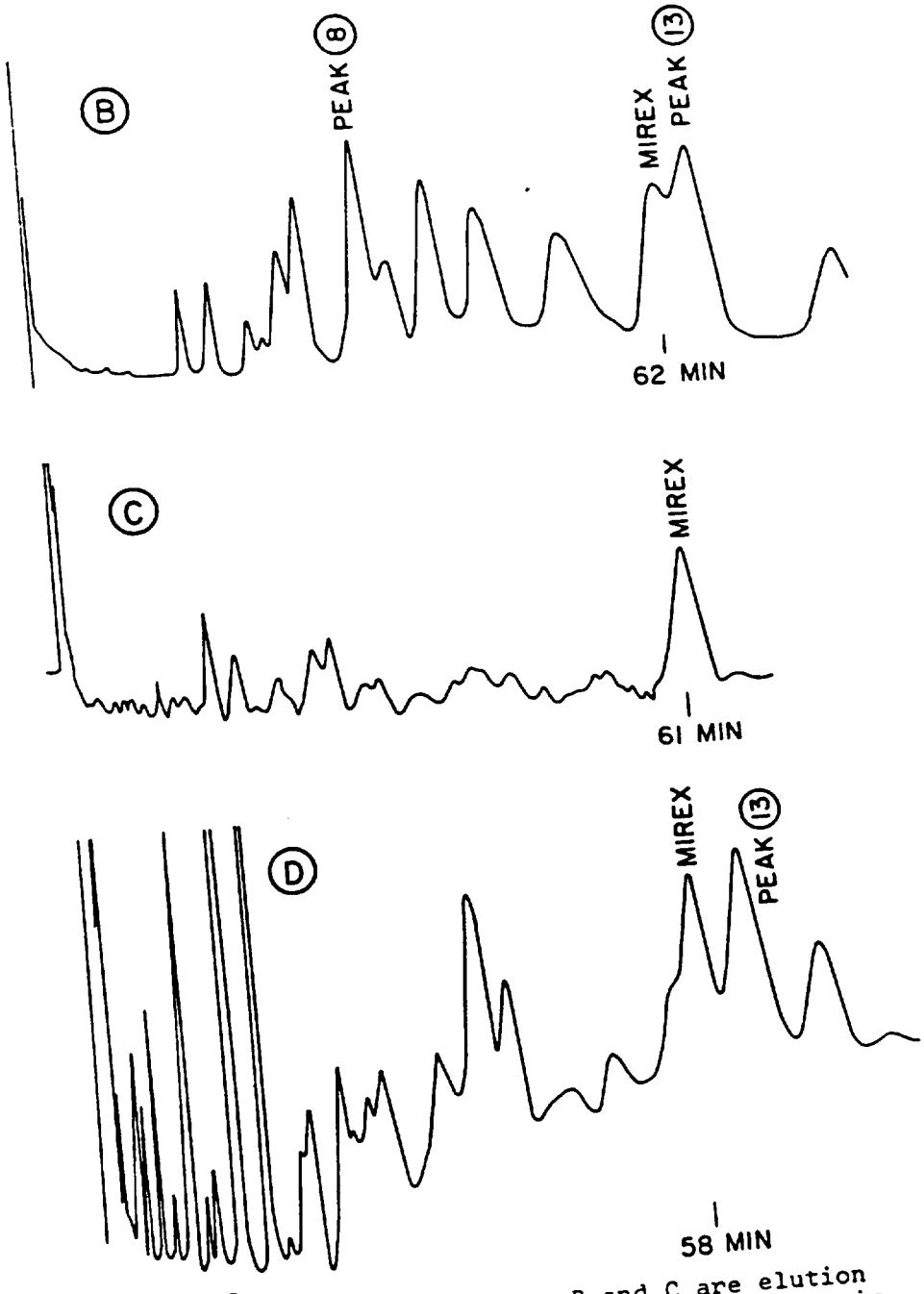

Fig. 2. The gas chromatograms B and C are elution patterns of standard solution C before and after irradition. Chromatogram D represents an irradiated human milk sample.

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Increasing the PCB content reduced Mirex recovery by approximately 10%. Examples of before and after U.V. irradiation are given in Fig. 2, chromatograms B and C.

The recovery of Mirex from spiked samples at the 0.1 and 0.01 ppm level were 88 and 77% respectively. The blank was free of interfering residues.

Mirex was found in 3 out of the 14 samples analysed (Fig. 3). Although Mirex can be identified by GC,

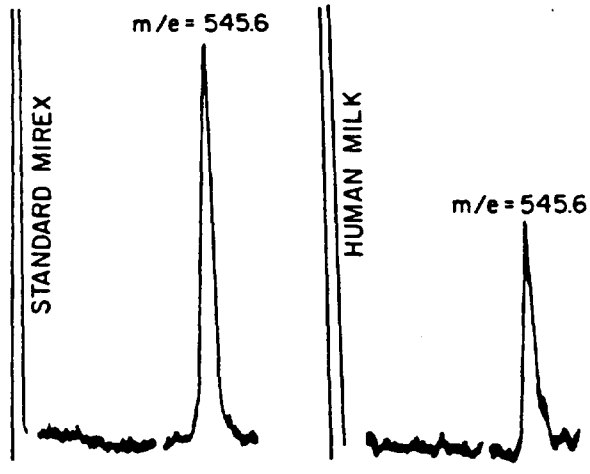

Fig. 3. Mass spectrogram of Mirex in human milk at $M/\Delta M = 2000$.

using irradiation to limit PCB interference and its presence confirmed by MS, quantitative results at these low levels obtained by GC remain questionable as indicated by the poor agreement between MS and GC data. The data in Table 4 are more an indication of the presence of Mirex than their absolute levels.

TABLE 4

Mirex in human milk samples

Sample	ng/g			
	MS		GC	
No.	wet weight	fat	wet weight	fat
1	0.6	15.3	0.4	9.3
2	0.3	21.5	0.2	11.1
3	0.2	6.4	0.1	2.3

All positive samples came from cities on Lake Ontario and Erie, where the Mirex problem was first identified (KAISER, 1974).
Chromatogram D in Fig 2 shows a human milk sample containing Mirex. At the <1 ppb level interference is still considerable.
The small amounts of Mirex do not account for the large ratio differences found in the human milk samples selected for this study compared to others previously analysed for PCB. This still remains an interesting question.

Acknowledgement

The authors wish to thank Drs. T. Panalaks and J. Lawrence for reviewing this paper.

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Effect of Polychlorinated Biphenyls on the Elimination Rate of Antipyrine from Plasma of Rats and Man

V. Krampl, and M. Kontseková

Institute of Hygiene & Occupational Medicine, Bratislava, Czechoslovakia

The ability of polychlorinated biphenyls /PCBs/ to induce the activity of hepatic microsomal enzymes that catalyze the metabolism of drugs and other xenobiotics has been well documented /VILLANEUVE et al.1971, BENTHE et al.1972, BICKERS et al.1972, LITTEREST et al.1972, CHEN and DU BOIS 1973, JOHNSTONE et al.1974, SCHMOLDT et al.1974, ECOBICHON and COMEAU 1974, GOLDSTEIN et al.1975, SANDERS and KIRKPATRICK 1975/. This phenomenon has been paralleled by an enlargment of the liver and proliferation of the endoplasmatic reticulum /NISHIZUMI 1970, ALLEN et al. 1973, BRUCKNER et al.1974, ALLEN 1975, KASZA et al. 1976/.

While these studies have provided information on the ability of PCBs to cause enzyme induction in various animals no data are available on the effect of exposure to PCBs on enzyme induction in man. Therefore the main objective of our investigation was to study whether occupational exposure to PCBs changes the half-life of antipyrine in plasma of workers in production of these important industrial chemicals. It has been shown /KOLMODIN et al.1968, VESELL and PAGE 1968/ that the measurement of plasma antipyrine half-life is a good indicator for assessing the activity of liver microsomal enzymes.

Present paper reports that a single dose as well as the short-term administration of Delor 103, a polychlorinated biphenyl, increased markedly the elimination of antipyrine from plasma of rats and that plasma half-life of antipyrine in workers occupationally exposed to a commercial mixture of PCBs was significantly shorter than in control subjects.

Materials and Methods

A n i m a l s. Male Wistar rats, 140 - 155 g

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maintained on Larsen diet were used in these experiments and were fasted overnight prior to killing.

There were two experimental groups. A group for subacute treatment received 5 mg/kg body weight of polychlorinated biphenyl Delor 103 /a commercial product that contains 42 % chlorine by weight/ daily for a period of 5 weeks. Rats in the so called acute group obtained 100 mg/kg body weight of Delor 103 in a single dose. Doses were administered in pure vegetable oil by means of oral tube. Control animals received only oil.

Antipyrine was administered orally, as an aqueous solution, in a single dose of 50 mg/kg body weight 72 hours after the last treatment.

S u b j e c t s. Three groups of subjects were investigated. A group of 26 workers-men, ranging in age from 18 to 54 years, who have been exposed to a mixture of commercial products PCBs /Delor 103, Delor 104 and Delor 106/ in the production these compounds for a period from 2 months to 19 years.

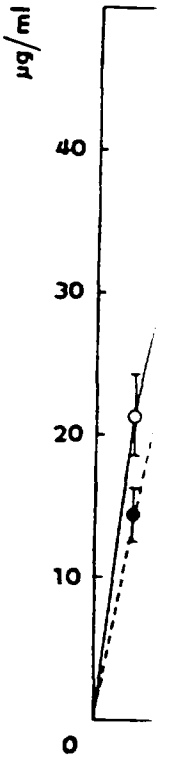
A control group of 18 not exposed men from the same factory, mainly research workers and office personnel in the age from 19 to 50 years. They have worked in the factory for a period 1 to 22 years. All subjects have been from the same geographical area.

A control group of 27 not exposed student volunteers from different geographical areas. All the volunteers were men between 20 and 27 years of age. No subject investigated received medication during or for one month preceding the investigation.

M e t h o d s. Antipyrine was given orally in a single dose of 1 g. Venous blood was collected in heparinized tube 3,6,9 and 12 hours after ingestion. Plasma concentrations of antipyrine were assayed by precipitation procedure according to method of BRODIE et al./1949/. The half-life of antipyrine in each subject was determined from the linear portion of a plot of the plasma values on semilogarithm paper.

Results

Animal model was used to find out whether PCBs alter the rate of disappearence of antipyrine from plasma. The elimination rate of antipyrine from plasma of rats treated with Delor 103 was significantly increased. The plasma concentrations of antipyrine in rats treated with Delor 103 in both acute and subacute groups were significantly lower /$P \leq 0.05 - 0.001$/ than those in controls at each time interval with the



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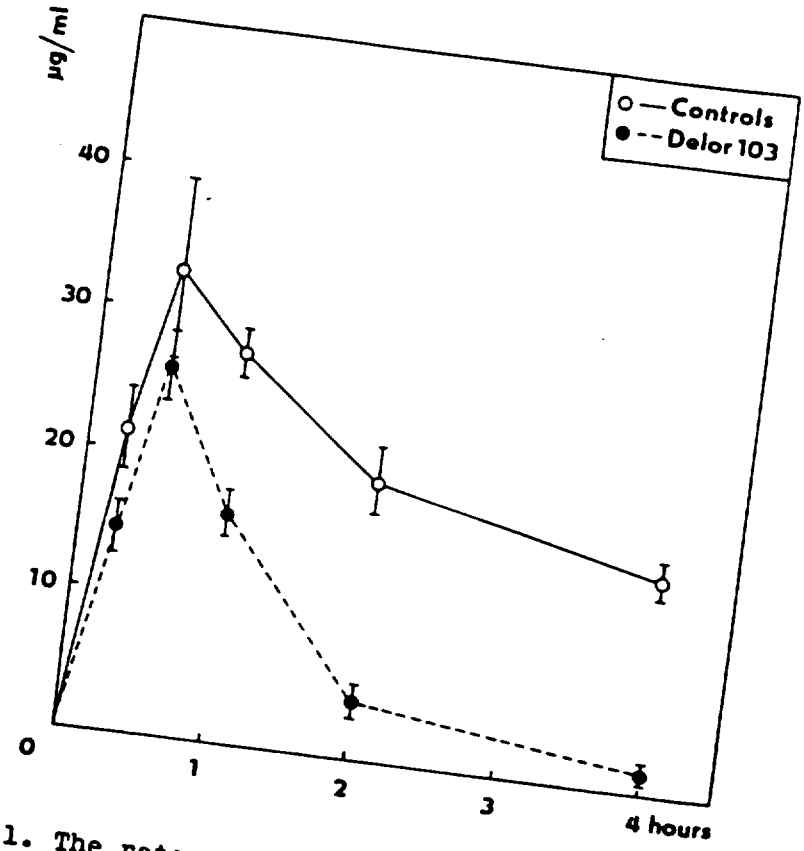


Figure 1. The rate of elimination of antipyrine from plasma of rats treated with a single dose /100 mg/kg B.W./ of Delor 103 and in control group. Antipyrine was administered orally 72 hours after treatment. Each point represents the mean value and S.D. of 6 animals.

exception of concentration at half an hour after ingestion of antipyrine in rats of subacute group. This concentration was not significantly higher than that in control rats. See Figures 1 and 2.

The plasma half-lives of antipyrine in workers occupationally exposed to PCBs and nonexposed control subjects are depicted in Figure 3.

For the exposed group the mean of antipyrine half-life was 10.2 hours, with a standard deviation of 4.1, and a range of 4.7 to 19.8 hours. In the nonexposed group of subjects from the same factory,

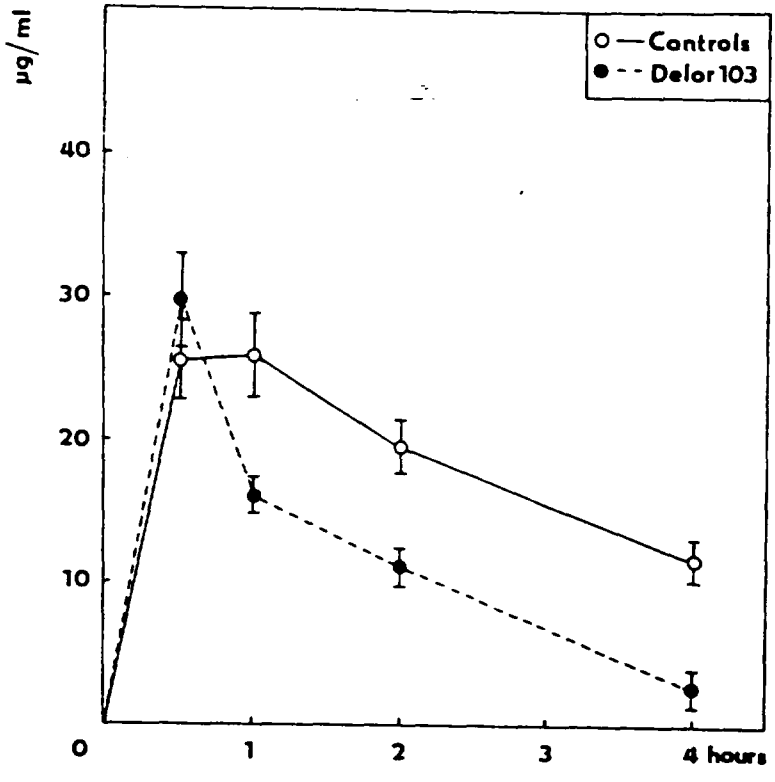
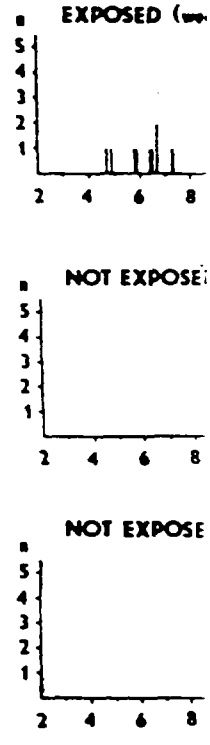


Figure 2. The rate of elimination of antipyrine from plasma of rats treated with the repeated daily doses /5 mg/kg B.W./ of Delor 103 and in control group. Antipyrine was administered orally in a single dose of 50 mg/kg B.W. 72 hours after treatment. Each point represents the mean value and S.D. of 6 animals.

there was a mean of 14.6 hours, with a standard deviation 1.7, and a range of 10.2 to 20.8 hours. For control group of student volunteers the mean of antipyrine half-life was 18.2 hours, with a standard deviation of 5.3 and a range of 9.4 to 29.1 hours.

The plasma half-life of antipyrine in workers exposed to PCBs was significantly lower than that in both control groups represented by subjects from the same factory /$P \leq 0.001$/ and the student volunteers /$P \leq 0.001$/, respectively. The difference in the values of antipyrine half-lives between control



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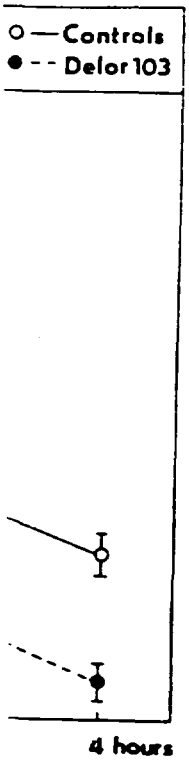

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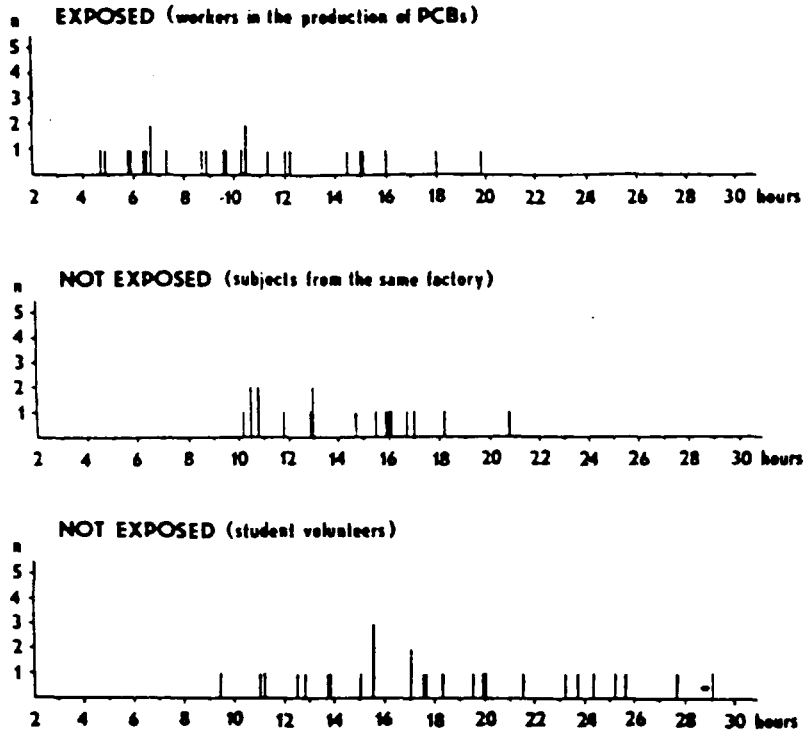

Figure 3. The plasma half-life of antipyrine in workers occupationally exposed to a mixture of commercial products of PCBs and in control subjects.

groups was statistically significant /$P \leq 0.02$/. In workers occupationally exposed to PCBs, no significant correlations was observed between duration of exposure and the plasma half-life of antipyrine.

Discussion

Our data from animal experiment indicate that Delor 103 increased markedly the elimination of antipyrine from plasma of rats following the acute as well as subacute treatment. These results confirm our previous unpublished observations demonstrating that

Delor 103 is a potent enzyme inducer. Since the elimination of antipyrine is totally dependent on its rate metabolism by liver microsomal enzymes we decided to study liver enzyme induction in workers occupationally exposed to PCBs by measuring of plasma half-life of antipyrine.

Plasma antipyrine half-life appears to be more sensitive indicator of liver enzyme induction than other methods proposed for examination of the drug metabolizing capacity of the liver in man. Thus, MORGAN and ROAN /1974/ have found no evidence that high tissue stores of DDT, DDE and dieldrin stimulate the hepatic synthesis of D-glucaric acid in exposed workers despite reports that the estimation of urinary excretion D-glucaric acid is a sensitive and reliable measure of hepatic-enzyme activity /HUNTER et al.1971, DAVIDSON et al.1974/.

KOLMODIN et al./1969/ have reported that workers occupationally exposed to various chlorinated hydrocarbon insecticides, mainly lindane and DDT, had a significantly shorter plasma half-life of antipyrine than control subjects from the same factory. In the present study it has been shown that PCBs, as further of chemical environmental factors, affect antipyrine metabolism in man. The plasma half-life of antipyrine was significantly shorter in workers exposed to PCBs than in control subjects for both the control group from the same factory and control group of student volunteers. However, in control subjects from the factory antipyrine had a significantly shorter half-life in plasma than in control subjects represented by student volunteers. Although the mean of half-life of antipyrine in students is 25 % longer than that in large group of 307 healthy male subjects reported by VESTAL et al. /1975/, the range in this our control group, 9.4 to 29.1 hours, is smaller than their data 4.8 to 41.7 hours.

Our observations suggest that hepatic enzyme induction by PCBs occurs in man. If the assumption is right, this phenomenon could affect significantly the toxicity of PCBs themselves by accelerating their metabolism. However, recently SHIMADA /1976/ and latest SEYMOUR et al. /1976/ have brought some evidence to previous assumptions of different investigators, that activated intermediate metabolites of PCBs can induce the chronic liver injury. Furthermore, our data demonstrate that these factors of industrial environment may play a role in regulating the rate of drug metabolism, what is important from a therapeutic aspect.

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Persistent Pesticides and Environmental Chemicals Are Taken Up to a Greater Extent than Non-persistent Compounds by Cultured Human Cells

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Pesticides and other man-made chemicals have played significant roles in increasing human needs such as food production and industrial uses, but in consequence of these uses some of these substances have entered the biosphere as persistent toxic chemicals. Therefore, the development of relevant test systems to evaluate the persistency of chemicals in the environment is required in order to eliminate the possibility of human exposure to such persistent chemicals. During the course of an examination of the uptake and persistence of pesticides in cultured human cells, we found that the rate of initial cellular incorporation of persistent pesticides, DDT, aldrin, and dieldrin was considerably greater than non-persistent insecticides, parathion and chlordimeform (MURAKAMI and FUKAMI 1976). The present communication presents cellular uptake data on eleven pesticides and environmental chemicals of different classes. The results demonstrate that persistent chemicals are taken up to a greater extent than non-persistent ones by cultured cells. This test method may be useful for evaluating the persistent potential of chemicals which have the possibility of environmental pollution.

Materials and Methods

Human embryonic lung diploid cells (HEL 299) from the American Type Culture Collection (CCL 137) (Rockville, Maryland) were used in these experiments. The cells were grown as monolayer cultures as described previously (MURAKAMI and FUKAMI 1976). Radioactive compounds used in this work are listed in Table 1.

To determine the cellular uptake of chemicals, cells in 5 ml of growth medium were planted in 24 cm^2 culture bottles. After 24 hours of incubation at 37° C the starter medium was replaced with 5 ml of medium and the cells were allowed to grow to near-confluence as indicated by microscopic examination. Radioactive chemicals (0.02 µmol) in 0.05 ml of ethanol were added to the cultures. The chemical concentration in the medium is about 4×10^{-6} M.

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TABLE 1

Radioactive Compounds Used in This Work

Common name	Chemical name	Specific activity mCi/mmol	Source**	Use
DDT	1,1,1-Trichloro-2,2-bis(p-chlorophenyl-^{14}C)ethane	29.7	RCC	Insecticide
Aldrin*	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene	69	RCC	Insecticide
Dieldrin*	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene	85	RCC	Insecticide
Malathion	O,O-Dimethyl-S 1,2-(diethoxycarbonyl) ethyl-1,2-^{14}C]phosphorodithioate	4.6	RCC	Insecticide
Parathion	O,O-Diethyl-1-^{14}C-O-p-nitrophenyl-phosphorothioate	19	RCC	Insecticide
Carbaryl	1-Naphthyl N-methyl-^{14}C-carbamate	57	RCC	Insecticide
Chlordimeform	N'-(2-Methyl-^{14}C-4-chlorophenyl)-N,N-dimethylformamidine	34.5	Schering	Insecticide
2,4-D	2,4-Dichlorophenoxy-1-^{14}C-acetic acid	54	RCC	Herbicide
2,4,5-T	2,4,5-Trichlorophenoxy-1-^{14}C-acetic acid	54	RCC	Herbicide
HCB	Hexachlorobenzene-^{14}C(U)	23.4	RCC	Seed disinfectant, intermediate for organic synthesis, plasticizer
PCB	2,4,5,2',4',5'-Hexachlorobiphenyl-^{14}C(U)	15.2	RCC	Industrial chemical

* Carbon 1,2,3,4, and 10 positions are labelled with ^{14}C.

** Abbreviation : RCC, Radiochemical Centre.

In controls, the same amount of chemicals was simply mixed with 5 ml of medium. Culture bottles were sampled at 4, 24, and 48 hours. At each period, the medium was removed and the cell monolayers were washed twice with cold 0.8 % NaCl solution and digested with 2 ml of 0.5 N NaOH at room temperature (ZIMMERMAN et al. 1975). Fifteen minutes later, 0.5 ml aliquots of the NaOH digest were placed into liquid scintillation counting vials. Radioactivity was measured on a Beckman LS-150 Liquid Scintillation System, after the addition of 15 ml of a mixture of 5 volumes of toluene with dimethyl POPOP (0.01 g/l) and PPO (6 g/l) and 1 volume of Triton X-100 (MENEGHINI 1974). Counting efficiencies were determined by reading directly from the quenching curve which was plotted against external standard ratio, and counts per minute were converted to disintegrations per minute.

Total protein per culture was determined by assaying 0.5 ml of the NaOH digest according to the method of LOWRY et al. (1951) as modified by OYAMA and EAGLE (1956) for tissue cultures.

The growth of the culture was measured by determination of total cell protein after 48-hour exposure to chemicals. The different compounds were applied at the concentration of 0.02 µmol /5 ml of culture medium. The growth of treated cultures was expressed as the percentage of the control growth (EtOH treated).

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Results and Discussion

Table 2 summarizes cellular uptake data obtained when HEL cells were exposed to ^{14}C-labelled chemicals over a period of 48 hours. The incorporation rates of DDT, dieldrin, and aldrin which are generally considered to be persistent pesticides, were greatest among chemicals tested. PCB which is also classed as a persistent chemical, was taken up markedly by the cells, but the rate was slightly lower than the chlorinated insecticides. HCB, another persistent chemical, was also accumulated significantly. Malathion, parathion, chlordimeform, 2,4,5-T, and 2,4-D, generally considered to be non-persistent pesticides, were taken up to a smaller extent than the persistent compounds. A considerable amount of carbaryl was taken up by the cells. This finding may suggest that the naphthalene ring in the carbaryl molecule is involved in the incorporation potential of this compound into the cells.

In a general way, it appears that an inverse relationship exists between the uptake of the test compounds by HEL cells and their solubility in water (Table 2). However, a close correspondence does not exist between them. This result may suggest that in addition to the lipophilic character, some factors such as binding capacity to proteins and other cellular constituents are involved in the uptake of chemicals by cultured cells.

In order to examine the state of the cells during the experiment, the effect of the chemicals on cell growth was investigated. Most compounds were not toxic at the same concentration and over the same experimental period as the uptake experiments. The herbicides, 2,4-D and 2,4,5-T, though less incorporated than the insecticidal and industrial chemicals, appear to be more toxic. It is therefore evident that differences in uptake are not the cause of the different degrees of cytotoxicity of the chemicals.

HAAG et al. (1975) reported that 2.7×10^{-6} M of 2,4-D caused a loss in cell differentiation of cultured chicken muscle cells. Using HeLa cells, LITTERST et al. (1969) and BLEVINS and DUNN (1975) found independently that carbaryl was more toxic than some other insecticides tested. Our findings on the inhibition of cell growth by chemicals are in accord with the results reported in the above papers. The drug-metabolizing system of the HEL cells was investigated by the measurement of the ability of the cell to convert the chemicals from a benzene soluble to an aqueous soluble form, as described by LOCKE et al. (1971), and any compounds tested were not metabolized by the cells.

TABLE 2

Uptake of ^{14}C-Labelled Chemicals into Cultured Human Embryonic Lung Cells
(Results are expressed as means ± the standard error of the mean.)

	Cell-associated chemical (pmol/mg of cell protein)			Water solubility*	Cell growth
	4 h	24 h	48 h	(mg/l)	(Per cent of control)
DDT	3690 ± 243	4060 ± 277	4830 ± 326	0.04	94
Dieldrin	3550 ± 175	3860 ± 122	4380 ± 240	0.1	97
Aldrin	3590 ± 369	3290 ± 224	3640 ± 383	0.01	99
PCB	1040 ± 80.8	1250 ± 40.4	1740 ± 98.0	0.001	111
HCB	993 ± 67.3	916 ± 78.6	778 ± 3.2	---	88
Carbaryl	278 ± 49.0	551 ± 110	945 ± 21.3	40	87
Malathion	323 ± 163	164 ± 111	180 ± 45.3	145	107
Parathion	70.7 ± 32.5	31.1 ± 2.5	22.6 ± 9.6	24	97
Chlordimeform	13.0 ± 2.8	18.7 ± 5.6	32.4 ± 5.7	250	96
2,4,5-T	6.6 ± 1.0	12.0 ± 0.4	9.9 ± 0.8	280	83
2,4-D	2.6 ± 0.3	5.0 ± 1.5	3.7 ± 1.0	730	79

* References for water solubilities quoted : DDT, Dieldrin, Aldrin, Carbaryl, and Parathion (LITTERST et al. 1969); PCB (HAQUE and SCHMEDDING 1975); Malathion (MELNIKOV 1971); Chlordimeform (NOYAKU NO KAGAKU TO ÔYÔ 1972); 2,4,5-T and 2,4-D (BÖHM and MÜLLER 1976).

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Conclusion

It can be concluded that persistent chemicals were taken up to a greater extent than non-persistent substances by cultured human cells. Increasing uptake of the chemicals by the cells accorded with decreasing water solubility in general, but a strict correspondence between them could not be obtained. We propose that the method employed in this study be used for detecting a compound with persistent character in the environment. It will be a useful addition to examinations of n-octanol-water partition coefficient and of accumulation in fish and other organisms which have been used for evaluating the persistent potential of chemicals in the environment.

We wish to thank Dr. K. Fukunaga for his interest and encouragement.

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Insecticides, Polychlorinated Biphenyls and Metals in African Lake Ecosystems. III. Lake Nakuru, Kenya

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Nakuru, Kenya

INTRODUCTION

Lake Nakuru is known as "The Lake of a Million Flamingos" and is famous for the large variety of birds and animals which inhabit the area. Because Lake Nakuru is a "closed system" with no outlet, it is especially vulnerable to pollution by toxic chemicals. In 1970 KOEMAN *et al.* (1972) examined the lesser flamingo (*Phoeniconaias minor*), white pelican (*Pelecanus onocrotalus*) and a cichlid fish (*Tilapia grahami*) for concentrations of insecticides and metals.

The present paper presents the results of the last of four studies on concentrations of insecticides, polychlorinated biphenyls (PCB's) and metals in African lake ecosystems. The results of the studies on the first two lakes, Hartbeespoort and Voëlvlei Dams, Republic of South Africa can be found in GREICHUS *et al.* (1977a) and the third lake, Lake McIlwaine, Rhodesia in GREICHUS *et al.* (1977b).

STUDY AREA

Lake Nakuru is typical of many of the Rift Valley Lakes in that it is a shallow, alkaline, eutrophic lake surrounded by agricultural land (Fig. 1). The lake lies entirely within the boundaries of Nakuru National Park. However the catchment area includes rich agricultural land which is subjected to insecticides and herbicides. It is situated just south of the city of Nakuru (population 50,000) which discharges its sewage effluent into the lake after treatment through a one stage digester. It covers an area of about 60 km^2 with an average depth of less than 1 m. The catchment area of 1938 km^2 includes several rivers which empty into the lake (Fig. 1). A number of fish-eating birds such as the white pelican and the white-breasted cormorant (*Phalacrocorax carbo lucidus*) were attracted to the lake after the introduction of a

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Figure 1

small cichlid fish in 1961. The fish have flourished apparently unbothered by the lake's high alkalinity (> pH 10). The lake is rich in blue-green algae (*Spirulina platensis*) which serves as the major food for the lesser flamingo.

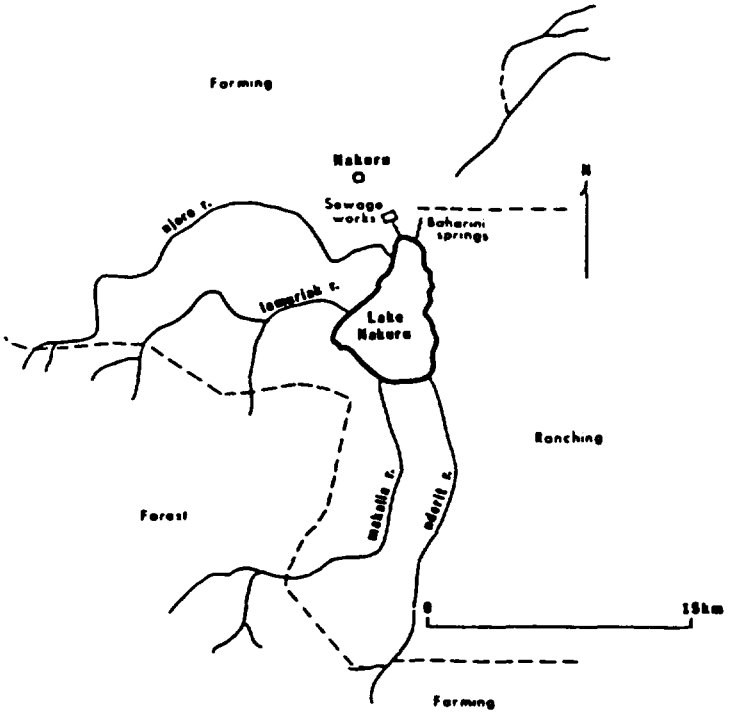

Figure 1. Lake Nakuru and catchment area.

METHODS AND MATERIALS

Samples were collected during April, 1975 and included water, bottom sediments, algae, aquatic insects (water boatmen, *Corixidae*), chironomids, and fish (*Tilapia grahami*). Unlike the studies on the other African lakes, no fish-eating birds were included as permission was not granted by the Kenyan government until it was too late for collection.

Collection and preservation of samples and methods of analysis for insecticides, PCB's and metals are described in GREICHUS *et al.*, 1977a. All samples were preserved in 10% formalin except water for metal analysis which was adjusted to a pH of < 2.0 with HNO_3. All samples were flown to the Pesticide Laboratory at South Dakota State University for analysis. Samples were analyzed for PCB's and the following organochlorine insecticide residues: lindane, heptachlor, aldrin, endrin, heptachlor epoxide, dieldrin, chlordane, endosulfan, methoxychlor, bayluseide, toxaphene, DDE, DDD and DDT. Metals included mercury, zinc, lead, arsenic, cadmium, copper and manganese. Average percent recoveries and standard deviations for samples fortified with insecticides, PCB's or metals are given in GREICHUS *et al.*, 1977a. Values for insecticides and PCB's were corrected for percent recovery, but metal values were not. In calculations of averages and totals, less than ($<$) values were included and given one-half the stated value, that is, a value of < 0.1 would become 0.05.

RESULTS AND DISCUSSION

Insecticides and PCB's

No samples from the Lake Nakuru ecosystem had levels of lindane, heptachlor, heptachlor epoxide, aldrin, endrin, chlordane, endosulfan, methoxychlor, bayluseide or toxaphene above the minimum analytical levels for this laboratory. The most prevalent residue was DDE followed by DDD, dieldrin and DDT (Table I). No water or bottom sediment samples had any discernible insecticide residues except a sediment sample collected at the inlet of a sewer from the city of Nakuru. This sample had 0.01 ppm of DDE and 0.02 ppm DDD. Plankton, chironomids, water

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TABLE 1

Average Concentrations of Insecticides and Polychlorinated Biphenyls in the Lake Nakuru Ecosystem

		ppm (ug/g)[a]					
Description	No.	DDE	Dieldrin	DDD	DDT	Total Insecticide	Total PCB
Waters	10	<.0001	<.0001	<.0001	<.0001	<.0002	<.001
Bottom Sediments	10	<.001	<.001	<.001	<.001	<.002	<.02
Plankton	1[b]	0.05	0.03	0.02	0.02	0.12	<.5
Chironomids	1[b]	0.02	<.01	0.02	0.01	0.06	0.59
Water Boatmen	1[b]	0.02	<.01	0.02	0.01	0.05	0.52
Fish	10[c]	0.02	0.02	0.01	<.01	0.05	<.5

a - All samples analyzed on a dry weight basis except water.
b - Each sample consists of a composite collected from all over the lake.
c - Each sample consists of a composite of 10 fish ranging from 5.4 to 9.6 g. Ten composite samples were analyzed.

boatmen and fish had residues of DDE and DDD. No dieldrin was detected in chironomids and water boatmen and no DDT was found in the fish. KOEMAN *et al.*, 1972 reported only residues of DDE and dieldrin in the same species of fish collected from Lake Nakuru. Although the values in this study are reported on a dry weight basis, it is possible to estimate the wet weight value of DDE and dieldrin to be about 0.003 and 0.005 ppm, respectively. The highest values reported by KOEMAN *et al.*, 1972 were 0.002 and 0.0024 ppm, respectively for DDE and dieldrin. Fish from Lake Nakuru had lower levels of average total insecticide residues than did sun-dried fish from the northern end of Lake Tanganyika which ranged from 0.69 to 2.04 ppm of DDE + TDE + DDT (DEELSTRA *et al*, 1976).

Only chironomids and water boatmen had levels of PCB's higher than the minimum analytical levels (Table 1). The pattern of PCB's more closely resembled those of Aroclor 1254 than those of Aroclor 1260 (Monsanto Chemical Co., St. Louis, MO). In general Lake Nakuru had lesser amounts of insecticides and PCB's in all types of samples analyzed, except water, than did Hartbeespoort and Voëlvlei Dams in the Republic of South Africa (GREICHUS *et al*, 1977a) or Lake McIlwaine in Rhodesia (GREICHUS *et al*, 1977b).

Metals

Average concentrations of metals in Lake Nakuru are given in Table II. In general levels of arsenic, manganese, lead, zinc and mercury did not appear unusual when compared to these metals in Hartbeespoort or Voëlvlei Dams in the Repbulic of South Africa (GREICHUS *et al*, 1977a) or to Lake McIlwaine, Rhodesia (GREICHUS *et al*, 1977b). Levels of cadmium in water, chironomids, aquatic insects, and fish were higher in Lake Nakuru than in the other three African lakes. It was noted that bottom sediments from the Nakuru sewage inlet were higher in cadmium (0.46 ppm) than the other two areas, Nsoro River inlet (0.16 ppm) and Nderit River inlet (0.17 ppm). KOEMAN *et al.*, 1972 reported fish in Lake Nakuru as having 0.086 ppm arsenic, 1.6 ppm copper, 19 ppm zinc, no detectable cadmium and 0.016 ppm mercury. Converting the values in this study to an approximate

TABLE II

Average Concentrations of Metals in Lake Nakuru F

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TABLE II

Average Concentrations of Metals in Lake Nakuru Ecosystem

ppm (ug/g)[a]

Metal	Water	Bottom Sediment	Chironomids	Insects	Fish[b]
No. Analyzed	3	3	Composite	Composite	3
Arsenic	0.006	35.	7.5	0.14	1.80
Cadmium	0.021	0.27	0.19	0.45	0.26
Copper	0.002	6.2	4.6	11.	10.
Manganese	0.024	550.	78.	12.	19.
Lead	0.005	34.	1.3	0.82	0.84
Zinc	0.049	140.	61.	70.	110.
Mercury	<.001	<.05	0.26	0.16	0.22

a - All samples analyzed on a dry weight basis except water.
b - Each sample consists of a composite of 10 fish ranging from 6.4 to 8.6 g. Three composite samples were analyzed.

wet weight value gives 0.33 ppm arsenic, 1.8 ppm copper, 20 ppm zinc, 0.05 ppm cadmium, and 0.04 ppm mercury. Although the fish in this study had higher copper concentrations than fish in the three African lakes in the previous studies, the levels in this study agree closely with those reported by KOEMAN *et al.*, 1972. Metals in Nakuru fish fell within ranges given for cadmium, lead and zinc for fish collected from the San Antonio Bay (SIMS and PRESLEY, 1976) but were higher in copper, mercury and arsenic. However some fish from the U.S. Southeast Coast (WINDOM, 1972) had as high or higher average levels of cadmium, copper, lead, zinc and arsenic than the average values for Nakuru fish.

In general concentrations of insecticide residues and PCB's in the Lake Nakuru ecosystem do not appear at present to be a pollution problem. This is probably also true for the metals but it is difficult to distinguish between naturally occurring concentrations and those introduced by man.

ACKNOWLEDGEMENTS

The authors wish to thank the personnel of the Baharini Wildlife Sanctuary who assisted us with this study. The hospitality of John and Sandy Hopcraft was greatly appreciated by the Greichus family during their stay in Kenya.

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Residues of Persistent Chlorinated Hydrocarbons in Human Tissues as Studied by Neutron Activation Analysis and Gas Chromatography

Alf Bjorseth,[1] Gulbrand Lunde[1] and Erik Dybing[2]

[1]*Central Institute for Industrial Research, Oslo 3 — Norway*

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Several persistent chlorinated hydrocarbons are found to be present in human adipose tissue and organs due to the widespread distribution of these compounds in the environment (KRAUL and KARLOG 1976, and references cited therein). Among the most dominant of these compounds are PCBs, DDT and DDE. The usual analytical technique for determination of organochlorinated compounds in such samples involves adequate clean-up and gas chromatography (GC) with electron capture (EC) detection. With this method it is inherent that non-volatile compounds do not pass the GC-column, thus the possibility exists that some organochlorine compounds escape detection.

By neutron activation analysis (NAA), it is possible to determine the total amount of chlorinated hydrocarbons present measured as chlorine (LUNDE and STEINNES 1975). In combination with NAA, GC-analysis results will give an estimate of the fraction of the total amount of chlorinated hydrocarbons present in the sample.

In the present paper we describe the determination of hexachlorobenzene (HCB), PCB and p,p'-DDE in liver and adipose tissue of 10 Norwegian subjects as determined by GC and NAA. The results are compared with similar studies from other countries.

EXPERIMENTAL

Samples. Samples of human autopsy material were provided by the Laboratory of Pathological Anatomy at the Oslo City Hospital (Ullevål sykehus). No special selection was made, with regard to age, sex or occupation. The samples were stored at -25 °C until analyzed.

Chemicals. All solvents used in this work were distilled in an all-glass distillation apparatus and their purity was carefully checked. Standards of the chlorinated hydrocarbons were purchased from commercial sources, and their purity checked by gas chromatography. All glassware used in the analysis were annealed by 550 °C.

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Clean-up. About 10 g of subcutaneous fat or liver was grinded and extracted twice by 25 ml cyclohexane/isopropanol (1:1) by vigorous shaking for 2 hours at room temperature. Water was added to the combined extracts and the cyclohexane phase transferred to a smaller tube. The oil yield was determined by removing the solvent in a water bath.

About 1 ml cyclohexane and 2 ml conc. sulfuric acid were added to 1 ml oil. The mixture was shaken for several minutes and centrifugated for 10 min. at 1500 rpm. The cyclohexane phase was transferred to a small column containing aluminium oxide activated at 800 °C and deactivated with 5 % water. Non-polar chlorinated hydrocarbons were eluted by 10 ml pentane. This solution was concentrated to about 3 ml in an aluminium heating block under a stream of highly purified nitrogen. About 2.5 ml was used for the NAA while the remaining part was used for the GC-analysis.

Gas chromatographic analysis. The GC-analysis was performed on a Perkin Elmer model 3920 GC equipped with a ^{63}Ni-electron capture detector. A stainless steel column of 2 m length and 2 mm inner diameter was packed with 3 % SE-30 on Supelcoport 100-120 mesh. Nitrogen was used as carrier gas and argon with 5 % methane as a make-up gas for the EC-detector. Injector- and detector temperatures were 275 °C and 300 °C, repectively, and the column temperature was programmed from 150 °C to 230 °C at 8 deg/min.

Identification and quantification of the peaks in the chromatograms were performed by comparing retention times and response factors with those of known standards. The GC-profile of PCB in the different samples was rather uniform and resembled most closely the commercial product Aroclor 1254, which therefore was used as a standard.

To confirm identification, samples were also chromatographed on a column filled with 1.5 % SP-2250/1.95 % SP-2401 on Supelcoport 100-120 mesh under the same chromatographic conditions.

Neutron activation analysis. The principles for determination of trace elements in non-polar extracts have been reported previously (LUNDE 1971). The samples were sealed in polyethylene vials and transferred to the reactor by means of a "rabbit" system, together with standards for chlorine, bromine and iodine. The neutron activation was carried out at a flux of about 1.5 x 10^{13} n/cm^2 sec. with an irradiation time of 5 minutes. The induced radioactivity was registered immediately after irradiation by means of a 33 cm^3 Ge-Li detector and a multichannel gamma spectrometer using ^{38}Cl (with 37.2 min. halflife) to quantify the chlorine. Simultanously, bromine and iodine were determined by ^{80}Br (halflife 17 min.) and ^{128}I (halflife 25 min.).

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RESULTS

Gas chromatography. Among the chlorinated hydrocarbons, residues of HCB, PCB and p,p'-DDE were present in all the samples. The results of the analysis of the fat and liver samples are given in Table I. The values in Table I are calculated on basis of extractable fat. For each compound, an average value is also given. Occasionally, other peaks appeared in the chromatogram, but no attempt was made to identify these.

TABLE I

Residues of PCB, DDE and HCB in fat and liver samples as determined by gas chromatography (in ppm)[a]

Sample Number	Age	Fat			Liver		
		PCB	HCB	DDE	PCB	HCB	DDE
1	81	1.39	0.14	0.40	1.11	0.15	0.35
2	65	0.69	0.05	0.32	1.68	0.14	0.79
3	72	1.70	0.09	1.08	1.86	0.11	1.21
4	68	4.44	0.19	1.68	2.06	0.12	0.90
5	29	1.61	0.07	0.63	1.93	0.11	0.84
6	48	2.40	0.19	0.59	2.29	0.16	0.60
7	73	1.25	0.10	0.78	1.42	0.12	1.09
8	67	2.06	0.11	0.60	2.28	0.11	0.70
9	86	0.37	0.03	0.40	2.03	0.23	0.88
10	78	1.87	0.16	1.12	2.01	0.20	0.60
Average		1.78	0.11	0.76	1.87	0.15	0.80

a) µg/g extractable fat

Neutron activation analysis. The results of the NAA are presented in Table II. The results are calculated on the basis of extractable fat and are corrected for blank values. For the sake of completeness, the table also contains the values of persistent organic brominated and iodated hydrocarbons.

TABLE II

Determination of the total organic bonded halogens in fat and liver, as determined by neutron activation analysis (ppm)[a)]

Sample Number	Age (Years)	Fat			Liver		
		Total Cl	Total Br	Total I	Total Cl	Total Br	Total I
1	81	1.19	0.005	0.001	1.06	0.047	0.001
2	65	0.62	0.012	0.001	2.17	0.005	0.001
3	72	1.73	0.005	0.001	1.60	0.014	0.001
4	68	4.99	0.017	0.001	-	0.043	0.001
5	29	1.64	0.010	0.001	1.68	0.005	0.001
6	48	1.72	0.005	0.001	-	0.005	0.001
7	73	1.05	0.005	0.001	1.80	0.005	0.001
8	67	2.58	0.005	0.001	2.57	0.005	0.001
9	86	0.47	0.029	0.001	2.03	0.013	0.001
10	78	1.69	0.005	0.001	2.67	0.005	0.001
Average		1.77	-	-	2.08	-	-

a) µg/g extractable fat

DISCUSSION

Only one investigation of residues of chlorinated hydrocarbons (DDE, PCBs) in Norwegian subjects has previously been carried out (BJERK 1972). Recently, similar studies have been performed in Denmark (KRAUL and KARLOG 1976) and German Federal Republic (ACKER and SCHULTE 1974). A comparison of these results is given in Table III. The results may be difficult to compare due to the lack of a standarized procedure for the analysis of these compounds, and to the limited number of samples studied in the present work. However, a few points may be made from Table III. The two Norwegian studies both show that, on an average, PCB and DDE fat levels in Norwegians are lower than in Danes and Germans. The most apparent reason for this, is that the Norwegian studies lack the extreme maximum values of the other studies. In liver samples, the Norwegian mean value is also lower than the Danish, however, the difference being smaller compared to the fat values.

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Only a limited number of determinations of HCB in human fat have been reported. As revealed by Table III, the level of HCB seems to be significantly lower in Norwegians than in German subjects.

TABLE III

Residues in human fat and liver in various countries (ppm)[a)]

Country Year	Sample	HCB	DDE	PCB	Reference
Denmark 1972-73	Fat	-	4.9 (0.31-18)	5.1 (1.0-49)	KRAUL & KARLOG 1976
	Liver	-	6.0[b)]	3.2 (0.94-27)	
GFR - Köln	Fat	2.9 (0.08-5.5)	2.9 (1.0-13)	6.9 (2.1-12)	ACKER & SCHULTE 1974
GFR - München	Fat	6.4 (1.2-21)	5.7 (1.1-19)	10 (2.1-52)	ACKER & SCHULTE 1974
Norway 1969-70	Fat	-	3.3 (0.19-12)	1.61 (0-6.87)	BJERK 1972
Norway 1975	Fat	0.11 (0.03-0.19)	0.76 (0.32-1.68)	1.78 (0.37-4.44)	This work
	Liver	0.15 (0.11-0.23)	0.80 (0.35-1.21)	1.87 (1.11-2.29)	

a) µg/g extractable fat
b) The actual value is Σ(DDT + DDE)

The NAA results show that persistent organochlorine compounds are present in human fat and liver averaging 1.77 ppm and 2.08 ppm, respectively, measured as chlorine on oil basis. The levels of persistent organobromine compounds are much lower, with all measured values less than 0.03 ppm. Persistent organoiodine compounds are not detected in this work. If present, the values are less than 1 ppb in the extractable fat.

It is also of interest to compare the total levels of organic bonded chlorine as determined by GC and NAA. In Table IV, the values of HCB, PCB and DDE given in Table I and II are converted to the equivalent amount of chlorine,

so that this number may be compared directly with the NAA results. In the last column the per cent of the NAA value that can be accounted for by the GC analysis is given. As shown in Table IV, the individual values range from 68 to 107 %, with an average of 80 %, for the fat and from 58 to 110 %, with an average value of 79 %, for the liver samples. These values are only estimates, and are given with rather large uncertainties. The major part of the chlorinated hydrocarbons are PCBs, and the choice of standard by quantification will influence the value of the chlorine equivalent. Nevertheless, the results indicate that most of the persistent chlorinated hydrocarbons present may be accounted for by PCB, DDE and HCB. Other compounds occasionally reported like DDT, DDD, dieldrin, the BHC-isomers, etc., apparently contribute only a small part of the total burden of chlorinated hydrocarbons.

TABLE IV

Comparison of results from neutron activation analysis and gas chromatography (ppm)a)

Sample Number	Sample	PCB as Cl	HCB as Cl	DDE as Cl	Total Cl by GC	Total Cl by NAA	Percent of NAA accounted for by GC
1	Fat	0.78	0.10	0.18	1.06	1.19	89
	Liver	0.62	0.11	0.16	0.89	1.06	84
2	Fat	0.39	0.04	0.14	0.57	0.62	92
	Liver	0.94	0.10	0.35	1.39	2.17	64
3	Fat	0.96	0.07	0.48	1.51	1.73	87
	Liver	1.04	0.08	0.54	1.77	1.6	110
4	Fat	2.50	0.14	0.75	3.39	4.99	68
	Liver	1.15	0.09	0.40	1.64	-	-
5	Fat	0.91	0.05	0.28	1.24	1.64	76
	Liver	1.08	0.08	0.38	1.54	1.68	92
6	Fat	1.35	0.14	0.26	1.75	1.72	102
	Liver	1.28	0.12	0.27	1.67	-	-
7	Fat	0.70	0.07	0.35	1.12	1.05	107
	Liver	0.80	0.04	0.49	1.38	1.80	77
8	Fat	1.16	0.08	0.27	1.51	2.58	59
	Liver	1.28	0.08	0.31	1.67	2.57	65
9	Fat	0.21	0.02	0.18	0.41	0.47	87
	Liver	1.14	0.17	0.39	1.70	2.03	84
10	Fat	1.05	0.12	0.50	1.67	1.69	99
	Liver	1.13	0.15	0.26	1.54	2.67	58

a) µg/g extractable fat

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AA	Percent of NAA accounted for by GC
	89
	84
	92
	64
	87
	110
	68
	-
	76
	92
	102
	-
	107
	77
	59
	65
	87
	84
	99
	58

It is noteworthy that, in variance with these results, less than 20 % of the organochlorinated compounds determined by NAA in fish and other marine organisms from relatively uncontaminated areas are accounted for by GC (LUNDE and OFSTAD 1976).

ACKNOWLEDGEMENT

The authors are indebted to Ing. M.B. Støbet Lande for skillful technical assistance. The human autopsy samples were kindly provided by Dr. L.A. Solberg. The financial support from the Norwegian Council for Scientific and Industrial Research is gratefully acknowledged.

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PCT, PCB and Pesticide Residues in Human Fat and Blood

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Shinjuku-ku, Tokyo 160, Japan

As descrived in the previous papers in this series (DOGUCHI et al 1974 ; DOGUCHI and FUKANO 1975), substantial amounts of PCTs were detected in human fat and blood. Especially, the levels of PCTs were almost equivalent to those of PCBs in human blood. In the end of 1960 or begining of 1970, the levels of total DDT in human fat were the same order of magnitude in Japan and other countries, but the levels of BHC in Japanese, especially of β-BHC, were 10 to 20 times higher than the levels found amoung the populations of other countries compaired(DOGUCHI et al 1970).

Our interest in the pollution by these substsnces led us to analyze the residues levels of PCTs, PCBs, DDT and BHC in human fat of 30 samples collected in 1974 in an atempt to compaire with the levels found in 1971 and to obtain additional information with PCT pollution. We also mentioned the residues of PCTs and PCBs in the blood collected again from 10 subjects in 1975 out of 27 subjects who had been already examined in 1973 to obtain some measure of changes in these residue status with time.

Materials and Methods

Thirty samples of the adipose tissues, 18 males and 12 females, obtained in Tokyo in 1974 were subjected to the analyses of PCTs, PCBs, DDT and BHC. Human blood were collected again from 10 subjects in 1975 out of 27 subjects from whom blood were obtained to estimate PCT and PCB levels in 1973(DOGUCHI and FUKANO 1975). Determinations of PCTs and PCBs were made with these blood samples.

The residues of PCTs, PCBs and pesticides in the fat were extracted with hexane-acetone(1:1) and cleaned up by fuming sulphuric acid treatment and column chroma-

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tography on florisil and silica gel. Elution of DDT and BHC with hexane containing 6% of ether followed elution of PCTs, PCBs and DDE from the column with hexane. Subsequent procedures, gas chromatography and quantitation, were the same as descrived previously(DOGUCHI and FUKANO 1975).

The analyses of PCTs and PCBs in the blood were made according to the method descrived in preceding paper(DOGUCHI and FUKANO 1975).

The contents of ortho, meta and para terphenyl in human fat and in KC-C, technical PCT product, were determined by treating them with $SbCl_5$ to yield perchlorinated terphenyls(DOGUCHI et al 1974) and by compairing the peak hights of these isomers with those of authentric samples on mass fragmentgrum of parent mass number(m/e = 714). GC-Mass instrumental conditions were as follows : Shimazu-LKB 9000 system was used with a 100 X 3 mm glass column packed with 2% OV-1 on 80/100 mesh Gas Chrom Q. The operating conditions were : column temperature, 280°C ; flow late of herium, 30 ml/min ; trup current, 60 μA ; ionization voltage, 70 eV.

Results and Discussion

The average levels of PCTs and PCBs in human fat were 1.11 ppm and 1.04 ppm on fat basis and respective standard deviations were 2.01 ppm and 0.59 ppm. Neither significant difference nor correlation were demonstrable statistically between the levels of PCTs and PCBs in human fat.

Table 1. Levels of PCTs and PCBs in Human Fat

	PCT Residues	PCB Residues
No. of Samples	30	30
Mean Value	1.11	1.04
Range	0.04 - 9.20	0.38 - 2.50
S.D.	2.01	0.59

(ppm on fat basis)

The peak profiles in the chromatograms of human fat and blood were quite similar to that of KC-C, as shown in previous papers(DOGUCHI et al 1974 ; DOGUCHI and FUKANO 1975). However, it was evidenced that the composition of PCTs in the fat was somewhat different from

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Table 2.

Age	Sex
24	F
28	M
23	F
44	M
26	M
29	M
25	F
32	M
29	M
28	M

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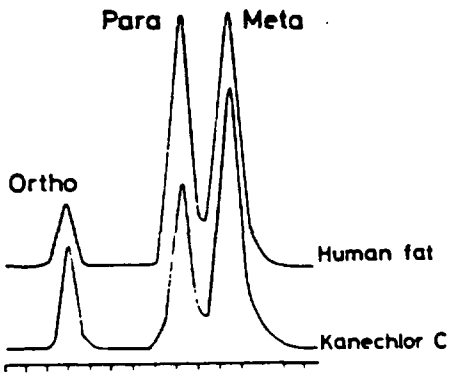
that of KC-C : the contents of ortho, meta and para isomers in the PCT residues in human fat were 8% ± 0.5, 47 % ± 1.4 and 45% ± 1.0 (n=4), and those in KC-C were 9%, 58% and 33% respectively. Metabolic behaviour or decomposition in the environment might be influenced by structural features.

Fig. 1. Mass Fragmentograms on m/e = 714 of Tetradeca Chlorinated Terphenyls in Human Fat and KC-C



With all subjects but one, no definitive changes as regards the levels of both PCTs and PCBs in the blood were recognizable between 1973 and 1975.

Table 2. Residue Levels of PCTs and PCBs in Human Blood Collected from Same Subjects in 1973 and 1975 (ppb on whole blood basis)

Age	Sex	PCT Residues 1973	PCT Residues 1975	PCB Residues 1973	PCB Residues 1975
24	F	19.6	8.4	4.0	1.8
28	M	11.8	9.4	3.0	2.0
23	F	7.8	7.6	2.3	2.2
44	M	7.2	8.0	2.5	2.5
26	M	7.0	8.4	3.1	2.9
29	M	4.8	4.4	3.0	2.4
25	F	2.6	2.7	3.2	3.8
32	M	1.9	2.0	3.5	3.7
29	M	1.1	1.1	2.2	1.9
28	M	0.7	1.2	5.1	2.7

The studies made by us(USHIO and DOGUCHI in press) on the diet and by other investigators(MINAGAWA and TAKIZAWA 1975 ; NISHIMOTO et al 1973) on the food stuffs revealed that the PCT residues in food were so low as to be practically negligible. In general population, ingestion of the residues in food is thought to be the major route by which persistent environmental pollutants enter into the body. However, as descrived above, the levels of PCTs in human fat and blood were shown to be almost equivalent to PCBs, despite negligible amount of PCTs was found in food. These facts led us to presume extremely longer period of biological half time of PCTs and/or other route responsible for accumulation of PCTs in human body than the route through ingestion of PCT residues in food.

Table 3. Pesticide Residues in Human Fat
(30 subjects, ppm on fat basis)

	α-BHC	β-BHC	γ-BHC	Total BHC	p,p'-DDE	p,p'-DDT	Total DDT
Average	0.015	2.32	0.026	2.36	2.91	0.68	3.59
Max.	0.064	5.41	0.131	5.43	6.20	1.44	7.64
Min.	nd	0.16	0.003	0.16	0.21	0.20	0.41
S.D.	0.012	1.42	0.031	1.42	1.61	0.33	1.87

The mean concentration of total BHC was 2.36 ppm and that of DDT was 3.59 ppm in human fat collected in 1974, as shown in table 3. About 98% of BHC was present in the form of β-BHC and 80% as DDE. The average level of BHC in 1971 on 27 subjects(DOGUCHI et al 1970) was 3.19 ppm and that of DDT was 3.60 ppm. The statistical analyses could not show any significant difference between the levels of DDT in 1974 and 1971, but lower level of BHC was demonstrated significantly in 1974. It was shown that the concentration of BHC in human milk tended to decrease gradually ; the level in 1973 reduced to about 1/2 of the level in 1969, but no changes were recognizable on the levels of DDE(YAKUSHIZI et al 1975). The results on human fat and milk suggest that BHC residues in human body are decreasing in Japan.

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p,p'- DDT	Total DDT
0.68	3.59
1.44	7.64
0.20	0.41
0.33	1.87

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Polychlorinated Biphenyl Residues in Maternal and Cord Blood in Tokyo Metropolitan Area

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Tokyo Metropolitan Research Laboratory of Public Health
and
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Tokyo University Hospital
Tokyo, Japan

INTRODUCTION

A number of reports indicate that PCBs are ubiquitously present in the biota of Japan (Tatsukawa and Isono, 1971, Mizutani et al, 1972, Hidaka et al, 1972, Kobayashi et al, 1972), although physiological significance is not known at this present level found in the fauna. An exposure to this substance for the human neonate starts at the commencement of breast feeding, however, the first exposure takes place as early as sometime during fetal stage, as indicated by the few incidences of "black babies" delivered by mothers with or without overt symptoms during the outbreak of PCB poisoning in Japan (Funatsu et al, 1971, Yamaguchi et al, 1971). Lipophilic chlorinated hydrocarbons such as DDT and PCB tend to remain in biota for years, once they are emitted to nature in substantial amount, even after termination of actual application. The production of PCBs was banned in 1972 in Japan and its use in the open system has also been forbidden. This report is a part of our project to monitor certain environmental chemicals found in the tissues of residents in Tokyo metropolitan area.

MATERIALS AND METHODS

Both maternal and cord blood was sampled at Tokyo University Hospital at the time of delivery during the period December, 1973 through February, 1974. Blood samples were taken in the blood container, pretreated with hexane and stored at 5°C in refrigerator until the time of analysis. For analysis of PCBs, we essentially followed the standard method recommended by the advisory committee for the Ministry of Health and Welfare (1972). Twenty g of whole blood in 50 ml centrifuge tube was shaken vigorously with 20 ml of ethanol. Five g of potassium hydroxide was added to this and heated at 90°C for one hour. After cooling, the content was extracted with three additional 20 ml portions of n-hexane. The hexane was washed three times with water, dried through the column of anhydrous sodium sulfate, and condensed

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in K-D condenser to the volume of 1 to 2 ml. The condensed sample was purified by letting it pass through the column consisting of 2 g of Florisin, 3 g of silica gel and anhydrous sodium sulfate. PCBs were eluated from the column with hexane and initial 180 ml of fraction was collected. The eluate was concentrated to 1 ml, shaken vigorously with 1 ml concentrated sulfuric acid and then hexane layer was buffered with potassium hydroxide pellets prior to gas chromatography.

The gaschromatograph used was Shimazu GC 5 AP equipped with ^{63}Ni Electron Capture Detector. The operative conditions for PCB analysis were as follows.

column dimensions	: 3 mm x 2m
column packing	: 2% OV - 1 on Gas Chrom. Q 80/100
column temperature	: 200°C
detector temperature	: 250°C
Nitrogen flow rate	: 60 ml/min.

The quantification of PCB residues followed the method provided by Ugawa (1973). Total blood lipids were determined by a modified method of Bragdon, i. e. 3 g of samples were treated twice in Folch's solvent with a dispersing mixer; the filtered solution was shaken with 0.05% sulfuric acid; the lower layer was evaporated to dryness and the residue was weighed.

RESULTS AND DISCUSSIONS

Our data is summarized in Table 1. The mean PCB level in adult blood (mother) obtained in our study (2.8 ppb) corresponds well to those reported by other workers in Japan (Inoue et al, 1974, Abe et al, 1974, Doguchi et al, 1975). On a whole basis, PCB levels in maternal blood are roughly threefold higher than those in cord blood. Because of its strong lipophilic tendency it was expected that PCBs were conveyed by the lipid fraction in the blood. Though the lipid levels in maternal blood were roughly twice as high as those in cord blood, the correction of the values to fat basis gave more or less similar results in terms of the concentration ratio between maternal and cord blood.

Gaschromatographic pattern of PCBs found in blood is fairly similar to the patterns noted in adipose tissues, namely closely resembling that of Kanechlor 500 and Kanechlor 600. The chlorine content of PCB residues in the present investigation is calculated roughly 53% which is very close to that of Kanechlor

500 (54.6%).

It is noteworthy that gaschromatographic patterns of PCB residues found in the samples of mother-baby pair are almost identical (Fig. 1), and this suggests that the placenta permits rather indiscriminate transmission of the organochlorine compounds of closely related chemical structure.

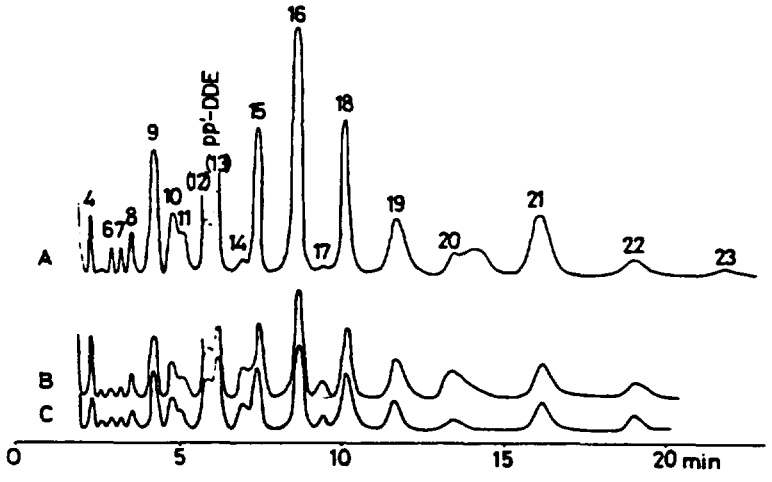

Figure 1: Gaschromatographic Patterns of PCB Residues.
A : Maternal blood
B,C : Cord blood of twins

Narafu (1973) noted an excellent correlation (0.972) as regards the PCB levels between maternal and cord blood, however we failed to see such relationship (-0.046 on a fat basis ; -0.018 on a whole basis). It appears that this issue remains unsettled in the cases of other organochlorine compounds such as BHC and DDT. (O'Leary et al, 1970, Yamagishi et al, 1972).

SUMMARY

PCB levels were determined with maternal and cord blood sampled at the time of delivery. All of the twentyfour mothers selected for this experiment are residents of Tokyo Metropolitan area. Gaschromatographic

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Av.

S. D.

#Twins

TABLE 1

PCB Levels of Maternal and Cord Blood-

Case	Wet Basis (ppb)		Fat Basis (ppm)	
	M.	C.	M.	C.
1	3.5	---	0.42	---
2	2.6	1.5	0.18	0.31
3	2.0	0.9	---	0.23
4	3.3	---	0.35	---
5	3.9	0.3	0.41	0.06
6	4.5	1.6	0.53	0.33
7	1.6	0.7	0.25	0.13
8	2.4	0.7	0.30	0.14
9	2.1	0.9#	0.23	0.22#
		0.6#		0.16#
10	4.2	0.6	0.57	0.17
11	0.3	1.4	0.05	---
12	3.0	0.9	---	0.22
13	1.8	1.2	0.29	0.24
14	1.4	0.8	0.19	0.13
15	1.0	0.4	0.14	0.06
16	1.9	0.8	0.28	0.25
17	2.9	1.3	0.39	0.24
18	---	1.2	---	0.36
19	3.6	1.2	0.43	0.17
20	---	0.8	---	0.22
21	2.6	3.3	0.65	0.35
22	7.6	0.3	0.80	0.08
23	1.8	1.6	0.23	0.49
24	3.5	0.8	0.43	0.21
Av.	2.8	1.1	0.36	0.22
S. D.	1.5	0.6	0.18	0.10

#Twins

patterns of PCBs in cord blood closely resembled those of mothers. The cord blood in general showed lower PCB levels on both whole and fat basis than those in the maternal blood. No correlation was noticeable on statistical basis as regards the concentrations of PCBs between the cord blood and maternal blood. Our data suggests that the passage of PCBs through the placenta follows general patterns observed in other organochlorine insecticides.

ACKNOWLEDGEMENT

We are indebted to Mr. K. Ohkawa for his technical assistance and to Mrs. S. T. Ohi for her useful critical discussions and typing of this paper.

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The Effect of DDT and PCB on Lipid Metabolism in *E. coli* and *B. fragilis*

by Douglas E. Greer and Julian E. Keil

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Lewis W. Stillway
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DDT and PCB, chlorinated hydrocarbons similar in structure and physiological effects (LICHTENSTEIN, et al, 1969), are of environmental concern because of their ubiquity, magnification and effects in natural food chains (COX, 1970; MACEK and KORN, 1970; GOULD, 1972; McLANE and HALL, 1972). Recent work has also suggested that DDT could be unrecognized source of PCB (MAUGH, 1973). These chemicals have been of concern in human health because of their accumulation in the fatty tissues of man (DEICHMAN, RADOMSKI, 1968). A number of workers have shown a statistical association between DDT and its metabolites to risk factors of heart disease (ROAN, MORGAN, 1973; SANDIFER, KEIL, 1972) and to other pathological states (HEINRICHS, et al, 1971; O'LEARY, et al, 1970a; O'LEARY, et al, 1970b). Conversely, DDT and its metabolites have been screened as chemotherapeutic agents for neoplastic diseases (WALKER, et al, 1970; TEMPLE, et al, 1969).

In this experiment Escherichia coli and Bacteroides fragilis were selected as models to observe the effects of DDT and PCB on lipid metabolism in bacteria. Since these species comprise about 90% of the intestinal bacteria in areas where lipid absorption occurs (DAVIES, et al, 1970), it was also of interest to study the metabolism of DDT and PCB in these organisms.

Methods

E. coli was cultured in one liter Erlenmeyer flasks containing 500 ml Trypticase Soy Broth. B. fragilis was grown in 60 ml Scott Anaerobic Broth Bottles. Toxicant treatment solutions were prepared with Geigy 99.9% pp'DDT and PCB (Aroclor 1242 ®) dissolved in acetone, (Nanograde, Mallinckrodt Chemical Works).

Each species of bacteria was treated with .01 ppm DDT, .1 ppm DDT, .01 ppm PCB, .1 ppm PCB and acetone (solvent control). Each treatment consisted of four replicates. E. coli and B. fragilis cultures were incubated at 35C for 24 and 49 hours, respectively. Culture flasks were agitated every four hours during the incubation period. Upon completion of incubation, each

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replicate culture was centrifuged at 2000 xg and the pellet was lyophilized and weighed. Total lipids were extracted from the lyophilized samples (VORBECK, MARINETTI, 1965) and the solvent was evaporated under a stream of nitrogen in tared vials. The residue was weighed, brought up to a volume of 5 ml in chloroform-methanol (2:1) and stored at 4C in Teflon-lined screw-cap vials. Total lipids were determined from aliquots containing approximately 100 ug of total lipid by chromic acid oxidation (PANDE, et al, 1963) using a standard consisting of a known concentration of total lipids extracted from a large culture of E. coli. All solvents were distilled.

DDT and its metabolites were quantitated by gas-liquid chromatography on two different columns. Aliquots of the samples in chloroform-methanol were evaporated to dryness under a stream of nitrogen and were made up to volume in hexane (Matheson, Coleman and Bell, nanograde). Determinations were obtained on a Tracor 220 gas chromatograph fitted with two 6 ft x 2 mm ID glass columns and tritium-foil electron capture detectors. Primary determinations were obtained with a column packed with a mixed stationary phase consisting of 1.5% OV-17 and 1.95% QF-1 on 100/120 Chromasorb WHP. Determinations were confirmed on another column packed with a mixed stationary phase consisting of 4% SE-30 and 6% QF-1 on 80/100 Chromosorb WHP. Operating parameters were: inlet 235C, column oven 200C, and detector 205C. The flow-rate of the nitrogen carrier gas was 70 cm^3/min for the OV-17, QF-1 column and 80 cm^2/min for the SE-30, QF-1 column.

Results

One way analysis of variance allowed calculation of least significant difference at the 95% probability level, for different numbers of replicate comparisons.

As shown in Table 1, only the .01 ppm DDT treatment group showed a significant increase in dry weight in the E. coli series. This parallels the findings of Keil, et al (KEIL, et, al., 1972). There was no significant difference in total lipid yields of any treatment groups as compared to the acetone control. PCB had no detectable effect on E. coli growth or lipid yield.

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TABLE 1

Harvest weights, toxicant residues, and lipid yields of *Escherichia coli* cultures treated with DDT and PCB

Treatment	No. Replicates	Mean Harvest Weight (mg)	Mean Toxicant Residues (ug)	Mean Lipid Yields (ug/mg dry wt)
.01 ppm DDT	4	311.3	2.05**	38.9
.1 ppm DDT	4	219.5	14.97**	36.6
.01 ppm PCB	4	257.3	N.D.	36.8
.1 ppm PCB	3	288.9	N.D.	37.7
Control	3	235.9	0	31.9
Acetone Control	4	220.1	0	35.3
LSD_{05}*	4/4	51.6		4.8
	3/4	31.8		9.5

*LSD = Least significant difference at 95% probability level calculated from one way variance analysis.
**Total DDT = DDT + 1.114 (DDE + DDD)
N.D. = Not Determined

As shown in Table 2, .01 ppm DDT significantly depressed the growth of *B. fragilis* and .1 ppm PCB significantly stimulated growth. Neither dosage of DDT had any effect on B. *fragilis* lipid synthesis, but PCB caused a significant and apparently dose-related depression of lipid synthesis.

TABLE 2

Harvest weights, toxicant residue, and lipid yields of *Bacteriods fragilis* cultures treated with DDT and PCB

Treatment	No. Replicates	Mean Harvest Weight (mg)	Mean Toxicant Residues (ug)	Mean Lipid Yields (ug/mg dry wt)
.01 ppm DDT	4	93.0	4.2**	36.9
.1 ppm DDT	4	99.0	68.38**	36.7
.01 ppm PCB	4	137.4	N.D.	23.2
.1 ppm PCB	2	210.4	N.D.	16.2
Control	4	119.7	0	34.9
Acetone Control	4	109.9	0	35.7
LSD_{05}*	4/4	26.0		7.8
	2/4	31.8		9.5

*LSD = Least Significant Difference at 95% probability Level Calculated from one way analysis of variance
**Total DDT = DDT + 1.114 (DDE + DDD)
N.D. = Not Determined

TABLE 3

Comparison of DDT metabolites in *E. coli* and *B. fragilis*

DDT Media Concentration	*Escherichia coli*		*Bacteroides fragilis*	
	DDD	DDE	DDD	DDE
.01 ppm DDT	95%	5%	100%	0%
.1 ppm DDT	98%	2%	93%	7%

Discussion

An important finding was that conversion of DDT by *E. coli* and *B. fragilis* was primarily to DDD, a metabolite easily hydroxylated and excreted in urine, thus demonstrating that these key aerobic and anaerobic human bacteria are capable of participating in the degradation of this insecticide.

Although neither DDT nor PCB significantly altered total lipid levels in these organisms, there remains the possibility of changes in ratios of lipid classes. There appears to be no relationship between DDT residues and lipid levels although both PCB treatments significantly depressed lipid production by *B. fragilis*. While these findings are not conclusive, it does suggest that if there is a relationship between DDT exposure and blood pressure and cholesterol, it may be via a mechanism other than stimulation of lipid synthesis.

Values approaching the extreme limits of $LSD_{.05}$ were discounted because of the inaccuracies arising from the small number of treatments.

Summary

Escherichia coli and *Bacteroides fragilis*, principal human enteric aerobic and anaerobic bacteria, were cultured with suitable controls in the presence of .01 and .1 ppm of DDT and PCB. The bacteria were assayed for dry weight and total lipids. DDT increased the growth of *E. coli* at the .01 ppm level but had no detectable effect at .1 ppm. Total lipid content was not significantly altered by either DDT or PCB.

DDT at .01 ppm depressed *B. fragilis* growth, but had no significant effect on lipid levels. PCB increased the growth of *B. fragilis* significantly at the .1 ppm level, but significantly depressed lipid synthesis at both concentrations. The effect was apparently dose dependent. All cultures metabolized DDT to DDD. Trace amounts of DDE were also found in several replicates.

DDT/PCB are statistically associated with cardiovascular disease risk factors, but the mechanism does not appear to be that of increased lipid anabolism by enteric bacteria.

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Comparison of Polychlorinated Biphenyls in Yusho Patients and Ordinary Persons

by

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Daiichi College of Pharmaceutical Sciences
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and

MASANORI KUHATSUNE
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Since 1966, it has been demonstrated that polychlorinated biphenyls (PCB) are widely distributed in ecosystems of fish, shellfish and birds (RISEBROUGH et al., 1968; JENSEN et al., 1969; ZITKO and CHOI, 1971). PCB in the human body has also been analyzed in the U.S.A. (BIROS et al., 1970; PRICE and WELCH, 1972), Germany (ACKER and SCHULTE, 1970) and Japan (MIZUTANI et al., 1972). During 1968, in western Japan was recorded a mass outbreak of "Yusho" due to a chronic ingestion of rice oil that had been contaminated with PCB (KATSUKI, 1969; HIGUCHI, 1971; TANAKA, 1972). It is therefore desirable to elucidate PCB concentration and components in human adipose tissue and milk of the western Japanese and to compare these values with those of Yusho patients.

EXPERIMENTAL

Samples were collected from hospitals in Fukuoka, Saga and Ishigaki cities. The general analytical procedure employed was essentially the same as the Japanese standard analytical method of PCB (KAWASHIRO et al., 1972). Hexane extract of the sample was refluxed in 1N-sodium hydroxide ethanol solution for 1 hour. The ethanol solution was extracted with n-hexane. The n-hexane solution was washed with purified water, chromatographed on silica gel (Wakogel S-1), concentrated into a small volume and subjected to gas chromatography using Shimadzu GC-5A with a detector of Nickel 63 electron capture. A glass column (3mm X 2m) packed with 5% SE-30 on Chromosorb W AW DMCS was used.

Retention times of many gas chromatographic peaks of all samples were identical to those of Kanechlors, commercial brand of Japanese PCB. Gas chromatographic patterns of these samples were similar to those of Kanechlor-500 (Cl 54%) and/or Kanechlor-600 (Cl 59%). Amounts of PCB were obtained by a calibration curve of PCB concentrations and total peak heights. The total heights were calculated by adding heights of all peaks except the height of the peak corresponding to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE).

Recovery rates of this experimental method were tested by adding 5μg of Kanechlor-500 and repeating the above mentioned analytical procedure and were estimated to be between 80 and 110%. To check the PCB concen-

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tration, a few samples of each group of test materials were chlorinated to decachlorobiphenyl (KAWASHIRO et al, 1972) by heating a glass tube (1 X 25cm) with sealed PCB and antimony pentachloride at 220°C for 1 hour. Amount of decachlorobiphenyl was estimated by gas chromatography and converted to amount of PCB. The PCB concentrations thus obtained were within the range of half and double the corresponding values obtained by standard method.

TABLE 1 PCB in human adipose tissue and milk

	Time of sample collection	Number of samples	PCB concentration Whole basis (Fat basis) ppm		
			Minimal values	Maximal values	Average
Adipose tissue (Saga)	Sept.-Nov. 1970	11	0.3 (0.5)	1.9 (5.3)	0.9 (2.6)
Milk (Fukuoka)	Jan.-March 1972	19	0.01 (0.3)	0.06 (5.6)	0.03 (1.2)
Milk (Ishigaki)	March 1972	12	0.01 (0.1)	0.02 (0.7)	0.014 (0.4)

Gas chromatographic patterns of the PCB were similar to those of Kanechlor-500 (Saga and Fukuoka) or Kanechlor-500 + Kanechlor-600(1:1) (Saga and Ishigaki).

TABLE 2 PCB in tissues of Yusho patients

Case	Age	Sex	Time of death (Time of operation)	PCB concentration Whole basis (Fat basis) ppm	
				Liver	Adipose tissue
Case 1	17	male	July, 1969	0.1 (9.5)	1.3 (3.7)
Case 2	25	male	July, 1969	0.2(10.4)	2.8(15.1)
Case 3	73	male	Nov., 1969	0.07(3.1)	3.8 (8.4)
Case 4	48	female	Dec., 1970	0.07(1.3)	0.7 (0.9)
Case 5	46	male	May, 1972	0.08(8.4)	4.3 (6.5)
Case 6	33	female	(Sept.,1972)	- -	1.9 (2.9)
Average				0.1 (6.5)	2.5 (6.3)

Values of PCB are calculated as Kanechlor-500 + Kanechlor-600(1:1).
The gas chromatograms of all samples were similar to C or D of Fig. 1 except Case 4.

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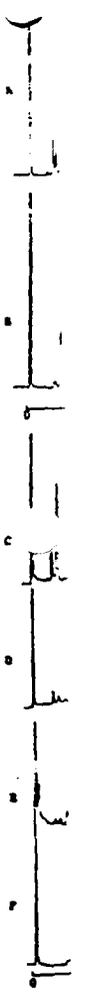

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.3	(6.5)
.9	(2.9)
.5	(6.3)

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RESULTS AND DISCUSSION

PCB concentration of human adipose tissue and milk in Saga, Fukuoka and Ishigaki are listed in Table 1. The values of Yusho patients are shown in Table 2. By the additional experiments of recovery test and decachlorobiphenyl method, the values in Table 1 and 2 were supposed to be quite precise, though the quantitative analysis of PCB is rather difficult due to the complex nature of these compounds.

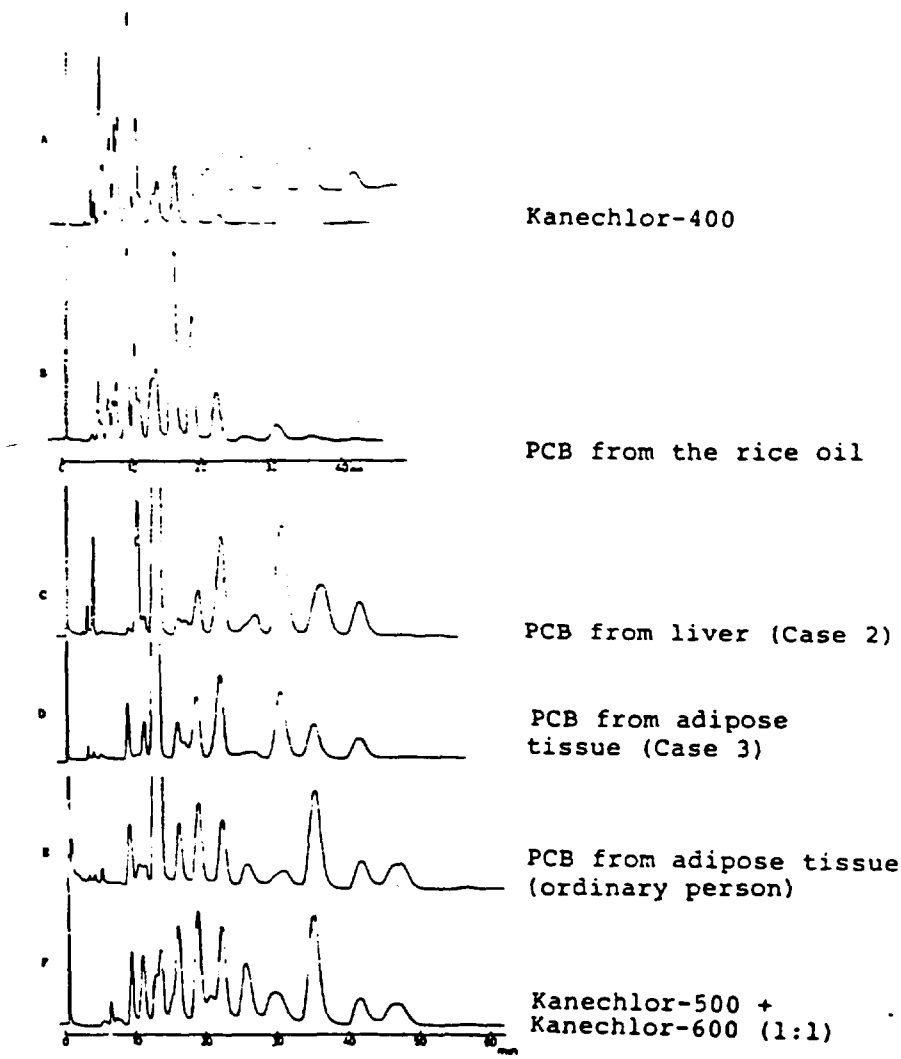

Figure 1. Gas chromatographic patterns of PCB

PCB concentration in adipose tissue of Yusho patients seemed to be a little higher than those of ordinary persons on the average. In the gas chromatograms of all samples, the early peaks corresponding to tetrachlorobiphenyls were seen very low in comparison with the later peaks (Fig. 1). This pattern suggests that the amounts of PCB in the body of Yusho patients were much decreased in a year after the accident.

When the gas chromatograms of Yusho patients and ordinary persons were carefully compared, it was discovered that the peak at retention time 31 min. was very high in the gas chromatograms of Yusho patients and low in those of ordinary persons as shown in Fig. 1. The same peak, probably due to hexa- or hepta-chlorobiphenyl, was noted in the rice oil which was contaminated with Kanechlor-400 (Cl 49%) and was taken by Yusho patients. It is therefore evident that this component of Kanechlor-400 still remains in the body more than 4 years after the accident.

Only one sample of milk from Yusho patient could be examined. The milk contained 0.06ppm of PCB and showed more similar gas chromatographic pattern to C or D than to E of Fig. 1.

ACKNOWLEDGEMENTS

We thank to dr. M. Kikuchi of Kyushu University and many other Drs. for their kind advices and co-operation.

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ACUTE HUMAN EXPOSURE TO TCDD IN SEVESO, ITALY

G. Reggiani

F. Hoffmann-La Roche Ltd., Basel, Switzerland

The children and adults who suffered injuries after exposure to chemicals that escaped in July 1976 from a reactor during the production of trichlorophenol in Seveso, Italy, have been repeatedly examined by a medical commission appointed by the judge holding the legal inquest. All patients suffered burnlike skin lesions and all but two recovered without residual effects. Chloracne occurred in 12 of these cases; it was mild in almost all and has now disappeared. Systemic functions were repeatedly evaluated in all cases and no evidence of visceral lesions was obtained. Immunocapability was normal and chromosome aberrations were within the normal range.

INTRODUCTION

On July 10, 1976, during the production of trichlorophenol in the Icmesa plant in Seveso, Italy, a runaway reaction resulted in the dispersion of chemical material over an area of about 700 acres (Garattini, 1977; Hay, 1976a, 1976b, 1977; Homberger et al., 1979). A few people, mainly children, were injured by contact with the escaped chemicals and were hospitalized. This report deals with findings obtained on examination of these people, who were referred to the judge holding an inquest on the accident. The judge had to determine the relationship of the injuries to the accident and assess their nature, extent, and consequences. Thus far, the injuries do not appear to be of a lasting nature.

SOURCE OF THE DATA

The 22 people referred to the judge, 7 adults and 15 children between 3 and 13 yr old, were hospitalized ($n = 18$) or admitted to outpatient clinics ($n = 4$) within the first 2 wk after the accident. All have been thoroughly examined by several independent clinicians, specialists, and consultants. They have been treated repeatedly for lengthy periods in

This paper was presented at the Conference on Scientific Resolution of Dispute in the Decision Making Process, Workshop on Human Exposure to 2,4,5-T and TCDD, Washington, D.C., June 4–6, 1979.

I am deeply indebted to Prof. Dr. K. H. Schulz, Dermatologische Universitätsklinik, Hamburg, for permission to use his pictures of the Seveso cases in this paper.

Requests for reprints should be sent to G. Reggiani, F. Hoffmann-La Roche Ltd., Basel, Switzerland.

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hospitals. The nature of the clinical symptoms that apparently resulted from the accident and the functions of all organs and systems of the body have been evaluated by all available methods. Finally, they have been examined twice by a group of medical experts appointed by the judge.

The hospital and examination records are the basis of this report. Only 17 of the original 22 cases referred to the judge have been retained. The remaining five have not been considered for the following reasons: three were eliminated from the inquest because their injuries had no relation to the accident and two because they refused to submit to a medical follow-up after the first examination.

ENVIRONMENTAL CONDITIONS OF CONTAMINATION

The following chemicals were charged into the reactor where the explosion occurred: ethylene glycol, tetrachlorobenzene, xylene, and sodium hydroxide. It can be assumed that the following products escaped: trichlorophenol, sodium trichlorophenate, ethylene glycol, sodium hydroxide, and the contaminant 2,3,6,7-tetrachlorodibenzodioxin (TCDD), which had been formed as a condensation product because the temperature in the reactor rose above 200°C. In the emergency situation caused by the accident, only the concentration of the latter substance surrounding the factory was measured. The aim was to assess the quantity of TCDD produced, estimate the risk of exposure for the population, and identify the contaminated area and have it evacuated and put under quarantine.

Samples of grass and vegetation were analyzed by gas chromatography coupled with mass spectrometry (Wipf et al., 1979). The values obtained are shown in Table 1.

DESCRIPTION OF THE OUTBREAK

Those exposed to the chemicals remained unaware of their exposure for the first few days after the accident. They were mainly children who lived in the vicinity of the factory. The first symptoms produced by the exposure were itching, redness, and swelling of the skin, which rapidly developed into lesions, resembling first- and second-degree burns, with the formation of blisters and later crusts. Lacrimation and the sensation of grit in the eyes were mentioned, but conjunctivities was not observed. Inhalation of the chemicals probably did not occur because the mucosa of the nose, pharynx, larynx, trachea, and larger bronchi was not affected. These skin lesions, whose onset was noted 3–7 d after the dispersion of the chemicals, were the complaint leading to hospitalization (Gianotti, 1977).

We informed the physicians in charge of these cases in the hospitals

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FIGURE 1. Child ($3\frac{1}{2}$ yr old) with first- and second-degree burnlike skin lesions of the parts of the body exposed to the chemicals (July 1976).

FIGURE 2. The same patient as in Fig. 1, with vesicles and pustules on the hands.

FIGURE 3. The vesicles, papules, and pustules of this case show crusts beginning to form (beginning of August 1976).

FIGURE 4. Comedones and cysts at a specific site, appearing about 2 mo after the exposure (September 1976).

FIGURE 5. The patient shown in Fig. 4, with eruption of chloracne.

TABLE 1. Results of Chemical Analysis of Vegetation Performed by Givaudan Laboratories, July and August 1976

TCDD (μg/100 g vegetation)[a]	Distribution of results for three measuring periods								
	July 16–July 23			July 24–July 30			July 31–August 6		
	No. of samples	ppm	μg/m²	No. of samples	ppm	μg/m²	No. of samples	ppm	μg/m²
> 100	11	1.3–15.8	1,300–15,840	–	–	–	–	–	–
10–99	15	0.1–0.9	100–970	–	–	–	–	–	–
1–9.9	11	0.02–0.09	21–90	2	0.01–0.03	11–35	5	0.01–0.23	10–23
0.1–0.99	5	0.002–0.007	2–7	10	0.001–0.075	1–7.5	24	0.001–0.004	1–4
< 0.1	2	< 0.001	< 1	9	< 0.001	< 1	19	< 0.001	< 1

[a] The original results were expressed as micrograms per 100 g vegetation as a direct measure of toxicological risk for humans and animals. Later results on soil samples were reported in micrograms per square meter, as a measure of general contamination. The conversion of the former unit to the latter (or to ppm) is based on the approximate value of 1 kg grass per square meter. As the vegetation was very variable deviations of a factor of ± 2 may occur.

that besides the acute toxic dermatitis caused by the caustic chemicals from the reactor, they should consider the possibility of other, more serious problems of potential systemic poisoning caused by the presence of TCDD. The physicians were provided with the medical literature pertinent to this substance. In due time most of the patients developed chloracne with different degrees of intensity. Analyses of the vegetation and plants carried out by our laboratories and by the Italian authorities (Adamoli et al., 1978) on samples of soil had shown that TCDD was present in the environment at concentrations up to 5000 $\mu g/m^2$. The TCDD was unevenly distributed in the area. It was therefore possible to subdivide the area into zones of different levels of risk (Bisanti et al., 1978), zone A being the most contaminated, as shown in Table 2.

Almost all of the cases dealt with in this report come from zone A. It can be assumed that they were exposed to the highest level of contamination. Careful and repeated questioning by qualified physicians established that the patients experienced continuous dermal exposure to the chemicals. Furthermore, many of them ingested the chemical by eating vegetables from contaminated gardens and by eating animals (chickens and rabbits) that had lived in the same area (Table 3).

PATTERN OF SYMPTOMATOLOGY

The dermal symptoms that were the reason for the hospitalization or for seeking medical advice were of two types:

1. Diffuse redness and swelling of the skin affecting mainly the exposed areas of the body. In some cases vesicles, pustules, and crusts were present and hyperkeratosis and vermicular atrophy occurred. Itching preceded and accompanied the other signs of acute inflammation, but

TABLE 2. TCDD Levels in Zones A, B, and R, December 1976

	TCDD ($\mu g/m^2$)		
Zone	Highest	Lowest	Average
A1	5477	ND[a]	580.4
A2	1700	6.1	521.1
A3 north	2015	1.7	453.0
A3 south	441	ND	235.5 { 93.0
A4	902	ND	139.9 }
A5	427	ND	62.8
A6	270	ND	29.9
A7	91.7	ND	15.5
B	43.8	ND	3.0
R	< 5.0	ND	0–0.5

[a] ND, not determined.

TABLE 3. Distribution of Cases by Zone of Contamination, Type of Exposure, and Frequency of Hospitalization

Case			Zone of contamination	Type of exposure	Hospitalization (frequency)
Initials	Sex	Age (yr)			
B.M.	M	13	A	Skin contact, ingestion	Yes (1)
B.F.	F	13	A	Skin contact, ingestion	Yes (1)
C.C.F.	M	3	B	Skin contact	No
C.E.P.	F	11	A	Skin contact	Yes (1)
C.P.R.	F	9	A	Skin contact	Yes (2)
C.C.	M	10	A	Skin contact, ingestion	Yes (2)
C.E.	F	6	A	Skin contact, ingestion	Yes (1)
C.K.	F	8	A	Skin contact, ingestion	Yes (1)
C.M.	M	12	A	Skin contact, ingestion	Yes (1)
S.A.M.	M	5	A	Skin contact	Yes (4)
S.A.	F	5	A	Skin contact, ingestion	Yes (4)
S.S.	F	3	A	Skin contact, ingestion	Yes (1)
S.M.	F	9	A	Skin contact, ingestion	Yes (1)
B.R.A.	F	18	B	Skin contact	No
R.S.	M	38	A	Skin contact	Yes (1)
S.J.	F	60	B	Skin contact	Yes (1)
V.A.	F	20	B	Skin contact	Yes (1)

acute initial pain, which is typical of thermal burns, was not mentioned. These skin lesions appeared between 3 and 7 d after the accident and healed without recurrence within 2–3 mo. These have been the only skin manifestations in six of the cases (Table 4).

2. Eruption of blackheads, usually accompanied by cysts of different sizes but commonly not larger than a lentil. In the majority of cases (Table 5) their onset could be dated between 2 wk and 2 mo after exposure. They started to appear when the other skin lesions were fading. In two cases they were the only skin lesions observed from the beginning.

EVOLUTION OF CHLORACNE

The severity of skin lesions was mild in all but in two cases, where inflammatory papules also appeared. Their distribution was typical for chloracne in the majority of the cases, with concentration around the eyes

TABLE 4. Distribution of Cases by Type, Onset, and Duration of Acute Skin Lesions

Case					
Initials	Sex	Age (yr)	Type of skin lesions	Date of onset of first skin lesions	Duration of acute skin lesions (mo)
B.M.	M	13	Acute dermatitis, chloracne	July 14	3
B.F.	F	13	Acute dermatitis, chloracne	July 14	2
C.C.F.	M	3	Acute dermatitis	July 30	2
C.E.P.	F	11	Chloracne	October 1976	–
C.P.R.	F	9	Acute dermatitis, chloracne	July 13	2
C.C.	M	10	Acute dermatitis, chloracne	July 13	3
C.E.	F	6	Acute dermatitis, chloracne	July 13	2
C.K.	F	8	Acute dermatitis, chloracne	July 13	2
C.M.	M	12	Acute dermatitis, chloracne	July 12	2
S.A.M.	M	5	Acute dermatitis	July 18	2
S.A.	F	5	Acute dermatitis, chloracne	July 12	2
S.S.	F	3	Acute dermatitis, chloracne	July 12	2
S.M.	F	9	Acute dermatitis, chloracne	July 17	1
B.R.A.	F	18	Acute dermatitis, chloracne	July 13	2
R.S.	M	38	Acute dermatitis	July 17	2
S.J.	F	60	Acute dermatitis	July 16	2
V.A.	F	20	Acute dermatitis	July 17	2

and on the ears, but they spread to the remainder of the face, neck, shoulder, chest, lower trunk, and limbs in a few cases. The classification recommended by the panel of chloracne specialists appointed for the Seveso accident by the Directorate of Health and Safety of the European Community was used to grade the severity of the skin lesions (Table 6). Only a few cases were moderately severe and none was very severe. Distribution to practically the whole body surface with formation of pustules and papules (grade 5) was not observed in these cases.

The chloracne lasted from 8 to 26 mo, the more severe cases lasting longer. Recovery was complete without scars in all but the two worst cases of the group. The residual scars still present at the time of the last examination by dermatologists (May 1979) in these two cases are limited to the face. They are not disfiguring and are therefore not aesthetically disabling to the subjects. They have continually improved in the course of the last $1\frac{1}{2}$ yr.

TABLE 5. Distribution of Cases by Onset, Intensity, Duration, and Sequelae of Chloracne

Case						
Initials	Sex	Age (yr)	Date of onset	Grade of intensity	Duration (mo)	Sequelae
B.M.	M	13	August 1976	3	12	None
B.F.	F	13	August 1976	2	8	None
C.C.F.	M	3	—	—	—	None
C.E.P.	F	11	October 1976	2	12	None
C.P.R.	F	9	September 1976	2	12	None
C.C.	M	10	August 1976	4	21	None
C.E.	F	6	August 1976	2	14	None
C.K.	F	8	August 1976	3	15	None
C.M.	M	12	August 1976	2	12	None
S.A.M.	F	5	—	—	—	None
S.A.	F	5	July 1976	4	26	Residual scars
S.S.	F	3	July 1976	4	26	Residual scars
S.M.	F	9	September 1976	2	9	None
B.R.A.	F	18	October 1976	2	12	None
R.S.	M	38	—	—	—	None
S.J.	F	60	—	—	—	None
V.A.	F	20	—	—	—	None

Complete recovery in these two cases can therefore be expected (Figs. 1–9). (Figures 1–5, reproduced in full color, may be found between pages 28 and 29.)

The only other symptoms observed in these cases were gastrointestinal tract disturbances of very short duration during the first days after the onset of skin lesions and some burning of the eyes. Temperature always remained normal. All cases required little more than regular cutaneous care by qualified dermatologists. Despite this favorable aspect of the symptomatology, the potential toxicity of TCDD dictated the course of the medical survey of these cases. The possibility of systemic lesions was always kept in mind, and the frequent examinations and laboratory

TABLE 6. Grades of Intensity of Chloracne

Grade	Description
0	No skin lesions
1	A few comedones (up to 10) at specific sites only (face, auricular region, neck, chest)
2	More comedones and no cysts at specific sites only
3	Numerous comedones and cysts at specific sites only
4	Numerous comedones and cysts at specific and other sites (limbs, scrotum, and other regions)
5	Comedones and cysts with inflammatory lesions (papules and pustules) at specific and other sites

analyses were designed to detect acute and subacute visceral toxic effects. Clinical and laboratory data have been used as the basis for the investigation of systemic toxicity.

CLINICAL AND ELECTROPHYSIOLOGICAL EXAMINATIONS

All patients were given repeated complete physical examinations. Liver size in particular was regularly evaluated and any evidence of hepatic enlargement recorded. This organ was slightly enlarged in five patients with higher grades of chloracne and remained so for several months, but there was no correlation with an increase of serum enzymes.

Detailed neurological examination (electroencephalography, electro-

FIGURE 6. Comedones and cysts were still present in the armpits in November 1977, after these symptoms had disappeared from the rest of the body.

FIGURE 7. The patient in Fig. 6 shown in May 1979, with some cysts still in the armpit.

myography, and motor and sensory conduction velocity) showed no evidence of peripheral neuropathy or disturbances of the central nervous system.

Immunocapability was examined by measuring the circulating level of complement, the serum immunoglobulin, and the ability of the circulating lymphocyte subpopulation T and B cells to react to nonspecific mitogens. The immunoresponse was within the normal range in all cases and no immunodeficiency was found.

Chromosome analysis was performed on the blood and the average frequency of gaps, breaks, and structural rearrangements found remained within the accepted normal limits (Table 7). The cardiovascular system was also normal in all cases.

During the observation period—that is, from the first hospitalization or

first medical examination in July 1976 to November 1978—no objective evidence for any visceral or systemic disease was found.

LABORATORY DATA

In all cases the laboratory tests performed, other than routine hemograms and urinalysis, were chosen to provide information about the status of hepatic function, protein, lipid, and carbohydrate metabolism, as well as porphyrin excretion. The hematologic values (blood counts including platelets and prothrombin time) and the urine values always

FIGURE 8. Inflammation of the skin resulted in formation of scars, which were still deep in November 1977.

FIGURE 9. The patient shown in Fig. 8 in May 1979, with the scars showing a tendency to become flat.

remained normal. Results of the relevant laboratory analyses are shown in Tables 8–12.

The values shown in Tables 8–12 are the ranges for each case as well as the mean or median values of all tests performed during the period of monitoring. The values for the two enzymes that express hepatocellular damage, serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT), and the enzyme, γ-glutamyl-transpeptidase (γ-GT), that may express cholestases, liver regeneration, and drug metabolism induction, all remained within the normal range. A temporary but modest increase was registered in some cases, but it was transitory and was below the level that is considered a biochemical manifestation of parenchymal hepatic injury caused by a hepatotoxic

TABLE 7. Functional Tests and Values (Cardiovascular, Central and Peripheral Nervous System, Immunocapability, Chromosome Aberrations)

Case						
Initials	Sex	Age (yr)	Electro-cardiogram blood pressure	EEG, EMG, motor and sensory trans-mission velocity	Immuno-capability	Chromosome aberrations
B.M.	M	13	Normal	Normal	Normal	Within standard range
B.F.	F	13	Normal	Normal	Normal	Within standard range
C.C.F.	M	3	Normal	Normal	Normal	Within standard range
C.E.P.	F	11	Normal	Normal	Normal	Within standard range
C.P.R.	F	9	Normal	Normal	Normal	Within standard range
C.C.	M	10	Normal	Normal	Normal	Within standard range
C.E.	F	6	Normal	Normal	Normal	Within standard range
C.K.	F	8	Normal	Normal	Normal	Within standard range
C.M.	M	12	Normal	Normal	Normal	Within standard range
S.A.M.	F	5	Normal	Normal	Normal	Within standard range
S.A.	F	5	Normal	Normal	Normal	Within standard range
S.S.	F	3	Normal	Normal	Normal	Within standard range
S.M.	F	9	Normal	Normal	Normal	Within standard range
B.R.A.	F	18	Normal	Normal	Normal	Within standard range
R.S.	M	38	Normal	Normal	Normal	Within standard range
S.J.	F	60	Normal	Normal	Normal	Within standard range
V.A.	F	20	Normal	Normal	Normal	Within standard range

TABLE 8. Results of Liver Enzyme Analyses Performed between July 1976 and May 1979

Case			SGOT (5-20 U/ml)[a]			SGPT (5-20 U/ml)[a]		
Initials	Sex	Age (yr)	Range	Mean	*n*	Range	Mean	*n*
B.M.	M	13	7-30	18.5	11	7-41	17.3	11
B.F.	F	13	11-36	20.7	8	10-42	20	8
C.C.	M	5	16-19	17.5	2	11-17	14	2
C.E.	F	14	12-17	14.3	3	6-13	8.5	3
C.P.	F	12	11-27	21	6	5-31	19.8	6
C.C.	M	14	11-28	16.3	9	7-21	12.3	9
C.E.	F	9	9-21	14.6	8	7-16	11.5	8
C.K.	F	11	19-57	32.4	7	14-50	31.8	7
C.M.	M	12	11-21	17.1	8	9-22	15.2	8
S.A.	M	5	9-24	15.25	4	8-18	11.5	4
S.A.	F	7	10-26	17.3	16	8-50	21.8	16
S.S.	F	5	13-48	20.2	10	7-36	13.4	10
S.M.	F	9	9-29	19.8	7	9-22	12.8	7
B.R.A.	F	16	10-25	16.4	5	8-24	13.8	5
R.S.	M	39	11-50	25.5	5	8-100	31	5
S.J.	F	63	13-17	15.3	3	13-16	14.3	3
V.A.	F	20	7-15	9.8	5	4-17	10	5

[a]Normal laboratory values.

TABLE 9. Results of γ-Glutamyltranspeptidase and Alkaline Phosphatase Analyses Performed between July 1976 and May 1979

Case			γ-GT (6–28 U/l)[a]			Alkaline phosphatase (28–48 U/l)[a] (38–138 U/l)[a]		
Initials	Sex	Age (yr)	Range	Mean	*n*	Range	Mean	*n*
B.M.	M	13	6–15	9.8	6	61–302	116.5	6
B.F.	F	13	6–23	13.3	3	58–64	61	2
C.C.	M	5	9–15	11	3	59–177	118	2
C.E.	F	14	6–12	8.6	3	100–123	111.5	2
C.P.	F	12	6–14	10.6	3	56–118	87	2
C.C.	M	14	9–20	14	4	83–305	150.5	4
C.E.	F	9	6–12	9.6	5	62–87	71.3	3
C.K.	F	11	11–56	26	6	105–144	124.6	3
C.M.	M	12	9–18	13.7	4	100–364	195.3	3
S.A.	M	5	7–18	10.5	4	67–346	163.6	3
S.A.	F	7	6–41	26.4	9	44–278	131.1	8
S.S.	F	5	9–12	10.5	4	54–257	167.2	5
S.M.	F	9	7–12	8.7	4	48–69	60	3
B.R.A.	F	16	5–15	8.2	5	44–70	33.9	4
R.S.	M	39	15–40	26.6	3	25–85	55	2
S.J.	F	63	22	–	1	110–115	112.5	2
V.A.	F	20	6–35	20.5	2	34–175	104.5	2

[a]Normal laboratory values.

TABLE 10. Results of Analyses for Serum Bilirubin and Cholesterol Performed between July 1976 and May 1979

Case			Serum bilirubin (0.3–1.1 mg/100 ml)[a]			Serum cholesterol (160–250 mg/100 ml)[a]		
Initials	Sex	Age (yr)	Range	Mean	*n*	Range	Mean	*n*
B.M.	M	13	0.25–0.7	0.43	8	142–252	190	5
B.F.	F	13	0.22–0.25	0.23	2	–	–	–
C.C.	M	5	0.4–0.5	0.45	2	–	–	–
C.E.	F	14	0.35–0.35	0.35	2	–	–	–
C.P.	F	12	0.25–0.35	0.31	5	152–200	174.6	3
C.C.	M	14	0.2–0.6	0.44	5	104–151	129.5	4
C.E.	F	9	0.25–0.5	0.36	4	165	–	1
C.K.	F	11	0.3–0.4	0.35	4	190	–	1
C.M.	M	12	0.3–0.6	0.4	4	121–135	128	2
S.A.	M	5	0.25–0.55	0.45	4	–	–	–
S.A.	F	7	0.2–0.4	0.3	8	–	–	–
S.S.	F	5	0.2–0.5	0.35	4	–	–	–
S.M.	F	9	0.2–0.5	0.4	6	142–196	180	5
B.R.A.	F	16	0.3–0.8	0.54	5	170–280	213.3	3
R.S.	M	39	0.4–0.85	0.73	4	171–690	377	10
S.J.	F	63	0.4–0.5	0.45	3	190	–	1
V.A.	F	20	0.25–0.7	0.45	–	–	–	–

[a]Normal laboratory values.

TABLE 11. Results of Analyses of Serum Triglycerides and Fasting Blood Glucose Performed between July 1976 and May 1979

Case			Serum triglycerides (50–150 mg/100 ml)[a]			Fasting blood glucose (60–100 mg/100 ml)[a]		
Initials	Sex	Age (yr)	Range	Mean	*n*	Range	Mean	*n*
B.M.	M	13	57–256	144.5	4	86–96	91	2
B.F.	F	13	158	–	1	75–90	82.5	2
C.C.	M	5	–	–	–	72–95	83.5	2
C.E.	F	14	–	–	–	85–93	89	2
C.P.	F	12	102–158	130	2	85–86	85.5	2
C.C.	M	14	86–106	97	3	77–95	86	2
C.E.	F	9	118–222	154	3	77–88	83.5	4
C.K.	F	11	91–176	122.3	3	76–112	92	4
C.M.	M	12	112–120	116	2	78–85	80.6	3
S.A.	M	5	–	–	–	72–95	83.5	2
S.A.	F	7	58–250	112.5	4	48–101	78.7	9
S.S.	F	5	47–185	116	2	40–108	69.6	5
S.M.	F	9	58–201	126.5	4	75–108	91.5	2
B.R.A.	F	16	113–162	132.6	3	–	–	–
R.S.	M	39	102–1700	642	7	104–160	130.4	5
S.I.	F	63	–	–	–	109–138	123.5	2
V.A.	F	20	–	–	–	80–88	82.6	3

[a]Normal laboratory values.

TABLE 12. Results of Coproporphyrin and Uroporphyrin Analyses Performed between July 1976 and May 1979

Case			Coproporphyrin (20–250 μg/24 h)[a]			Uroporphyrin (10–30 μg/24 h)[a]		
Initials	Sex	Age (yr)	Range	Mean	*n*	Range	Mean	*n*
B.M.	M	13	42–74	58	2	–	–	–
B.F.	F	13	–	–	–	14–20	17	2
C.C.	M	5	36	–	1	–	–	–
C.E.	F	14	–	–	–	–	–	–
C.P.	F	12	–	–	–	24	–	1
C.C.	M	14	–	–	–	10–19	14.5	2
C.E.	F	9	24	–	1	16	–	1
C.K.	F	11	30	–	1	9	–	1
C.M.	M	12	–	–	–	–	–	–
S.A.	M	5	24	–	1	–	–	–
S.A.	F	7	20–41	30.5	2	9–20	14.5	2
S.S.	F	5	20	–	1	9	–	1
S.M.	F	9	–	–	–	–	–	–
B.R.A.	F	16	10	–	1	10	–	1
R.S.	M	39	15	–	1	–	–	–
S.I.	F	63	–	–	–	–	–	–
V.A.	F	20	–	–	–	–	–	–

[a]Normal laboratory values.

agent (Czaja and Summerskill, 1978; Leevy and Tygstrup, 1976; Wright et al., 1977).

Serum bilirubin was not increased. The elevation of alkaline phosphatase in some cases may be related to age (children in the period of growth). Blood glucose, when measured under fasting conditions (hospitalized cases), was normal except in those (B.P., C.P.R., R.S., S.J.) who were known to be diabetic or who had an abnormal glucose tolerance test. As for lipid metabolism, both serum cholesterol and serum triglycerides remained normal. In the cases where the test was performed, there was no increase of either uroporphyrin or coproporphyrin in the urine.

PATHOGENESIS OF THE SKIN LESIONS

Chloracne has been described as a refractory acne seen in workers involved in the production of chlorinated aromatic compounds. Kimmig and Schulz (1957) described this dermatologic condition in workers in a trichlorophenol factory and showed that trichlorophenol and its salts, when applied to cutaneous areas, are not acnegenic. They showed, however, that the contaminant TCDD isolated from trichlorophenol is a potent acnegenic agent. It can therefore be assumed that among the chemicals dispersed in the environment in the accident of July 1976, TCDD was the cause of chloracne in the cases analyzed in this report.

The other skin lesions (erythema, acute dermatitis) were most likely caused by the other chemicals. In the cases where chloracne did not subsequently develop, the level of contamination and therefore of exposure to TCDD was not sufficiently high and the sensitivity of the subjects was lower than in the other cases. (These cases were adults, and may have been less sensitive to acnegenic activity because of their age.) The fact that chloracne did not appear in these cases does not exclude the possibility that they absorbed a minimal amount of TCDD by skin contact or ingestion.

Analysis of blood and urine samples to detect TCDD were performed in five of the cases (S.R.A., C.E., C.K., C.M., S.M.) but no detectable quantities of TCDD were found. The negative results may be due to the limits of detection of the methods of analysis used.

Tissue levels were analyzed in a 55-yr-old woman who died of pancreatic carcinoma 7 mo after the accident. She lived close to the factory and was certainly exposed to TCDD through ingestion if not by direct contact. Two members of her family (S.A. and S.S.) developed severe chloracne. Neither she nor the parents of the children developed chloracne or had any skin lesions. The TCDD levels found in the tissues are reported in Table 13.

From these values, one can speculate about the TCDD levels that lead to skin, liver, and neurological lesions, but these data provide irrefutable evidence of the presence of TCDD in the population.

TABLE 13. Levels of TCDD Found in Human Tissues, July 28, 1977[a]

Sample	Origin	Quantity (g)	Limit of detection (ppt)	Recovery (%)	TCDD[b] (ppb)
Liver	Autopsy	10	10	64	0.15
Fat		10	10	59	1.84
Pancreas		5	10	59	1.04
Lung		10	10	60	0.06
Kidney		10	10	60	0.04
Brain		10	10	60	0.06

[a]Vacuum Generator Micromass Laboratory, Altrincham (Manchester, England).

[b]For a total body weight of 70 kg the calculated total amount at time of death is 40 μg.

CONCLUSIONS

Twenty-two people who suffered injuries after exposure to the released chemicals during the first days after the Seveso accident were referred to the judge holding the inquest. They were 7 adults and 15 children. The judge appointed a medical commission, who examined the cases and prepared a report for the judge's office. Of the 22 original cases, 3 were excluded because their injuries were not associated with the accident and 2 because they did not submit to the medical examinations. Of the remaining 17 who suffered burnlike skin lesions, all but 2 recovered without residual effects. Chloracne occurred in 12 of these cases; it was mild in almost all cases and has now disappeared. Systemic functions were repeatedly evaluated in all cases and no evidence of visceral lesions was obtained. Immunocapability was normal and chromosome aberrations were within the normal range.

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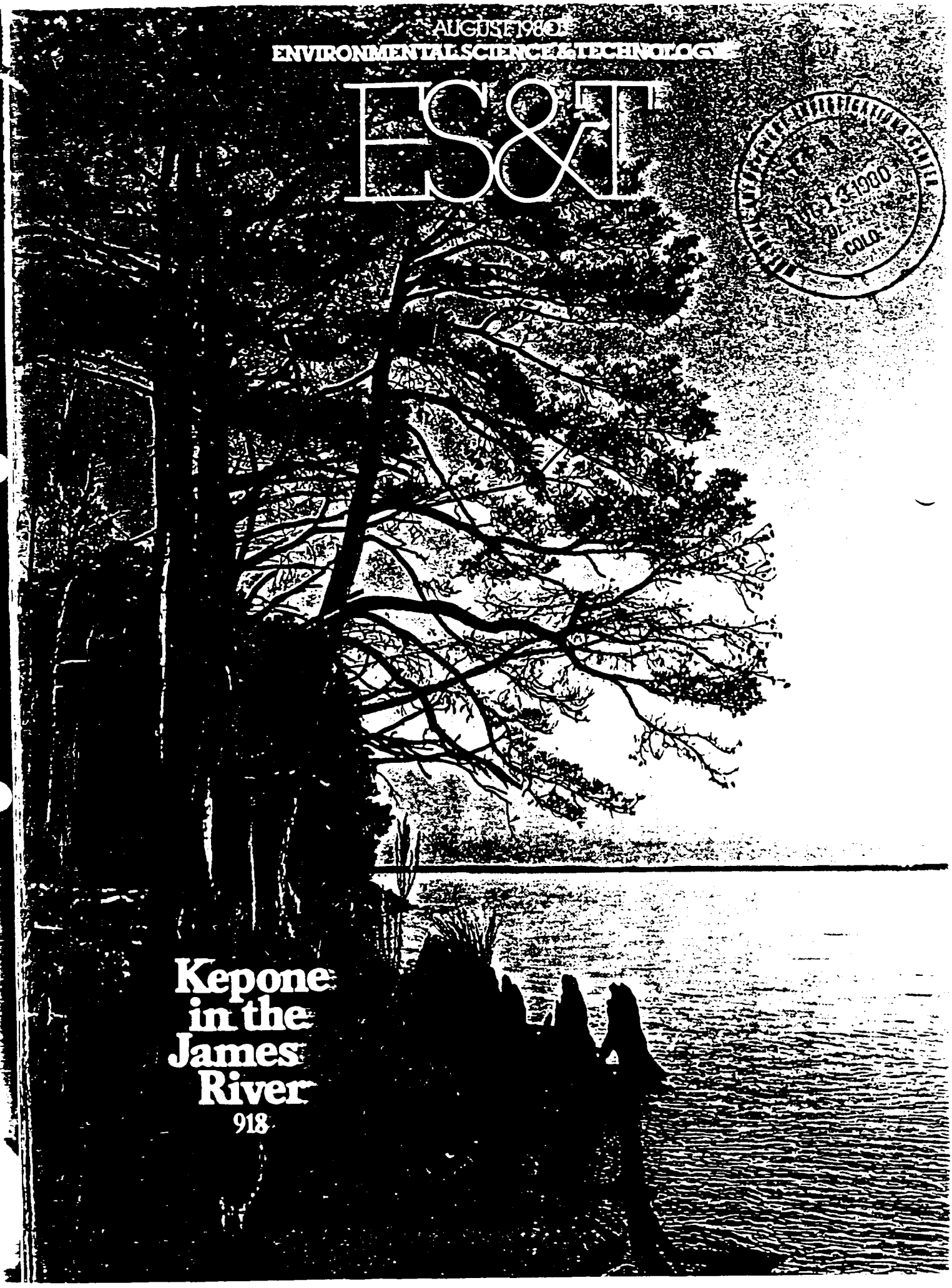

Kepone in the James River

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Experiences in Human Milk Analysis for Halogenated Hydrocarbon Residues

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Factors affecting the different stages of human milk analysis for halogenated hydrocarbon residues are explored. The variation in milk fat during breast feeding and its consequences for sampling are outlined. Extraction efficiency is discussed in terms of fat content, residue recovery and sample size.

The importance of clean-up procedures is illustrated by the removal of fat from the extract and unknown contaminants from adsorbents. Polychlorinated biphenyls (PCBs) are partially separated from other halogenated hydrocarbon residues on a Florisil-silicic acid column.

Both packed and capillary gas chromatographic columns can be used for identification and quantification of residues in human milk.

Confirmation by chemical derivation and mass spectrometry is illustrated by the perchlorination of PCBs and PCB isomer distribution respectively. Fortification of samples and the use of a collaborative study are described as a means for method evaluation.

KEY WORDS: Human milk, residue, recovery, effect.

INTRODUCTION

Halogenated hydrocarbon residues in human milk, such as polychlorinated and polybrominated biphenyls, Mirex, polychlorinated terphenyls and many organochlorine pesticides, have been the subject of many studies.[1-9] The purpose of most of these studies was to gather residue data to determine the extent of infant exposure and to evaluate the potential health hazard. Few studies dealt with the analytical problems and limitations of analyzing halogenated hydrocarbon residues in human milk. Nevertheless, the residue data from such monitoring studies are often used to help to determine an acceptable level of infant exposure.

In this paper some of the analytical aspects of human milk analysis will be considered from the point of view of sampling, extraction, clean-up, separation, identification, quantification, confirmation and evaluation.

SAMPLING

Sampling is an important part of any analytical procedure, but especially so in the case of biological samples. A representative sample of human milk is difficult to obtain, both because of its limitation in sample size and possible variation in fat composition. It has been reported, that the fat content of human milk not only increases during the feeding of the infant, but changes from feeding to feeding during the day.[8] Hytten reported a difference in the fat content of the milk between the right and left breast.[10] This author also observed, that the fat content was at its lowest and highest at the 6 and 10 a.m. feedings, respectively. Table I shows some of

TABLE I

Variation in fat content of human milk during feeding

Sample code[a]	% Fat	
	Donor 1[b]	Donor 2[c]
M1	2.21	0.97
M2	1.86	0.54
M3	2.55	0.66
M4	3.80	0.55
M5		0.73
M6		0.63
MD1	0.55	1.10
MD2	0.89	1.09
MD3	1.93	1.34
MD4	3.55	1.52
MD5	4.69	1.85
MD6		1.48
E1	1.35	3.19
E2	1.59	3.21
E3	2.47	3.43
E4	3.78	3.63
Average	2.40	1.62

[a] M = morning feeding
MD = mid-day feeding
E = evening feeding
[b] = 20 ml fractions
[c] = 10 ml fractions

this variation in the fat content of human milk. Both donors expressed milk from the right breast only and did not feed the infant on that breast between morning and evening milk expressions. Donor 1 expressed milk at 7 a.m. and 1 and 6.30 p.m. on her 126th day of lactation, while donor 2 expressed milk at 5.30 a.m. and 11.30 and 5.30 p.m. on her 177th day of lactation. The importance of the fat content, for the determination of halogenated hydrocarbons, lies in the lipophilic nature of these contaminants and its use for calculating residue levels on a fat basis. Therefore, the timing in milk sampling is important. At the present state of our knowledge it seems prudent to take a random sample.

The mother should be asked to express her milk at different times during a particular feeding, at all feedings during the day and alternating between right and left breast. The amount of sample to be collected depends on the requirements of the analytical procedure, expected residue levels and availability. A 50-ml sample, which represents approximately less than 10% of the total volume of milk available per day, would be ample, but not necessarily representative. The milk should be collected without external contamination.

In our laboratory a special kit is prepared, consisting of a 4-oz brown bottle with Teflon-lined cap, a 16-oz large-mouth jar, a 50-mm glass funnel and instruction sheet with questionnaire. All glassware in the kit is washed and heated at 350 C for 1 hr and finally rinsed 5 × with acetone and 3 × with hexane. The funnel is wrapped in aluminum foil, of which the non-shiny side (in contact with the funnel) has been rinsed with hexane. The coded questionnaire covers such questions as age of mother, fish eating habits, pesticide exposure, menstrual irregularities, number of babies breast fed, etc. The mother is requested to express the milk manually into the large mouth jar, transfer it to the brown bottle via the glass funnel and keep the sample refrigerated between collections. After the final collection the milk should be frozen, and kept that way during transport and storage until analysed.

EXTRACTION

Halogenated hydrocarbon residues are lipophilic and may be isolated by separation or extraction of the milk fat. This can be done by direct solvent extraction of the human milk or by a combination of physical separation of fat from milk and solvent extraction as shown in Figure 1. The choice of extraction method will depend on the extraction efficiency of a particular residue or residues, required accuracy and available time and equipment. In our laboratory, PCBs in human milk have ben analyzed, using several extraction techniques and solvents, including saponification

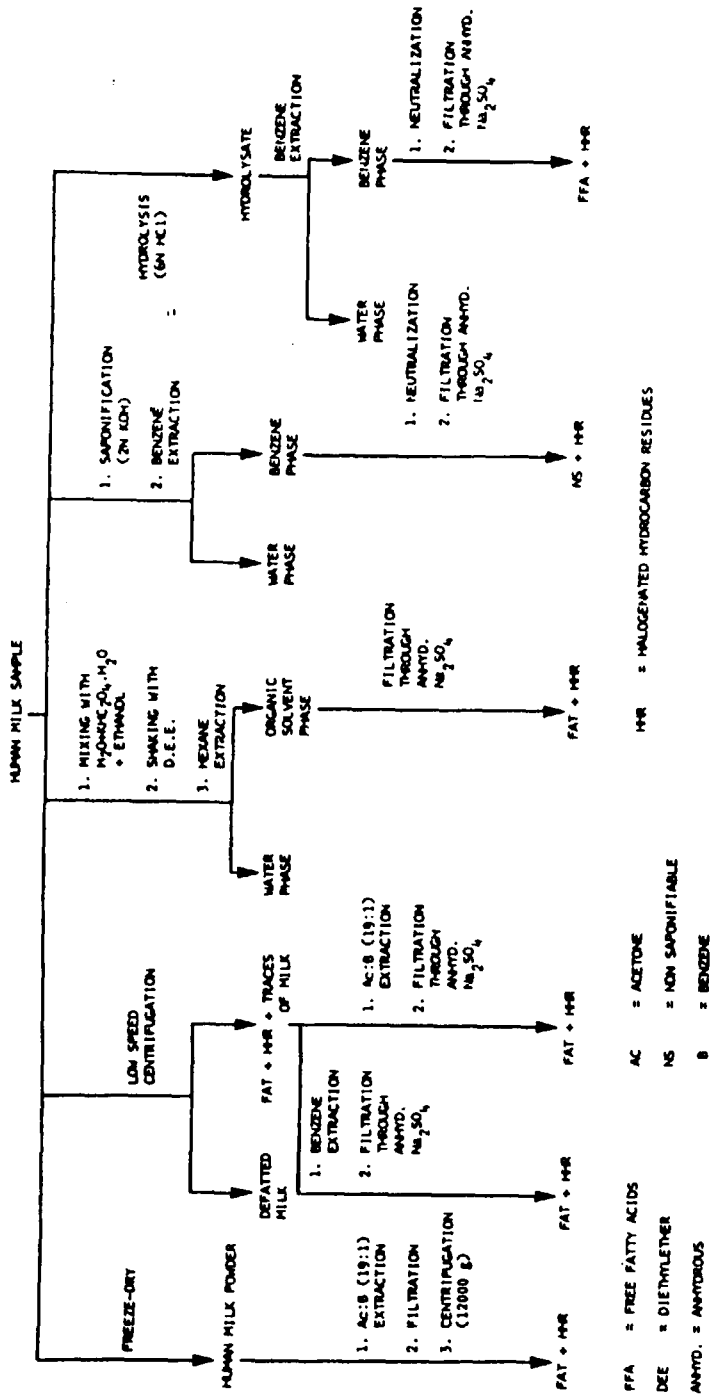

FIGURE 1 Scheme for the extraction of halogenated hydrocarbons from human milk by various methods.

and acid hydrolysis. The latter two procedures have the advantage that halogenated hydrocarbon residues can be extracted with considerable less lipid material, but the disadvantage that some residues may be destroyed or altered.

PCBs however are an important exception. In the saponification method, the fat content can be determined by acidifying the alkaline water layer after benzene extraction. The results in Table II are based on 25 g samples, but for hydrolysis and saponification only 10 g samples were used. Although the "direct extraction" method gave the highest PCB level, it also showed the greatest variation (coefficient of variation = ~ 30)[11]

Small samples (< 2 ml) would eliminate the need to physically separate the fat by centrifugation prior to extraction.[12]

TABLE II

The effect of residue isolation techniques on the fat and PCB content of human milk

Method	Extraction solvent	% fat	C.V.	ng PCB per g whole milk[a]	C.V.
Freeze-drying	Ac:C_6H_6 (19:1)[d]	1.22	0.8	7.0	6.2
Centrifugation	C_6H_6	1.17	3.8	7.4	2.3
Centrifugation	Ac:C_6H_6 (19:1)	1.13	0.5	7.2	2.9
Direct extraction	EtOH-DEE[c]-Hex	1.34	2.2	8.6	32.6
Acid hydrolysis	C_6H_6	N.A.		6.6[b]	3.2
Saponification	C_6H_6	N.D.		7.0[b]	7.1

[a] Triplicate determinations
[b] Duplicate determinations
[c] Ethanol-diethylether
[d] Acetone
N.A. = not applicable
N.D. = not determined
C.V. = coefficient of variation.

Table III compares the fat and PCB content obtained by using the macro (25 g) and micro (2 g) methods. There seems to be little difference in the fat content. The lower values for the micromethod may have partly been caused by losses incurred during the fat determination step, where the entire extract is transferred to and evaporated in an aluminum weighing dish before column chromatography. In the macromethod, only an aliquot of the extract is removed for fat determination. However, both macro- and micro-values may well fall within the expected variation at these low residue levels.

TABLE III

The effect of sample size on the extraction efficiency of fat and PCBs from human milk

Extraction method[a]	Extraction solvent	% Fat[b]				ng PCB/g whole milk[a]			
		Macro	C.V.	Micro	C.V.	Macro	C.V.	Micro	C.V.
1	Ac:C_6H_6 (19:1)	1.22	0.8	0.92	0.0	7.0	6.2	6.9	4.3
2	C_6H_6	1.17	3.8	0.77	5.1	7.4	2.3	4.4	4.5
3	Ac:C_6H_6 (19:1)	1.13	0.5	1.13	5.3	7.2	2.9	5.1	5.9
4	DEE-Hex	1.34	2.2	1.25	0.8	8.6	32.6	6.7	6.0

[a] 1: freeze-drying for both macro- and macromethod
2 and 3: centrifugation and homogenization for macro- and micromethod, respectively
4: direct extraction for both macro- and micromethod
[b] Triplicate determinations

What is not apparent from Table III is the background problem during gas chromatography. Figure 2 compares chromatograms obtained with the macro and micromethods. The negative background differed in magnitude. From this example it is evident, that quantification poses a problem.

However, using a capillary GC system good agreement and acceptable chromatograms were obtained comparing macro and micromethods (Table IV and Figure 3). Extraction solvents were not the same: acetone:benzene (19:1 v/v) was used in the micro and benzene in the macromethod. The differences between results in Table III and IV may lie in the magnitude of the residue levels measured. The lower the residue level the more difficult the determination will be.

Although there is no strong indication, which of the two methods is superior, the higher concentration of residues obtained with the macromethod would give less background problems in GC analyses.

CLEAN-UP

Clear-up procedures are very important, when minute quantities of residues have to be analyzed. Both the sample itself and all other media with which the sample comes in contact should be free of interfering contaminants. In human milk the fat and extraneous matter, such as colors, have to be removed in order to clearly identify the residues by GC. In our laboratory two approaches have been used: precipitation of the fat at −72°C (freeze-out)[1,2] and/or retention on a Florisil column. Table V shows to what extent fat can be removed on a semi-micro combined Florisil-silicic acid column. The latter can take up to 350 mg of fat before contaminating the PCB fraction (35 ml Hexane). A combination

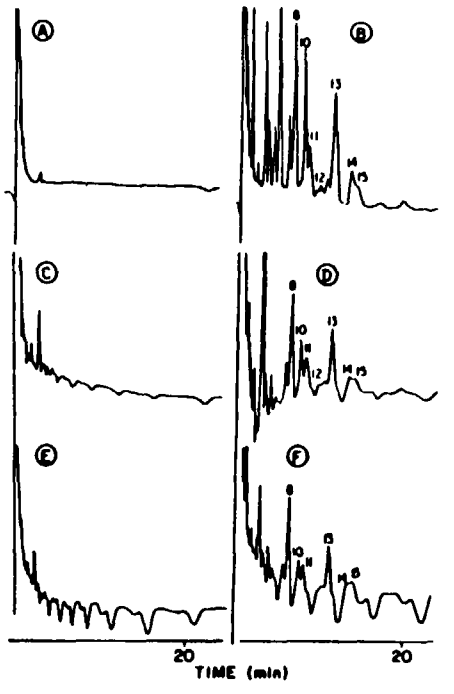

FIGURE 2 The GC elution pattern of PCBs in human milk (B, D, F) and blanks (A, C, E) on packed column (183 × 0.6 cm; 6% OV-210 + 4% SE-30 on Chromosorb W (AW) 100/120, column temperature 208°C). Chromatograms A and B: macromethod (freeze-dried sample), C and D: micromethod (freeze-dried sample); E and F: micromethod (centrifuged sample)

of the freeze-out and column techniques is required for large samples with high lipid content.

Adsorbents and solvents are two of the most important contributors to sample contamination. Glass distilled solvents, although free of interfering residues under normal conditions (concentration 250:1), show contaminants when large volumes are concentrated to 1 ml or less. Adsorbents, such as Florisil and silicic acid should be cleaned to remove all traces of contamination, that could interfere at the level of sample concentration expected during the analysis.

Figure 4 shows a slightly modified soxhlet extraction set-up for washing adsorbents. This modification allows the collection of extraction solvent (CH_2Cl_2) during the siphoning cycle via a two-way stopcock. A 50-ml aliquot is collected and concentrated to 0.2 ml. Figure 5 shows contaminants found in successive washings.

TABLE IV

A comparison of the PCB levels in human milk between the macro- and micromethods

Sample no.	ng PCB/g whole milk		Ratio
	Macromethod	Micromethod	Macro/micro
1HM/7	32	32	1.00
1HM/14	25	29	0.86
1HM/28	24	24	1.00
1HM/42	14	13	1.08
1HM/70	13	15	0.87
2HM/14	36	29	1.24
2HM/28	26	32	0.81
3HM/7	6	14	0.43
5HM/14	26	27	0.96
5HM/28	20	30	0.67

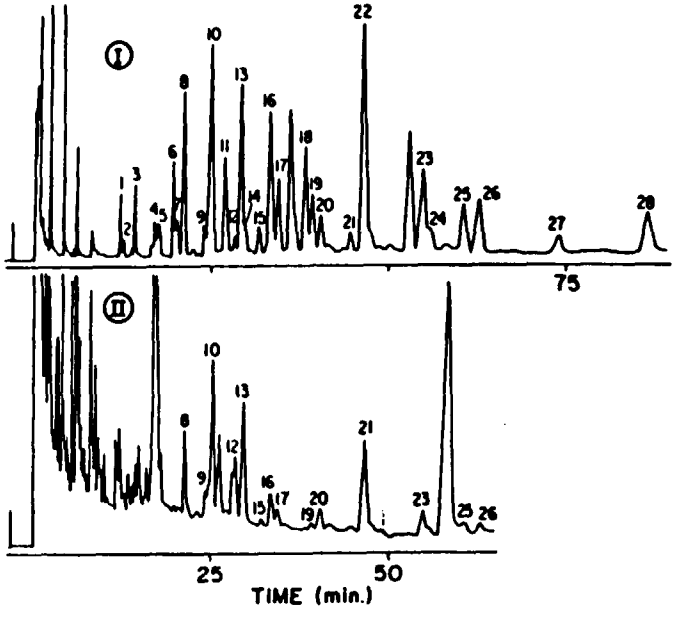

FIGURE 3 The GC elution patterns of standard Aroclor 1260 (I) and PCBs in human milk (II) on capillary column (36 m × 0.5 mm; SP 2100; column temperature 224°C).

TABLE V

Retention of rapeseed oil on a combined semi-micro Florisil-silicic acid column

Fraction[a]	% Recovery[b]					
	Lipid load					
	50 mg	C.V.	100 mg	C.V.	200 mg	C.V.
I	0.2	0	N.D.		N.D.	
II	0.3	33	0.1	0	0.1	0
III	0.2	0	0.5	20	0.8	13
IV	7.0	37	12.1	12	53.9	15
V	83.0	3	80.9	2	41.6	19
VI	N.C.		8.4	2	5.2	104

[a] I = 35 ml hexane; II to V = 40 ml of 20, 40, 60 and 80% $CHCl_3$ in hexane respectively, VI = 40 ml of 20% ethylacetate in CH_2Cl_2

[b] Average of 3 determinations

N.D. = not detected, N.C. = not collected

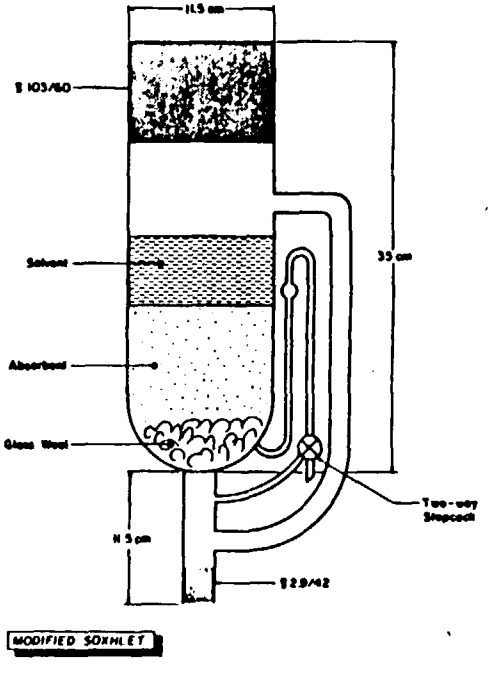

FIGURE 4 Modified soxhlet apparatus for washing adsorbents.

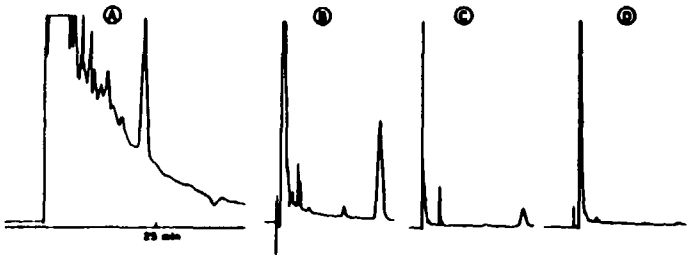

FIGURE 5 Contamination in Florisil washings (250:1). Chromatogram A = after 1st siphoning; B = after 4 hrs; C = after 8 hrs; D = after 12 hr.

SEPARATION

Human milk is often monitored for residues of different polarity such as PCBs, DDTs, Dieldrin and Heptachlorepoxide. A pre-gas chromatographic separation is not only desirable, but often necessary. In our laboratory a combined semi-micro Florisil-silicic acid column is used to separate halogenated hydrocarbons into several fractions. Table VI shows the elution pattern of a selected number of halogenated hydrocarbons, many of them appearing in human milk. It is evident from Table VI, that many compounds elute in more than one fraction, especially Aroclors. It is fortunate, that the PCB pattern in human milk closely resembles that of Aroclor 1260, which elutes 95% in one fraction. Recently however, lower chlorinated biphenyls have been reported in human milk.[13] These lower chlorinated biphenyls would elute in more than one fraction. Nevertheless, many other compounds can still interfere with the PCB analysis, such as p,p'-DDE and Mirex.

IDENTIFICATION AND QUANTIFICATION

Individual compounds can be identified by gas chromatography. The type of column and/or stationary phase to be used depends on the analytical requirements and available equipment. For example, PCBs can be identified on both packed and capillary columns. Figure 6 shows PCBs in human milk chromatographed both ways. In our laboratory PCBs in human milk are estimated on a packed column, using peaks 8 and 10 15, according to the numbering system of Reynolds[14] and Jensen and Widmark.[15] On the capillary column, peaks 8–13, 15–17, 19, 20, 22, 23, 25, 26 and 28 were used according to our own numbering system. Quantification will only be as accurate as the separation is good. No

doubt the capillary column gives better separation than the packed column, but from experience in our laboratory there was no quantitative difference. This could indicate, that compounds other than PCBs were not interfering on the packed column. Capillary gas chromatography holds the

TABLE VI

Elution pattern of some halogenated hydrocarbons from a combined Florisil-silicic acid column

Compound (2.5 μg each)	% Recovery									
	Fraction*									
	I	C.V.	II	C.V.	IV	C.V.	VI	C.V.	Total	C.V.
Aroclor 1016	62	5	43	3					105	3
Aroclor 1242	54	9	47	10					101	4
Aroclor 1254	88	2	16	21					104	4
Aroclor 1260	95	1	7	14					102	1
Aroclor 5460	36	4	74	6					110	5
Mirex	88	8	*t*		2	140			90 +	9
Photomirex	107	3	3	13					110	3
p,p'-DDE	19	1	88	3	2	5	*t*		109	2
pentachlorobenzene	107	3	1	50					108	2
hexachlorobenzene	95	2	*t*						95 +	2
heptachlor	22	94	79	43	1	140			102	11
Polybrominated biphenyl	84	6	22	57	4	68			110	9
Methylated pentachlorophenol			101	6	*t*				101 +	6
heptachlor epoxide			1	40	87	3	*t*		88 +	2
o,p'-DDT			102	6					102	6
p,p'-DDT			107	7					107	7
oxychlordane			113	5	*t*				113 +	5
γ chlordane			90	2	*t*				90 +	2
α hexachlorocyclohexane			96	3	*t*		*t*		96 +	3
β hexachlorocyclohexane			26	7	74	3	*t*		100 +	1
γ hexachlorocyclohexane			93	3	7	16	*t*		100 +	3
toxaphene			90	2	8	13	*t*		98 +	3
dieldrin					96	2	*t*		96 +	2
methoxychlor					94	7	12	45	106	2
octachlorodibenzo-p-dioxin					5	36	85	7	90	9
octachlorodibenzofuran					8	6	94	4	102	4

*Fractions are the same as in Table V

†t = trace (< 1%)

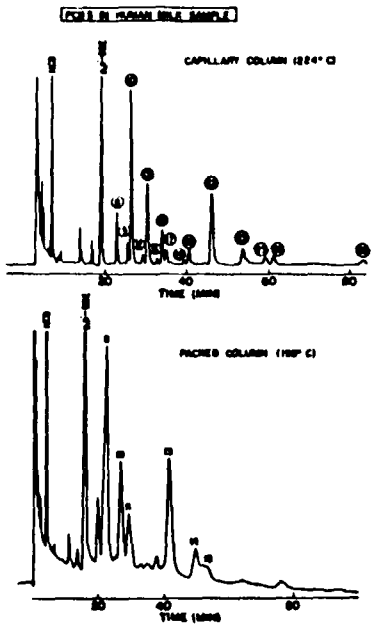

FIGURE 6 GC elution patterns of PCBs in human milk on capillary and packed columns.

possibility of eliminating pre-gas chromatographic fractionation, because it can separate many more compounds at a given time. The simultaneous separation of 17 organochlorine pesticides is shown in Figure 7. This figure also illustrates that caution is needed in identification, since column temperature can affect elution time on capillary columns, as indicated by p,p'-DDE and dieldrin.

CONFIRMATION

At present PCBs are the most important industrial contaminant in human milk and when identified, their presence should be confirmed, if at all possible. In a single sample the residue level is often low for mass spectrometry or chemical derivation, and pooling of samples may be necessary. The determination of PCBs by conversion to the decachlorobiphenyl by means of perchlorination, seems to compare favourably to direct measurement of the PCB pattern by GC (Table VII).

The success of this derivation technique may partly depend on the amount of higher chlorinated biphenyls in the human milk. The lower chlorinated biphenyls are more volatile and easily lost, and recently,

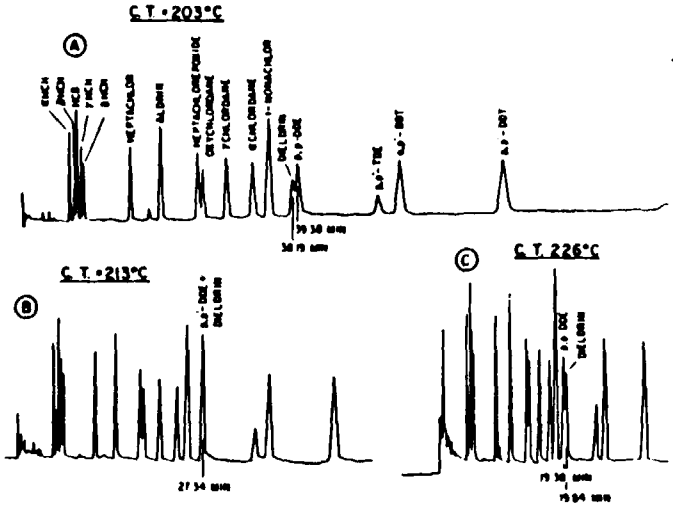

FIGURE 7 The effect of capillary column temperature on the elution time of p,p'-DDE and dieldrin.

TABLE VII

Comparison of PCB quantification in human milk between gas chromatographic and perchlorination techniques

Sample code	ng PCB/g whole milk		
	PC[a]	GC[b]	PC/GC
M1	5	6	0.83
M2	5	6	0.83
M3	9	6	1.50
M4	9	9	1.00
MD1	6	3	2.00
MD2	4	4	1.00
MD3	6	6	1.00
MD4		8	
MD5	16	15	1.07
E1	5	5	1.00
E2	6	5	1.20
E3	7	7	1.00
E4	8	8	1.00

[a]PC = perchlorination (corrected for blank)
[b]GC = gas liquid chromatography

substantial quantities were found in human milk.[5] It is our experience that better results were obtained when extracts were immediately perchlorinated after the GC pattern was established.

Although the quantitative value of this technique may be questioned, the validity of the confirmatory aspect remains, if no biphenyl is present.

Preliminary results on the possible variation within the PCB isomer distribution due to extraction technique, have been reported[11] and are shown in Table VIII. These results indicate the need for further investigation into the effect of extraction technique on isomer distribution, especially since the data are based on a single MS determination. Nevertheless, the first three methods in Table VIII gave almost identical isomer composition, while two of these methods used the same extraction solvent. Aroclor 1260 contains 12% penta-, 38% hexa-, 41% hepta- and 8% octachlorobiphenyl.[16] This composition closely approximates that found for the first three methods, except for hexachlorobiphenyl.

TABLE VIII

Variation in the isomer distribution of PCBs extracted from human milk by various techniques

Extraction[a]		% [b]					
Method	Solvent	Cl_3	Cl_4	Cl_5	Cl_6	Cl_7	Cl_8
Freeze-dry	Ac:C_6H_6	7	25	12	14	35	7
Centrifugation	C_6H_6	4	26	13	14	34	8
Centrifugation	Ac:C_6H_6	4	25	12	15	36	8
Direct	EtOH:ether:hex	10	28	16	12	27	7
Acid hydrolysis	C_6H_6	7	17	10	13	44	9
Saponification	C_6H_6	4	15	11	14	49	8

[a] For details of extraction techniques, see Reference 11.
[b] Percent of tri- (Cl_3), tetra- (Cl_4), penta- (Cl_5), hexa- (Cl_6), hepta- (Cl_7) and octachlorobiphenyl (Cl_8) present.

EVALUATION

It is evident from the foregoing, that the analyst has to make many decisions before a routine analytical procedure is established. He must decide how best sampling, extraction, separation, identification and quantification should be carried out and to what extent his results needs confirmation. In some cases objective judgements cannot be easily made, e.g. does the PCB pattern in human milk resemble Aroclor 1254 or 1260? How many and which peaks to use for quantification?

The reliability of the methodology may be supported by fortification experiments, where known amounts of halogenated hydrocarbons are

added to the human milk. However, even excellent fortification results do not necessarily give an accurate representation of the residue level in the sample, or indicate the suitability of the method.[17] Nevertheless this technique is the only guide for the residue chemist in evaluating his methodology, without extensive research.

Table IX shows recoveries of chlorinated hydrocarbons from human milk fortified at the 100 ppb level. Acetone:Benzene (19:1 v/v) was used as extraction solvent and the fat removed by precipitation. Recoveries of PCB and HCB are not only lower, but also show much greater variation than for other compounds.

Recovery of PCBs at low levels of fortification (10 ppb), using a micromethod (no freeze-out procedure), was >95% as shown in Table X

TABLE IX

Recovery of chlorinated hydrocarbons from human milk samples fortified at 100 ppb

Compound	% Recovery ± S.D. ($N = 5$)[a]
PCB (Aroclor 1260)	70.6 ± 15.9
HCB	78.4 ± 17.5
βHCH	87.3 ± 5.2
heptachlor epoxide	97.6 ± 10.0
oxychlordane	88.7 ± 5.2
p,p'-DDE	91.1 ± 11.8
dieldrin	88.1 ± 8.0
o,p'-DDT	89.3 ± 5.7
p,p'-DDT	85.9 ± 13.0

[a] S.D. = standard deviation, N = number of samples

TABLE X

Effect of various parameters on the recovery of PCB from human milk, fortified at 10 ppb

Sample no.	Fortification		Extraction solvent	ng PCB/g whole milk[a] (as Aroclor 1260)	% Recovery[b]
	Solvent	Volume (μl)			
1	—		Benzene	3.1	
2	Acetone	20	Benzene	7.4	43
3	Acetone	100	Benzene	7.7	47
4	Hexane	100	Benzene	12.3	95
5	Acetone	100	Acetone:C_6H_6	13.4	106

[a] Average of 3 determinations (1 ml samples)
[b] Corrected for background
Not detected

Recovery is apparently affected by the right combination of extraction and fortification solvents. This apparent increase in PCB recovery from human milk with decrease in fortification level (100 vs. 10 ppb) lies more in the evolution of analytical technique than in the level of fortification.

The reliability of a method and consequently its usefulness may be verified by a collaborative study. Table XI shows the results of a limited study of PCBs in human milk, using a modified "Direct extraction" method.[2] The extracted milk fat was mixed with 25 g of 2% deactivated Florisil, which was then added to 25 g Florisil already in a 22-mm I.D. glass column. The PCBs were eluted in 300 ml hexane. This hexane eluant was treated with 0.5 N KOH before GC analysis. The coefficient of variation for both fat and PCB level was ~30. However, the results do give a certain degree of confidence, considering the level of contamination, the problems of quantification and the inexperience of some laboratories.

TABLE XI

PCB and fat levels in a human milk check sample

Laboratory no.	% Fat	ng PCB/g whole milk
1	3.4	27
2	3.9	67
3	4.9	20
4	2.9	22
5	3.3	13
6	3.0	11
7	1.9	16
Average ± S.D.[a]	3.3 ± 0.9	17 ± 5[b]

[a] S.D. = standard deviation.
[b] Laboratory no. 2 excluded.

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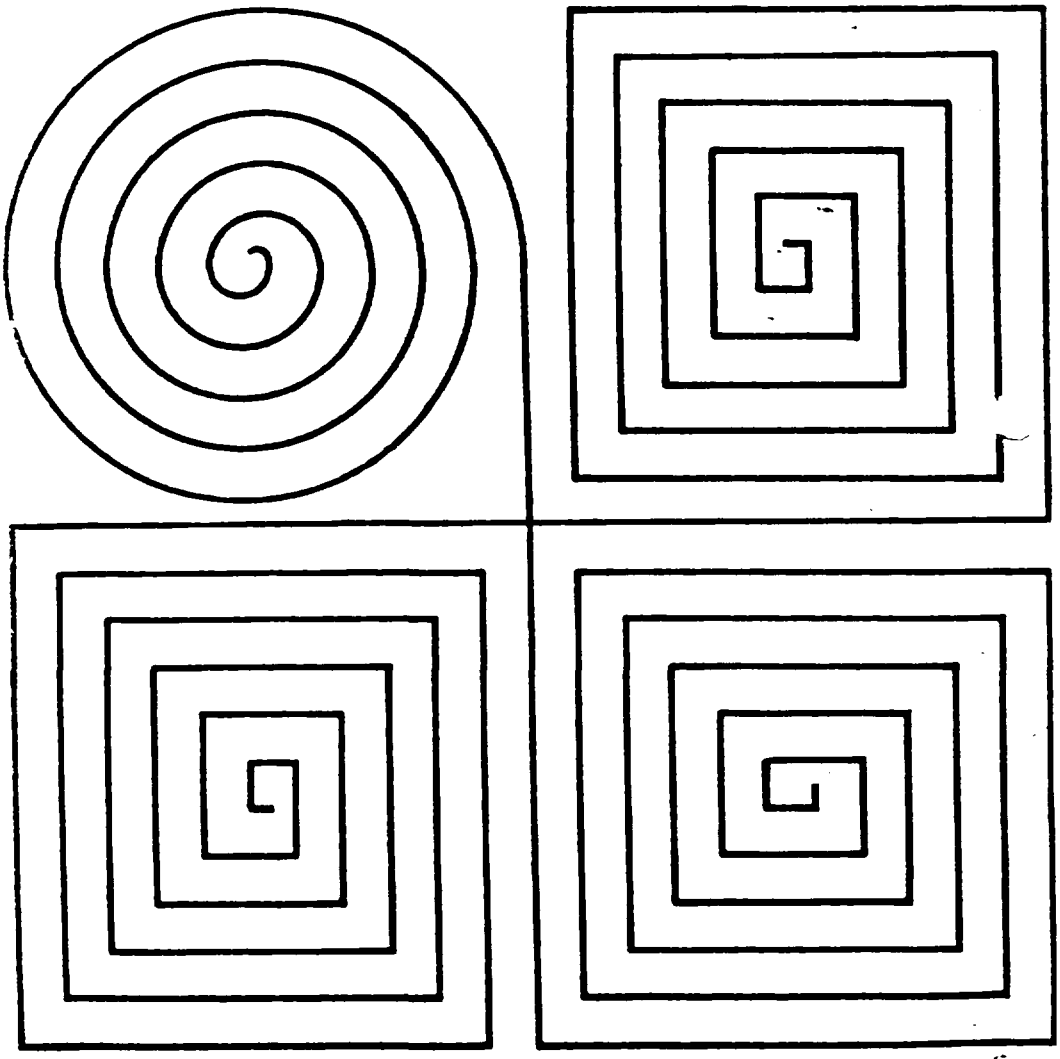

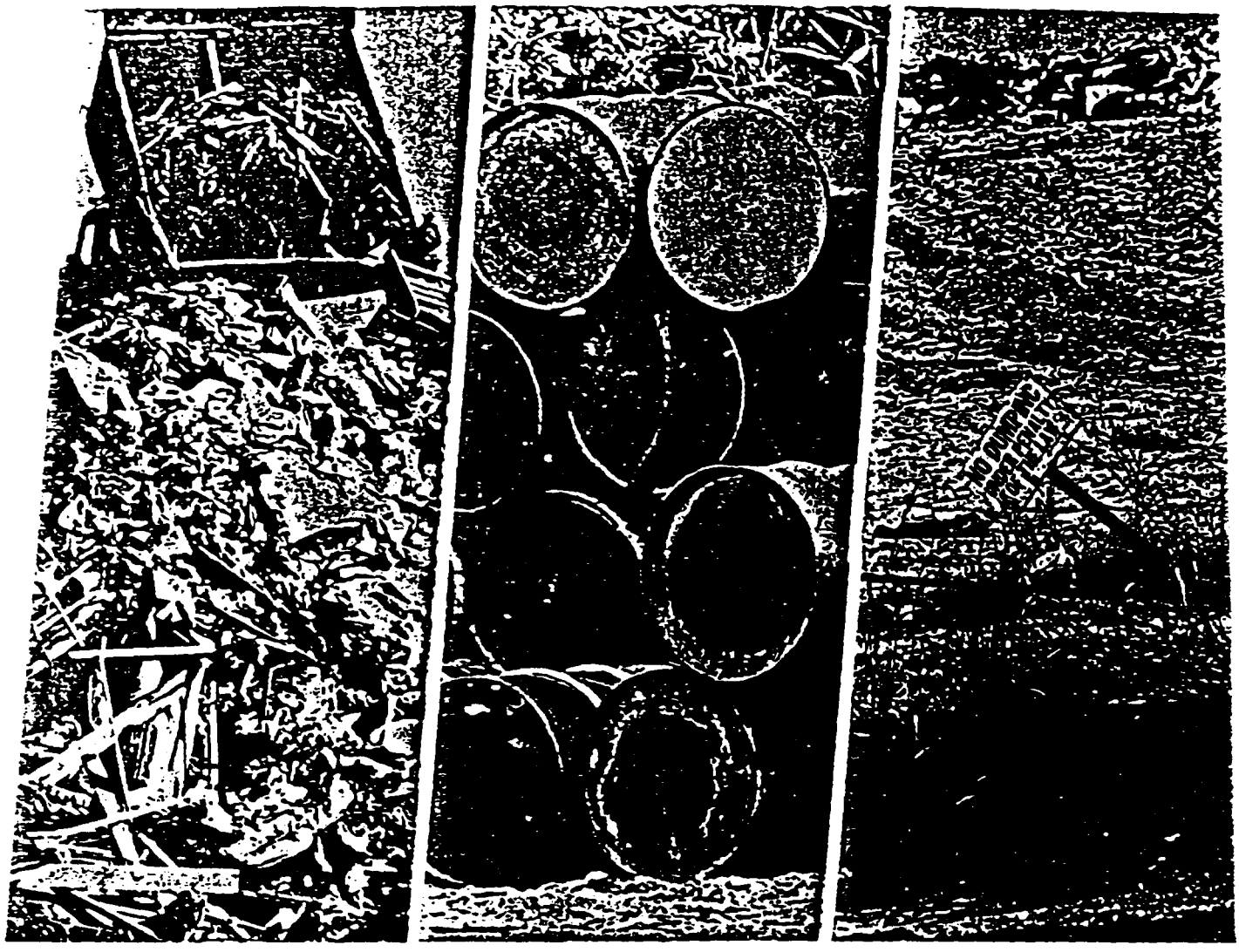

Here is a survey of some of the technical and institutional strategies needed for

Developing solutions to hazardous-waste problems

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There can be little doubt that hazardous waste is on everybody's mind these days. As with so many other public issues, it is amazing that attention can be so clearly focused on the problem, yet there is so little consensus on the potential solutions.

Table I lists some of the hazardous-waste issues that surfaced in a recent survey of the 50 state governments. There seems to be a great deal of philosophical uncertainty about how to determine what is or is not hazardous.

For example, much of the "hazardous" waste consists of acidic and alkaline solutions which, if properly treated, present very little public health risk. Yet Congress broadly defined the term "hazardous waste" in the Resource Conservation and Recovery Act (RCRA). By this definition, road salt can be hazardous when its storage is mismanaged. This is possible because sodium could be leached into the groundwater, which could cause aggravation of hypertension and other health disorders in the people who drink the water.

Recently the U.S. Environmental Protection Agency (EPA) issued a list of waste sources, waste streams, and some specific wastes that it defines as hazardous. Also released were four characteristics of hazardous wastes: ignitability, corrosivity, reactivity, and toxicity, as determined by a specified extraction procedure. There is still considerable debate as to whether this

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termining what is a hazardous waste.

Even when everyone agrees that a waste is hazardous in nature, they cannot always agree on how it should be treated and disposed. A number of disposal techniques have been discussed (*ES&T*, July 1979, p. 810). Moreover, Gary Dietrich of EPA's Office of Solid Waste has been quoted as saying, "Our knowledge about land disposal, treatment, and incineration is almost as a black art."

Once adequate treatment and disposal technology is found for a particular waste, the problem remains to find the environmentally, geologically, geographically, economically, sociologically, and politically proper site for this activity. Public education is desperately needed to provide the facts, so that the affected people can make informed judgments about the proposed facilities.

However, education is not to be confused with propaganda. Because of human errors in operation and maintenance of facilities in the past, they can never pose zero risk to the population. Thus, the public's concern about waste chemicals must be addressed and resolved. There must be adequate facilities sited, coupled with strong regulatory and enforcement activities. According to EPA Administrator Douglas M. Costle, "Despite public fears, America needs hundreds of waste disposal sites and state officials must choose where they will be."

RCRA's definition of hazardous waste

"A solid waste, or combination of solid wastes, which because of its quantity, concentration, or physical, chemical, or infectious characteristics may cause . . . increase in mortality or an increase in serious illness . . . or . . . pose a substantial present or potential hazard to human health or the environment when improperly treated, stored, transported, or disposed of, or otherwise managed."

Research initiatives

Given the magnitude of the problem, there has been no long-standing, organized, well-funded effort to develop solutions. This does not mean that there was a lack of specific activity or research publication, only that the focus was not present. For instance, under the authorization of the Intergovernmental Science, Engineering, and Technical Advisory Panel (ISETAP) in the Executive Office of the President, The American Association for the Advancement of Science (AAAS) organized and held a workshop on hazardous-waste management. In this key forum, members of the scientific community identified researchable issues in three areas: analytical procedures, technologies for waste treatment and disposal, and siting new disposal facilities. A report was issued on this activity.

After the workshop, ISETAP and the Office of Science and Technology Policy (OSTP, Executive Offices of the President) developed specific recommendations and set priorities for major U.S. funding agencies for future hazardous-waste research. This follow-up also looked to the health research community to identify priority research activities for determining health defects and other risks associated with hazardous waste. The recommendations of this activity are summarized in Table 2.

The EPA's Office of Research and Development has also prepared work plans in each of the areas described above. That is important, because a concentrated, well-financed program is necessary in order to provide timely solutions to this previously inadequately handled problem.

Analytical developments

The analysis of hazardous-waste samples poses many difficult problems for the analytical chemist. Consider the range of sample types sent to the laboratory. These samples may be from a waste stream at an industrial site, from wastes being transported from a generator, from contained wastes located at a treatment or disposal site, or from soil and water in or near a waste treatment or disposal operation. Wastes may be in a number of forms, such as solids, sludges, liquids, gases, or a mixture of these forms.

TABLE 1
Specific issues of concern to states[a,b]

Public acceptance of new sites/public participation in siting
Identification of the sources, quantity, movement, and final disposition of hazardous materials
Overall strategy for hazardous-waste control
Adequate disposal capacity
Locating abandoned facilities
Criteria for new site selection
Criteria and test procedures for identifying hazardous wastes
Assessing the nature and severity of problems at abandoned facilities
Classification of waste by degree of hazard
Inspection and monitoring/assessing the nature and severity of problems at facilities in operation
Locating operating facilities
Determining effects of hazardous wastes on human/animal health
Financing emergency cleanup at abandoned facilities when no responsible/capable party
Federal preemption of state and local authorities in siting new facilities
Immediate mitigation of exposure/containment of wastes at abandoned facilities
Methods for reducing amounts of hazardous wastes and increasing reuse/recycling/recovery
Data on the capabilities and costs of various treatment options
Establishing legal liability for abandoned facilities
State preemption of local authorities in siting new facilities
Safety and risk assessment of new facilities

[a] In order of priority.
[b] From a study initiated by the Intergovernmental Science, Engineering and Technology Advisory Panel (Executive Offices of the President).

TABLE 2
Research, development, and demonstration needs

Sampling and analytical procedures development
Health effects data evaluation
Fate and transport studies
Improve technologies for cleanup of existing sites
Refine technologies for improved management of current and future wastes
Quantify socioeconomic aspects of new facility siting

Policy needs

Develop procedures for assessment of hazardous wastes and waste sites
Institute an environmental research center dedicated to hazardous waste
Disseminate information
Increase trained personnel pool

variety of containers and locations, such as fiber and steel drums, sacks, buckets, bins, barges, soils, trucks, waste piles, storage tanks, wells, ponds, pits, or lagoons. Analysis problems are further complicated by the special methods necessary to obtain a representative sample from each of these locations.

Most of the existing analytical techniques and procedures available are expensive because they require extensive laboratory work, sophisticated instrumentation, and highly trained personnel. Development of new or approved analytical methods and protocols is extremely complex because of the large number of possible components, their differing properties, and their wide range of concentrations. Complex separation processes and involved interpretation of the analytical data are required for most waste samples. A large amount of quality assurance is required, in order to ensure that the data meet the original objectives of the investigation.

The data produced in the analytical program should be annotated to include information as to the purpose of the analysis, that is, semiquantitative screening or detailed quantification for specific compounds. Detailed analytical information is important to any hazardous waste management program. This information is required in four key areas:

- identification of wastes as hazardous
- determination of the degree of hazard
- identification of wastes' compatibility for handling
- identification of appropriate treatment methods.

While much work has been done in the area of analytical chemistry, the methods and protocols needed to provide the necessary information for effective waste management are still not available. Existing analytical methodology usually cannot be directly applied to these problems. Many analytical chemists have never been faced with a problem of this magnitude and complexity.

The EPA has implemented a high-priority program to deal with this problem; its high-priority status is geared toward upgrading certain technologies, on a short-term basis, to support RCRA activities. Program highlights are described in Table 3.

Additional areas requiring action were identified by the AAAS/ISE-TAP/OSTP deliberations. These groups felt that more rapid and less expensive methods for the qualitative and quantitative determination of specific chemical components or classes of chemicals isolated from samples should be developed and tested. Specific needs are the following:

- protocols for sample analysis including improvement in throughput and cost per sample, and development of a tiered approach to sample analysis
- procedures for differentiating between inorganic hazardous waste and natural background
- semiquantitative tests that can be performed in the field
- assessment of the feasibility of using surrogate parameters for the estimation of a waste's hazard potential
- determination of sample stability (How long can the sample be held for analysis? Is preservation required?).

Several more general areas require attention to improve the analytical aspect of hazardous-waste management. To begin with, all protocols of waste analysis should provide adequate safety procedures to protect personnel, public health, and the environment.

Good laboratory facilities and practices appear to have been developed for handling hazardous materials. However, they need to be rigorously applied, and more research needs to be conducted to improve laboratory safety protocol. Secondly, it has been argued that there is a shortage of trained analytical chemists with interest and expertise to handle this hazardous waste identification program. Assistance from the federal government may also be needed to improve the size and quality of this trained personnel pool by providing direct training and research grants to universities and by supporting continuing-education courses in this topic area.

Finally, an analytical data base should be developed for all hazardous compounds found in the hazardous-waste notification forms sent to the EPA. It should include access to health effects and other data collected by the federal government for other purposes, as well as data collected in hazardous-waste operations. Special attention should be paid to including references to analytical methods needed to analyze for the various compounds.

There have been some strides made in the analytical area. Many of these have been funded by EPA's Solid and Hazardous Waste Research Division (Cincinnati, Ohio). Work is presently being conducted by the EPA Laboratory in Las Vegas, Nev. However, this activity must be stepped up dramati-

TABLE 3

EPA research goals—hazardous-waste identification . . .

Develop, evaluate, and validate proposed hazardous waste and waste disposal site sampling and analytical procedures

Develop monitoring guidelines for hazardous waste disposal facilities

Develop and operate a quality assurance program for the uncontrolled hazardous waste site investigation program

Develop biological screening methods

Identify, sample, and characterize current industrial hazardous wastes and waste streams and current methods of disposal

. . . and waste technology

Short-term needs

Develop data base and prepare guidance manuals for "Best Engineering Judgment" evaluations by the permit writer for landfills, surface impoundments, and land treatment facilities

Develop protocols for evaluating hazardous-waste fixation and encapsulation methods

Develop methods for prediction of volatiles from surface impoundments and land-disposal facilities

Develop test burn data base and procedures for "Best Engineering Judgment" evaluations of incineration facilities

Develop, evaluate, and validate measurement methods to determine the destruction efficiency and air pollution control efficiency of incineration facilities

Medium-term needs

Determine the compatibility of industrial hazardous wastes and various disposal technologies

Develop and demonstrate cost-effective technologies for the treatment, detoxification, or destruction of industrial hazardous waste

Determine the potential for recycle or reuse of industrial hazardous waste

Determine the potential for regional hazardous waste management facilities

Long-term needs

Develop data on options to improve land disposal site design and management for treated wastes

Develop and demonstrate alternatives to landfill disposal

Determine methods to increase recovery and use of secondary materials

cally, because analyses provide the crux of the entire hazardous waste management strategy.

Technological developments

Many operation and maintenance problems within the treatment and disposal industry have been made public during the past year. Some problems have been caused simply by sloppy operations. In other cases, business pressures, such as competition with dumping, force firms to "cut corners" in their daily operation.

Also, the disposal industry has suffered a loss of credibility, especially after almost daily releases by the media of information on past practices. State and federal regulatory agencies must also share some of the blame for these past operations. For example, many past and present regulations assume that if a site meets design criteria, it is acceptable. To concur with this position, the public must have faith in the regulator who adopted those criteria. The public must also believe that the criteria are sufficiently universal to apply to all circumstances.

To date, the public has not shown this faith. Regulatory groups have also lost credibility as the result of instances such as the contamination of the Hudson River with PCBs, even though the discharges were made in full compliance with a permit issued by the state. Site-specific and waste-specific methods of determining the acceptability of present and proposed facilities are fundamental in evaluating the adequacy of waste-management technology and in building public acceptance of it.

Unfortunately, with the exception of solidification technology developments, there have been but few innovative and widely used treatment technologies in the past 30 years. In large measure, newer developments have been held back because of the lack of current demand. This lack of demand was fostered by a widespread inability to recognize improper administration of hazardous-waste disposal as a major public health and environmental problem.

Actually, a variety of processes exist for the treatment and disposal of hazardous waste. But no process can be universally applied to all different types of waste streams. Therefore, considerable judgment must be used to select the proper treatment and disposal option for each specific industrial waste. A number of disposal options, in the order of preference for employing them, are briefly discussed below.

Many industrial processes can be modified so that the volume or toxicity of the waste is reduced. This is often called source reduction and is regarded by many to be the most preferable way of handling hazardous waste. Feasibility study and retrofitting costs may be high; however, they can be justified in terms of having a smaller volume of waste to dispose of in the future and commensurately lower costs.

Many regulators and regional hazardous-waste studies are examining waste exchange. Existing exchanges provide information on waste products available from a primary user that sometimes can be reused in another industrial process before becoming a waste. Reuse can constitute a net reduction of the volume of waste needing disposal.

Other recycle/reuse possibilities for certain waste products should be thoroughly investigated by the generator. For example, many solvents can be reclaimed by distillation or other chemical treatment for use by another industry in processing or as a fuel. Moreover, many recycle/reuse applications will be exempted from regulation under RCRA.

If none of the above possibilities are applicable to a particular waste stream, the next consideration should be treating the waste. Neutralization, detoxification, and solidification may be used to convert the waste, chemically and physically, to an operationally defined nonhazardous waste. Disposal requirements for these treated wastes would be nowhere as rigorous as those for an untreated waste. Incineration also can be looked upon as a treatment process to destroy certain chemical wastes, provided that proper air-scrubbing equipment is utilized.

In all of these treatment processes, a reduced volume of hazardous waste may be generated as a by-product. However, solidification of waste to reduce the hazard may be used to convert the waste to a nonhazardous material. A last option is the placement of certain untreated chemical wastes in a secured chemical landfill, deep well, or other geologically formed deposit.

The economic attractiveness of this low-priority disposal option will disappear, once post-closure and financial responsibility regulations are promulgated. In addition, the impact of the Wilsonville (Ill.) court rulings will continue to make responsible disposers wary of this approach.

Some of the approaches discussed above may take considerable time to reduce to practice, and have thus been given medium-term and long-term developmental priority. The AAS/ISETAP, OSTP program cited several needs for technologies involved in the approved management of the current and future hazardous-waste streams. These include:

- the identification of process changes that will reduce waste production for those industries which produce extremely hazardous wastes and have limited abilities to manage their wastes according to RCRA regulations
- the development and demonstration of new treatment and detoxification methods, particularly for "problem" industries
- incineration parameters and technologies for hazardous wastes, including fail-safe, on-line monitoring and emission-control technology, and conditions for coincineration of hazardous wastes in conventional combustion operations
- identification of wastes most amenable to resource recovery.

Other activities in the area of developing waste-treatment technologies include formation of a committee on waste disposal (Committee D-34) by the American Society of Testing and Materials (ASTM). The proposed mission of this group is to promote knowledge, to stimulate research, and to develop test methods, specifications, nomenclature, classifications, and definitions relating to waste disposal. Its goal is to provide a measurement foundation to allow for acceptable disposal. These activities are being coordinated in cooperation with other relevant ASTM committees and with outside organizations. The various subcommittees in this group are concerned with the following:

- sampling and monitoring
- extraction and leachate testing
- health and environmental effects
- transportation and containerization
- evaluation of line of materials
- disposal sites
- management of specific waste
- incineration
- treatment technology.

In light of the RCRA regulations and the development of the hazardous waste management industry in general, there are four items that must be addressed in order to have more operational facilities. First, firms involved in hazardous-waste management must generally be large enough to cover potential liability that could arise from facility operation. Also, because some processes have been developed by smaller firms, there must be a means of bringing together many smaller

firms at a larger location in order to use these processes.

The hazardous waste disposal business generally involves high capital entry costs; therefore, a means of financing these operations has to be implemented. Moreover, an enterprise requires a high degree of technical sophistication and managerial confidence if it is to be run correctly. As mentioned earlier, there is a need for more analytical chemists and also well-trained facility managers and workers.

Finally, the firms must generally be large enough to demonstrate a firm commitment to staying in the business and shouldering financial responsibility for the operation. In the case of a hazardous waste treatment facility presently being developed by the Gulf Coast Waste Disposal Authority (Houston, Tex.), this responsibility is being handled by an independent agency, with the processes being run by vendors who have short-term lease commitments on the site.

Because it has been hard to find these key characteristics in firms, many large companies have opted for on-site disposal. This choice is justified because of rapidly escalating costs of legal off-site disposal, a shortage of legal off-site facilities within reasonable transportation distance, fear of legal liability for waste improperly disposed of by off-site contractors, and public relations problems with firms connected with off-site problems. These industries further justify on-site disposal because it gives them complete control. However, availability of land and capital are crucial to the determination that on-site disposal is the way to go.

Siting developments

Without question, siting is a major problem in the development of future hazardous waste disposal facilities. People are against these facilities for many reasons:

- fear of groundwater contamination
- expectation of air and land pollution problems, especially with dusts and odors
- worry about safety problems, such as fire and explosions
- dislike of heavy traffic and possibility of spills
- fear of property value decline
- questions about the financial ability of the waste contractor to provide for long-term facility maintenance and closure.

Some communities just do not want their town to be known as a "dumping ground" for nonlocal industry's wastes. Indeed, opposition to any hazardous-waste facility is virtually inevitable. People will generally resort to lawsuits and civil disobedience to stop these facilities.

Many states have conflicting concerns. The success of the hazardous-waste regulatory approach depends upon the establishment of new facilities. Economic development branches of the states want to promote retention and development of industry. However, in order to meet the public's concerns, the states must guarantee environmentally sound operations and post-closure management of the facilities. This limits the public's share of financial risk, a factor of opposition to these developments.

However, the states suffer a tremendous credibility problem with most people. Also, in cases in which the states seek to preempt local controls, the desire for local automony may foster additional opposition to proposed facilities. Thus, the states have to decide which role they wish to play in the siting of hazardous-waste facilities.

The siting process itself is quite complex, and involves a large number of possible steps. These potential steps are indicated in Table 4. A state's role in the siting process will depend upon the policy legislated within that state.

The ideal way to deal with the siting of a hazardous-waste facility is to attempt to make people in the vicinity indifferent to the proposed facility. The only hope that one has to accomplish this aim is to ascertain their concerns by talking with them and genuinely respond to their concerns by negotiating agreements with them. These agreements will seek to minimize adverse impacts; make sure people have information they can trust (for instance, independent monitoring and consulting, financed by the disposal contractor or the state); and compensate people for the costs that cannot be avoided (such as reduced property evaluations, handling emergencies and traffic patterns, and the like).

TABLE 4
Steps involved in siting a hazardous-waste facility[a]

Public information
Demand assessment
Site criteria establishment
Site nomination
Land acquisition
Local information
Local approvals
Appeal of local approval
State approval
Negotiations
Extralegal opposition
Project operation
Enforcement of conditions
Response to accidents
Post closure

[a] As proposed by M. O'Hare, Massachusetts Executive Office of Environmental Affairs

EPA's Office for Solid Waste and its internal task force on hazardous waste facilities siting are exploring the use of incentives and compensation payment plans as part of the negotiation process. A number of types of payments to the local community, and especially to affected individuals, can be included in the agreement:

- additional safety precautions, such as increased frequency of monitoring, annual environmental audits by independent consultants, more sophisticated cleanup equipment, detailed accident contingency plans, and training of local police and fire personnel
- cleanup and rehabilitation of existing abandoned dump sites in the locality
- purchase of local bonds at interest rates more favorable to the locality than prevailing market rates.

Ideally, these payments would be made by the disposal facility and charged back to the consumers of the products that have hazardous-waste by-products, rather than be assumed by society at large. The states, however, may get involved in the compensation plan by giving cooperative localities a greater share of state funds for parks, highways, highway maintenance, police, or even education. Also, the state may require developers to give incentives to localities in the form of cash payments of tipping fees.

There are a number of forms of direct payments that can be made to the affected individuals. These include:

- payment to abutting or neighboring land owners to compensate for diminished property values
- gross receipt taxes on facility revenues, in lieu of taxes or "tipping fees" with payments made to a local government's general fund
- payment of any additional costs the community bears because of the facility, such as upgrading roads, special training or equipment for the fire department, or periodic independent monitoring

• one-time lump sum payment to residents or local governments.

In fact, some indirect payments are also common and quite versatile. These may be:

• land value guarantees, whereby a developer could agree with local landowners to purchase their property if its value is below market rate when they are ready to sell. This would include a provision that after a given period of time, say five years, an assessor could determine the difference between actual and probable value, and cash payments could be made to compensate the owners.

• provision of jobs, job training, and tax-base expansion

• purchase of buffer zones or development rights of adjacent land

• provision of recreational areas and facilities

• provision of aesthetic improvements such as additional landscaping, earth berms, street scraping, appropriate signs and lighting, and attractive color, design, and texture

• limitations on hours of operation or receiving materials

• rerouting of traffic generated by the facility

• free disposal for the local industries.

Unfortunately it would be difficult, if not impossible for a facility to offer all these incentives. Even if it could, there are no guarantees that the facility would be welcome by the community. Further, an incentive program at the state level would have to be carefully designed to encourage siting of facilities without unnecessarily increasing the demand for incentives in other areas.

Mediation

The objective of the mediation function is twofold: first, to ensure that many groups with an interest in the proposed facility would have an opportunity to express their concerns; and second, to provide a forum where competing or opposing groups could negotiate their differences. The state may become involved in the mediation process by acting as an initiator and facilitator, and by making certain that groups with opposing views and interests are brought together to discuss their conflicts.

As an impartial mediator between a firm wishing to site a facility and citizens opposed to a proposed site, the independent state agency could also provide a variety of services. It could assist in defining the areas of conflict and in identifying mutually acceptable means of resolving these conflicts. In addition, the media could encourage other state groups to initiate actions to help opposition to a site.

Finally, the state could see to it that agreements negotiated among the interested parties are fully implemented. EPA and a number of the states are currently exploring the use of incentives, compensation payment plans, and mediation techniques to be used by the private sector and by states in the siting of hazardous-waste facilities. Handbooks for these purposes are to be completed for the EPA Office of Solid Waste and made available in early 1981.

Convincing the citizen

From the discussion above, it should be clear that there are specific areas of research which, if undertaken, would potentially provide some solutions to the current hazardous waste disposal problem. However, even with successful completion of this work, it must be recognized that the public may never accept hazardous waste disposal sites. There may be absolutely no way to convince the average citizen that a well-designed, operated, and regulated site poses minimal risk, especially when compared to illegal dumping. Indeed, there are sites with *good* operating records which are undergoing public challenges. This has resulted in some being removed from service. Unfortunately, that trend may continue, even in light of increasing demands upon current waste generators to ensure adequate storage and disposal of their hazardous-waste streams.

In a move which remarkably parallels the drive for energy conservation, there must be a supreme effort in industry for waste-source reduction. In the uncertain light of disposal capacity, at least in the short term, every firm generating hazardous waste must modify its processes to encourage the reduction of wastes which may be deemed hazardous at each site. Smaller industries may group together and fund research in this area through their trade organizations.

Another issue involved here is the need for every state to become involved in some way in the siting process for new or expanded hazardous waste disposal facilities. It is particularly important to develop facilities which encourage treatment, rather than rely solely on untreated disposal in a "geologically secure" facility. Recycle/reuse must be encouraged but cannot become a loophole for illegitimate activities involving waste disposal. This area will have to be closely regulated, even though it was originally designed to avoid regulations concerning disposal of the wastes.

Moreover, both the EPA and the states must be quite careful about what they define as hazardous. It would be foolish to consume scarce site capacity with materials of low intrinsic hazard. Thus, legitimate exemptions should be provided whenever they can be justified.

There must be close cooperation between those involved in public policy questions and those concerned with technical issues of waste disposal to provide a balanced program for investigating means for hazardous-waste handling in the future. Forums such as the AAAS/ISETAP/OSTP meetings must be encouraged and held frequently. There must also be a mechanism of communicating their findings to the funding agencies and especially to Congress, which supplies the funding agencies with the wherewithal to conduct meaningful programs for improving hazardous-waste disposal and treatment. These programs should be well publicized so that those involved will have ample opportunity to express their concerns and opinions to the same audiences, with the public focus on hazardous waste, and the funding agencies' priorities reflecting this focus. The time is indeed ripe to develop solutions to the hazardous-waste problem.

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Reduction of Polychlorinated Biphenyl Toxicity and Uptake of Carbon-14 Activity by Plants through the Use of Activated Carbon

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The use of soil-applied activated carbon in reducing the phytotoxicity and uptake from soil of polychlorinated biphenyls (PCB's) by a variety of crop plants was investigated. Reductions in growth parameters resulted at the highest rate of PCB for soybean [*Glycine max* (L.) Merr.] and beet (*Beta vulgaris* (L.)]. Growth parameters taken at harvest showed no apparent inhibition of corn (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moensch] by PCB. The activated carbon treatment substantially reduced growth inhibition caused by PCB. Treatment with soil-applied activated carbon reduced ^{14}C uptake into foliage of beet, corn, sorghum, and peanut (*Arachis hypogaea* L.) in studies using a mixture of U-^{14}C-labeled polychlorinated biphenyls mixed with unlabeled PCB and applied to soil at 20 ppm (total PCB). Activated carbon continued to be effective in reducing ^{14}C uptake over three croppings of fescue (*Festuca arundinacea* Schrib).

Land application of municipal and industrial sludge is becoming an increasingly acceptable method of disposal. Application to agricultural land is viewed as an inexpensive method to exploit the nitrogen and other nutrients of these sludges for use as fertilizer (Overcash and Pal, 1979). However, concern has been voiced over the effect of the organic chemical content of these sludges on crops, and the potential for their uptake by crops needs to be determined (Weber, 1978; Pahren et al., 1979). The PCB content of sludges has been reported to range from <0.01 to 1700 ppm dry weight (Furr et al., 1976; Lawrence and Tosine, 1976; Bergh and Peoples, 1977; Pahren et al., 1979). With application to agricultural land of 2250–22 500 kg of sludge hectare^{-1} as dictatd by nitrogen content (Black and Kronis, 1974), an application range of ≪1 to 1500 kg of PCB ha^{-1} year^{-1} could potentially occur. Since PCB's are resistant to degradation, in particular those which are highly chlorinated (Gustafson, 1970; Kalmaz and Kalmaz, 1979; Pal et al., 1980), and do not leach readily in soils (Tucker et al., 1975; Scharpenseel et al., 1977a; Moza et al., 1979a; Weber, 1980), sludge application over a 3-year period could result in PCB levels ranging from ≪1 to 1000 ppm in the upper 7.6 cm of soil. At more reasonable application rates of 11 200–44 800 kg/ha/year of sludges containing 1–200 ppm of PCB's, one could anticipate a range of <1 to 24 ppm accumulating in the upper 7.6 cm of soil over a 3-year period. PCB levels in soils receiving dried sludge have been reported to range from 0 to >50 ppm (Bergh and Peoples, 1977), and PCB levels as high as 1200 ppm have been reported in some Japanese agricultural soils (Fujiwara, 1975).

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Uptake of PCBs by various crops and weeds from soils containing low amounts of PCB's has been reported for carrots (Iwata et al., 1974), carrots and radishes (Wallnöfer et al., 1975), carrots and sugar beets (Moza et al., 1976, 1979a,c), soybean (Suzuki et al., 1977), soybean and fescue (Weber and Mrozek, 1979), and pigweed and panicum (Strek and Weber, 1980). PCB concentrations in the soils ranged from 0.05 to 100 ppm and levels in the crops reached a maximum mean (average of five isomers) of 13.9 ppm (fresh weight) in carrots grown outdoors for 72 days in soil treated with 100 ppm of Aroclor 1254 (Iwata et al., 1974). Uptake over 4 years of growth by spruce trees from soil fortified with [^{14}C]PCB-treated sludge totaled 0.401 and 0.231 ppm (dry weight) in the needles and stems, respectively (Moza et al., 1979b), showing that plants can take up PCB's from contaminated sludge applied to soil. Disruption of growth in plants has been reported for an aquatic plant *Spirodela oligorrhiza* (Mahanty and Fineran, 1976; Mahanty and McWha, 1976) and for soybean and fescue (Weber and Mrozek, 1979). Aroclor 1242 is reported to inhibit photosynthesis in isolated spinach chloroplasts (Sinclair et al., 1977).

Volatilization appears to be an important route for the loss of PCB's from the soil, despite the moderate to low volatilities of 7×10^{-3} to 4×10^{-6} mmHg at 25 °C reported for Aroclors 1221–1260 (Pal et al., 1980). Losses from a woodland soil ranged from 79.2 to 41.5% of that applied in a single cropping season under outdoor conditions for single isomers possessing two to five chlorines (Moza et al., 1976, 1979a,c). The amount of loss through volatilization appears to depend upon the degree of chlorination, with the more highly chlorinated PCB's being lost to a lesser degree (Iwata et al., 1974; Kilzer et al., 1979) and also more readily adsorbed by the soil (Haque et al., 1974).

The effectiveness of activated carbon as an adsorbent of organic molecules has long been recognized (Mattson and Mark, 1971; Cheremisinoff and Ellerbusch, 1978). Although early investigation of this adsorptive property

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focused on agricultural chemicals, particularly on pesticides applied to agricultural lands (Ahrens, 1968; Anderson, 1968; Lichtenstein et al., 1968; Coffey and Warren, 1969; Gupta, 1976), recent work seems to be directed to investigating the activated carbon adsorption of other chemicals in both agricultural lands and other parts of the environment. Activated carbon has been shown to reduce the availability of PCB's to plants (Weber and Mrozek, 1979) and to goldfish (Shea et al., 1980) when applied to contaminated soil. Adsorption of PCB's from aqueous solution by activated carbon has been demonstrated (Lawrence and Tosine, 1976; Hiraizumi et al., 1979).

The research reported herein was conducted to determine (a) the potential effect of a polychlorinated biphenyl mixture (Aroclor 1254) on several important crop plants, (b) the potential residue levels in crops, and (c) the potential of activated carbon treatments to soil to reduce toxicity and residue levels in crop plants.

MATERIALS AND METHODS

Soil. Soil used in the experiments was taken from the 0–15-cm region of the A horizon of a virgin Lakeland sand (*Typic Quartzipsamments*; siliceous, thermic, coated, pH 4.0; cation-exchange capacity, 1.5 mequiv/100 g; 1% organic matter, 5% clay, 6% silt) obtained from a Johnston County, North Carolina, site which had not been subjected to agricultural practices for least 40 years. The soil was air-dried and sieved through a 0.5-cm screen prior to potting. Plants were grown in 350-mL styrofoam pots containing 300 g of soil, which was limed to approximately pH 6.0 with $CaCO_3$.

Aroclor Studies. Analytical-grade PCB (Aroclor 1254), obtained from the Food and Drug Protection Division Laboratory of the North Carolina Department of Agriculture (Lot AM 51, originally obtained from Monsanto Co. in 1971), was dissolved in 95% ethanol. Aliquots of the stock solution were thoroughly mixed with the soil and 3 days were allowed for solvent evaporation. This procedure yielded PCB concentrations of 0, 1, 10, 100, and 1000 ppm. All PCB and carbon concentrations in the soil reported in ppm are based on dry weights. The pots were then separated into two treatment groups; one received no carbon, while the other received 3.7 t/ha (3333 ppm) activated carbon (Nuchar SA, Westvaco, Inc.), which was thoroughly mixed with PCB-treated soil.

The pots were planted to soybean [*Glycine max* (L.) Merr Ransom], beet (*Beta vulgaris* L. Detroit Dark Red), corn (*Zea mays* L. Pioneer 3369A), and sorghum [*Sorghum bicolor* (L.) Moensch Savanna 4 19833-5134]. The crops were grown in controlled environment chambers (200 hlx, 16-h day, 30 °C day, 18 °C night), and the soil was kept at ~80% field capacity and fertilized weekly with a modified Hoagland's nutrient solutin (Weber, 1977). Height, root, and top fresh weight measurements were taken at harvest or as indicated. Cumulative water use measurements were taken periodically using unplanted pots to account for water lost to evaporation from the soil. The second and third soybean plantings followed the original planting reported in a previous study (Weber and Mrozek, 1979) and represent a continuation of this particular study.

^{14}C-Labeled PCB Studies. A ring-labeled polychlorinated biphenyl mixture resembling Aroclor 1254 [specific activity = 31.3 mCi/mmol, obtained from New England Nuclear (lot no. 872-193), average molecular weight = 326.25] was mixed with Aroclor 1254 in 95% ethanol. The labeled material was an isomeric mixture of U-^{14}C-labeled polychlorinated biphenyls ~54% chlorine by weight having a greater Cl/biphenyl range than the unlabeled material. The PCB rates used for these studies were 0 and 20 ppm, and activated carbon rates were 0 and 3.7 t/ha (3333 ppm). PCB and activated carbon applications were made according to the previously described protocol. The pots were planted to fescue (*Festuca arundinacea* Schrib. Kentucky 31), beet, sorghum, peanut (*Arachis hypogaea* L. NC-2), and corn. Plants were continuously cropped in the same pots (without additional PCB additions) under the same controlled environment chamber conditions described in the unlabeled PCB studies. The sequence of planting and days of growth were as follows: fescue-1 (50), beet (39), sorghum (39), peanut (78), fescue-2 (25), corn (13), and fescue-3 (48). The plantings followed one another in the same pots after accumulated toxins were leached out through the bottom of the pot.

At harvest, plant height and top fresh weight were determined. ^{14}C activity was extracted from freshly cut plant tops by homogenizing them in 25–35 mL of a 1:1 hexane–acetone mixture for 1 min with a homogenizer at 25 000 rpm. The extract was separated from the homogenate by suction filtration through a glass fiber filter and washed with an additional 10–15 mL of extractant. The filtrate was evaported to near dryness under a hood and then redissolved in 5 mL of hexane by sonication for 1 min. A 1-mL aliquot was removed and added to a scintillation vial containing 20 mL of cocktail (5.0 g of 2,4-diphenyloxazole, 0.1 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)] benzene, 1000 mL of Triton X-100, and 2000 mL of toluene). For removal of quenching due to chlorophyll, 5–10 drops of sodium hypochlorite (chlorox) was added. Radioactivity was determined in a liquid scintillation spectrophotometer for 20 min and was corrected for background levels in plants and for counting efficiency. All ^{14}C content of plants is reported on a fresh weight basis. Recoveries of spiked samples ranged from 86 to 99%. Activities which were converted to ppm represent a best approximation.

Random duplicate soil samples of ~5 g were taken from the pots 1.5 years after initial treatment and continuous cropping and extracted by shaking with 20 mL of hexane for 24 h. Following removal of the supernatant by suction filtration through a glass filter, a 2-mL aliquot was taken and counted in a manner similar to that described for the plant samples. Recovery of spiked samples averaged 93 ± 1% after the first extraction; therefore, no conversion factor was used in the final calculation of recovery.

Statistical Treatment. Analyses of variance were performed on the randomized complete block experiments with the carbon and no carbon treatments analyzed as complete blocks, PCB rates as the main treatment, and PCB X carbon as an interaction effect. When the PCB X carbon interaction F test proved to be significant, LSD's were calculated for mean pairs. Analyses of variance were also performed within the carbon treatments. When the PCB treatment effect proved to be significant, differences from the respective controls within the block were indicated. Three replications were used for the unlabeled PCB studies. All tests for significant differences were made at the 0.05 level of probability of error unless otherwise noted.

RESULTS AND DISCUSSION

After the first crop of soybeans grown in the PCB-treated pots showed significant reductions in height and top fresh weight at 100 and 1000 ppm and reduction of 5-day cumulative water use at all PCB rates (Weber and Mrozek, 1979), it was decided to continue cropping the same pots. This procedure allowed us to study the longevity effect of soil-applied PCB on plants as well as the

Table I. Height and Top Fresh Weight and Cumulative Water Use for Two Crops[a] of Soybean, As Affected by Soil-Applied PCB (Aroclor 1254) and Protection by Activated Carbon Applied to Lakeland Sand

carbon, t/ha	PCB, ppm	height				top fresh weight[b]				cumulative water use (18-22 days)[b]			
		second crop		third crop		second crop		third crop		second crop		third crop	
		cm	% I[b]	cm	% I	g	% I	g	% I	mL	% I	mL	% I
0	0	35.6	0	69.0	0	11.30	0	7.04	0	239	0	149	0
	1	34.8	1	55.3[d]	20	10.83	4	7.22	-3	214	10	146	2
	10	35.6	0	49.3[d]	29	10.96	3	7.82	-11	219	8	147	-5
	100	35.0	1	53.8[d]	22	11.02	2	7.19	-2	202	15	141	5
	1000	27.9	22	56.7[d]	18	8.64	24	4.41[d]	37	126	47	101[d]	32
3.7	0	36.7	0	48.0	0	12.42	0	7.10	0	258	0	127	0
	1	37.1	-1	58.3	-21	13.11	-6	7.81	-10	253	2	130	-2
	10	36.8	0	54.5	-14	13.06	-5	7.41	-4	254	2	139	-9
	100	34.9	5	56.5	-18	12.21	2	7.15	-1	239	7	140	-10
	1000	36.9	0	59.0	-23	12.55	-1	6.53	8	235	9	95	25
	LSD 0.05	NS[c]	NS	10	21	NS	NS	NS	NS	NS	NS	NS	NS

[a] Grown for 23 and 24 days, respectively. [b] Percent inhibition compared to respective controls; negative inhibition indicates stimulation. [c] PCB X carbon effect not significant at the 5% level of probability. [d] PCB effect within carbon treatment is significant and particular mean is different from control at 5% level of probability.

Table II. Height and Top Fresh Weight at Various Times and Cumulative Water Use for Beets, As Affected by Soil-Applied PCB (Aroclor 1254) and Protection by Activated Carbon Applied to Lakeland Sand

carbon, t/ha	PCB, ppm	height at						top fresh weight[b] at				cumulative water use	
		14 days		28 days		56 days		28 days		56 days		32-53 days[a]	
		cm	% I[c]	cm	% I	cm	% I	g	% I	g	% I	mL	% I
0	0	3.2	0	5.0	0	13.5	0	5.1	0	6.3	0	392	0
	1	3.5	-9	5.0	0	14.3	-6	5.4	-6	8.3	-32	374	5
	10	3.2	0	5.0	0	12.0	11	5.3	-4	5.7	10	348	11
	100	2.8	13	5.0	0	15.6	-16	5.0	2	7.5	-19	304	22
	1000	1.2[d]	63	1.5[d]	73	0[d]	100	0[d]	100	0[d]	100	17[d]	96
3.7	0	3.0	0	5.2	0	14.9	0	5.0	0	7.4	0	414	0
	1	3.7	-23	5.3	-2	16.5	-11	6.1	-22	7.5	-1	476	-15
	10	3.8	-27	5.5	-6	16.8	-13	5.1	-2	7.5	-1	469	-13
	100	3.3	-10	5.0	4	16.5	-11	6.1	-22	7.9	-7	440	-6
	1000	3.3	-10	5.3	-2	15.1	-1	5.2	4	8.3	-12	425	-3
	LSD 0.05	0.9	28	1.4	9	3.6	27	NS[c]		3.4	54	90	23

[a] Mean of two plants, each pair harvested from the same plot at 28 and 56 days. [b] Percent inhibition compared to respective controls; negative inhibition indicates stimulation. [c] PCB X carbon effect not significant at the 5% level of probability. [d] PCB effect within carbon treatment is significant, and particular mean is different from control at 5% level of probability.

effectiveness, over time, of the activated carbon treatment to protect the crop from injury.

Inhibition of plant height caused by PCB X carbon effects were significant only for the third crop of soybean (Table I), where inhibitions of 29 and 22% were reached at the 10- and 100-ppm rates, respectively. When heights were analyzed within the carbon treatments (blocks), significant difference due to PCB treatment was reached only in the no carbon treatment of the third soybean crop where heights were significantly lower at all levels of PCB. Top fresh weight and cumulative water use inhibitions were not significant for PCB X carbon effects for either crop of soybean. However, effects of the PCB treatment on fresh weight of the third crop and cumulative water use for both the second and third crops of soybean were significant, but only at the 1000-ppm rate in the no carbon treatment. The 47% inhibition of water use at the 1000-ppm rate for the second crop of soybean was significantly lower at the 10% level of probability. Root fresh weight differences caused by PCB were not significant and are not reported.

Height inhibitions of 22 and 18% and top fresh weight reductions of 24 and 37% at the 1000-ppm rate for the second and third crops of soybean, respectively (Table I), can be compared to the 15% reduction in height and 22% reduction in top fresh weight reported in the first study (Weber and Mrozek, 1979). Inhibitions of water use at the 1000-ppm rate of PCB for the second and third crops reached 47 and 32%, respectively, also comparing to the 52% reduction reported in the first study. However, significant reductions in water use, which occurred at all PCB rates in the first study, were noted only at the highest rate in either later study. Significant top fresh weight reductions were not observed at the 100-ppm rate as they were during the first study. No malformations of leaves at the 1000-ppm rate in the no carbon treatment were noted for the second and third crops of soybean, as was previously reported for the first crop. These trends indicate that soil-applied PCB's diminish in toxicity to soybean with increasing time and subjection to cropping. The activated carbon treatment eliminated all significant differnces in height, top fresh weight, and cumulative water use caused by PCB in the no carbon treatment. This protective effect continued through three crops of soybean, illustrating the protectiveness, over time, given by the activated carbon treatment.

Beets were chosen as experimental plants to determine the effectiveness of activated carbon in protecting a root crop from PCB's. All PCB, carbon, and PCB X carbon effects were significant for heights and top fresh weights at all sampling dates and for water use, with one exception (Table II). The PCB X carbon interaction effect of beet

Table III. Height Inhibition at 5 Days and Recovery by 18 Days for Corn by PCB (Aroclor 1254) Applied to to Lakeland Sand

carbon, t/ha	PCB, ppm	height at 5 days, cm	5 days, % I[a]	height at 18 days, cm	18 days, % I
0	0	4.7	0	46.7	0
	1	5.6	2	48.7	−4
	10	5.8	3	49.3	−5
	100	4.0[c]	30	48.7	−4
	1000	2.9[c]	49	45.7	2
3.7	0	5.4	0	46.0	0
	1	5.8[c]	−7	42.7	7
	10	5.8[c]	−7	43.3	6
	100	5.9[c]	−9	43.7	5
	1000	5.9[c]	−9	43.7	5
	LSD 0.05	0.3	5	NS[b]	NS

[a] Percent inhibition compared to respective controls; negative inhibition indicates stimulation. [b] PCB *X* carbon effect not significant at 5% level of probability. [c] PCB effect within carbon treatment is significant, and particular mean is different from control at 5% level of probability.

top fresh weights taken at 28 days was significant only at the 10% level. When heights, top fresh weights, and water use were analyzed within blocks, effects due to PCB treatment were significant only at the 1000-ppm rate in the no carbon treatment.

By 14 days, beet foliage height was inhibited by 63% at the 1000-ppm rate, increasing to 73% by 28 days and finally to 100% with the death of the plants between 28 and 56 days (Table II). Top fresh weight was reduced to nearly 100% after 28 days of growth in the 1000-ppm rate of PCB (only one plant out of six survived). Cumulative water use reductions exhibited a progressive reduction with increasing PCB treatment, becoming significant at only the highest PCB rate (however, approaching significance at the 100-ppm rate). Beets are apparently extremely susceptible to soil-applied Aroclor 1254 at the 1000-ppm rate, yet activated carbon protected the beets from the PCB's. In fact, significant growth stimulation resulted from the carbon treatment. Possible explanations for the improved plant growth from the carbon addition include the possibility that the activated carbon adsorbed nonspecific toxic substances normally found in soils or that the activated carbon acted to buffer the availability of applied nutrients.

There was no significant effect of PCB or PCB *X* carbon interaction on corn height and top fresh weight taken at 26 days or cumulative water use taken between 12 and 26 days of growth (data not shown). Similarly, there was no significant effect of PCB on sorghum height and top fresh weight, taken at 69 days, and cumulative water used taken between 65 and 68 days (data not shown). At harvest the crops appeared healthy despite PCB treatment; even differences between carbon and no carbon treatments also appeared minimal. The only effect observed was a significant reduction in corn height at the 100- and 1000-ppm rates after 5 days of growth (Table III). Corn plants appeared to recover and no differences from the controls could be seen after 18 days. These data suggest that corn and sorghum can withstand relatively high rates of PCB in the soil without permanent effects on growth.

In order to determine the influence of activated carbon on the uptake of soil-applied [^{14}C]PCB by plants, a nontoxic rate of 20 ppm was chosen. Amounts in the plants were <1 ppm (fresh weight basis) (Table IV), which concurs with the low values reported in the literature. Reductions in [^{14}C]PCB uptake due to activated carbon treatment ranged from 81 to 100%. The lowest reduction (81%) for corn probably resulted from the high amount of variability in [^{14}C]PCB content at extremely low levels of uptake. Reductions at relatively higher amounts of PCB uptake are much more meaningful than at lower amounts of PCB uptake. These data indicate activated carbon was highly effective in reducing [^{14}C]PCB uptake by these crops.

Bioaccumulation factors are included in Table IV. Bioaccumulation is generally taken to be the factor, expressed as a ratio, at which level a chemical is present in an organism relative to the level found in its environment, either soil or water. A factor greater than 1 means the organism contains a greater concentration of the chemical than the soil or water it inhabits. The bioaccumulation factors for the crops are much lower than 1; none were greater than 0.041. This signifies that amounts in the plants were very low relative to the concentration in the soil. The connotation implied by the terms bioaccumulation factor or biomagnification factor is often misleading. In aquatic systems bioaccumulation factors are ofter large, which is predictable for chemicals of such low water solubility. The amount of chemical actually dissolved in the water is so low that any amount taken in by an organism becomes substantially larger than that found in its surroundings. In soil systems, bioaccumulation factors do not account for the water solubility of a chemical or its adsorption to a particular soil constituent, which may greatly decrease the amount of chemical in the soil solution and influence the availability of chemicals to organisms (Weber, 1972; Weber and Weed, 1974). Bioaccumulation factors are generally reported on a weight basis. Most soil weights are reported as dry weights while plant weights may be reported as either dry or wet weights, further allowing for difficulty in interpretation. The calculated PCB content reported in Table IV represents a theoretical amount of PCB in the plant which is extractable. By combustion of a sample of redroot pigweed plants (*Amaranthus retroflexus* L.) grown in [^{14}C]PCB-treated soil and entrapment of the ^{14}C activity, an estimate of unextractable residue

Table IV. ^{14}C, Estimated PCB Content of Plant Tops, and Bioaccumulation Factors of Various Crops Grown in Lakeland Sand Treated with [^{14}C]PCB[a] (Aroclor 1254) and Activated Carbon

crop	growth period, days	^{14}C and PCB content: no carbon, dpm/gfw[b]	no carbon, ppb[c]	no carbon, b.f.[d]	carbon, dpm/gfw	carbon, ppb	carbon, b.f.	reduction in uptake by carbon, %
beet[e]	39	149.1	815	0.041	18.6	102	0.005	88
sorghum[e]	39	12.5	68	0.003	0	0	0	100
peanut[f]	78	86.5	473	0.024	6.8	37	0.002	92
corn[e]	13	0.32	2	0.001	0.06	0.3	0.00002	81

[a] Applied 0.5 μCi to 300 g of soil; total [^{14}C]- and [^{14}C]PCB concentration was 20 ppm. [b] Disintegrations per minute per gram fresh weight. [c] Based on fresh weight. [d] Bioaccumulation factor based on fresh weight. [e] Mean of two replications. [f] Mean of three replications.

Table V. ^{14}C Extracted from Fescue Grown for Three Different Periods on Lakeland Sand Treated with [^{14}C]PCB[a] (Aroclor 1254) and Activated Carbon

sampling date[b]	^{14}C content: no carbon, dpm/gfw[c]	^{14}C content: carbon, dpm/gfw	reduction in uptake by carbon, %
Nov 3, 1978[d]	309.1	0	100
July 5, 1979	73.5	0.2	100
Feb 6, 1980	28.6	2.2	93

[a] Applied 0.5 μCi to 300 g of soil; total [^{12}C]- and [^{14}C]-PCB concentration was 20 ppm. [b] After 50, 25, and 48 days of growth, respectively. [c] Disintegrations per minute per gram fresh weight. [d] Originally reported in Weber and Mrozek (1979).

was obtained (Mrozek, 1980). The total residue was determined to be 500 dpm/g dry weight, whereas the level of extracted activity was 234 dpm/g dry weight. This represents an underestimation of ~50%, which is comparable to the value of unextractable residues reported in the literature (Moza et al., 1976, 1979a,c). Furthermore, the form of the PCB in the plant may not be in the parent isotype but in the form of conjugates and phenols, as reported by Moza et al. (1976, 1979a–c).

Three crops of fescue were grown in one set of treated pots at various periods to determine the long-term effectiveness of the activated carbon treatment in reducing [^{14}C]PCB uptake (Table V). Though the growing periods of the three crops of fescue varied in length, the fresh weight production of each crop was comparable, averaging 2.25, 2.26, and 3.50 g, respectively, for the three crops. ^{14}C content of fescue in the no carbon treatment decreased with each sampling over a 15-month period. Of 3667 dpm/g ^{14}C originally applied to the soil, after 1.5 years of cropping, 1939 and 3248 dpm/g could be extracted from the no carbon and carbon treatments, respectively. This corresponds to a 53% recovery in the no carbon treatment and a 89% recovery in the carbon treatment of applied ^{14}C activity after 1.5 years. The majority of loss of PCB's can probably be explained as resulting from volatilization. Discounting losses due to uptake by plants in each treatment (only a total of 0.36% of applied activity was taken up in the no carbon treatment), the activated carbon apparently inhibited the disappearance of PCB from the soil. However, the possibility remains that upon volatilization or microbial breakdown of the PCB's, the PCB or $^{14}CO_2$ may have adsorbed to the activated carbon surface and provided an underestimate of the disappearance of the parent molecule (Helweg, 1975). Another possibility is that with increasing time in the soil, PCB's may become less extractable but may remain extractable from activated carbon. Recovery of ^{14}C-labeled PCB from a forest soil averaged 80% after extraction with hexane and centrifugation, 6 months after application, whereas an acetone Soxhlet extraction was able to recover 95% of that applied (Seidl and Ballschmiter, 1976). It has been our experience that centrifugation of PCB's in hexane or water greatly reduces the amount in solution, which may explain this discrepancy in recoveries. No activity was detected in the collected leachate of carbon-amended pots; very little (less than 0.1% of applied activity) was detected from the no carbon pots. Also, the decrease in ^{14}C activity extracted from fescue tops was greater than could be explained by the decrease in ^{14}C content in the soil. This suggests that with continued residence in soil, less PCB is available to plants, possibly due to complexing by organic soil colloids. PCB's are reported to have a high affinity for the organic component of soils (Haque et al., 1974; Scharpenseel et al., 1977a,b). Lower chlorinated biphenyls are reportedly taken up in greater amounts than higher chlorinated biphenyls (Iwata et al., 1974; Wallnöfer et al., 1975; Suzuki et al., 1977; Moza et al., 1979a). Perhaps continued cropping of these pots caused a depletion of the more mobile, lower chlorinated PCB's, leaving behind less mobile, higher chlorinated PCB's. Depite the fact that the absolute amount of ^{14}C activity (either PCB or metabolites) remaining in the soil was greater and decreased less rapidly in the carbon treatment than in the no carbon treatment, the activated carbon still reduced [^{14}C]PCB uptake by 93–100% over a 15-month period.

CONCLUSIONS

Soil-applied PCB (Aroclor 1254) caused reductions of height, top fresh weight, and cumulative water use for soybean and beet at the 1000-ppm rate. Corn and sorghum appeared tolerant to high rates of PCB in the soil (1000 ppm in these studies). Little phytotoxic damage is likely to result from application of sludge containing moderate levels of PCB (1–50 ppm) applied at reasonable rates to soil (11 200–44 800 kg ha^{-1} $year^{-1}$). Measurable growth effects due to soil-applied PCB were eliminated by activated carbon.

[^{14}C]PCB studies showed a reduction in uptake of soil-applied [^{14}C]PCB by beet, corn, sorghum, and peanut due to soil treatment with activated carbon. Reduction of ^{14}C uptake by fescue in the no carbon treatment continued over a 15-month period. Calculated estimates of PCB levels in the crop foliage were low (<1 ppm fresh weight) when applied to soil at 20 ppm. Activated carbon appeared to inhibit the disappearance of [^{14}C]PCB or metabolites from soil over a 1.5-year period. These studies demonstrate that activated carbon could be effectively used to adequately reduce PCB uptake, should PCB levels in crops grown on sludge-amended soil reach high levels.

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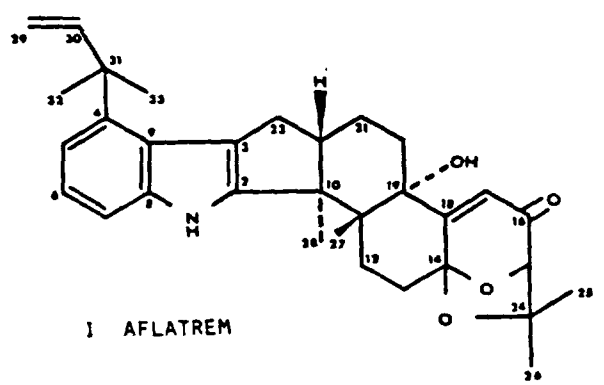
Indole Metabolites from a Strain of *Aspergillus flavus*

Richard J. Cole,* Joe W. Dorner, James P. Springer, and Richard H. Cox

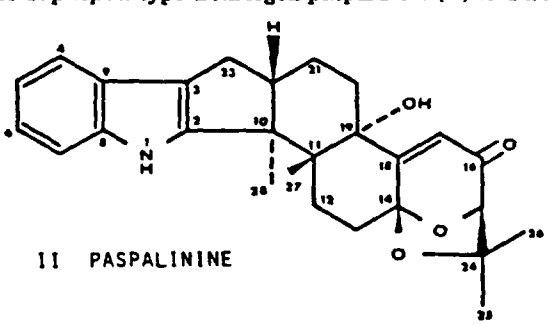
The X-ray structure of dihydroxyaflavinine, a new diterpene indole metabolite from *Aspergillus flavus*, is reported. The fungal tremorgens aflatrem and paspalinine were also isolated from the *A. flavus* isolate. Previously, paspalinine has only been reported from sclerotia of *Claviceps paspali*.

The tremorgenic indole metabolites of *Claviceps paspali* are closely related chemically to aflatrem (I), which is



I AFLATREM

produced by some strains of *Aspergillus flavus* (Cole et al., 1977; Gallagher et al., 1980a). In addition, aflatrem represents the most logical distal product in the biosynthesis of this group of fungal metabolites (Cole, 1980). Because some *A. flavus* isolates can produce this distal product, some of the *C. paspali* type indoles may be present in *A. flavus* cultures. These relationships prompted us to critically examine the indole metabolites of an aflatrem-producing strain of *A. flavus* to determine if the *C. paspali* type metabolites are also metabolites of *A. flavus*. We now report the isolation and identification of the *C. paspali* type tremorgen paspalinine (II) and a new



II PASPALININE

unrelated nontremorgenic indole diterpene metabolite, 20,26-dihydroxyaflavinine (III), from *A. flavus*.

MATERIALS AND METHODS

The *A. flavus* (NRRL 3251) isolate was obtained from the Northern Regional Research Center Culture Collection. The fungus was mass cultured in 2.8-L Fernback flasks,

National Peanut Research Laboratory, U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, Dawson, Georgia 31742 (R.J.C. and J.W.D.), Merck Institute of Therapeutic Research, Department of Biophysics, Rahway, New Jersey 07065 (J.P.S.), and National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709 (R.H.C.).

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COMPARATIVE STUDY OF 3,4,3',4'-TETRACHLOROBIPHENYL IN MALE AND FEMALE RATS AND FEMALE MONKEYS

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3,4,3',4'-Tetrachlorobiphenyl (TCB) is thought to be the most toxic chlorinated biphenyl present in commercial polychlorinated biphenyl (PCB) formulations. A comparative study of the distribution and excretion of TCB has been done in male and female rats and female rhesus monkeys in an effort to account for the toxicity of this PCB. The distribution and clearance of TCB were similar in male and female rats, and although TCB was cleared more slowly by monkeys than by rats, the monkeys were able to clear it at an appreciable rate so that tissue levels were negligible 6 wk after TCB administration. The results indicate that the toxicity of TCB cannot be attributed to inability of the animals to metabolize and excrete it or to its persistence in any tissue, with the possible exception of blood. TCB and/or TCB metabolites appear to be more persistent in blood than any PCB studied to date.

INTRODUCTION

Polychlorinated biphenyls (PCBs) were manufactured, marketed, and used as complex mixtures of congeners and isomers for approximately 30 yr before it was discovered that they are ubiquitous contaminants of the environment (Hutzinger et al., 1974). They have been identified as the causative agents in one incident of large-scale human poisoning (Kuratsune et al., 1975) and several incidents of livestock contamination (Kimbrough, 1972). Thus, a considerable effort has been exerted to elucidate the toxicology of PCBs.

Studies of the toxicity of PCBs have shown considerable variation with species. Many species, including laboratory rats and mice, are only moderately sensitive to intoxication by most PCBs (Grant and Phillips, 1974; Biocca et al., 1975), whereas the monkey appears to be quite sensitive (Allen and Norback, 1976). Two PCBs, 3,4,3',4'-tetrachloro- and 3,4,5,3',4',5'-hexachlorobiphenyl, have been highly toxic to all species

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tested and are the only PCBs known to induce the dramatic atrophy of the lymphatic organs and phorphyrin accumulation characteristic of intoxication by the chlorinated dioxins and furans (Biocca et al., 1975; McKinney et al., 1980). However, the effects of much higher doses of other PCBs are unknown. These toxic PCBs are also the only chlorinated biphenyls known to induce increased formation of cytochrome P-448 and aryl hydrocarbon hydroxylase (Goldstein et al., 1977; Poland and Glover, 1977). 3,4,3',4'-Tetrachlorobiphenyl (TCB) has been shown to be a potent teratogen, inducing behavioral and kidney abnormalities in rats, whereas similar treatment with 2,4,5,2',4',5'-hexachlorobiphenyl produced no such developmental defects (Lucier and McDaniel, 1979). Furthermore, the behavioral effects induced by TCB in perinatal exposure persisted into adulthood (Tilson et al., 1979).

As opposed to the toxic hexachlorobiphenyl, TCB is found in commercial PCB formulations and has been detected in environmental samples (Albro and Parker, 1979). It was therefore of interest to make a comparative study of the disposition of this PCB in both sexes of the rat and in the female monkey. The objective of the study was to determine whether the unique toxicity of this PCB could be attributed to a slower rate of clearance or to a pattern of tissue distribution that differed from that observed previously for a series of other PCBs (Matthews and Anderson, 1975a; Matthews and Tuey, 1980; Van Miller et al., 1975; Peterson et al., 1976).

MATERIALS AND METHODS

Carbon-14-labeled TCB (New England Nuclear, Boston, Mass.) of specific activity 15.1 mCi/mmol was diluted with unlabeled TCB, dissolved in an equal mixture of ethanol and Emulphor EL-620 (GAF Corp., New York, N.Y.), and made up to the required volume with 4 parts water to give a concentration of 0.6 mg/ml and a specific activity of 3.33 μCi/ml. Young adult rats weighing 210-270 g (CD strain, Charles River Breeding Laboratories, Wilmington, Mass.) were injected iv with [^{14}C]TCB solution as a single dose of 0.6 mg/kg body weight. At each of a number of predetermined time points, groups of three rats were sacrificed and samples of blood, liver, femoral muscle, adipose tissue, and skin plus 1 cm from the tail around the injection site were taken from each animal and stored in a freezer (−16°C) until they could be analyzed for TCB-derived radioactivity. Animals having 5% or more of the dose at or around the injection site were discarded and additional animals were treated and sacrificed at the appropriate time points as replacements. Rats held for 1 d or more were housed in individual metabolism cages with food and water *ad libitum* and urine and feces were collected daily. Estimates of tissue volumes and methods used for tissue and feces oxidation, analysis of urine and bile, and extraction of tissue and excreta have been reported (Matthews and Anderson, 1975b).

Adult female rhesus monkeys (5.2–6.8 kg) obtained from the NIH Primate Colony (Bethesda, Md.) were anesthetized with ketamine (Bristol Laboratories, Syracuse, N.Y.) and given an iv injection of TCB (0.6 mg, 20 μCi/kg) prepared in Emulphor-ethanol-water as described for the rats. After the injection venous blood samples were taken at 1, 3, and 24 h, at 2, 7, and 14 d, and at sacrifice. Monkeys were housed in individual metabolism cages and feces and urine were collected daily from treatment to sacrifice 42 d after administration. At sacrifice the monkeys were subjected to a complete necropsy and samples of the major tissues were taken for radioactivity assay as previously described (Sipes et al., 1980). Methods used in extraction and analysis of monkey urine and feces were also described by Sipes et al. (1980).

RESULTS

Tissue Distribution

The distribution of TCB-derived radioactivity in the tissues of male and female rats at various times after administration is presented in Tables 1 and 2. The data show that most of the TCB was rapidly removed from the blood and at approximately the same rate in both sexes. The amount of TCB-derived radioactivity in the blood did not vary significantly with sex at any time point for which comparable data were available. Liver and muscle accounted for storage of a significant portion of the dose during the first 4 h after administration; however, by 24 h after administration most of the TCB had been cleared from these tissues. Clearance of TCB from liver was relatively rapid and showed very little variation with sex. Clearance from muscle was also relatively rapid; however, the data for muscle were more variable than the data for liver, and sex-related differences could not be clearly established or dismissed. The peak TCB concentration in the skin in both male and female rats was observed

TABLE 1. Average Percent of Total Tetrachlorobiphenyl Dose[a] in Male Rat Tissue with Time

	Percent of dose[b]				
Time	Blood	Liver	Muscle	Skin	Adipose tissue
1 h	3.18 ± 0.26	11.74 ± 3.10	23.27 ± 1.74	13.81 ± 0.54	15.62 ± 2.71
2 h	2.74 ± 0.07	8.78 ± 4.10	18.51 ± 1.56	16.32 ± 1.17	24.51 ± 1.62
4 h	4.09 ± 0.54	6.85 ± 1.05	6.71 ± 1.20	14.05 ± 0.63	22.89 ± 0.95
24 h	3.41 ± 0.47	2.13 ± 0.10	5.58 ± 0.67	7.97 ± 0.62	18.29 ± 1.73
2 d	1.79 ± 0.35	0.97 ± 0.14	1.46 ± 0.05	2.84 ± 1.06	13.83 ± 6.37
7 d	0.8 ± 0.14	0.45 ± 0.15	0.5 ± 0.03	2.23 ± 0.42	2.74 ± 0.45

[a] Dose = 0.6 mg/kg.
[b] Each value is the mean ± SD obtained with three animals per group.

TABLE 2. Average Percent of Total Tetrachlorobiphenyl Dose[a] in Female Rat Tissues with Time

	Percent of dose[b]				
Time	Blood	Liver	Muscle	Skin	Adipose tissue
30 min	7.18 ± 1.98	16.0 ± 0.50	33.32 ± 10.14	12.30 ± 4.40	7.46 ± 5.19
3 h	4.95 ± 2.37	9.0 ± 1.30	22.28 ± 6.97	19.90 ± 2.96	14.68 ± 2.63
4 h	3.93 ± 2.06	8.8 ± 4.10	16.20 ± 9.33	16.22 ± 0.99	12.20 ± 0.57
24 h	3.93 ± 0.39	2.1 ± 0.15	12.83 ± 9.76	8.28 ± 1.92	9.60 ± 2.4
7 d	1.00 ± 0.63	1.3 ± 0.30	3.38 ± 2.63	1.80 ± 0.83	7.40 ± 1.31

[a] Dose = 0.6 mg/kg.
[b] Each value is the mean ± SD obtained with three animals per group.

approximately 2 h after administration and the concentration decreased steadily thereafter. Neither the magnitude of the peak concentration in skin nor the rate of decay from the peak concentration indicated a significant variation with sex. The data in Tables 1 and 2 indicate that female rats accumulated a smaller portion of the TCB dose in adipose tissue than did males, but females also cleared TCB from adipose tissue more slowly than did males. At 7 d the TCB stored in adipose tissue showed more variation with sex than that in any other tissue.

Excretion by Rats

The pattern and route of excretion of TCB-derived radioactivity in male and female rats varied only slightly (Figs. 1 and 2). The major route of excretion in both sexes was via the feces. Cumulative excretion of TCB and/or its metabolite(s) in urine accounted for less than 2% of the dose during the 7-d holding period. Approximately 1% of the dose was excreted in urine during d 1 of the study, followed by less than 1% during the remaining 6 d (Fig. 1). As with urine, the largest single excretion of TCB-derived radioactivity in feces occurred on d 1, but in feces the rate of TCB excretion decreased more slowly than in urine (Fig. 2). The values for cumulative 7-d excretion of TCB-derived radioactivity in the feces of male and female rats were 79.9 ± 11.2 and 88.4 ± 6.8% of the total dose, respectively. Extraction and analysis of the feces and urine (Matthews and Anderson, 1975b) indicated that more than 90% of the TCB-derived radioactivity excreted by either route was in the form of various metabolites of this PCB. Results from studies of bile duct cannulations indicated that the major source of the TCB-derived radioactivity excreted in feces was the bile.

Tissue Extraction

Since the distribution and clearance of TCB showed only minor variations with sex, it was assumed that the nature of the TCB-derived

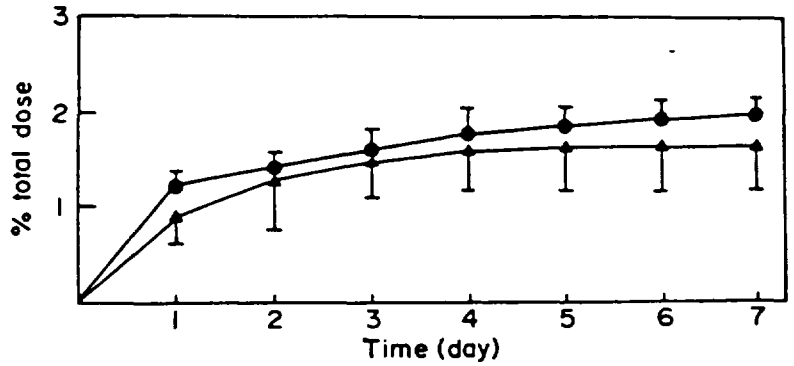

FIGURE 1. Cumulative excretion of TCB in urine by male (▲) and female (●) rats. Each point represents the average ± SD obtained with three animals that received an iv dose of 0.6 mg/kg.

radioactivity in tissues would also be quite similar, and analysis of radioactivity in the tissues at various time points was restricted to tissues from male rats. The results of tissue extraction and analysis are presented in Table 3. It is seen that, with the exception of blood, most of the radioactivity in rat tissues was unmetabolized TCB. In blood, the concentration of metabolites of TCB equaled or exceeded the concentration of the parent compound at every time point assayed.

Tissue Distribution in Monkeys

Three adult female rhesus monkeys were treated with a single dose of TCB, as described under Methods. Monkeys were held in individual

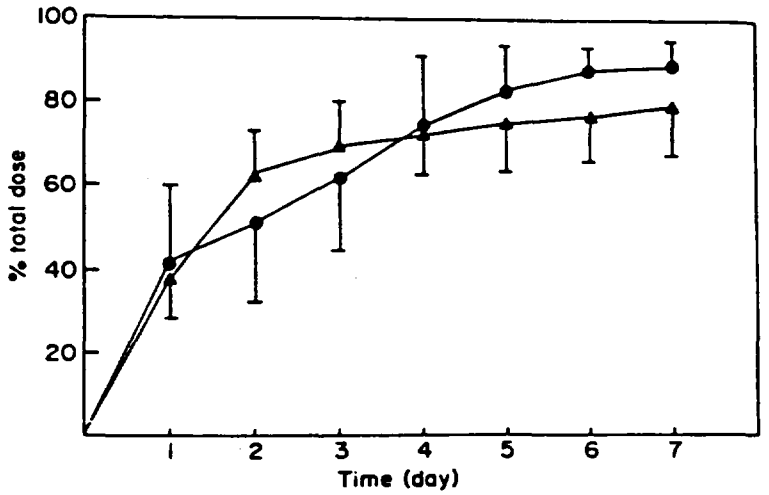

FIGURE 2. Cumulative excretion of TCB in feces of male (▲) and female (●) rats. Each point represents the average ± SD obtained with three animals that received an iv dose of 0.6 mg/kg.

TABLE 3. Tetrachlorobiphenyl Metabolites in Male Rat Tissues

	Metabolites (% of total radioactivity)[a]				
Time	Blood	Liver	Muscle	Skin	Adipose tissue
1 h	50	–	–	–	–
2 h	–	5.3	1.6	>1	>1
4 h	72.9	–	–	–	–
8 h	91.0	35.4	7.7	3.2	1.2
24 h	86.5	10.7	8.9	2.2	1.5

[a]Values represent the percent of total radioactivity in tissue that did not cochromatograph with parent compound or was not extracted by organic solvents.

metabolism cages with food and water *ad libitum*. Blood samples (~3 ml) were taken from each monkey at six time points and at sacrifice. Each blood sample was extracted as described under Methods to determine the relative amounts of parent TCB and TCB metabolites. These results are presented in Table 4. Approximately 95% of the TCB dose was removed from the blood within 1 h after it was administered. The amount of TCB in blood continued to decrease for the first 24 h after administration and then increased to a small secondary peak between 2 and 7 d. After 7 d the TCB in blood decreased slowly to nearly negligible levels at sacrifice on d 42. The relative amount of TCB metabolites increased steadily until it accounted for all but a trace of the TCB-derived radioactivity in blood

TABLE 4. Tetrachlorobiphenyl in Monkey Blood with Time

Time	Percent of total dose[a]	Percent of metabolite[b]
1 h	4.38 ± 0.51[c]	Trace
3 h	2.32 ± 0.36	22
1 d	1.84 ± 0.13	52
2 d	3.23 ± 0.57	53
7 d	3.49 ± 0.80	>98
14 d	1.81 ± 0.22	>98
42 d	0.14 ± 0.07	ND[d]

[a]Dose = 0.6 mg/kg.
[b]Percent of radioactivity in blood that did not cochromatograph with parent compound.
[c]Each value is the average ± SD obtained with three animals.
[d]ND, too little radioactivity to determine percent of metabolite.

by d 7. At necropsy, the only signs of PCB intoxication were thickened meibomian glands of the eyelid, in which histology showed atrophy of sebaceous elements and enlarged central ducts impacted with keratin (McConnell and Moore, 1979; Moore et al., 1979). Tissues sampled at sacrifice included blood, liver, kidneys, heart, lungs, muscle, intestines, adrenals, skin, and adipose tissue. By d 42 most of the TCB had been cleared from all tissues except adipose tissue, which contained 2.1 ± 1.8% of the total dose compared to only 1% for all the other tissues combined. These low levels of radioactivity precluded determination of the relative amounts of parent and metabolite TCB in monkey tissues.

Excretion by Monkeys

During wk 1 of the holding period, excretion of TCB-derived radioactivity in the urine was appreciable and accounted for 6.6% of the total dose (Fig. 3). During the next 5 wk of the holding period the excretion of TCB-derived radioactivity in urine was very slow and accounted for only an additional 1.9% of the total dose. Extraction and analysis of the radioactivity excreted in urine (Sipes et al., 1980) indicated that 97% was in the form of metabolites of TCB.

As with rats, the major route of TCB excretion by monkeys was the feces. However, fecal excretion of TCB by monkeys was somewhat erratic because the monkeys, apparently stressed by the physical manipulations

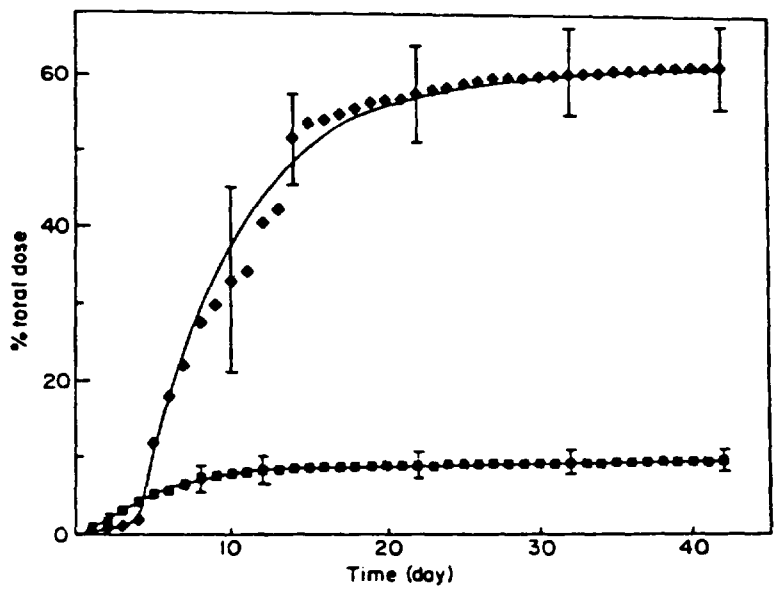

FIGURE 3. Cumulative excretion of TCB in urine (■) and feces (●) of female rhesus monkeys. Each point represents an average obtained with three animals. Standard deviation calculations were limited to simplify the figure.

associated with TCB administration and blood sampling, excreted little or no feces before d 5, and one monkey failed to excrete feces before d 8. Once the excretion pattern became more regular, TCB-derived radioactivity was excreted in the feces more rapidly than in the urine and accounted for more than 50% of the total dose in 14 d (Fig. 3). During the next 4 wk the monkeys excreted an additional 10% of the dose in the feces, for a total of 61.4 ± 5.8% of the total TCB dose in 6 wk (Fig. 3). Extraction and analysis of the TCB-derived radioactivity excreted in feces (Sipes et al., 1980) indicated that 97% was in the form of TCB metabolites.

DISCUSSION

With the exception of the unexplained variation in the accumulation and clearance of TCB from adipose tissue, there were no apparent sex-related differences in the distribution of TCB in the rat. The patterns of excretion of TCB by male and female rats were similar, and the lower total excretion of TCB-derived radioactivity by male rats (Figs. 1 and 2) was more indicative of lower total recovery of the administered radioactivity, approximately 89.5% for males compared to 97.1% for females, than of any sex-related difference. The observation that most of the TCB-derived radioactivity in tissues was parent compound (Table 3) whereas most of that excreted was in the form of TCB metabolites is consistent with reports that PCBs are not excreted to an appreciable extent before metabolism to more polar compounds (Matthews and Anderson, 1975a; Matthews and Tuey, 1980). Therefore, it may be inferred from the similar rates of TCB excretion by male and female rats that there is little sex-related difference in the rate of TCB metabolism by rats.

A comparison of the data presented here with those reported for disposition studies of other PCBs in the rat (Matthews and Anderson, 1975a; Van Miller et al., 1975; Tuey and Matthews, 1977) indicates that TCB was cleared from most tissues at rates that might have been anticipated, given the degree and position of TCB chlorination. That is, the rate of TCB clearance from most tissues was intermediate between that of less highly chlorinated and that of more highly chlorinated PCBs and intermediate between that of 2,5,2',5'-tetra- and that of 3,5,3',5'-tetrachlorobiphenyl. However, TCB-derived radioactivity remaining in blood at the later time points, 1 d and longer, exceeded that reported for any other PCB that has been studied in a similar manner. This observation was constant despite the fact that some PCBs were much more persistent in all tissues except blood. For example, in similar studies of TCB, 3,5,3',5'-tetrachlorobiphenyl, and 2,4,5,2',4',5'-hexachlorobiphenyl administered at 0.6 mg/kg to groups of three male rats each, the mean blood levels 7 d after administration were 0.8, 0.4, and 0.19% of the total dose for the three compounds, respectively, whereas at the same time

point, mean values of 6.9, 52.7, and 75.0% of the total dose, respectively, remained in all the other tissues combined (Table 1) (Matthews and Anderson, 1975a; Tuey and Matthews, 1977). Less than 0.01% of the total dose of 4-chloro-, 4,4'-dichloro-, or 2,5,2',5'-tetrachlorobiphenyl remained in the blood 7 d after administration (Matthews and Anderson, 1975a; Van Miller et al., 1975).

Sipes et al. (1980) studied the disposition of PCBs in male cynomolgus monkeys (*Macaca fascicularis*) and reported that they do not readily metabolize and excrete PCBs that are chlorinated in the 4,4'- positions. In the work described here we used female rhesus monkeys and the results indicate that 50% of the TCB dose was metabolized and excreted within the first 14 d of the study (Fig. 3). Therefore, the monkeys used in this study excreted TCB more rapidly than anticipated and the TCB-derived radioactivity remaining in tissues at sacrifice was quite low. Apart from confirming that in rhesus monkeys, as in other species, TCB was most persistent in adipose tissue, the data were limited in usefulness. Radioactivity recovered from these monkeys averaged 73% of the total dose. Incomplete collection of feces is thought to be the major source of lost radioactivity; if the amounts of TCB excreted in feces (Fig. 3) were actually 27% greater, approximately 50% of the TCB dose would have been excreted within the first 8–10 d of the study. This excretion is significantly slower than that in rats, which take approximately 1.3–1.5 d to excrete 50% of a similar TCB dose, but significantly more rapid than that in male cynomolgus monkeys, which take 19 d to excrete 50% of a similar dose of 4,4'-dichlorobiphenyl (Sipes et al., 1980). Thus monkeys metabolize and excrete PCB less rapidly than do rats, but it is not certain whether the variance in PCB clearance observed with monkeys was due to the species, sex, or PCB studied. It is our opinion, based on previous experience with studies of PCB disposition, that the different rate of PCB clearance observed in monkeys was due to the species of monkey used.

The results of this comparative study of TCB disposition corroborate the observation of Sipes et al. (1980) that PCBs are cleared more slowly by monkeys than by rats. These observations may explain, in part, the greater sensitivity of monkeys to intoxication by PCBs (Allen and Norback, 1976). Furthermore, since at 1 d and later most of the TCB-derived radioactivity remaining in the blood in both rats and monkeys was in the form of one or more metabolites (Tables 3 and 4), the present study also demonstrated for the first time that a metabolite of this uniquely toxic PCB is more persistent in blood than any individual PCB or PCB metabolite studied to date. Yamamoto and Yoshimura (1973) reported that the major metabolite of 2,4,2',4'-tetrachlorobiphenyl is approximately five times as toxic as the parent PCB. One is tempted to speculate that a higher concentration of a toxic TCB metabolite in blood might result in a higher concentration of toxicant at the site of action and thus account for the unique toxicity of TCB. However, metabolites of

TCB have not been isolated and studied, and the toxicity of any proposed TCB metabolites will remain a matter of speculation until this additional work is done.

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NEW CONCEPTS OF DESIGN AND UTILITY OF LARGE-SCALE CARCINOGENICITY STUDIES

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This review summarizes salient features of the recently completed large-scale, long-term animal carcinogenicity study conducted at the National Center for Toxicological Research.

INTRODUCTION

It is generally acknowledged that animal experiments to test for the possible carcinogenicity of chemicals provide, to date, the best and only deeply researched method for the detection of environmental carcinogens for humans. This does not preclude further research and development of short-term tests (individual, battery, or tier concepts), but for the present and the foreseeable future, long-term animal tests are the principal predictive tools for safety evaluation.

Although results of chronic toxicity and carcinogenesis tests have a major impact on regulatory and societal decisions, these tests have limitations, and efforts must be made to improve their reliability and the assessment of the validity of experimental results. While the predictive value of animal bioassays is good, there are serious limitations that must be dealt with in applying the test results to best assess human risk.

A broad spectrum of guidelines or recommended procedures have been suggested or reviewed, primarily in the last 5 yr. These include the following: Ad Hoc Committee on the Evaluation of Low Levels of Environmental Carcinogens (1971); Arcos et al., (1968); Berenblum (1969); Canada, Ministry of Health and Welfare (1973); Food Assessment Scientific Committee (1978); Food and Drug Administration (1976); Golberg (1974, 1975); IARC (1977, 1978, 1979); IRLG (1979); Magee (1970); Munro (1977); NAS (1975); Page, 1977a, 1977b); Rall (1977); Roe and Tucker (1973); Shubik and Clayson (1976); Sontag (1977); Sontag et al., (1976); Tomatis (1977, 1978); World Health Organization (1969, 1974, 1978); Zweickey and Davis (1975).

The view is widely held that there are a number of severe biological, pharmacological, and statistical problems involved in utilizing data from animal experiments to estimate the cancer risk to humans from long-term exposure to very low doses of chemicals (Crump et al., 1976; Food Assessment Scientific Committee, 1978; Gaylor and Shapiro, 1977a; Guess and Crump, 1976; Hoel et al., 1975; Hoel, 1976, 1977; IRLG, 1979; Mantel and Bryan, 1961; NAS, 1975; Weinhouse, 1977).

A major statistical problem is that of extrapolating the animal dose-response relation from the high-dose range where animal test data are most available to the low doses that humans are most likely to encounter. In the past it has been considered impractical, mostly because of constraints on resources, to test more than a few hundred animals at a given dose

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level. Hence, the doses at which most tests have been conducted have been high enough to produce positive responses in a test group of approximately 50 animals per dose; the sensitivity of the test varies with the incidence of spontaneous tumors. Thus, although current testing methodologies can establish the carcinogenicity of a chemical, they provide no information on the nature of the dose-response curve at low doses. Weinhouse (1977) observed that the assessment of risk in humans requires extrapolation of animal data obtained at high doses to a human population approximately 10^6 times larger with exposure levels likely to be approximately 10^4 times lower and with a life-span 30 times longer.

It has been a major goal of the National Center for Toxicological Research (NCTR) to develop improved methodologies and test protocols that will facilitate the extrapolation of toxicological data from laboratory animals to humans (Fishbein, 1976). This review is intended to highlight the salient features of a recently completed NCTR large-scale complex chronic low-dose response (ED01) study. A major objective of this study was to establish a quantitative experimental base for evaluating excessive carcinogenic risk produced by a chemical in variously exposed populations. Integral to this objective was the determination of the mathematical characteristics of the low dose-response curves for tumor incidence and the time to tumors in terms of time, dosage, rate, and total dose.

These considerations should help clarify the possibilities of extrapolating from high to low dosages and hence facilitate an approximate determination of the populations at risk from exposure data. A vital requirement of the study was the use of sufficient numbers of animals to permit an accurate mathematical description of the nature of the curve at low dosages (e.g., down to a 1% prevalence of bladder tumors). The major aspects of this study were described at the NCTR Symposium on Innovations in Cancer Risk Assessment, Washington, D.C., June 1979 (Cairns, 1979; Farmer et al., 1979; Frith et al., 1979a, 1979b, 1979c; Gaylor, 1979; Greenman, 1979; Henry and Johnson, 1979; Kodell and Nelson, 1979; Kodell et al., 1979; Littlefield et al., 1979; Sheldon and Greenman, 1979).

DESIGN AND LOGISTICS

The ED01 study employed approximately 24,000 BALB/c female mice, which were fed 2-acetylaminofluorene (2-AAF) in 81 different treatment groups. The mice were randomly assigned to the dose groups, housed 4 per cage under specific pathogen-free/defined flora (SPF/DF) barrier conditions, and fed the carcinogen *ad libitum* in the diet at various concentrations from weaning until they were sacrificed, became moribund, or died.

Part of the study included serial sacrifices to investigate the progression of tumorigenesis. Another part of the study was devoted to lifetime exposure to 2-AAF to study time to tumor and life shortening, and a third part included removal of the carcinogen from the diet to study the initiating and promoting effects of 2-AAF. Sufficient animals were used in the treatment groups to provide adequate statistical precision. This was the largest single study ever conducted with a chemical carcinogen.

A large complex chronic study of this scope required the development of an adequate supply of suitable animals, carefully controlled diets and environment, long-term animal care, automatic data collection systems, verification and timely retrieval of data, and high-quality pathology on a large scale. Controls on chemicals and microbiological contamination of the feed were maintained by constant surveillance. Proper animal care was provided daily; animal feeders, water bottles, bedding, and cages were changed weekly. Environmental conditions of the animal rooms were maintained throughout the study (Gaylor, 1979).

The automated data system support for the ED01 study was of necessity quite extensive. It was found that most of the data to be generated could be directly associated with the specific test animal to which the data were related and could be simplified into

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TABLE 1. Resu
Analyzed Prior t

Param
Aflatoxin B_1, B_2
Lindane, ppb
Heptachlor, ppb
Malathion, ppm
DDT (total), ppb
PCB, ppb
Dieldrin, ppb
Cadmium, ppb
Arsenic, ppm
Lead, ppm
Mercury, ppb
Selenium, ppm
Calcium, %
Copper, ppm
Zinc, ppm
Vitamin A, 101
Vitamin B_{12}, mg,
Estrogenic activi
Total protein, g
Total fat, g/100

[a] NA, not appli
[b] Diethylstilbes

three major systems: (1) breeding information system (BIS) (Holland et al., 1978), (2) experiment information system (EIS) (Lawrence et al., 1978), and (3) postexperiment information system (Herrick et al., 1979) incorporating the pathology data collection system (Frith et al., 1976). The support of the study (from protocol approval through final report generation) by these systems produced the largest volume of research data of this type ever collected (Henry and Johnson, 1979; Taylor and Johnson, 1979).

We are becoming increasingly aware that impurities or manufacturing by-products present in exceedingly small concentrations can exert profound toxic effects that may be associated with a test substance [e.g., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T); chlorinated dibenzofurans in polychlorinated biphenyls (PCBs)]. Hence, a comprehensive chemical surveillance and quality assurance program (Oller et al., 1979) to augment the ED01 study included initially determining the purity of 2-AAF; identifying any trace contaminants; and evaluating the homogeneity, stability, and dosages of the agent in the commercial diet (approximately 250 batches of laboratory animal chow per month).

Other elements of the program included testing for the presence of deleterious concentrations of specific chemical and elemental contaminants in the animal feed (each 3-ton lot), water, hardwood chip bedding, and cardboard feeder boxes. This was done for various chlorinated and organophosphorus pesticides, PCBs, and pentachlorophenol to ensure that concentrations were within program guidelines. In addition, the feed and water were analyzed for toxic metals such as Cd, Pb, Hg, As, and Se, and the feed was analyzed for fat, protein, vitamins A and B, Cu and Zn, aflatoxin contamination, and estrogenic activity. Table 1 shows analytical results for lots of feed assayed for selected constituents over a 5-yr

TABLE 1. Results from 148 Lots of Purina Autoclavable Rodent Laboratory Chow Analyzed Prior to Autoclaving

Parameter	Min.	Max.	Mean	SD
Aflatoxin B_1, B_2, G_1, G_2, ppb	–	5	5	NA[a]
Lindane, ppb	–	100	1.67	3.6
Heptachlor, ppb	–	20	1.07	2.2
Malathion, ppm	–	5	0.33	0.52
DDT (total), ppb	–	100	27.7	48.4
PCB, ppb	–	50	8.7	15.1
Dieldrin, ppb	–	20	2.4	4.6
Cadmium, ppb	–	250	87.3	33.2
Arsenic, ppm	–	1.0	0.31	0.18
Lead, ppm	–	1.5	0.47	0.38
Mercury, ppb	–	200	0.024	0.018
Selenium, ppm	0.5	0.65	0.34	0.15
Calcium, %	0.75	–	1.16	0.18
Copper, ppm	8	–	15.0	2.8
Zinc, ppm	75	–	108.2	9.7
Vitamin A, 101 U/g	15	75	41.6	36.9
Vitamin B_{12}, mg/g	0.075	0.125	9.1	1.25
Estrogenic activity[b], ppb	–	5	5	NA
Total protein, g/100 g	21.0	28.0	24.2	2.4
Total fat, g/100 g	4.3	6.7	5.54	0.58

[a] NA, not applicable.
[b] Diethylstilbestrol equivalent.

TABLE 2. Approximate Data Volumes

Test animals	24,192
Study duration	43 mo
Animal weights	1.98 million
Animal observations	2.16 million
Tissues examined (histology)	830,000

period (Oller et al., 1979). The variability of nutrient concentrations is much less than of pesticides or heavy metals (the latter levels may be dependent on geographic and seasonal variations attendant on grain procurement).

Room air, wastewater (from autoclaving, cage washings, etc.), and environmental surfaces were monitored at 3-mo intervals. Urine specimens from individuals potentially exposed to the test compound were monitored. The analytical procedures used in personnel and environmental surveillance were reported by Bowman and King (1974) and Bowman et al. (1976), respectively.

A comprehensive surveillance program was also conducted to provide as much information as possible on the microbiological condition of the test animals and their environment throughout the course of the study (Tortorich et al., 1979). The main objective of this program was to ensure the detection of specified microorganisms, primarily pathogenic bacteria, fungi, parasites, and viruses, which could adversely affect the study. The microbiological program consisted of frequent and repetitive analyses of samples from animal and nonanimal sources in the breeder and test facilities. The following were examined: (1) SPF/DF experimental mice; (2) animal diets (for total count of *Salmonella*, *Bacillus subtilis*, and *B. stearothermphalis*); (3) drinking water (total bacterial count and *Pseudomonas aeruginosa*); (4) environmental air samples, bedding (total bacterial, mold, coliform counts, pathogenic fungi, *Salmonella* species), cages and accessories (bacterial and mold contamination); (5) support equipment such as carts, trays, balances, computer terminals, and closed-circuit TV components (bacteria and mold); (6) clothing (specific bacterial pathogens); (7) walls and floors (approximately every 3 mo for bacterial and mold contamination); and (8) personnel authorized for entrance into the controlled areas (semiannually for viral, bacterial, and fungal contamination) (Tortorich, 1979).

From these considerations, the logistical problem encompassed five major categories: (1) raw materials, (2) scheduling, (3) study initiation, (4) study maintenance, and (5) data collection and utilization. The scope of this undertaking could be gleaned from two considerations: approximate data volumes (Table 2) and diet preparation (Table 3) (Henry and Johnson, 1979).

TABLE 3. Diet Preparation

Year	Feed mixed (tons)	Batches	Six-packs of feed
1974[a]	16.9	770	17,707
1975	46.6	2123	48,820
1976	24.1	1099	25,279
1977[b]	2.4	111	2,545
Total	90	4103	94,351

[a] Figures from April 29, 1974.
[b] Figures through April 4, 1977.

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TABLE 5. Experimental Des

2-AAF (ppm)	9	12
150	144[a]	144
100	144	144
75	144	144
60	288	288
45		
35		
30		
0	144	144

[a] These numbers refer to the

TABLE 4. Estimated Bladder Tumor Prevalence with 2-AAF in Feed from Pilot Study

2-AAF in feed (ppm)	Bladder tumor prevalence (ED %)
150	50
100	28
75	16
60	10
45	4
35	2
30	1

Before the initiation of the large-scale ED01 study, a pilot study with dosages of 100, 250, and 500 ppm 2-AAF was conducted with BALB/c female mice obtained from a commercial source to establish dosages for the large-scale study at the low end of the dose-response curve (Cairns, 1979; Littlefield et al., 1979). Bladder tumor prevalence at 18 mo was high at 250 and 500 ppm; the most precise data point was obtained at 100 ppm, where 28% of the animals had bladder tumors at 28 mo. Since it was difficult to establish the slope of the dose-response curve because of the high dosages used, a dose range of 30–100 ppm was selected for the large-scale study to span the range of tumor rates from 1 to 50% (Table 4). It was also determined that under barrier conditions with an enclosed feeder and BALB/c female mice bred at NCTR, the bladder tumor rate was only about 4% at 18 mo. This strain of mouse also has a relatively low background tumor incidence and is long-lived.

It took 9 mo to allocate the 24,192 mice to 6 SPF/DF barrier rooms (replications). The mice were derived from the breeding colony at NCTR from a Charles River substrain of BALB/c mice. Exposure to 2-AAF began after weaning at 3–4 wk of age (Cairns, 1979; Littlefield et al., 1979).

The experimental design is shown in Table 5, which gives the numbers of animals allocated to various treatment groups. There are 7 dose levels ranging from 30 to 150 ppm 2-AAF plus a control. The lowest and highest doses are separated by only a factor of 5,

TABLE 5. Experimental Design of 2-AAF ED01 Study: Serial Sacrifice

2-AAF (ppm)	Time (mo)								
	9	12	14	15	16	17	18	14	Life
150	144[a]	144	120	96	96	72	144	336	144
100	144	144	120	96	96	72	144	336	144
75	144	144	120	96	192	144	288	600	288
60	288	288	240	192	288	216	288	792	288
45					288	288	432	864	432
35						432	864	1296	864
30							1728	1728	1728
0	144	144	120	96	192	144	432	744	432

[a]These numbers refer to the number of animals assigned to a specific test group.

which gives a very compact experimental design. The number of test animals is significantly larger at the lower dosages, where effects are more difficult to detect. There are 8 sacrifice intervals commencing at 9 mo and ending at 24 mo. Most of the sacrifices are between 12 and 18 mo, where the pilot study showed a rapid increase in tumor incidence. Although the study was originally designed to terminate at 18 mo, it was noted that approximately 90% of the animals in the study were alive at the 18-mo sacrifice since the lower doses were not producing neoplastic growths at this time. It was thus decided to reschedule sacrifices in three of the six replications to 24 mo. Since the death rates were low, a large number of mice were available for this transfer. This 24-mo sacrifice was incorporated into the design after the study had progressed to the 18-mo sacrifice in two of the replications. The design also contained a group of animals allocated to a life-span study, which were to remain in the study until they were dead or moribund; an additional sacrifice interval occurred when this group was terminated at 33 mo.

Table 6 represents a subgroup designated as a serial treatment group. Mice at the four highest dose levels (60, 75, 100, and 150 ppm) were treated for 9, 12, or 15 mo, then sacrificed at 18 or 24 mo. A key objective of this part of the design was to examine the regression or progression of the tumors after the administration of 2-AAF was stopped. All other conditions were the same as in the rest of the study.

RESULTS AND DISCUSSION

The results consist primarily of data on the incidence of primary hepatocellular carcinomas (liver neoplasms) and transitional cell carcinomas (bladder neoplasms) of the urinary bladder (Frith, 1979a, 1979b; Littlefield et al., 1979).

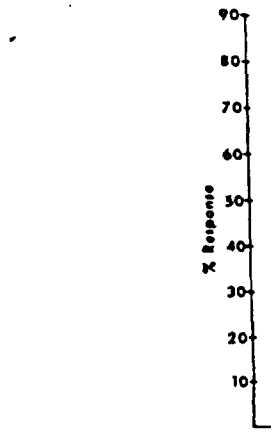
Figure 1 shows the occurrence of bladder neoplasms in sacrificed animals plotted against time in months. Neoplasms occurred at the 60, 75, 100, and 150 ppm doses. The incidence of tumors at 150 ppm was low at 9 mo, but increased rapidly between 12 and 18 mo; at 100, 75, and 60 ppm, the growth and induction of bladder neoplasms were much lower and later in time. Figure 2 shows the same data as Fig. 1, but plotted against dose, for the 18, 24, and 33 mo sacrifices. The apparent effect of extending the sacrifices beyond 18 mo is evidenced by the displacement of the curve. Although there are substantial differences in the response at the 100 and 150 ppm dose levels at the 24 mo sacrifice, changes in bladder tumor incidence at 75 and 60 ppm were not apparent until the 33 mo sacrifice. Data plotted in this manner suggest a possible minimal effect at 45 ppm.

Figure 3 shows the occurrence of liver neoplasms in sacrificed animals plotted against time. The data show a striking dose-response effect at all seven doses. In contrast to the bladder neoplasms, the liver neoplasms appear very late in the life-span of the test animals. The additional data derived by extending the study to 24 mo are evident.

TABLE 6. Experimental Design of 2-AAF ED01 Study: Serial Treatment

Time of sacrifice (mo):	18	24	18	24	18	24
Time on 2-AAF (mo):	15	15	12	12	9	9
150 ppm	72[a]	72	72	72	72	72
100 ppm	72	72	72	72	72	72
75 ppm	144	144	144	144	144	144
60 ppm	216	216	216	216	216	216

[a] These numbers refer to the number of animals assigned to a specific test group.



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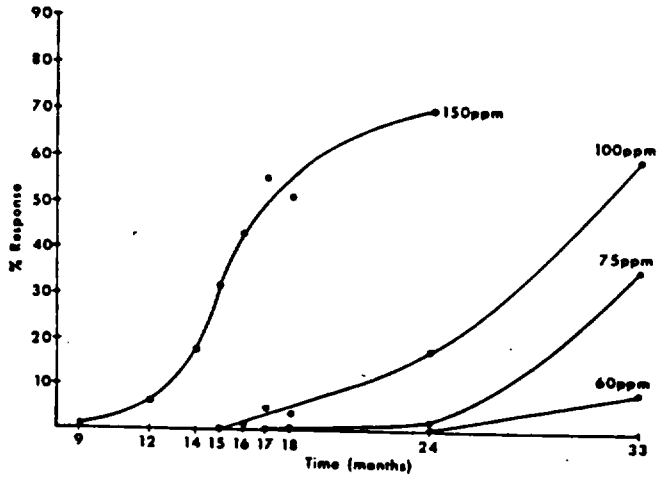

FIGURE 1. Prevalence of bladder neoplasms in sacrificed mice with respect to time on study.

Figure 4 shows the same data as Fig. 3, but plotted against dose, using the 18, 24, and 33 mo sacrifices. In contrast to the bladder neoplasm data, there does not appear to be any minimal effect level in any sacrifice group. The data are nearly linear and appear to extrapolate directly to zero in the 18, 24, and 33 mo groups.

The effects on bladder neoplasms of discontinuing 2-AAF after 9, 12, and 15 mo are shown in Figs. 5, 6, and 7, respectively. For example, Fig. 5 shows that after 9 mo on 2-AAF at 150 ppm, although the induction of bladder neoplasms continued to increase to 18 and 24 mo, the rate was much lower than if the treatment was continued to the sacrifice times. This suggests that bladder tumorigenesis is not complete after 9 mo on 150 ppm 2-AAF. It should also be noted that an increase in bladder neoplasm incidence was not apparent among mice fed 2-AAF for 9 mo at levels of 100 ppm or less. Hence, it is suggested that the detection of bladder neoplasms could well be missed with short exposures at low dosage levels.

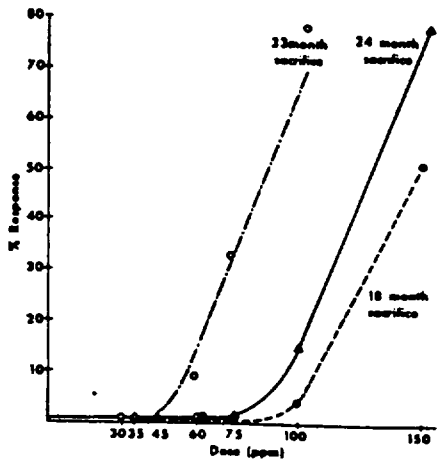

FIGURE 2. Prevalence of bladder neoplasms in sacrificed mice with respect to dose.

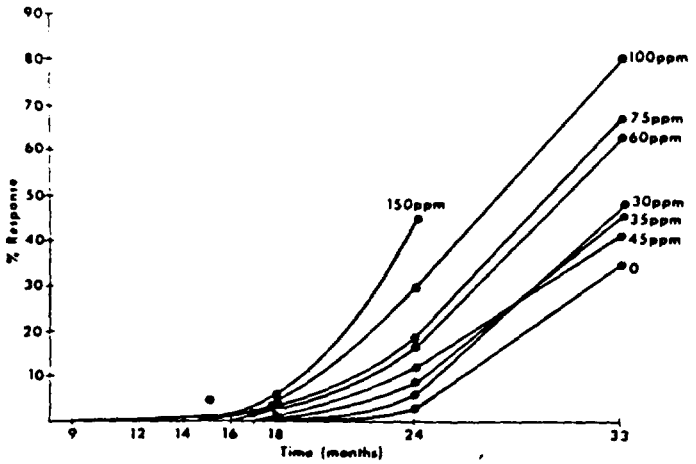

FIGURE 3. Prevalence of liver neoplasms in sacrificed mice with respect to time on study.

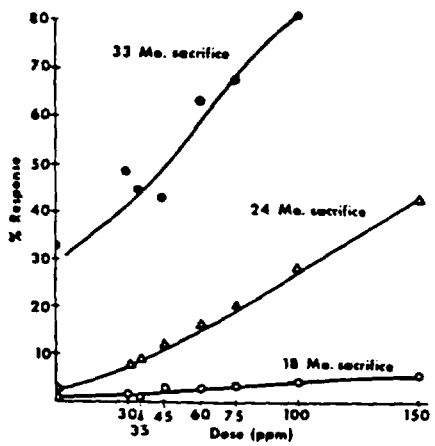

FIGURE 4. Prevalence of liver neoplasms in sacrificed mice with respect to dose.

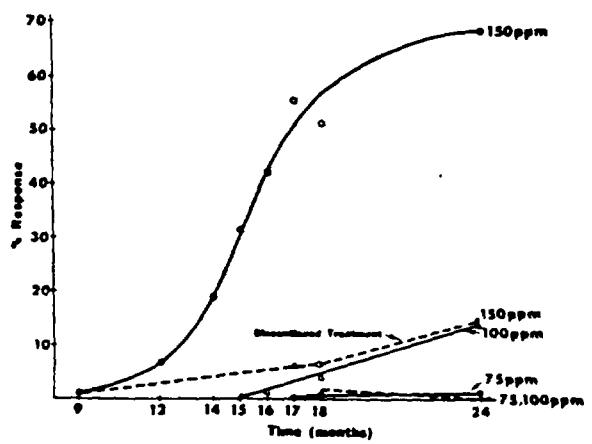

FIGURE 5. Prevalence of bladder neoplasms in mice fed 2-AAF for 9 mo and sacrificed at 18 and 24 mo.

FIGURE 6. Prevalence

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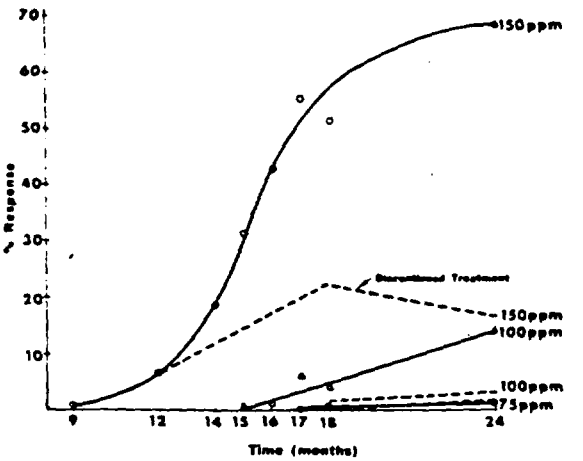

FIGURE 6. Prevalence of bladder neoplasms in mice fed 2-AAF for 12 mo and sacrificed at 18 and 24 mo.

Figure 6 depicts essentially the same response as Fig. 5—that is, bladder tumorigenesis occurred in animals exposed for 12 mo to 150 ppm 2-AAF, but at a much lower rate than in animals that were treated until the 18 and 24 mo sacrifices.

As shown in Fig. 7, animals that were treated for 15 mo and sacrificed at 18 and 24 mo showed no change in percent response during the 3 sampling periods, suggesting that no further bladder tumorigenesis occurred after treatment was discontinued.

The effects of discontinuing 2-AAF in the feed (after 9, 12, and 15 mo) on the development of liver neoplasms are depicted in Figs, 8, 9, and 10. For example, as shown in Fig. 8, after 9 mo on 2-AAF, tumor incidence continued to increase with time for all dose levels (60, 75, 100, and 150 ppm). The rates of increase are, in most cases, only slightly to moderately lower than in the animals that were treated for the entire 18 or 24 mo. This would appear to indicate that the induction of liver neoplasms is nearly complete after 9 mo of exposure to 2-AAF. The data for mice fed 2-AAF for 12 and 15 mo and sacrificed at 18 and 24 mo (Figs. 9 and 10, respectively) show essentially the same results.

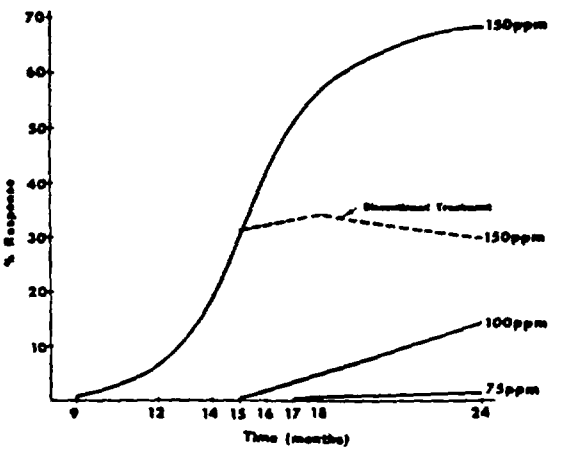

FIGURE 7. Prevalence of bladder neoplasms in mice fed 2-AAF for 15 mo and sacrificed at 18 and 24 mo.

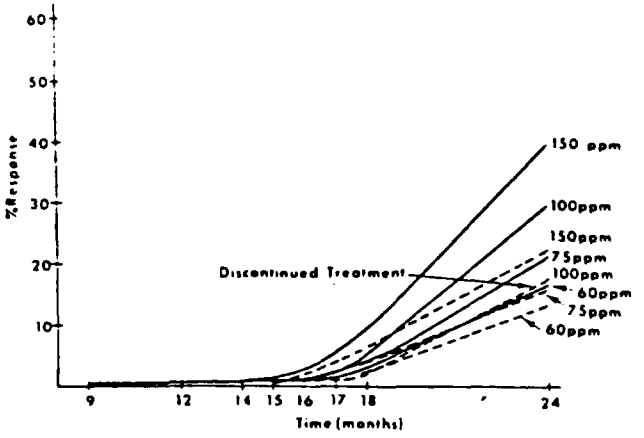

FIGURE 8. Prevalence of liver neoplasms in mice fed 2-AAF for 15 mo and sacrificed at 18 and 24 mo.

A comparison of the development of liver and bladder neoplasms is striking. Bladder neoplasms develop much sooner than liver neoplasms but require the presence of 2-AAF if the incidence is to increase. Liver neoplasms, in contrast, becoming apparent much later in the life-span of the mice, but do not require the continued presence of 2-AAF beyond 9 mo for the incidence to continue to increase at nearly the same rate.

Dose- and time-response models for the incidence of bladder and liver neoplasms found in the ED01 study have been reported by Farmer et al. (1979). The modeling of the experimental data was done in three steps for each of these neoplastic end points. A dose-response model was first attempted for each time interval, then a time-response model for each dose level, and finally a model that would relate the incidence to both dose and time. Since there was a low incidence of liver and bladder neoplasms at all but the highest dose, there was no attempt to fit a dose-response model before 18 mo. Table 7 shows the parameters of the model for the incidence of bladder and liver neoplasms at 18, 24, and 33 mo. Figure 11 illustrates a probit log dose model for bladder neoplasms; an estimate of the

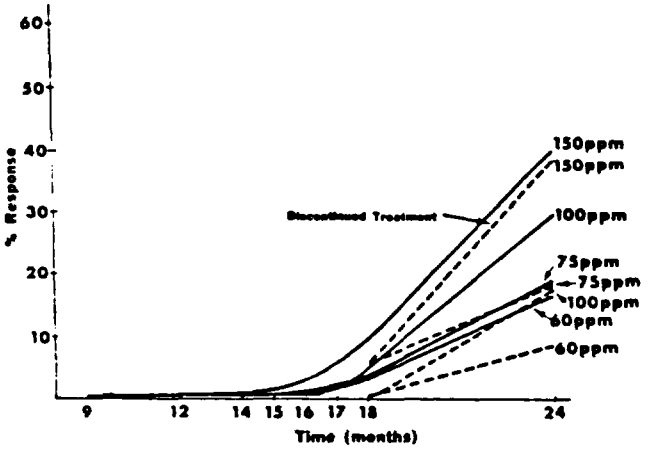

FIGURE 9. Prevalence of liver neoplasms in mice fed 2-AAF for 12 mo and sacrificed at 18 and 24 mo.

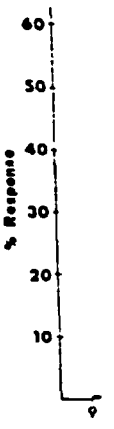

FIGURE 10. Prevalence

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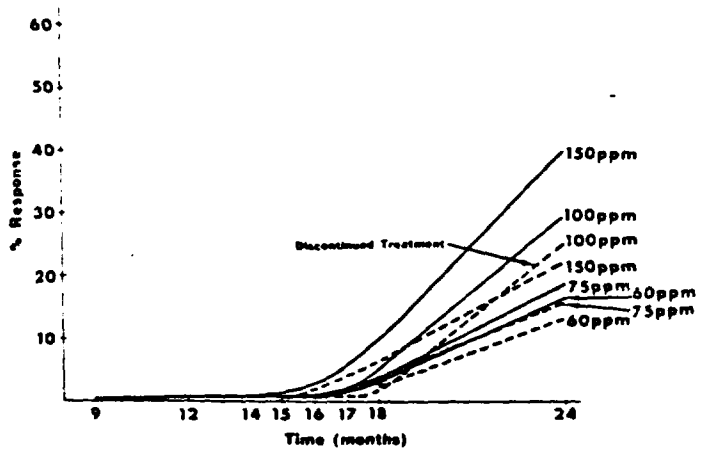


FIGURE 10. Prevalence of liver neoplasms in mice fed 2-AAF for 15 mo and sacrificed at 18 and 24 mo.

common slope was 9.4. The steepness of the slope of these regression lines indicates that 2-AAF is a potent bladder carcinogen, and the relatively small difference between the estimated ED50 values for 18 mo (149 ppm) to 33 mo (91 ppm) suggests that it is a fast-acting carcinogen.

A probit log dose model for liver neoplasms is shown in Fig. 12. The regression lines fit to the liver neoplasms at 18, 24, and 33 mo were also parallel. The slopes, however, were shallow (the common slope was 1.86) and the estimated ED50 was reduced 8-fold from 674 ppm at 18 mo to 85 ppm at 33 mo. This indicates that the liver responded to 2-AAF late, but once the neoplasms began they developed rapidly.

A probit log time model was used to describe the development of liver and bladder neoplasms for a given dose level of 2-AAF in the second stage of modeling (Farmer et al., 1979). Table 8 shows the parameters of the model for probit log time. The incidence of bladder neoplasms at dose levels of less than 60 ppm 2-AAF was insufficient to fit the model. Slopes ranged from 1.47 at 60 ppm to 6.80 at 150 ppm. Figure 13 illustrates the probit log time model for bladder neoplasms.

TABLE 7. Probit Log Dose (ppm) Model on Incidence of Bladder and Liver Neoplasms

Time (mo)	Intercept	Slope	ED01	ED50
		Bladder		
18	−15.08	9.24	83	149.2
24	−13.86	8.97	69.6	126.5
33	−17.89	11.67	57.8	91.5
		Liver		
18	−0.27	1.86	38.1	673.9
24	0.64	1.84	12.6	233.6
33	0.84	2.15	7.1	84.8

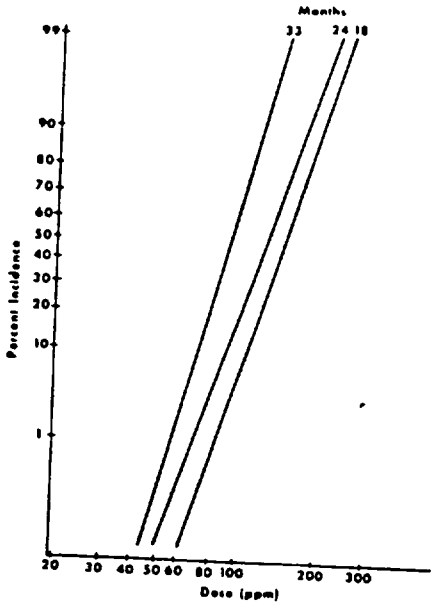


FIGURE 11. Probit log dose model for bladder neoplasms.

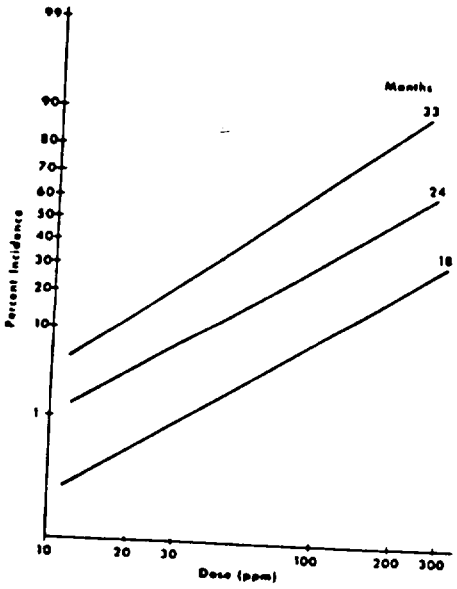


FIGURE 12. Probit log dose model for liver neoplasms.

TABLE 8. Probit Log Time (mo) on Incidence of Bladder and Liver Neoplasms

Dose (ppm)	Intercept	Slope	ET01	ET50
		Bladder		
60	0.65	1.48	23.3	880.4
75	−2.41	4.03	18.2	68.8
100	−5.01	6.64	14.4	32.2
150	−3.58	6.80	9.4	19.2
		Liver		
0	−3.84	5.09	19.1	54.8
30	−4.88	6.15	16.9	40.5
35	−5.40	6.60	16.8	37.8
45	−4.87	6.29	15.8	37.1
60	−4.22	5.95	14.4	35.5
75	−4.61	6.35	14.0	32.6
100	−4.23	6.24	12.8	30.1
150	−6.54	8.17	13.4	25.8

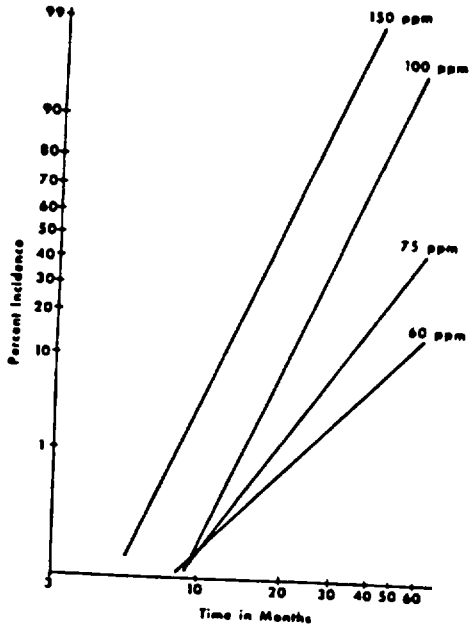


FIGURE 13. Probit log time model for bladder neoplasms.

Figure 14 shows that the incidence of liver neoplasm can be represented by a probit log time model for all dose levels including zero and the regression lines are parallel with a common slope of 6.33.

The parallelism of the dose-response and dose-time regressions for the liver neoplasms suggested that these data might be represented by one model. A model describing the probit of the incidence of liver neoplasms in the plane of both log dose and log time was fit to the 21 points represented by 18, 24, and 33 mo and 30, 35, 45, 60, 75, 100, and 150 ppm 2-AAF. The estimated equation of the plane was:

$$\text{Probit (incidence of liver neoplasms)} = -7.55 + 1.52 \log \text{dose} + 6.44 \log \text{time}$$

The chi-square goodness of fit for this model with 21 degrees of freedom was 12.62 and the probability of a larger chi-square was 0.81, suggesting the excellent fit of the model. The 1% and 50% response contours for this model and the 95% fiducial limits are depicted in Figs. 15 and 16, respectively.

The parallelism of the time regression lines for the liver neoplasms (Fig. 14) suggests that the mechanism of action is the same regardless of the dose of 2-AAF. For example, any level of exposure to 2-AAF would result in the development of liver neoplasms if the mice survived long enough. This, coupled with the linear fit of the incidence of liver neoplasms in the dose and time plane, supports the concept of no dose or time threshold.

In regard to bladder neoplasms, the steepness of the slopes of the dose-response curves suggests that 2-AAF is a potent bladder carcinogen. The small displacement of the regression line from 18 to 33 mo (Fig. 11) suggests that 2-AAF is a fast-acting bladder carcinogen as well. The nonparallelism of the time-response regressions (Fig. 13) suggests that the mechanism of action of 2-AAF in inducing bladder neoplasms may be more dependent on the amount present than the length of exposure (Farmer et al., 1979).

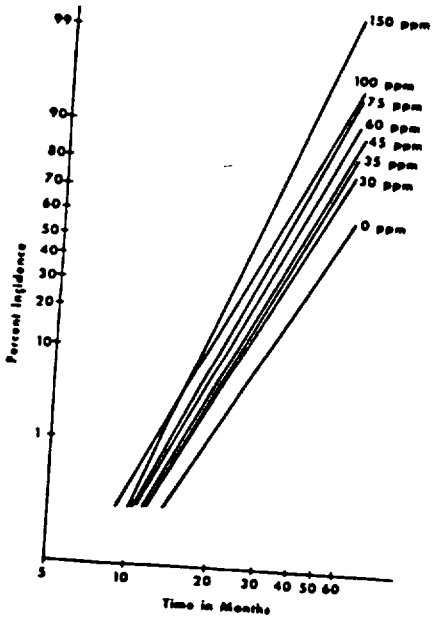
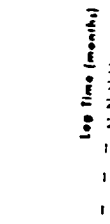


FIGURE 14. Probit log time model for liver neoplasms.



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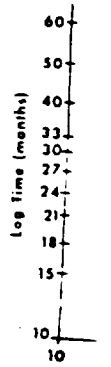

FIGURE 16. The 50%

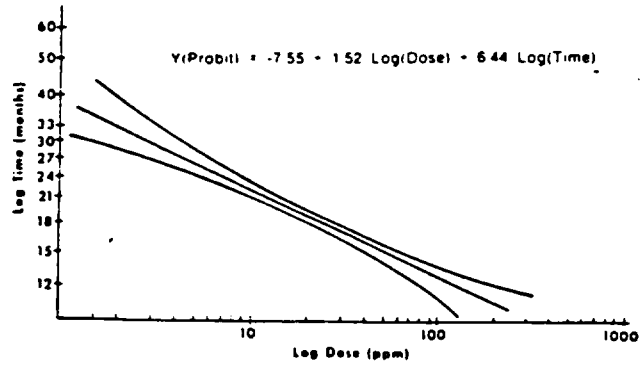

FIGURE 15. The 1% contour for liver neoplasms related to log dose and log time.

Estimates of time to appearance of tumor and time to death after the appearance of tumor from the interval sacrifice and survival information in the ED01 study were made by Kodell et al. (1979). This was done for five types of tumors—two dose-related (bladder and liver neoplasms) and three non-dose-related (reticulum cell sarcoma, lymphoma, and lung tumor)—at each of 8 dose levels of 2-AAF (0, 30, 35, 45, 60, 75, 100, and 150 ppm) administered to a total of 20,880 mice.

The statistical method employed by Kodell et al. (1979) for the analysis of interval sacrifice data to estimate time to appearance of tumor was developed by Kodell and Nelson (1979). It consisted of estimating two independent Weibull probability distributions, one for time to appearance and the other for time to death from tumors after their appearance. The distributions were corrected for competing risks and hence represent net probabilities of occurrence of the events of interest. Each distribution is characterized by a scale parameter (b) and a shape parameter (c). For a given distribution with parameters b and c, the median is calculated from:

$$m = b(-\log_e 0.5)^{1/c}$$

For bladder neoplasms, except at 100 and 150 ppm, the estimated median times to tumor were well beyond the normal life-span of the animals. However, a very steep

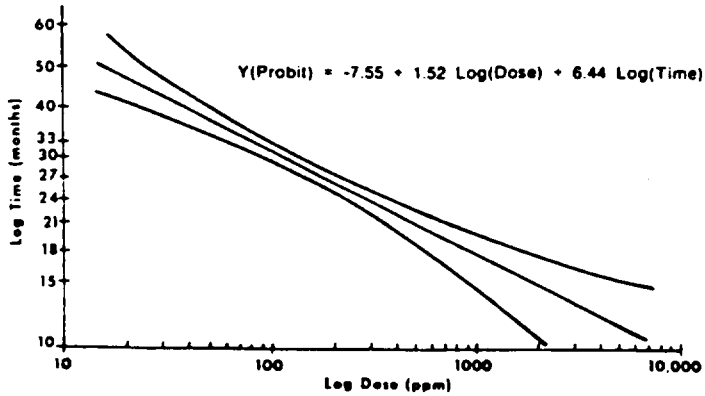

FIGURE 16. The 50% response contour for liver neoplasms as related to log dose and log time.

dose-response relation was demonstrated. Estimated times to tumor ranged from 20 mo at 150 ppm to 1877 mo at 0 ppm. It should be noted that the median time to death from a bladder carcinoma after its appearance did not follow a dose-response pattern but was approximately 7 mo regardless of dose.

Similarly, for liver neoplasms most of the estimated median times to tumor were near or beyond the normal life-span of the animal. Although a definite dose-response relation was observed, it was more gradual than that found for bladder neoplasms. Estimated median times to tumor for liver neoplasms ranged from 25 mo at 150 ppm to 38 mo at 0 ppm. The median time to death from a liver carcinoma following its appearance also indicated that 2-AAF did not stimulate the growth of liver neoplasms after they became observable. An overall estimated median time to death following appearance of a liver neoplasm was 5–7 mo.

Reticulum cell sarcomas occurred on the average at 24 mo, while the median times to occurrence of lymphomas and lung alveolar cell tumors were 41 and 43 mo, respectively, regardless of dose level. The estimated median times to death after appearance of these non-dose-related tumors were: reticulum cell sarcoma, 3.0 mo; lymphoma, 2.7 mo; and lung tumor, 3.3 mo.

Gaylor (1979) used linear extrapolation of the ED01 bladder and liver carcinoma data to estimate risk. The dose response for liver was nearly linear, indicating absolutely no tendency for a threshold dose. Linear extrapolation from the lowest experimental dose (30 ppm) predicts that the risk of liver tumors in BALB/c female mice fed 0.4 ppb 2-AAF for a lifetime is less than 1 in 10^6. The corresponding dose corresponding to a lifetime risk of bladder tumor of no more than 1 in 10^6 is 10 ppb. The risk at this level is expected to be considerably less than 1 in 10^6, but because of the uncertainty in the extrapolation process, a dose level of 10 ppb was suggested by Gaylor (1979) to be cautiously low. This is 3000 times lower than the lowest level (30 ppm) used in the study.

Gaylor (1979) also cautioned that although the steepness of the dose-response curves for bladder tumors may suggest that a threshold exists, the compatibility of the data with a threshold model does not constitute proof as nonthreshold models also fit the data.

Bladder tumors were apparent by 12 mo at 150 ppm, by 18 mo at 150 ppm, by 18 mo at 100 ppm, and by 24 mo at 75 ppm. It should be noted that as time was extended, bladder tumors appeared at lower doses with no indication of a threshold within the limits of observation of the study. At the lowest dose studied, 30 ppm, 1.2% (29 of 2457) of the dead or moribund animals had bladder tumors compared with 1.0% (8 of 783) of the controls. The upper 95% confidence limit on the excess bladder rate at 30 ppm is 0.9% over a lifetime. Gaylor (1979) concluded that even with these large sample sizes it could only be established that the lifetime bladder tumor rate at 30 ppm was less than 9 per 1000, not the practical equivalent of a threshold effect.

SUMMARY AND RECOMMENDATIONS

The salient features of the recently completed NCTR complex chronic study (ED01) are as follows:

1. The study consisted of approximately 24,000 BALB/c female mice fed 2-AAF in 81 different treatment groups.
2. The study was designed to estimate with precision the effective dose producing a 1% tumor rate (ED01) so that the shape of the dose-response curves could be studied with about an order of magnitude better precision than is generally available from carcinogenesis studies.

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3. The study required 9 mo to allocate 24,192 mice to 6 SPF/DF barrier rooms (replications). The mice were derived from a Charles River substrain of BALB/c mice. Exposure to 2-AAF was begun after weaning, at 3–4 wk of age.
4. Seven dose levels were employed (30, 35, 45, 60, 75, 100, and 150 ppm) plus a control—an extremely compact design since the lowest and highest doses are separated by only a factor of 5.
5. The experimental design included eight sacrifice intervals (9–24 mo) plus a lifetime group. An additional subgroup involved serial treatment at 60, 75, 100, and 150 ppm for 9, 12, or 15 mo, then sacrifice at 18 or 24 mo. The purpose of the latter was to examine the regression or progression of the tumors after administration of the carcinogen was stopped.
6. For animals tested at the highest dose level (150 ppm), liver tumors were observed in 5.8% of the animals sacrificed at 18 mo and in 39% at 24 mo. Had a typical number of 50 animals been used and the study terminated at 18 mo (not uncommon for safety tests with mice), liver tumors would have gone undetected. Hence, the tumor end point (mostly hepatocellular carcinomas) that posed the greatest risk at low doses would have been overlooked. Even bladder tumors would not have been detected by 18 mo with 50 animals at the highest dose of 150 ppm, as the bladder tumor rate in animals fed 100 ppm for 18 mo was only 3.8%.
7. These results indicate the need to conduct safety tests in mice for at least 24 mo. Spontaneous background rates of bladder and liver tumors did not increase sharply with age. For types of tumors where the spontaneous background rate does rise sharply with age, tumor rates can be studied in animals dying before the end of an experiment.
8. Discontinuing 2-AAF administration could result in failure to detect bladder tumors or in a serious underestimate of the risk; hence the practice of discontinuing administration of a chemical is not recommended as a planning part of a screening test. For example, the incidence of bladder tumors began to approach zero when 2-AAF was removed from the diet. The bladder tumor rate in animals fed 100 ppm 2-AAF for 12 mo and sacrificed at 24 mo was 3.0%, whereas the rate for animals fed 2-AAF continuously for 24 mo was 15.6%.
9. As demonstrated in many studies, including this one, the best point from which to extrapolate for risk assessment at extremely low exposure levels is generally near or below the lowest experimental dose level where excess tumors can be detected. This dose would depend on the background rate, number of animals used, and shape of the dose-response curve. Since the shapes of dose-response curves in general are not known, it is difficult to select an experimental dose level close to the optinum dose for risk extrapolation. Hence, it would be prudent to conduct safety tests at several dose levels in order to obtain at least one level near the optimum dose from which to extrapolate. Because of the critical role of dosage in safety evaluation, it is recommended that several dose levels be used even if fewer animals per dosage group are used. A recommendation for risk assessment would be to consider several dose levels at the low end of the dose-response curve, perhaps 5 dose levels differing by steps of no more than a factor of 2, plus controls, with 25 animals per group. For screening and risk assessment, the recommendation would be 25 animals each at the maximum tolerated dose (MTD), 1/3 MTD, 1/9 MTD, 1/27 MTD, 1/81 MTD, and controls.
10. The ED01 study provides a large data base, relatively free of wide statistical fluctuations, that can be used both for elaborating processes of carcinogenesis and for comparing strategies of risk assessment.

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Health risks associated with land application of municipal sludge

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The disposal of municipal wastewater sludge has increasingly become, and justifiably so, of public concern. Sludges produced at municipal wastewater treatment plants in the U. S. amount to approximately 4.5 billion dry kg/y.[1] As the number of facilities converting to secondary treatment increases, this figure is expected to reach 8 billion dry kg annually by the early 1980s. The current national breakdown of municipal wastewater sludge disposal is estimated as 35% incineration, 15% ocean disposal, 25% landfill, and 25% land application. Incineration not only contributes to air pollution, but is energy dependent. Because the federal government has prohibited ocean dumping of sludge after 1981, land application is being considered by many as a feasible alternative, which also allows reclamation of otherwise lost plant nutrients.

With increasing amounts of sludge being applied to land, there have been a number of discussions regarding such practices,[2, 3] including questions concerning the potential for human health problems. In an attempt to answer some of these questions, the authors have made an assessment of some potential problems, with emphasis on cadmium and parasites, and tried to put the information into a rational perspective.

TRACE METALS

General. Cadmium seems to be the metal of most concern to human health when applying municipal sludges to land. Exceptions naturally occur in individual situations in which an industry contributes an unusually high quantity of a metal to a sewerage system. An excellent overview of metal contaminants in sludge was recently published.[4]

Copper, molybdenum, and selenium are potential problems in a few small geographical areas where natural soils are already sufficient high in these elements to result in the sporad poisoning of livestock. A potential limitin factor for animals, rather than man, exists sludge containing high concentrations of thes elements is applied to soils where the livestod graze. In fact, the average American die probably contains less than the 2 mg/d o copper recommended by the National Acad emy of Science.[5] Neither nickel nor zinc represent any realistic hazard, and the lat e also seems to be in need of supplementati n in many human diets. Mercury, when add-d to soil in large quantities, may be taken up y plants but generally is bound by clay and or ganic matter in unavailable forms. Fortu nately, mercury concentrations tend to be lo w in municipal sludges and would rarely lim it sludge application.

Lead and tin do not appear to be a proble available data suggest they are not appreciab y translocated by plants. Little data exist f r antimony, but it does not seem critical in sludges. Manganese, iron, and aluminum a e not hazardous at normal soil pHs and hav e caused no known problems in soils when foun l at high concentrations. Municipal sludge s contain little, if any, hexavalent chromium, which is reduced to the trivalent state durin digestion. Plants do not accumulate trivalen chromium even when it is present in soil a high levels, and there is evidence that diets ar deficient in chromium in certain areas. Waste water sludge generally contains low levels o arsenic and application of sludge usually wil not be limited by its arsenic content. Once incorporated into soil, arsenic is strongly held by the clay fraction.

Cadmium. The human kidney is the pri mary target organ for chronic health effects from cadmium. Results from animal studies and studies of humans exp long period to cadmium ind tration of 200 μg cadmium kidney cortex may cause tubular dysfunction.[6, 7] P cadmium results in an molecular-weight serum β2-microglobulin,[8] in the exposure beyond the thres results in proportionally gr ulin excretion. Whether th the kidney are reversible creases is not yet known nificance of minimal renal also unknown.

Level in kidneys. To cadmium level that is nece disease, tissue samples ha autopsy and analyzed. C crease in the kidney fro mately age 50, and then is important. One of t metals in tissues was r and Cook,[9] who obta adult accident victims in U. S. Values were report have been converted to using original data. The centration of cadmium weight, with 90% of the μg g and the highest val

Indraprasit *et al.*[10] exa specimens from normal s Angeles area along with s or chronic renal failure. 31 to 80. Based on the had a mean cadmium of while the concentration i failure was approximately was no evidence of trace- ney disease.

The effect of age was by Gross *et al.*,[11] w h tex cadmium levels f wet weight at birth to a age 50 and then decrease found was 101 μg/g in doubtedly was occupatio mium-containing fumes

A study of trace meta completed by the Soutl tute,[12] was carried out research institutes in Jap mean kidney cortex cadm to 59 age group was 26 highest value encounter the next highest, 59 μg/

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natural soils are already sufficiently elements to result in the sporadic of livestock. A potential limiting animals, rather than man, exists if ıtaining high concentrations of these s applied to soils where the livestock ı fact, the average American diet contains less than the 2 mg/d of commended by the National Acad- ;cience.[5] Neither nickel nor zinc any realistic hazard, and the latter ; to be in need of supplementation uman diets. Mercury, when added arge quantities, may be taken up by generally is bound by clay and or- ter in unavailable forms. Fortu- rcury concentrations tend to be low ɔal sludges and would rarely limit ɔl ion.

d do not appear to be a problem; lata suggest they are not appreciably y plants. Little data exist for but it does not seem critical in Manganese, iron, and aluminum are lous at normal soil pHs and have known problems in soils when found concentrations. Municipal sludges ttle, if any, hexavalent chromium, educed to the trivalent state during Plants do not accumulate trivalent even when it is present in soil at , and there is evidence that diets are ı chromium in certain areas. Waste- ge generally contains low levels of d application of sludge usually will ıited by its arsenic content. Once d into soil, arsenic is strongly held y fraction.

m. The human kidney is the pri- et organ for chronic health effects ıium. Results from animal studies

and studies of humans exposed industrially for long period to cadmium indicate that a concentration of 200 μg cadmium/g wet weight in the kidney cortex may cause the appearance of tubular dysfunction.[6, 7] Renal damage from cadmium results in an abundance of low-molecular-weight serum proteins, especially B_2-microglobulin,[8] in the urine. Continued exposure beyond the threshold for proteinuria results in proportionally greater B_2-microglobulin excretion. Whether the earliest effects on the kidney are reversible when exposure decreases is not yet known. The clinical significance of minimal renal tubular damage is also unknown.

Level in kidneys. To determine the renal cadmium level that is necessary for the onset of disease, tissue samples have been obtained at autopsy and analyzed. Cadmium tends to increase in the kidney from birth to approximately age 50, and then decrease[6]; thus, age is important. One of the early studies of metals in tissues was carried out by Tipton and Cook,[9] who obtained tissues from normal adult accident victims in various parts of the U. S. Values were reported as ash weight and have been converted to a wet weight basis using original data. The median kidney concentration of cadmium was 32 μg/g wet weight, with 90% of the values less than 56 μg/g and the highest value 88 μg/g.

Indraprasit *et al.*[10] examined kidney cortex specimens from normal subjects from the Los Angeles area along with subjects having acute or chronic renal failure. The age range was 31 to 80. Based on the data, normal tissue had a mean cadmium of 22 μg/g wet weight while the concentration in subjects with renal failure was approximately 15% lower. There was no evidence of trace-element-induced kidney disease.

The effect of age was clearly demonstrated by Gross *et al.*,[11] who showed that kidney cortex cadmium levels rose from less than 1 μg/g wet weight at birth to a peak of 39 μg/g at age 50 and then decreased. The highest value found was 101 μg/g in a plumber, who undoubtedly was occupationally exposed to cadmium-containing fumes from solder.

A study of trace metals in tissues, recently completed by the Southwest Research Institute,[12] was carried out in cooperation with research institutes in Japan and Sweden. The mean kidney cortex cadmium value for the 40 to 59 age group was 26 μg/g wet weight. The highest value encountered was 109 μg/g and the next highest, 59 μg/g. Smokers had a cadmium level 50% higher than nonsmokers. This is consistent with calculations that show smoking a pack of cigarettes a day for 35 years results in 30% higher renal cortex levels compared with nonsmokers.[6]

Hammer *et al.*[13] in a North Carolina study of males age 40 to 79 reported the cadmium in the kidney cortex to average 18 μg/g wet weight for nonsmokers, 30 μg/g for those smoking less than one-half pack of cigarettes daily, and 39 μg/g for those smoking more than one-half pack daily. They calculated the total body burden to be 17.8 mg cadmium for nonsmokers and 37.8 mg for heavy smokers.

Daily cadmium intake. Data published by the Food and Drug Administration (FDA),[14] based on market basket surveys over 7 years, show that the average cadmium intake, including that found in water, of 15- to 20-year-old males is 39 μg/d. If this figure is adjusted by the recommended daily calorie intake for various age groups,[15] the average daily cadmium intake from birth to age 50 would be 33 μg/d for men and 26 μg/d for women. These figures are consistent with intake data calculated by measuring cadmium in feces and adjusting for cadmium absorption. Such cadmium intake calculations range from 13 to 39 μg/d.[12, 16, 17]

Drinking water contributes relatively little of the daily intake. A survey of 969 community water supply systems showed the average cadmium concentration to be 1.3 μg/l.[18, 19] This source results in an average of 3 μg/d.

Ambient air is not a significant source of cadmium exposure for the vast majority of the U. S. population. Less than 0.2 μg/d may be expected by this route. Although persons living in the vicinity of a metal smelter may be at a slightly increased risk, controls being placed on emissions are expected to hold intake by way of the respiratory route for this group to less than 1 μg/d.[20, 21]

Approximately 100 000 persons in the work force are potentially exposed to cadmium.[7] The spectrum of occupational exposure varies from the negligible to situations that may produce acute or chronic toxicity. The authors' appraisal is not intended to apply to this group. Efforts are being made by those involved with occupational health to reduce the exposure to a minimum and eliminate adverse health effects.

All data agree that 0.1 to 0.2 μg of cadmium are inhaled for each cigarette smoked.[6] Thus, smoking 20 cigarettes per day will result in the inhalation of about 3 μg.

Although total intake by way of the respiratory route is far less than intake by way of the gastrointestinal route, the body absorption from the former is far greater. Elinder *et al.*[22] calculated respiratory absorption to be 46% of the cadmium inhaled. Limited data from humans indicate gastrointestinal absorption of cadmium to be about 4 to 5%. Similar data for rats and mice indicate a cadmium absorption of 2%, with 50 to 400% higher values if the animals had a calcium deficient diet.[6, 23]

Excretion. Urine is considered the major route for body burden related cadmium excretion.[6] In the U. S., the urinary cadmium excretion is 0.5 to 2 μg/l.[6, 12] Based on these data, the excretion rate of cadmium may be calculated to be 0.005 to 0.012% of the total body burden.

Comparison with intake limits. The simplified metabolic model outlined by Friberg *et al.*[6] predicts that a total daily cadmium ingestion of 250 to 400 μg/d over 50 years will result in the critical kidney level of 200 μg/g wet weight in nonsmokers. Smokers would reach the critical level with proportionately less intake by ingestion. Kjellstrom and Nordberg[24] developed a more elaborate model in order to overcome some of the uncertainties associated with the earlier version. Using this model they conclude that the average renal cortex concentration would reach 200 μg/g at an average daily cadmium intake through food of 440 μg for a European-American population.

There are no official limitations in the U. S. for cadmium in food. Comparisons can be made, however, to recommendations addressing the cadmium issue.

The Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives has proposed a provisional tolerable weekly intake of 400 to 500 μg of cadmium.[25] This is based on an intake of 1 μg/kg body weight per day. In the U. S. for a 70 kg "Standard Man," this would result in a daily intake of 70 μg. The committee indicated that with this intake limitation, it is unlikely that the level of cadmium in the renal cortex will exceed 50 μg/g wet weight, a safety factor of four. The simplified metabolic model was apparently used in their calculations.[24]

For purposes of calculating the amount of cadmium passing through the food chain, a figure of 110 μg cadmium/d as a maximum safe intake from foods is reasonable. It is based on one-fourth the amount calculated by Kjellstrom and Nordberg,[24] with their more sophisticated metabolic model, as being the level at which proteinuria is initiated. The authors do not intend that the cadmium level be allowed to increase to 110 μg/d, an approximate 3- to 4-fold increase over existing levels, but believe it is a reasonable figure from which to calculate theoretical cropland loading.

The Occupational Safety and Health Administration recently proposed, on the recommendation of the National Institute for Occupational Safety and Health,[5] a time-weighted average limit for work place air of 40 μg total Cd/m³. One of the principal studies considered in recommending this limit was that of Lauwerys *et al.*[26] They found reduced pulmonary function and proteinuria in workers, all of whom happened to be smokers, exposed to total cadmium at concentrations of 66 μg/m³ for up to 40 years. Respirable cadmium was measured at 21 μg/m³. Stokinger and Woodward[27] outlined how threshold limit values of a substance in air may be used to arrive at approximate limiting concentrations for ingestion. Assuming Lauwreys' respirable concentration is the threshold cadmium limit, and considering the differences in absorption and exposure time,

$$21\ \frac{\mu g}{m^3} \times \frac{10\ m^3}{day} \times \frac{5\ d\ work\ week}{7\ d\ week} \times \frac{0.46\ lung\ absorption}{0.045\ intestine\ absorption} = 1530\ \mu g$$

A safety factor of 10 to protect the general population would result in a maximum permissible ingestion of 150 μg/d. A larger safety factor than that used by FAO/WHO is justified because we are starting with inhalation data. Notwithstanding the obvious problems associated with different routes of cadmium intake, it is interesting that results are the same order of magnitude.

Cadmium in food classes. As part of its market basket survey, the Food and Drug Administration (FDA) determines the cadmium levels of composite samples in each of 12 food classes. The 117 food items included in the survey were determined by using the 1965 data of the U. S. Department of Agriculture for 15- to 20-year-old males. The latest published results, for 1974, are shown in Table I.[28] In a correlative 1974 study, the FDA determined the cadmium content of 71 samples of 32 foods. When the median value for each type of food was used to calculate the dietary intake of the teenage male, the cadmium intake was found to be 33 μg/d.

TABLE I. Cadmium in

Dairy pro	
Meat, fish	
Grain and	
Potatoes	
Leafy veg	
Legume v	
Root vege	
Garden fr	
Fruits	
Oils, fats,	
Sugars an	
Beverages	
Total	

[a] Trace values assumed a
[b] Calculated from concen

When the arithmetic calculations, the d 72 μg. The FDA conclu sensitivity for cadmium in the total diet approac basket samples more median food concentrati frequent but more hig dividual samples that mean upward.[28]

When municipal slud cultural lands, only cer as grains, forage grasse likely to have increased c grown on these lands. C as dairy products or bev erally show increased m of sludge application t siders the theoretical sit containing cadmium is lands in such a way t class possible wou be foodstuff concentra a each class. A multipli determined to bring the 110 μg. The calculatio Table I, is

$$\frac{110 - 4.0 - 4.0 -}{36 - 4.0 - 4.0 -}$$

Intake for the four food impacted by sludge sho The meat class should cause cadmium does n with the exception of

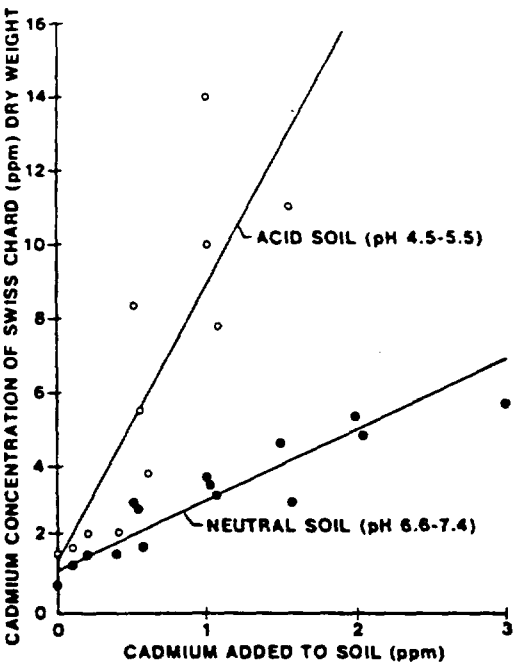

FIGURE 1. Relationship of cadmium uptake in Swiss chard to cadmium in soil.

ment. To understand the system, each of these processes and their interactions must be understood.

It is apparent that a number of variables can be the controlling factor for a given situation. In general, however, it appears that soil solution concentration of the micronutrients is the major limiting factor in plant uptake.[28, 30]

It has generally been assumed that the total amount of an element in a soil is directly related to the amount in the soil solution. Therefore, there has been a desire to limit the total amount of metal added. However, it has been demonstrated that the form of metal added (for example, valence, organic versus inorganic) affects this relationship because of the slow rates of precipitation and dissolution that dictate that both kinetic and thermodynamic factors are important.[32, 33] This has been further verified by the data of Bates,[34] Hinesly,[35] and the C.A.S.T. report [4] that indicate that for repeated annual applications of sludge or inorganic metals to soil cropped to corn, the amounts applied in a given year influence the cadmium content in the crop to a far greater extent than the total cumulative amounts of cadmium applied. This implies that the annual loading rate is more important than the total amount of cadmium applied and that th cadmium applied by previous applications i less available to plants. However, when cad mium application stops, the formerly applie cadmium remains available for extended pe iods of time, which indicates that total so cadmium is also important.[30, 31]

Presently, there is no satisfactory explanatio consistent with the observations on the additiv ity (crop cadmium is a linear function of cad mium applied when the different rates ar applied at one time) or lack of additivity (cro cadmium is not a linear function of adde cadmium when the cadmium is applied ove time) of cadmium application. This contro versy causes a dilemma in that if cadmiu applications are additive, the total amount o cadmium applied is of critical importanc whereas, if cadmium applications from year t year are not additive, the annual applicatio rate is of critical importance. This reduces t two opposing application systems in that limit on total cadmium encourages applicatio to all available land, whereas an annual app li cation limit encourages application to the sa.n land for extended periods of time.

The conservative approach would be to st x application of cadmium to soil because a ce pendence on either the total or annual load. y rate could be wrong. However, because of th immediacy of the issue, the realization th a some of the questions will be answered in th near future, and that in the interim either approach would be acceptable, it would appe a logical to apply limits on either or both.

In Table II, where it is assumed that 1 l(μg cadmium/d is acceptable, the concentrati n of cadmium allowable in the various fo x classes is calculated. This calculation assun e all food consumed is grown on land amend x by sludge containing cadmium.

With this in mind, the relationships betwe n annual loading rates of cadmium and cr y concentration have been constructed for Sw s chard (leafy vegetable) at two pHs.[4] The u a of Swiss chard will represent the worst c a because it is a maximum accumulator crop a c shows the most response to a given level x cadmium. These data are for one soil a c thus have limitations, but do illustrate how t a data on crop uptake and acceptable conce n trations in crops can be used.

Assuming that the food class distributi n given represents the diet of concern, the co n centration of cadmium allowed in leafy veg e tables is 161 ppb (wet weight), which is 2(ppm dry weight assuming 92% moistur e

Under poor management ppm level of cadmium is chard with 0.1 ppm cadn (Figure 1). This is 0.2 k management (neutral pH) of cadmium is reached in 0.50 ppm (1 kg/ha) cadn (Figure 1).

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$$(0.016 \times 0.5) + (0.026 \times ($$

$$= 0.0$$

$$\frac{225}{0.0132} = 17$$

Using the good managemen annual cadmium applicati concentration of cadmium t mitted in municipal sludge ing leafy vegetables is 60 p the cadmium of the sludge i under good management, i to land at essentially maxim of nitrogen and produce a contents in vegetables. A quire sludge with less than under the same nitrogen lo

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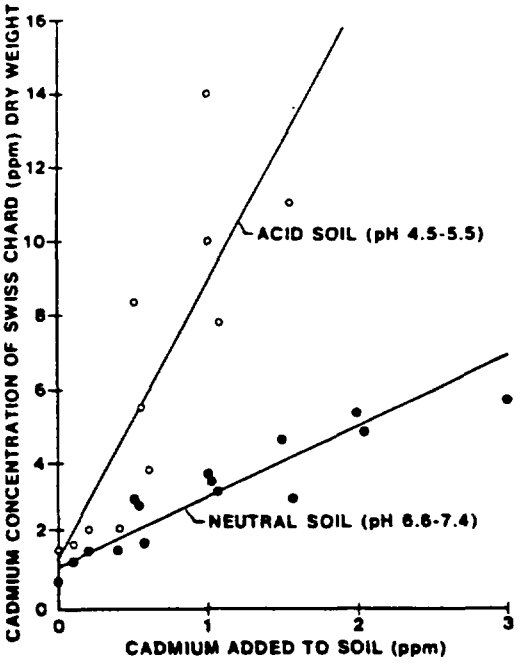

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Under poor management (low pH), the 2.0 ppm level of cadmium is reached in Swiss chard with 0.1 ppm cadmium added to soil (Figure 1). This is 0.2 kg/ha. Under good management (neutral pH), the 2.0 ppm level of cadmium is reached in Swiss chard with 0.50 ppm (1 kg/ha) cadmium added to soil (Figure 1).

If one assumes that cadmium concentration in crops is related to yearly cadmium application, this means that these maximum rates of cadmium could be applied per year. From the work at the University of Illinois,[38] it appears that the discrepancy between annual and total loading of cadmium holds for at least 8 years, but where the relationships between total and annual amounts converge cannot be predicted yet. If this annual loading rate is used until the relationship between total and annual application of cadmium is more clearly defined, there is a 5-year lead time until the amount of cadmium applied exceeds that recommended for soils with a cation exchange capacity of 0 to 5 meq/100 g.[30]

The acceptable concentration of cadmium in a sludge for a given nitrogen content can be calculated. The calculations assume 4.2% total nitrogen and 1.6% ammonia-nitrogen in the sludge, and 225 kg available nitrogen/ha are to be applied (rates for corn production are double the rate that would be applied for vegetable production). According to the data on sludge nitrogen, 50% of the ammonia-nitrogen is lost by volatilization on surface application and 20% of the organic nitrogen is available the first year. Therefore

$$(0.016 \times 0.5) + (0.026 \times 0.2) = 0.0132 \frac{\text{kg available N}}{\text{kg sludge}}$$

$$\frac{225}{0.0132} = 17\,000 \text{ kg sludge/ha}$$

Using the good management figure of 1 kg/ha annual cadmium application, the maximum concentration of cadmium that should be permitted in municipal sludge used on soils growing leafy vegetables is 60 ppm. Therefore, if the cadmium of the sludge is less than 60 ppm, under good management, it could be applied to land at essentially maximum agronomic rates of nitrogen and produce acceptable cadmium contents in vegetables. Acid soils would require sludge with less than 12 ppm cadmium under the same nitrogen loading rates.

Good management (neutral pH) should be emphasized. Data indicate that the amount of cadmium in crops, where no cadmium has been added to the soil, can be reduced by raising the pH.[4] Further, where low cadmium sludges are used along with good pH management, there appears to be no increase in the cadmium content of the crop.[4]

TABLE II. Maximum cadmium concentration by food class.

Food Class	Maximum Intake (μg/d)	Maximum Concentration (ppb)
Dairy products	4.0	6
Meat, fish, poultry	4.0	15
Grain and cereal products	39.1	92
Potatoes	33.6	190
Leafy vegetables	8.7	161
Legume vegetables	1.6	24
Root vegetables	3.9	122
Garden fruits	5.1	58
Fruits	2.8	13
Oils, fats, shortening	4.3	60
Sugars and adjuncts	0.8	10
Beverages (including water)	2.1	3
Total	110	

Municipal sludges can vary widely in cadmium content. Concentrations ranging from 3 to over 3 000 ppm have been found.[16] The majority of municipal sludges would be less than 20 ppm.

This assessment leads the authors to conclude that if a person eats a normal diet and uses municipal sludge to amend the soil where virtually all of his food is grown, it would be prudent to assure that such sludge contains less than 60 ppm cadmium.

The calculations above represent the worst case because if wastewater sludge is applied at fertilizer rates based on nitrogen content, only 0.5% of the agricultural lands would be affected. Further, some of the food classes would not be affected because application to soils for production of certain crops (vegetables) would be unlikely. However, if sludge application will not result in unacceptable levels of cadmium in crops under these assumptions, it is much less likely (factor 100 to 200 times) to happen in the real world. For the vast majority of the U. S. population, subject to the existing food marketing system, the dilution effect that results from the small proportion of agricultural land that could receive sludge would minimize any po-

TABLE III. Parasites of concern for possible transmission from sludge amended soils.

Organism	Disease	Reservoir(s)	Range(s)
Protozoa			
Balantidium coli	Balantidiasis	Man, swine	Worldwide
Entamoeba histolytica	Amebiasis	Man	Worldwide[a]
Giardia lamblia	Giardiasis	Man, animals?	Worldwide
Toxoplasma gondii	Toxoplasmosis	Cat, mammals, birds	Worldwide
Nematodes (roundworms)			
Ascaris lumbricoides	Ascariasis	Man, swine?	Worldwide-Southeastern U. S
Ancylostoma duodenale[b]	Hookworm	Man	Tropical-Southern U. S.[c]
Necator americanus[b]	Hookworm	Man	Tropical-Southern U. S.[c]
Ancylostoma brasiliense (cat hookworm)	Cutaneous Larva Migrans	Cat	Southeastern U. S.
Ancylostoma caninum (dog hookworm)[b]	Cutaneous Larva Migrans	Dog	Southeastern U. S.
Enterobius vermicularis (pinworm)	Enterobiasis	Man	Worldwide[a]
Strongyloides stercoralis (threadworm)[b]	Strongyloidiasis	Man, dog	Tropical-Southern U. S.[a]
Toxocara cati (cat roundworm)	Visceral Larva Migrans	Carnivores	Probably Worldwide
Toxocara canis (dog roundworm)	Visceral Larva Migrans	Carnivores	Sporadic in U. S.
Trichuris trichiura (whipworm)	Trichuriasis	Man	Worldwide[a]
Cestodes (tapeworms)			
Taenia saginata (beef tapeworm)	Taeniasis	Man	Worldwide-U. S.[a]
Taenia solium (pork tapeworm)	Taeniasis	Man	Rare in U. S.
Hymenolepis nana (dwarf tapeworm)	Taeniasis	Man, rat	Worldwide[a]
Echinococcus granulosus (dog tapeworm)	Hydatid disease	Dog	Far North-Alaska
Echinococcus multilocularis	Alveolar Hydatid disease	Dog, carnivores	Rare in U. S.

[a] Has been identified in domestic sludges.
[b] Man infested through skin contact.
[c] Reported in foreign sludges only to date.

tential problem. In addition, as mentioned earlier, most municipal sludge would be used on soils devoted to production of crops that would have a much lower uptake of cadmium than Swiss chard. Still another factor to consider is the mobility of the population of the U. S. About 6.7% of the U. S. population makes an out-of-county move each year. Less than 2% of the population remains in the same county over a 50-year period.[37] If a community happened to draw much of its food from agricultural land containing excessive cadmium, the number of persons impacted for a long period of time would be expected to be relatively small.

PATHOGENS

Bacteria, viruses, and parasites associated with municipal sludge applied to agricultural lands present potential problems of plant surface contamination.

Bacteria are the most fragile of the three groups of pathogens and are greatly reduced by sunlight, drying, or soil competition when sludge is applied to land. Contamination of plants can occur by direct ground contact and rain splashes, but survival is only a week or so. Bacteria can survive longer if lodged in cracked or split vegetative surfaces, as they are protected against light and drying.

Viruses may persist in soils and on vegetation for several weeks or months. If exposed to sunlight and desiccation, viruses will eventually be inactivated. Most enterovirus infections occur early in life and are mild an usually subclinical. For man, the virus o greatest potential concern appears to b Hepatitis A, a serious disease with an .p preciable potential for long-term liver dama e

Relatively little is known about the po si bility of parasitic disease transmission by v. a of sludge-amended soils. Parasitic ova a x cysts generally are quite resistant to dis n fectants and adverse environmental conditio s Many, in fact, require a period of free liv: y existence in the soil to develop infectiousn s for man. While often thought of as tropi a diseases, many of these pathogens are of c s mopolitan distribution (see Table III) a x commonplace within the U. S.

As outlined in Table III, only a limit x number of parasites need be considered a potential problems in sludge management ev s though numerous other species are present i the feces or urine of parasitized humans x domestic animals and are normally acquir x through the oral or cutaneous routes of ent y The blood flukes (schistosomes) and otl = trematodes require one or more intermedi: t hosts for development to an infectious sta e The intermediate hosts are usually speci i species of aquatic organisms such as sna: s crayfish, crabs, or fish. Although repeatec y introduced into this country and of immer a public health importance in much of the r s of the world, these parasites have not becor e established because they lack the proper inte r mediate host(s) essential for the completion o their life cycle and are delimited. Those parasiti specific intermediate host have simple fecal-oral tra have been the most succ achieving worldwide distri concern in sludge manage

Protozoa. The protozoa cysts in fecal material and active, feeding stage of th resides in the intestine. waterborne and seem to b to adverse environmental ing and elevated temperat of helminths. *Entamoeba* apparently unable to survi tion, even at low tempera stroyed within 3 days on Under optimum conditions moisture they survive 6 to Much less is known about bilities of the other prot potential interest.

Toxoplasmosis is a fection caused by a prot *gondii*. The parasite exhi trophozoite or actively pr cyst form containing hunc and found in brain and m infected animals; and the form found in cat feces. T infection are not clear. Ir meats have been suggestec way. Because the parasit reproduction only in cats, the major reservoir for p disease.[40] In humans, the primarily a subclinical in comes to medical attention. transmission from mother t sults in severe consequenc fants often exhibit permane system damage, blindness, a tion. The risk to s e litter seems small, but can regarded until studies on o cate that treatment proc organism.

Both *Balantidium coli* a are capable of causing d and carriers may shed large in the feces. The cysts of chlorination, but data on sludge are lacking.

Helminths (worms). T todes (roundworms) and worms) are probably of gr

1 from sludge amended soils.

·servoir(s)	Range(s)
;wine	Worldwide
	Worldwide[a]
:nimals?	Worldwide
ammals, birds	Worldwide
wine?	Worldwide-Southeastern U. S.
	Tropical-Southern U. S.[a]
	Tropical-Southern U. S.[a]
	Southeastern U. S.
	Southeastern U. S.
	Worldwide[a]
log	Tropical-Southern U. S.[a]
ores	Probably Worldwide
ores	Sporadic in U. S.
	Worldwide[a]
	Worldwide-U. S.[a]
	Rare in U. S.
at	Worldwide[a]
	Far North-Alaska
nivores	Rare in U. S.

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A, a serious disease with an ap
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their life cycle and are thus geographically delimited. Those parasitic species that lack specific intermediate host requirements and have simple fecal-oral transmission pathways have been the most successful in terms of achieving worldwide distribution, and are of concern in sludge management.

Protozoa. The protozoan agents appear as cysts in fecal material and the trophozoite or active, feeding stage of the organism usually resides in the intestine. Cysts are normally waterborne and seem to be more susceptible to adverse environmental effects such as drying and elevated temperatures than the eggs of helminths. *Entamoeba histolytica* cysts are apparently unable to survive anaerobic digestion, even at low temperatures, and are destroyed within 3 days on vegetable surfaces.[38] Under optimum conditions of temperature and moisture they survive 6 to 8 days in the soil.[39] Much less is known about the survival capabilities of the other protozoan parasites of potential interest.

Toxoplasmosis is a worldwide zoonotic infection caused by a protozoan, *Toxoplasma gondii*. The parasite exhibits three forms: a trophozoite or actively proliferating form; a cyst form containing hundreds of organisms and found in brain and muscle of chronically infected animals; and the oocyst, an infective form found in cat feces. The routes of human infection are not clear. Inadequately cooked meats have been suggested as a major pathway. Because the parasite undergoes sexual reproduction only in cats, they appear to be the major reservoir for perpetuation of this disease.[40] In humans, the acquired disease is primarily a subclinical infection and rarely comes to medical attention. However, uterine transmission from mother to unborn child results in severe consequences. Surviving infants often exhibit permanent central nervous system damage, blindness, and mental retardation. The risk to sludge workers from cat litter seems small, but can not be totally disregarded until studies on oocyst survival indicate that treatment processes destroy the organism.

Both *Balantidium coli* and *Giardia lamblia* are capable of causing dysenteric symptoms and carriers may shed large numbers of cysts in the feces. The cysts of both species resist chlorination, but data on their survival in sludge are lacking.

Helminths (worms). The intestinal nematodes (roundworms) and cestodes (tapeworms) are probably of greater public health significance than the protozoan agents in terms of sludge disposal.

Nematodes (roundworms). None of the intestinal nematodes require an intermediate host and most produce prodigious numbers of eggs, for example, a single female *Ascaris* produces 200 000 eggs daily. The eggs, particularly those of *Ascaris* and *Trichuris*, are frequently very resistant to environmental effects. Many are cosmopolitan in distribution and infestation rates are fairly high. They exhibit several categories of life cycle: a direct type (*Trichuris* and *Enterobius*) in which adults develop directly from eggs reaching the alimentary canal; a modified direct type in which eggs require an external period for embryonation before hatching in the intestine, entering the circulation, passing to the lung, migrating to the pharynx from which they are again swallowed to complete maturity in the intestine (*Ascaris*); a skin penetrating type in which the eggs hatch in the soil and the larvae enter the circulation through the skin and traverse the cardiopulmonary-pharyngeal route before being swallowed and reaching full maturation (hookworms and *Strongyloides*). These species are all found within U. S. borders although those with external maturational requirements are more common in the warmer, humid portions of the country. It is estimated that over a million persons harbor ascarids, and in children their presence may cause serious surgical problems.[41] When the number of migrating larvae is sufficiently large, their passage through the lung causes a peculiar form of pneumonia known as Loeffler's syndrome consisting of fever, cough, chest pain, shortness of breath, and eosinophila. *Ascaris* transmission by sludge is an extremely important consideration because of the disease's prevalance and the extreme resistance of the eggs to adverse influences. Infectious eggs can live for years in the soil. Recent experiments in which conditions simulated anaerobic digestion have demonstrated that *Ascaris* ova survive such treatment and remain infectious. While sludge appeared to inhibit development, it apparently protected the ova from the effects of 38°C temperatures.[42]

Because a significant amount of animal wastes reach municipal sludges, parasites of animal origin are also of concern. Two zoonotic roundworms, *Toxacara canis* and *T. cati*, are nearly universal in our pet population and have a life cycle in their normal host identical to *Ascaris* in man. When ingested by a child these worms are unable to complete their nor-

mal developmental cycle and continue to wander through the tissues for prolonged periods before eventually succumbing. This results in a chronic and usually mild disease (visceral larva migrans) lasting up to a year. Occasionally, more serious manifestations are seen and the larvae are particularly dangerous if they enter the eye because blindness may result.

Another roundworm with extremely hardy eggs is the whipworm (*Trichuris trichiura*), principally a problem in the humid tropics where in small children with heavy worm loads it causes severe anemia and hemorrhagic colitis. While not the medical problem of *Ascaris*, *T. trichiura* is a common parasite in Latin American migrants living in the U. S. and large numbers of eggs can be anticipated in some municipal sludges.[43]

Pinworms. Pinworms (*Enterobius vermicularis*) are extremely common in all strata of our population. The female worm migrates to the anus and lays her eggs on the peri-rectal skin resulting in itching and discomfort. Other than this, the effects are principally psychogenic in adults. Pinworm infestations spread largely within the family or within groups living in close quarters. While the eggs may occasionally be found in sludge and are relatively resistant to desiccation they are probably more susceptible to adverse effects than *Ascaris* and *Trichuris*.

Hookworms. Two species of hookworms occur in the U. S., but infection with the New World species, *Necator americanus*, is the most common. The larvae of these worms must embryonate for about one week in moist, warm soil before they penetrate the skin and pass into the circulation, eventually reaching and maturing in the small intestine. They do not actually ingest blood, but are tissue feeders. In feeding on tissue they secrete an anticoagulant that promotes bleeding from the site of attachment. This may eventually result in iron deficiency anemia and debility. Actually, persons with adequate diets are little affected, but the disease may be serious in pregnancy and in other conditions associated with depleted iron stores. Reportedly, up to 16% of rural school children in Georgia are infested; control is difficult in areas where poor sanitary practices and going barefoot are common.[41] The eggs are said to be fairly sensitive to adverse influences, and because proper sludge disposal practices should preclude the access of the public to the site until sufficient time has passed to destroy any remaining eggs o larvae, the risk from sludge seems a small one

Threadworms. Strongyloides stercoralis, o the threadworm, is very widespread in th tropics, but is also seen in more temperat areas. Dogs are also commonly infected Children in orphanages and schools are fre quently infected, as is the case with pinworms These parasites may follow a life cycle simila to the hookworm (feces, soils, skin penetration and so on) or because the eggs are fully em bryonated when passed by the female worm autoinfection with the larvae penetrating th rectal area tissues may occur. This leads t extremely large worm loads, and symptoms o weight loss, anemia, abdominal pain, and diar rhea may occur. Patients receiving steroi medications for other reasons may develop fata infections, as the worms leave their normal sit in the bowel and migrate terminally into man vital organs.

Cat and dog hookworms (*Ancylostom braziliense, A. caninum*) are another exampl of aberrant helminthic infections; that is, i an abnormal host they fail to complete thei life cycles. While they can penetrate th human epidermis they are unable to enter th circulation and thus wander aimlessly for month or more before dying. This cause condition known as creeping eruption, famil a to physicians along Florida's coasts wh n beachcombers encounter animal feces.

Cestodes (tapeworms). In contrast to th nematodes, tapeworm infections are usua l solitary and of great chronicity, with ma species living for decades. With the excepti of *Hymenolepis nana*, tapeworms require a intermediate host and are normally acqui by ingesting incompletely cooked beef or p containing the partially developed parasi Man serves as the definitive host and harb the adult self-fertile worm. The hazard i then, principally to stock grazing on sluc amended soils containing the eggs; epidem in cattle have been traced to contaminat irrigation water.[44] Man can also act as t intermediate host of *Taenia solium*, the p tapeworm, by ingesting the eggs, and the cysted intermediate larvae may give rise t various serious symptoms, depending on th location. Fortunately, this tapeworm is r in the U. S. *H. nana* is an exception, requiri no intermediate host, and infested persons f quently harbor multiple worms. It is our m common tapeworm, especially in the Sou The fish tapeworm, *Diphyllobothrium latu* is not deemed a threat because it requires t

aquatic intermedia ness for man. We sites should precl Similarly, the rat *diminuta*, can para but poses no realist ingest an intermec fleas, roaches, or n worm.

The intermediate dog tapeworms, *Ec E. multilocularis*, m problems if man in of these species. Th occupying cysts (hy and occasionally othe incidental host, with ing maintained by th mediate hosts (inclu and voles). The dis continental U. S., bu Alaska, particularly in animal offal to r cycle in close pro it for several years in th

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aquatic intermediate hosts to attain infectiousness for man. Well constructed land disposal sites should preclude this from happening. Similarly, the rat tapeworm, *Hymenolepsis diminuta*, can parasitize both the rat and man, but poses no realistic hazard because man must ingest an intermediate insect host (such as fleas, roaches, or mealworms) to acquire the worm.

The intermediate host or larval form of two dog tapeworms, *Echinococcus granulosus* and *E. multilocularis*, may cause serious medical problems if man incidentally ingests the eggs of these species. The larvae form large space-occupying cysts (hydatids) in the liver, lungs, and occasionally other organs. Man is only an incidental host, with the disease normally being maintained by the infection of other intermediate hosts (including sheep, pigs, moose, and voles). The disease is rarely seen in the continental U. S., but occurs in Canada and Alaska, particularly in areas where natives feed animal offal to their dogs, perpetuating the cycle in close proximity to man. The eggs live for several years in the soil.

It seems obvious that tapeworm infections are of less concern, as a threat to man from sludge, than are the nematode parasites, although tapeworm eggs will undoubtedly find their way into municipal sludges. The protection of stock grazing on sludge amended soils is a more pressing problem and it seems wise to prohibit grazing until present sludge treatment methods can be shown to destroy tapeworm eggs.

ORGANICS

There is little information available regarding organics in municipal sludges. A systematic survey has not been made. It is reasonable to assume that any organic compound discharged into a sewerage system could end up in the sludge. Therefore, a limited amount of persistent organics must be added to soil if wastewater sludge is applied to land. The most likely substances of concern are polychlorinated biphenyls (PCBs), insecticides, and herbicides.[45]

The maximum dichloro-diphenyl-trichloroethane (DDT) concentration found in seven municipal sludges in 1971 (Table IV), was only one-tenth the mean concentration reported in corn soils in 1954 after a 6-year period of application of this insecticide.[46, 47]

Any high concentration of PCB is undoubtedly associated with electrical manufacture or paper recycling plants. A survey in Ontario, Canada, of digested sludge at 33 plants showed a range of 0.6 to 77 ppm PCB as dry weight. All but the highest value were below 6 ppm.[48] PCB use is being phased out in both the U. S. and Canada, so even though it may continue to be found in the environment for many years, the levels discharged to wastewater treatment plants should progressively decrease to insignificance.

TABLE IV. Pesticides and PCB content of dry sludges.

Contaminant	Range (ppm) Min.	Max.	Sludges Examined
Aldrin[a]	ND	16.2	5
Dieldrin[b]	<0.03	2.2	21
Chlordane[a]	3.0	32.2	7
DDT + DDD[a]	0.1	1.1	7
PCBs[c]	ND	352.0	83

Note: ND = not detected.
[a] Examined in 1971.
[b] Examined in 1971, 1972, and 1973.
[c] Examined 1971, 1972, 1973, and 1975.

Plants do sorb many organic pesticides through roots and translocate them within the plant.[49] The most important factors controlling uptake are water solubility, solute concentration, size and polarity of pesticide molecules, organic matter content, pH, clay, and microbial activity of the soil. Climatic factors also play a role. Some organophosphate insecticides are applied to soil to be taken into foliage of plants to control pests. Organochlorine pesticides are also sorbed by plants and translocated to areal portions, but at a very low rate compared to the concentration in the soil.

Beetsman *et al.*[50] reported that not more than 3% of dieldrin from the soil passed into the foliage of corn. Concentrations in root tissue were 20 to 80 times the concentrations in the above-ground portion of the plants. Although organochlorine pesticides can reach seeds of fruits in small amounts, the concentration in roots is much less than the concentration in soil.[51]

PCBs are chemically and biologically stable and are adsorbed by soils. Absorption by plant roots and translocation within plants is very unlikely, but two studies have reported low levels of plant uptake under high application rates of the material. Iwata[52] used carrots, an avowed scavenger of organochlorine pesticides residues, on field plots treated with

100 ppm of PCB Aroclor 1254 to study plant uptake. PCBs were absorbed by carrot roots at very low levels and 97% of absorbed residue remained in the peel. Translocation within the plant was very slight. Boxes of sand treated with PCB to achieve 100 ppm concentration resulted in soybean uptake of 0.15 ppm (wet weight basis) in sprouts after 2 weeks growth.[53] Jacobs *et al.*[54] found little or no uptake of polybrominated biphenyls in orchard grass and carrots grown on contaminated soils.

Generally, organics with large molecules reaching agricultural lands do not tend to pass the semipermeable membrane of plant roots. For example, using the FDA tolerance level for infant foods of 0.2 ppm and a typical sludge application of 15 700 kg/ha (7 tons/acre) as dictated by nitrogen limitations, concentrations up to 2 800 ppm PCB could be permitted in sludge. The problem is not so much one of uptake by the plants, but one of adherence to the plant. In addition, if sludge is applied to grazing land, the animal could ingest the sludge while grazing. The organics could then concentrate in certain animal tissues, such as fats.

There is insufficient information to make definitive judgments, but caution should be exerted in applying sludge, high in PCB or similar hazardous organics, to land where the material may be ingested by food animals. In the Metropolitan Denver Sewage Project, where sludge is being incorporated into the feed of cattle, PCB with a range of 2 to 5 ppm was higher than any of the pesticides present in the sludge.[55] Chlordane was 1 to 2 ppm, dieldrin 0.1 to 0.2 ppm, followed by p, p'DDE and oxychlordane. The muscle samples of the cattle fed 15% sludge showed trace levels of alpha-BHC, p, p'DDE and dieldrin but no PCB. Fat samples showed trace levels of hexachlorobenzene, alpha-BHC, p, p'DDE, dieldrin, and PCBs. The PCB, at 0.4 to 1 ppm, was much higher than the pesticide levels in the fat. Oxychlordane and transnonachlor were also found in some of the fat samples.

Generally, municipalities without a significant source of PCB would be expected to have a PCB concentration in their sludge of less than 10 ppm. It would appear that caution should be exerted before sludge containing more than 10 ppm PCBs is applied to the surface of grazing lands, where the sludge may be ingested. Incorporation of the sludge into the soil prior to planting grasses would reduce the chance for ingestion. Because of the tendency of many organics to accumulate in lipid-rich tissues and fluids (milk), dairy cows should not be permitted to ingest municipal sludges.

It appears unlikely that crops grown on insecticide-contaminated soil contain enough insecticide to be harmful to animals or men that consume the crop.

CONCLUSIONS

- A large quantity of municipal sludge is placed on land in the U. S. and this quantity is expected to increase.
- Cadmium appears to be the metal of most concern to human health when applying municipal sludge to land.
- To date, there is no evidence from autopsy data that nonoccupationally exposed persons in the U. S. have kidney cadmium concentrations close to the critical level for onset of disease.
- Persons on a typical American diet of foods obtained in the market place ingest approximately 30 μg/d cadmium, as an average over 50 years. This could be higher, or lower for persons who limit themselves to only certain classes of foods.
- Available data indicate that cadmium in the diet of the typical nonsmoking American is approximately one-thirteenth to one-seventeenth of the level at which kidney disease is expected to begin.
- A procedure is presented for estimating the passage of a sludge contaminant through the food chain so that health effects may be estimated.
- In general, because of marketing practices and types of crops commonly grown on sludge amended soils, health effects from cadmium in municipal sludge applied to agricultural lands are not expected to be a problem.
- If a person uses municipal sludge for many years to amend the soil where virtually all his food is grown, it would be prudent to assure that such sludge contains less than 60 ppm cadmium and that the soil is maintained near neutrality.
- Pathogenic bacteria, viruses, and parasites found in municipal sludges raise the specter of potential public health problems from plant or soil contamination.
- Densities of bacteria and viruses in raw wastewater are greatly reduced in most conventional sludge treatment processes. Those reaching plants or soil have rather limited survival times and fairly quickly reach low titers. Reasonable area entry limitations and site de-

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- Several parasite species normally possess free living phases in their life cycles that have resulted in greatly enhanced tolerance to adverse environmental factors. They are, therefore, capable of prolonged survival on plants or in soil. This suggests that parasitic loads in soils may be augmented by wastewater sludge above naturally occurring background levels and thus present a somewhat enhanced risk of transmission to man.
- Despite the possibility of communicable disease transmission from land disposal of municipal sludges, a practice which has markedly accelerated in recent years, there is no epidemiologic evidence, to date, suggesting that this practice has resulted in actual human illness where sludge has been properly treated and applied.
- Organics are not so much a problem of uptake by plants, but of adherence to the plant. Because of the tendency of many organics to accumulate in lipid-rich tissues and fluids (milk), dairy cows should not be permitted to ingest municipal sludges.

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Pollution Control

Organochlorine Pesticides and PCBs in Cod-Liver Oil of Baltic Origin, 1971–80

Jerzy Falandysz [1]

ABSTRACT

Organochlorine pesticides and polychlorinated biphenyls (PCBs) in cod-liver oil of Baltic origin were monitored during 1971–80. Residues of DDT and its metabolites, PCBs, and hexachlorobenzene were present in all samples. Generally, ΣDDT residues declined, but the reason for the decline is unknown.

Introduction

Use of technical DDT as an insecticide in countries surrounding the Baltic has been prohibited since 1971. The organochlorine pesticides presently used for agricultural purposes in Poland are toxaphene, methoxychlor, endosulfan, and lindane. DDT was widely used in agriculture and has been detected at high levels in tissues of Baltic marine mammals, fish, and birds (*7*). The prohibition on the agricultural use of DDT in Poland was reflected in the nearly tenfold reduction of ΣDDT residue levels in adipose fat of slaughtered animals during the last decade (A. Niewiadowska, Veterinary Institute, Pulawy, 1980, personal communication). However, so far there have been no reports showing a decline of ΣDDT residue levels in animals of the Baltic Sea. Another class of organochlorine compounds, polychlorinated biphenyls (PCBs), occurs throughout the Baltic environment. Also, polychlorinated terphenyls (PCTs) have been found recently in Baltic marine organisms (*2, 13, 15*). Information on production and industrial use of PCBs and PCTS in countries surrounding the Baltic remains obscure. Some quantities of PCBs are produced and available in Poland under the trade name Chlorofen (*11, 12*). PCBs have also been produced in West Germany as Clophen, in the Soviet Union as Sovol, and in Czechoslovakia as Delor. This paper presents the results of analyses of cod-liver oil of Baltic origin for residues of hexachlorobenzene (HCB), ΣDDT, and PCBs.

Analytical Methods

Samples of cod-liver oil were obtained from a factory in Gdynia. The procedures for isolation and determination of the organochlorines have been presented elsewhere (*4*). Two cleanup processes were used. The oil sample was dissolved in *n*-hexane; 1 ml *n*-hexane was used for every 20 mg fat extracted. Twenty mg fat was cleaned with 1 ml of a 1:1 mixture of fuming 20–25 percent sulfur dioxide and concentrated sulfuric acid in a screw-capped, Teflon-lined test tube (*2, 4, 8*). The colorless hexane layer was injected directly into the gas chromatograph. In addition, a 4-ml aliquot of cleaned sample was subjected to alcoholic potassium hydroxide hydrolysis in a screw-capped, Teflon-lined test tube. The test tube was immersed for 15 minutes in the water bath at 50°C. After cooling, the mixture was shaken with 4 ml distilled water. Following separation, the upper hexane layer was injected into the gas chromatograph. The residues were quantitated by electron-capture gas chromatography. Instrument parameters and operating conditions follow:

Chromatograph:	PYE 104
Detector:	^{63}Ni
Column:	glass, 150 cm long by 4 mm ID packed with a 2:1 mixture of 8 percent QF-1 and 4 percent SF-96 on 100–120 mesh Gas-Chrom Q
Temperatures, °C:	detector 210 column oven 195
Carrier gas:	argon flowing at ml/min

PCBs were quantified by comparing sample peaks with that of PCB standard Clophen A 50, which appeared on the gas chromatogram after *p,p'*-DDE. DDE content was calculated from the total height of the peak with retention time equal to that of standard *p,p'*-DDE. *p,p'*-DDT and *p,p'*-TDE were calculated from the difference in height of peaks with retention times equal to those of *p,p'*-DDT and *p,p'*-TDE before and after hydrolysis with alcoholic potassium hydroxide. In recovery experiments, the calculated values and standard deviations, in ppm, were as follows: HCB, 0.27 ± 0.015; DDE, 5.8 ± 0.25; TDE, 5.1 ± 0.39; DDT, 1.3 ± 0.14; and PCBs, 11 ± 0.84 (*4*).

Results and Discussion

The levels of organochlorine residues in cod-liver oil are presented in Table 1. DDT and its metabolites were present in all samples. DDE levels ranged from 1.1 to

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Residues of Polychlorinated Biphenyls and DDT in Water and Sediment of the Indian River Lagoon, Florida—1977–78[1]

Tsen C. Wang, Robert S. Johnson,[2] and Joe L. Bricker

ABSTRACT

Water and sediment samples collected during 1977–78 from the Indian River lagoon between Vero Beach, Indian River County, and Fort Pierce, Saint Lucie County, Florida, were analyzed for PCBs and DDT. Sample locations were chosen on the basis of proximity to major tributaries, sewage outfalls, or municipal areas. Concentrations in water samples were below 0.01 ppb ΣDDT and 0.5 ppb PCBs. Small amounts of PCBs and DDT were found in most sediment samples, ranging from <1.0 ppb to 0.63 ppm Aroclor 1254 and from <0.1 ppb to 0.081 ppm ΣDDT. Samples from the Taylor Creek tributary and from the Fort Pierce power plant and municipal docking area contained higher PCB concentrations then did samples from other locations. DDT and PCB levels in most samples indicate little contamination by these compounds of the Indian River Waterway between Vero Beach and Fort Pierce.

Introduction

Polychlorinated biphenyls (PCBs) and ΣDDT are ubiquitous environmental contaminants. Reports of widespread distribution of organochlorine pesticide residues in the global ecosystem are increasing (*3–5, 8, 9, 11*). However, little information is available in the literature regarding organochlorine levels in the water and sediment of the Indian River lagoon.

The Indian River is a lagoonal estuary situated along the east central coast of Florida. This estuary extends 190 km. from Titusville, Brevard County, in the north to Saint Lucie Inlet, Martin County, in the south (Fig. 1). Authors investigated the occurrence of organochlorines in the Indian River lagoon between Vero Beach, Indian River County, and Fort Pierce, Saint Lucie County (Fig. 2), during 1977 and early 1978.

Methods and Materials

Figure 2 indicates approximate locations of seven sampling stations which were chosen on the basis of proximity to major tributaries, sewage plant outfalls, or municipal areas.

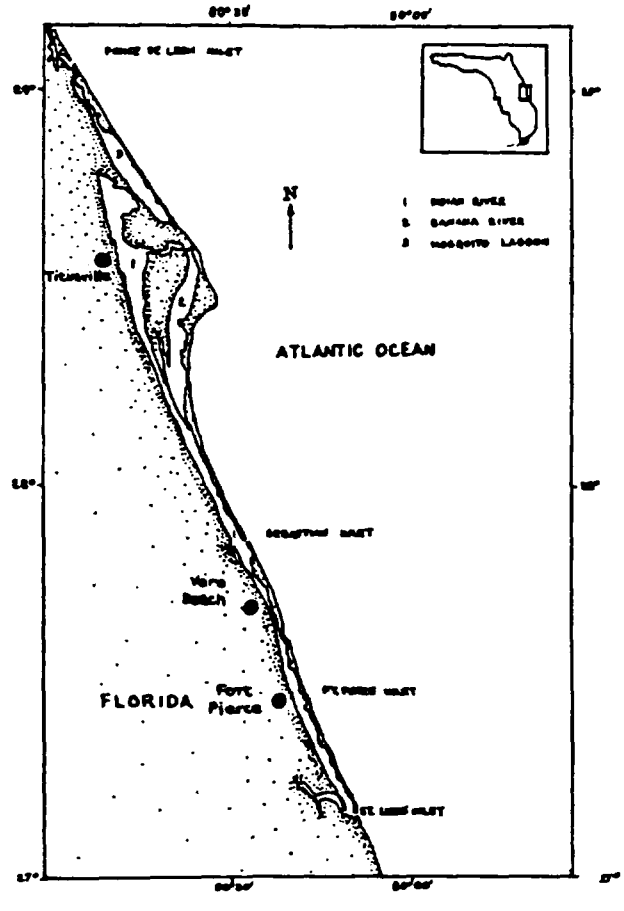

FIGURE 1. *Location of Indian River lagoon, Florida*

Surface water samples were collected at each station in a precleaned solvent bottle. The water samples were brought back to the authors' laboratory and refrigerated at 4°C overnight. Samples were analyzed the day after collection.

Each 3-liter water sample was drained at 250 ml/minute through a glass column containing 50 ml Amberlite XAD-2 resin (*2*). The column was eluted with 200 ml

[1] Harbor Branch Foundation, Inc., RR 1, Box 196, Fort Pierce, Fla. 33450. Contribution No. 163.
[2] Present address: Varian Corporation, Boston, Mass.

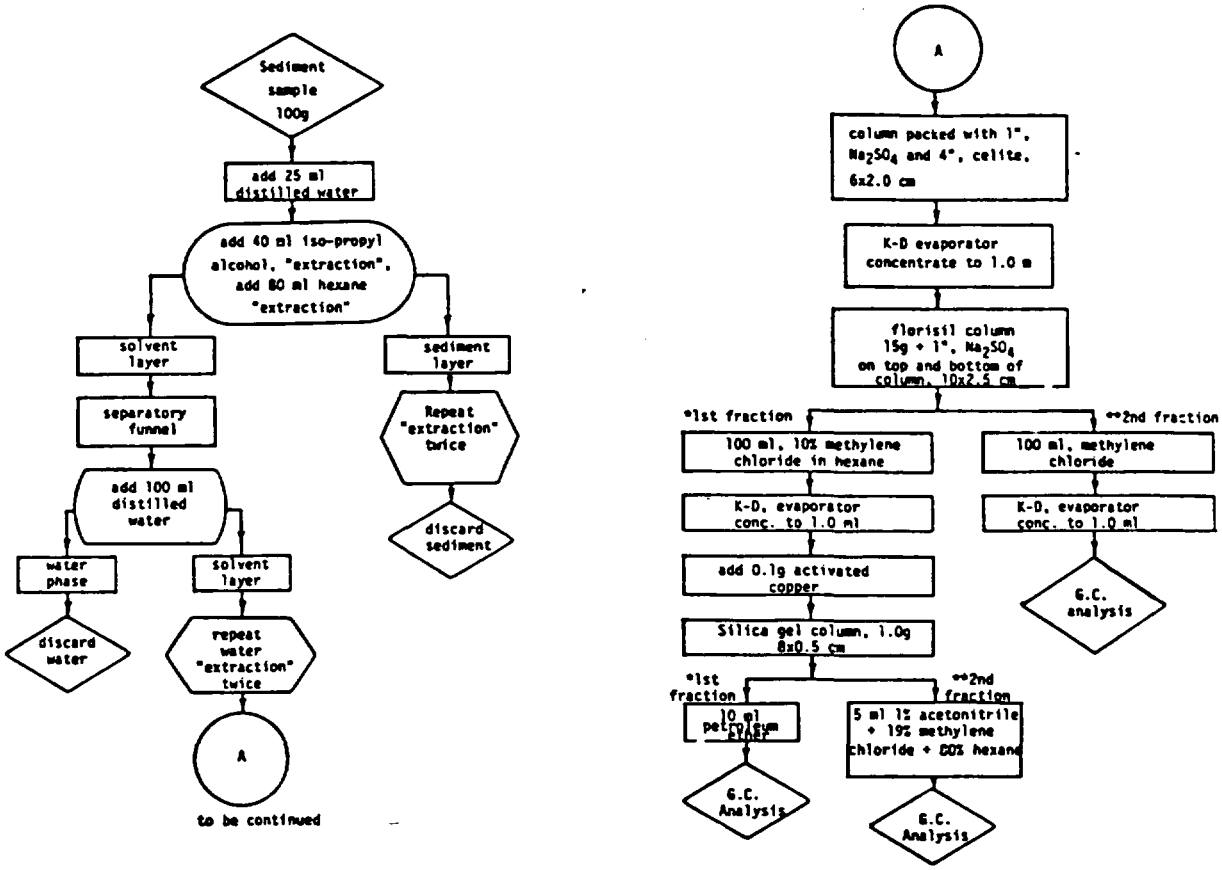

FIGURE 3. *Analytical scheme for cleanup and extraction of organochlorines from sediment samples from the Indian River lagoon, Florida*

than did samples from the South Canal tributary. PCB and ΣDDT residue concentrations in the Main Canal samples ranged from not detectable to 210 ppb and from not detectable to 5.62 ppb, respectively. Residue concentrations in South Canal samples ranged from not detectable to 8.67 ppb PCBs and from not detectable to 0.50 ppb ΣDDT. The frequency with which PCB and DDT appeared at both sampling sites was 60 percent. The Taylor Creek tributary in Saint Lucie County is another major source of agricultural runoff to the lagoon. Samples from Taylor Creek had the highest PCB residues in the study area. Residues ranged from 20 to 570 ppb Aroclor 1254 and from 0.96 to 19.9 ppb ΣDDT. Fort Pierce power plant and municipal docking area also had relatively high PCB and ΣDDT residues. Aroclor 1254 ranged from 39 to 278 ppb and ΣDDT from 1.90 to 81.0 ppb in the sediment samples. Both compounds were found in all samples from Taylor Creek and Fort Pierce municipal docking area. Samples from near the Vero Beach sewage outfall contained 1.0–16.2 ppb Aroclor 1254 with 100 percent frequency occurrence; ΣDDT levels ranged from not detectable to 0.38 ppb in 25 percent of samples. Residues in samples from near the Fort Pierce sewage outfall ranged from not detectable to 11.1 ppb Aroclor 1254 and from not detectable to 1.70 ppb ΣDDT. Of six samples analyzed, 83 percent contained both compounds.

Conclusions

Low concentrations of PCB and ΣDDT residues were found only in the sediment samples. Sediment samples collected from Taylor Creek tributary and Fort Pierce municipal docking area had higher PCB and ΣDDT residue levels than did other sample sites. The source of the compounds is not known. The data from this survey do not show any clear correlation between sampling site and sampling date.

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PCB's in Fish from Selected Waters of New York State [1]

John J. Spagnoli and Lawrence C. Skinner

ABSTRACT

PCB residues in fish from 41 stations throughout New York State were monitored in 1975. Nearly all fish contained PCB's in detectable amounts although the levels of contamination and specific Aroclor varied. The Hudson River contained the highest known PCB concentrations within the United States; levels often exceeded 100 ppm. Other waters and fish which were significantly contaminated include Lake Ontario salmonids and Cayuga Lake lake trout. Onondaga Lake, previously closed to fishing because of mercury contamination, also appears to have abnormally high levels of PCB's approaching in some instances the action level of the Food and Drug Administration, U.S. Department of Health, Education, and Welfare. Samples from marine waters generally have contaminant levels substantially below 5.0 ppm.

A summary of PCB data collected during 1970–75 is presented.

Introduction

The Division of Fish and Wildlife, New York State Department of Environmental Conservation, began analyzing fish flesh for DDT in the early 1960's. This work was conducted in response to data suggesting that chlorinated hydrocarbons may interfere with reproduction of fish and wildlife resources. Results of the investigation led to State restrictions on use of DDT in 1965. During this time, research indicated that DDT analyses could be biased through sample contamination with similar compounds, polychlorinated biphenyls (PCB's) (*8*). To minimize errors in future work, both DDT and PCB's were extracted and analyzed to obtain separate values. From 1971 through 1975 PCB's were evaluated in all fish flesh analyses as a part of DDT monitoring.

The recent concern over the impact of PCB's on human health and their possible effect on New York's natural resources as well as reports of their ubiquitous nature (*6, 13, 20*) precipitated a shift from low-level monitoring to the statewide monitoring described in this report. The new program promulgated by the Department of Environmental Conservation in August 1975 revised all existing directions. Specific fish species and desired sizes were defined (Table 1). Stations were located in most large waterways throughout the State where PCB contamination could occur (Figure 1, Table 2) and, when possible, where earlier samples had been taken. Several Adirondack streams were included to ascertain statewide ambient conditions. Where contamination was known to exist sampling was intensified. This was the case of the Hudson River where inordinately high concentrations of the contaminant in fish were reported by the U.S. Environmental Protection Agency (EPA (*16*).

The objectives of this program were to determine the level and extent of PCB contamination in fish inhabiting or migrating through major waterways in New York State and to isolate contaminated portions or reaches of waterways, thereby identifying specific areas to be addressed by agencies which control water pollution.

TABLE 1. *Desired species and size distribution of fish from New York waters analyzed for PCB content, 1975*

Species	Small [1]	Medium [2]	Large [3]
	Length, in.		
Smallmouth bass	5–7	10–12	14–16
Largemouth bass	5–7	10–12	14–16
Walleye	7–9	11–13	19–21
Bullheads	4–6	8–10	11–13
Carp	5–7	13–15	23–25
Goldfish	4–6	7–9	10–12
Suckers	6–8	10–12	17–19
Striped bass	11–13	16–18	28–32
	Weight, lb. [4]		
Salmon (coho)	1–2	4–6	8–12
(chinook)	3–5	8–12	15–20
Rainbow or steelhead trout	1–2	3–5	5–8
Lake trout	1–2	4–6	8–12

[1] Minimum number of fish per sample was 6.
[2] Average number of fish per sample was 5.
[3] Maximum number of fish per sample was 5.
[4] Minimum number of fish per sample was 5.

[1] New York State Department of Environmental Conservation, Bureau of Environmental Protection, 50 Wolf Road, Albany, N.Y. 12233

TABLE 2. *Statewide sampling stations for PCB analysis, New York—1975*

Station No.[1]	Watershed	Waterway	Location
1	Upper Hudson River	Hudson River	Above Corinth
2			Above Glens Falls
3			At Fort Edward
			below General Electric Co.
4			5 miles below Fort Edward
5			Waterford area
6	Lower Hudson River		Coxackie-Catskill area
7			Lower Hudson
			Mouth of Rondout Creek
			Mouth of Wappingers Creek
			Mouth of Esopus Circle
			Kingston Rhinecliff Bridge
			Tappan Zee Bridge area
			West Point
			George Washington Bridge area
			Highland Poughkeepsie area
8		Rondout River	Rondout River above first dam
9	Mohawk River	Mohawk River	Below Schenectady
10			Below Otiskany
11	Seneca/Oneida/Oswego Rivers	Seneca/Oneida Oswego Rivers	Below Fulton
12		Erie Canal	Below Clyde
13	Lake Erie/Niagara River	Niagara River	Below Lewiston
14	Lake Erie/Niagara River	Lake Erie	Dunkirk
15	Lake Ontario	Lake Ontario	Off Rochester
16			Oswego
17			Pulaski (salmonids)
18	Genesee River	Genesee	Below Industry
19	Chemung River	Chemung	Chemung
20	Susquehanna River	Susquehanna	Smithboro
21	Delaware River	Delaware	Below Pt. Jervis (State line)
22	Lake Champlain	Lake George	Bolton Landing
23		Lake Champlain	Ticonderoga
24			Plattsburg
25	St. Lawrence River	St. Lawrence	Cape Vincent
26			Massena
27	Black River	Black River	Watertown
28	St. Lawrence River	Raquette River	Norfolk
29		Fulton Fish Market	
30		Albany Fish Market	
31	Seneca/Oneida/Oswego Rivers	Onondaga Lake	
32	Upper Hudson River	Walloomsac/Hoosick Rivers	
33	Long Island	Marine Waters	
34	Upper Hudson River	Champlain Canal	Lock 8
35	*Genesee River*	Hemlock Lake	
36		Canadice Lake	
37	Seneca/Oneida/Oswego Rivers	Canandaigua Lake	
38		Keuka Lake	
39		Seneca Lake	
40		Cayuga Lake	
41	"	Seneca River	Montezuma National Wildlife Refuge

[1] See map, figure 1.

or storage center. Common names of fishes used throughout this report are those adopted by the American Fisheries Society (*1*) and are presented with their scientific nomenclature in Table 3.

TABLE 4. *Concentrations of PCB's in fish and shellfish in New York State, 1975*

Water	Station	Laboratory [1]	Species	Aroclor	No. Fish Analyzed	Number of Analyses	Concentration of PCB's, ppm wet weight [2]		
							Weighted Average	High	Low
Hudson River	1, 2	DEC–R	Smallmouth bass	1242/1016	26	12	0	0	–
				1254			TR	TR	0
			Walleye	1242/1016	2	2	0	0	–
				1254			TR	TR	0
			Yellow perch	1242/1016	15	11	0	0	–
				1254			TR	TR	0
			White sucker	1242/1016	4	4	0	0	–
				1254			TR	TR	0
Hudson River (Fort Edward below General Electric Company)	3	DEC–R	Walleye Whole fish [3]	1242/1016	14	2	91.01	104.21	81.12
				1254			36.01	41.80	28.29
			Edible flesh	1242/1016	4	1	9.88	9.88	–
				1254			8.31	8.31	–
			Yellow perch Whole fish	1242/1016	30	2	164.36	236.40	128.33
				1254			37.14	62.88	24.27
			Edible flesh	1242/1016	10	1	61.10	61.10	–
				1254			18.24	18.24	–
			Rock bass	1242/1016	2	1	27.35	27.35	–
				1254			ND		
			Alewife	1242/1016	1	1	20.35	20.35	–
				1254			ND		
			White sucker	1242/1016	9	2	41.74	82.88	8.83
				1254			23.10	47.57	2.72
Hudson River (5 miles below Fort Edward)	4	DEC–R	Smallmouth bass	1242/1016	1	1	100.27	100.27	–
				1254			22.64	22.64	–
			Walleye	1242/1016	3	3	56.73	157.27	5.19
				1254			10.67	20.74	5.56
			Yellow perch Whole fish	1242/1016	26	2	87.29	88.18	34.31
				1254			30.69	34.21	18.95
			Edible flesh	1242/1016	3	1	35.54	35.54	–
				1254			15.01	15.01	–
			White sucker [3]	1242/1016	10	5	47.30	78.00	28.03
				1254			19.82	41.10	8.13
			Brown bullhead	1242/1016	5	2	77.04	85.30	73.24
				1254			37.23	38.52	36.38
Hudson River (Stillwater)	4A	DEC–R	Largemouth bass Whole fish	1242/1016	8	2	11.65	14.31	8.99
				1254			8.42	9.84	6.99
			Edible flesh	1242/1016	4	1	19.25	24.85	12.40
				1254			10.59	15.97	4.44
			American eel [4]	1242/1016	2	2	207.33	403.38	11.28
				1254			83.47	155.87	11.07
			Brown bullhead [5]	1242/1016	1	1	4.66	4.66	–
				1254			7.99	7.99	–
			White sucker	1242/1016	6	2	58.32	154.28	10.34
				1254			15.93	35.92	5.94
Hudson River (Waterford)	5	DAM	Smallmouth bass	1242/1016	10	2	30.60	36.0	27.0
				1254			15.70	17.5	14.5
			Walleye	1242/1016	2	1	23.30	23.3	–
				1254			9.10	9.1	–
			White sucker	1242/1016	11	2	27.44	36.0	20.3
				1254			10.15	12.9	7.9
Hudson River (Below Troy Dam)	6, 7a–h	DEC–R	Striped bass Whole fish	1242/1016 [6]	21	2	0.10	2.19	0
				1254			1.75	1.96	1.66
			Edible flesh	1242/1016	42	31	12.05	56.42	0
				1254	48	37	11.41	50.06	1.66

(Continued next page)

TABLE 4 (cont'd.). *Concentrations of PCB's in fish and shellfish in New York State, 1975*

Water	Station	Laboratory [1]	Species	Aroclor	No. Fish Analyzed	Number of Analyses	Concentration of PCB's, ppm wet weight [2]		
							Weighted Average	High	Low
			Carp	1242/1016	10	2	2.00	4.0	0
				1254			5.60	7.2	4.0
Erie Canal	12	DH	Largemouth bass	1242/1016	1	1	0	0	–
				1254			0.80	0.8	–
			Northern pike	1242/1016	1	1	2.00	2.0	–
				1254			1.30	1.3	–
			Carp	1242/1016	8	2	0.60	1.6	0
				1254			0.30	0.8	0
Niagara River	13	DEC–R	Goldfish	1242/1016	5	1	0	0	–
				1254			TR	TR	–
			Largemouth bass	1242/1016	1	1	0	0	–
				1254			3.10	3.10	–
			Smallmouth bass	1242/1016	1	1	0	0	–
				1254			3.30	3.30	–
			White bass	1242/1016	1	1	0	0	–
				1254			11.31	11.31	–
Lake Erie	14	DEC–R.DAM	Smallmouth bass	1242/1016	19	4	0.19	0.4	0
				1254			0.72	1.0	0.6
			Walleye	1242/1016	19	9	0.19	0.6	0
				1254			0.70	1.45	TR
			Yellow perch	1242/1016	10	2	0.40	0.4	0.4
				1254			0.60	0.6	0.6
			Goldfish	1242/1016	4	1	1.00	1.0	–
				1254			1.40	1.4	–
			Brown trout	1242/1016	20	9	0.23	2.4	0
				1254			1.60	3.23	0.57
Lake Erie	14	DEC–R	Rainbow trout	1242/1016	10	9	0	0	–
				1254			2.03	3.77	0.76
			Chinook salmon	1242/1016	8	8	0	0	–
				1254			1.71	2.71	0.98
			Coho salmon	1242/1016	15	4	0	0	–
				1254			2.36	5.06	0.68
Lake Ontario	15–16	DEC–R	Coho salmon	1242/1016	23	15	0	0	–
				1254			8.05	18.65	2.09
			Chinook salmon	1242/1016	29	17	0.21	6.07	0
				1254			14.48	24.57	4.62
			Rainbow trout	1242/1016	9	9	0	0	–
				1254			5.76	12.35	1.47
			Brown trout	1242/1016	12	12	0	0	–
				1254			5.25	16.76	0.96
			Lake trout	1242/1016	19	14	0	0	–
				1254			9.38	14.62	1.35
			White perch	1242/1016	27	5	0	0	
				1248			0.05	0.48	–
				1254			4.12	12.06	2.14
			Yellow perch	1242/1016	25	4	0.38	0	
				1254			0.38	0.69	–
			Northern pike	1242/1016	17	10	0	0	–
				1254			1.79	4.02	0.96
			Largemouth bass	1242/1016	5	5	0	0	–
				1254			1.79	4.25	0.42
			Smallmouth bass	1242/1016	7	2	0	0	–
				1254			1.81	2.69	1.15
			Rock bass	1242/1016	11	2	0	0	0
				1254			0.83	3.58	0.55
			Brown bullhead	1242 1016	10	2	0	0	–
				1254			0.57	0.75	0.39

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TABLE 4 (cont'd.). *Concentrations of PCB's in fish and shellfish in New York State, 1975*

Water	Station	Laboratory[1]	Species	Aroclor	No. Fish Analyzed	Number of Analyses	Concentration of PCB's, ppm wet weight[2]		
							Weighted Average	High	Low
			Yellow perch	1242/1016	17	3	0.40	0.4	0
				1254			0.59	0.8	0.2
			Brown bullhead	1242/1016	14	3	0.34	0.7	0
St. Lawrence River	25, 26	DEC-R		1254			0.45	1.2	0
			Smallmouth bass	1242/1016	16	9	0	0	–
				1254			4.58	6.85	1.62
			Walleye	1242/1016	11	3	0	0	–
Black River	27	DH		1254			1.83	2.05	1.58
			Walleye	1242/1016	8	2	1.25	2.0	0
				1254			3.11	4.5	0.8
			Brown bullhead	1242/1016	10	2	0.95	1.5	0.4
Raquette River	28	DH		1254			2.50	4.0	1.0
			Smallmouth bass	1242/1016	15	4	0.30	0.4	0.2
				1254			0.90	1.7	0.3
			Shorthead redhorse	1242/1016	14	3	0.32	0.4	0.2
Onondaga Lake	31	DEC-R		1254			0.44	0.6	0.3
			Smallmouth bass	1242/1016	9	3	1.82	1.9	1.2
				1254			3.13	4.0	1.2
			Walleye	1242/1016	1	1	3.10	3.1	–
				1254			2.50	2.5	–
			White perch	1242/1016	11	2	2.28	4.3	0.6
				1254			2.33	4.9	0.2
			White sucker	1242/1016	3	1	0.70	0.7	–
				1254			0.20	0.2	–
			Carp	1242/1016	7	2	2.20	3.0	0.2
				1254			6.04*	7.5	2.4
			Brown bullhead	1242/1016	5	1	3.10	3.1	–
Walloomsack-Hoosic River	32	DH		1254			5.30	5.3	–
			Largemouth bass	1242/1016	5	2	2.62	2.8	2.5
				1254			2.98	3.5	2.2
			Yellow perch	1242/1016	4	1	2.00	2.0	0
				1254			3.40	3.4	0
			White sucker	1242/1016	7	3	1.73	2.9	0.7
				1254			1.58	2.9	0.6
Atlantic Ocean	33	DEC-SB	Bluefish	1242/1016	26	15	0	0	–
				1248			0	0	–
				1254			1.43	8.39	0.21
			Striped bass	1242/1016	29	17	0	0	–
				1248			0	0	–
				1254			1.05	3.59	TR
			Porgy	1242/1016	12	2	0	0	–
				1248			0	0	–
				1254			0.29	0.32	0.25
			Atlantic menhaden	1242/1016	18	7	0	0	–
				1248			0	0	–
				1254			1.07	1.53	0.44
			Spiny dogfish	1242/1016	60	4	0	0	–
				1248			0	0	–
				1254			0.25	0.27	0.22
			Bluefin tuna	1242/1016	7	7	0	0	–
				1248			0	0	–
				1254			1.42	6.68	0.19
			Atlantic mackerel	1242/1016+	5	5	0.46	0.52	0.31
				1254					
			Weakfish	1242/1016	1	1	0	0	–
				1248			0	0	–
				1254			0.21	0.21	–

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TABLE 4 (cont'd.). *Concentrations of PCB's in fish and shellfish in New York State, 1975*

Water	Station	Laboratory[1]	Species	Aroclor	No. Fish Analyzed	Number of Analyses	Concentration of PCB's, ppm wet weight[2] Weighted Average	High	Low
			White sucker Whole fish	1242/1016	5	3	0	0	-
				1254			0.59	0.92	TR
			Pumpkinseed Whole fish	1242/1016	5	1	0	0	-
				1254			2.00	2.00	-
Hemlock Lake	35	DEC-R	Lake trout	1242/1016	10	10	TR	TR	0
				1254			TR	TR	0
			Rainbow trout	1242/1016	2	2	0	0	-
				1254			0	0	-
			Chain pickerel	1242/1016	9	5	0	0	-
				1254			0	0	-
			White sucker	1242/1016	3	1	TR	TR	-
				1254			0	0	-
			Carp	1242/1016	4	1	0	0	-
				1254			0	0	-
			Cisco	1242/1016	3	1	TR	TR	-
				1254			0	0	-
			Alewife	1242/1016	5	1	0	0	-
				1254			0	0	-
			Rainbow smelt	1242/1016	5	1	TR	TR	-
				1254			0	0	-
			Yellow perch	1242/1016	7	2	TR	TR	-
				1254			0	0	-
Canadice Lake	36	DEC-R	Lake trout	1242/1016	7	7	0	0	-
				1254					
			Rainbow trout	1242/1016	5	5	0	0	-
				1254					
Canandaigua Lake	37	DEC-R	Lake Trout	1242/1016	17	12	3.11	25.78	0
				1254			5.38	21.69	1.12
			Brown trout	1242/1016	1	1	0	0	-
				1254			2.58	2.58	-
Keuka Lake	38	DEC-R	Lake trout	1242/1016	18	8	TR	TR	0
				1254			0.09	1.77	0
			Rainbow smelt	1242/1016	6	1	0	0	-
				1254			0	0	-
			Alewife	1242/1016	9	2	0	0	-
				1254			0	0	-
Seneca Lake	39	DEC-R	Lake trout	1242/1016	10	10	0	0	-
				1254			6.42	15.99	2.62
Cayuga Lake	40	DEC-R	Lake trout	1242/1016	7	7	0	0	-
				1254			6.15	8.23	1.76
Montezuma National Wildlife Refuge	41	DEC-R	Carp	1242/1016	3	3	0.66	1.59	TR
							1.67	3.53	0.29

[1] Laboratory code: DEC-R = Department of Environmental Conservation, Rome Laboratory, New York State.
DAM = Department of Agriculture and Markets, Food Laboratory, New York State.
DH = Department of Health, Griffin Laboratory, New York State.
DEC-SB = Department of Environmental Conservation, Stony Brook Laboratory, New York State.

[2] - indicates absense of data, or no value less than 0 as reported in column titled "High."
TR = trace.
ND = not determined.

[3] Includes only fish analyzed for both aroclors; 4 fish not included.

[4] Sample includes one highly contaminated specimen.

[5] Sample does not include 6 fish for which Aroclor 1242/1016 was not determined.

[6] Sample does not include 1 fish for which Aroclor 1254 was not determined.

[7] Sample does not include 2 fish for which Aroclor 1254 was not determined.

[8] Sample does not include 12 fish for which Aroclor 1254 was not determined.

[9] Data in parentheses are weighted average PCB concentrations excluding two fish with exceptionally high concentrations.

TABLE 5 (cont'd.). *Concentrations of PCB's in fish, 1970–74*

Water	Species	Year Collected	Aroclor	No. Fish Analyzed[1]	Number of Analyses	Concentration of PCB's, ppm wet weight: Weighted Average	High	Low
Lake Ontario	Coho salmon	1971	1254	9	9	6.67	21.97	0.66
		1974	1254	29	29	6.26	10.72	2.00
	Chinook salmon	1971	1254	12	12	21.45	34.48	9.24
		1974	1254	9	9	7.15	14.77	1.54
Lake Ontario	Rainbow trout	1974	1254	3	3	1.97	2.81	1.45
	Brown trout	1974	1254	4	4	7.69	14.50	2.70
	Northern pike	1970	1254	4	4	5.32	12.86	1.38
	Smallmouth bass	1970	1242/1016	3	3	7.11	8.81	4.38
			1254	2	2	3.88	4.52	3.24
	Largemouth bass	1970	1254	1	1	2.84	2.84	–
	Rock bass	1970	1254	2	2	0.49	0.59	0.40
	White bass	1970	1254	3	3	10.26	15.46	6.37
	White perch	1970	1254	3	3	3.42	5.15	2.12
	Carp	1970	1254	1	1	0.71	0.71	–
	Rainbow smelt							
	Whole	1972	1254	10	10	4.02	7.12	2.26
	Edible	1972	1254	11	11	2.16	3.42	1.15
Lake Ontario (Salmon River Pulaski Weir)	Coho salmon	1970	1254	17	17	7.92	12.10	5.01
		1971	1254	20	20	10.35	21.43	0.83
		1972	1254	20	20	4.65	13.29	0.82
		1974	1254	5	5	7.05	11.29	3.48
Lake Ontario (Salmon River Pulaski Weir)	Chinook salmon	1971	1242/1016	2	2	19.38	28.11	10.65
			1254	4	4	9.37	12.10	5.98
		1973	1254	51	51	5.81	13.14	1.45
	Rainbow trout	1971	1254	3	3	7.24	8.38	5.76
Lake Ontario (Little Salmon River)	Coho salmon	1971	1254	3	3	2.60	6.10	0.76
	Chinook salmon	1971	1254	4	4	15.75	29.36	4.68
Lake Ontario (Sprang Brook)	Coho salmon	1972	1254	1	1	6.81	6.81	–
St. Lawrence River	Smallmouth bass	1971	1254	7	7	5.35	9.76	1.31
	Walleye	1971	1242/1016	1	1	3.42	3.42	–
	Sturgeon (species not known)	1971	1242/1016	1	1	11.89	11.89	–
	Channel catfish	1971	1242/1016	2	2	65.85	81.79	49.91
Allegheny River	Smallmouth bass	1971	1242/1016	2	2	2.21	2.36	2.06
	Muskellunge	1970	1242/1016	1	1	2.21	2.21	–
Genesee River-Lower portion	Smallmouth bass	1971	1254	2	2	7.36	10.59	4.12
	White bass	1971	1254	1	1	7.76	7.76	–
Chemung River	Smallmouth bass	1971	1254	3	3	0.46	0.62	0.23
	Walleye	1971	1254	2	2	1.02	1.09	0.94
	Yellow perch	1971	1254	2	2	0.14	0.28	TR
Cohocton River (above Bath)	Smallmouth bass	1971	1254	1	1	0.90	0.90	–
	Brown trout	1971	1254	1	1	2.80	2.80	–

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TABLE 5 (cont'd.). *Concentrations of PCB's in fish, 1970–74*

Water	Species	Year Collected	Aroclor	No. Fish Analyzed[1]	Number in Analyses	Concentration of PCB's, ppm wet weight: Weighted Average	High	Low
	Black crappie	1971	1254	3	3	TR	TR	0
Atlantic Ocean	Striped bass	1972	1254	5	2	0.57	0.59	0.57
	Bluefish	1972	1254	5	5	1.82	8.49	0.07
	Winter flounder	1973–74	1254	180	9	0.13	0.26	0
	Atlantic silverside	1973–74	1254	900	9	0.16	0.30	0
	Mummichog	1973	1254	11	1	0.30	0.30	-
	Grubby	1973	1254	20	1	0.19	0.19	-
	Spiny dogfish	1972	1254	4	4	0.25	0.27	0.21
	Northern lobster	1974	1254	1	1	0.15	0.15	-
	Oyster	1974	1254	36	3	0.34	0.75	0.12

NOTE: Results tabulated only for those fish for which the specific PCB was reported. All analyses conducted by Department of Environmental Conservation Laboratory, Rome, N.Y., except Atlantic Ocean analyses which were conducted by the Stony Brook Laboratory
- indicates absense of data.
TR = trace
[1] Parenthetic numbers exclude one individual fish with high PCB concentration.

TABLE 6. *Laboratory comparisons of single aroclors in hexane and iso-octane solutions*

Sample Solution	Aroclor	Residues, µg/ml [1,2]: Known Standard	DAM	DEC-SB	DEC-R	DH	EPA
Hexane	1016	0	0	0	0	0	NS
	1242	0	0	0	0	0	NS
	1254	0	0	0	0	0	NS
Hexane	1016	0.80	0.89	1.09 [3]	1.15	0.71	NS
	1254	0	NV	NV	NV	NV	NS
Hexane	1016	0	NV	NV	NV	NV	NS
	1254	0.95	0.92	1.32	1.01	0.97	NS
Iso-octane (combined standards)	1016	55.5	73.3 [3]	NV	39.8 [4]	54.2(43.8)	26
	1254	72.0	92.8	NV	90.4	72.2(90.0)	91
	1016+1254	127.5	166.1	177 [3]	130.2	126.4(133.8)	117
Iso-octane (combined standards)	1016	61.0	70.0	NV	NV	NV (103.4)	38
	1242	64.5	50.0	NV	70.3	125 NV	60
	1016+1242	125.5	120.0	145 [3]			98

[1] Laboratory code: DAM = Department of Agriculture and Markets, Food Laboratory, New York State.
DEC-SB = Department of Environmental Conservation, Stony Brook Laboratory, New York State.
DEC-RL = Department of Environmental Conservation, Rome Laboratory, New York State.
DH = Department of Health, Griffin Laboratory, New York State.
EPA = U.S. Environmental Protection Agency Laboratory, Region II, New York City.
[2] NV = no value reported for this Aroclor.
NS = no sample sent to this laboratory.
[3] Residue reported as Aroclors 1242 and 1254.
[4] Residue reported as Aroclor 1242.

This model can be modified to show direct uptake by each biotic community from the aqueous phase (*11, 18, 19*). Through this avenue, a steady state may be achieved within a short period of time (±30 days) in highly contaminated waters (*23, 24*). Therefore, monitoring fish populations and analyzing properly composited samples for PCB contamination can indicate whether it is safe to consume fish flesh based on the Food and Drug Administration action level of 5 ppm, and to what degree a waterway is contaminated by PCB's. Indirectly, monitoring studies can show the need for specific studies to address the impact of PCB's on natural communities and/or populations, and the need to re-evaluate existing discharge permit limits.

The 1975 analyses confirmed that PCB's expressed as Aroclor 1016/1242 and/or 1254 were present in detectable amounts in most fish sampled. The level of contamination and specific Aroclor vary according to waterway size, location, and peripheral land use. Table 4 summarizes the data for edible flesh except in the cases indicated. Following is a general discussion of the findings in each waterway.

HUDSON RIVER; Stations 1–8, 32, 34. This major river system contains fish with the highest average concentration of total PCB's of any waterway sampled. Sampling nearly the entire extent of the river shows that contamination is widespread from Hudson Falls to the mouth of the river. The average level of contamination decreases in the lower reaches of the Hudson, perhaps through dilution from tributary waters, deposition in the sediments and aquatic organisms, and the effects of marine waters. Similar results occurred in fish collected from 1970 and 1973 (Table 5).

MOHAWK RIVER; Stations 9 and 10. Concentrations in 5 of 13 analyses in 1975 exceed the Food and Drug Administration action level of 5 ppm. Highest average concentrations appear upstream at the Little Falls station rather than at Schenectady, indicating a source of contamination above the town of Little Falls. The problem appears localized and does not affect the entire waterway as indicated by the Schenectady samples.

Earlier samples (Table 5) were either absent or too small to be compared.

SENECA/ONEIDA/OWSEGO RIVERS; Stations 11 and 12. PCB's are present, averaging 4 ppm. Concentrations are lowest at the Clyde station.

NIAGARA RIVER; Station 13. Largemouth and smallmouth bass have concentrations below 5 ppm; one white bass had levels above 10 ppm. The station was located below Lewiston, thus indicating no major source of PCB contamination from the Buffalo/Niagara Falls industrial complex. Earlier data are too sparse for direct comparison. However, additional study is warranted based on the 1971 analytical results and the single white bass noted above.

LAKE ERIE; Station 14. Samples from Lake Erie were taken at two locations, Dunkirk and the mouth of Cattaraugus Creek at Irving. The several species of fish contained levels of total PCB's well below 5 ppm. This is consistent with the 1973–74 data which indicated the presence of the contaminant at low concentrations.

LAKE ONTARIO; Stations 15–17. Generally, low levels of PCB's were found in warm water species, in contrast to the elevated concentrations commonly found in three species of salmonids taken from the lake proper. However, reduced levels were noted in the salmon that had migrated upstream in one major tributary, the Salmon River (Station 17). A reduction of the body fat through metabolic activities such as spawning and migration apparently caused the loss or displacement of some of the PCB burden in the edible flesh.

Comparison of the 1975 data with earlier findings is difficult. General trends indicate that concentrations similar to the present levels existed as early as 1970.

GENESEE RIVER; Station 18. Analysis of fish taken from the lower portion of the river below the village of Industry indicates a potential PCB problem in large fish. In two samples, one each of smallmouth bass and silver redhorse, levels were noted at or near 5 ppm. However, the 1971 samples, which were analyzed only for Aroclor 1254, indicate levels higher than those found in 1975. Due to the size of the earlier samples no trends can be identified except that PCB's (Aroclor 1254) are still present in substantial concentrations.

CHEMUNG/SUSQUEHANNA/DELAWARE RIVERS; Stations 19–21. These three independent river systems have been grouped for discussion since they reflect low levels of contamination, averaging 1 ppm or less. These figures are comparable to the samples collected in 1971 in the Chemung River.

LAKE GEORGE; Station 21. The larger, fatty lake trout of Lake George contain levels of PCB's (primarily Aroclor 1254) above 5.0 ppm. Lake George lake trout are known to have elevated levels of DDT (*4*). The analytical methodology used converted only DDT metabolites to dichlorobenzophenone rather than DDT itself. Therefore, PCB levels in lake trout and other species in Lake George, particularly ciscos, may be overstated. Further study of the lake is warranted.

LAKE CHAMPLAIN; Stations 23 and 24. These stations were located at Ticonderoga and Plattsburgh, respectively.

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PESTICIDES MONITORING JOURNAL

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FEDERAL WORKING GROUP ON PEST MANAGEMENT
(RESPONSIBLE TO THE COUNCIL ON ENVIRONMENTAL QUALITY)

ser that emits 1.06-micrometer light. It has just been shut down while an even larger laser is built. Peter Hagelstein, a recent MIT graduate whose Ph.D. thesis was to develop x-raser designs while working in Wood's group, notes that no experiment was ever done, although theoretical work was extensive on two neon systems that would lase at 460 and 82 angstroms.

In a second laser-based project, Robert Carman of Los Alamos and Chapline plan to use the eight-beamed Helios laser at Los Alamos. Helios is a carbon dioxide gas laser that emits light at 10.6 micrometers. Chapline reported at New Orleans that there may be experiments with copper targets this year and that additional theoretical work was needed even to predict what the lasing wavelengths would be in the range from 220 to 327 angstroms.

The third x-raser project with Livermore's participation is also based on a high-temperature plasma, but it does not use a laser to produce it. Richard Fortner is working with Glen Dahlbacka and Ray Dukart of Physics International in San Leandro, California. The company has a machine called Pithon, originally built for nuclear weapons effects simulations, that generates extremely high bursts of electric current (up to 3.5 million amperes) into a gas or solid laser medium and thereby creates a plasma. A first round of experiments has been completed, but only one test produced any evidence of x-rays being emitted at the correct wavelength. The plasma was very inhomogeneous in both temperature and density, and the prospects for achieving lasing in future tests with krypton and molybdenum that emit at 149 or 97 angstroms are viewed as uncertain.

Livermore, of course, is not the only laboratory in the world with x-raser projects. But, as with the laboratory's unclassified work, all are far from fruition or else are concentrating on wavelength ranges far from those required for x-ray holography in the soft x-ray region of interest for biological imaging.

How interesting is x-ray holography? John Sedat of the University of California at San Francisco, who has also been contacted by Livermore about collaborating on a holography project, says that the technique would "really change the whole field of structural biology." For example, the importance of the aqueous environment in maintaining structures without distortions is crucial, and x-ray holography would allow this. Sayre at IBM is a bit more cautious in noting that there are two or three other methods that promise three-dimensional imaging capabilities similar to holography. Both researchers enthusiastically hold that x-ray holography should be tried. Now it looks as though it will, although under rather unusual circumstances. Meanwhile, laser scientists hope that the details of the putative x-raser will soon be declassified so that work can begin on the possibility of making a laboratory version of the device that will use a more conventional pumping source.

—ARTHUR L. ROBINSON

Just How Hazardous Are Dumps?

Everyone knows toxic wastes are hazardous, but there is little agreement about how to assess the potential risks to health

In October, the United States Environmental Protection Agency (EPA) finally issued a list of 115 hazardous waste storage sites that represent the most serious potential danger to human health. These sites were among the first candidates to be cleaned up with support from Superfund, a $1.6-billion kitty accumulated primarily from taxes on industrial chemicals. In November, however, the Congressional Office of Technology Assessment (OTA) sharply criticized that list, arguing that EPA had used inappropriate criteria in preparing the rankings. States and environmental groups have had their own thoughts about which dump and storage sites deserve first call on the funds.

These conflicting opinions symbolize the most crucial problem confronting individuals and agencies that must deal with the hazardous waste problem: There is no firm consensus about how to determine the potential hazard of chemicals at a dump site, the risk of exposure, and the potential health effects from such an exposure. The problems are not simply academic. A 1979 survey by EPA of 100 waste disposal sites produced an average estimated cost for cleanup of more than $8 million, with the cost at some sites running as high as $25 million. There are hundreds of such sites—perhaps thousands—and even $1.6 billion will not go very far. Hard choices are going to have to be made—and soon.

Part one of two parts

A good example of the problems involved is the highly publicized Love Canal area in Niagara Falls, New York, where homes were built immediately adjacent to a waste disposal site that was disturbed. Despite the great amount of time and effort that has been devoted to this volatile problem, it has been extremely difficult to document the extent of exposure of area families to the chemicals. There has been no conclusive evidence linking any such exposure to health effects in humans—particularly since some potential effects may not become apparent for at least another 15 years.

Investigators have even had problems defining precisely which questions should be asked about hazardous waste sites. There is consensus about only a few of the most general:

- What do we know about how chemical waste disposed onto and into the land contributes to pollution of air and ground water?
- In sampling and analyzing air, water, and other media, what strategies are most promising?
- How can human exposure best be estimated?
- What is the extent of normal human exposure to the chemicals in dumps?
- How can exposure to chemicals be related to human health problems?
- What are the health effects of mixtures of chemicals? Are they additive or synergistic?
- Are there existing technologies that have not been applied to the problem? What new technologies need to be developed?

These questions and others have been addressed at three major meetings during the past year. Proceedings of two of the meetings are now available in book form

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got correct values (within 15 percent) for more than 70 percent of the samples. After an intensive training program at CDC and much work, about 65 percent of the laboratories now get more than 70 percent of the determinations correct. It is clear, however, that the laboratories are putting special emphasis on the CDC samples. When identical samples are sent to the laboratories from a disguised source, the number getting more than 70 percent of the answers correct drops by ılf.

> "The private testing industry is simply too small to deal with the uncontrolled waste site problem."

CDC recently began a similar study with polychlorinated biphenyls (PCB's) in cow's blood, but only about half of the state laboratories and only five of the 114 largest private laboratories invited to participate did so—"They can't be convinced that we are nonregulatory," says Liddle. The samples were spiked with other halogenated hydrocarbons commonly found in humans so that they represented "the worst case" that the laboratories might encounter. Only three of the 29 participating laboratories produced values of PCB concentrations that were within 2 standard deviations of the correct value. Both lead and PCB's are compounds that have been studied for a long time and should be readily measurable, Liddle says; the implication is that results may be much worse for other chemicals with which there is less experience. There is a "desperate need" for more work on interlaboratory comparability, he concludes, so that it will be possible to believe published results and accurately relate them to health effects.

Even when the concentration of contaminants in air, water, and soil is known, it is still difficult to determine whether humans have been exposed to them and, if so, at what concentrations and for how long. "The only objective measures of this so far," says Heath, are for chemicals that collect and persist in the body, particularly chlorinated aromatics such as PCB's and pesticides. Even for these, it is necessary to know baseline levels in the population at large before the extent of exposure can be determined. In some cases, those baseline concentrations are quite high.

One of the few sources of such information is the Health and Nutrition Examination Survey (HANES) conducted by the National Center for Health Statistics. For HANES, physical examinations were conducted on more than 15,000 people, ages 12 to 74, at 64 locations throughout the country. As part of the survey, says study director Robert S. Murphy, blood and urine samples from many of the participants were examined for pesticides and related chemicals.

Among some 6000 subjects whose blood was examined, more than 99 percent had significant quantities of DDT. The proportion with other halogenated pesticides in their blood ranged from 4 to 14 percent. Among some 4000 individuals whose urine was examined, 79 percent had significant quantities of the pesticide-related compound pentachlorophenol. Other halogenated intermediates were present in 4 to 7 percent of the samples, carbamate pesticides were present in 2 to 4 percent of samples, and organophosphate pesticides were present in 6 to 12 percent.

The center has also conducted a Human Adipose Tissue Survey, says Murphy, in which samples of fat were obtained from cooperating pathologists. Some 93 percent of 785 samples contained significant quantities of the pesticides DDT, chlordane, and dieldrin, as well as hexachlorobenzene. A third of the samples also contained PCB's.

Other studies have shown significant regional variations. A survey in the Southeastern United States showed that 23 percent of the individuals studied had trace levels of the potent carcinogen aflatoxin in their blood, according to Renate Kimbrough of CDC; aflatoxin is produced by a microorganism that grows on peanuts. Studies in Michigan show that 99 percent of individuals in the lower peninsula and 85 percent in the upper peninsula show significant quantities of polybrominated biphenyls in their adi-

Battelle's Columbus Laboratories

Some dump sites are obvious . . .

Piles of corroding barrels may or may not represent a hazard, depending on their contents and location.

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Human Health Risk? Using Sludge For Crops

by George Braude, Bernard P. Sagik, and Charles A. Sorber
U. S. Food and Drug Administration
The Center for Applied Research and Technology
The University of Texas at San Antonio

SEWAGE DISPOSAL

One solution to the dilemma of accumulating wastewater and sewage has been application of sludge to agricultural lands. Several notable benefits are: recycling of nutrients, improvement of marginal soils with organic matter, and direct costs comparable to alternatives.

Land application, however, may also involve risks to human health: Municipal sewage contains a variety of chemical compounds and human and animal pathogens, including viruses, bacteria, fungi, and parasites.

Pathogens

Several authorities have addressed the problem of pathogen transmittal to humans by way of land-applied wastes. Theodore G. Metcalf has conducted studies following the progress of pathogens along the food chain in simulated aquatic and terrestrial ecosystems.[1] Results showed that enteric viruses were transmitted from wastes to animals in mariculture and on a soil system utilizing macrovinvertebrates. Since the animals served as food for others, viruses were recovered from an increasing variety of organisms.

Sequential virus carriage was established in the mariculture system, extending through polychaete worms, shellfish and the bottom-feeding flounder. This is shown schematically in Figure 1.

In the terrestrial system, five species of soil macroinvertebrates were given food contaminated with virus. Accumulation, survival and elimination of virus were determined. All species shed viruses, although at different rates.

The animals studied in these aquatic and terrestrial systems represented a hypothetical food chain through which pathogenic viruses could pass sequentially from simpler to increasingly more-complex life forms. These were experimental systems, though extrapolation from such data to a situation in which viruses in municipal wastes were passed along a food chain—eventually reaching the human consumer—is possible.

Edward P. Larkin has researched the potential for microbiological contamination of food and soils.[2] He has pointed out that all treated wastewaters, unless sterilized, retain some before-treatment organisms. Complete removal of microbial pathogens by present treatment methods is not possible. Fecal contamination of crops by way of soil and water is an obvious health hazard. Fecal-indicator organisms have been detected on field-grown vegetables. Larkin has noted that other microbes and pathogens also may survive the waste treatment process and contaminate the food chain via land disposal of sludge and effluents.

Larkin has also reported his study of effluents seeded at $> 10^7$ organisms/ml with *Salmonella typhimurium*. Soils and vegetables irrigated with the seeded effluents were sampled weekly. The concentration of *S. typhimurium* in soil decreased linearly to week[6] then leveled off erratically to week 12 (Figure 2). The concentrations of fecal coliform and fecal streptococcal bacteria decreased at similar rates. Total coliforms were covered at levels of 10^6/g of soil at the end of the sampling period. The levels reported are 2 log 10 units higher than those reported by others, but seeding levels were higher.

Implicit in Larkin's studies is the knowledge that some sewage treatment plants operate beyond their capacity. Thus, some wastes are not treated until a plant can accommodate. Obviously in these circumstances bacteria and viruses persist longer and in larger numbers. The risk to human health is great when such wastes are applied to land for crop production. For this reason, the Food and Drug Administration recommends that food crops that are to be consumed raw should not be planted within three years after the last land application of wastes. In addition, unless sewage is shown to be free of pathogens, it should not be used on foods that may enter homes or food establishments in the raw state for cooking because other foods and utensils may become contaminated.

Wastes may contain microinvertebrate animals, some of which are parasites of man and other animals. G. L. Jackson has described a possible environmental circuit of the parasitic roundworm *Ascaris*.[3]

Jackson has pointed out that the layered structure of the *Ascaris* egg and the composition of its shell allow it to remain viable through repeated freezing-thaw cycles. Temperatures as high as 50–60 degrees C (120–140 degrees F) or more for 10 minutes are recommended for complete inactivation. Its durability permits some *Ascaris* eggs to survive the sewage-treatment process and the application of sludges to agricultural lands may cause some cases of parasitic infestation. Jackson described methods for obtaining *Ascaris* eggs for seeding and compared three different methods for recovering *Ascaris* from sludge. He has reported that *Ascaris* were recovered from seeded radishes and lettuce for 6 days and from the soil for 21 days after seeding. He noted, however, that survival times vary widely and are dependent on environmental conditions.

Although it is difficult to prove that individual cases of parasitic infection are caused by food contaminated with recycled refuse, *Ascaris* and other parasitic animals do withstand sewage treatment and thus have the potential for remaining viable and infective in soils and crops. Variable weather, agricultural conditions and the limits of recovery methods hinder predicting how long and where these organisms can be detected in the field and marketplace.

Heavy Metals

Because variable quantities of nearly any metallic element may eventually find their way into municipal sewage and sludges and thus be applied to crops by land application of wastes, the effects of such elements on the food chain must be

considered. Herbert R. Pahren has addressed this issue in his study of the effects of metals on the human food chain.[4]

The following results are cited from Pahren:

Copper, molybdenum and selenium are known to have caused sporadic poisoning of livestock. In general, however, Pahren noted that the risk to humans from these three elements is very small by way of the human food chain. Nickel, frequently found in sludges in substantial amounts, may cause phytotoxicity to plants growing in acid soils. Zinc also is found in large quantities in many sludges and plants.

Neither nickel nor zinc, however, presents a realistic hazard to humans because both are readily excreted, are of low toxicity, and are not accumulated.

Most municipal sludges contain mercury in low concentrations, but generally it is bound by clay or organic matter in an unavailable form and would not be a risk to human health.

The uptake of lead by grazing animals on land fertilized with sludges be a problem.

Manganese, iron and aluminum are not hazardous at normal soil acidities because of their low solubility.

Hexavalent chromium is seldom present in sludges, since it is reduced to the trivalent state during digestion. Trivalent chromium is not accumulated by plants and is of low toxicity.

Arsenic is present at low levels in some sludges but tends to be excluded from aerial plant tissues. It is also bound strongly by clay fractions of the soil.

In contrast, cadmium is present in significant amounts in municipal sludges, is absorbed appreciably, translocated and accumulated by many food crops (particularly by leafy green vegetables), and accumulates in human kidneys where it can cause severe damage. Food is the primary source of cadmium for the general population, although smoking is a contributor.

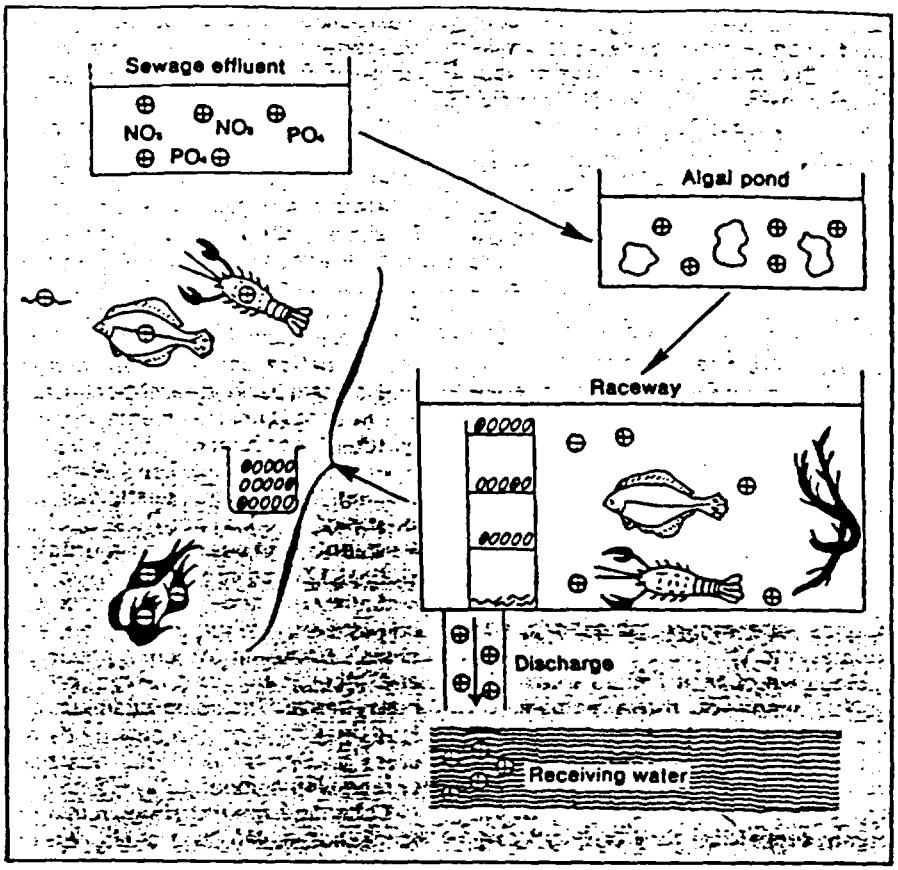

Figure 1. Virus Survival and Transmission Potential within Mariculture System.

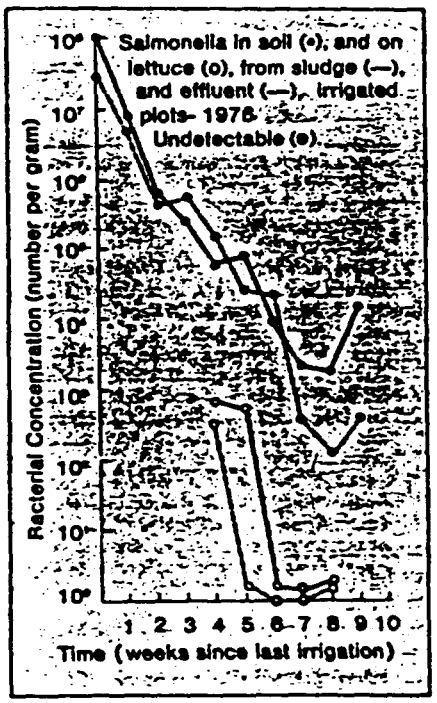

Figure 2. Concentrations of *S. typhimurium* in Soil.

Some crops, especially green vegetables, accumulate cadmium. Figure 3 shows the uptake of cadmium by Swiss chard from acid and neutral soil containing various levels.

Based on these plant uptake data, estimates of acceptable cadmium content of vegetables, and calculations of the amount of sludge re-

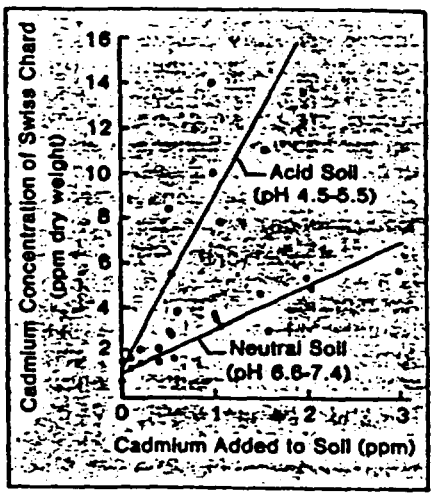

Figure 3. Uptake of Cadmium by Swiss Chard from Acid and Neutral Soil.

Table 1
Maximum Acceptable Daily Intakes[4]

Organic	Limit (mg/kg body wt.)
Aldrin	0.0001
Dieldrin	0.0001
Endrin	0.0002
Chlordane	0.001
DDT	0.005
Heptachlor	0.0005
Lindane	0.0125
2,4-D	0.3

quired to provide nitrogen for corn production. Pahren outlined a method of calculating a safe level of cadmium content in sludge. The conclusion reached was that

> if the cadmium content is less than 60 ppm, it can be applied at maximum rates of nitrogen and produce an acceptable cadmium content in vegetables. At these same nitrogen loading rates, acid soils would require sludges with less than 12 ppm.

Organic Chemicals

A large number of organic compounds can be found in waters, including drinking water.* Many of these organics have been identified in raw and chlorinated domestic sewage. Jack Dacre has divided organic chemicals into four classes: organochlorine insecticides, chlorinated phenolics, polyaromatic-ring compounds, and others (phthalates, sulfonates, etc.).[5]

Because of their limited water solubility, many of these compounds are found in both wastewaters and sludges. The organochlorine compounds are especially important, Dacre has observed because of two inherent properties: they are highly resistant to degradation in the environment and fat-soluble. They bioaccumulate and translocate to animals and man via the food chain. Dacre also has pointed out that many of the chlorinated organic compounds found in wastewater and sludges are known or suspected animal carcinogens. Some are also teratogens. None have been shown to be human carcinogens except the benzopyrenes, which may be categorized as suspected human carcinogens.

According to Dacre, because of the potential danger to human health from consumption of these chemicals, the World Health Organization has estimated acceptable daily intake values for many of them. Similarly, soil pollutant limitation values are being developed for some insecticides (Table 1). Although these values were derived for soil, they can be used to control the level of toxic chemicals in sludges before application to land.

Summary

The application of sludge and wastewater on agricultural lands and crops, although desirable in many ways, may present hazards to human health.

It has been shown that these waste materials may contain viruses, pathogenic bacteria, and viable forms of such parasitic worms as *Ascaris*. Evidence exists that these pathogens may survive land application of wastes and thus enter the food chain.

Among the heavy metals present in sludge, cadmium is of most concern. It accumulates readily in plants and foods and when chronically ingested will accumulate in body tissues, especially in the kidneys. Studies to better define safety margins and determine long-term health risks are needed. Other elements, generally, are of less concern.

Persistent organic chemicals, including chlorinated pesticides and polychlorinated biphenyls (PCBs) are frequently present in municipal sludges. These are highly toxic, potentially carcinogenic materials that can accumulate in the human food chain. Both the organic pesticides and chemicals, and some of the heavy metals including cadmium and lead, may be ingested directly by animals grazing on land that has been spread with sludge.

Collective results show that contamination of the food chain can occur unless measures are taken to establish tolerable levels of pathogens, heavy metals, and organic materials.■

*A summary listing of some 1,000 organic compounds in effluent waters has been published in a technical report of the World Health Organization International Reference Centre for Community Water Supply.

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State Capital News

(continued from page 16)

Hawaii

Delegates to the Hawaii Constitutional Convention, by an overwhelming vote, gave preliminary approval to the state's "obligation to protect, control and regulate the use of Hawaii's water resources for the benefit of all the people." It also called for the state legislature to set up a statewide agency to "set overall water conservation, quality and use policies."

Supporters of the proposal cited numerous water problems in Hawaii, as well as the lack of overall coordination in water planning and policies.

Maine

A near record $62 million in federal subsidy money is slated to be available to Maine cities and towns for the construction and planning of municipal sewage-treatment plants during the fiscal year that started Oct. 1. It is expected the money will generate water cleanup projects totaling about $100 million. The funds will provide enough money to complete the construction of facilities for all the major sources of untreated pollution in the Kennebec River. Major construction grants are also slated for Cumberland, Brunswick, Kennebunkport, Bath, Saco and Limerick. The amount includes $33.7 million expected to be appropriated by Congress this fall and $28 million made available last year but not spent.■

Water & Sewage Works

A SCRANTON GILLETTE PUBLICATION
DECEMBER, 1978

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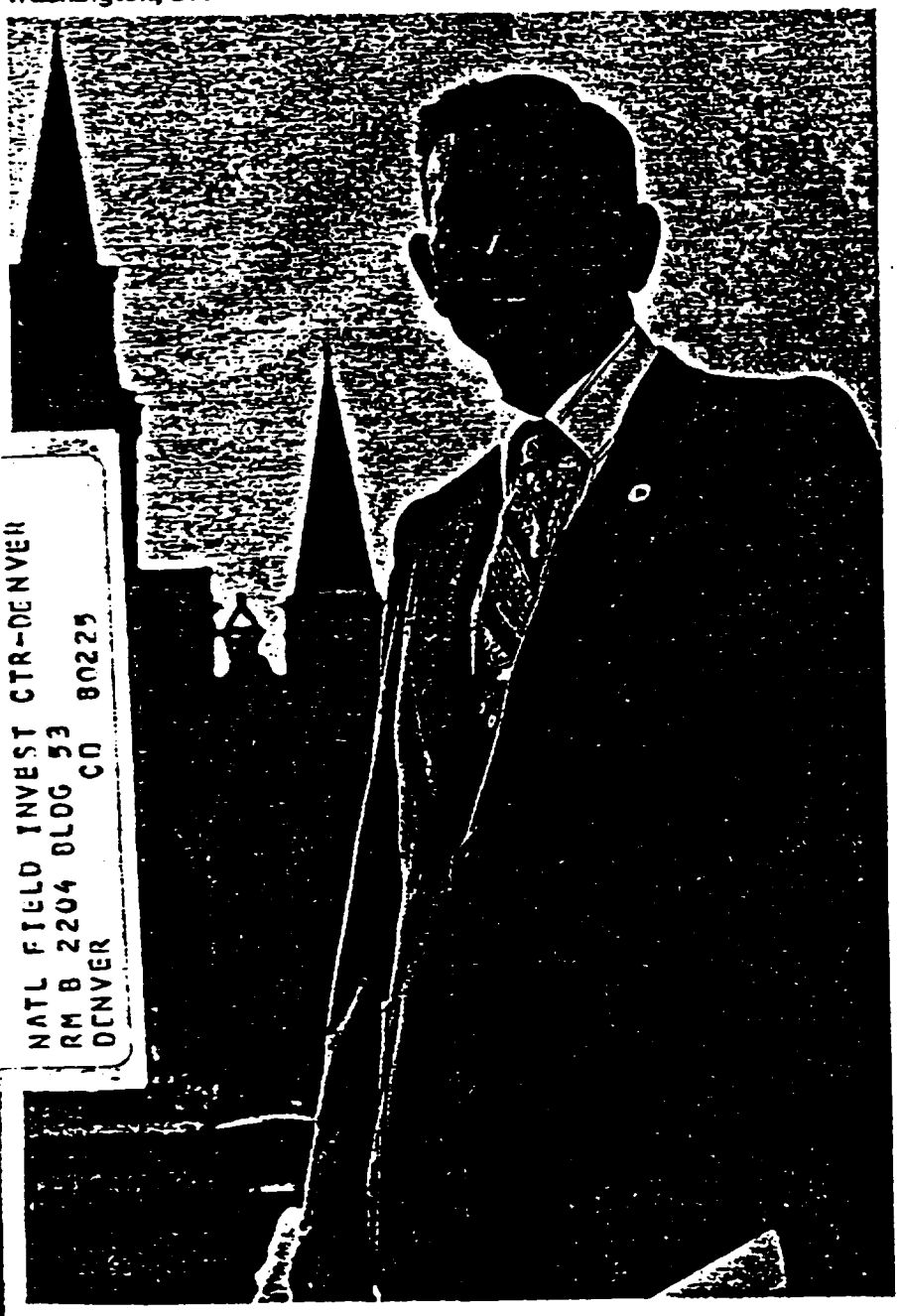
NUCA: Finish Plants First

by Ken Goodwin, President
National Utility Contractors Association Inc.
Washington, D.C.



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The line must be drawn. Somewhere we've got to turn away from seemingly easy money—the federal handout. That is why the board of the National Utility Contractors Association says, "No," to H.R. 13624, a proposed bill that would provide grants for operation and maintenance of waste-treatment works. It advocates its position not in spite of the facts but because of them.

The town of Greenville, Maine, with a population of about 2,000 people, built a $4.3 million AWT facility and faces a $125,000 annual O&M cost—about $200 per family.

Pensacola, Florida, with a population of 60,000, has annual operating costs of $6 million for its AWT facility.

As EPA's massive Construction Grants Program continues to pick up momentum and more and more communities begin to satisfy the requirements of the act, concern is mounting over the capabilities of communities to meet continuing operation and maintenance costs.

EPA estimates that if categories I-IV (secondary treatment, more stringent treatment, infiltration inflow, replacement and/or rehabilitation and new collectors and interceptors) are funded per the 1976 needs survey, annual operation and maintenance costs nationwide by 1990 will be $4.39 billion.

If EPA persists in continuing to promote widely criticized "gold-plated" advanced waste-treatment plants that need more tech-

(continued on page 5)

secondary sedimentation tanks, and at the gravity thickeners.

The use of women in this previously held male stronghold has not been completely without problems for plant management, but can be handled.

Not all the male opposition to the introduction of females into the plant work force has been overcome. The experience in Detroit, to date, with women on the plant operations and maintenance staffs, is that this is a change for the better.

SEWAGE MANAGEMENT

The Great Lakes Cleanup

by Kenneth H. Walker
Great Lakes Regional Office
International Joint Commission
Windsor, Ontario

General and specific water quality objectives to ensure that pollution of the boundary waters does not occur were set out in the 1972 Great Lakes Water Quality Agreement. The goals are to be maintained or achieved in the boundary waters, through effective pollution control programs in both Canada and the U.S. Based on the concept of protection of the most sensitive beneficial uses of the waters, they are intended to be the minimum basis for formulating provincial and state water quality standards, and programs for achieving the levels of water quality necessary for designated uses.

Phosphorus Controlled

Reduction of phosphorus inputs to control eutrophication has been identified as a critical issue for the Great Lakes. One control strategy has been to reduce the phosphorus concentration to 1.0 mg/l or less in the effluent from municipal wastewater treatment plants discharging in excess of 3.8 x $10^3 m^3/d$ (1.0 MGD). Unfortunately, all of the treatment plants are not yet meeting this goal.

A recent Research Advisory Board study to evaluate various strategies for phosphorus control revealed that, of 44 major municipal wastewater treatment plants investigated, only 9 were achieving annual average phosphorus concentrations of 1.0 mg/l or less in their effluents. The indication was that this might be due to either inadequate facilities or problems with the operation of municipal treatment plants.

A preliminary assessment of the study results indicates that phosphorus levels of 0.5 mg/l in wastewater treatment plant discharges are technically and economically feasible. Phosphorus loadings to Lake Erie and Lake Ontario decreased from 1975 to 1976, but are still higher than the target loadings called for in the Agreement.

The importance of phosphorus contributions to the lakes from the atmosphere, sediments and land drainage and other nonpoint sources has been identified. For the most part, specific control programs have yet to be developed.

Because of the concern about the impact of phosphorus on the lakes, the use of non-phosphorus compounds in detergents is of interest. The Research Advisory Board reported to the Commission this year on its evaluation of the health implications and ecological effects of sodium nitrilotriacetate (NTA), which is widely used in Canada as a replacement for phosphorus in laundry detergent formulations. NTA is not presently used in the United States in detergent formulations, due to concerns expressed by the Surgeon General of the United States Public Health Service in 1970.

Surveillance Programs

Detailed surveillance plans are currently being developed for Lakes Erie and Ontario. Additional plans will be developed for the other lakes as soon as possible. When implemented, these coordinated plans will provide the data for determining water quality conditions and trends, and assessing compliance with objectives in the Agreement.

Toxic Substances

The occurrence of persistent toxic substances in the Great Lakes has been a matter of growing concern in recent years.

PCBs continue to be a problem in most Great Lakes fish. Mirex is present in some Lake Ontario fish but has not been found in the fish of other Great Lakes. DDT and mercury levels continue to decline. Concerns for human health have resulted in the closure of some commercial and sport fisheries in portions of the Great Lakes.

Some jurisdictions have issued warnings that certain fishes from some areas should be eaten only once a week, or should not be eaten at all. The Environmental Contaminants Act of Canada and the Toxic Substances Control Act of the United States have been passed and are designed to provide protection against environmental contaminants. Their prompt, thorough implementation and stringent enforcement are imperative.

The Research Advisory Board has embarked on a project designed to provide an "early warning system" for environmental contaminants. Early identification of chemicals, not previously recognized as problems within the Great Lakes Basin, would enable jurisdictions to take preventive measures before a "Crisis of the Month" emerged. The Board is developing, on a pilot scale, an inventory of chemicals used, manufactured, or discharged within the Basin.

By using existing knowledge on the structural and physical characteristics of these chemicals, the Board expects to be able to predict their bioaccumulation potential, and possibly toxic effects.

The Board also expressed concern about the lack of knowledge of organic chemical residues accumulated in the tissue of persons residing in the Great Lakes Basin and is beginning a survey to identify these contaminants.

Ecosystem Quality

In its presentation to the IJC, the Research Advisory Board expressed concern over the limited scope of present planning and management activities for the Great Lakes. Traditionally, such activities have been based on compliance with objectives for chemical and physical water quality parameters. The Board urged that the lakes be evaluated on a much broader concept of "ecosystem quality," which considers the diverse interactions which occur within the lakes' chemical, physical, biological and societal components.

To aid in the understanding of the ecosystem and subsequently in the formulation of sound resources management decisions, the Board endorsed the development of environmental maps. It suggested that a pilot study of environmental mapping be carried out in a subarea of the Great Lakes to determine the cost/benefits, the potentials and the liabilities of such an effort, as a basis for future mapping.

Agreement Being Reviewed

This past April marked the end of the fifth year of the Agreement, and the Governments are now busily involved in the review process. Review teams in both the United States and Canada are consolidating opinions from many sources into an official position. Once this is completed, negotiating teams will start work on any necessary revisions to the Agreement. It appears that these may be rather extensive, but it is premature to attempt to speculate on just what changes will be made.

The determined efforts of the United States and Canada to restore and enhance the water quality of the Great Lakes are reflected in the unique international approach called for in the provisions of the 1972 Great Lakes Water Quality Agreement.

The final judgment on the effectiveness of this program of binational cooperation and effort to clean up the Great Lakes may not be made for years. The stakes are so high that we must ensure successful attainment of the goals established to save the multiple resources of the Great Lakes for future generations. The Great Lakes Water Quality Agreement provides a workable mechanism for reaching these goals. ■

WATER & SEWAGE WORKS

VOLUME 124 NO. 11 NOVEMBER, 1977

EDITORIAL

(from cover)

mittee on the Environment and Public Works at the request of the Committee chairman, Senator Randolph. Dr. Edwin Gee, a presidential appointee to the Commission who is also a senior vice president of the Dupont Company, had appeared before the Subcommittee on Environmental Pollution, primarily to discuss the question of toxics control. Lt. General Frederick J. Clarke and I were invited to testify on July 14, 1977, during the mark-up sessions and just one week before a bill was reported.

The House Committee on Public Works sought testimony from Vice President Rockefeller without success, but did hear from Dr. Gee and Mr. Raymond Kudukis, director, Department of Public Utilities, Cleveland, Ohio, as representatives of the Commission. I also testified at a different time.

Of the five senators and seven representatives who served on the Commission, eight are still in Congress, and seven have been actively involved in formulating amendments to PL 92-500—Senators Baker, Benson, Muskie and Randolph, and Representatives Cleveland, Harsha, and Johnson. In addition, several former NCWQ staffers now serve on Senate or House committee staffs or those of individual members.

Thus, the influence of the NCWQ may be ubiquitous and not easily measured. Even Senator Muskie, who strenuously objected to some of the Commission recommendations, has consistently endorsed the work of the staff. Language in the Senate Committee Report which suggests that it "reviewed the work of the Commission and heeded its advice in this bill" (S. 1952) may be more a tribute to the persistence of Senator Randolph than a statement of the Committee's commitment.

The following discussion compares the provisions of H.R. 3199 and S. 1952. The two are not exactly comparable, since the Senate bill contains amendments to some sections not amended by the House version. To provide testimony on matters it had not addressed earlier and proposals from the Senate as the basis for Conference Committee meetings, the House Committee on Public Works and Transportation held hearings in September, 1977.

The 1983 deadline—for the Commission's recommendations, the one which fulfilled the specific statuatory charge in Section 315 of PL 92-500 and is, in my view, the most significant, was stated as follows:

"Maintain the 1983 interim water quali-

(continued on page 76)

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United States
Environmental Protection
Agency

December 1979

Toxic Substances

EPA

Polychlorinated Biphenyls:

An Alert for Food and Feed Facilities

United States
Environmental Protection
Agency
Washington D C 20460

TS-799

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...nalty for Private Use $300

Polychlorinated Biphenyls:

An Alert for Food and Feed Facilities

This booklet has been publish[illegible]y the Environmental Protection Agency in cooperation with the Department

Important Notice: PCB Incinerators

At the time this booklet went to press, there were still no approved PCB incinerators available for commercial use in the United States. *Until incinerators are available, it is not advisable to remove PCB fluids or intact PCB-containing equipment unless safe storage facilities are available off the site.*

Several food contamination incidents have been caused by PCBs in storage. These incidents indicate that removing PCB equipment or draining fluid from a PCB transformer and storing the equipment or fluid may result in an increased risk of contamination. Therefore, instead of removing this equipment from service at this time, carefully examine the risk of leaks or spillage and monitor continued operations closely. (See suggestions in paragraph C of checklist.) If there is potential for leaks or spills, corrective measures must be taken, including removal of the equipment and fluid to a safe storage area away from the premises. If you have any questions, consult with your regional EPA or FDA office or local USDA inspector.

At the present time, we anticipate that the first incinerator will be available in spring 1980. EPA will publicize information on commercial PCB incinerators as soon as they are approved. In addition, you may check on the availability of incinerators by either calling EPA's Office of Industry Assistance toll-free (800) 424-9065 or calling your regional EPA or FDA contact or local USDA inspector. (See list of names and numbers in the booklet.)

December 1979

FOREWORD

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

December 1979

The food and feed industries are particularly vulnerable to contamination from polychlorinated biphenyls (PCB). PCBs are ubiquitous, persistent industrial chemicals that are known to cause serious health and environmental effects. The Environmental Protection Agency has responsibility for regulatory control of PCBs and other toxic substances under the Toxic Substances Control Act of 1976. We are working cooperatively with the Food and Drug Administration, which regulates chemical substances in food and their use in food and animal feed plants, and the Department of Agriculture, which has responsibility for assuring the safety of meat and poultry, to protect our food supply from PCB contamination.

This booklet has been prepared to:

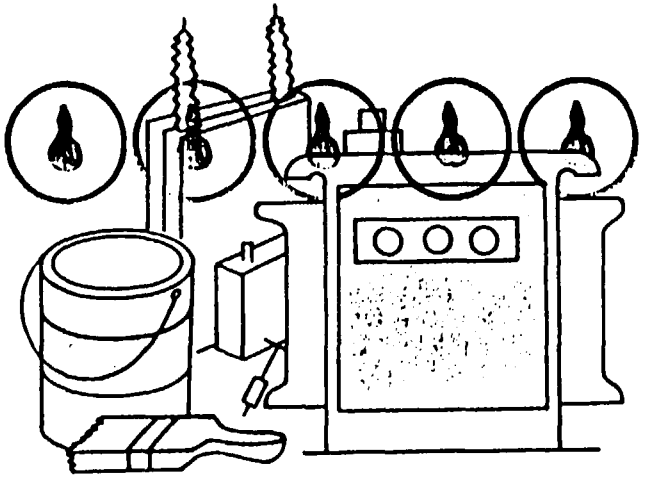
- Alert you to the serious potential problems associated with the use of PCB-containing equipment in the food and feed industry,
- Provide information that will help you establish a program for contamination prevention in these facilities, and
- Provide information on current PCB government regulations and a reference of sources of additional information.

PCB contamination has already caused costly destruction of large quantities of food and uncountable damage to our environment. We urge you to alert your managers and employees to the problem of PCB contamination and to institute a program for preventative action without delay. This brochure will assist you in your efforts.

Steven D. Jellinek

Why the concern?

In recent years, there have been a number of incidents where food has been contaminated with polychlorinated biphenyls (PCBs) and other toxic chemicals. This contamination has resulted from accidental spills, improper disposal, and unintentional misuse of PCB-containing materials and equipment. PCBs are now known to have extremely hazardous health and environmental effects, even at low levels of concentration in the food chain. PCB-containing equipment, such as electrical transformers, has been extensively used in food and feed processing facilities. We are especially concerned that future accidental contamination be prevented. This booklet has been prepared to help you identify potential problems and prevent costly future incidents that could have adverse effects on health and the environment.



What are PCBs — why, how, and where are they used?

PCBs are a class of chemicals called chlorinated hydrocarbons. PCBs range in consistency from heavy oily liquids to waxy solids. Their most important properties are chemical stability, low flammability, high boiling points, and low electrical conductivity.

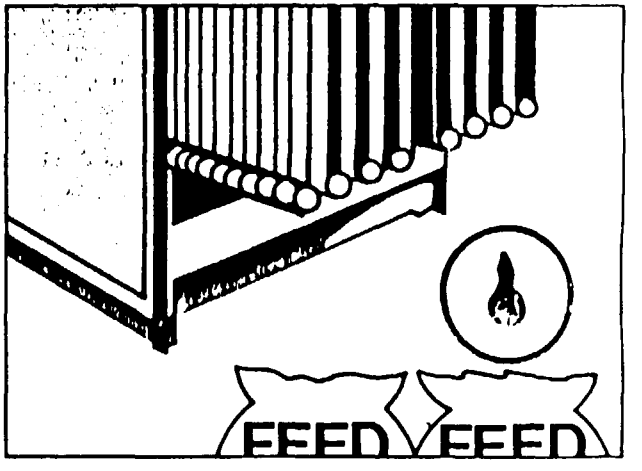
Since their development in 1929, PCBs have been used in a variety of industrial applications. Hundreds of millions of pounds of PCBs hav en used as fluids in electrical transformers, capacitors, and electromagnets,

PCBs have also been used as plasticizers in paints, adhesives, and caulking compounds; fillers for investment casting waxes; and dye carriers in carbonless copy paper.

Concern over PCBs' toxicity and persistence in the environment led Congress to prohibit their manufacture, processing, or distribution in commerce. Although PCBs have not been manufactured in the United States since 1977, the chemical has been permitted to remain in older electrical transformers and other industrial machinery.

Why are PCBs hazardous?

Knowledge of PCB toxicity in humans is based primarily on an incident which occurred in Yusho, Japan in 1968 when PCBs leaked from a heat exchanger and contaminated rice oil. Among the many symptoms observed were chloracne (skin rash), discoloration of the gums and nailbeds, swelling of joints, waxy secretions of glands in the eye lids, as well as more general effects such as lethargy and joint pain. There are also well documented tests on laboratory animals that show PCBs cause reproductive failures, gastric disorders, skin lesions, tumors, and other effects of concern.

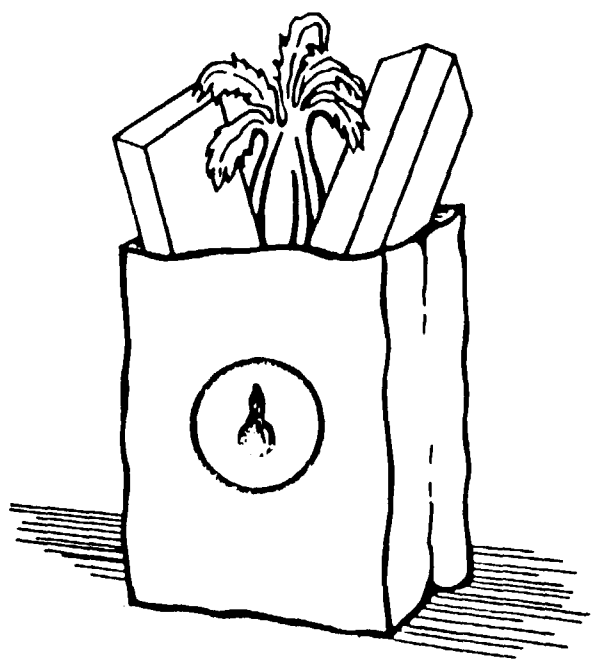


Why should food and feed related industries be especially concerned about PCBs?

The Yusho incident illustrates the serious human health and economic consequences of PCB contamination in food or feed related industries. Several other incidents underline the need for special attention to prevent similar costly contamination in the future. These include:

- Discovery of PCBs in fishmeal used as a feed ingredient in North Carolina as a result of a leaking heat exchanger,
- Contamination of fishmeal in Puerto Rico resulting from a fire involving stored electrical transformers in the same warehouse,
- Death and severe illness of feedlot cattle in Kansas caused by inadvertent use of PCB-contaminated oil as a carrier for insecticide,
- Contamination of animal feed ingredients with PCBs leaking from a transformer in storage at a packing company in Billings, Montana.

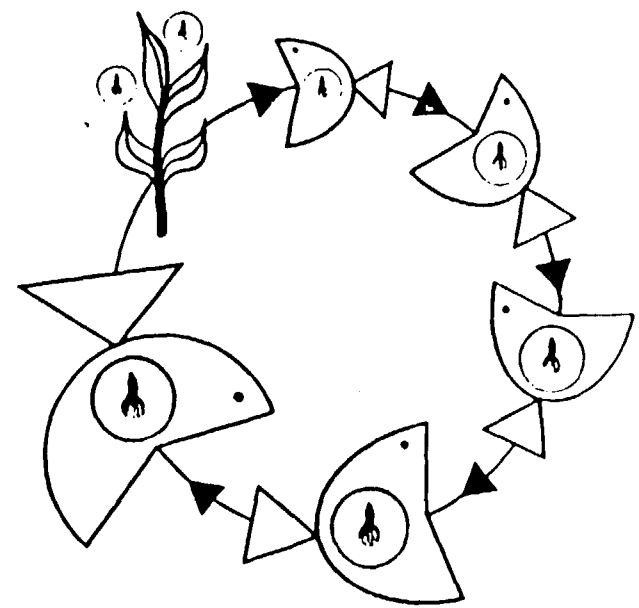
These incidents have resulted in the destruction of large quantities of food, feed, and farm animals, and major disruption of the companies involved.



PCBs in the environment

Unlike most organic chemicals which break down fairly rapidly in the environment, PCBs are extremely stable. The potential for biomagnification in the food chain is of special concern. Biomagnification is the process through which small amounts of toxic substances reach higher levels of concentration at each stage of the food chain.

Microorganisms and plants take in traces of PCBs from the environment. These microorganisms and plants provide the food for small fish and other animals which are in turn eaten by higher forms of animal life. At each step of the sequence, the PCB content increases until human food supplies may be contaminated above the levels considered to be safe for consumption.

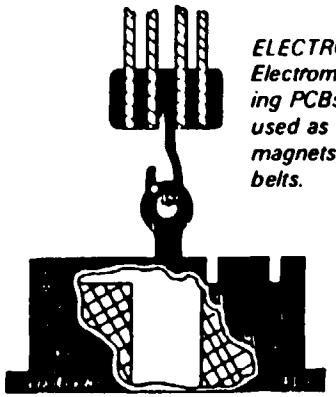


Where might PCBs be found in food industry facilities?

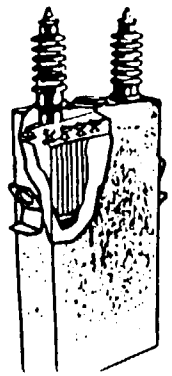
The following types of equipment may contain PCB fluids or other liquids such as mineral oil that are contaminated with PCBs:

- ***Transformers***
- ***Large capacitors (over 3 lbs. of fluid)***
- ***Electromagnets***
- ***Heat transfer and hydraulic systems***
- Small capacitors
- Equipment containing small capacitors including: electrical switch gear, fractional HP motors, and ballasted lighting fixtures.

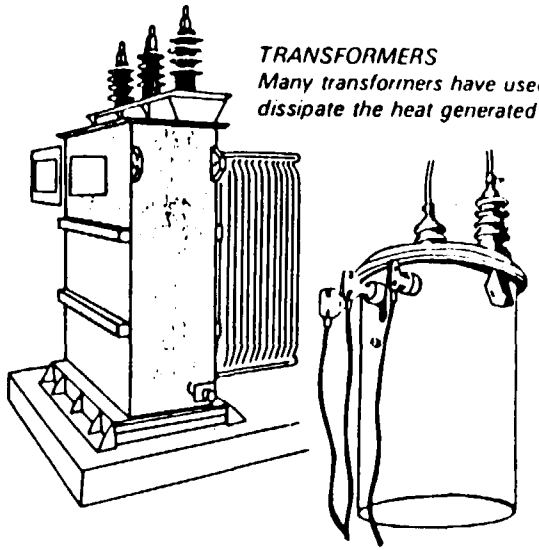
Transformers, large capacitors, electromagnets, and heat transfer and hydraulic systems are of primary importance because they contain free flowing liquid. Small capacitors are of less concern because the PCBs are usually in a nonliquid, nonmobile state.



ELECTROMAGNETS
Electromagnets containing PCBs have been used as separator magnets over conveyer belts.



CAPACITORS
Capacitors have been manufactured in a wide range of sizes for various industrial uses. Capacitors may contain PCB dielectric fluids.



TRANSFORMERS
Many transformers have used PCB liquids to dissipate the heat generated by transmission

HEAT TRANSFER SYSTEM
Heat transfer systems containing PCBs have been used in food processing equipment.

Existing federal regulations concerning PCBs and food

Concern about highly toxic PCBs contaminating human food has led to a number of regulatory actions by the Food and Drug Administration (FDA), the U.S. Department of Agriculture (USDA), and the Environmental Protection Agency (EPA). Under FDA regulations foods and feeds are considered to be contaminated and may not be sold in interstate commerce if they contain PCBs in excess of certain prescribed concentrations. (See Table 1.) The use of PCBs in food and animal feed processing facilities, except enclosed use in transformers and capacitors, is prohibited by FDA regulation (21 CFR 110.40, 500.45).

EPA has issued rules governing the use of PCBs. In general, PCB mixtures with concentrations less than 50 parts per million (ppm) or 0.005 percent are not regulated. The use of intact, non-leaking transformers, electromagnets, capacitors, and capacitor-containing equipment with PCB concentrations greater than 50

The EPA rule permits the continued use of PCBs in servicing of electromagnets and transformers and in heat transfer and hydraulic systems until July 1, 1984. As of November 1, 1979, PCBs may no longer be used in heat transfer systems in plants manufacturing or processing food, drugs, and cosmetics.

EPA rules include provisions for marking, disposal, storage, and recordkeeping, portions of which are discussed later in this booklet.

The potential for further regulatory action exists. EPA, FDA, and USDA are considering prohibiting the use of PCBs in all transformers, capacitors, and other electrical equipment in food and feed related industries.

The following Checklist for Action has been prepared to assist you in conducting a full safety check of your facility. We urge you to use this checklist to implement a program for safety and contamination prevention in all your food and feed related facilities.

Checklist for Action
What can I do?

A. Determine if you have PCB equipment.

1. Locate any potential PCB source.

You should inventory your plant for transformers, capacitors, heat transfer systems, hydraulic systems, and fluid stored for use in this equipment. After you have located these items, . . .

2. Examine the equipment for caution labels

A caution label specifically identifying the equipment as containing PCBs may be present. If so, skip to Section B of this checklist. If not, . . .

3. Transformers and large capacitors may bear nameplates.

If your transformer or capacitor has a manufacturer's nameplate with a trade name, refer to Table 2 at the end of this booklet. If the trade name does not appear there, the equipment may still contain PCBs. Contact the manufacturer, referencing the serial number to determine if the equipment contains PCBs. If there is no nameplate, or if this approach is unsuccessful . . .

4. Seek assistance from a servicing expert.

Call on your local transformer servicing company or the engineering service department of your utility company. They can assist you to obtain a sample of your transformer fluid for analysis. You may also want to sample your heat transfer systems, hydraulic systems, or other suspect oil. (Do not attempt to sample fluids in capacitors.) Then . . .

5. Have the samples analyzed.

This is the only way to be certain whether or not your equipment contains PCBs.

If you determine that you have PCB equipment, . . .

B. Consider replacing PCB-containing equipment with non-PCB equipment.

EPA, FDA, and USDA are considering prohibiting the use of PCBs in all electrical equipment in food and feed related industries. Although the prohibition on PCB-containing equipment is not yet in effect, *we urge voluntary removal of this equipment and replacement with non-PCB equipment to prevent the possibility of future food contamination.*

As an alternative in transformers, you may want to consider replacing the PCB fluid with non-PCB fluid. You should be aware that the transformers will contain residual PCB concentrations after replacement and, therefore, may be subject to future federal regulation.

EPA disposal regulations require incineration of all PCB liquids with concentrations of 500 ppm (0.05%) or greater. The first approved incinerators will be available sometime in 1980. Until incinerators are available, PCBs removed from service must be stored for later disposal.

If it is not possible for you to replace your PCB-containing equipment, you will want to . . .

C. Take special precautions for continued use.

The risk of PCB contamination can be minimized by analyzing the risk of spillage associated with each individual PCB unit and by taking steps to contain the potential uncontrolled loss of PCBs from the unit. You should consider the following questions:

1. Is there any evidence of an active leak from the PCB unit? If so, take whatever steps are necessary to protect your product or usable byproduct. Of course, leaking equipment should be repaired immediately. If your product is being contaminated, contact the appropriate agency to determine the best way to destroy it.

2. Is the PCB unit located in an area where there is a way that leaking fluid from the unit could find its way into your process? If so, these units should be given priority in the development of spill prevention measures. Special consideration should be given to potential leaks resulting from accidents as discussed in the following.

3. Is any liquid ever pumped from the vicinity of the PCB unit to the process area? If so, can the PCB unit be isolated from the pump?

4. If the PCB unit is pad mounted, are there curbs or pans to serve as a barricade against loss of fluid?

5. If the PCB unit is located in an area surrounded by porous cinderblock walls, have they been sealed with material which will not dissolve in solvents such as PCB and trichlorobenzene? Is the wall/floor interface tight?

6. Is the floor in the vicinity of a PCB unit free from cracks? Cracks that look like they would not prevent the loss of fluid should be grouted and painted with solvent resistant materials, or government approved materials if required.

7. Is the PCB unit in a location where vehicular traffic might be a hazard? If so, a vehicle barrier might be useful.

8. Is the PCB unit located near machinery which might throw projectiles with sufficient energy to damage the unit? If so, a shield, such as a fence, may provide the necessary protection.

9. Is the PCB unit mounted on a surface which might be difficult to seal, such as unwelded steel plates or in a mobile machine? If so, a catchbasin might be installed under the unit.

10. Are there floor drains in the vicinity of a PCB unit? If so, can they be sealed or otherwise isolated from the unit?

11. If you have PCB equipment or drums of PCB fluid in storage for future use, can they be removed from the process area? Are they marked or labeled to warn of PCB presence? If you have PCB equipment, you may be subject to mandatory labeling and storage requirements.

D. Be familiar with mandatory labeling and storage requirements.

1. Labeling requirements.

The EPA label for PCBs looks like this.

You must label the following items: transformers containing liquids with more than 500 ppm of PCBs, PCB capacitors with 3 (three) or more pounds of fluid operating at 2,000 volts or more, containers such as bags, barrels, or drums used to hold PCBs, electric motors using coolant with 50 ppm or more of PCBs, hydraulic systems or heat transfer systems containing 50 ppm or more of PCBs, and the storage areas described in the following.

In addition, labels must be placed on the following items when they are removed from service: PCB capacitors containing more than 3 pounds of fluid operating at less than 2,000 volts and equipment containing a PCB transformer or large high voltage capacitor.

2. Storage requirements.

PCBs and PCB items which you remove from service for disposal must be stored in a location meeting EPA criteria to insure that they are properly contained. This location should not be near your process area. It should be isolated so that it is not vulnerable to damage and puncture from vehicular, forklift, and other moving equipment. For assistance with the storage design criteria, contact the EPA regional office for your state. A list of EPA regional offices appears at the back of this booklet. (See Appendix B.)

E. Obtain further information.

If you do not yet have the EPA regulations on PCBs, you can obtain a free copy from EPA by calling toll free (800) 424-9065 (in Washington, DC, 554-1404) and asking for a reprint of the PCB Ban Regulation, *Federal Register,* May 31, 1979, and for a list of the approved PCB Disposal Facilities.

If you want more background information on the regulation, ask for a free copy of the EPA Support Document to the PCB Ban Regulation at the same phone number. The mailing address is: Industry Assistance Office (TS-799), Office of Toxic Substances, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460. You may also contact the nearest EPA Regional Office. (See Appendix B.)

Other useful background information on PCBs is listed in Appendix A.

Table 1.

FDA Limits on PCB Concentration in Food

Food	Concentration (parts per million)
Milk and manufactured dairy products	1.5 ppm (fat basis)
Poultry	3 ppm (fat basis)
Red meat	3 ppm (action level) (fat basis)
Eggs	0.3 ppm
Fish and shellfish	5 ppm (edible portion)*
Feed for food producing animals	0.2 ppm (except concentrates, supplements, and premixes)
Infant and junior foods	0.2 ppm
Animal feed components of animal origin	2.0 ppm
Paper food packaging in direct contact with food	10 ppm (action level)

Source: Title 21, Code of Federal Regulations, Section 109.30. Federal Register, Vol. 44, No. 127, June 29, 1979, pp. 38330-38340.

**A regulation establishing a new level of 2 ppm was promulgated but was stayed on October 5, 1979 until further notice.*

Table 2.
PCB Manufacturers and Trade Names

PCBs have been marketed and used by manufacturers in their products under various trade names. This list is not necessarily complete since many companies have gone out of business and information is no longer available.

Manufacturer	Country	Trade Name
Aerovox	US	Hyvol
Allis-Chalmers	US	Chlorextol
American Corp.	US	Asbestol
Axel Electronics	–	
Bayer	Germany	Clophen
Caffaro	Italy	Dk
Caffaro	Italy	Fenclor
Caffaro	Italy	Inclor
Capacitor Specialists	US	
Chemko	Czechoslovakia	
Cornell Dubilier	US	Dykanol
Dings Co.	US	
Electrical Utilities Corp.	US	Eucarel
Electro Engineering Works	US	
Electromagnetic Filter Co.	US	
Envirotech Buell	US	
Eriez Magnets	US	Pyranol
ESCO Mfg. Co.	US	Askarel*
Ferranti-Packard Ltd.	US	Askarel*
General Electric	US	Pyranol
Geneva Industries	US	
H. K. Porter	US	
Helena Corp.	US	
Hevi-Duty Electric	–	Askarel*
ITE Circuit Breaker	US	Non-Flammable Liquid
Jard Corp.	US	Clorphen
Kanegafuchi	Japan	Kennechlor
Kuhlman Electric	US	Saf-T-Kuhl
McGraw Edison	US	Elemex
Maloney Electric	US	
Monsanto	UK and US	Aroclor
Monsanto	UK and Japan	Santotherm FR
Monsanto	UK and Europe	Pyroclor
Monsanto	US	Therminol**
Monsanto	US	Pydraul***
Monsanto	US	Santovac 1 and 2
Niagara Transformer Corp.	US	Askarel*, EEC-18
P. R. Mallory & Co.	US	Aroclor B
Power Zone Transformer	US	EEC-18
Prodelec	France	Phenoclor
Prodelec	France	Phyralene
R. C. Uptegraff	US	
R. F. Interonics	US	
Reliance Electric Co.	US	
Research-Cottrell	US	Askarel*
Sangamo Electric	US	Diaclor
Sovol	USSR	
Sprague Electric Co.	US	Chlorinol
Standard Transformer Corp.	US	
Stens Magnetics	US	
Tobe Deutschmann Labs.	US	
Universal Mfg. Corp.	US	Askarel*
Van Tran Electric	US	
Wagner Electric	US	No-Flamol
Westinghouse Electric	US	Inerteen
York Electronics	US	
–	US	Nepolin
–	–	Apirolio
–	–	Kaneclor

**Askarel is the generic name used for nonflammable insulating liquid in transformers and capacitors.*

***Various products used as heat transfer fluids, such as Therminol FR-0, were manufactured under this name.*

****Various products used as hydraulic fluid, such as Pydraul A-200, were manufactured under this name.*

Appendix A

Other Available Information on PCBs

This booklet does not go into detail about the health and environmental effects of PCBs or the chemicals now being used as substitutes for PCBs. The following reports cover these technical areas. The NIOSH Criteria Document is particularly recommended for its discussion of health issues for servicing PCB equipment or cleaning up PCB spills.

"Criteria for a Recommended Standard . . . Occupational Exposure to Polychlorinated Biphenyls (PCBs)." DHEW (NIOSH) Publication No. 77-225, September 1977. Order from Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. Price $4.00 plus postage.

"Assessment of the Use of Selected Replacement Fluids for PCBs in Electrical Equipment." Report No. EPA 560/6-77-008, March 1979. Order from National Technical Information Service, Springfield, Virginia 22161. Report No. NTIS-PB-296 377.

"Polychlorinated Biphenyls and the Environment." 1972. Order from National Technical Information Service, Springfield, Virginia 22161. Report No. NTIS COM-72-10419. This is a thorough discussion of the properties and uses of PCBs.

"PCBs in the United States: Industrial Use and Environment Distribution." 1976. Order from National Technical Information Service, Springfield, Virginia 22161. Report No. NTIS PC 252-012.

Appendix B

Where Can I Seek Assistance?

Assistance from EPA or FDA may be obtained by contacting your nearest regional office. Plants regulated by the Food Quality Service of USDA may obtain help from their local inspector. The following table lists by the state the ten Federal regional offices for FDA and EPA.

REGION I: Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, Vermont

EPA	FDA
Mr. Robert Dangel	Mr. A. J. Beebe
Toxic Substances Coordinator	Regional Director
John F. Kennedy Federal Building	585 Commercial Street
Boston, MA 02203	Boston, MA 02109
(617) 223-0585	(617) 223-1278

REGION II: New Jersey, New York, Virgin Islands, Puerto Rico

EPA	FDA
Mr. Ralph Larsen	Mr. Caesar A. Roy
PCB Coordinator	Regional Director
26 Federal Plaza	830 3rd Avenue
New York, NY 10007	Brooklyn, NY 11232
(212) 264-1925	(212) 965 5416

REGION III: Delaware, Maryland, Pennsylvania, Virginia, West Virginia, District of Columbia

EPA	FDA
Mr. Charles Sapp	Mr. R. J. Davis
Toxic Substances Coordinator	Regional Director
Curtis Building (3AH20)	2nd and Chestnut Street
6th & Walnut Street	Room 900
Philadelphia, PA 19106	Philadelphia, PA 19106
(215) 597-4058	(215) 597-4390

REGION IV: Alabama, Florida, Georgia, Kentucky, Mississippi, North Carolina, South Carolina, Tennessee

EPA	FDA
Mr. Ralph Jennings	Mr. M. D. Kinslow
Toxic Substances Coordinator	Regional Director
345 Courtland Street, NE	880 W. Peachtree Street
Atlanta, GA 30308	Atlanta, GA 30309
(404) 881-3864	(404) 881-4266

REGION V: Indiana, Illinois, Michigan, Minnesota, Ohio, Wisconsin

EPA	FDA
Mr. Karl Bremer	Mr. L. R. Claiborne
Toxic Substances Coordinator	Regional Director
230 South Dearborn Street, Room 1165	175 W. Jackson Boulevard
Chicago, IL 60604	Room A-1945
(312) 353-2291	Chicago, IL 60604
	(312) 353-1047

REGION VI: Arkansas, Louisiana, New Mexico, Oklahoma, Texas

EPA
Mr. John West
PCB Coordinator
First International Building
1201 Elm Street
Dallas, TX 75270
(214) 767-2734

FDA
Mr. P. B. White
Regional Director
3032 Bryan Street
Dallas, TX 75204
(214) 749-2735

REGION VII: Iowa, Kansas, Missouri, Nebraska

EPA
Mr. Wolfgang Brandner
Toxic Substances Coordinator
324 East 11 Street
Kansas City, MO 64106
(816) 374-6538

FDA
Mr. Clifford G. Shane
109 Cherry Street
Kansas City, MO 64106
(816) 374-5646

REGION VIII: Colorado, Montana, North Dakota, South Dakota, Utah, Wyoming

EPA
Mr. Dean Gillam
Toxic Substances Coordinator
1860 Lincoln Street
Denver, CO 80295
(303) 837-3926

FDA
Mr. F. L. Lofsvold
Regional Director
721 19th Street
US Customhouse Room 500
Denver, CO 80202
(303) 837-4915

REGION IX: Arizona, California, Hawaii, Nevada, American Samoa, Guam, Trust Territories of the Pacific, Wake Island

EPA
Mr. Gerald Gavin
PCB Coordinator
215 Fremont Street
San Francisco, CA 94105
(415) 556-4606

FDA
Mr. I. B. Berch
Regional Director
UN Plaza
Federal Office Bldg.
Room 526
San Francisco, CA 94102
(415) 556-2062

REGION X: Alaska, Idaho, Oregon, Washington

EPA
Dr. Jim Everts
Toxic Substances Coordinator
1200 6th Avenue
Seattle, WA 98101
(206) 442-5560

FDA
Mr. J. W. Swanson
Regional Director
909 1st Avenue
Room 5003
Seattle, WA 98174

For additional information you may call the Industry Assistance Office toll free at (800) 424-9065 (in Washington, DC 554-1404), or write to Director, Industry Assistance Office (TS-799), Office of Toxic Substances, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460

ES&T
FEATURES

Toxic substances in the Great Lakes

Here is an overview of the origin, distribution, and fate of some heavy metals and organics, and some suggestions as to what may be done about them

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Concern over the presence of chemicals in the Great Lakes has increased in recent years. Alfred Beeton reviewed historical trends of many common anions and cations, and found that their concentrations increased slowly but continuously in the past half century (*Eutrophication*, National Academy of Sciences, 1969). These changes were accompanied by changes in the biota, particularly the algae, as populations evolved from so-called clean-water species to those indicating eutrophic symptoms. Beeton hypothesized that man's activities were influencing the limnology of the Great Lakes, resulting in decreased water quality. These conditions were noted particularly in Lakes Erie and Ontario and in the southern basin and Green Bay in Lake Michigan.

An international conference, held in Madison, Wis., in 1967, focused on eutrophication and spurred greater research interest. Numerous additional conferences have been held on this topic in the past 12 years. However, environmental issues rarely remain simple, and in the early 1970's the eutrophication problem was confounded by concern over the potential effects of thermal pollution in the Great Lakes, a topic reviewed some years ago by Arthur Levin and others (*ES&T*, March 1972, p 224). For instance, as demands for electric power increased, especially in the Midwest, many power plants were constructed on Great Lakes shorelines.

Citizens and regulatory agencies, already alarmed by the specter of eutrophication-influenced water quality changes, attempted to restrict the discharge of power-plant cooling water into the lakes. By 1972, these two issues had created sufficient attention that an interstate enforcement conference on Lake Michigan pollution was convened. Also in 1972, the Great Lakes Water Quality Agreement between the United States and Canada was first signed. It established water-quality objectives and timetables, and espoused a nondegradation philosophy.

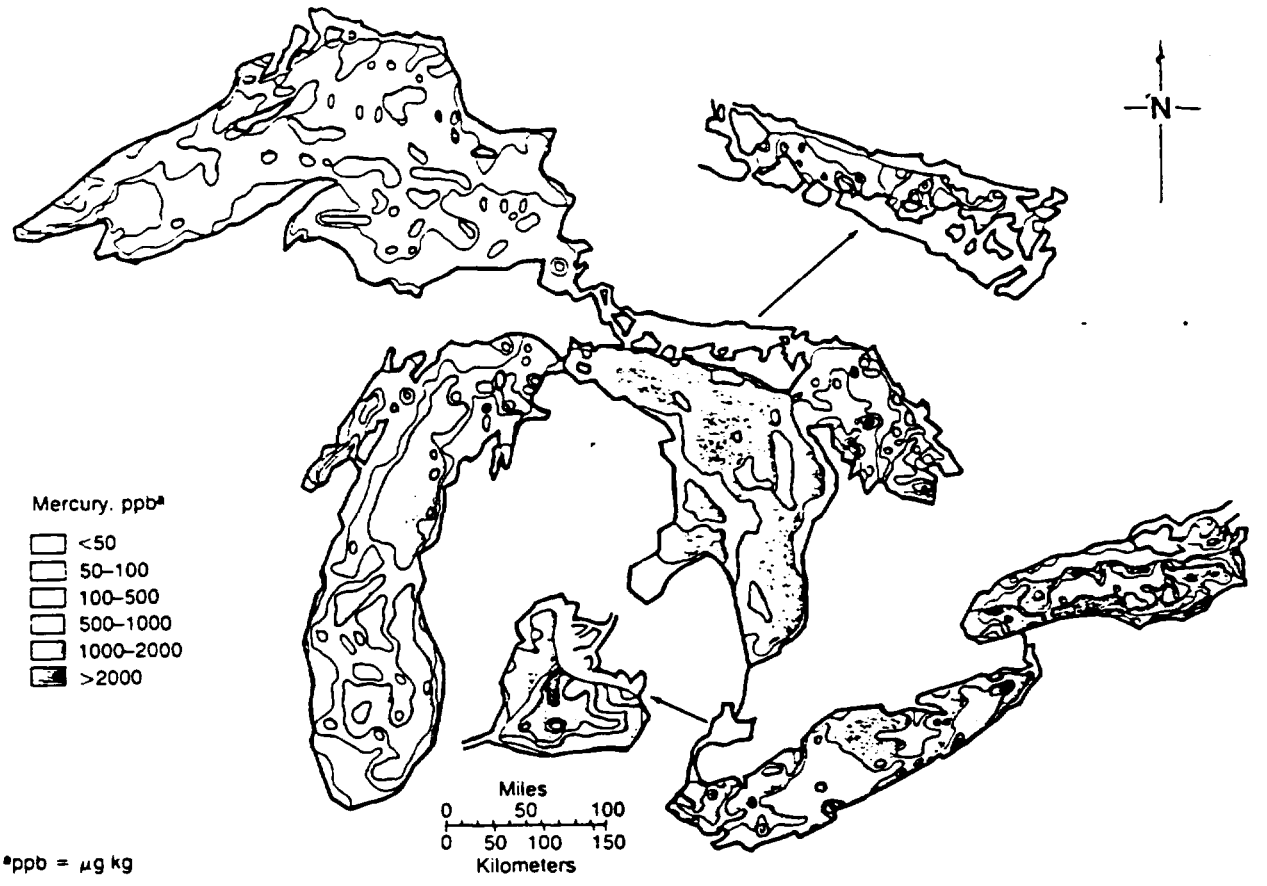
While these environmental issues in the early 1970's originally emphasized phosphorus loading and thermal pollution in the Great Lakes, the toxic-chemical problem was also developing. Although the first serious alert concerning toxic chemicals, particularly pesticides, had been sounded by Rachel Carson in *The Silent Spring* in 1962, it was not until the late 1960's and early 1970's that the combination of increased environmental awareness and laboratory analytical capability led to the discovery of pesticides throughout the Great Lakes Basin. The chemical stability of many pesticides, coupled with their capacity to bioaccumulate, resulted in high concentrations of compounds such as DDT and dieldrin in fish. Fish that exceeded U.S. Food and Drug Administration (FDA) tolerance levels for pesticides could not be sold commercially.

Feature articles in ES&T have by-lines, represent the views of the authors, and are edited by the Washington staff. If you are interested in contributing an article, contact the managing editor.

0013-936X/79/0913-1462$01.00/0 © 1979 American Chemical Society

FIGURE 1

Mercury concentrations in surface sediments of the Great Lakes



During the time that extensive effort was mounted to document DDT levels in Great Lakes fish, chemists noted that additional compounds were also present. In fact, some of these other substances created significant problems in the analysis of DDT. It was soon recognized that these troublesome compounds were polychlorinated biphenyls (PCB's), and that they were also present at levels as high as 25 μg/g. These historical levels have been documented by scientists from the U.S. Fish and Wildlife Service (FWS), the U.S. Environmental Protection Agency (EPA), and particularly by Gilman Veith at EPA's Duluth, Minn., laboratory. Thus, what was first a problem involving nondegradable pesticides, such as DDT, soon blossomed into a major issue of contamination by all types of toxic chemical substances.

To be sure, there is still concern about the input of phosphorus and thermal discharges into the Great Lakes. But, according to the Pollution from Land Use Activity Reference Group (PLUARG) of the International Joint Commission (IJC), toxic chemicals have now become the predominant water-quality issue in the Great Lakes Basin. This concern is also shared at the national level by the President's Council on Environmental Quality.

What are toxic substances?

Webster's *Seventh New Collegiate Dictionary* defines toxic as something "of, relating to or caused by a poison or toxin", where a poison is "a substance that through its chemical action usually kills, injures, or impairs an organism." The Congress of the United States defined toxic substances in October 1976, when it enacted the Toxic Substances Control Act (Public Law 94-469). That law defines toxic substances as those chemical substances "whose manufacture, processing, distribution in commerce, use, or disposal may present an unreasonable risk of injury to health or the environment." More specific definitions of a toxic substance and a hazardous polluting substance were included in the Great Lakes Water Quality Agreement of 1978, signed by the U.S. and Canada on Nov. 22, 1978.

Numerous industrial chemicals, such as PCB's, have been entering the environment for many years and, like DDT, have accumulated in living organisms including Great Lakes fish and in the people that consume them. PCB's have been identified essentially everywhere in the world, despite the fact that they were produced for uses that should not have resulted in such widespread distribution. These uses included capacitors, transformers, and hydraulic fluids. Their careless use and disposal have led to ubiquitous contamination of the environment.

Details about certain toxic chemicals, including lists of the so-called "priority pollutants", were published recently by Larry Keith and William Telliard (*ES&T*, April 1979, p 416). These should be consulted for additional information.

Distribution in water

The extent of toxic-substances distribution in the water, sediments, and fish of the Great Lakes Basin is only now beginning to be understood. For example, a comprehensive inventory of information on the distribution of or-

ganic and trace-metal contaminants in the basins of all of the Great Lakes, except Lake Ontario, has been prepared for a committee of the IJC's Water Quality Board by Dennis Konasewich and his co-workers. This inventory will serve as a basis for an assessment that will determine those contaminants that may present a hazard to human health and the environment. Data were obtained from municipal, state, provincial and federal agencies in the U.S. and Canada, as well as from reports and papers published by university researchers, government scientists and consulting firms. Among those groups that have performed considerable work and compiled extensive data on the Great Lakes are the Canada Centre for Inland Waters, the Ontario Ministry of the Environment, various EPA laboratories, the National Oceanic and Atmospheric Administration, and the natural resources departments of the Great Lakes states.

Chemicals in Lake Michigan

The most complete interpretive compilation of historical data for chemicals in Lake Michigan was published by Marguerite Torrey in 1976. No similarly thorough treatise for the other Great Lakes has been prepared.

At one time, organochlorine pesticides were widely used in the Lake Michigan Basin and were applied to forest and agricultural lands, and to noncrop lands. Pesticides were also used in industry and for household-pest control. According to a 1972 EPA report, the extensive use of DDT and dieldrin in Wisconsin led to the transport of these chemicals to Lake Michigan via tributaries, land runoff, and wastewater-treatment plants. Atmospheric precipitation was also implicated because of the wind-borne transport of these pesticides following aerial spraying.

The concentrations of pesticides in the waters of the Great Lakes is relatively low, due partly to their low aqueous solubility. As reported by Torrey, typical concentrations were generally below 1 μg/L, and often near 1 ng/L. In addition to DDT and dieldrin, numerous other compounds were detected by gas chromatography, including PCB's, lindane, heptachlor, heptachlor epoxide, endrin, aldrin, and methoxychlor. Much of the early DDT and dieldrin data from Lake Michigan and other waters, prior to the late 1960's, are now considered only approximate because of the likely interference of PCB's in the analysis of these pesticides.

Torrey demonstrated that the concentrations of the main toxic inorganic materials (Hg, Pb, As) are quite low in Lake Michigan. There, inorganic substances do not appear to pose significant problems, except for As in the Green Bay area (currently being studied by Marc Anderson and associates at the University of Wisconsin-Madison), and Pb in the southern basin.

Distribution in sediments

Great Lakes sediments are the ultimate sink for many, but not all, of the toxic contaminants that enter the lakes. Some of the contaminants, such as mercury, can be converted to more biologically active forms. Also, compounds such as PCB's are taken up by bottom organisms that either live or feed in the sediments, and which are subsequently eaten by larger carnivores. This results in an eventual increase in the concentration of this contaminant in carnivorous fish. Thus, despite the fact that the sediments are many meters below the water's surface, they do not always act as the ultimate sink for toxic substances.

The presence of many contaminants in the Great Lakes sediments was summarized in the 1978 PLUARG *Final Report to the IJC*. Of particular concern were Hg, Pb, and PCB's. A contour map prepared by PLUARG, showing the approximate distribution of mercury (Hg) in Great Lakes sediments, is reproduced as Figure 1.

The highest Hg concentrations generally appear in Lakes Erie, Ontario, and St. Clair. Previous industrial discharges, apparently current atmospheric deposition into the Great Lakes, and runoff from the land surface appear to be sources of Hg.

Prior to 1970, major inputs into the lakes' system were discharges from the St. Clair and Detroit Rivers. On the basis of the sediment patterns, Lake St. Clair appears to be a continuing source of Hg to Lake Erie, despite the elimination of the chloralkali-plant source.

The transport of Hg-enriched sediments from Lake St. Clair, through the Detroit River, and into western Lake Erie, continues to be a problem. The resuspension of Hg-contaminated sediments from western Lake Erie, and subsequent in-lake transport, has carried Hg along the southern shoreline and led to its ultimate deposition in the eastern basin. The western basin of Lake Erie is an active area of sediment resuspension because of wind-induced wave action.

In Lake Ontario, Hg appears to be carried by the Niagara River, with eventual dispersal throughout the eastern basin. The sharp decrease in point-source inputs of Hg to the lakes should result in a gradual decrease in Hg content of the surface sediments, according to PLUARG.

The concentrations of most organic contaminants in Great Lakes sediments have not been well mapped. PCB contours, however, were compiled by PLUARG for Lakes Huron, Erie, and Ontario. These indicate a general occurrence of PCB's in the sediments, with higher loadings in the western and southern areas of Lake Erie and in the south central area of Lake Ontario. The PLUARG Report estimated that nonpoint sources, including atmospheric inputs, account for the major reflux of PCB's to the lakes.

Distribution in fish

Data for PCB's in Great Lakes fish were compiled in the PLUARG Report with additional data provided by the State of Wisconsin. These appear in Table I. The most significant PCB contamination occurs in Lake Michigan, according to data now available.

One interesting aspect of PCB, DDT, and dieldrin contamination of fish in Lake Michigan is the slightly decreasing trend in concentrations that has developed over the past five to eight years. Data from the FWS and EPA indicate such a trend, but further analysis and monitoring will be required in the future to verify the trend. The PCB concentrations in fish can

A toxic substance means "a substance which can cause death, disease, behavioral abnormalities, cancer, genetic mutations, physiological or reproductive malfunctions or physical deformities in any organism or its offspring, or which can become poisonous after concentration in the food chain or in combination with other substances." A hazardous poiluting substance is defined to mean "any element or compound which, if discharged in any quantity into or upon receiving waters or adjoining shorelines, would present an imminent and substantial danger to public health or welfare. For this purpose, public health or welfare encompasses all factors affecting the health and welfare of man including but not limited to human health, and the conservation and protection of flora and fauna, public and private property, shoreline and beaches."

Source: Great Lakes Water Quality Agreement of 1978, International Joint Commission

vary from one season to another, as well as during spawning periods. Thus, fish monitoring programs must be conducted in a consistent manner to avoid bias in the interpretation of the data.

One reason that PCB's continue to appear in relatively high concentrations in Lake Michigan fish is that the chemical is easily bioaccumulated; that is, passed through the food chain as smaller organisms are consumed by larger ones, ultimately reaching the primary carnivores in the lake (salmonids) which are highly prized sport fishery species. This process is illustrated in Figure 2.

Mercury concentrations in Great Lakes fish are not too much of a concern, except for those caught in Lake Erie. Concentrations from fish in that lake indicate that the FDA consumption guideline of 1.0 μg/g is still being exceeded, although trends of decreasing concentrations have been noted. The PLUARG Report noted that lead (Pb) concentrations are nowhere close to the recommended consumption-advisory limit of 10 μg/g in any of the Great Lakes.

Effects of toxic substances

One effect on citizens in the Great Lakes Basin, particularly those who consume relatively large amounts of locally caught fish, is contamination from certain species by chemicals, especially PCB's. Most of the species that have sport fishery importance, particularly the salmonids, consistently exceed the proposed FDA tolerance guideline of 2 μg/g in Lake Michigan. However, sportsmen are not prevented from consuming their catch, and many of these people are unwilling to abandon their sport (which involves considerable financial investment), particularly since toxicological studies have not conclusively linked the dietary intake of low levels of PCB's with human health problems.

Despite the lack of firm human health evidence, there is ample documentation that the intake of PCB's near the FDA guideline significantly affects rhesus monkeys. James Allen and co-workers at the University of Wisconsin-Madison have shown that infants born to monkeys that were fed 2.5–5.0 μg/g of PCB's developed facial acne and edema, swelling of the eyelids, loss of facial hair including eyelashes, and hyperpigmentation of the skin. Half of the PCB-exposed infant monkeys died within eight months following birth, assumedly from PCB intoxication. Surviving infant monkeys proved to be hyperactive in locomotor tests, and to be slow in learning how to

Toxic substances: classes and sources

According to the Great Lakes Basin Commission, the general classes of toxic substances that are presently of concern in the Great Lakes Basin are:

- halogenated organic compounds (PCB's, PBB's, chlorophenols),
- radioactive substances,
- pesticides,
- heavy metals and toxic nonmetals (Hg, Pb, As), and
- petroleum products.

There are many sources for the toxic substances that enter the Great Lakes. The Great Lakes Basin Commission and others have itemized these as:

- point sources (municipal- and industrial-effluent discharges),
- nonpoint sources (land application of toxic materials followed by runoff; the atmosphere),
- urban runoff (Pb from automobile exhausts; grease and oil; metals; household pesticides),
- toxic and hazardous waste disposal sites or "phantom" disposal activities (legal or illegal waste disposal that results in the pollution of the Great Lakes; transportation accidents),
- *the atmosphere* (*ES&T*, November 1979, p 1337) (can be considered a nonpoint source but represents a major point of entry of PCB's, Pb and other toxic substances to the lakes),
- agricultural runoff (nonpoint source but emanates from specific useage of chemicals in agriculture).

TABLE 1
PCB concentrations in Great Lakes fish.

Lake	Sampling period	Mean PCB concentrations[a] µg/g	Range µg/g
Superior	1968–1975	0.61	<0.1–3.7
Michigan	1972–1974	10.2	2.1–18.9
	1974–1978	NC[b]	1.2–37[c]
Huron	1968–1976	0.82	<0.1–7.0
Erie	1968–1976	0.88	<0.1–9.3
Ontario	1972–1977	2.37	<0.1–21.1

[a] The FDA tolerance level is 2 µg/g
[b] Not calculated
[c] Based on the analysis of 35 fish consisting of chubs, lake trout and coho salmon. Data from the State of Wisconsin Department of Natural Resources
Source: PLUARG, 1978

The PLUARG Report

The 1978 PLUARG Report summed up the toxic substances control problem very well and the following points are excerpted from that document. In order to control toxic substances and reduce their inputs to the Great Lakes:

- Toxic substances must be controlled at their source.
- Closer cooperation between the U.S. and Canada in implementing toxic substances control legislation must occur.
- Proper management control, and the means of ultimate disposal of toxic substances presently in use, must be developed.
- Identification and monitoring of historic and existing solid waste disposal sites (where there is an exisiting or potential discharge of toxic substances) must be done, and control programs at those sites must be executed as needed.
- Joint U.S.-Canadian expansion of efforts to assess the cumulative and synergistic effects of increasing loads of toxic contaminants on environmental health must be given high priority.
- The rapid translation of these assessments into revised water-quality objectives must be made; moreover, for certain toxic substances, a zero loading to the Great Lakes must be a requirement.

solve various types of discrimination problems, when compared to control animals.

The linkage of rhesus monkey health effects to potential human health problems is still not firm. However, the animal studies suggest that humans should limit their consumption of PCB-contaminated fish taken from the Great Lakes, especially Lake Michigan, until epidemiological research determines the extent, if any, of low-level dietary intake effects of PCB's on humans.

The most serious effect of toxic substances in the Great Lakes seems to be the diminished value of the Great Lakes as a natural resource, particularly in the production of fish that are suitable for human consumption. Commercial fishing has already been impaired. Sportsmen now enjoy their pastime with the caveat that their catch is tainted, and that their fish intake should be limited to no more than one meal per week. Pregnant or nursing women have been advised to eliminate the PCB-contaminated fish entirely from their diets.

Problems in the Lake Michigan basin

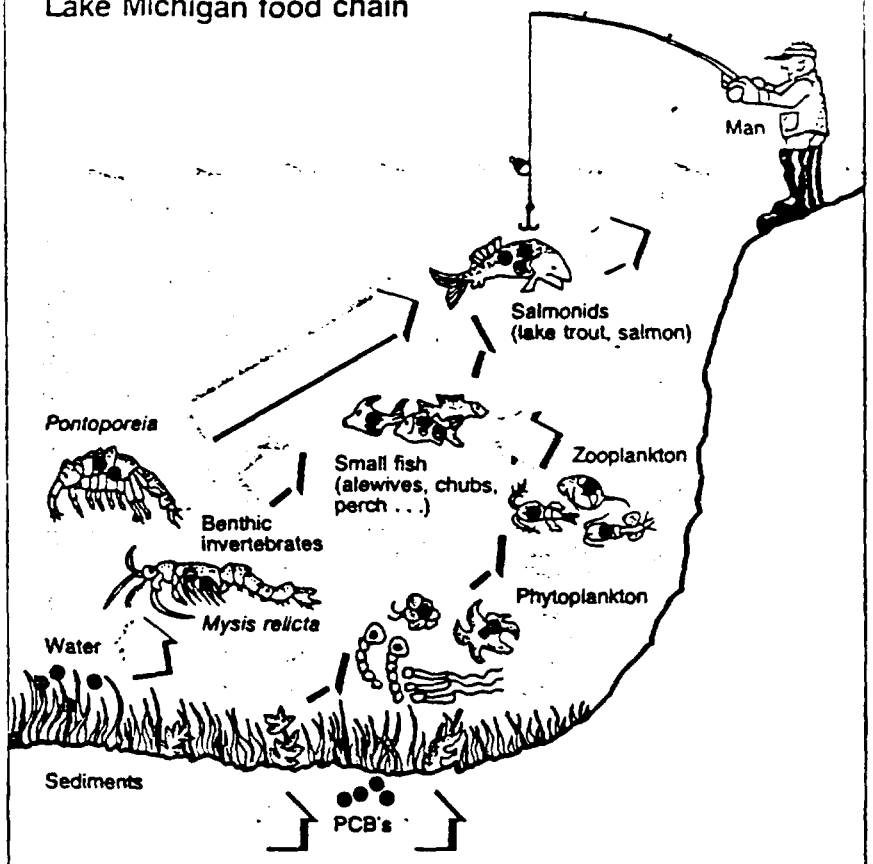
In recent years, numerous toxic and potentially hazardous organic compounds have been identified in the Wisconsin waters of the Lake Michigan basin. The findings in Wisconsin are representative of experiences in the other Great Lakes states, and will be discussed further here to provide an overview of some current problems.

Perhaps the best studied of the toxic organic compounds are the PCB's. A National Conference held in Chicago, Ill., in 1975 (*ES&T*, February 1976, p 122) addressed this industrial contaminant in detail. The unusual stability of the PCB's in the environment, and their capability to bioaccumulate, have contributed to the fishery problems mentioned above.

Contamination of Lake Michigan waters by PCB's has been a matter of concern since the late 1960's when the Wisconsin Department of Natural Resources (DNR) found that PCB's in fish collected from the lake exceeded the FDA guidelines (which were set at 5 μg/g at that time). PCB sources were found to be industrial and municipal effluents; but also, perhaps more significantly, the atmosphere was implicated as a possible major contributor of PCB's to the lake. Indeed, as the point-source contributions of PCB's are eventually closed off, nonpoint sources, such as the atmosphere, will take on a more important role.

Nevertheless, according to Stanton Kleinert of the Wisconsin DNR, the PCB problem in Wisconsin is chiefly a fishery problem, in terms of natural-resources management. Public water supplies in the state, for example, do not contain detectable amounts of PCB's, while nearshore waters of Lake Michigan and its tributaries contain only small concentrations of the contaminant.

FIGURE 2
How PCB's are passed through the Lake Michigan food chain



Source: University of Wisconsin Sea Grant Program. Published by permission of the copyright owner.

PCB's in the Sheboygan River

In March 1978, very high concentrations of PCB's were reported in fish collected in the Sheboygan River, a Lake Michigan tributary in northeastern Wisconsin. The fish, originally collected in late 1977, showed PCB concentrations ranging from 26 to almost 1000 μg/g. Recall that the FDA guideline is 5 μg/g; lowering it to 2 μg/g is proposed.

Because of these very high concentrations, an intensive sampling program was begun. The results verified the earlier findings that the PCB concentrations in the Sheboygan River fish were significantly elevated above the values normally found in the Lake Michigan basin.

These findings indicated the probable existence of a previously unidentified source of PCB's. Investigative work by the Wisconsin DNR quickly identified a metal-casting plant as the PCB source. The plant had been disposing PCB-contaminated wastes behind its building and along a dike adjacent to the Sheboygan River. High water, runoff, and erosion deposited the PCB's in the river sediments, and through food-chain accumulation, contaminated numerous fish species.

The PCB-contaminated shoreline material has now been isolated and placed in sealed drums, and awaits final disposal. The sediments, however, are still loaded with PCB's and continue to contaminate fish. They also are washed out into Lake Michigan through normal stream sediment transport processes. The question of dredging the sediments has not been resolved, since dredging might mobilize the PCB's, and lead to more contamination of the nearshore Lake Michigan area. Also, an adequate dredge spoil disposal site would have to be developed, and such sites can be placed only on land or in contained shoreline areas. The disposal of con-

contaminated dredge spoils in Lake Michigan waters is unlawful, posing the dilemma that both action and inaction will result in continued PCB pollution problems. This case is an example of the type of toxic substance control problem that is likely to arise again in the future.

The company involved in the Sheboygan River case has changed ownership since the period when PCB's were used in the hydraulic systems of the casting machinery. PCB's are no longer in use, but their careless disposal in the past haunts the present owners, as well as the environment near the casting plant. A similar case is now the object of considerable research and regulatory interest in the Waukegan, Ill., harbor area.

Wastewater: the Lower Fox River

Probably the most industrialized drainage basin entering into Lake Michigan is the Lower Fox River, a tributary to the waters of Green Bay in northeastern Wisconsin. Leading from Lake Winnebago to Green Bay, it receives wastewaters from many pulp and paper mills, and other industrial and municipal wastewater treatment facilities.

A study initiated in 1976 sought to identify the sources of PCB's and other chlorinated organic compounds in the river. Many organic and chlorinated organic compounds were identified in the wastewaters, river water, sediment, and fish collected throughout the watershed. Included in these results were 20 of the "priority pollutants" that were discussed by Keith and Telliard (*ES&T*, April 1979, p 416).

Representative data from this study appear in Table 2. They indicate that the Lower Fox River system receives considerable loadings of chemicals from a variety of sources. The heavy use of chlorine as a bleaching agent in pulp-mill operations, the recycling of paper products contaminated with PCB's, and chlorination of municipal treatment plant effluents are sources of many of the chlorinated organic compounds.

The environmental significance and ultimate fate of these materials are not fully known at present, except of course, for PCB's which are found in high concentrations in fish taken from the Lower Fox River. Research sponsored by state and federal organizations, including the University of Wisconsin Sea Grant Program, is underway and will help to resolve many of the current uncertainties.

TABLE 2
Selected organic compounds identified in the Lower Fox River watershed in Wisconsin[a]

Compound	Concentration ranges		
	Wastewaters (μg/L)	Sediments (μg/g)	Fish (μg/g)
Anisole,			
Tetrachloro-	0.04–0.08	—[b]	—
Pentachloro-	0.05–0.38	—	0.005–0.06
Benzothiazole	10–30	—	—
Hydroxy-	10–30	—	—
Methylthio-	10–40	—	—
Dehydroabietic acid	100–8500	2.7	—
Dieldrin[c]	—	—	0.008–0.022
Guaiacol,			
Trichloro-	10–60	—	—
Tetrachloro-	10–50	—	—
Hexachlorocyclohexane[c]	0.04	—	—
Phenol(s),			
Dichloro-[c]	15–40	—	—
Trichloro-[c]	5–100	—	—
Tetrachloro-	2–20	—	—
Pentachloro-[c]	0.1–40	0.22–0.28	—
Polychlorinated biphenyls (PCB's)[c]			
(Aroclors 1242, 1248 and 1254)	0.1–56	0.05–61	0.5–90
Polycyclic aromatic			
Hydrocarbons (PAH's)[c]	0.5–10	—	—

[a] Source: Wisconsin Department of Natural Resources, 1978
[b] Not determined or not detected
[c] Compound on EPA priority pollutant list

PCB's in lake trout

A recently completed study has added substantially to knowledge of how PCB's are taken up by salmonid species, particularly lake trout in Lake Michigan. This research was performed by David Weininger, under the direction of David Armstrong, at the University of Wisconsin-Madison. In his Ph.D. thesis, Weininger developed a bioenergetic model to account for the accumulation of PCB's by Lake Michigan lake trout. With some modification, the model might apply to related species in other lakes that have similar feeding habits and metabolic processes.

The primary source of PCB's accumulated in the lake trout is adult alewives. Furthermore, for an average adult lake trout, the PCB concentration is more related to its age than its size, signifying that accumulation is more a function of time or exposure than of diet alone.

Of major importance in Weininger's work is the prediction that greater than 50% of the PCB's in adult lake trout have cycled through Lake Michigan sediments. This emphasizes food-chain processes, since benthic invertebrates are eaten by adult alewives which, in turn, are consumed by adult lake trout.

To reduce future concentrations in lake trout, the PCB content of the alewives must be reduced. Weininger speculated that if the alewife population could be reduced, then lake trout might switch to smelt as a primary food source. Smelt have much lower PCB concentrations; thus, lake trout PCB levels should decrease rapidly. This might result in lake trout eventually meeting the FDA tolerance limit in the future.

Weininger's hypothesis is intriguing, and fish resource managers have now been challenged to take innovative steps to reduce the PCB content of prized Lake Michigan stocked species (salmonids). The alternative is to abandon salmonid stocking for many years until PCB's in the sediments are buried beyond those levels at which they would be available to invertebrate organisms.

Control of contamination

Resource-management strategies in the Great Lakes Basin must contend with the toxic-substances issue. This

involves human and environmental health, thereby elevating itself to a position of prominence above some of the earlier Great Lakes problems, such as eutrophication and thermal pollution. The task will not be easy because it requires resource managers to adopt nontraditional concepts. However, progress can and must be made.

A toxic substances control plan has been developed by the Great Lakes Basin Commission. It draws on numerous sources for its background information including publications and policies of the IJC, PLUARG and EPA. Some of the important cornerstones of the Basin Commission's plan are the many existing environmental statutes, including the following (*ES&T*, February 1978, p 154):

- Toxic Substances Control Act,
- Federal Insecticide, Fungicide and Rodenticide Act,
- Federal Water Pollution Control Act Amendments,
- Resource Conservation and Recovery Act,
- Clean Air Act,
- Hazardous Materials Transportation Act,
- Ports and Waterways Safety Act,
- Safe Drinking Water Act,
- Atomic Energy Act,
- Marine Protection, Research and Sanctuaries Act, and
- Food, Drug and Cosmetic Act.

Despite the laws that have been enacted to control pollution, serious contamination incidents still occur in the Great Lakes Basin. These problems indicate the need for continued concern and cooperation on the part of those who produce, use, and discharge toxic substances. Regulatory surveillance and enforcement must continue, and will remain a major stimulus in controling the release of toxic substances in the basin.

In addition to surveillance, increased emphasis must also be placed on research. During the course of the analysis of environmental samples, numerous unknown compounds still appear as recorder traces on gas chromatographs. Resources must be made available to government and university scientists so that the number of these "unknowns" can be reduced. Expanded research efforts should result in a decrease in the number of incidents that were at one time sarcastically referred to as "pollutants of the month".

It is known that many toxic substances are transported to the Great Lakes Basin from other areas of the U.S. and Canada. This emphasizes the need for environmental-protection personnel to learn more about atmospheric chemical transport and reaction phenomena. Atmospheric chemistry is now receiving attention in a number of Great Lakes area universities. A symposium on atmospheric chemistry at the ACS meeting in September (*ES&T*, November 1979, p 1337) highlighted much of the current research in this area.

The Great Lakes states and the EPA should ultimately agree on a common list of priority pollutants that will receive primary emphasis for monitoring, research, and eventually control in the basin. The Great Lakes Basin Commission has stated that federal and state cooperation is critical in solving toxic-substances problems. And, importantly, research is also needed to determine the human health implications and environmental significance of the toxic substances that have been found distributed throughout the area.

Another item involves the analytical detection of lower and lower concentrations of chemicals which were not previously anticipated in the Great Lakes Basin. Should government regulate these substances "just because they are there", or should they await appropriate toxicity testing to determine their potential environmental and human health impacts? It is an important question that may require the wisdom of Solomon to answer to everyone's satisfaction.

In summary, it should be clear that there are toxic-substances problems in the Great Lakes Basin, and that steps are now being taken to resolve them. This process will take considerable time and money, but for the more than 15% of the U.S. population that lives in the Great Lakes area, these investments will provide long-term dividends in terms of improved water quality and enhanced utilization of the Great Lakes as a natural resource.

Why controls would be difficult

- A number of toxic substances are already present in the Great Lakes.
- Some toxic pollutants in the Great Lakes are derived from sources well removed from the basin and are carried hundreds of kilometers by atmospheric processes, illustrating the need for national and international planning.
- The national goal of zero discharge of toxic substances into natural waters must be vigorously pursued, particularly in the Great Lakes where residence times can be long.
- Methods must be sought for the attenuation or removal of those toxic substances already present in the Great Lakes Basin.
- Existing laws must be enforced to reduce and ultimately eliminate the discharge of toxic substances to the aquatic environment.

Note: This article is a condensed version of a report that was originally prepared for a course on water-quality issues entitled, "Decisions for Lake Michigan", funded by the U.S. Office of Education as part of the Interstate Water Quality Training Program, and coordinated by the Purdue University Calumet Campus and the Lake Michigan Federation.

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THE NEXT 20 YEARS

1451

A Survey of Polychlorinated Biphenyls in Paper Recycling Mill Treated Wastewater

PCBs and Wastepaper

by Kevin J. Walter and John J. Zambrano

Polychlorinated biphenyls (PCBs) emerged in the 1970s as a significant water contaminant and serious hazard to human and animal health. Prior to this general period, it was not realized by anyone that PCBs could be as hazardous as they turned out to be. The presence of this chemical is widespread, but it appears that New York might very well have more contamination by these persistent chlorinated hydrocarbons than any other state. Reports of high levels of PCB contamination in fish caught in New York state waters became more and more common in the mid 1970s. Levels often exceeded the FDA limit of 5 ppb.

A gas chromatograph installation typical of those used for testing.

The initial concern of the State Department of Environmental Conservation, was elimination of the direct sources of PCB wastewaters. In September 1976 the Department and General Electric Company, the state's major user of PCBs for capacitor production, reached a settlement concerning their wastewater discharge. The company was permitted a one gram per day discharge based on an achievable concentration of 1 ppb.

In the aftermath of the General Electric case, the Department became concerned with potential discharge of wastewater with indirect contamination of PCBs. Those of us associated with the paper industry had been aware for some time of the contamination of recycled paper from at least two uses of this chemical in years prior to 1971. What was not known, however, was the level of this contaminant in treated paper mill wastewater discharges and specifically in the large number of mills using recycled paper. This state has long led the country in total number of paper mills in operation even though the tonnage produced is well below that of many states.

For these reasons it was believed necessary to institute PCB monitoring in wastewater discharges from mills using recycled paper. The purpose of the monitoring survey was to establish the individual levels of PCBs that are discharged from state paper mills. The information would be used to determine if specific receiving water quality problems might be attributed to these discharges and whether treatment, controls or additional monitoring would be necessary or practicable at the levels measured.

New York has approximately 61 operating paper mills. Of these, approximately 40 or 66 percent process some amount of recycled papers. The requirement for PCB self-monitoring was to be implemented through the state's SPDES permit program for wastewater discharges. The survey was restricted to mills with wastewater discharge permits.

The number of mills using recycled paper and discharging directly is approximately 29. A further reduction in the survey population resulted from the elimination of mills that could trace recycled paper to a source of virgin fiber without possible PCB contamination. This left approximately 18 mills for permit modification and self-monitoring of PCBs.

The survey was conducted over a one-year period with samples collected and analyzed once a month. This frequency and number of samples were judged satisfactory to determine a reliable level and cover a sufficient period, to be indicative of processing variations and sources of recycled paper. The sampling and analysis, although expensive, was not prohibitive for the number of paper mills involved.

PCBs and Recycled Paper

PCBs were first manufactured in

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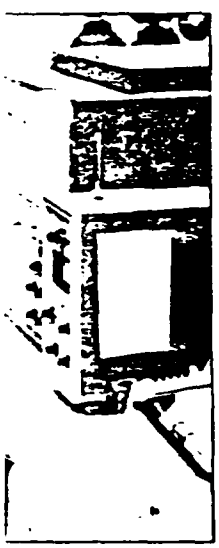

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PCBs and Recycled

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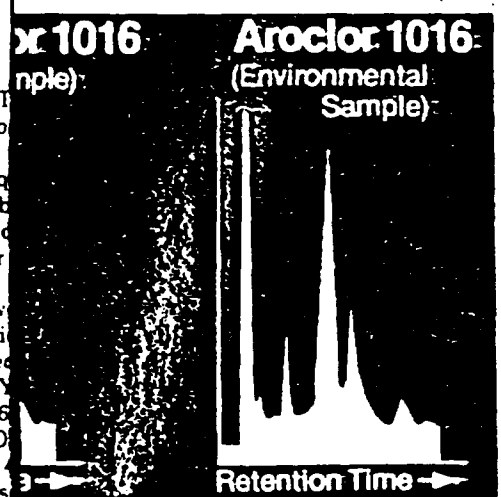

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In all except one case, where in-company analytical facilities were used, the survey mills selected private laboratories to conduct PCB testing. A total of eight laboratories were utilized. All of the laboratories used gas chromatography (GC) with the electron capture detection method. Extraction, sample cleanup, concentration, and chromatogram integration techniques varied considerably between laboratories. Although 1.0 ppb detectability was specified by the state, much of the data submitted was performed to a detectability of 0.1 ppb, or as low as 0.01 ppb.

PCB concentrations were reported in accordance with state instructions as total PCB by most survey mills, rather than as various aroclors. In order to calculate total PCBs though, each lab compared the sample to standard aroclor chromatograms. In all survey mills except one, the most commonly found Aroclors 1242 and 1254 were utilized as standards at a minimum. **Figure 1** shows that PCB components undergo environmental alterations.

Importantly, it is noted that all analytical results submitted were gross, not net, values. Therefore, any background PCB concentrations in mill intake waters were reflected in the final discharge concentrations. No allowance was made for intake waters, even in the few cases where mills analyzed for it.

Mill Production and Wastewater Treatment

Included in the survey were eight

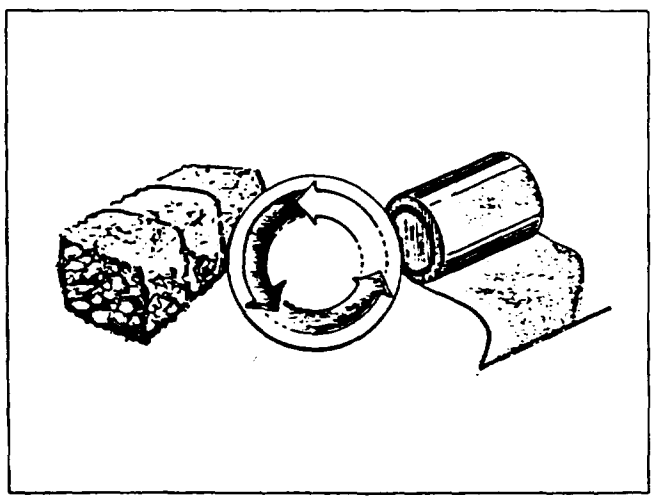

Figure 2. Twenty-five percent of all paper produced in New York state is made from recycled paper.

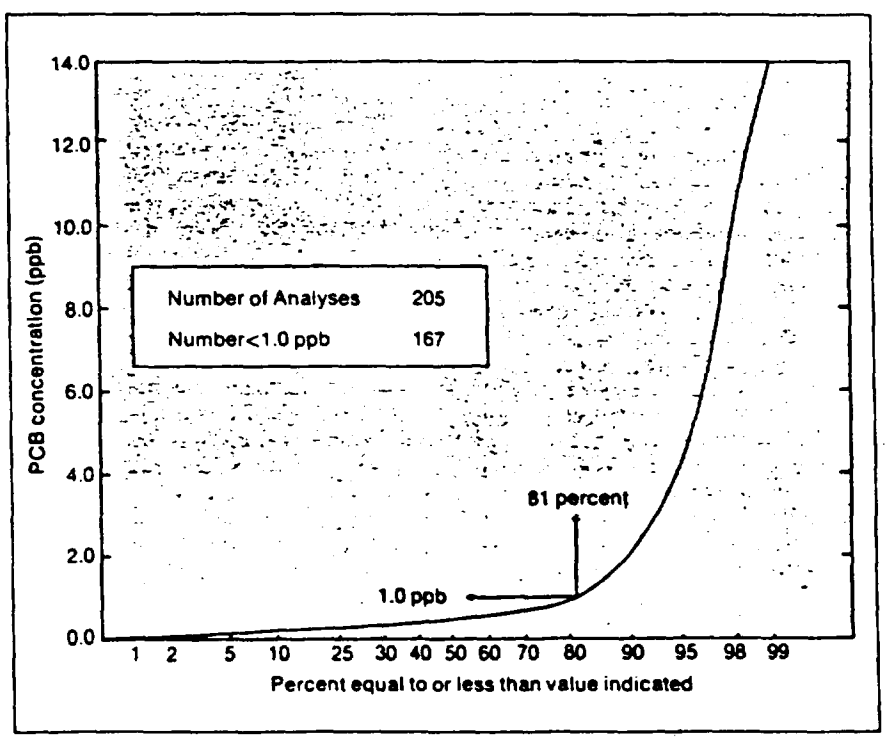

Fig. 3. PCB concentration cumulative frequency distribution.

boardmills, seven tissue mills, one fine paper mill, one molded products mill and deinked market pulp mill (Table 1).

The fine paper mill and one of the tissue mills process deinked pulp for a portion of their pulp. The types of wastewater treatment ranged from no treatment with high solids losses to biological treatment with chemically aided secondary clarification.

Total paper production of the surveyed mills is 2,100 tons/day (tpd) of which 1,225 tons or 58 percent is recycled paper (Table 1). The production of 2,100 tpd at the surveyed mills, over the period of the study was

"Many types of paper currently being recycled today were possibly originally manufactured with PCB contaminated paper products."

an average of 88 percent of peak capacity.

Detailed paper production and recycled paper use was not available for the 11 state mills using recycled paper and discharging to municipal systems. It is estimated, however, that their total paper production is about 1,000 tpd, and the recycled paper use is about 600 tpd. Total New York recycled paper use is, therefore, approximately 1,800 tons/day, and this survey would represent 68 percent of the state's recycled paper use tonnage.

The 1,800 tpd of recycled paper is approximately 25 percent of all, i.e., virgin and recycled paper production in the state and compares closely with the 1976 production in the state and the 1976 nationwide rate of 23 percent of 13.9 million tons/year of recycled paper[2] (Figure 2).

Table 1
Survey Mills
Recycled Paper Consumption

Mill	Mill Type	Total Pulp/ Paper Produced (tpd)	Recycled Paper Processed (tpd)*	Recycled Paper Production (days/year)	Consumption of Recycled Paper (ton/year)
A	Board (felt)	450	33	68	2,240
B	Tissue	9	10	300	3,000
C	GW pulp/ board	40/65	27	300	8,100
D	Board	70	74	300	22,200
E	Board	100	106	350	37,100
F	Tissue	30	2	350	700
G	Tissue	20	4	300	1,200
H	Board	160	170	350	59,500
I	Board	90	94	300	28,200
J	Board	96	103	300	30,900
K	GW pulp/ molded products	43/50	8	300	2,400
L	Tissue	70	11	300	3,300
M	Deinked pulp/ fine paper	75/400	88	350	30,800
N	Deinked pulp/ tissue	50/125	117	350	40,950
O	Deinked pulp	125	147	350	51,450
P	Tissue	45	24	350	8,400
Q	Tissue	85	90	350	31,500
R	Board	110	117	350	40,950
Totals, survey mills		165/2,100	1,225		402,890
Totals, all New York state mills using recycled paper		/3,100	1,800		600,000

**Recycled paper consumption obtained from paper production using known shrinkage or assumed shrinkage of 6 percent.*

Results

A summary of the analyses is presented in Table 2 and includes 205 of the 220 analyses received. Fifteen analyses were conducted with detectabilities above 1 ppb and are not adequate for our purpose. Fifty-nine of the analyses were reported as positive (not as less than) results. The remainder were reported as less than the indicated value. The range of concentrations was <0.01 to 18 ppb.

A high percentage of tests are reported as less than the indicated value, hampering computations of average values and frequency distributions. A large number of analyses are reported as less than 1.0 ppb. A useful computation, therefore, is the percentage of tests less than 1 ppb PCB. This value is 81 percent for the results from all mills (Figure 3).

Three mills in the survey group had no treatment, and one mill inappropriately sampled raw waste. Elimination of the analyses from these mills results in 85 percent of the remaining analyses being less than 1.0 ppb.

In order to characterize each mill's average concentration, median values were utilized. For the purpose of computing medians, analyses reported as less than the indicated number were considered as equal to the number. Therefore, median values represent a maximum median. A summary of the medians is presented in Table 2. With the exception of Mill E, all mills had a median concentration of less than 1 ppb. The average of all the mill median concentrations is 0.76 ppb.

Elimination of the data of the mills without treatment results in an average concentration of 0.61 ppb, with all mills below 1.0 ppb median concentration.

A number of mills reported all results with detectabilities of <0.25 ppb or lower. Calculations with these mills would reduce the error in the computation of median and may result in a closer approximation of a true average median concentration for paper mills. As indicated in Table 2, these eight mills had an average median concentration of 0.45 ppb which is significantly lower than the 0.76 ppb concentration using all mills.

When the analyses of the two mills without treatment in this group are eliminated, the average median concentration is substantially reduced to 0.10 ppb.

The concentration of PCBs in wastewater effluents is significant from the considerations of detectability, background water levels and the practicality of treatment. The mass of PCBs discharged, however, is significant for receiving water and overall ecological effects. As shown, the total discharge from all 18 mills in the survey is 43 grams per day or 14 kilograms (30 lb) per year. It should be noted that these values are calculated using median rather than arithmetic average concentrations from each mill and that the median values are approximations as explained previously. PCBs discharged

Table 2
Median PCB Concentrations
of Individual Mills*

Mill	Number of Analyses	Number of Positive Analyses	Range (ppb)	Median Concentration (ppb) Mills with Detectability<1.0 or Lower	Median Concentration (ppb) Mills with Detectability<0.25 or Lower
A	18	3	<0.01−0.38	0.10	0.10
B	13	2	<0.5−3.0	1.0	
C	12	6	<0.06−5.0	0.4	0.4
D	12	5	<0.06−2.6	0.05	0.05
E	10	10	<0.7−5.7	2.6	2.6
F	14	6	<0.06−12.0	1.0	
G	5	1	<1.0−13.0	1.0	
H	11	3	<1.0−14.0	1.0	
I	10	1	<1.0−10.5	1.0	
J	10	1	<0.1−1.0	1.0	
K	13	0	<0.1−0.3	0.2	0.2
L	11	3	<0.01−18.0	0.1	0.1
M	12	4	<0.05−0.26	0.05	0.05
N	11	6	<0.1−4.5	1.0	
O	12	2	<1.0−0.89	1.0	
P	13	4	<1.0−1.5	1.0	
Q	4	2	<1.0−2.7	1.0	
R	14	0	<0.01—<1.0	0.1	0.1
Total 1**	205	59	<0.01−18.0 average 1**	0.76	0.45
Total 2**	178	59	<0.01−18.0 average 2**	0.61	0.10

Median is defined as the 50 percent cumulative frequency value, however, analyses reported as less than indicated numbers were considered as equal to the indicated number. Therefore, the median values represent maximum possible medians. True medians values will be lower.

***Average 1 includes all mills. Average 2 eliminates mills B, C, E, Q whose results are for untreated wastewater.*

per ton of recycled paper varied widely with no correlation apparent.

Conclusions

A cumulative frequency distribution of results from all mills shows about 85 percent of the analyses are 1 ppb or less. Above 1 ppb the distribution increased rapidly to a high of 18 ppb. With the exception of Mills E and N, the high values are distributed somewhat uniformly among the mills and their occurrence is not readily understood. They may be a result of individual highly contaminated purchases of paper or testing errors. Because they are relatively infrequent and distributed among most of the mills, we have not considered them highly significant. A PCB concentration of less than 1 ppb is considered an accurate characterization of all mill's average discharge, with the exception of Mill E.

Conclusions from this survey are summarized as follows:

- The average PCB concentration of every mill with primary or secondary treatment is less than 1 ppb.
- The weighted average of discharges from all mills, using individual median concentrations is 0.4 ppb or less.
- Data from the group of mills using analytical techniques with increased minimum detectability indicates that the average concentration of PCBs in recycled paper mill wastewater may be as low as 0.1 ppb for mills with treatment.
- The absence of treatment at one mill (E) may be responsible for its relatively high average PCB concentration of 2.6 ppb.
- The New York State Department of Environmental Conservation had previously concluded that the practical limit of carbon treatment for PCBs was 1 ppb.

Based on the survey results PCB wastewater treatment is not practical for mills with primary or secondary treatment.

- The total discharge of PCBs from all survey mills is a maximum of 30 lb/year. The total discharge from all recycle paper mills in New York state is estimated at a maximum of 45 lb/year.
- The New York State Department of Environmental Conservation does not have a water quality standard for PCBs; however, a standard of one part per trillion has been previously considered. This receiving water standard, if adopted, may necessitate a reevaluation of the need for PCB monitoring and discharge limitations.
- The Environmental Protection Agency is investigating the need for limitations for the paper industry. Through the EPA's contractor for pulp and paper standards development, it has been indicated that PCB effluent standards will not be proposed. This is a preliminary judgment; and if standards are eventually adopted, they will have to be dealt with by recycled paper mills nationally. ■

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INDUSTRIAL WASTES

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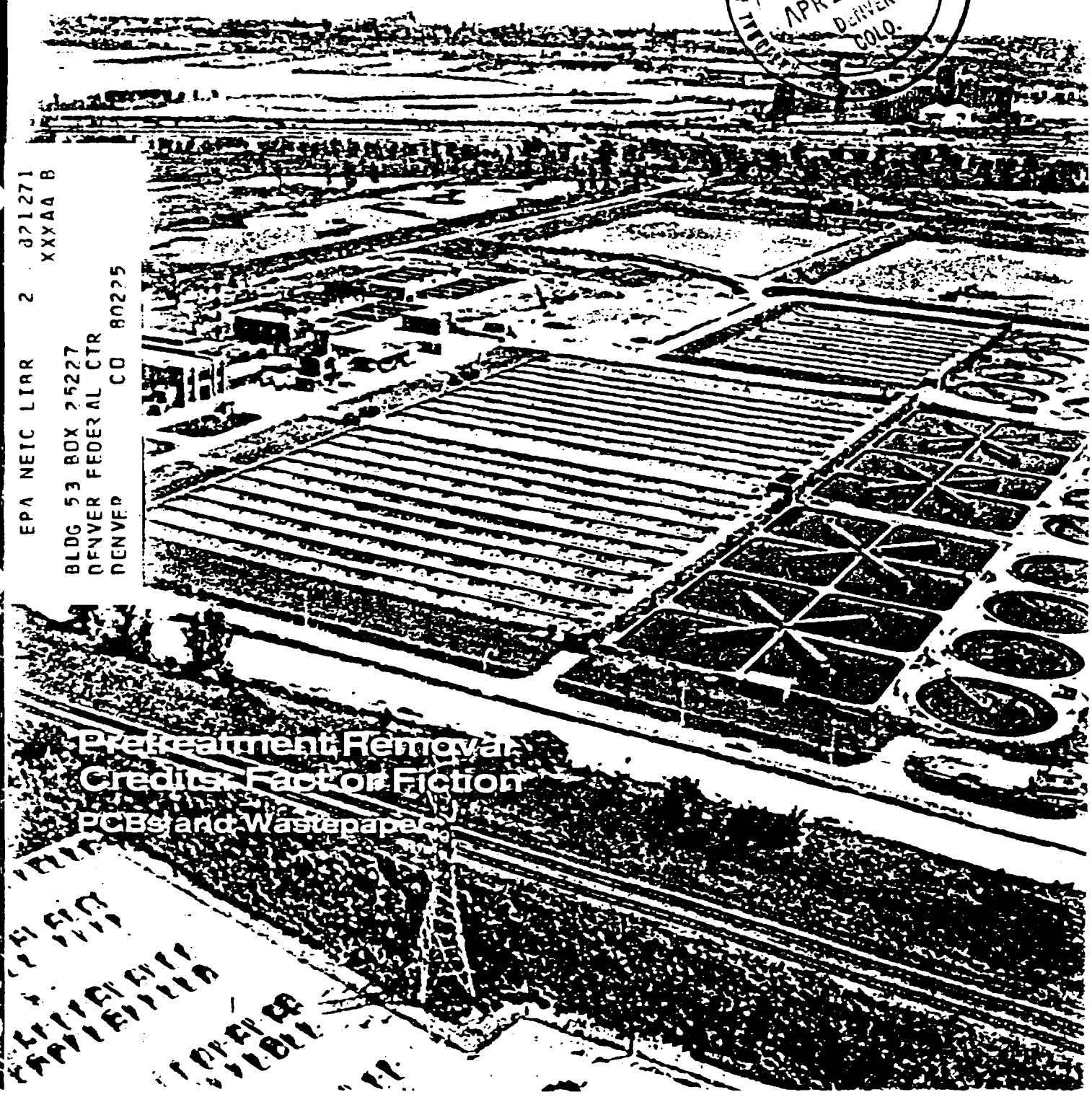
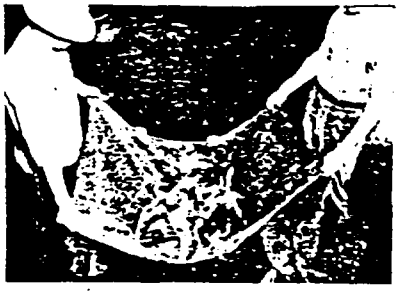


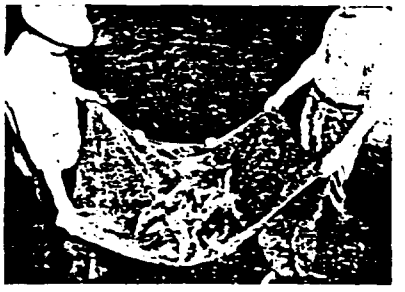
Pretreatment Removal Credits: Fact or Fiction

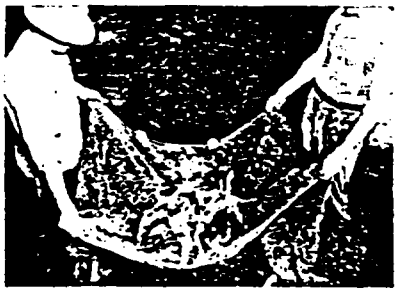
PCBs and Wastepaper

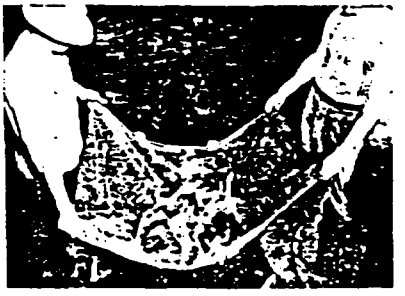
The Persistence of PCBs

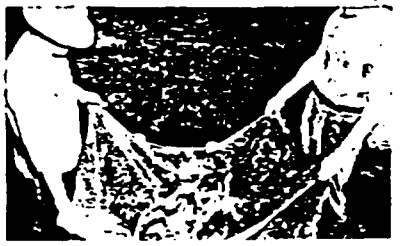
BY ROBERT H. BOYLE and JOSEPH H. HIGHLAND











Interior Dept.

DURING THE HOUSE DEBATE on the Toxic Substances Control Act in 1976, Representative Gilbert Gude, a Maryland Republican, called polychlorinated biphenyls—PCBs—a "mad dog" of the environment. Out of the sea of chemicals that surround us PCBs alone were singled out by name: the Toxic Substances Control Act prohibits the manufacturing, processing, and sale of all PCBs in the United States starting in 1979. As extraordinary as this ban might seem to some, it still does not even begin to solve the problem.

In many ways PCBs can serve as the model for the situation that exists for hundreds of chemicals that now contaminate the environment and man. Their careless spread is a horrifying lesson in what can happen when government fails to regulate the use of toxic substances. The United States will continue to pay the price of PCBs for years: some of the higher chlorinated PCBs may persist for decades.

PCBs are probably the most widespread chemical contaminant known to mankind. They are tough chemical cousins of DDT, but unlike DDT they have not been sprayed deliberately but have instead entered the world through the back door, by discharge, vaporization, leaks, and spills. They are carcinogenic in laboratory rats and mice.

Widely used by industry because of their resistance to heat, PCBs are toxic to a wide range of life. Ingestion of PCBs has caused a host of afflictions in humans, including numbness of limbs, deformities of bones and joints, and stillbirths. Thanks to the ignorance and recklessness of industry and government, PCBs have even been found in animals 11,000 feet down in the North Atlantic.

Fish and wildlife in many parts of the United States are contaminated with PCBs. Many of the fish stocks of the Great Lakes and numerous river systems are now officially too dangerous to eat because they contain 5 parts per million (5 ppm) or more of PCBs, the Food and Drug Administration's current "temporary tolerance" level for fish products. As of this writing, the FDA has proposed reducing the temporary tolerance to 2 ppm. This will eventually mean the loss of even greater supplies of fish.

PCBs are literally almost everywhere in the United States. Indeed, the U.S. Environmental Protection Agency calculates that 91 percent of all Americans have detectable levels of PCBs in their adipose tissues, and 40.3 percent have at least 1 ppm. Both the percentage of Americans showing

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detectable levels of PCBs and the extent of contamination have been increasing over the last several years.

Fifty Years of Ignorance

PCBs did not become a matter of urgent national concern until 1975, even though they had been in use for almost fifty years. In 1927 Theodore Swann, of the Federal Phosphorus Company, set up a pilot plant to supply biphenyl for use as a heat transfer fluid in the refining of lubricating oils. Biphenyl is a derivative of benzene, a liquid aromatic hydrocarbon found in coal tar. By 1929 Swann developed polychlorinated biphenyls, and in 1930 Monsanto took over production. Monsanto, which discontinued production of PCBs in 1977, has sold the chemical under the trademark name of Aroclor. PCBs have also been manufactured abroad under various trade names.

PCBs are made by controlled chlorination of biphenyl with anhydrous chlorine. When biphenyl is chlorinated, chlorine atoms replace the hydrogen atoms. Since each biphenyl has ten hydrogen atoms, as shown in Figure 1, PCBs may have any number of chlorine atoms up to ten.

PCBs are among the most stable organic compounds known to science. This stability makes them useful to industry and depresses ecologists. Highly chlorinated PCBs are able to persist in the environment for years because enzymes are essentially unable to shear the bonding between the chlorine atom and the biphenyl structure. There are only two feasible methods known for destroying PCBs. One is to burn them in a special incinerator generating temperatures in the range of 2,000 to 3,000°F. The other is to expose them to ozone and ultraviolet light. Of course, neither of these methods can be used to eliminate PCBs dispersed in the environment.

Because of their low flammability, high heat capacity, and low electrical conductivity, PCBs have obvious advantages as dielectric and heat transfer fluids. Other properties regarded as favorable by industry include low solubility in water and low vapor pressure. PCBs are also resistant to acids, alkalies, and corrosive chemicals. They are insoluble in ethyl alcohol and water but soluble

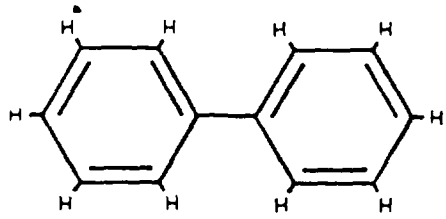


FIGURE 1. Biphenyl Molecule.

in fats. Hence, they tend to become stored in the fatty tissues of organisms.

Because of their industrial properties PCBs have been used in an extraordinary variety of products, so broad that almost no one in the United States has escaped contact (see box p. 8). Besides deliberate uses, PCBs also turn up accidentally. They are a by-product of paper recycling. They have also been found in toilet soaps, barrier creams, hydrated lime used to manufacture glass, water-treatment chemicals, primer paints, coloring compounds, industrial degreasers, additives in zinc alloys, and waxes used in the tool and die-casting process. According to EPA calculations, 10 million pounds of PCBs contaminate the environment each year through vaporization, leaks, and spills.

In 1971 Monsanto announced it would restrict PCB sales to so-called closed-cycle systems, such as capacitors and transformers. U.S. industry manufactures more than 100 million capacitors a year for use in air-conditioning units, refrigerators, television sets, and fluorescent light ballasts. PCBs can be and have been directly discharged into rivers and streams, municipal sewage systems, and the atmosphere during the production of capacitors and transformers. When any of these products are discarded, they are ordinarily taken to dumps, where PCBs can leach through the ground into water or vaporize into the air. In New York and Michigan, several companies drained PCBs from old transformers, mixed them with crankcase oil, and sold them to municipalities as a dust suppressor on roads.

Worker or Industry Lassitude?

As far back as the 1930s, medical reports showed that PCBs were detrimental to the health of workers. As Dr. Barry Commoner, Director of the Center for the Biology of Living Systems at Washington University in St. Louis, has written, "There is much irony—and tragedy—in the PCB story, for it shows that had the scientific community given enough attention to its earlier effects on workers, the present ecological hazard could have been avoided and the long-term effects of industrial exposures would now be much better understood."

In a paper published in 1936, Drs. Jack W. Jones and Herbert S. Alden, of Atlanta, reported on the case of "O.D.," a 26-year-old Negro who began working in the distillation of chlorinated diphenyl (biphenyl) in April 1930. In three years O.D. developed a severe skin disease, chloracne, and his body was covered with pustules. By October 1933, twenty-three of the twenty-four men working in the manufacturing plant had acneform eruptions on the face and body. In December 1933 O.D. complained of lassitude, loss of appetite, and loss of libido, now classic symptoms of PCB poisoning, but as Drs. Jones and Alden reported, "On examination he seemed in good general health. His complaint of lassitude was not borne out by anything more than the usual temperament of the Negro toward work."

In the August 1943 issue of *Industrial Medicine* Dr. Leonard Greenburg, executive director of the Division of Industrial Hygiene of the New York State Department of Labor, reported on outbreaks of chloracne among workers handling electrical equipment containing PCBs and the chemically related chlorinated naphthalenes. "During the past year," Dr. Greenburg wrote, "the Division of Industrial Hygiene of the New York State Department of Labor has conducted an investigation in two cable plants using chlorinated naphthalenes and diphenyls. In this investigation, a large number of cases of dermatitis were found, and several deaths due to liver damage among workers in the industry." One of Dr. Greenburg's main conclusions was that "chlorinated naphthalenes and diphenyls are in general highly toxic compounds and must be used with extreme care. Industrial hygienists should make every effort to see that such exposures are controlled, in so far as humanly possible." Nothing was done.

FROM SALMON TO SEALANTS

Even as U.S. consumers must curb their appetites for those PCB-polluted fish products rated by FDA as too dangerous for the dinner table, their frequent contact with a variety of common household and office products involuntarily exposes them to other sources of polyclorinated biphenyl contamination. In 1975, Karl E. Bremer, of the EPA Region V office in Chicago, listed these past and present uses of PCBs:

They may be found in
- *transformer and power capacitors*
- *heat transfer applications*
- *washable wall coverings and upholstery fabrics*
- *envelope and tape adhesives*
- *coatings for ironing board covers*
- *waterproofers for canvas*
- *film casting solutions*
- *insulating tapes*
- *protective lacquers and epoxy resins*
- *"carbonless" carbon paper*

PCBs are also used as
- *hydraulic fluids*
- *plasticizers*
- *ceramic and metal laminates*
- *flameproofers for synthetic yarns*
- *additives in xerographic processing*
- *additives in caulking and fireproofing sealants*

Swedish Detective

Although PCBs began entering the environment soon after they were first manufactured, they were not detected in the environment until 1966. The detection took place not in the United States but in Sweden. In 1964 the Royal Swedish Commission on Natural Resources asked Dr. Sören Jensen, an analytical chemist at the University of Stockholm, to determine the levels of DDT and other chlorinated pesticides in human fat and in wildlife. Using gas chromatography, in which peaks traced on a graph represent individual chemical components, Dr. Jensen soon discovered, by his own account, "numerous peaks whose retention times did not agree with any of the known chlorinated pesticides." None of these peaks was described in any of the literature, so Jensen had to play detective.

He asked the assistance of ecologists at the Swedish Museum of Natural History. They collected fish, in this instance pike, from the south of Sweden and from Lapland, in the relatively unpolluted north. All the pike showed traces of the unknown compounds, but the farther north the samples of pike, the lower the levels of residues. Reasoning that the compounds might possibly be unknown metabolites of pesticides, Jensen tested this hypothesis by analyzing single feathers from white-tailed eagles collected by the museum from 1888 to the present. The unknown compounds were found in the feather of an eagle collected in 1942 and in eagle feathers from subsequent years, but since chlorinated pesticides had not been introduced until 1945, Jensen knew that the compounds could not be their metabolites.

By chance a white-tailed eagle was found dead in the Stockholm archipelago, and Jensen had the opportunity to examine the whole bird. He discovered that it contained enormous amounts of the unknown compounds. He was able to recognize the presence of chlorine from the unusual isotopic pattern, and by using a series of equations he determined the molecular weights of the parent hydrocarbons. Given the molecular weights, the most plausible formula for the parent hydrocarbon was biphenyl. Logically, then, the unknown substances were polychlorinated biphenyls. Jensen confirmed the identification by injecting a PCB mixture into the mass spectrometer. Gas chromatographic studies also showed that the mixture gave peaks "with retention times identical to those of the unknown peaks from the white-tailed eagle and the other species investigated." Jensen published his findings in *New Scientist* in 1966.

Second Thoughts

After the appearance of Jensen's paper, scientists in the Netherlands, England, Scotland, and the United States began their own investigations. Dr. Robert Risebrough, of the University of California at Berkeley, did important work on West Coast peregrine falcons. A paper of his prompted Monsanto to issue a statement scoffing at both his research and Jensen's. "The conclusions of these scientists are puzzling from several aspects," Monsanto said. "Polychlorinated biphenyls are stable chemical compounds which are essentially insoluble in water. Their use does not make them easily released into the natural environment. . . . It has also been implied that polychlorinated biphenyls are 'highly toxic' chemicals. This is not true."

In the January/February 1970 issue of *Environment*, Risebrough published an article summarizing knowledge to date about PCBs. He called for the establishment of tolerance levels of PCBs in food, and he warned: "The possible PCB hazard, like so many environmental hazards, is one of long-term, low-level exposure and perhaps of effects from its combination with other poisons. There is also the more indirect, but no less real danger of destroying other forms of life—part of the vast interconnected web of species of which man is but one part and on which he depends."

Risebrough took particular note of the ability of PCBs to stimulate or induce the production of enzymes in the livers of birds and rats. "Induction of these enzymes would be one of the expected effects of the polychlorinated biphenyls in people," he wrote.

In the early spring of 1970, John R. Clark, then assistant director of the U.S. Marine Gamefish Laboratory at Sandy Hook, New Jersey, suggested to Robert H. Boyle, a senior writer at *Sports Illustrated*, that the magazine have the eggs and flesh of coastal gamefish tested for residues of DDT and other chlorinated hydrocarbon pesticides. It was high levels of DDT in Lake Michigan coho salmon that caused Clark's concern. Boyle spoke to the editors at the magazine, and they assented. The WARF (Wisconsin Alumni Research Foundation) Institute in Madison, Wisconsin, a leading analytical laboratory, agreed to test the fish, and in May 1970 *Sports Illustrated* correspondents collected fish from the Atlantic, Gulf, and Pacific coasts and shipped them to the WARF Institute. As a result of reading Risebrough's PCB article in *Environment*, Boyle asked the WARF Institute to check the flesh and eggs for PCBs. He also asked that the fish be tested for methyl mercury.

The October 26, 1970, issue of *Sports Illustrated* carried a long article by Boyle reporting on the findings of the WARF Institute. The tests revealed that a number of fish species contained PCBs. Striped bass from the Hudson had especially high levels. They averaged 11.4

ppm of PCBs in their eggs and 4.01 ppm in the flesh.

Because Boyle held a scientific license to seine fish from the Hudson River, he was required to make an annual report to the state conservation department, and on February 17, 1971, he wrote to Carl Parker, chief of the Bureau of Fish, and reported on the levels of PCB and DDT in the striped bass eggs. "These are grim figures," Boyle wrote, "and I certainly think the state should warn fishermen not to eat striped bass eggs." He also informed Parker that the levels in the flesh were "not great reading" and suggested he read the article in full. In reply, Parker scoffed at the WARF findings as unproven allegations.

"Deep Trout"

Despite Parker's response, documents later obtained from the Department of Environmental Conservation (DEC) revealed that the state had begun collecting Hudson River fish as early as the summer of 1970, although PCB analysis was not conducted until 1972. Tests conducted then showed that eleven of twenty-nine largemouth bass from the Hudson had PCB levels in excess of 5 ppm in their flesh. One largemouth had 53.81 ppm. Other 1972 findings included a smallmouth bass with 17.58 ppm of PCBs, a striped bass with 10.48 ppm, a northern pike with 17.78 ppm, and four sturgeon with levels ranging from 5.73 to 7.03 ppm. Out of fifteen white perch tested, seven had excessive levels. Not a word was said to the public. Nor was the public informed that, in 1974 and 1975, the DEC found excessive residues of PCBs in Hudson River striped bass. The highest level was 49.63 ppm.

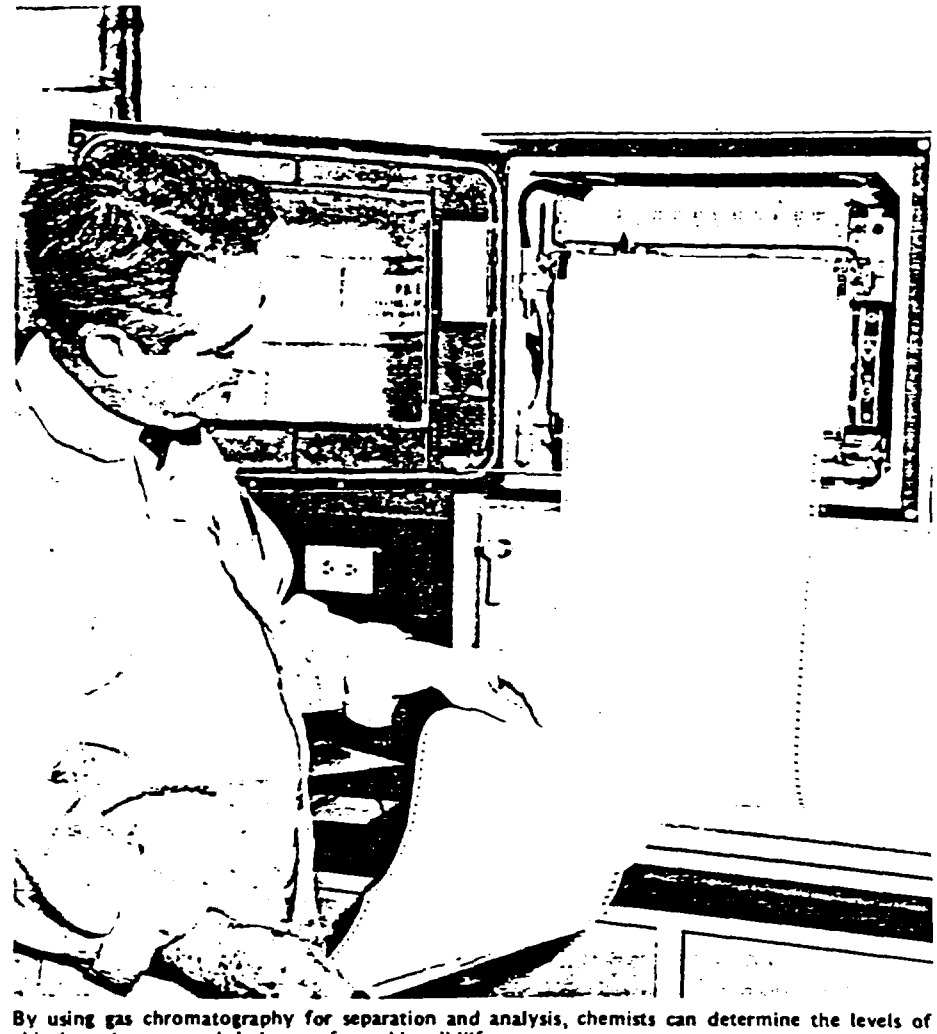
The September 29, 1972, issue of *Science* carried a paper by four Cornell scientists reporting that all lake trout tested from Cayuga Lake contained PCBs and that there was a "highly significant" correlation between the levels of PCBs and the age of the fish. Although Cayuga Lake is well known in New York for its trout fishing, no one in the DEC bothered to call the Cornell scientists to ask about their findings.

The conservation department suppressed information on excessive levels of PCBs detected in Lake Ontario coho and Chinook salmon in the early 1970s. In 1975 a scientist, who requested anonymity (and thus received the code name of Deep Trout), gave Boyle copies of analyses performed on the salmon by the department's Rome Pollution Laboratory between December 23, 1971, and September 26, 1973.

All fish except one had PCB levels in excess of the 5.00 ppm tolerance level established by FDA. There was not a word from the state to anglers. The DEC was busy extolling the wonderful salmon fishery it had established for anglers in Lake Ontario.

Undoubtedly there were other reasons why the state did not go public about PCB contamination of waters in New York. For one, it might have hurt license sales, and the DEC is dependent upon fishing license revenues for income. For another, the truth of the situation might have reflected poorly on Governor Nelson A. Rockefeller and other members of his administration, who had promised in 1965, after voters approved the billion-dollar "Pure Waters" bond issue, that all the waters of the state would be clean by 1972.

Throughout all this there was one recalcitrant scientist within the conservation department. Wildlife pathologist Dr. Ward B. Stone had warned his superiors for years that PCBs were a menace. He had fired off warnings as



Interior Dept.—Sport Fisheries & Wildlife/R. G. Schmidt

By using gas chromatography for separation and analysis, chemists can determine the levels of chlorinated compounds in human fat and in wildlife.

ROBERT H. BOYLE is a senior writer at *Sports Illustrated*. **JOSEPH H. HIGHLAND** is the chairman of the Toxic Chemicals Program at the Environmental Defense Fund in Washington, D.C. This article is an abridged version of a chapter from the Environmental Defense Fund's new book, *Malignant Neglect*, about environmental health hazards and what can be done about them.

far back as 1970, but despite all his memos, his efforts were, in Stone's word, "futile." "No one really cared," says Stone. In one very typically blunt memo, he advised: "I hope the department begins to take some of these toxic problems seriously . . . because it is doubtful these problems can be put off. In fact, they already have been put off far too long." The state continued to put off any response to the problem.

Sounding the Warning

In May 1974 the EPA held a hearing on toxic pollutant effluent standards; PCBs were a major issue. By now industry was beginning to have second thoughts about PCBs, and some indication of the threat that PCBs were posing to the environment and human health can be gleaned from an answer given by Dr. James H. Wright, a chemical engineer with the Westinghouse Electric Corporation. Asked by EPA attorney Alan Eckert, "When you took these steps to prevent the discharge of PCBs, did you attempt to flush out or clean sewers contaminated with PCBs from old operations?" Dr. Wright answered, "I would be the most frightened man in the world to recommend the flushing out of old lines and old drains."

Dr. Wright's startling reply drew no public attention, but the answers given by the next industry witness, Dr. Edward L. Simons, who appeared for General Electric, eventually helped give rise to national public alarm. In the course of his testimony Dr. Simons testified that a GE transformer plant in Rome, Georgia, was discharging PCBs into the Oostanaula River "on the order of one pound per day." The effect on organisms living in the river was not known because, as Dr. Simons admitted: "We have made no measurements of biota for any facility of the General Electric Company."

Simons also testified that another GE transformer plant, in Pittsfield, Massachusetts, released PCBs into the Housatonic River. In the vicinity of the outfalls, according to "occasional samples" by GE, the river's water contained between 1 and 3 ppb (parts per billion) of PCBs, while levels in the river sediments varied from 130 to 1,300 ppm. At "a distance of something between one and six miles" downstream, PCBs were "not detectable" in the water, but PCB levels in the river sediments were between 30 and 60 ppm. Simons pointed out that the Pittsfield plant was releasing an average of 0.1 pound per day of PCBs into the Housatonic, with "occasional excursions as high as 0.8 pounds per day." These answers were horrendous enough to anyone acquainted with the toxic properties of PCBs, but the most dramatic moment in the hearing came when Eckert asked Simons about two GE capacitor plants at Fort Edward and Hudson Falls, New York, on the Hudson River. Simons replied that the two plants were discharging twenty-five to thirty pounds a day to the river on the average.

Interior Dept.—Nat'l Park Svc.

National Breakthrough

This was to be the breakthrough nationally on PCBs, although GE's dumping of PCBs into the Hudson was to be subject to more bureaucratic twists and turns. In a memo dated May 31, 1974, Sandra P. Kunsberg, an attorney in the Water Envorcement Branch of the EPA Region II office, wrote to Dr. Richard Spears, chief of the Surveillance and Analysis Division:

Following the [toxic pollutant effluent standards] hearings [at which GE admitted dumping PCBs], in a memorandum from Alan Eckert to Meyer Scolnick, the Office of Enforcement and General Counsel in New York recommended that this office undertake a careful investigation of PCB discharges from the Fort Edward and Hudson Falls facilities to determine whether or not there exists a health hazard within the meaning of Section 504 of the FWPCA [Federal Water Pollution Control Act].

Section 504 authorizes the administrator of the EPA to sue to restrain persons whose pollution of waters causes "substantial endangerment to the health of persons or to the welfare of persons, where such endangerment is to the livelihood of such persons." Dr. Royal J. Nadeau and Robert F. Davis, of the Region II EPA Laboratory, conducted the recommended investigation, and they reported, based on GE figures, that the Fort Edward plant was discharging up to 30 pounds a day of PCBs into the Hudson River, while the Hudson Falls plant was discharging up to 17.6 pounds. Minnows they collected below the GE discharge averaged 78 ppm of PCBs, while a rock bass contained 340 ppm of PCBs. Nadeau and Davis wrote, "The PCB level in the rock bass is greater than the maximum level documented for fish taken from any industrial river in the U.S. . . . This represents a new record for PCB contamination of fresh water fish." They noted that youngsters from Fort Edward fished areas downstream and that "ingestion of these fish by the populace would certainly lead to contamination of specific tissues in their bodies."

Nadeau and Davis submitted their report to the EPA Region II office, but effective action was slow in coming. Richard Flye, the chief of the Region II Water Enforcement Branch, apparently saw little reason to raise a fuss. As he remarked afterward, "From some of the evidence I've seen, you can eat PCBs just like an ice cream cone."

Months passed, but the Nadeau-Davis report was not forgotten. It shocked scientists at the EPA National Water Quality Laboratory in Duluth, Minnesota, and when no action was forthcoming, Dr. Gilman Veith of the lab wrote a "review" of the Nadeau-Davis report on July 7, 1975.

This field investigation has revealed the largest source of PCBs into the aquatic environment that I know of. . . . It is

my opinion that this report has been written in a conservative manner and does not by itself adequately describe the significance of the G.E. discharge into the Hudson River. . . . The river for many miles downstream from the G.E. discharge could be contaminated with PCBs at levels one hundred times over the 10 ppt [parts per trillion] guideline set forth by EPA in 1972 and a thousand times higher than the recommended water quality criteria [of 1 ppt].

Veith's review was forwarded to the Region II office, and the bureaucrats there finally realized that the Nadeau-Davis report was too explosive to contain. The regional administrator, Gerald Hansler, thereupon finally passed it on to Ogden R. Reid, the new commissioner of the Department of Environmental Conservation.

The report astounded Reid, who began a preliminary investigation of his own. He soon learned that the department had known for several years that striped bass from the Hudson and coho and Chinook salmon from Lake Ontario were heavily contaminated by PCBs. Reporter Richard Severo, of the *New York Times*, persuaded Reid to give him the story, and on August 8, 1975, Severo broke it on the front page. In the story Reid warned the public against eating stripers from the Hudson or salmon from Lake Ontario because they contained more than 5 ppm of PCBs, the FDA's temporary tolerance for fish. With the appearance of that warning on the front page of the *New York Times*, PCBs at last became a national issue.

Complaints and Compliance

Even after Reid's warning, EPA bureaucrats were less than helpful. Thomas Whyatt and John Harris-Cronin, of the Hudson River sloop *Clearwater*, began an investigation. When Harris-Cronin went to the EPA office in Manhattan to request a copy of the Nadeau-Davis report, Richard Flye told Harris-Cronin he would have to submit his request in writing. Aware of EPA runaround tactics, Harris-Cronin had had the foresight to bring stationery with him, and he immediately wrote out the request on the spot. Flye looked at the letter and then said to Harris-Cronin that under the Freedom of Information Act "we have 10 days to decide whether or not to honor your request, and the tactic we're going to employ with you is to use them all."

In Albany, Commissioner Reid learned, to his shock and dismay, that the New York State Department of Health had given the GE plants a water quality certification in 1971, and in 1973 the chief of the enforcement section of the Division of Pure Waters in the DEC had done the same. Still Reid had the DEC bring a complaint against GE, accusing the company of violating state water quality standards and the Environmental Conservation Law. Professor Abraham Sofaer, of Columbia University Law School, served as the hearing officer in the case, which began in the fall of 1975.

The case did not go well for GE. In an affidavit the company admitted that 65 employees had become ill in the two plants in the course of a fifteen-year period under conditions that "may have been caused or aggravated by exposure to PCBs." Of approximately 1,300 employees, 49 had complained of "allergic dermatitis," and 16 others had reported suffering from fungus, nausea, asthmatic bronchitis, and nasal and eye irritations. Another setback for GE came when Dr. Gerald J. Lauer, vice-president of Ecological Analysis Inc., later characterized by Professor Sofaer in his decision as "a firm that engages for profit in preparing testimony for corporations in environmental litigation," testified that the average concentrations of PCBs in the edible portions of fish sampled from the river were well below the FDA guideline of 5 ppm. During cross examination by Sarah Chasis, of the Natural Resources Defense Council, and Philip Gitlen, of the DEC, Lauer admitted that there had been some omissions and errors in his data. The fish sampled had been tested by Woodson-Tenent Laboratories in Memphis, Tennessee, and as Lauer put it, "I got most of my data over the phone." He admitted that levels in four fish were well over the FDA limit of 5 ppm, with levels of 66 ppm, 75 ppm, 112.1 ppm, and 143.2 ppm. GE's lawyer, N. Earle Evans, Jr., was so taken aback at "the cloud" over the data that he offered to consent to a withdrawal motion of the data. GE also agreed to request that Woodson-Tenent prepare a complete analysis of all its Hudson River samples. The laboratory did, and several fish had more than 100 ppm in their edible flesh, while concentrations in what GE called "non-edible" tissues reached as high as *1,178 ppm*!

U.S. Fish & Wildlife Svc./George B. Kelez

In February 1976 Professor Sofaer rendered his decision and found GE guilty of violating state water quality standards. "PCBs are toxic substances," Sofaer wrote in his decision, "capable in sufficient quantities of causing skin lesions, destroying cells in vital body organs, adversely affecting reproduction, and inducing cancer and death."

A new hearing was ordered to determine what steps the company should take to rid the Hudson of an estimated 500,000 pounds of PCBs lodged in the riverbed silt and backwaters. The case was settled by Reid's successor, Peter Berle, in September 1976, when GE agreed to pay the state $3 million in cash and contribute an additional $1 million in research. The DEC for its part agreed to contribute $3 million of its own, making the total clean-up package $7 million.

Inasmuch as estimates for dredging the entire contaminated section of the upper Hudson now range up to $204 million, $7 million might seem a paltry sum. But legally pursuing the case further posed problems, since the state

NO END IN SIGHT

With passage of the Toxic Substances Control Act (TSCA) and the curtailment of commercial PCB production, focus on the PCB problem has shifted away from the problems associated with the discharge of PCBs into the nation's waterways during the manufacturing process to the hazards posed by the continued use and eventual disposal of PCBs. While PCBs and PCB-containing products were being commercially produced, most of the concern about environmental degradation and adverse effects on human health centered on the direct contamination of streams and rivers, and the subsequent bioaccumulation of PCBs in fish. Many streams, rivers, and lakes throughout the United States have been so contaminated that fish from these bodies of water continue to exceed FDA tolerance limits.

During the first six months of 1979, two major lawsuits were filed as part of a continuing effort to make the federal government handle the PCB problem more responsibly and effectively. The first, filed in January by the Environmental Defense Fund (EDF) in the U.S. Court of Appeals for the District of Columbia, seeks to compel the promulgation of a final regulation revising the current tolerances for PCBs in food. The Food and Drug Administration (FDA) first promulgated tolerances for PCBs in food in 1972; in 1977 they proposed to lower these tolerances because new scientific data showed that PCBs were cancinogenic and caused learning and behavioral difficulties in infant monkeys nursed on PCB-contaminated milk. However, after more than two years, the FDA still has not finalized these regulations. EDF is therefore seeking a court order directing the FDA to promulgate final revised tolerances.

In a related matter, EDF has asked the U.S. Court of Appeals for the District of Columbia to review the Environmental Protection Agency's (EPA) new regulations governing the future use and disposal of PCBs. Under § 6 of TSCA, EPA was given authority to put an end to the manufacture and use of PCBs over a two-and-a half year period, and to make sure that future disposal was conducted in an environmentally safe manner. After months of delay, EPA has at last promulgated its final ban regulations. But because of their obvious inadequacy, EDF is now seeking a court order sending the regulations back to the Agency for revision. It is clear from the new PCB ban regulations that most current uses of polychlorinated biphenyls will continue as long as the present PCB equipment remains in service. While most of the continued use authorizations are limited to five years, EPA has indicated a willingness to grant further extensions where the equipment remains usable. Of the estimated 750,850,000 pounds of PCBs still in use, only 5,430,000 pounds are banned immediately from further use. Thus, more than 99 percent of the PCBs currently in existence will continue to be used. In addition, many millions of pounds of PCBs will go totally unregulated. It is estimated, for instance, that there are 450,000,000 pounds of PCBs in capacitors of varying size. Of this total, 282,500,000 pounds are covered by the regulations—albeit inadequately. This means that 167,500,000 pounds of PCBs will go *totally unregulated*. Neither their use nor their disposal will be regulated to ensure that they do not add to the already high environmental burden of PCBs.

itself was culpable for having given GE water quality certifications.

GE agreed to discontinue the use of PCBs at Hudson Falls and Fort Edward on or before July 1, 1977. Independent scientists and environmentalists were appointed to serve on an advisory board to explore ways to rid the river of PCBs, but barring some miracle, the Hudson will not be free of PCBs for decades.

Beyond the Hudson

A number of the fish species in the Hudson migrate in and out of the river system and a fish can quickly become contaminated by PCBs by taking in bottom sediments, eating lesser organisms that are polluted, or simply swimming through contaminated water. PCBs bioaccumulate in fish, so that the levels of contamination can become far higher than the PCB concentrations to which the fish are exposed.

Interestingly, stripers that spawn in the Hudson migrate as far north as Massachusetts, possibly Maine, and as far south as New Jersey, possibly Delaware. The Oak Ridge National Laboratory has estimated that Hudson-spawned stripers constitute from 50 to 90 percent of the bass caught in Long Island Sound and off the south shore of Long Island and the northern New Jersey coast. These Hudson River fish pose a threat, then, to fishermen, who may eat them after they are caught in supposedly "clean" far from the Hudson.

Although the Hudson is the most dramatic case of PCB pollution, there are numerous other bodies of water with severe problems. The U.S. Fish and Wildfire Service, which regards the presence of one-half part per million (0.5 ppm) in a fish egg as a source of biological trouble, maintains one hundred monitoring stations across the United States, and trouble spots have ranged from the Merrimack River in Massachusetts to the Saint Johns River in Florida on the Atlantic Coast, from the Apalachicola in the Florida panhandle to the Rio Grande on the Gulf Coast, and from the Sacramento to the Columbia and the Snake on the Pacific Coast. Inland drainage systems, such as the Mississippi, Missouri, and Ohio system, have their trouble spots. Even the Yukon River in Alaska is affected, as are parts of the Atlantic, Gulf, and Pacific coasts.

Much of the Great Lakes system, the single largest supply of fresh water in the world, is contaminated. The Great Lakes states restricted the use of DDT in 1970, and by 1975 DDT levels had dropped 87 percent in Lake Michigan fish. By contrast, PCB levels have remained ominously high. According to scientists at the EPA National Water Quality Laboratory in Duluth, all Lake Michigan trout and salmon greater than twelve inches in length exceed the FDA limit on PCBs of 5 ppm. PCBs enter Lake Michigan by various routes, of which atmospheric transport is probably the most important. Dr. Thomas J. Murphy, of DePaul University, estimates that almost half the estimated 2,800

USDA

The FDA and U.S. Department of Agriculture have reported incidents of PCB contamination of poultry. When inspection reveals high levels of PCBs in the birds, their products must be kept from the market and destroyed.

pounds of PCBs that contaminate the lake each year enter through precipitation. Up to 0.20 ppb of PCBs have been found in snow falling on Milwaukee.

Even Lake Superior, which has the largest surface acreage of any freshwater body in the world and relatively little industry along its shores, has a PCB problem. Large lake trout often contain more than 5 ppm of PCBs. Two-thirds of the Superior basin is the lake itself, and the belief is that PCBs are entering the lake via atmospheric transport.

Testing Mother's Milk

In addition to PCB contamination of fish and wildlife the U.S. Food and Drug Administration and the U.S. Department of Agriculture have reported numerous specific incidents of PCB contamination of food. For instance:

In 1970 the Campbell Soup Company detected high levels of PCBs in chickens raised in New York State. As a result, 146,000 chickens were destroyed.

In July 1971 the Monsanto Chemical Company informed the FDA that large amounts of fish meal might have become contaminated with PCBs during pasteurization as a result of a leak in a heating system in Wilmington, North Carolina. The FDA discovered that the leak began in April and continued through July. More than 123,000 pounds of egg products and 88,000 chickens had to be destroyed.

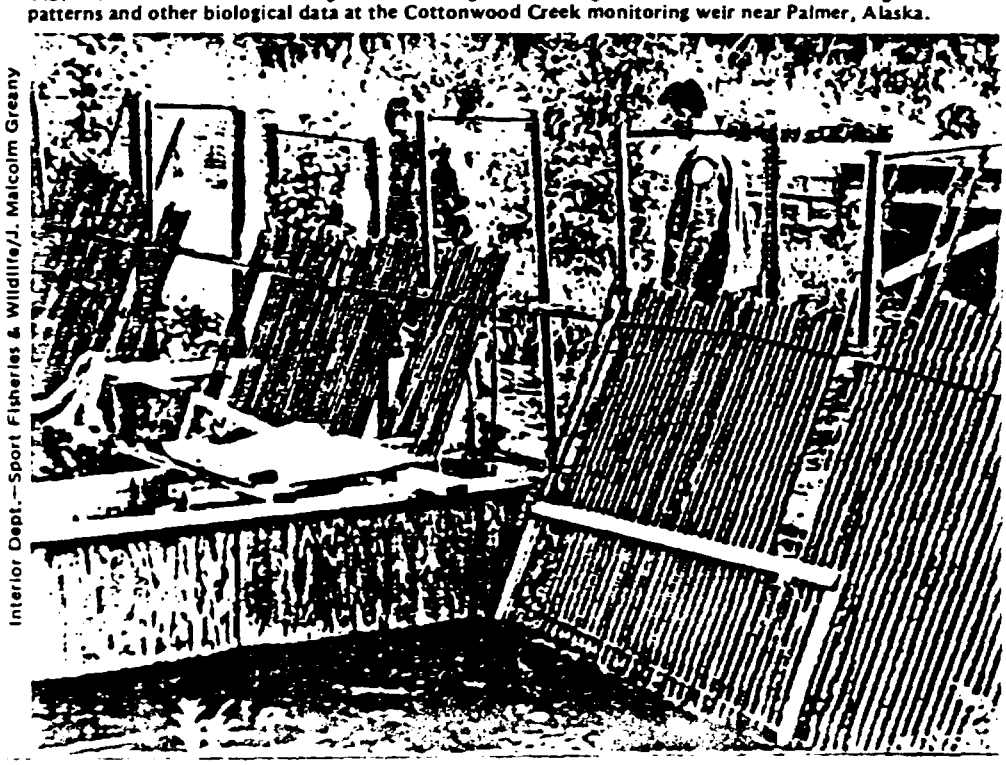
In 1971 the FDA also learned that high levels of PCBs, up to 20 ppm, had been found in turkeys by the USDA and Swift and Company. About 50,000 birds were kept from market.

Research of the utmost significance has been done by Dr. James R. Allen and his colleagues at the University of Wisconsin Medical School. This research, which has been underway for several years, involves the use of rhesus monkeys, nonhuman primates, as the test animals, and their close relationship to man is of importance. Allen and his colleagues fed six rhesus monkeys food containing 25 ppm of Aroclor 1248 for two months. The monkeys developed facial swelling, loss of hair, including their eyelashes, and acne lesions within a month. One of the monkeys died from PCB intoxication two months after going off the PCB diet.

In another experiment the scientists fed two groups of female rhesus monkeys food containing 5 ppm and 2.5 ppm of Aroclor 1248. Within two months both groups of monkeys had lost hair from the face and neck, and their skin was of "a sandpaperlike texture." Both groups developed acne, although the 2.5 ppm group developed the lesions later in time as the PCBs accumulated. In six months' time all the monkeys in both groups were bred to control males that had not been fed PCBs. Six of the eight 5 ppm females became pregnant. Four of the six pregnant females aborted or resorbed the fetus, one gave birth to a stillborn, and

(continued on page 37)

U.S. Fish and Wildlife Service gamefish management biologists collect information on migration patterns and other biological data at the Cottonwood Creek monitoring weir near Palmer, Alaska.



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PCBs

(continued from page 13)

one to a very undersized infant. All eight monkeys in the 2.5 ppm group became pregnant, and of these, two resorbed the fetus, one aborted, and five gave birth to undersized infants. These five infants and the one survivor of the 5 ppm group were permitted to nurse on their mothers. The survivor of the 5 ppm group and two of the 2.5 ppm infants died while nursing. After weaning, the three remaining infants exhibited both behavioral and learning disabilities. They were hyperactive and did not learn various tasks as well as control monkeys under the same conditions.

The Environmental Protection Agency began testing the milk of human mothers in 1976. Preliminary results indicated that essentially all samples of mother's milk had detectable levels of PCBs. Indeed, the average, 1.8 ppm, gives an infant seven times the amount permitted in cow's milk by the Food and Drug Administration. One nursing mother in Michigan had 10.6 ppm of PCBs in her milk, and that dosage approaches the levels that caused learning disabilities and hyperactivity in two groups of monkeys studied by Allen.

No one knows what effect PCBs may have as an additive or synergist with other contaminants widely present in mother's milk, such as aldrin, dieldrin, or hexachlorobenzene. Indeed, any level of any carcinogenic agent in a nursing infant or any other human should be viewed with concern.

A Continuing Problem

Under the U.S. Toxic Substances Control Act, which became law in the fall of 1976, PCB sale, manufacture, or use in anything but closed systems was prohibited in 1978, and for all uses in 1979. But this ban, as noted previously, does not even begin to solve the problem. There is, for instance, no comprehensive national recovery program for the 758 million pounds of PCBs currently in use. There is no program to deal with the 290 million pounds in dumps and landfills and the 150 million pounds in soils, water, and air. The EPA has even needlessly delayed action it proposed to take months ago under the Toxic Substances Control Act.

A number of agencies are remiss in affording more immediate protection to the public. In 1973 the Food and Drug Administration set temporary tolerances for PCBs in food and food packaging materials. In light of current scientific evidence on the carcinogenicity of PCBs and their effect on nonhuman primates, these action levels must now be considered woefully obsolete. In more recent times the FDA has been reviewing the latest scientific information to determine if the action levels should be revised, and the agency in 1977 put forth the proposal that the action level in fish be reduced from 5 ppm to 2 ppm. No final action on this proposal has been taken. In addition, 2 ppm of PCBs would still be unsafe. Even existing FDA coverage must be considered incomplete. There is, for example, no standard for PCBs in beef, or in water used in food or drug manufacturing.

For several years the EPA has been aware of air pollution problems occurring from the use of PCBs, but to date the agency has taken no action to curtail emissions. Neither has the EPA taken action to set standards with respect to the level of PCBs permissible in human drinking water. Indeed, the EPA has suggested that it *not* regulate the disposal of approximately 150 million pounds of PCBs in small capacitors and light ballasts.

Given the record of government failure and industrial negligence, PCBs are free to poison the environment and human beings for decades to come. A new and vigorous approach to the problem is imperative.

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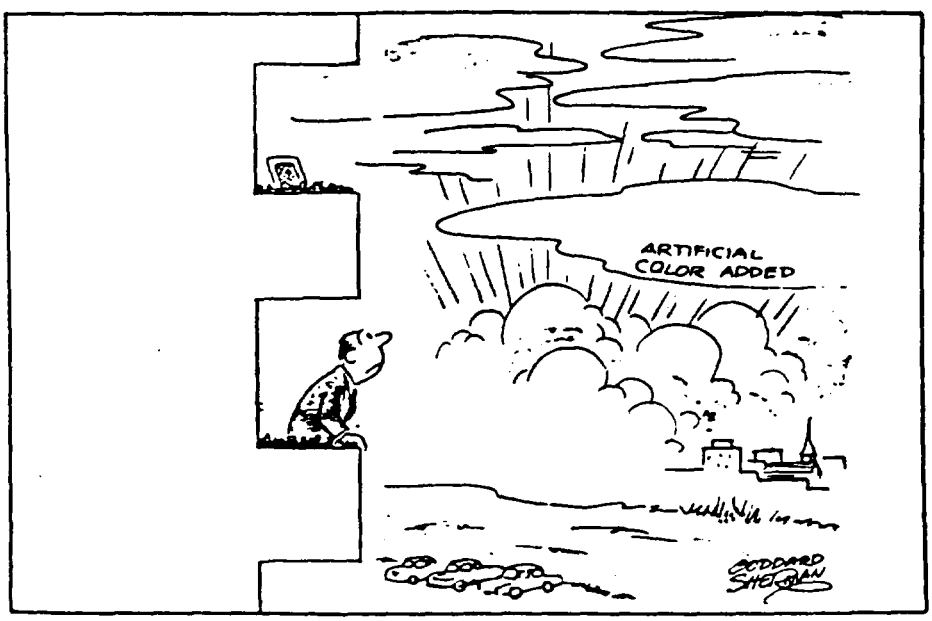

Overview—China

(continued from page 5)

mountain areas are interspersed with industrial plants emitting dense plumes.

Third, EPO is dealing with current pollution problems by encouraging modification of raw materials, process change, and by-product recovery; supporting the research needed to solve certain problems; and monitoring industrial activities. Fourth, it proposes new environmental initiatives whenever necessary.

The environment management problem we discussed most often was how to obtain industrial compliance with pollution control plans. Apparently many plant managers are not convinced that the additional costs of production entailed by the installation of pollution control equipment are a worthwhile investment. They are more influenced by directives calling for keeping production costs low than by the public relations campaign calling for industry to serve the best interests of the people.

The EPOs at the central and provincial levels of government do not have the authority to levy a fine on an industrial plant which does not comply with pollution control plans. In any case, a fine would probably not be a very strong inducement because it would just be a transfer from one government unit to another rather than a reduction in profit. The EPO is attempting to gain some compliance by emphasizing the economic importance of by-product recovery (called "multiple use" by the Chinese) and monitoring the enforcement of the current law which does require plants whose residuals cause damage to fields and animals to compensate the owners. The EPO is considering such new inducements as deductions from the salary of a plant manager and having him demoted for failing to install and operate pollution control equipment, but these inducements will probably be difficult to implement if a plant manager is successful in meeting his production targets.

Another pressing problem for the EPOs is setting appropriate ambient

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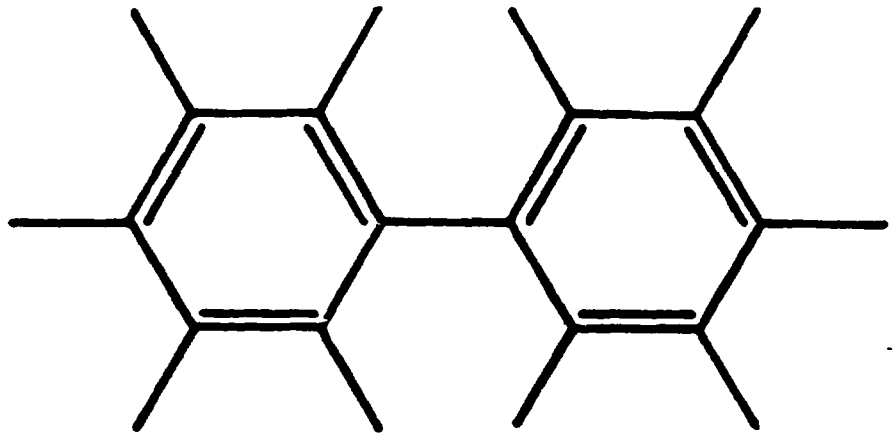

BY A. KARIM AHMED

The accumulation continues

POLYCHLORINATED BIPHENYLS (PCBs) were first introduced into commercial use more than 45 years ago, and for a long time the widely used industrial chemicals were considered to be relatively nontoxic. Their potential threat to the environment, however, was recognized in 1966, when the Swedish scientist, S. Jensen, observed the presence of the compounds in fish and wildlife samples while analyzing for chlorinated hydrocarbon pesticide residues.[1,2] Since that initial discovery, PCBs have been detected in numerous aquatic and animal species and have been found in high concentrations in industrial waste discharges, river-bottom sediments, food packaging materials, and in foods such as poultry, fish, and dairy products. Detectable levels of the chemical substance have been found in marine life ranging in size from small (plankton and zooplankton)[3,4,5] to large species (tuna).[6] High levels of PCBs have been found in fish-eating and predatory birds,[7,8,9,10] demonstrating the process of concentration of the substance along the food chain from lower to higher animals,[11] a process which unfortunately has become all too well known in the era of modern industrial and agricultural activity. Relatively large concentrations of PCBs also have been discovered in the eggs of wild birds.[6,9,12]

During the past ten years, PCB residues have been analyzed in wildlife and fish samples in Holland, Germany, and the Baltic Sea.[7,13] Off the coast of Scotland, PCB residues have been found in several marine species, the contamination presumably having arisen from sewage wastes dumped into the open sea.[14] Nevertheless, despite global incidence of the chemical in the environment, residues are generally found mostly in regions of the world where there is intense industrial and urban activity. In the U.S., PCBs in increasing amounts have been detected in the eastern and western coastal regions, in highly urbanized areas (for instance, in and around the Chesapeake Bay, San Francisco Bay, and Puget Sound), and in regions where industrial discharges are known to occur, such as Escambia Bay, Florida, and the Hudson River.[8,15,16,17]

The environmental accumulation of PCB residues continues despite attempts by both industry and the federal government to improve the situation. As will be explained, industry's countermeasures have been

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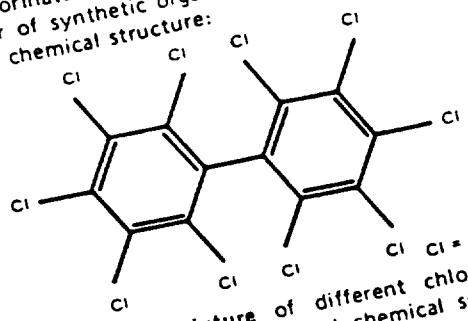
STRUCTURE OF PCBS

Polychlorinated biphenyls (PCBs) is a generic term used for a number of synthetic organic compounds that possess the following chemical structure:



PCBs are a mixture of different chlorinated biphenyl homologs (very closely related chemical substances). Thus, different PCB formulations or products may contain mixtures of biphenyls which have different percentages of the many possible chlorinated homologs, each containing a different number of chlorine atoms per biphenyl molecule. Monsanto Company, the sole U.S. producer of PCBs, sells its product under the trade name Aroclor. This product is sold under different formulations: Aroclor 1268, 1262, 1260, 1254, 1248, 1242, 1232, 1221, 1016, and so on. The last two digits in this biphenyl series denote percentage of chlorine in the mixture. Aroclor 1016 is an exception to this nomenclature, since it contains 41 percent chlorine and is, in fact, very similar in composition to Aroclor 1242. Trade names used by foreign manufacturers include: Phenoclor and Pyralene (Prodelec, France), Kannechlor and Santotherm (Kanegafuchi Chemical Company and Mitsibushi-Monsanto, respectively, Japan), Clophen (I. G. Farben-Industrie A. G., Germany), Fenclor (Caffaro, Italy), and Sorol (U.S.S.R.).

ment action has

mpact of PCBs on best illustrated by amination of sport heries in the Great lsewhere. According Michigan Department ces, levels of PCBs in ho salmon from Lake a sharply increasing . By 1974, the values ore than 10 to nearly llion (ppm), well above d and Drug Administra- maximum allowable of 5 ppm for fish and n sharp contrast, DDT same species of fish clining trend since 1970. consumption in the U.S. d.[18] Not only are the eco- ces of continued PCB ion ominous, but the potenic implications are serious. total annual value of sport Lake Michigan is as high as llion.[19] In addition, the com- catch of fish is now worth .5 million each year.[20] Michigan studies are supported ies made by the Wisconsin Dent of Natural Resources. Fish s collected in 1974 showed ex- e contamination.[21] Affected included Chinook salmon, Coho n, brown trout, tiger trout, lake , whitefish, and carp. Generally, residue levels ranged well above 5 , with lake trout (up to 43.8 ppm) carp (up to 51.6 ppm) showing highest concentrations.

In Upper Fox River, samples ken at one station by Wisconsin re- archers in 1975 showed high concen- trations of PCBs in white suckers (32.7 ppm), carp (average 35.9 ppm), and northern pike (average 15.4 ppm). Samples from several areas of the Mississippi River analyzed by the department in 1973 showed similar residues of PCBs in walleyed pike, largemouth bass, northern redhorse, and carp. An interagency task force on PCBs, formed of federal and several state agencies of Minnesota and Wisconsin, sampled fish in the Mississippi River during the early summer of 1975 and again found similar levels of PCBs in several fish species.[22]

Elsewhere, the Division of Fish and Game of the Commonwealth of Massachusetts has in the past detected high PCB levels in fish in several rivers and streams in the state.[23] From other data collected by the agency, it is clear that in 1972 there was extensive contamination of a large variety of fish species in nearly all the rivers which were sampled in the state, with mean PCB residue levels ranging between 1.11 and 197.0 ppm.

To date, however, the most contaminated fish in the U.S. have been found in the upper reaches of the Hudson River, near and below two General Electric plants in the Fort Edward-Hudson Falls area in the upper Hudson River more than 40 miles north of Albany. The two plants manufacture electrical capacitors in which PCBs are used as insulating fluids. In 1974, sampling by the U.S. Environmental Protection Agency (EPA) showed yellow perch and shiner minnows to contain average PCB residues of 17 and 78 ppm, respectively, with one rock bass sample containing 350 ppm.[24] It appeared that the two General Electric plants had been discharging approximately 30 pounds of PCBs into the river each day; in recent months, this amount seems to have declined to less than 10 pounds per day. Fish samples collected in 1975 by the New York State Department of Environmental Conservation confirm the earlier findings. The composite PCB averages in fish caught near one plant show typical values well above 20 ppm, with one American eel sample yielding residue values of 403.4 ppm (concentration measured as Aroclor 1016/1242).[25]

As large amounts of PCBs were being discovered in the upper Hudson River, New York State Department officials also were finding high residues of PCBs in fish species of recreational and commercial importance farther south in the Hudson River.[25] Striped bass caught near the Albany area showed total PCB residue values of between 11.08 and 89.76 ppm. Fish caught in the lower reaches of the river had a relatively higher concentration of an older PCB formulation (Aroclor 1254), indicating long-range transport of PCBs discharged by industrial activities in the past. Striped bass sampled near the West Point and Tappan Zee area of the Hudson River (50 miles north of New York City and several hundred miles south of the Fort Edward-Hudson Falls area where the General Electric plants are located) had total PCB residues of from 1.16 to 7.54 ppm. Similarly, a sample of American shad in the Poughkeepsie area showed PCB residue levels of 9.0 ppm.

Disruption of the striped bass commercial fishing industry on the Hudson

Environment — Robert C. Smith

High levels of PCBs have been detected in aquatic birds.

River would have serious economic consequences, since the value of the annual commercial catch appears to be about $1.2 million.[20,27,28] In addition, the annual take of striped bass by sportsmen exceeds the annual commercial catch.[28] Furthermore, the overall problem is serious in its relation to reproduction in striped bass. The striped bass is a migratory fish which has stock that intermingles in various locations such as the Chesapeake Bay, the Delaware River, and the Hudson River regions. As a result, PCB contamination may affect a large segment of the entire North Atlantic stock of striped bass.

Bottom Buildup

Of equal concern is the accumulation of PCBs in river-bottom sediments. PCBs, like most chlorinated hydrocarbons, are highly insoluble in water and tend to settle and then be adsorbed on silt and fine particles on the bottom.[15]

Data collected by the U.S. Geological Survey (USGS) on the Hudson River during the years 1973 and 1974 show the presence of PCBs in the water and in the bottom sediments of the river.[29] Values ranging between 0.3 and 3.0 parts per billion (ppb) PCBs have been reported in water samples collected at several stations on the Hudson River. The sampling sites include locations near Poughkeepsie, Waterford, Chelsea, and Rhinebeck.

In contrast to water samples, PCB concentrations analyzed from the river-bottom sediments showed considerably higher values characteristic of the insoluble nature of PCBs. The USGS data are summarized below:[29]

PCB Concentrations in Hudson River Sediments, USGS

Date	Station	PCB in Sediments (ppb)
7/74	Chelsea	3,200
7/74	Poughkeepsie	11,000
7/74	Waterford	13,000
7/74	Winebrook Hills	0.1
9/74	Chelsea	1,800
9/74	Poughkeepsie	3,600
9/74	Waterford	640
8/75	Roger's Island, south of Glenn Falls	18,000

More recently, the EPA conducted water and sediment analyses for PCBs in the vicinity of Ford Edward, New York, near one of the General Electric plants. Water samples collected directly south of the plant's waste-water discharge pipes had PCB values of 2.2 to 3.1 ppb. Nearby sediments, in contrast to the water, contained 540,000 to 2,980,000 ppb (540 to 2,980 ppm), with 6,600 ppb being detected several miles downstream of the discharge pipes.[24] These concentrations obviously are much larger than those at the more distant sites checked by USGS personnel, even though the latter concentrations are themselves excessive. One immediate practical problem is that the largest concentration of PCBs is likely to exist in the deep navigation channel of the Hudson, and dredging in this channel will further disperse the material.[15,30]

PCBs, like most chlorinated hydrocarbons, are highly insoluble in water and tend to settle and then be adsorbed on silt and fine particles on the bottom.

Toxic Effects of PCBs

Based on the high lethality of PCBs to marine and freshwater aquatic species, the National Academy of Sciences-National Academy of Engineering Committee made the following recommendation:[11]

"Aquatic life should be protected [that is, safe from toxic effects] where the maximum concentration of total PCB in unfiltered water does not exceed 0.002 microgram per liter [0.002 ppb] at any time or place and the residues in the general body tissues of any aquatic organism do not exceed 0.5 microgram per gram [0.5 ppm]."

These levels obviously are exceeded in areas of the Hudson River and elsewhere.

The danger to aquatic life is a warning of the danger to human life. In fact, the toxicity of PCBs to humans has been clearly and tragically demonstrated. In 1968, a severe form of skin disease was diagnosed in Japanese families who consumed rice oil contaminated with Kanechlor 400 (PCB with 48 percent chlorine content).[31,32] The disease, known as the "Yusho syndrome," is characterized by swelling of the upper eyelids, visual impairment, acne-like formations, and heightened pigmentation of the skin,[31] and a total of 1,057 poisoning cases have been recorded to date in Japan.[33] Patients with this disease also have exhibited neurological disorders and have shown signs of hearing loss. Babies born to several patients had many of the symptoms of the disease, indicating a placental transport of PCBs; most fetuses of the victims were smaller than the national average. Another disturbing feature of the disease was the observation that most patients recovered very slowly, which suggested that PCBs were retained in their bodies over a very long period.[33] PCBs have been detected in human adipose tissue (connective tissue in the body containing stored cellular fat) in such widespread occurrence that over 40 percent of the U.S. population contains one part per million or more.[34]

A number of studies have conclusively shown the effect of PCBs on the reproduction processes of some birds and animals, including monkeys.[31,35,36,37,38,39,40] It is important to note that PCBs with lower chlorine content may have a greater effect on reproduction than biphenyls with higher chlorine content, even though the former substances are now being suggested as substitutes for the latter. In a recent review article on the toxicology of PCBs, Renate Kimbrough of the Center for Disease Control in Atlanta, Georgia, stated:[31]

"The results obtained from the reproduction studies in birds particularly seem to indicate that the lower chlorinated biphenyls affect reproduction more than the higher chlorinated biphenyls, and it is very important to determine whether the lower chlorinated biphenyls that have been suggested as replacements for presently employed chlorinated biphenyls have an effect on

reproduction."

The recent PCB toxicity tests in monkeys are particularly relevant to human health considerations. In an extensive series of tests carried out by James Allen and his colleagues at the University of Wisconsin School of Medicine, PCBs in the diet of rhesus monkeys caused significant effects that mimicked both the symptoms of known human toxicity and reproduction failures in experimental animals. When female rhesus monkeys were given 25 ppm of Aroclor 1248 for a period of two months, severe symptoms of facial swelling and skin eruptions were manifested.[38] At lower dietary levels (5.0 ppm and 2.5 ppm) of the same substance, similar clinical symptoms were observed after a few months; more significantly, these low levels of PCBs had a marked effect on reproduction. After three matings with male monkeys, only 12.5 percent of the 5 ppm PCB dietary group and 37.5 percent of the 2.5 ppm group were pregnant as compared to 90 percent pregnancies in a comparable control group.[39] At the same time, offspring were significantly smaller in size; their health was further threatened since they continued to ingest high PCB levels from the nursed milk of their mothers.[40] One notable consideration related to the experiments in monkeys is that the present FDA tolerance limit of 5 ppm[41] for PCB residue in fish and shellfish is based on the assumption that toxic effects occur at levels much higher than is actually the case. On this basis, then, the FDA tolerance limit is too high.

Also of serious concern is a recent study conducted by a group of research investigators at the Center for Disease Control in Atlanta, the EPA, the National Cancer Institute, and the Johns Hopkins University School of Medicine.[42] They observed that when experimental female rats were fed a diet containing 100 ppm of PCB (Aroclor 1260) for about 21 months, 26 of 184 of the experimental animals examined had malignant liver tumors (Hepatocellular carcinoma), whereas only 1 of 173 control animals had the disease. At the same time, 146 of the experimental and none of the control animals had tumorous lesions (neo-plastic nodules) in the liver.

Other effects of PCBs that have been reported in the scientific literature include: (1) increased production of certain enzymes in the livers of human beings and laboratory rats, (2) induction of liver porphyria in several animal species, (3) interference with the immune defense mechanisms in rabbits, (4) neurological disorders in rats, (5) increased PCB residue in fatty tissues, serum, and milk, and (6) toxic effects in chick embryos.[43,44]

There has been some concern that reported PCB effects may be attributed in part to the presence of chlorinated dibenzofurans as a contaminant in PCB mixtures.[31] Chlorinated dibenzofurans have been shown to be acutely embryotoxic and have been detected in several PCB preparations (Clophen A 60, Phenoclor DP-6, Aroclors 1248, 1254, 1260).[45] Recent reports by M. Kuratsune and his associates have indicated that the "Yusho" patients apparently had been exposed to PCBs in rice oil contaminated with chlorinated dibenzofurans and showed detectable levels of this compound in their body tissue.[46] However, it was not determined from this study whether the contamination occurred during manufacture of the PCBs or during the use of the PCB mixture as a heat exchange fluid in the rice oil plant, since high temperatures may have led to its formation through the hydroxylation and oxygen ring bridging of chlorinated biphenyl molecules. At present, the possible link of PCB toxicity to the presence of chlorinated dibenzofuran contaminants remains highly speculative, as there appears to be no direct experimental evidence that dibenzofuran derivatives have long-term chronic effects similar to those which have been amply demonstrated with PCBs.[31,46]

Control of PCBs

The attack on PCB contamination has taken two forms, one a voluntary response by manufacturers of the substance and the other a response by various government bodies. Both responses have been inadequate, as will be shown in what follows.

In 1971, over 106 million pounds of PCBs was produced in the industrial countries of the world, with a third of this, nearly 40 million pounds, being manufactured in the U.S.[47] During the late 1950s and into the 1960s, U.S. domestic sales of PCBs increased nearly threefold, reaching a peak of approximately 72 million pounds in 1970.[48] Prior to 1970, about 60 percent of PCB material sold domestically in the U.S. was used for closed electrical systems such as capacitors and transformers. Another 25 percent was used as plasticizers and in the production of carbonless duplicating paper.[48] In September 1970, Monsanto Company, the sole manufacturer of PCBs in the U.S., restricted its sale of PCBs because of environmental contamination by the chemicals. This restriction consisted of limiting sales to those materials earmarked for closed electrical systems. Thereafter, U.S. consumption was reduced considerably. It is important to note, however, that these reductions occurred mainly in the use of PCBs as plasticizers and as hydraulic and lubricating fluids, since the bulk of PCBs (60 percent), as mentioned above, had been used in electrical systems. This chemical substance is still being used almost exclusively as the insulating fluid for large, high-voltage capacitors (as well as for certain small ones) and for electrical transformers. In addition, the EPA estimates that approximately one-half million pounds of PCBs is being imported into this country each year[49] and presumably is being used in products other than electrical equipment.

As to government response to the PCB problem, a federal Interdepartmental Task Force was convened in 1972. The group concluded that because of the highly persistent nature of PCBs and their bioaccumulation in the food chain, these chemical compounds posed a serious threat to human health. The group recommended restriction of the use of PCBs to closed electrical systems such as electrical transformers and capacitors.[43] However, since Monsanto Company actually had already begun restricting domestic sales in this fashion, it is not clear whether the task force's recommendations, issued a year and a half after Monsanto's action, had any influence on the company's decision (although this has recently been suggested by a federal official).[50]

In July 1973, the FDA established PCB tolerance limitations in a variety of food products, as follows:[41]

FDA Tolerances for PCBs in Food and Food Packaging (ppm)

Milk*	2.5
Dairy products*	2.5
Poultry*	5.0
Fish and shellfish**	5.0
Eggs	0.5
Infant and junior food	0.2
Complete and finished animal feed	0.2
Animal feed components	2.0
Paper food-packaging material	10.0

*On fat basis
**Edible portion

Toxicity data obtained during the past year showing severe reproductive failure with low concentrations of PCBs in primates (5.0 and 2.5 ppm) and the recent demonstration of carcinogenicity with rats[49,42] indicate the need to substantially revise the present FDA tolerance limitations of PCBs in foods. Although the present tolerance standards are deficient, the FDA has in recent months taken seizure action based on these standards

against several fish shipments earmarked for commercial markets in Lake Michigan, since PCB concentrations in the fish exceeded the present 5 ppm guideline.[51] Similarly, the FDA seized a shipment of carp this summer in Lake Pepin on the Mississippi River.[52] In view of a recent legal decision that apparently allows the FDA to define pesticide residues in foods as an indirect food additive, the FDA is expected to enforce its guidelines more forcefully in the future. FDA officials in the Great Lakes region have warned that shipment of chubs, Coho salmon, and lake trout may be disallowed in interstate commerce, since the fish continue to contain high residue levels of PCBs.[51] It is apparent that with a more thorough monitoring and surveillance program, many species of fish in the Great Lakes region and in several rivers in the East Coast area will be essentially unavailable for human consumption for many years.

One of the great ironies of the present situation is that *more than three years ago* the Federal Water Pollution Control Act (FWPCA) directed the federal government (specifically the EPA) to take swift action to reduce the discharge of PCBs and other toxic water pollutants to safe levels. The fact that today there is no meaningful national program to regulate PCB discharges is a result of the federal government's failure to carry out the act as written.

The congressional authors of the FWPCA believed that the "hazards posed by toxic substances" made their effective regulation "especially urgent."[53] Accordingly, they established in the act a "national policy that the discharge of toxic pollutants in toxic amounts be prohibited"[54] and added to the act a far-reaching special provision, Section 307(a), to ensure that this goal was fully implemented. If the section's mandatory deadlines had been met, toxic pollutant effluent standards covering PCBs and other toxic pollutants would have been promulgated by the EPA roughly two years ago and complied with by dischargers about one year ago. Yet, to date, the EPA has failed to promulgate a single standard under this provision.

Despite shortcomings in federal enforcement, several states have taken administrative and legislative initiatives to control the discharge of PCBs into lakes and rivers. Earlier this year, the Michigan Natural Resources Commission endorsed the recommendation of the Lake Michigan Toxic Substances Committee, calling for a ban on PCB imports and their use in the U.S. and for a rapid replacement of PCBs in electrical capacitors and transformers.[51] At the same time, a bill has been introduced in the state legislature to place strict control on the use and sale of PCBs in Michigan.[51]

In Wisconsin, the Division of Health of the State Department of Natural Resources has issued warnings on consumption of large lake trout and salmon from Lake Michigan and the upper Mississippi River. In August 1975, the department held a hearing with a view to developing a strategy to control the sale and distribution of PCBs within the state.[51] In Minnesota, an Interagency Task Force was established, principally to broaden the monitoring of PCBs in fish in several rivers and streams in the state.[22]

In terms of administrative remedies, the strongest action was taken this year in New York state, after it was discovered that the two General Electric plants on the Hudson River appeared to be the major dischargers of PCBs in upper regions of the river. On September 23, 1975, the New York State Department of Environmental Conservation issued an abatement order requiring the General Electric plants to achieve zero discharge of PCBs by September 30, 1976. Administrative hearings were begun on October 6, 1975.[55] At the same time, Ogden Reid, commissioner of the department, publicly warned consumers against eating striped bass from the Hudson River and salmon caught in Lake Ontario.[56]

On the international front, the Organization for Economic Cooperation and Development (OECD) a few years ago recommended that its member countries which are involved in PCB production (including the U.S., West Germany, France, the United Kingdom, Japan, Italy, and Spain) restrict the commercial use of PCBs to closed systems.[57] Only Japan has taken fur-

Large amounts of PCBs have been found in fish in the Hudson River, especially in those of recreational and commercial value.

Photo by Wendy Holmes

ther strict measures to completely eliminate the use of PCBs in all commercial applications, including capacitors and transformers. Japan also prohibits the import and export of the compound, and, since September 1972, there has been a virtual elimination of almost all applications of PCBs in that country.[50]

Most recently, following the National Conference of PCBs which was held in Chicago on November 19 to 21, 1975, the EPA on December 22, 1975, announced a far-reaching federal policy of "totally eliminating the use of polychlorinated biphenyls (PCBs) as rapidly as possible" in the U.S.[49,58] In a more specific action, the EPA has ordered the two General Electric plants on the Hudson River to achieve zero discharge by June 1977.[59] It is clear from these actions that the federal government has recognized the gravity of the PCB issue and at last has taken definitive steps to address the problem.

The title of this article as originally submitted was "A Review of Federal and State Government Roles in Controlling Impacts of PCBs on the Environment." The Publisher and Editors of Environment *are responsible for the published titles and subtitles, selection of photographs and lead-in excerpts, photo captions, and preparation of most graphs and illustrations which appear in* Environment *articles.*

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ENVIRONMENT

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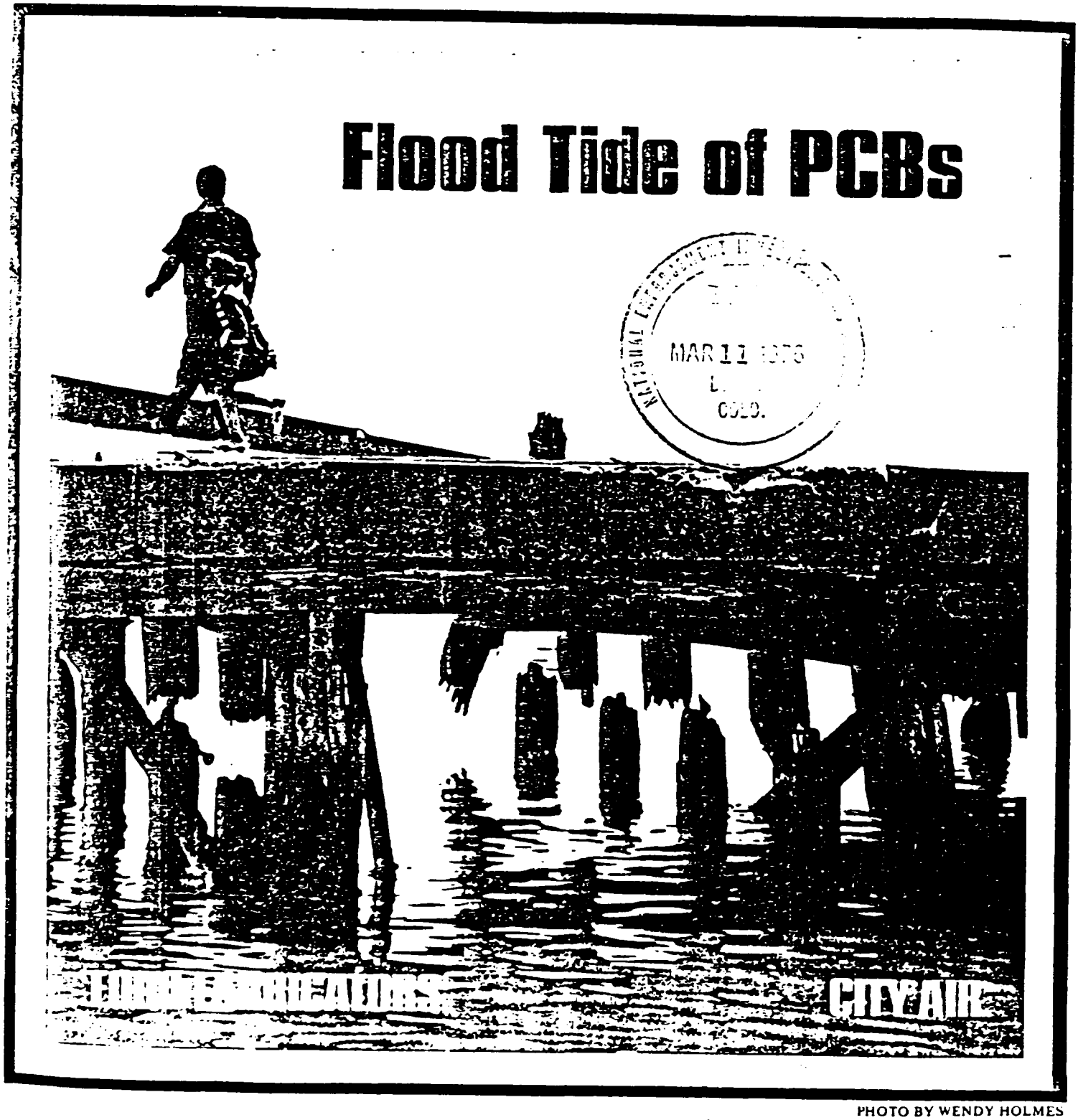
Flood Tide of PCBs



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EPRI, the research arm of the electric utility industry, announced a $7 million, five-year research program to find ways to manage . . .

The persistent PCB problem

On the one hand, PCBs are a very small segment of any hazardous waste management scheme. On the other hand, they are persistent environmental contaminants whose use has been controlled. They are the only group of chemicals specifically mentioned by the Toxic Substances Control Act, now five years old.

Between 1929 and 1977, about 1.4 billion lb of PCBs were produced in the U.S. Although its chemical manufacture has now ceased, 750 million lb are still in service, but only 162 million lb are associated with the electric utility industry.

PCBs are used in two types of sealed electric equipment:

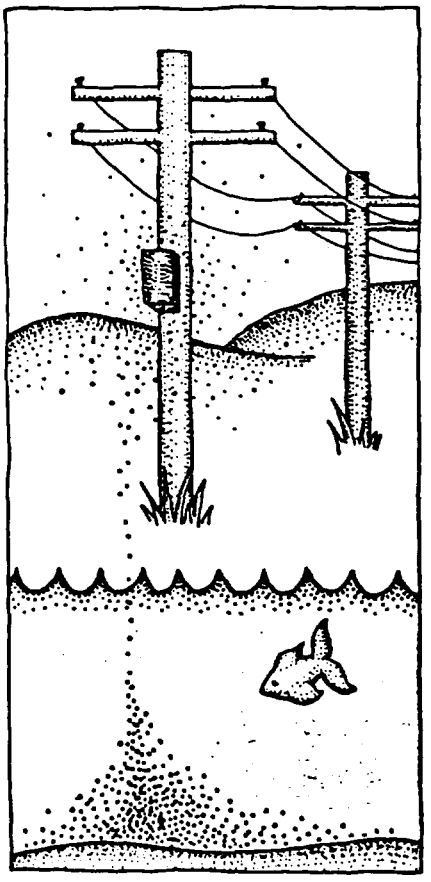
- transformers that raise or lower the voltage of a power line
- capacitors, the bread box-sized cans on utility poles that help maintain constant voltage in homes. Their good electrical properties (for capacitors) and low flammability (for transformers) make polychlorinated biphenyls especially suitable for these uses.

Of the other half of the PCBs, 3.6% have been destroyed; another 10.7% were exported. Some 21.4% are buried in landfills and 10.7% have escaped into the environment, much of that portion coming to rest at the bottom of the ocean.

The EPA regulations on PCBs have caused trouble for the electric utility industry. These regulations address two types of equipment: that containing PCB concentrations over 500 ppm, and so-called PCB-contaminated equipment containing 50-500 ppm PCBs. Concentrations below 50 ppm are not regulated.

Extent of the problem

The electric utilities estimate that fewer than 22% of the 750 million lb are the responsibility of their industry. According to estimates of the Edison Electric Institute, Askarel transformers contain 75 million lb of PCBs and PCB capacitors another 87 million lb. Askarel contains PCBs with up to 50% trichlorobenzene. Together, the 162 million lb account for essentially all PCBs in the electric utility industry.



In addition, however, there are another 262 000 lb of PCBs in other transformers. These other transformers are filled with mineral oils. Approximately 10% of the utility transformers are contaminated with PCBs in the 50-500 ppm range. This includes 1.9 million distribution transformers and 25 000 power transformers. The remainder, below 50 ppm, are considered free of contamination.

PCBs are an obvious problem for the electric utilities, though not exclusively, since fewer than 22% of the remaining PCBs still in use are found in their industry. Other industries are said to have a PCB problem; for example, the metal die-forming industry. Another use is in electrical equipment in high-rise buildings, industrial plants, and commercial operations. Here the equipment is not the responsibility of the electric utility industry per se, but rather that of other industry.

The regulations have caused such concern to the electric utility industry that the Electric Power Research Institute (EPRI) has launched its research effort to find ways to manage this PCB equipment. A recent three-day seminar in Dallas was devoted to the detection, extraction, destruction, and cleanup aspects of the problem. Now nine years old, EPRI operates on the premise that the research it conducts will save the utilities more money than the cost of the research.

A look at the statistics from another angle reveals that there are about 150 000 PCB transformers now in service. Some 40 000 are owned by utilities. Most are relatively small, and each contains about 200-600 gal of PCB liquids. Many are found in high-rise buildings where they were installed for fire safety reasons.

In practice, many are allowed to stay in service until they become faulty. Nevertheless, real problems exist. There is no reliable way to forewarn of incipient failure of transformers; however, there is partial discharge detection or measure of gas evolution. Using present methods, mainly incineration, disposal costs might be as much as $2000 per unit, in which case the total cost to this industry might be $300 million.

PCB-contaminated transformers, those with 50-500 ppm PCBs, repre-

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sent a headache for the utilities. The location of these units is unknown. Finding faulty transformers is difficult and expensive. The most frequently used method of checking transformer oil is to take a sample, send it to the analytical laboratory, and have the analysis performed by gas chromatography with an electron capture detector. The cost of this analysis is $50-100 per sample, so the total cost of testing 20 million transformers could reach $1-2 billion. Eventually the contaminated oil must be drained, disposed of, or decontaminated. One of the goals of the research program is to find better and lower-cost ways of reducing PCB-contaminated transformer oil to acceptable (less than 50 ppm PCBs) levels while saving the oil.

The problem with capacitors differs. There are an estimated 2.8 million capacitors in use; about 2000 of them rupture each year, spilling PCBs into the environment. EPRI research goals in this area are to find a way to predict capacitor failure and to develop a mobile device for testing soil samples for PCB contamination. Unlike transformers, there are no valuable materials to be recovered from capacitors. In capacitors, PCBs are absorbed into paper insulation; at present this solid PCB-containing material must be shredded before it is fed into an incinerator.

Research on new capacitors is needed because exhaustive toxicological tests have not been made on the liquids now being used. Research is also needed for a mobile facility that could be used to destroy PCBs from the old capacitors. Most capacitors made before 1977 contained PCBs.

Detection and extraction

The electric utilities need a number of industry-specific detection and analytical devices. There is a need for a device to detect incipient failures in capacitors. Also needed is a portable device for testing transformer oil for PCB content in the 50-500-ppm range. Another device is needed to detect the PCB content of soils in the field so that cleanup completion could be declared when the level of PCBs is less than 50 ppm.

There are prototype instruments or ideas being developed in each of these problem areas, but they are just beginning to be tested in the field. Commercial instruments are not here today.

A portable X-ray fluorescence instrument for screening transformer oil for 50 ppm PCBs or less by measuring total chlorine content is being field-tested by the Salt River Project, a utility in Arizona. Capacitors that are about to fail typically emit ultrasonic sounds. An acoustical detector (Dunnegan-Endevco) being field-tested alerts utility management to incipient capacitor failure. Another device, an infrared scanner, is being used to measure the temperature of operating capacitors. Capacitors that are about to fail may show a higher-than-normal operating temperature.

Spills

In the case of spills of either transformer oil after rupture or capacitor material after failure, it is necessary to

EPRI's Gil Addis: managing PCB destruction

take samples of the contaminated soil and send them to a laboratory for analysis. A more expedient approach would be to have a field instrument. EPRI is working with the Oak Ridge National Laboratory on a field device for on-the-spot analysis of PCB spills. A technique using a portable infrared spectrometer looks promising for Askarel spills from capacitors.

Destruction

Although there are several semicommercial processes for destroying or removing PCBs in oil, none have been fully tested for treating transformer oil. A number of proposed processes use sodium in a reactive chemical to combine with the chlorine in the PCBs. A possible unknown in these latter processes is that the sodium treatment may also damage or remove some components of the mineral oil, making it unfit for continued use in transformers. Tests are under way to check this point.

Sunohio, based in Canton, Ohio, has constructed a mobile PCB chemical destruction unit (*ES&T*, November 1980, p. 1278) and has demonstrated that its system is capable of reducing the PCB concentration in contaminated mineral oil to a nondetectable level. Sunohio has received approvals for the unit in EPA Regions 1, 4, and 7. Acurex Corp. has also constructed a mobile PCB destruction system and demonstrated it for EPA.

Incineration on land and at sea seems to be the best option for disposal of Askarels (high-level PCB concentrations) at present. Two land-based incineration disposal sites are now licensed by the EPA—a Rollins incinerator in Texas and the Ensco (Energy Systems Co.) incinerator in El Dorado, Ark.

The ocean incinerator ship, *Vulcanus*, is another option. The ocean incineration record with *Vulcanus* included EPA-approved test burns in 1974 and 1975 at designated sites in the Gulf of Mexico. In 1977, the *Vulcanus* incinerated the stockpile of Agent Orange, the defoliant used in Vietnam, in an area west of Johnston Island. More recently, an EPA permit, dated Oct. 23, 1981, granted permission for four separate burns in an area known as a near-biological desert, 200 miles south of Galveston, Tex. PCBs will be incinerated on *Vulcanus* for the first time. The *Vulcanus* can burn 4000 gal/h and has a holding capacity of 850 000 gal; the waste must be liquid and pumpable.

Other processes for removing PCBs from transformer oil are being investigated by EPRI in lab studies. These include solvent extraction, several additional alkali metal dechlorination processes (removing chlorine atoms from the PCB molecule), electron irradiation, and super critical fluid extraction.

Since a number of alkali metal processes are already under way, EPRI may sponsor scale-up of an extraction process, the most attractive of the alternative processes. Other destruction processes include Rockwell International's molten saltbath and the plasma arc process of Dr. Barton, Imperial College, Ontario, Canada.

Prognosis

The EPA regulations for PCBs are the classic example of a moving target. The EPA regulation has been challenged in a court case brought by the Environmental Defense Fund. The case has been remanded to the agency, which must now show cause for setting the 50 ppm cutoff. The preliminary agency response should appear in the *Federal Register* in March, but the final ruling is not expected until about September 1982. Originally, the cutoff value was made to distinguish between two disposal options—incineration or landfilling. —Stanton Miller

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ES&T

Acid deposition

110A

Bull. Environm. Contam. Toxicol. 24,527-534 (1980)

Toxicity of Arsenic and PCBs to Fry of Deepwater Ciscoes (*Coregonus*)[1]

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The deepwater ciscoes (known commercially as chubs) are among the native fish of the Great Lakes that have declined or disappeared during this century (CHRISTIE 1974). The ciscoes of Lake Michigan, now primarily bloaters (Coregonus hoyi), are only about 1% as abundant as they were in 1960-61 (GREAT LAKES FISHERY LABORATORY 1977). Stress factors that may have interacted to cause the decline of ciscoes include overfishing, invasion of the lake by exotic species, and pollution. Compared with other fish species from Lake Michigan, ciscoes are relatively high in contaminants, especially arsenic and polychlorinated biphenyls (PCBs). Although the maximum concentration of PCBs in the open waters of Lake Michigan is only 10 ng/L (GREAT LAKES WATER QUALITY BOARD 1975), the average concentration of PCBs was 4.1 µg/g in ciscoes taken off Saugatuck, Mich., in 1976 (GREAT LAKES WATER QUALITY BOARD 1978). COPELAND et al. (1973) reported arsenic values of 0.26, 0.77, and 1.7 µg/g for three bloaters collected near Ludington, Mich., and 0.56, 1.1, and 2.5 µg/g for three ciscoes (Coregonus sp.) taken near Cook, Mich.

Information on the toxicity of arsenic and PCBs to ciscoes is needed to evaluate the relative importance of these potential stress factors. Because fry are usually the most sensitive life stage, toxicity data on the fry can often indicate threshold values for the entire life cycle (SCHIMMEL et al. 1974, MCKIM et al. 1978). Few data are available on the toxicity of arsenic and PCBs to fry of other fish species. The 96-h LC50 for newly hatched fathead minnows (Pimephales promelas) was 7.7 µg Aroclor 1254/L (NEBECKER et al. 1974). SPOTILA and PALADINO (1979) reported that 50 µg arsenic (As^{+3})/L caused 100% mortality in muskellunge fry (Esox masquinongy) during the swim-up stage. No other data are available for toxicity of arsenic to fish fry. Water quality criteria and objectives (U.S. ENVIRONMENTAL PROTECTION AGENCY 1976, GREAT LAKES WATER QUALITY BOARD 1975) have been established on the

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[1]Contribution 543 of the Great Lakes Fishery Laboratory, U.S. Fish and Wildlife Service, Ann Arbor, Michigan 48105. This article was written by employees of the United States Government as part of their official duties and therefore cannot be copyrighted.

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basis of such data as above, although possible additive effects of metals and organochlorines found in Lake Michigan are generally unknown.

As the initial step in determining the potential role of arsenic and PCBs in the decline of Lake Michigan ciscoes, our objectives were to measure the acute toxicity (96-h LC50) of arsenic and PCBs, singly and in combination, for cisco fry; to determine the additive index (MARKING 1977) for arsenic and PCBs; and to evaluate the adequacy of water quality criteria for these contaminants relative to protecting ciscoes.

MATERIALS AND METHODS

Mature ciscoes (Coregonus sp.) were captured by gillnet from a commercial fishing vessel, December 1976, at 130-150 m, 16 km NE of Marquette, Mich., in Lake Superior, because of the greater availability of ciscoes in Lake Superior compared with Lake Michigan. Four male Coregonus hoyi, four female C. hoyi, and one male C. zenithicus were stripped on board the vessel by personnel of the Michigan Department of Natural Resources and all eggs and sperm were mixed. After 80-84 days of incubation at the Great Lakes Fishery Laboratory in running well water (total hardness 400 mg/L as $CaCO_3$) at 4-5 C, 95% of the eggs hatched. Newly hatched fry were placed in floating baskets in flowing, 6-8 C well water and were fed brine shrimp (Artemia) nauplii, beginning when the fry were 1 week old. For 1 week prior to toxicity tests, fry were gradually acclimated to reconstituted soft water (total hardness 40-48 mg/L and alkalinity 30-35 mg/L as $CaCO_3$) (COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS 1975).

Stock solutions of toxicants were prepared as follows. Arsenic trioxide (As_2O_3) was dissolved in warm water by adding 10 N NaOH and then neutralized with concentrated HCl. PCBs (Aroclor 1254 from Monsanto, St. Louis, Mo.)[3] were dissolved in 100% acetone.

Fry, randomly pipetted into battery jars containing 2.5 liters of reconstituted soft water, were acclimated overnight to the test chambers. The battery jars, each containing 10 fry, were randomly arranged in a trough of running water at about 7 C (6.8-7.8). Toxicants were then added to the battery jars and mixed by gentle stirring. A control containing 0.4% acetone, the highest concentration of acetone in the experimentals, was included. Toxicant concentrations were selected by progressive bisection of intervals on a logarithmic curve (AMERICAN PUBLIC HEALTH ASSOCIATION 1976, p. 715). After beginning the tests, we counted and removed dead fry at 3, 6, 12, 24, 48, 72, and 96 h. At 96 h, we also noted any

[3]Use of trade names or manufacturers' names does not imply U.S. Government endorsement of any commercial product.

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moribund individuals. During the extended bioassay, the water and toxicants were renewed at 96 h and daily observations of mortality were made until day 14.

Water samples were taken from the battery jars at 96 h for analysis of Aroclor 1254 and total arsenic to determine possible losses during that time. For Aroclor 1254 analysis, 100-mL samples were extracted twice with petroleum ether and dried over anhydrous Na_2SO_4. The volume of the combined extracts was reduced to about 1-2 mL by heating on a hot plate. The sample was allowed to cool before transferring to a test tube, and the volume was adjusted to 10.0 mL with isooctane. After additional dilutions in isooctane, the samples were injected into a gas chromatograph (WILLFORD et al. 1976). The samples were quantitated against standard solutions of Aroclor 1254. Recovery after 96 h in the battery jars was 73% for 1.0 mg /L and 80-89% for 1.8 to 10 mg/L. All of the concentrations were above the solubility of Aroclor 1254 in water (WIESE and GRIFFIN 1978), even in the presence of 0.4% acetone. The 10 mg PCBs/L "solution" was a cloudy suspension.

We saponified (REINERT 1970) 10-g samples from homogenized whole ciscoes before analysis for PCBs (WILLFORD et al. 1976) and p,p'DDE (saponified p,p'DDT plus p,p'DDE) (HESSELBERG and SCHERR 1974). We quantitated PCBs with standards containing a 1:1:1 mixture of Aroclors 1248, 1254, and 1260, the Aroclors most common in environmental samples. Based on isomer analysis of fish from Lakes Michigan and Superior, typically the p,p'DDT represents about 25% of the saponified mixture, which includes at least 80% of the total DDT complex present (HESSELBERG 1979). The p,p'DDE for the standards was obtained from Analabs, New Haven, Conn.

Using nondestructive neutron activation (DESOETE et al. 1972), the Phoenix Memorial Laboratory, University of Michigan, analyzed water samples for arsenic. The analysis included 10-h irradiation in the Ford Nuclear Reactor, counting by lithium drifted germanium detector, and data reduction by a computerized analyzer system (NICHOLSON and RENGAN 1979). After 96 h, the recovery was 104 ± 0.9(SE)% for 19 samples containing 10 to 100 mg arsenic/L.

Analysis of arsenic (NICHOLSON and RENGAN 1979) in tissue samples of eggs and adult ciscoes required additional steps to remove bromine, which is normally present in environmental samples and which interferes with determination of arsenic. After passage of irradiated and acid-digested samples through a tin dioxide column, we counted the radioactivity of the arsenic-76 with the tin dioxide, using a Ge(Li) detector.

We analyzed bioassay data by probit analysis (FINNEY 1952) with a computer program (a modified version of IBM SSP package) from the Dow Chemical Company, Midland, Mich. The probit slope, b, is related to the classical slope function, S (LITCHFIELD and WILCOXON 1949), as follows: $b = (\log S)^{-1}$. The additive indexes for mixtures of arsenic and PCBs were calculated by the method of MARKING (1977).

TABLE 1

PCBs, DDT + DDE, and Arsenic in Lake Superior Ciscoes

Species	Sex	Number[a]	Total length mm $\bar{x} \pm SE$	PCBs[b] µg/g tissue[c] $\bar{x} \pm SE$	DDT + DDE µg/g tissue[c] $\bar{x} \pm SE$	Arsenic µg/g tissue[c] $\bar{x} \pm SE$
Coregonus hoyi	M	4	304 ± 7	2.3 ± 0.2	1.4 ± 0.3	0.75 ± 0.11
C. hoyi	F	4[d]	295 ± 14	1.2 ± 0.3	0.67 ± 0.20	0.84 ± 0.05
C. zenithicus	M	1	276	1.8	0.74	0.81
Chub eggs		e		0.51	0.25	0.26

[a]The fish were analyzed individually.
[b]Quantified by using a mixed standard of 1:1:1 Aroclor 1248:1254:1260.
[c]Wet weight.
[d]Eggs were obtained from four females, but tissue sample of one female was lost during analysis for PCBs and DDT + DDE. The mean length for the three females was 298 ± 20 mm.
[e]Ten grams were used for analysis of PCBs and DDT + DDE, and 2.2 g for analysis of arsenic. Eggs were analyzed shortly before hatching.

RESULTS AND DISCUSSION

The difference in organochlorine concentration between male and female C. hoyi was highly significant ($P<0.01$) for PCBs and significant ($P<0.05$) for DDE (Table 1). In view of the lower concentrations of both PCBs and DDE in the females and eggs, these contaminants are probably not preferentially stored in the eggs. Concentrations of PCBs tended to be lower in Lake Superior ciscoes than in those from Lake Michigan, but DDE concentrations were similar in 1976 (GREAT LAKES WATER QUALITY BOARD 1978).

Other subsamples of the same fish homogenates and eggs yielded the data for total arsenic shown in Table 1. The difference in arsenic concentration between male and female C. hoyi was not significant, and arsenic was not preferentially stored in the eggs.

Duplicate acute toxicity tests (96 h) with arsenic (As_2O_3) were performed when the fry were 15 to 19 days old. The mean LC50 was 26 mg/L and the mean slope 5.6 probit/log (mg/L).

Acute toxicity tests (96 h) of arsenic and PCBs (Aroclor 1254), singly and in combination, when the fry were 22 to 26 days old, yielded the results shown in Table 2. Only 20% of the fry died in 10 mg PCBs/L; however, all living fry were abnormally lethargic. Accurate acute toxicity values for PCBs could not be determined because of the low solubility of these compounds.

The LC50 values from Table 2 show that PCBs in the presence of arsenic (LC50 = 3.5 mg PCBs/L) were more toxic than arsenic either singly (17 mg As_2O_3/L) or in combination (11 mg As_2O_3/L). The slopes of these three toxicity curves were not significantly different (i.e., the 95% confidence limits overlapped). The presence of hybrid C. hoyi x C. zenithicus in the experimental population probably contributed to the variability in the data.

TABLE 2

LC50 Values and Slopes of Toxicity Curves
(95% confidence limits in parentheses) for Arsenic and
PCBs, Singly and in Combination, after 96 Hours
(22-Day-Old Fry)

Toxicant	LC50 mg/L	Slope probit/log (mg/L)
Arsenic	17 (13-22)	5.39 (2.61-8.18)
Arsenic (PCBs)	11 (8-15)	3.90 (2.17-5.64)
PCBs	>10	
PCBs (Arsenic)	3.5 (2.6-4.7)	3.92 (2.16-5.68)

To determine the additive toxicity of the mixture according to the method of MARKING (1977), we needed an LC50 value for PCBs individually. Because 20% mortality occurred and all the fry appeared moribund at 10 mg PCBs/L and because the low solubility of PCBs made tests at higher concentrations unrealistic, the value of >10 mg PCBs/L was substituted in the equation below to estimate the sum of biological activity:

$$(A_m/A_i) + (B_m/B_i) = S$$

where A and B = chemicals, i and m = toxicities (LC50s) of the individual chemicals and mixtures, respectively, and S = sum of biological activity. With our data, S $\leq$1.01, indicating simple additive toxicity. Estimating the additive index by (S)(-1) + 1.0, yields $\geq$-0.01, again indicating simple additive toxicity.

When the above toxicity test (Table 2) was extended to 5 days after renewal of solutions at 96 h, a marked dose-related increase in mortality occurred between days 4 and 5, especially for PCBs (Table 3). Apparently handling stress contributed to the increased sensitivity of the fry. Based on data in Table 3, the sum of biological activity, S, was 1.07, and the additive index was 0.07, with 95% confidence limits of -1.73 to 1.95. Since these confidence limits overlap zero, simple additive toxicity is assumed, confirming the estimated results at 96 h. When the exposure of these fry to arsenic was extended to 14 days, the LC50 value was 6.7 mg/L (C.L. = 5.31 to 8.46), and the slope was 7.71 probit/log concentration (C.L. = 3.29 to 12.12). After 14 days' exposure, 100% mortality was observed for all fry at 1.0 to 10 mg PCBs/L, alone and in combination with 3.2 to 32 mg arsenic/L.

TABLE 3

LC50 Values and Slopes of Toxicity Curves
(95% confidence intervals in parentheses) for Arsenic and PCBs, Singly and in Combination after 5 Days (22-Day-Old Fry)

Toxicant	LC50 mg/L	Slope probit/log (mg/L)
Arsenic	14 (11-19)	4.56 (2.30-6.82)
Arsenic (PCBs)	6.3 (3.1-10)	2.32 (0.73-3.90)
PCBs	3.2 (1.9-5.5)	1.98 (0.83-3.13)
PCBs (Arsenic)	2.0 (0.96-3.3)	2.27 (0.69-3.85)

Since additive toxicity was indicated for the mixture of PCBs and arsenic, we selected the 96-h LC50 values for the mixture to calculate safe concentrations (WARREN 1971). Using an application factor of 0.01 (NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF

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ENGINEERING 1973), we estimated safe concentrations to be 35 µg PCBs/L and 110 µg arsenic/L. These values are well above the reported levels of <10 ng PCBs/L and 1 µg arsenic/L for Lake Michigan waters (GREAT LAKES WATER QUALITY BOARD 1975, COPELAND and AYERS 1972). Hence, the water quality criteria and objectives for PCBs (1.0 ng/L) and arsenic (50 µg/L) recommended by the U.S. ENVIRONMENTAL PROTECTION AGENCY (1976) and the GREAT LAKES WATER QUALITY BOARD (1975) would appear to be adequate to protect ciscoe populations in Lake Michigan. Subacute tests at ambient (Lake Michigan) and calculated safe concentrations should be performed to test the no-effect hypothesis for arsenic and PCBs on ciscoes.

ACKNOWLEDGMENTS

We thank James W. Peck (Michigan Department of Natural Resources) for collecting the ciscoes, Colin Park (Dow Chemical Company, Midland) for providing the probit computer program, and Wayne A. Willford for helpful discussions and review of the manuscript.

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ES&T
CRITICAL REVIEW

The effect of environmental pollutants on human reproduction, including birth defects

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Nothing can cause greater fear in an expectant mother than the prospect of her unborn child being defective. And nothing touches on a man or woman more closely or personally than their reproductive capacity. In recent years, the public has become alarmed that inadvertent or careless exposure to chemicals in the environment might predispose birth defects or reproductive failure. This fear and awareness is reflected by the titles of articles in the popular press, such as "The Poisoning of America" (*1*), and the admission into our household vocabulary of a whole new set of names that reflect this problem: toxic wastes, Love Canal, Agent Orange, dioxin, PCBs. Chemicals from a range of environmental sources have been implicated in birth defects and reproductive failure. We will concern ourselves here with chemicals in the workplace, chemicals in the environment (from air pollution and from carelessly disposed materials that contaminate the land, water supply, or food chain), and food additives or contaminants. Chemicals knowingly ingested for therapeutic (pharmaceuticals) or other reasons (alcohol, cigarettes, drugs) are covered in other reviews (*2*).

Feature articles in ES&T have bylines, represent the views of the authors, and are edited by the Washington staff. If you are interested in contributing an article, contact the managing editor.

Concepts in teratology

The study of the induction of birth defects by exogenous agents constitutes the discipline of teratology. *Terat* derives from the French meaning "monster." In terms of environmental factors, there are four classes of known teratogens: radiation, viruses, drugs, and chemicals. We will concern ourselves only with this last class.

Birth defects are known to occur in 2-3% of all births (*2*). Of these, 25% have underlying genetic causes, while 5-10% result from the influence of the above classes of teratogens (*3*). The remaining 60-65% arise from unknown causes, but may follow from an interplay of multiple environmental agents with genetic factors. Of those birth defects caused by environmental agents, an estimated 4-6% are due to chemicals (*3*); yet of all the sources of birth defects, this is potentially the most preventable. To date, only about 25 chemicals are known to be teratogenic in humans, compared to over 800 in laboratory animals (*4*). It is unknown if this discrepancy rests with a greater resistance of humans to these agents or a failure in our ability to determine sources of teratogenicity in humans. The potential danger is great and growing, however, because of the estimated 2000 new chemicals synthesized each year and introduced into the environment.

In terms of the development of a newborn, there are three periods of development during which the fetus is at risk for injury (*5*): fertilization to implantation, the embryonic period, and the fetal period. The period of fertilization and implantation spans from conception to 17 days, through the blastocyst and early gastrula stages. An injury during this stage results in cell death, from which the aggregate of still totipotential cells can recover and multiply, or if a lethal dose is given, abortion or resorption of the developing organism results. If recovery follows, no structural deformity in the embryo is seen. The embryonic period (18-55 days) is when organogenesis takes place; therefore, this period is one of extreme sensitivity to teratogens (*6*). Within this time, each organ system or morphologic trait has its own critical period of development (Figure 1). In later stages, the fetus is

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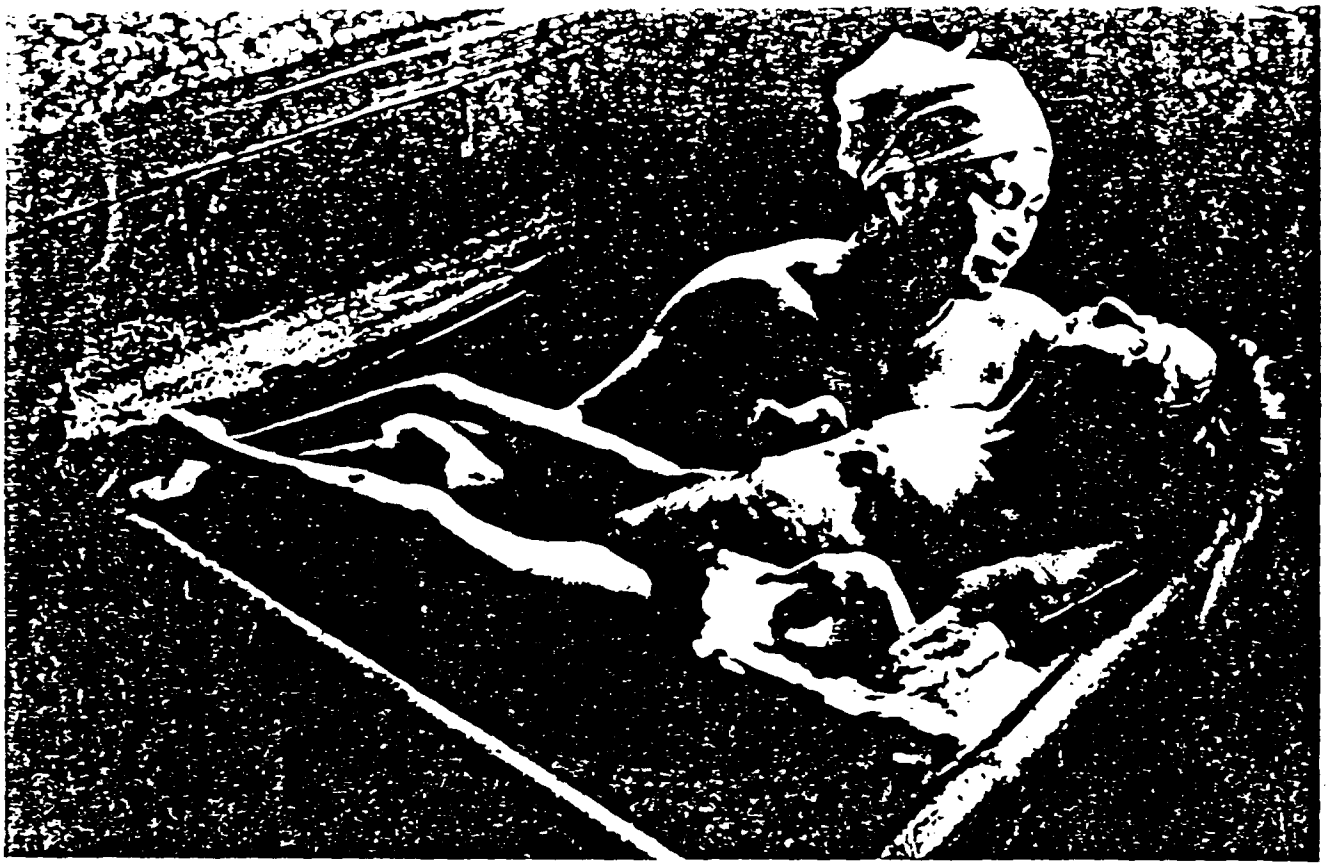


Chisso–Minamata disease: *the effect of mercury poisoning in a small Japanese fishing village*

less sensitive, but not immune to environmental influences. During the fetal period (56 days to term), toxicity is mainly manifested as a reduction in cell size and number. Growth retardation or functional deficits (e.g., central nervous system injury or retarded development) usually result from injury during this stage of development, since growth in size is the primary process active at this time. The developing brain is still prone to injury, since its development (e.g., myelination) is incomplete even at birth.

Over the years, a number of factors have been determined to play a role in the susceptibility of a fetus to the teratogenic effects of an agent (*7*, *8*). These include:

1. the *time of administration* of the agent during gestation, with the most sensitive period being that of organ differentiation

2. the *route of administration* and *dose*, which involve the chemistry of the compound, its toxicity, metabolism, and toxicology of its degradation products. As in other types of toxic responses, the teratogenic effects of most compounds follow a dose response. However, the dose thresholds for teratogenic effects are generally not known. Safe thresholds are currently determined by both epidemiologic and laboratory studies. Manson (*5*) has proposed that in teratology, unlike mutagenic and carcinogenic effects, safe threshold levels of a chemical may exist; much work is being expended in determining these levels of exposure.

The unborn fetus is exposed to chemical agents by hematogenous transplacental transfer. The routes of administration to the mother, on the other hand, are by the usual routes, including inhalation, absorption by contact with the skin and mucous membranes, and, most importantly, ingestion.

3. the *number of doses*, with a single dose being most effective in producing defects. It is assumed with the single high dose that the mother is not given the chance to adjust to the agent and to mobilize her detoxification mechanisms. These mechanisms may be protective in chronic low level exposures.

4. *species variation*, most strikingly demonstrated by the thalidomide incident, in which exposure produced limb defects in man, monkeys, and rabbits, but not in mice and rats, on which the drug was tested (*7*). Therefore, the susceptibility to a chemical depends on the genotype of the conceptus, with different effects being seen in different species and different strains of the same species. The difficulty this poses in the animal testing of compounds and in the interpretation and extrapolation of the results to humans is obvious. In humans, racial and familial differences may even play a role in susceptibility.

5. *other factors*, including the extrinsic effects of maternal metabolic diseases, maternal age (the young are more likely to have defects), and stresses such as nutritional deficiencies (protein or vitamin A, E, and B_2 deficiencies). Lastly, an embryo may be more susceptible than the mother to the effects of interaction between multiple agents to which they have been concurrently exposed (*5*). Experiments in animal systems have demonstrated the possibility of producing additive, synergistic, or antagonistic effects between multiple agents.

Manifestations of damage to the developing organism caused by a chemical agent follows the injury or death of certain cells; this interferes with the orderly sequence of development, giving rise to morphologic aberrations. A description of various histologic and physiologic processes whose alteration may give rise to aberration can be found elsewhere (*2*). Depending on the dose and stage of embryonic development, the effect

may be teratogenic or fetotoxic. Fetotoxicity is usually seen at doses higher than those expected for teratogenicity; at high doses, fetotoxicity may culminate in fetal death. Although teratogenic agents are usually also fetotoxic, as we shall see, not all fetotoxic agents are teratogenic (*9*).

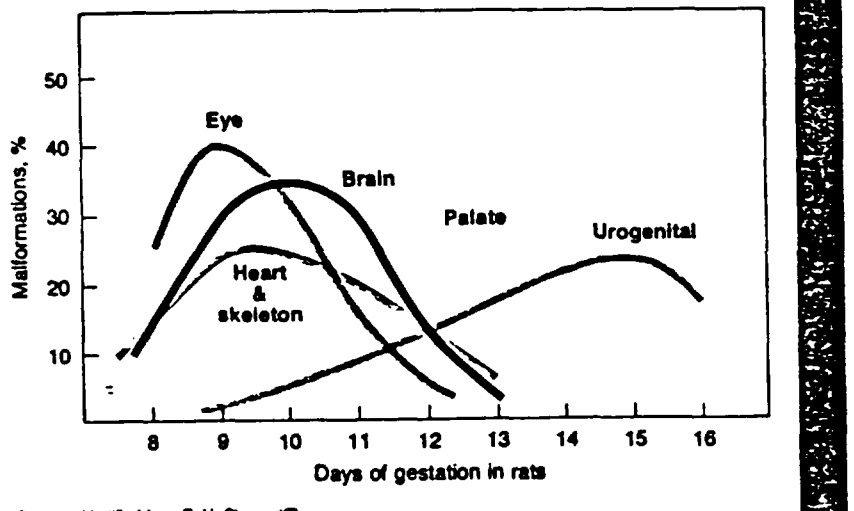
The mechanisms by which chemicals induce teratogenic actions are in general unknown. Unlike mutagenic chemicals, which usually belong to the alkylating class, teratogens may belong to many classes of chemicals; their mechanisms of action are therefore different. Malformations and mutations thus cannot be equated (*10*), nor can carcinogens and teratogens (*11*). Chemicals may act directly on the embryo, fetus, or neonate, or indirectly via interference with maternal, placental, or fetal membrane functions (*12*). Through laboratory animal studies it has been shown that, as a rule, chemicals lack specificity and may affect many structures depending on the time of administration (*9*). For example, trypan blue in rats affects 15 structures, resulting in nine defects. Conversely, different teratogens may produce the same malformations.

It has been proposed that a fetus may be more susceptible to damage by chemical exposure than an adult, depending in part on the development of its liver enzyme conjugating systems and on its greater blood-brain permeability (*13*). Indeed, for a given exposure, there may be no maternal signs of toxicity and the fetus may be the only victim of the toxicant. This has been verified in animal studies as well as in human accidents, for example, chronic low level exposure to methylmercury. Since all substances cross the placenta to some extent, susceptibility to fetal damage may be related to the effective dose reaching the fetus. Some chemicals, such as methylmercury, are preferentially concentrated in the fetus, resulting in injury to the central nervous system. In most other instances, however, the fetus is separated from maternal circulation by the placenta, which permits only a fraction of the concentration of chemical to reach the developing offspring. The placenta therefore serves a protective role in reducing the dose to the fetus. Transfer of a compound across the placenta decreases with increasing molecular weight, increasing electrical charge, and decreasing lipid solubility. Transfer is by simple diffusion for unbound chemicals or for those with high dissociation constants; in other cases, the mechanisms of transfer include active transport and pinocytosis. In some cases, the placenta may also be involved in biotransformation of the compound.

The fetus may also be protected by maternal homeostatic mechanisms that lower the dose reaching the fetus via detoxification by the liver (although some metabolites may be more toxic), excretion by the kidneys or in the bile, or removal of the chemical via binding to plasma proteins which are less likely to cross the placenta. Peak concentrations in maternal circulation may also be decreased by deposition into maternal storage depots. DDT and other lipid-soluble compounds, for example, are deposited in the adipose tissue, while some heavy metals are deposited in bone. It has been suggested that variation in the dose of chemical that reaches the fetus, controlled by the factors outlined above, may underlie the variability in observed species' susceptibility.

Much of what we know about teratology in humans comes from clinical observations and epidemiologic studies (*8*). The bulk of information, however, derives from animal studies. In the discussion to follow, we will not limit ourselves to evidence linking chemicals with birth defects alone, but also will consider general aspects of fetal damage, fetal loss, and reproductive impairment in both the female and male. Fetal damage may be in the form of birth defects or neonatal sequelae of fetal toxicity. This toxicity may be compounded by continued exposure during the neonatal period (postpartum) to residues present in breast milk. Fetal loss may be postimplantation ("early"), manifested by spontaneous abortion, or by a late fetal loss, called a stillbirth. Death may also follow in the postpartum period due to either severe life-threatening malformations or to cumulative toxicity to vital organs. Reproductive impairment is the most difficult entity to document. In females, this may take the form of preimplantation loss (damage to the blastocyst, or even damage to the zygote) or menstrual irregularities. In males, impaired fertility may take the form of decreased sperm counts, decreased motility, or abnormal morphology. In addition, reproductive failure may be manifested as an increased frequency of spontaneous abortions in unexposed females due to mutations in male gametes (*5*).

FIGURE 1
The critical periods for teratogenicity



Source: Modified from T. H. Shepard[7]

Air pollutants

Air pollutants commonly include those compounds produced by combustion of fossil fuels, such as the oxides of nitrogen and sulfur and incompletely oxidized hydrocarbons such as carbon monoxide. The most serious source of air pollution to a pregnant woman is cigarette smoke because it exposes the unborn baby to high levels of CO, HCN, cadmium, nicotine, and polycyclic aromatic compounds such as benzo[*a*]pyrene, which can cross the placenta (*14*). Exposure to harmful gases may also follow from occupational exposures, such as to ozone, anesthetic gases, or solvent vapors.

Ozone. A constituent of the earth's upper atmosphere, ozone is considered hazardous in exposures to concentrations over 1 ppm. In areas prone to smog, such as Los Angeles, levels may reach 0.1 ppm, at which point an alert

"The most serious source of air pollution to a pregnant woman is cigarette smoke because it exposes the unborn baby to high levels of CO, HCN, cadmium, nicotine, and polycyclic aromatic compounds such as benzo[*a*]pyrene, which can cross the placenta."

may be called. Ozone concentrations in high-altitude aircraft have been measured at 1.2 ppm, four times the permissible occupational exposure level set by OSHA. Some flight attendants have claimed that they suffer higher rates of spontaneous abortions and birth defects due to ozone exposure (*14*), but no confirmation of these claims is available. However, Fetner (*15*) has shown that ozone is mutagenic to human cell lines in culture, producing chromosomal breaks (at 8 ppm) equivalent to that produced by 200-roentgen x-rays.

Carbon monoxide. More information exists on the effects of carbon monoxide (CO) on the fetus than any other air pollutant. Industrial combustion, automobile exhaust, and cigarettes are the major sources of exposure to CO. Although "mean" urban CO concentrations are 3–10 ppm, near factories or at busy street intersections levels may be 50–100 ppm. With these high CO concentrations, the blood concentration of carboxyhemoglobin can be 4–6% (the equivalent of smoking a pack of cigarettes per day), and smoking may raise these levels an additional 4–5% per pack per day (*14*).

Carbon monoxide diffuses across the placenta and has a half-life of 1.5–2 hours. Under steady-state conditions, the carboxyhemoglobin concentration in the fetus exceeds that in the mother by 10–15%; the fetal arterial oxygen tension, which is much less than that in the adult, is decreased further. The decreased oxygen tension is associated with a redistribution of fetal blood to the brain, heart, and adrenals. Experiments with CO on rats and rabbits have shown a decrease in fetal weight and increase in perinatal mortality (*14*). Brain damage with decreased brain concentrations of protein, nucleic acids, and neurotransmitters has been demonstrated and is assumed to follow from cellular hypoxia. Studies in rats show that reproductive failure via reabsorption of the embryo and early spontaneous abortions (*16*) is concentration-dependent. Teratogenic limb deformities were produced in chicks (*17*) and rabbits (*16*) exposed to high concentrations (180–250 ppm). Brain damage occurred in Long-Evans rats (*16*). In animal studies in which the concentration of carboxyhemoglobin exceeded 15% for 30 minutes, the incidence of fetal death was 57%. In a survey on human carbon monoxide poisonings, Longo (*16*) reported an increased rate of stillbirths when the concentration of carboxyhemoglobin was 20–49%. Some of the surviving infants from these poisonings had neurologic sequelae including mental retardation, seizures, spasticity, and retarded psychomotor development. Autopsies on the stillborns confirmed brain damage (cerebral atrophy).

The 1970 amendment to the Clean Air Act recommended a maximum allowable concentration of CO of 9 ppm for eight hours; however, a pregnant woman may be more susceptible to the effects of CO since in pregnancy oxygen consumption is increased 15–25%, and the blood oxygen capacity is decreased. Safe levels for pregnant females therefore are expected to be lower than this.

Other air pollutants. Hydrogen cyanide may be significant in its effects on the fetus and is primarily found in cigarette smoke. Its mechanism of action is expected to be similar to that of CO, and it may inhibit the development of the fetal central nervous system. No data is available on the effects in the pregnant woman.

Certain heavy metals may be found in polluted air and may act as teratogens. Primarily these include lead and cadmium and are discussed below.

Vapors from occupational exposure. Many vapors one may be occupationally exposed to are toxic, but few are known to be associated with teratogenic or fetotoxic effects in humans. The only two implicated so far are tetrachloroethylene and, with less certainty, nitrous oxide.

It has been known for many years that female anesthesiologists and anesthetists, as well as the unexposed wives of male anesthetists, have a significantly higher risk of spontaneous abortions, infertility, and birth defects. A review by Smithells (*18*) noted that the rate of spontaneous abortions was elevated twofold (29.7–37.8%); a study by the American Society of Anesthesiologists found a higher malformation rate (60% increased) in those exposed, as well as in the wives of exposed males. The identity of the anesthetic gas has not been established, but the effects may be due to nitrous oxide (N_2O). In animal studies, Fink (*19*) found N_2O to be lethal to chick embryos, and both fetotoxic and teratogenic to Sprague-Dawley rats. These results were confirmed by Lane et al. (*20*), who noted a fourfold increase in fetal resorption as well as ocular, central nervous system (CNS), and skeletal defects in rats. It has been hypothesized that N_2O or its metabolites may interfere with organogenesis by inhibiting vitamin B_{12}-dependent reactions, with inactivation via oxidation of cobalamine taking place. Apparently in addition to acting as a teratogen, the offending gas also is a mutagen, causing abortions in the unexposed female via mutations in the male gametes. In animal studies on other anesthetics, isoflurane (*21*) has not been shown to have fetotoxic or teratogenic effects.

Exposure to various solvent vapors (such as benzene and vinyl chloride) are considered hazardous to the pregnant woman, but no data on teratogenic effects in humans are available. Benzene and its methyl derivatives were found to be fetotoxic in rats (*22*), mice, and rabbits (*23*) at high doses, but Watanabe has described teratogenic effects (cleft palate and micrognathia at 3 cm^3/kg subcutaneous) in mice (*24*). Vinyl chloride was found to cause CNS defects in rats (*25*), and trichloroethylene is toxic and teratogenic in rats (*26*).

Tetrachloroethylene, a solvent present in dry-cleaning fluid, has been reported to cause cholestatic jaundice in a breast-fed infant (*27*). The mother was exposed to the vapors chronically, which accumulated in her breast milk. This chlorinated hydrocarbon is therefore fetotoxic, but its teratogenic capability is undetermined. An extensive list of solvents shown to be fetotoxic in various animal systems was tabulated by Wilson (*13*); a few others

include 1,1,1-trichloroethane, carbon tetrachloride, chloroform, and methylene chloride.

Food additives

Food additives are compounds knowingly added to food products to color, preserve, or enhance the taste of the product. In a broader context, they may also include some natural constituents of food that have teratogenic potential, as well as inadvertent additives or contaminants. These may be natural products, contaminants of processing, or residues, either on grains or stored in animal tissues. Examples of these are pesticides, PCBs, and heavy metals; these will be discussed later.

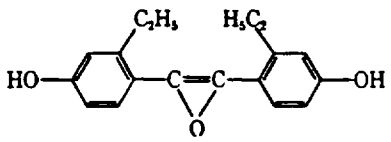
Although the Delaney Clause of the Food, Drug, and Cosmetics Act protects the public from additives that may be carcinogenic based on animal studies, no such clause exists with respect to teratogens. Cognizant of the difficulties involved in performing teratogenic testing and in interpreting the sometimes contradictory results, the Teratology Society has felt it inadvisable to enact such regulations (*28*).

Food dyes. No food coloring has come under closer scrutiny or criticism than Red Dye #2, or Amaranth, a dye used since the turn of the century without reported hazards to humans. The dye was used in soft drinks, gelatins, cereals, candy, lipsticks, pill coatings, etc. One study on Osborne-Mendel rats reported a dose-related embryotoxicity with both early and late deaths (*29*). No teratogenic effects were seen, but it was also carcinogenic in rats. Subsequent studies in the same and other mammalian species revealed a mild tendency for preimplantation loss and increased embryo resorption (*30-36*). The variability of the results among investigating groups brought the significance of these results under question. Again, no teratogenic effects were seen. The dye was removed from the market because of its potential carcinogenicity. However, subsequent work has not verified this azo compound's carcinogenicity (*37*).

Artificial sweeteners. Calcium cyclamate is an artificial sweetener first introduced in 1950 and widely used without ill effects. In 1969, one case report (*38*) appeared describing two infants born with a rather rare combination of cleft lip, cleft palate, and a lobster claw deformity. Allegedly, the only positive factor in the history of both mothers was the use of artificial sweeteners (cyclamates) in low-calorie foods. No other information of teratogenic effects of artificial sweeteners in humans is available. Studies on cyclamate, saccharin, and sucrose have failed to show teratogenic or embryotoxic effects in rats, mice, rabbits (*39*), or zebra fish (*40*). Studies with ^{35}S-cyclamate (*39*) show that it does cross the placenta and that its metabolite, cyclohexylamine, causes preimplantation loss, early fetal death, and growth retardation in Swiss mice (*41*). Because cyclamate caused bladder tumors in rats, its use was limited in 1969.

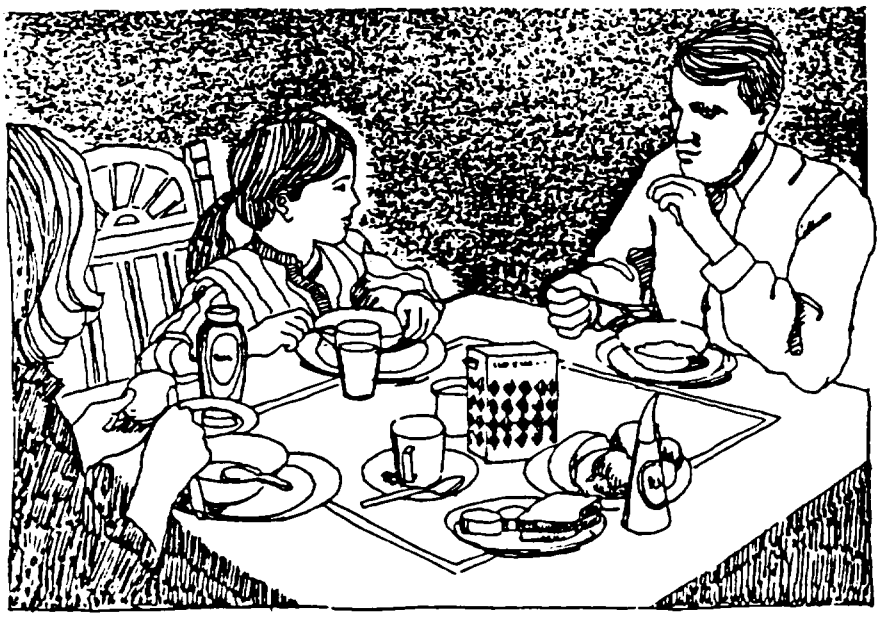
DES. Few people today are unaware of DES (diethylstilbestrol), a drug prescribed between 1940 and 1970 to prevent miscarriages. Herbst (*42*) showed that the infants exposed in-utero suffered carcinogenic and teratogenic effects in the females, and teratogenic effects in the males. The birth defects centered around the genitalia, with the females having cervical and vaginal adenosis and possible hypoplastic cervixes and uterine anomalies (*43*). These females later in life have a higher incidence of premature deliveries due to these defects. The exposed males have a higher incidence of sperm abnormalities (and therefore possible infertility), undescended testes, hypospadias, and epididymal cysts. DES also has the distinction of being the only known transplacental carcinogen in humans, giving rise to vaginal clear-cell adenocarcinoma in select females. About 350 recorded cases of this cancer in young women are known, 80% with documented exposure to DES. Significantly, Herbst pointed out that the effects of DES seem to be independent of dose, unlike most teratogens. Besides exposure to the pharmaceutical, the population at large may have been exposed to DES as a contaminant in food because of its use to fatten beef and poultry. Before its use for this purpose was discontinued, an estimated 50 million people were exposed to this compound in trace amounts. The effects, if any, of such an exposure have not been determined. Birth defects similar to those found in humans have been reproduced in mice (*44*).

The mechanism of action of DES proposed by Vorherr et al. (*44*) involves the unstable cis form, which attracts electrons to form an epoxide at the double bond that can then attach to DNA. A DES metabolite, hydroxydienestrol:



may also form an epoxide that is highly reactive. This may explain the carcinogenic action.

Preservatives. Sodium nitrite, a food preservative with antibacterial properties, has come under fire because of its tendency to be converted by bacteria in the gut to nitrosamines. By itself, $NaNO_2$ has mutagenic action in bacteria, fungi, and phages. Studies in mice (*46*), as well as our long-term experience in humans, have failed to show reproductive or teratogenic effects. The only demonstrable effect of $NaNO_2$ on the mammalian fetus (CD-1 mice) is its involvement in the oxidation of ferrous iron in hemoglobin to produce methemoglobin, with a resultant decreased blood oxygen carrying capacity. This oxygen deprivation in the fetus is manifested by an increased liver production of



"Studies with radiolabeled cyclamate (an artificial sweetener used as a food additive) show that it does cross the placenta and that its metabolite, cyclohexylamine, causes preimplantation loss, early fetal death, and growth retardation in Swiss mice. . . . its use was limited in 1969."

fetal red blood cells. The effect is dose-dependent (*46*) and is probably insignificant in humans at usual doses of exposure.

Nitrosamines are of concern because of their mutagenic and carcinogenic action in lower animals. Several nitrosamines are also known to be transplacental carcinogens in animals. In humans, they are formed in the colon by bacterial conversion of $NaNO_2$ and enzymatic reduction of nitrates. Independent of their carcinogenic effect, the ethyl and methyl derivatives are embryotoxic in rats, chicks, and hamsters (*47*); malformations have been demonstrated in BD-rats (*48*) only at near-lethal doses (ocular, brain, skeletal, and limb defects). Effects on humans are unknown. In addition to the above, *N*-nitrosodimethylamine may be found outright in six out of seven Scotch whiskeys and in 18 brands of beer (*14*). Beer may be the major dietary source of this compound to some pregnant women. FDA has estimated the levels of nitrosamines in beer to be at or below 1 ppb (*49*).

Another bactericidal food preservative, ethyl-*p*-hydroxy benzoate, was found to be without reproductive effect in studies on rats (*50*).

Chemicals naturally occurring in foods and inadvertent food additives. That natural products may be embryotoxic in lower animals is well known. For example, to the unlucky and unwary swine, wild black cherry, poison hemlock, jimsonweed, and tobacco stalks all are embryotoxic (*13*). Little is known of the teratogenic potential of natural products in humans. For centuries, Mexican women have terminated unwanted early pregnancies by drinking tea brewed from the zoapatle leaf; the active components, zoapatanol and montanol, have oxepane diterpene structures (*51*). Labor can also be induced and early pregnancies terminated by ingestion of ergot alkaloids produced by the fungus *Claviceps purpurea* (*52*), which may be a contaminant of grain, especially rye. Epidemics of ergot poisoning still occur. But in both of these cases, the action of the natural products is probably to induce abortion by eliciting labor, rather than by being embryotoxic. Mycotoxins may also be embryotoxic and teratogenic in lower animals, the most notorious of these being aflatoxin B_1 and G_1, ochratoxin A, rubratoxin B, and T-2 toxin (*53*). There has been speculation that a toxic product of the fungus *Phytophthera infestans* on blighted potatoes (*52*) may cause the higher incidence of neural tube defects (anencephaly and spina bifida) in humans seen in Ireland and the British Isles. This theory remains unproven.

Indirect additives may also take the form of trace chemicals migrating from food packaging, such as vinylidine chloride from plastic wrapping. Studies of this compound in rats and rabbits have shown embryotoxicity (at 80 ppm and 160 ppm), but no birth defects (*54*). One of the more studied possible food contaminants is nitrilotriacetate (NTA)—a compound used in detergents to replace sodiumtripolyphosphate, and which can enter food by adhering to eating utensils. The compound is a weak chelating agent able to sequester divalent cations, e.g., Ca^{2+}. The compound was voluntarily discontinued by its manufacturers in 1970 due to preliminary data suggesting teratogenic capacity by its ability to mobilize heavy metals, perhaps inactivating enzymes (e.g., containing Zn^{2+}), and its ability to concentrate these heavy metals in the fetus. It was speculated that it could perhaps enhance the teratogenic effects of cadmium and mercury (*55*). Studies by Nolan (*56, 57*) on rats and rabbits failed to substantiate fears of embryotoxicity or teratogenicity. In addition, Scharpf (*58*) showed that NTA had no effect on accumulation of cadmium in the fetus and that, in fact, this chelator offered some protection against the toxicity of cadmium (*59*) by inhibiting the deposition of this metal in maternal and fetal tissues in rats (*58*). In humans, however, chelating agents are useful in the treatment of acute inorganic lead and mercury poisoning, but not in the treatment of chronic mercury or cadmium toxicity (*60*). Likewise, chelating agents have not been effective in protecting the unborn human fetus in select cases of heavy-metal poisoning (Pb, Hg).

Caffeine is an additive to soft drinks, chocolate, and analgesics, but a natural constituent of coffee and tea. In September 1980, FDA issued a warning to pregnant women to stop or limit their use of caffeine (*61*), based on its observations of teratogenic effects. Some rats given the equivalent of 12–24 cups of coffee per day had missing digits, delayed skeletal development, and growth retardation. Concern was raised because these defects were seen at doses corresponding to the higher levels of human caffeine consumption. About 100 000 pregnant women are estimated to consume eight or more cups of coffee per day, and the intake from all sources is about 193 mg/day. More severe defects (limb reduction, cleft palate) were seen in higher doses in three strains of mice and two strains of rats (*62*). However, other studies (*63*) at low caffeine doses failed to duplicate these findings in rats.

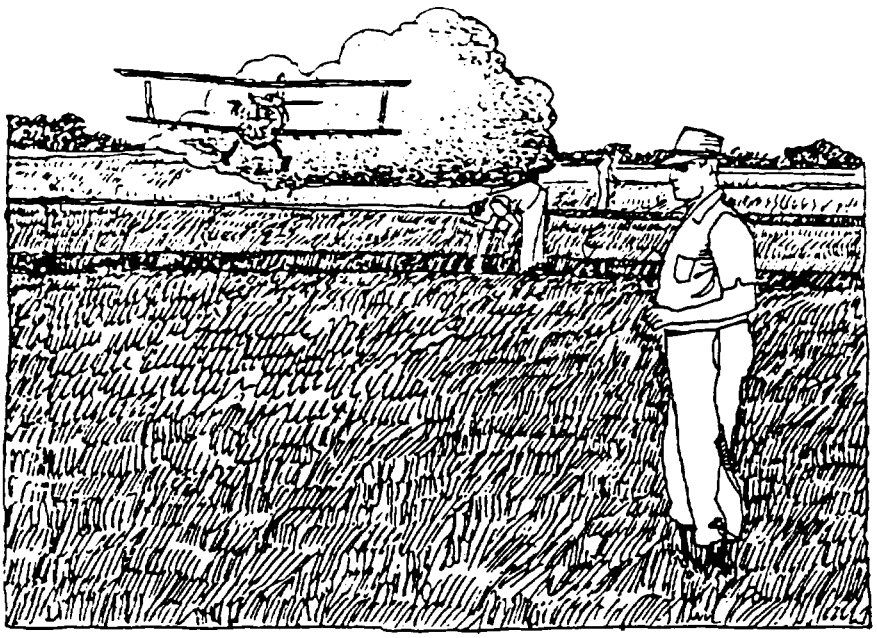
Caffeine is structurally a purine and is mutagenic in bacteria. It crosses the human placenta and is distributed to all fetal tissues. Uptake by the blastocyst has been demonstrated.

No effects of caffeine on humans are proven. However Lechat (*64*) studied 202 cases of birth defects in humans and found a statistically significant difference in the coffee consumption in the group with defective babies. An increase in the frequency of birth defects was seen in those who tended to be heavy drinkers (over seven cups per day). The study was controlled for age and for cigarette and drug consumption.

Caffeine (as well as calcium cyclamate and dimethylnitrosamine), although a mutagen, has also been demonstrated to be without effect in inducing sperm abnormalities in male mice (*65*).

Polyhalogenated biphenyls

PCBs. Polychlorinated biphenyls are a family of compounds largely used as heat exchangers (for example, in



transformers). Over the years, careless disposal of PCBs has contaminated the surface soil and aquatic system of this country. Its deleterious effects on health and reproduction were not fully appreciated until this last decade. Gradually, a picture of embryotoxicity and teratogenicity in many species evolved. In fish, spinal defects and decreasing numbers of striped bass in the Hudson River (*66*) and East Coast waters was seen, and the commercial salmon industry in the Great Lakes was virtually destroyed (*67*). Accidental poisoning of chickens (*68*) with fish meal resulted in decreased hatchability, and CNS and ocular defects. Growth retardation, liver damage, and neonatal deaths were observed (*69*). In mammals, an increase in premature deliveries with neonatal deaths were observed in California sea lions (*70*) which had a two- to eightfold increase in concentration of PCBs and organochlorine pesticides in the mothers and pups. Reproductive failure in ranch minks (*70*) and embryotoxicity in rabbits (*47*) have also been recorded. In 1968, teratogenicity in humans was established.

PCBs tend to persist and accumulate in the environment because of its slow or nonexistent biodegradation. The compound is readily passed through the marine food chain and accumulates in fish tissues. This accumulation is related to the compound's relative insolubility in water and high lipid solubility, which causes tissue levels to be elevated by conceivably a factor of a million or more. This is called bioconcentration. PCBs are usually sequestered in fatty tissues such as adipose and in the breast. Long-term, low-level exposures to PCB therefore give rise to high concentrations in breast milk, especially in fish eaters, since human milk is 1–4% fat and exists in equilibrium with the body's other fat stores in terms of chemicals present. PCBs have been detected in human breast milk at mean concentrations of about 87 ppb (*71*).

Human experience with PCBs stemmed from a large-scale poisoning from ingestion of contaminated rice (cooking) oil in southern Japan in 1968 (*72*). Leakage of the heat exchanger from a pinhole in the heating coils contaminated the rice oil to levels of 2000–3000 ppm. The poisoning dose in this incident amounted to 0.5–2 g/day. One thousand fifty-seven recorded victims had symptoms which included an acnelike rash, headache, nausea, and diarrhea. Thirteen infants were born to exposed women—two were stillborn and the others showed growth retardation; dark cola-colored pigmentation of the skin, gingiva, and nails; conjunctivitis; and neonatal jaundice at birth. Infants who were breast-fed had higher serum PCB levels, and a nine-year follow-up revealed some neurologic and developmental impairment. A 13-year follow-up found PCBs in the tissues of children who had been breast-fed, the level varying with the duration of breast feeding (*71*).

In studies with rats, transfer of PCBs by breast milk exceeded transplacental transfer by a factor of 10 (2% vs. 0.28%) (*73*). Therefore, for women with high exposures to PCBs, the American Academy of Pediatrics has recommended assaying the breast milk before suckling the infant. Also, eating sports fish is discouraged during pregnancy since this is the major dietary source of PCBs. Dieting during pregnancy is discouraged since this may encourage mobilization of chemicals from the fat stores.

This clinical entity related to PCB ingestion has been variously named "Yusho" (oil disease), "cola-colored baby syndrome," and "PCB-induced fetopathy." The mechanism of action is unknown. PCBs are a family (*68*) of 210 possible chlorinated compounds, and some indication exists that the less-chlorinated species may be more toxic (*69*). Although the original heat exchanger in the Japanese poisoning was known to contain contaminants such as polychlorinated dibenzofurans, which are embryotoxic and more potent than PCBs (*68*), the full spectrum of the disease was still produced in rhesus monkeys with purified PCBs (*74*).

PBBs: Little is known of the teratogenic potential of polybrominated biphenyls, used as fire retardants. Only one accident with this compound is recorded. In 1973–1974, PBB was accidentally mixed with cattle feed i Michigan (*14*) and an increase in th incidence of stillbirths in the fed cattle was noted. Subsequently 30 000 cattle, sheep, swine, and poultry were slaughtered. Unfortunately, the cattle carcasses were used as pig food and the manure was used for fertilizer throughout the state. PBBs at 68 ppb have since been detected in the breast milk of women in Michigan (*71*). No harmful effects on human pregnancies or infants have been reported, however.

Pesticides

Organic pesticides become pollutants when they enter our food supplies as inadvertent additives; they are present in trace amounts on grains in our surface and ground drinking wa and even in the tissues of lower animal forms. Pesticides by definition include insecticides, herbicides, and fungicides. These in turn can be variously subclassified by chemical groups, such as the methyl carbamates, organophosphates, organochlorides, ureas, and phenoxyacetic acids. The teratogenic capability of pesticides is not limited to any particular grouping, but rather transcends all groups; the same is true of pesticides and their mutagenic action.

That pesticides were associated with reproductive failure in lower animals became apparent in the 1960s with decreased hatchability of avian species ("The Silent Spring"). Since then, the teratogenic action of pesticides has been confirmed, and extensive listings

"In 1969, scientists reported that DDT, DDE, lindane, dieldrin, and heptachlorepoxide were present in cord blood (fetal) and tissues of 10 stillborn infants, in levels equal to that of adults. This confirms the transplacental transfer of these compounds, which we now know can also reach the newborn via breast milk."

of pesticides and their embryotoxic and teratogenic effects on various lower species have been published: see, for example, Wilson (*13*). Good studies and well-documented cases of teratogenic effects of pesticides in humans, on the other hand, are lacking. Only the organomercurials are conclusively known to be teratogenic in humans (*13*).

Because of their lipid solubility and resistance to degradation, the presence of organochlorine pesticides have also been well documented in human adult tissues (*75*). In 1969, Curley et al. (*76*) reported that DDT, DDE, lindane, dieldrin, and heptachlorepoxide were present in the cord blood (fetal) and tissues of 10 stillborn infants, in levels equal to that of adults. This confirms the transplacental transfer of these compounds, which we know can also reach the newborn via breast milk. Whether these compounds reaching the fetus exert toxic or teratogenic effects is speculative. One Japanese report (*12*) notes increased fetal deaths and malformations following acute high-level spraying of organophosphorus insecticides on field workers. Another contention of birth defects in humans from such sprayings was raised by Nora (*77*), who reported two infants with limb defects (talipes equinovarus) from 50 mothers with first trimester exposure, and 125 with later exposures. However, in this poor study, the pesticides were not identified, nor was the history of concurrent drug exposure explored.

An interesting observation by O'Leary et al. (*78*), noting elevated pesticide levels in premature infants, may have some implications with respect to human fetal toxicity. Measuring DDE levels in fetal whole blood, they found that premature infants had levels elevated three- to fourfold relative to control, term infants. Although these elevations could be explained by the absence of body fat to store the compound, which is common in preterm infants, a causative role of chlorinated pesticides in inducing premature deliveries cannot be ruled out. Other supporting data comes from animal studies. DeLong (*70*) reported that California sea lions which delivered their pups prematurely had an eightfold increase in fat levels of chlorinated pesticides (DDT, DDD, DDE, and PCB) relative to those which delivered pups at term. The premature pups themselves had a twofold elevation in fat levels of these compounds. Similarly, in New Zealand, in white rabbits fed DDT (50 mg/kg), a premature delivery rate of 57% was noted, with a 25% early fetal loss (*79*).

Two classes of compounds of particular interest are phenoxyacetic acids (2,4-D and 2,4,5-T) and dioxins (2,4,7,8-TCDD). The question of their teratogenic potential arose following their use in Vietnam, where an increase in birth defects was reported (*80*) with the initiation of the defoliation program. "Agent Orange" is a mixture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D), herbicides that also contain the contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), formed from chlorophenols in the synthesis of the herbicides (*9*). Questions have been raised about the effects of these chemicals on the fertility of 3000 exposed veterans and about possible mutagenic effects on their gametes. Evidence linking 2,4,5-T with defects in mice was first provided by a Bionetics study (*80*). Subsequent work by Courtney (*81*) showing increased incidences of cleft palate and cystic kidneys in mice and rats was later shown to be primarily due to the contaminant TCDD (*82, 83*). TCDD is an extremely potent fetotoxicant and teratogen, and can induce cleft palate in mice with doses as low as 1 $\mu g/kg \cdot d$ (*84*). Purified 2,4,5-T in high doses has been found to be teratogenic also (*85*); data on 2,4-D is as yet inconclusive. Of all the isomers of dioxin, only the 2,3,7,8-tetrachloro- form has a high embryotoxic effect; an important aspect of TCDD's effect may be to potentiate the teratogenic effect of other compounds (*86*).

The National Toxicology Program's latest study on male mice fed 2,4-D, 2,4,5-T, and TCDD has been unable to show an effect on male fertility, reproduction, germ cell toxicology, or survival or development of offspring (*87*). However, the results of the teratologic studies in animals with 2,4,5-T and TCDD are clearly positive. In humans, on the other hand, a relationship between exposure to these compounds and teratogenic or fetotoxic effects as listed below are suggestive but largely circumstantial:

1. An inordinate number of abortions were noted in women in Alsea, Ore., in 1978 after the area had been sprayed with 2,4,5-T containing TCDD (*88*).
2. A similar spraying in New Zealand resulted in two children with meningomyeloceles from mothers who drank rainwater collected from the roofs (*89*). The rainwater was not assayed for TCDD, however, and so this association was implied but unproven.
3. An elevated incidence of cleft palate was noted in Australia in areas following sprayings with 2,4,5-T (*90*).
4. An elevated rate of spontaneous abortions was noted in the Love Canal district. Dioxin, along with many other chemicals, was present in the toxic wastes at the dump site, but direct cause and effect has not been shown.

Another pesticide that gives evidence of human neonatal toxicity is hexachlorobenzene, a fungicide. Although human fetotoxicity or teratogenicity has not been established, the compound's presence in breast milk is known, and incidents of newborn poisonings following breast-feeding have been documented in Turkey. Skin pigmentation and a syndrome of toxicity resembling porphyria cutania tarda was described (*91*).

The class of pesticides called the phthalimides is of interest because of their structural similarity to thalidomide, a known teratogen. Captan, folpet, and difolatan, fungicides in wide usage, are mutagenic in bacteria, and captan has alkylating capability. Adducts of $SCCl_3$ with guanine have been isolated and usually result in base pair substitutions (*92*). Although birth defects are seen in chicks, none have

wise, no adverse effects have been seen in humans. As described below, the mechanism of action of teratogenicity of thalidomide in terms of structure-function relationships is unsolved (*93*).

Metals and inorganic compounds

Of all classes of chemical compounds, the metals are said to have the greatest potential for embryotoxicity and teratogenicity. Wilson summarized the experience of animal testing with metals (*13*) and found that of 25 metals, 20 were associated with increased fetal death and 17 were teratogenic. Some of what we know of the teratogenic potential of metals in animals is given in Table 1. Metals which are definitely embryotoxic or teratogenic in humans include mercury and lead. Those for which a high index of suspicion exists include lithium, selenium, and copper. These compounds as well as tellurium, cadmium, zinc, and chelates will be discussed below.

Alkyl mercury. Of all human teratogens, no other compound has been involved in more poisoning incidents or claimed more victims than mercury. Its organic form is used as an antifungal grain seed dressing, and multiple accidental poisonings have occurred when contaminated grain was mistakenly consumed. The first such incident occurred in Sweden in 1952 when methylmercury dicyandiamide (panogen) was ingested, resulting in intrauterine toxic effects (*94*). The infant born had brain damage manifested by mental retardation, incoordination, and inability to move. Poisonings with the same antifungal compound occurred in Guatemala in 1963-1965 (*95*). In 1963 in Pakistan, 100 cases of poisoning were recorded following ingestion of seed treated with Agrosan-GN, a mixture of phenylmercury acetate and ethylmercury chloride (*96*). Large-scale poisonings occurred in Iraq (*95*) in 1956 and 1960, with 6530 victims and 459 documented hospital deaths. In this case, flour was prepared from wheat seed treated with the antifungal seed dressing ethylmercury *p*-toluene sulfonanilide. Thirty-one pregnant women were victims and 45% died. Infants born with blood mercury concentrations of 2500 ng/mL suffered severe brain damage.

Also, infants born before the epidemic were exposed to organic mercury in breast milk, where its concentration was 3% of that in the mother's blood. One poisoning in the U.S. was noted when a pregnant woman in New Mexico ate pigs that had been fed contaminated grain (*97*). Her infant was born with tremors and convul-

TABLE 1
Teratogenic metals

Element	Species	Effect on fetus	Reference
Arsenic	Mice	Increase in males born	*117*
	Hamsters	Eye defects, exencephaly, renal agenesis	*115*
Cadmium	Mice	Abortions, many defects	*125*
	Rats	Abortions, many defects	*126, 127*
	Hamsters	Brain, eye, limb, craniofacial defects	*128*
Calcium	Hens	Increased anomalies	*47*
Cobalt	Chicks	Eyes, lower limb defects	*156*
Copper	Mice	Abortions	*13*
Gallium	Hamsters	Spina bifida, limb defects, exencephaly	*155*
Germanium	Hamsters	Spina bifida, limb defects, exencephaly	*155*
Indium	Hamsters	Digital defects	*155*
Lead	Rats	Early & late deaths	*152*
	Mice	Abortions, many defects	*152*
	Chicks	Growth retardation, eye and neck defects, micromelia	*153*
	Hamsters	Sacral vertebral defects, spina bifida, eye defects, exencephaly	*113, 151*
Lithium	Primates	Cardiac anomalies	*13*
Mercury	Birds	Fetotoxic	*47*
	Mice	High fetal deaths, cleft palate, retardation in growth and brain development	*102*
	Rats	Abortions, brain damage	*100*
	Hamsters	Poor head ossification	*9*
Molybdenum	Mice	Slight toxicity	*125*
Nickel	Chicks	Abortions, growth retardation, eye and limb defects, exencephaly	*149*
	Rats	Abortions, growth retardation, eye defects	*150*
	Hamsters	Abortions	*149*
Selenium	Chicks	Abortions, many defects	*118*
	Mice	Abortions, growth retardation	*46*
	Sheep	Limb, eye defects	*119*
Strontium	Rats	Abortions, growth retardation, increase in anomalies	*47*
Thallium	Chicks	Abortions, growth retardation, achondroplasia	*154*
	Rats	Growth retardation hydronephrosis, ossification defects	
Titanium	Rats	Abortions, growth retardation	*125*
Zinc	Hamsters	Abortions, exencephaly	*47*

"No other compound has been involved in more poisoning incidents or claimed more victims than mercury. At Minamata, Japan, 700 cases of human poisonings were recorded with a 38% mortality rate. . . . The lack of maternal symptoms attests to the ease with which methylmercury crosses the human placenta."

sions, and at the age of one year was blind and could not sit up.

Other mercury poisonings have followed from discharge of this element into natural bodies of water. For example, the wood pulp industry has been guilty of discharging phenyl mercury compounds into fresh water bodies, and chlorine-alkali plants have discharged metallic and inorganic mercury compounds (*95*). Discharge into our salt waters has essentially destroyed the swordfish industry, because of the high flesh alkyl mercury content. Currently, FDA limits for methylmercury in fish for consumption is 0.5–1.0 ppm. Large-scale poisonings of humans who ate fish contaminated with methylmercury were recorded in Japan at Minimata Bay (1953) and at Niigata (1964) (*95, 98*). The fetal encephalopathy or neurological damage that resulted from exposure during intrauterine development has since been named Minimata disease.

At Minimata Bay, inorganic mercury compounds used as catalysts at a plastics plant were dumped into the bay. The metallic and inorganic mercury salts were converted to alkyl compounds by anaerobic bacteria. The compounds were then taken up into the aquatic chain and by bioconcentration attained high levels in fish flesh. About 700 cases of human poisonings, and a 38% mortality rate, were recorded. In one area, the excess of newborn victims (17.3% of victims) relative to pregnant victims underscored the greater susceptibility of the newborn to mercury poisoning (*47*). In fact, in 17 of these infant victims with cerebral palsy, the mother had no symptoms to warn her of danger, and only a few had numbness of their fingers or fatigue (*99*). The lack of maternal symptoms attests to the ease with which methylmercury crosses the human placenta. The sequelae in these infants has been brain damage, manifested as mental retardation, spasticity, chronic seizures, and blindness. A follow-up study of surviving infants in one area from 1955–1959 showed that 6% of the children developed cerebral palsy (*14*).

Minimata disease has been reproduced in animals (*100*); the resulting brain damage involves diffuse neuronal degeneration in all regions of the brain, although the cerebellum and calcarine cortex appear most sensitive to methylmercury (*101*). Segmental atrophy of the axons, thinning of the myelin sheaths, and abnormal synaptic complexes also are seen. Methylmercury is able to produce such toxicity because of several factors: It has an affinity for the CNS (*47*); the dose is cumulative over time (*102*); and there is the phenomenon of fetal mercury "trapping" (*101*)—mercury crosses the placenta by facilitated transfer, at which point it is "trapped" by the fetus. The mercury is bound tightly to receptors forming a concentration gradient. The concentration of mercury in the fetal brain is four times that of the maternal brain, and the level in the fetal blood is 28% higher than in the maternal blood (*101*), indicating preferential accumulation in the fetus. The ease in crossing the placenta is greatest for alkyl compounds, aryl, and then inorganic compounds with lower transfer rates (*47*). With alkyl mercury, chronic exposures are more effective in causing deformities than single doses, since accumulation of the metal results in larger effective doses (*102*).

The chemical mechanism of alkyl mercury toxicity is unknown, but it may rest with its affinity for ligands, e.g., sulfhydryl groups. In this way it may affect a cell's permeability by action on the enzymes on the cell surface. Mercury is known to interrupt the normal function of mitochondria and lysosomes (*103*).

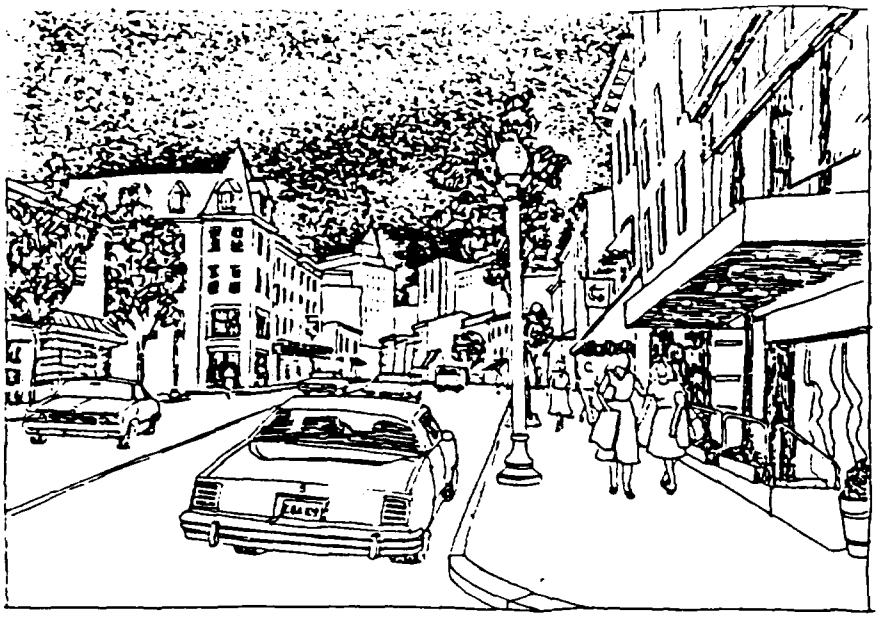
An effect of mercury and methylmercury in decreasing male fertility is well known (*37*). Whether a mutagenic effect on human gametes also can occur is not established. The frequency of chromosomal breaks in lymphocytes of 18 men and women in Sweden who chronically ate fish contaminated with methylmercury (*104*), was proportional to the mercury concentration in their erythrocytes. Concurrent studies of effects on human spermatocytes by such exposure and the outcome of the pregnancy have not been conducted.

Lead. Lead, like methylmercury, is embryotoxic and teratogenic in humans. Its milder effects may be due to a lower transplacental transfer or to differential potency. In pregnant women, its main effect is to increase the rate of spontaneous abortions. Its effect on the fetus may cause low birth weight or brain damage with convulsions and other CNS disorders (*105*). If poisoning takes place in the third trimester, fetal toxicity is below 25% and living infants are underweight, anemic, and develop slowly (*106*). Decreased reproductive ability in men occupationally exposed to lead has been documented via toxicity to sperm (*5, 107*) (decreased counts), which has also been confirmed by animal studies.

The association of high occupational lead exposure with sterility, abortions, and high fetal neonatal loss is well documented. Angle and McIntire (*105*), summarizing data from the turn of the century, cited a 60% stillborn and abortion rate among female lead factory workers and a 70% newborn mortality by the age of three. Industrial exposure data from Japan also cites a doubling of the spontaneous abortion rate, with blood lead levels of 0.11–0.317 mg% (*106, 108*).

At the turn of the century, the ability of lead to induce abortions was well recognized and was used illegally to terminate unwanted pregnancies. Hall (*109*) described 30 cases of maternal ingestion of a lead-containing compound (Diachylon) to induce abortions in England. The abortion rate was high, but so was maternal toxicity, including brain damage and blindness. In the same era in Denmark (*105*), lead oxide poisoning was reported in 25 cases of self-inflicted abortions. In this series, the abortion rate was 60% in the first trimester, with four abortions beyond 20 weeks. Three live births resulted from unsuccessful abortion attempts and one of these children died at the age of two months, possibly from toxic effects.

More recently, lead contamination of acidic drinking water from lead pipes has been implicated in Scotland (*110*), where there was a late fetal



death (lead was found in the fetal kidneys and the maternal urinary lead concentration was 86 mg/24°), one mother with frequent miscarriages and bleeding disorders of pregnancy, and two infants with anomalies (congenital nystagmus and hemangiomata). Transplacental lead intoxication has also been reported from maternal inhalation of fumes from the burning of battery cases and consumption of moonshine whiskeys (*111*). In fact, Palmisano (*111*) reported that 53% of these whiskeys contain lead concentrations over 1 mg/L and may be a common cause of lead intoxication in the southeastern U.S.

Lead crosses the placenta only if given in high concentrations; it tends to concentrate in the fetal bone and liver (*105*). Lead accumulates in the placenta and may induce abortions. Catz and Jaffee (*106*) reported a tendency for abortion associated with placental hemorrhage.

The chemical mechanism of lead teratogenesis and embryotoxicity is assumed to be related to its effects on the metalloenzymes involved in embryonic differentiation. Cole (*112*) suggested an inhibitory effect similar to that which it exerts on the enzymes of the heme synthetic pathway, e.g., delta amino levulinic acid. Lead interacts with other metals in teratogenesis in animal studies. The frequency and severity of lead-induced malformations in hamsters is potentiated by the presence of cadmium, which, when acting in concert, introduces new malformations (sympodia) (*113*).

Arsenic. Arsenic (Na_3AsO_4) is the only teratogenic member of the Group V elements. In rats, ewes, and hamsters, it causes cleft palate, exencephaly, anophthalmia, microphthalmia, and renal agenesis (*114*). In humans, however, organic arsenicals do not cross the placenta, but rather accumulate there. These compounds were used in the past in the treatment of syphilis without report of fetotoxicity (*115*). In the anionic form, however, arsenic does cross the mammalian placenta to some extent. One case (*116*) reports a human fetal death resulting from a mother's suicide attempt. She ingested a toxic dose of rat poison (As_2O_3) and subsequently delivered a live (30 week) infant. The infant soon died of severe intraalveolar pulmonary hemorrhages and had high arsenic levels in its liver, kidneys, and brain. No birth defects were noted. Arsenic therefore has the potential to be fetotoxic in humans.

The effect of arsenic can be reduced by administering selenium (*117*). Presumably, selenium acts by competitive inhibition at the sites where arsenic exerts its activity.

Copper. Copper ($CuCl_2$) is known to be lethal to mice embryos in the blastocyst and late preimplantation stage (*13*). Considering the effective use of metallic copper in intrauterine contraceptive devices in preventing pregnancy in humans, it is interesting to speculate whether embryolethal effects of copper may hold true in humans also. Prevention of implantation by endometrial inflammation from the device has also been proposed.

Selenium. Selenium (Na_2SeO_3) is embryotoxic and teratogenic in hens, sheep, and calves; among other defects, shortened limbs are produced (*118*, *47*). One case describes reputed effects of selenium in humans whereby five pregnancies in women employed in handling selenium gave rise to four abortions and to one live birth of a child with clubbed feet (*119*).

A contention that the high content of selenium in Ireland's soil is causally related to the high incidence of neural tube birth defects in that country is also unproven.

In hamsters, selenium blocks the teratogenic effects of cadmium and arsenic (*120*). It has been suggested that this may be due to its chelation to cadmium that is bound to the sulfur on sulfhydryl groups.

Tellurium. There is no evidence that tellurium (Na_2TeO_3) is embryotoxic or teratogenic in humans. In fact, at one time it also was used to treat syphilis. Overdoses of tellurium in men were detected by the classical sign of a garlic odor on the breath, due to dimethyltelluride, a metabolic product (*121*). No effect on human pregnancies was ever recorded, probably due to low transplacental transfer. Tellurium does cross the placenta and the blood-brain barrier in other mammals, however; this is of interest because it causes a very specific defect: nonobstructive hydrocephalus (*122*). Elemental tellurium is crystallized as a black precipitate in the brains of rats, and toxic changes are seen in the ependymal cells of the lateral ventricles, which perhaps breaks down the blood-brain barrier. Neurologic damage has similarly been seen in rabbits (ataxia). Since tellurium belongs to the same group as sulfur and selenium, its effect may be mediated by interaction with sulfur-metabolizing mechanisms (*123*).

Cadmium. Although cadmium poisoning is known to occur in humans, (the Japanese named it "Itai, Itai," meaning "agony"), embryotoxic or teratogenic effects in humans have never been documented. We do kn however, that cadmium crosses t... human placenta and is retained in the fetus, mainly in the liver and kidneys, where it accumulates (*124*). Cadmium is embryotoxic in mice (*125*), and teratogenic in the rats (*126*, *127*), in which it causes growth retardation, hypoplastic lungs, clubbed feet, and cleft palate. In hamsters (*128*), it causes craniofacial, brain, eye, and limb defects.

The mechanism of cadmium teratogenicity is believed to involve competition with zinc for divalent cationic sites in metalloenzymes (*129*, *130*). It has been observed that Zn^{2+} antagonized the action of Cd^{2+} (*131*), and a high Cd^{2+}/Zn^{2+} ratio is needed for toxicity (*124*). Barr (*127*) has made the observation that the limb-reduction anomalies associated with cadmium

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exposure bore a resemblance to those seen with acetazolamide exposure. Since acetazolamide inhibits carbonic anhydrase, a zinc-containing metalloenzyme, Barr proposed that cadmium may exert its teratogenic effect in animals by competing with zinc and affecting the activity of carbonic anhydrase in the developing fetus. Zinc (*127*) and selenium (*120*), therefore, are antagonists of cadmium; lead and mercury (*132*) potentiate the action of cadmium (in hamsters).

In a study of pregnant rats, Parizek (*133*) noted that as they approached term they became increasingly sensitive to the toxic effects of cadmium. He described the effects observed as resembling "toxemia," or hypertensive disease of pregnancy in the human. Pathological changes in the animals included renal cortical necrosis, venous congestions, adrenal hemorrhage, and convulsions. An association of hypertension with high renal cadmium levels may follow from the nephrotoxicity of cadmium (*124*). Parizek observed that cadmium chloride or acetate causes destruction of the fetal part of the placenta in rats (0.04 mmol/kg · d subcutaneous), with necrosis of the placenta and decidua, and intrauterine or external hemorrhage (*134*, *135*). In humans, heavy cigarette smoking is associated with an increased incidence of bleeding due to placental separation (abruption), with underlying placental and decidual necrosis (*136*). Whether this results from the cadmium present in cigarette smoke (*124*) or from the vasospastic action of nicotine (*136*) or some other factor is yet undetermined.

Zinc and chelates. Zinc deficiency in rats is associated with gross malformations (*137*). Therefore, any situation simulating zinc deficiency, whether by antagonism of other divalent cations as described above or by binding by chelating agents, is expected to produce teratogenic effects. Strong chelators such as ethylene diamine tetraacetic acid (EDTA), as expected, produce such defects in rats as cleft palate, malformed digits, clubbed feet, and brain defects, as well as increased abortions (*137*, *138*). Weak chelators such as trisodium nitrilotriacetate (*56*) and trisodium citrate (*59*) are without teratogenic effects. On the other hand, one might expect chelates to be useful tools to counter the effects of teratogenic divalent cations (e.g., Hg, Pb, Cd). Although in animals this has been confirmed with nitrilotriacetate (*58*) and trisodium citrate (*59*) (which are weakly efficacious in preventing fetal accumulation of cadmium and mercury, respectively), chelating compounds have not been found to be efficacious in humans to protect the fetus. Such experience is limited, however.

Other concerns

A relatively new concern that was unrecognized until recently is the adverse effects on human reproduction secondary to male exposure in the workplace. Previously, industry sought to protect the unborn fetus by removing it and the mother from the toxic source. But for effects produced through the male partner, it is clear that this policy is inadequate; reduction of occupational exposures for both sexes is the key.

Male sterility, fetal loss, and birth defects in their offspring was first recognized in the 1970s with exposure to Kepone and DBCP (1,2 dibromo-3-chloropropane) (*139*). Kepone, a chlorinated insecticide used to control fire ants, was implicated in 1975 when exposed workers suffered CNS changes, liver damage, and testicular damage (*3*). Sperm counts decreased and infertility resulted. Although production of Kepone was subsequently halted by the EPA, sources of exposure still exist in the environment (such as the contaminated James River (*140*)) and it is still produced by breakdown of other pesticides, such as Mirex. Similarly, DBCP, a soil fumigant used to control nematodes, decreases sperm counts in exposed workers and results in infertility (*5*). Production of DBCP was discontinued in 1977.

To date, compounds that decrease male fertility by toxic action on the testes and sperm include lead (*107*), DBCP (*5*), Kepone (*3*), and chloroprene (*5*). In addition, chloroprene exerts mutagenic effects on male gametes, manifested by a threefold increase in spontaneous abortions in the unexposed female. Possible mutagenic effects of vinyl chloride (*5*) are an increase in late fetal deaths, miscarriages, and stillborns. In addition to its effect on male sterility, DBCP may also induce birth defects; one case of penile abnormalities in an offspring has been noted.

The effect of male exposure to hydrocarbons remains controversial. A report of an increased cancer mortality rate in the children of exposed males has not been verified by further studies (*139*). Also, the contention that male exposure to the herbicide Oryzalin (*141*) results in an increased abortion rate and congenital cardiac defects has not been substantiated to date.

Another recently recognized problem in toxicology is poisoning of the newborn via chemicals present in breast milk. It is an avenue for neonatal toxicity for those compounds that cannot cross the placenta and for continued exposure and toxicity extending into the postpartum period and later stages of development. Pollutants in breast milk are generally characterized by high lipid solubility, resistance to degradation, and slow excretion rates. Long-term low-level exposure results in accumulation; lactation is the only way in which large amounts of the chemicals can be excreted.

Only three known compounds exerting such toxicity have been documented and these have already been discussed: hexachlorobenzene, PCB, and tetrachloroethylene. In addition to these, Rogan et al. (*71*) have recently confirmed and quantitated the levels of DDT (50–2000 ppb) and its primary metabolite DDE (2130 ppb) in whole milk, as well as the less-common pesticides dieldrin, heptachlor and its epoxide, chlordane, and oxychlordane. Concentrations of these chemicals in whole milk were 91–164 ppb. Human toxicity of these compounds is low, especially at these levels, but the possibility of increased susceptibility of newborns has not been studied. No

transmission of these chemicals has been reported.

The job ahead

Teratology had its birth as a science with the thalidomide disaster. It then became more than just an academic curiosity. It is unfortunate that much of what we know of chemical teratology in humans stems from disasters, rather than from testing. The toxicologic testing of chemicals is a reality, in spite of its critics who quote Karnofsky's law (*142*): Any compound can be teratogenic if given to the right species, at the right dose, at the right time. To be certain, the task is monumental, but the moral responsibility cannot be shaken.

Currently, teratologic screening as recommended by FDA consists of three segments (*142*), each to be applied to two or more species and at several concentrations:

1. Fertility and general reproductive performance is studied for both sexes over three generations from premating on.
2. Embryotoxicity and teratogenicity is studied in pregnant females (Somer's Test) during the period of organogenesis.
3. Peri- and postnatal studies in females are carried out to assess late injuries through the period of lactation.

This present mode of screening is expensive, time-consuming, and, according to some, low in predictive value. The future in teratologic screening will be in more rapid, inexpensive mass screening of chemicals. These methods, some still experimental, include:

1. in-vitro embryo cultures (*143*)—in rats to the early limb bud stage (48 hours)
2. post-abortive investigations on human embryos (*8*)—correlating malformations in the abortus with events during the pregnancy or in the maternal history
3. developmental enzyme patterns (*144*)—to assess late (i.e., other than first trimester) toxicity giving rise to functional deficits
4. epidemiologic (population) studies (*145*)
5. behavioral teratology—to study neurotoxicity from late exposures.

As chemists, we would think that structure-function relationships would hold great promise to predict teratologic potential. Unfortunately, no great insights have followed from such studies on two promising classes of teratogens: trypan blue and the diazo group of teratogens (trypan blue, nitrofen and azovan blue) (*146*), and the phthalimide family of compounds. Although thalidomide is a potent teratogen, none of the other *N*-substituted phthalimides (Figure 2), all containing the *o*-dicarboximido structure (captan, folpet, difolatan, etc.), have shown such a tendency (*147*). The active site or responsible functional group is still unknown. Only in terms of predicting a compound's alkylating ability have theoretical studies borne fruit; but, as already mentioned, this is more true in predicting a compound's mutagenic rather than teratogenic capability.

Through toxicologic testing of chemicals, our goal is to prevent future mishaps. But an important lesson must be learned from incidents like the ban on spray adhesives in 1973 (*148*). The ban, following one study implicating industrial exposure of women with increased chromosomal breaks and birth defects, prompted nine women to have abortions, only to see the ban lifted six months later when the associations could not be confirmed by further experiments. Testing and reporting must be accurate and responsible or the end result will be the hysteria and human anguish that we sought to prevent.

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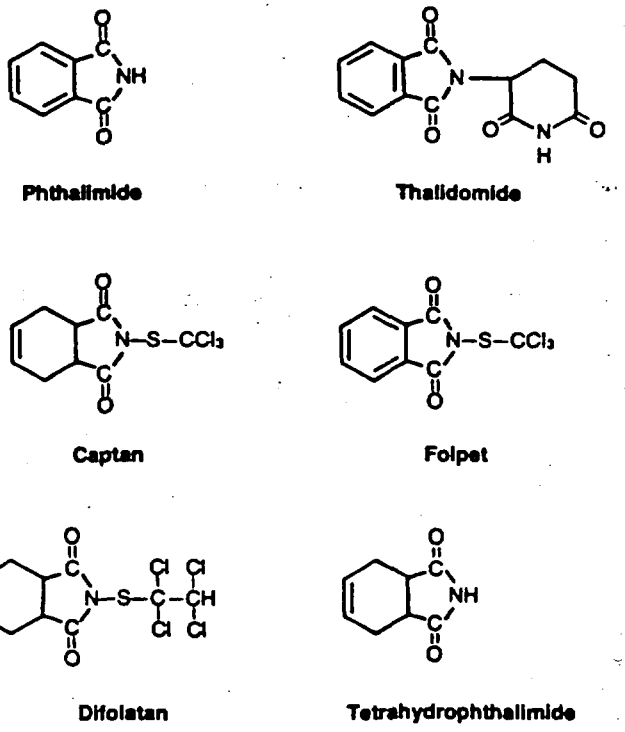
FIGURE 2
The phthalimide family



Among this family of compounds, only thalidomide is teratogenic. This teratogenic potential is assumed to be due to the particular functional group at the nitrogen of the phthalimide moiety.

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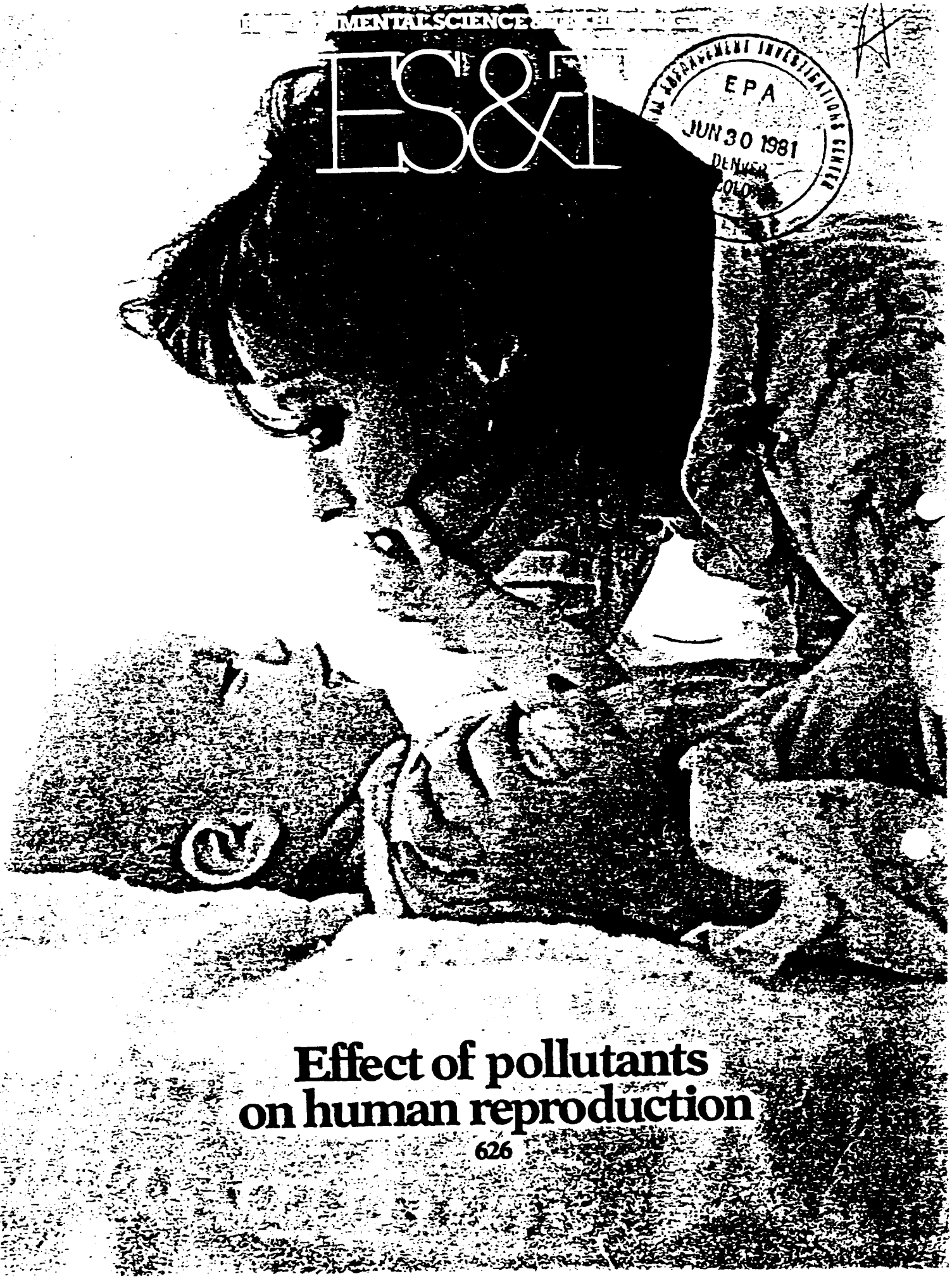

Effect of pollutants on human reproduction

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PESTICIDES IN PEOPLE

Organochlorine Pesticide Residues and Polychlorinated Biphenyls in Human Milk, Colorado—1971-72 [1]

E. P. Savage, J. D. Tessari, J. W. Malberg, H. W. Wheeler, and J. R. Bagby

ABSTRACT

Organochlorine pesticide residues and PCB's were determined in 40 human milk samples collected from women in rural Colorado during 1971-72. The highest levels found were of p,p'-DDE which ranged from 19 to 386 ppb; p,p'-DDT ranged from 7 to 109 ppb. β-BHC, o, p'-DDT, dieldrin, heptachlor epoxide, and p,p'-DDD ranged from a trace to 38, 13, 11, 5, and 5 ppb, respectively. PCB's ranged from 40 to 100 ppb. P,p'-DDE and p,p'-DDT were found in all milk samples, β-BHC in 87.5%, and PCB's in 20%.

Introduction

Laug *et al.* (5) reported DDT in 30 of 32 human milk samples collected in the Washington, D.C., area in 1951. Since that time, a number of investigators in the United States and other areas of the world have reported organochlorine compounds in individual and pooled human milk samples. Recent interest in the polychlorinated biphenyls (PCB's) has also resulted in several investigators reporting on PCB's in human adipose tissues, plasma, and human milk. Dyment *et al.* (2) were unable to find PCB's in human milk collected from participants living in Texas and New Guinea. Finklea *et al.* (3), in a study of 723 plasma samples collected from volunteers living in Charleston County, S. C., found *p,p'*-DDT and DDE almost universal, DDD in 84%, dieldrin in 63%, and PCB's in 43% of the study participants. Newton and Greene (7) analyzed 67 rural and urban human milk samples collected in Australia for organochlorine pesticides. All samples contained DDT and DDE, 12 contained DDD, and 29 contained dieldrin; they did not report on PCB's.

The current study reports levels of the organochlorine pesticides β-BHC, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDD, dieldrin, heptachlor epoxide, and the industrial pollutant PCB's in 40 human milk samples collected from women in rural Colorado during 1971-72.

Sampling and Analyses

Participants were asked to manually express milk samples into glass tubes equipped with plastic screw caps and teflon liners. The filled tubes were kept frozen until time of extraction.

Analytical standards for the chlorinated pesticides were obtained from the pesticide repository of the U.S. Environmental Protection Agency Laboratory, Perrine, Fla. The Aroclor analytical standards were obtained from the Monsanto Chemical Co., St. Louis, Mo. Solvents were redistilled in glass prior to use. The evaluation, storage, activation, and use of the Florisil followed the recommended procedures described in the "Manual of Analytical Methods" (8).

The extraction procedure used was a modification of those described by Guiffrida *et al.* (4) and Curley and Kimbrough (1). The procedure consisted of 3 parts: (1) isolating the fat from the milk, (2) extracting the Aroclors and pesticides from the fat, and (3) cleaning up the extract.

Whole milk (4.5 g to 24.3 g) was weighed into clean glass centrifuge bottles. Glass wool was added to adhere to the coarse precipitate of the milk solids. After addition of 100 ml of acetone, the sample was shaken manually for 1 minute and centrifuged for 2 minutes. The acetone layer was then transferred to a 1-liter separatory

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funnel. The extraction procedure was repeated three more times with equal volumes of 25 ml of acetone each time. All four acetone extractions were combined in the 1-liter separatory funnel.

Twenty-five milliliters of n-hexane was then added to the coarse precipitate of milk solids, shaken, centrifuged, decanted, and combined with the acetone extracts in the 1-liter separatory funnel. This procedure was repeated, and then a volume of 125 ml of 2% sodium sulfate solution and 50 ml n-hexane was added to the acetone and hexane extracts. The separatory funnel was shaken manually for 1 minute and the lower aqueous layer was discarded. The 2% sodium sulfate washing was again repeated, the lower aqueous layer discarded, and the n-hexane layer poured into a 500-ml concentrator flask. The sample extract was taken through the Florisil procedure as described in the "Manual of Analytical Methods" (*8*).

Primary identification and quantification of the pesticides was accomplished on a MicroTek 220 Gas Chromatograph equipped with a Ni^{63} electron capture detector and Coulson conductivity detector. Two columns having different resolution characteristics were utilized on all samples. Instrument parameters were as follows:

Columns:	(A) Borosilicate glass, 6' x ¼", packed with 1.5% OV-17 and 1.95% QF-1 on Gas Chrom Q 100-120 mesh Applied Science
	(B) Borosilicate glass, 6' x ¼", packed with 4% SE-30 and 6% OV-210 on Gas Chrom Q 100-120 mesh Applied Science
	(C) Borosilicate glass, 6' x ¼", packed with 3% OV-1—column obtained from Tracor
Detectors:	(A) Electron capture, having 14.5 mc Ni^{63} ionizing source
	(B) Coulson conductivity operated in the chloride mode
Temperatures:	(A) Electron capture
	Injector 245° C
	Column 200° C
	Detector 300° C
	(B) Coulson conductivity
	Injector 245° C
	Column 200° C
	Furnace 820° C
	Block 230° C
Carrier gas:	High purified nitrogen
Flow rates:	60 cc/min for Column A
	100 cc/min for Column B
	Approx. 60 cc/min for Column C

Primary quantification of the Aroclors was accomplished by thin layer chromatography (TLC). TLC provides a sound approach for the semi-quantitation of the stated PCB's because the various compounds of the series have similar Rf values and therefore produce a single spot. Six percent ethyl ether/n-hexane eluate, in concentrate form, is treated with KOH to effect dehydrochlorination of DDT and DDD to their olefins, thus eliminating the problem of separating these pesticides from the PCBs'. Oxidative treatment is then applied to convert any interfering DDE to *p,p'*-dichlorobenzophenone which has an Rf value different from the PCB's. The PCB's are then determined by thin layer chromatography. The procedure used is a modification of the one described in the "Manual of Analytical Methods" (*8*) which is based on the method used by Mullhern *et al.* (*6*).

Gas chromatographic sensitivity limits for *p,p'*-DDT and Aroclor 1254® were 20 and 500 picograms, respectively. TLC sensitivity limits for the Aroclors were based upon visual comparison of sample spot intensity to the intensities of Aroclor standards at 25, 50, 100, and 400 ng/μl. Recovery rates were established by spiking separate milk samples with known amounts of standards as follows: Arocolor 1254® at 0.5 ppm and Aroclor 1260® at 1.25 ppm; β-BHC, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD, and dieldrin at 1 ppb; *p,p'*-DDT, α-BHC, aldrin, lindane, heptachlor, and heptachlor epoxide at 4 ppb. These samples were then analyzed utilizing the described procedures. Recoveries from the spiked pesticide samples were based on unspiked control samples. The pesticide recoveries ranged from 85.2 to 118%. Recoveries for the Aroclor samples based on five major peaks ranged from 75 to 83%. For further verification, the PCB samples were spotted on thin layer plates and compared with gas chromatographic data. The TLC recoveries ranged from 75 to 90%. Residues were not corrected for percent recovery.

All samples were placed on two different columns for electron capture detection and then concentrated to 1.0 ml or below for analyses on the Coulson detector.

Results

The percent of human milk samples with residues of organochlorine pesticides and PCB's are presented in Table 1. *P,p'*-DDE and *p,p'*-DDT were found in all 40 milk samples. β-BHC was found in 87.5% and PCB's in 20% of the samples.

TABLE 1.—*Percent of total milk samples with organochlorine pesticide residues and PCB's, based on 40 samples, Colorado—1971-72*

Compound	Percent Positive Samples
p,p'-DDE	100.00
p,p'-DDT	100.00
β-BHC	87.5
o,p'-DDT	72.5
p,p'-DDD	35.0
Dieldrin	45.0
Heptachlor epoxide	25.0
PCB's	20.0

The levels of selected organochlorine pesticides and PCB's in each of the 40 milk samples are listed in Table 2. The highest levels found were of *p,p'*-DDE which ranged from 19 to 386 ppb and *p,p'*-DDT, from 7 to 109 ppb. β-BHC, *o,p'*-DDT, dieldrin, heptachlor epoxide, and *p,p'*-DDD ranged from a trace to 38, 13, 11, 5, and 5 ppb, respectively. PCB's ranged from 40 to 100 ppb.

Values for total organochlorine pesticides and PCB's in each sample are given in Table 3 with selected epidemiological data for each donor: age, occupation, birthplace, number of years lived in Colorado, and whether the participant had ever lived in a city of 50,000 population or greater. The total organochlorine levels ranged from a high of 521 to a low of 27 ppb. The age of participants ranged from 19 to 33 years.

Discussion

All samples contained *p,p'*-DDE and *p,p'*-DDT; β-BHC was found in 87.5%, *o,p'*-DDT in 72.5%, dieldrin in 45%, and PCB's in 20% of the samples. Milk from one study participant (No. 20) contained quantifiable levels of all compounds except heptachlor epoxide. Heptachlor epoxide and PCB's were never found in the same sample in the study, and *p,p'*-DDD and PCB's were found in the same sample on only one occasion. The total organochlorines per sample ranged from 27 to 521 ppb. The sample with the lowest level of organochlorines contained *p,p'*-DDE and *p,p'*-DDT. The sample with the highest level contained β-BHC, *p,p'*-DDE, *p,p'*-DDT, dieldrin, and a trace of *o,p'*-DDT, *p,p'*-DDD, and heptachlor epoxide. The highest level of total organochlorines occurred in the sample from a 24-year-old housewife who had spent all but 1 year of her life in Colorado. Only nine women had not lived in a city of 50,000 population or greater. Two of these nine were positive for PCB's. The fact that *p,p'*-DDE and *p,p'*-DDT were found in all samples was not surprising as Laug *et al.* (5) found DDT in 30 of 32 milk samples collected in the Washington, D. C., area in 1951.

Newton and Greene (7) found DDT and DDE in all of 67 human milk samples collected in Australia. Dieldrin was recovered in approximately 43% of the Australian samples and in 45% of the samples in our study. Dyment *et al.* (2) did not find PCB's in human milk collected from residents of New Guinea and Texas, but Finklea *et al.* (3) in a study of plasma collected from volunteers living in Charleston County, S. C., found PCB's in 43% of the study participants. In the current study, we found PCB's in 20% of the samples.

Results of this study and other studies indicate that organochlorines are widespread in human milk. Although *p,p'*-DDE and *p,p'*-DDT are found almost universally, *p,p'*-DDD, dieldrin, heptachlor epoxide, and PCB's are not as widely distributed.

See Appendix for chemical names of compounds discussed in this paper.

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TOXICOLOGY AND APPLIED PHARMACOLOGY 62, 294-306 (1982)

Disposition of Polychlorinated Biphenyl Congeners in Occupationally Exposed Persons[1]

MARY S. WOLFF, JOHN THORNTON, ALF FISCHBEIN, RUTH LILIS, AND I. J. SELIKOFF

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Disposition of Polychlorinated Biphenyl Congeners in Occupationally Exposed Persons. WOLFF, M. S., THORNTON, J., FISCHBEIN, A., LILIS, R., AND SELIKOFF, I. J. (1982). *Toxicol. Appl. Pharmacol.* 62, 294-306. Relative concentrations of 37 polychlorinated biphenyl (PCB) congeners and the adipose-plasma partition of 28 PCB congeners were investigated in 26 persons occupationally exposed to various PCBs (20 to 54% chlorine). Concentrations of PCBs in adipose and plasma were related to duration and intensity of exposure in the workplace. PCB concentration in adipose tissue was proportional to that in plasma, with a partition for total PCBs of approximately 190:1 indicated from regression analysis. PCB congeners with chlorines in both 4-positions of the biphenyl ring were the major components in plasma and adipose tissue. Congeners with unsubstituted 3,4-positions (e.g., 2,5-chlorine substitution) on at least one of the biphenyl rings were observed at lower concentrations and had lower adipose-plasma partition than other congeners. In contrast, those compounds with substituents at the 2,4- and/or 3,4-positions on both rings were present in much higher proportions in blood or adipose than in the PCB mixtures in use. These components also had higher adipose-plasma partition than those with unsubstituted 3,4- positions, regardless of the degree of chlorination. PCBs with 2,4-substitution patterns on both rings, including 2,4,4'-tri- and 2,4,5,4'-tetra-CBs, had somewhat higher adipose-plasma partition than congeners with 3,4-substitution on at least one of the biphenyl rings (e.g., 2,4,3',4'-tetra-CB).

The complexity of PCB[2] mixtures introduced into the environment results in the presence of many individual PCB congeners in human tissues. Usually, these are reported s the sum of several gas chromatographic eaks relative to a commercial PCB mixture. Individual PCB components have been identified in blood or adipose tissue in a few studies. Jensen and Sundstrom (1974) determined 40 PCB congeners in human adipose tissue. PCBs with no chlorines at the 3,4-position of one ring (e.g., 2,5-dichloro-), which were major constituents of commercial PCB mixtures, constituted about 20% of PCBs in human adipose tissue. Two compounds, 2,4,5,2',4',5'- and 2,3,4,2',4',5'-hexachlorobiphenyl, accounted for 36% of the PCBs in tissue. Similar findings for blood and adipose tissue were reported by Kuroki and Masuda (1977).

Identification of specific PCB structures in human tissues may be important not only for assessment of long-term persistence but also for evaluation of potential health effects. This has been suggested by toxicological studies of individual PCB isomers, which have demonstrated the specificity of toxicity and inducibility of mixed-function oxidase enzymes (i.e., cytochrome *P*-448 or *P*-450)

[1] Presented in part, at the Second International Congress on Toxicology, Brussels, July 1980.

[2] Abbreviations used: PCB, polychlorinated biphenyl; *p,p'*-DDE, 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene; CB, chlorobiphenyl.

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Recoveries of Aroclor 1254
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1254 in DMSO) 93 ± 7% (n

with respect to chlorine substitution patterns. The simplest PCB inducer of cytochrome *P*-448, 3,4,3',4'-tetrachlorobiphenyl, has a stereoelectronic molecular configuration consistent with requirements for binding to the postulated receptor, including an energy barrier favorable to achievement of coplanarity of the biphenyl rings (McKinney and Singh, 1981; Poland *et al.*, 1979). Addition of chlorines at both *ortho*- positions, as in 2,4,5,2',4',5'-hexachlorobiphenyl, results in preferential induction of cytochrome *P*-450. Recent studies have demonstrated the inducibility of both *P*-448 and *P*-450 by PCB congeners with 3,4-substitution on one ring, e.g., 2,4,5,3',4'-pentachlorobiphenyl (Parkinson *et al.*, 1980a,b; Yoshihara *et al.*, 1979). Many PCB congeners with such substitution are present in commercial PCBs and in human tissues (Sissons and Welti, 1971; Jensen and Sundstrom, 1974).

As part of a larger study of PCB exposure among capacitor manufacture workers (Fischbein *et al.*, 1979), the disposition of individual PCB congeners in adipose tissue and plasma was investigated in 26 cases.

METHODS

Plasma and adipose samples were obtained from workers in a capacitor manufacturing facility using PCBs, who participated in a clinical health survey in 1976. Clinical aspects of this study have been reported (Fischbein *et al.*, 1979), and a detailed report of the relation of PCB exposure to PCB concentrations in adipose and plasma (determined using packed column gas chromatography) will be reported elsewhere. From this study, 26 paired adipose and plasma samples were analyzed by high-resolution gas chmatography (HRGC) with electron-capture detection, each pair being run successively on the same day. Sample cleanup was identical with that previously reported for polybrominated biphenyls (Wolff *et al.*, 1979a). A 2 ml plasma sample (Florisil cleanup) was analyzed from a final volume of 0.05 to 0.1 ml. Adipose samples (gel-permeation chromatography cleanup; lipid equivalent 30 to 60 mg) were analyzed from a final volume of 0.1 to 0.5 ml. Recoveries of Aroclor 1254 from adipose were (mean ± SD) 97 ± 7% (n = 8); from hexane 95 ± 5% (n = 8; Florisil cleanup); from plasma (fortified with Aroclor 1254 in DMSO) 93 ± 7% (n = 18). Recovery of individual PCB congeners (Table 1, peaks, 1, 4, 8, 11, 17, 22, 25, 29, 32, 37 and 3,4,3',4'-tetraCB) from adipose was 93 ± 7%. From plasma, recovery was 92 ± 7% for 9 of these compounds; 85% for peak 29; and 15% for 3,4,3',4'-tetra-CB, the latter two due to retention on Florisil. Retention of Aroclor 1254 peaks was less than 10%. The lower recovery of 3,4 (than of 2,4-)-substituted congeners from plasma (but not adipose) would cause the adipose–plasma partition of 3,4-substituted congeners to be lower than those reported in Table 1, thus not affecting the conclusion that 3,4-substituted congeners have lower partitions than 2,4-substituted congeners.

Standards (Aroclors and 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene [*p,p'*-DDE]) were obtained from the EPA reference library, Research Triangle Park, North Carolina. Individual PCB congeners were obtained from RFR, Inc., or Analabs. Each of these standards had the correct retention time (see below) and only one major peak (more than 99%).

PCB concentration of individual peaks was calculated using 2,4,4'-trichlorobiphenyl, 2,4,5,2',5'-pentachlorobiphenyl (which had a response factor similar to most of the tetrachloro isomers), and 2,4,5,2',4',5'-hexachlorobiphenyl for the tri-, tetra- and penta, and hexa- and hepta- isomers, respectively. Other isomers were not available at the time the analyses began. The use of the latter two standards was based on analyses of standards later available, which showed (peak No. from Table 1, response factor): 1, 1.9, 3, 2.3; 7, 1.56; 8, 1.58; 11, 1.73; 18, 1.61; 17, 1.25; 25, 0.73; 29, 1; 32, 0.77.

Response factors are relative to DDE = 1.0. The response factor for DDE (pg/(μl × area), uncorrected for split ratio and volume injected), varied from 0.32 to 0.44 during the course of the analyses. Response factors for the standards (relative to DDE; 12 runs) were (mean ± SD): 2.01 ± 0.06 (peak 1); 1.59 ± 0.08 (peak 11); 0.98 ± 0.11 (peak 22). Although the quantitative data from these analyses can only indicate relative concentrations, the use of the data for adipose-plasma partitions is not dependent on absolute concentrations, since adipose and plasma samples were run side-by-side the same day, and thus response factors were the for both.

HRGC was performed on a modified Perkin-Elmer 3920 instrument, with a split ratio of 60, with a 70-m WCOT OV17 glass capillary column (0.21-mm i.d., 210,000 theoretical plates) operated isothermally. The column temperature was adjusted so that *p,p'*-DDE eluted at ca. 30 min.

GC-mass spectroscopy (GC-MS) was accomplished on a Finnigan 4000 instrument interfaced with a 50-m OV101 or 25-m OV17 glass capillary column. Masses 200 to 360 were scanned in the electron impact mode at 0.5 to 0.7-sec intervals.

Identification of PCB congeners (peaks 2–5, 7, 9–12, 14–37, Table 1) was by Dr. Goran Sundstrom, Wallen-

TABLE 1

ANALYSIS OF PCB CONGENERS IN ADIPOSE TISSUE AND PLASMA AMONG CAPACITOR MANUFACTURE WORKERS

Peak No.	Structure assignment	Concentration in Adipose (μg/g) Median	Adipose (μg/g) Range	Plasma (ng/ml) Median	Plasma (ng/ml) Range	Adipose-plasma partition
1A	4,4'-	0.08	0.002-0.2	0.7	0.03-2	90
1	2,4,4'- (2,5,4'-)	9	0.5-183	27	1-850	200
2	2,5,2',5'-	0.5	0.07-3	4	0.5-23	80
3	2,4,2',5'-	0.3	0.06-4	2	0.2-42	90
4	2,4,2',4'-	0.6	0.09-11	3	0.2-24	310
5	2,3,2',5'-	0.5	0.1-5	3	0.4-55	80
5A	(2,3,4,2'-; 3,5,3',5'-)	0.3	0.04-3	1	0.2-42	60
6	2,4,5,4'-	7	0.5-36	26	0.7-180	200
7	2,5,3',4'-	0.4	0.1-2	2	0.2-27	70
8	2,4,3',4'-	2	0.2-18	7	0.3-113	160
9	2,3,6,2',5'-	0.2	0.05-2	1	0.3-9	60
10	2,3,5,2',5'-	0.1	0.08-0.3	0.9	0.6-2	
11	2,4,5,2',5'-	0.1	0.07-0.5	0.8	0.4-6	50
12	2,4,5,2',4'-	1	0.07-5	4	0.4-12	240
13	(2,3,4,4'-; 3,4,3',5'-)	0.9	0.03-6	3	0.7-29	190
14	2,3,6,2',3'-					
15	2,3,5,2',3'-					
16	2,4,5,2',3'-					
	p,p'-DDE	2	0.3-8	7	0.6-35	170
17	2,3,4,2',5'-	0.2	0.08-0.5	1	0.6-2	
18	2,3,6,3',4'- (3,4,3',4'-)	0.1	0.07-0.3	0.5	0.3-3	
19	2,3,5,3',4'-	0.1	0.09-0.2	0.6	0.4-1	
20	2,4,5,3',4'-	2	0.09-5	8	0.7-41	180
21	2,3,5,2',4',5'-	0.1	0.05-0.7	0.7	0.2-2	300
22	2,4,5,2',4',5'-	1	0.09-4	5	0.7-12	270
23	2,3,4,3',4'-	1	0.2-4	2	0.6-20	130
24	2,3,4,2',3',5'-	0.1	0.05-0.3	0.4	0.2-2	
25A	(2,3,4,5,3',5'-)	0.3	0.05-1	1	0.4-4	360
25	2,3,4,2',4',5'-	1	0.06-4	4	0.5-17	110
26	2,3,5,6,2',4',5'-	0.2	0.05-0.7	0.6	0.2-2	370
27	2,3,4,6,2',4',5'-	0.1	0.06-0.2	0.3	0.2-0.8	160
28	(2,4,5,3',4',5'-)	0.1	0.05-0.4	0.5	0.2-2	120
29	2,3,4,2',3',4'-	0.07	0.05-0.2	0.8	0.7-0.8	
30	2,3,4,5,2',3',6'-	0.09	0.05-0.2	0.3	0.2-0.3	
31	2,3,5,6,2',3',4'-	0.07	0.05-0.3	0.4	0.2-0.5	
32	2,3,4,5,3',4'-	0.4	0.08-2	2	0.2-5	310
33	2,3,4,5,2',4',5'-	0.6	0.05-3	3	0.6-8	270
34	2,3,5,6,2',3',4',5'-	0.2	0.05-0.5	1	0.2-2	260
35	2,3,4,5,2',3',4'-	0.2	0.08-1	1	0.5-3	360
36	2,3,4,5,2',3',4',6'-	0.1	0.05-0.2	0.7	0.2-1	
37	2,3,4,5,2',3',4',5'-		0.1			

Note. Concentrations were calculated from response factors of standards for peaks 1(1); 11(2-20, 23); peak 22 (21,22,24-36). Peaks 14-16 were negligible in fat and plasma. Partition was derived from regression coefficient (slope) of adipose vs plasma levels. Peaks with no partition values had fewer than four cases with detectable values in both adipose and plasma, or the correlation was not statistically significant (peaks 17, 18, 24). The number of cases with detectable peaks in both adipose and plasma were (peak, *n*): 1A, 14; 1, 25; 2, 16; 3, 9; 4, 17; 5, 17; 5A, 16; 6, 26; 7, 7; 8, 20; 9, 8; 10, 3; 11, 9; 12, 23; 13, 17; 20, 22; 21, 16; 22, 23; 23, 19; 25A, 17; 25, 23; 26, 16; 27, 7; 28, 10; 32, 19; 33, 22; 34, 4; 35, 11.

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Retention times of 4,4'
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were 2162 (1; 2,4,4'-tri-C
2368 (8; 2,4,3',4'-tetra-C
2,3,4,4'- or 3,4,3',5'-tetra-
tion was verified by HR
for peaks 1 and 8 corrob

Peak 13 was a tetrac
signed as either 2,3,4,4'-
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A peak at this retention t
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column was closer to that
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For purposes of compar
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47; 2,4,5,4'-, 2,5,3',4'-, and
2,4,5,2',4'-, and peak 13 to
also coelute at these retent
tion times were 125, 2,4
5,2',4',5'-hexa-CB + 2,3,4,
4',5'-hexa-CB; 203, 2,3,4,2'
3',4'-hexa-CB.

Statistical analysis was
University of New York c
by the Statistical Packag
(SPSS) or the Statistical A
Institute, Raleigh, N.C.).

AMONG CAPACITOR

ma (ng/ml) Range	Adipose-plasma partition
0.03–2	90
1–850	200
0.5–23	80
0.2–42	90
0.2–24	310
0.4–55	80
0.2–42	60
0.7–180	200
0.2–27	70
0.3–113	160
0.3–9	60
0.6–2	
0.4–6	50
0.4–12	240
0.7–29	190
0.6–35	170
0.6–2	
0.3–3	
0.4–1	
0.7–41	180
0.2–2	300
0.7–12	270
0.6–20	130
0.2–2	
0.4–4	360
0.5–17	110
.2–2	370
.2–0.8	160
0.2–2	120
0.7–0.8	
0.2–0.3	
0.2–0.5	
0.2–5	310
0.6–8	270
0.2–2	260
0.5–3	360
0.2–1	

s 1(1); 11(2-20, 23); peak 22
d from regression coefficient
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s 17, 18, 24). The number of
; 2, 16; 3, 9; 4, 17; 5, 17; 5A,
: 25A, 17; 25, 23; 26, 16; 27,

berg Laboratory, University of Stockholm, from synthesized standards using a 50-m OV17 capillary column. Retention times of 4,4'-dichlorobiphenyl and peaks 1, 2, 4, 5, 7, 8, 11, 17, 18, 22, 25, 29, and 32 (Table 1) were verified with standards on the 70-m OV17 column and also checked by coinjection with Aroclor 1254 or 1016 and with actual adipose or serum samples. Retention indices (RI) for individual PCB congeners were calculated for the OV17 or OV101 column according to Albro *et al.* (1977). These RIs were linearly correlated with retention times of congeners identified from standards on both OV17 columns. For peaks with retention times less than *p,p'*-DDE, the linear regression of RI versus retention time gave predicted values within 10 of that calculated with r^2 0.99 (50-m OV17 column with temperature program) or r^2 0.97 (70-m OV17 column, 250°C isothermal). For isomers differing only with respect to 2,4- or 2,5-substitution on one ring, e.g., peaks 7, 8 or 11, 12 the order of elution was reversed from the calculated RIs. Similar results for RI vs retention time were obtained with GC-MS using two columns.

Four major unidentified LPCB peaks (1, 6, 8, 13) had extrapolated RIs of 2174, 2333, 2352, 2413 (OV-17 column, respectively). RIs for the proposed structures were 2162 (1; 2,4,4'-tri-CB), 2325 (6; 2,4,5,4'-tetra-CB), 2368 (8; 2,4,3',4'-tetra-CB), and 2401 or 2407 (13; 2,3,4,4'- or 3,4,3',5'-tetra-CB). The degree of chlorination was verified by HRGC-MS. Standards available for peaks 1 and 8 corroborated their assignments.

Peak 13 was a tetrachlorobiphenyl, tentatively assigned as either 2,3,4,4'- or 3,4,3',5'-tetra-CB. Both are present in Aroclor 1242 (0.2%; Albro and Parker, 1979). A peak at this retention time was also observed in Aroclor 1254 (Fig. 3). RI data obtained by GC-MS with two capillary columns (OV-17, OV-101) gave corroborative results. The RI for peak 13 on the OV101 column (2062) was closer to that for 4,2',3',4'-TCB (2050 actual) than for 3,4,3',5'-TCB (2095 actual). Similarly, the RI for unidentified peak 5a (1982) on the OV101 column was closer to that for 2,2',3',4'-TCB (1974) than for 3,5,3',5'-TCB (2046).

For purposes of comparison with data calculated by the method of Webb and McCall (1973), 2,4,4'-trichlorobiphenyl corresponded to retention time 37 (relative to *p,p'*-DDE = 100); 2,4,2',4'-tetrachlorobiphenyl to 47; 2,4,5,4'-, 2,5,3',4'-, and 2,4,3',4'- to 70; 2,4,5,2',5'-, 2,4,5,2',4'-, and peak 13 to 84. Other components may also coelute at these retention times. The HPCB retention times were 125, 2,4,5,3',4'-penta-CB; 146, 2,4,-5,2',4',5'-hexa-CB + 2,3,4,3',4'-penta-CB; 174, 2,3,4,2',-4',5'-hexa-CB; 203, 2,3,4,2',3',4'-hexa-CB; 232, 2,3,4,5,-3',4'-hexa-CB.

Statistical analysis was performed through the City University of New York computer center (IBM 370) by the Statistical Package for the Social Sciences (SPSS) or the Statistical Analysis System (SAS; SAS Institute, Raleigh, N.C.). Because the PCB concentrations were distributed similar to the lognormal, medians were used as central valves.

RESULTS

Packed column GC analysis of plasma or adipose samples from these workers showed as many as 14 peaks corresponding to tri- through heptachlorobiphenyls, while HRGC showed more than 40 peaks (Figs. 1 to 4). Total PCB residues were calculated as Aroclor 1248 (for peaks with retention times less than *p,p'*-DDE, hereinafter denoted as LPCB) and Aroclor 1254 residues (for peaks with retention times greater than *p,p'*-DDE, hereinafter denoted as HPCB). Data from HRGC (Table 2) showed total PCB values comparable to those obtained from packed column GC analyses by the method of Webb and McCall (1973). These two methods of quantitation gave lower values than those found by the sum of peak areas (Table 2).

Adipose concentrations of LPCB and HPCB, as the sum of individual HRGC peaks, were correlated with levels in plasma (Figs. 5 and 6). The adipose-plasma partition, determined as the slope (regression coefficient) from linear regression of adipose versus plasma concentrations, was 185 for LPCB. For HPCB, the adipose-plasma partition was 190. The PCB peaks, observed by HRGC of the human samples, were consistent with peaks in Aroclors 1016, 1242, and 1254. For 40 peaks, structures are listed in Table 1. At the time of the study (1976), Aroclor 1016 had been the most commonly used PCB, with some Aroclor 1221 since 1971. Aroclors 1242 and 1254 had been used from 1959 until 1971, and Aroclor 1254 had been used from 1946 until 1959.

In Aroclor 1016, peak 1 contained both 2,5,4'- and 2,4,4'-trichlorobiphenyls, which were resolved on the OV101 (GC-MS), but not the OV17, column. Adipose tissue samples showed only 2,4,4'-trichlorobiphenyl. The proposed structure and the retention time for peak 6 corresponded to that re-

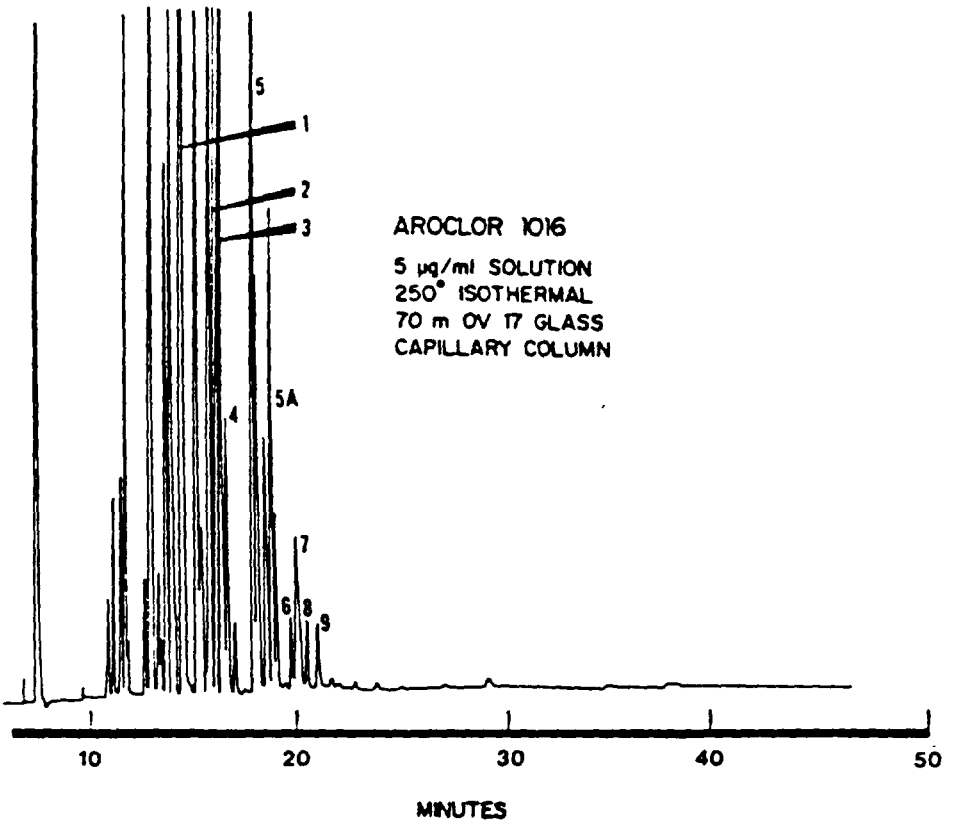

FIG. 1. Typical chromatogram from HRGC analysis of standard PCB solution (Aroclor 1016). Peak numbers refer to structure designation in Table 1.

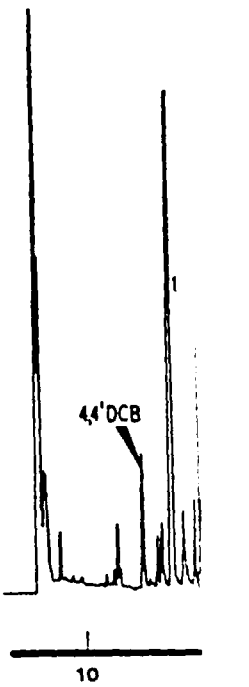

FIG. 2. Typical ch
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ported by Japanese workers (Yakushiji *et al.*, 1978; Kuwabara *et al.*, 1978; Watanabe *et al.*, 1977). These peaks have been reported in Aroclor 1016 and 1242 (1, 13–14%; 6, 1–2%; 8, 0.1–0.8%) (Albro *et al.*, 1977; Albro and Parker, 1979). Peak 20 (2,4,5,3',4'-penta-CB) has not been reported in Aroclor 1242 (Albro and Parker, 1979) and could not be strictly differentiated from 2,4,3',4',5'-penta-CB (reported as 0.36% in Aroclor 1242) on the basis of retention indices on only two columns; standards were not available. However, this peak has been reported in commercial PCBs with 54% chlorine as 2,-4,5,3',4'-pentachlorobiphenyl (Jensen and Sundstrom, 1974; Sissons and Welti, 1971).

LPCB peaks 1 and 6, 2,4,4'-tri-CB and 2,4,5,4'-tetra-CB, were the most commonly detected and had the highest concentrations of all PCB congeners in adipose tissue and plasma (Table 1). The major LPCB peaks, 1, 6, 8, 12, and 13 were present in 26 of 26 adipose tissue samples. Some samples (5 of 26) demonstrated only these peaks, with barely perceptible peaks 2 and 4, as in Fig. 4. An additional 11 of 26 samples were similar except that peaks 5, 5a, 9, and 10 were also detectable. In 6 of these 11 cases, peak 6 was greater than peak 1, the reverse of the usual pattern. Thus, altogether 16 of 26 samples showed a well-differentiated LPCB pattern, with mainly peaks 1, 6, 8, 12, and 13. For these 16 cases, the adipose LPCB level (geometric mean; GM) was 26 ppm. The group included 2 retired persons; 8 persons with no direct exposure to PCBs; 6 from jobs

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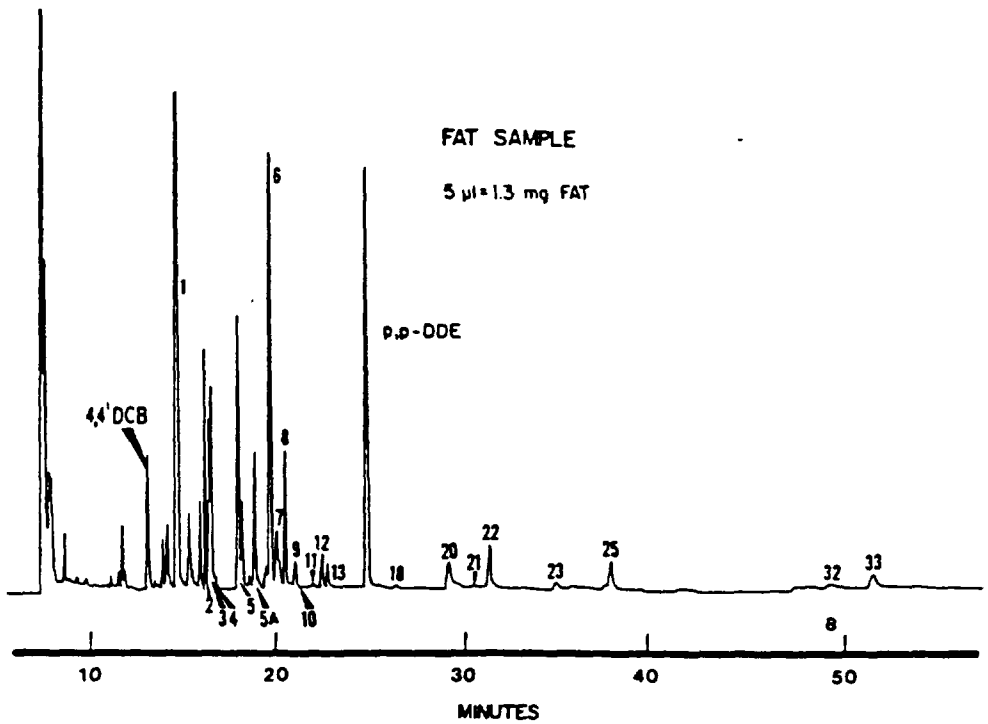

FIG. 2. Typical chromatogram from HRGC analysis of adipose sample from a PCB-exposed worker with current high exposure to Aroclor 1016 and with HPCB (peaks 20 to 33) typical of the general population.

involving direct exposure to PCBs, such as capacitor filling. The remaining 10 samples showed more LPCB peaks, including 4,4-dichlorobiphenyl, and resembled Fig. 2. These 10 persons all had direct PCB exposure and a level of adipose LPCB (GM) of 64 ppm. Even those samples with LPCB traces similar to Aroclor 1016, however, showed enhanced levels of the major peaks 1, 6, and 8, reflecting an accumulation of these congeners superimposed on the current exposure.

Of the HPCB congeners, peak 20 was usually the most concentrated, and the intensity of this peak, greater than peak 22 (2,4,5,2',4',5'-hexa-CB), constituted a clear pattern, even on packed column GC analysis, among these workers (Fig. 4). Among 26 adipose samples, 18 demonstrated this pattern, similar to Aroclor 1254 (Fig. 3). The concentration of peak 20 was correlated with that of peaks 12, 13, and 23 (r = 0.7–0.9), consistent with the idea of a common origin, from either Aroclor 1242 or 1254. The concentrations of peaks 20, 21, 22, 24 to 26, 28, and 32 to 35 were also correlated with duration of employment (r = 0.5–0.7). For those persons employed for less than 10 years, the correlation was stronger (r = 0.9 for peaks 20 and 22). Peaks 14 to 18, prominent in Aroclor 1254, were absent (<0.01 ppm in adipose); these peaks all had the 2,5-substitution pattern amenable to metabolism (Jensen and Sundstrom, 1974; Lutz *et al.*, 1977; Matthews and Tuey, 1980).

By contrast, in the general population, or in Aroclor 1260-exposed persons and in Aroclor 1260 itself, peak 20 is ordinarily much smaller than peak 22 (unpublished data). Peaks 22 and 25 have been the major peaks

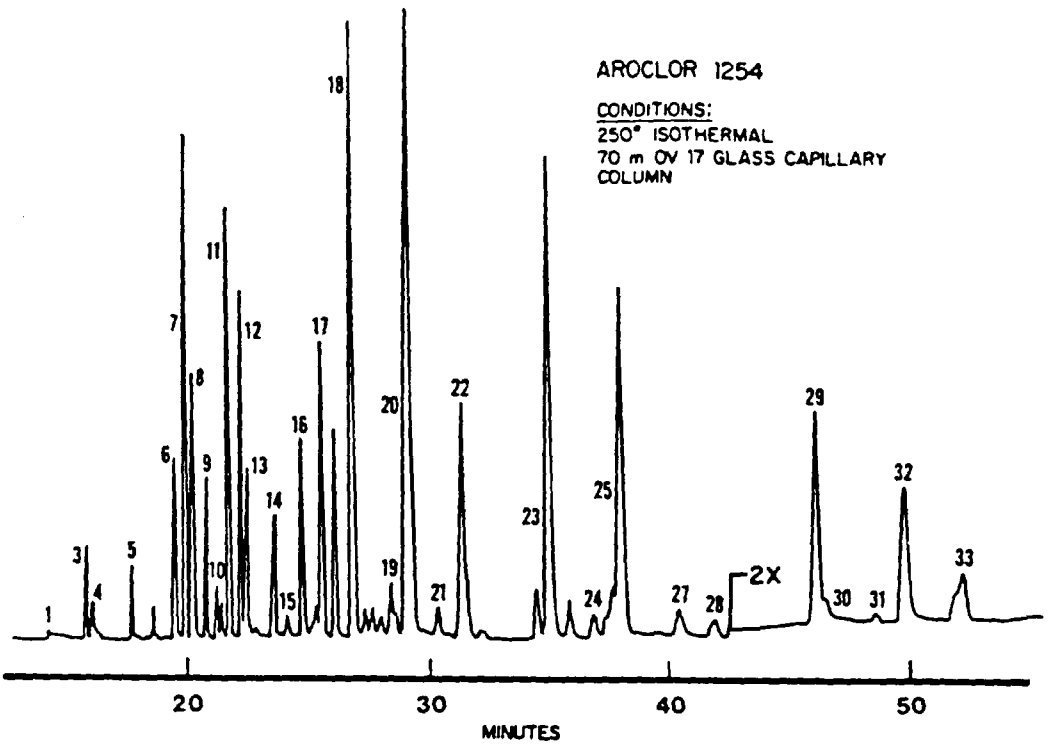

FIG. 3. Typical chromatogram from HRGC analysis of standard PCB solution (Aroclor 1254). Peak numbering refers to structure designation in Table 1.

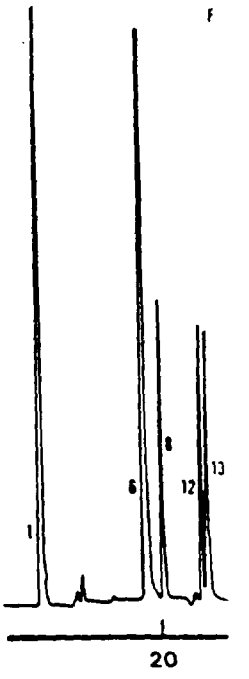

FIG. 4. Typical ch showing well-differe preceding peak 17 i

in general population samples analyzed in this laboratory, consistent with the findings of Jensen and Sundstrom (1974). The worker whose adipose tissue analysis is depicted in Fig. 2 had 18.3 ppm LPCB in adipose; this worker had been employed for less than 2 years working in a job with direct PCB (Aroclor 1016) exposure.

The lower PCB peaks resembled those in Aroclor 1016, but the HPCB pattern and level (2.4 ppm) were consistent with a general environmental exposure. Hence, the low concentrations of peaks 12, 13, and 20 were consistent with the lack of exposure to Aroclor 1242 or 1254, which had been used prior to this person's employment. For the paired adipose-plasma samples, the patterns of PCB peaks from the HRGC analysis were practically superimposable. The adipose-plasma concentrations from the HRGC analysis were linearly correlated for every individual congener ($r = 0.7$–0.98) where more than three cases had detectable peaks, except for peak 24 (Table 1; $r = 0.4$, $n = 6$). The correlation was not statistically significant for peaks 17 ($r = 0.7$, $n = 4$) and 18 ($r = 0.8$, $n = 4$).

The partition values [slope (regression coefficient) of adipose vs plasma concentrations] for individual PCB congeners (Tables 1, 3) could be classified into three groups: <100, 100 to 190, 200 to 300+. The PCB congeners so divided were characterized more by chlorine substitution pattern than by degree of chlorination (Table 3). The group with lowest partition values (<100) included congeners with 2,5-substitution on one ring (peaks 2, 3, 5, 7, and 11) and 4,4'-DCB. Congeners with 3,4-substitution on one ring had partition ratios (linear slope)

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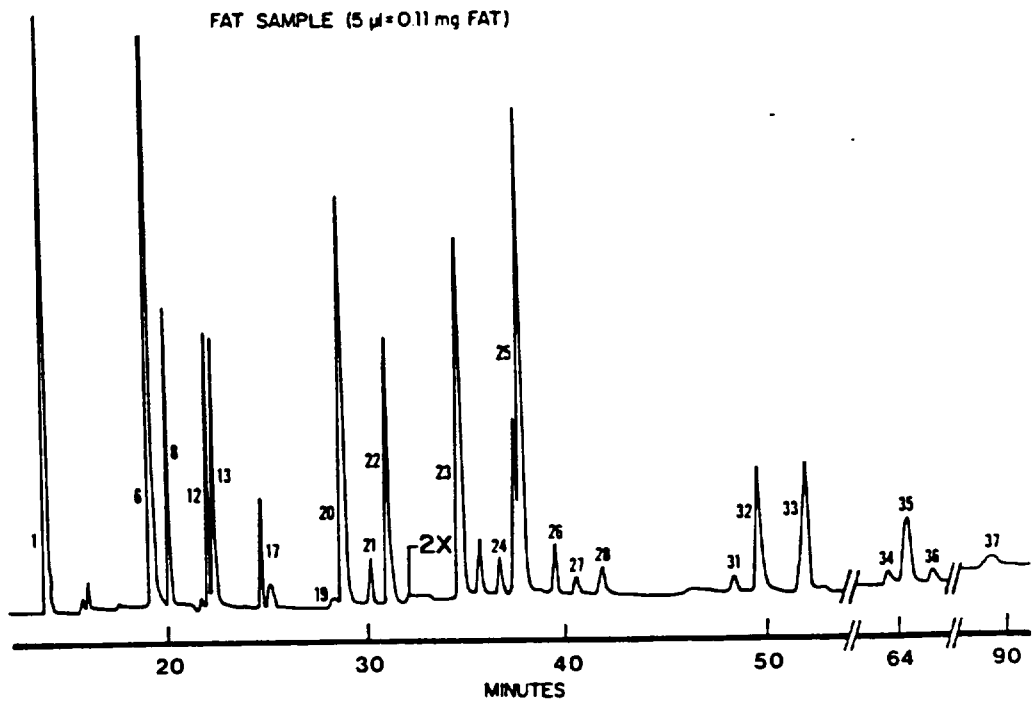

FIG. 4. Typical chromatogram from HRGC analysis of adipose sample from a PCB-exposed worker showing well-differentiated PCB pattern. Peak numbering refers to Table 1. The peak immediately preceding peak 17 is *p,p'*-DDE.

from 100 to 190 (peaks 8, 19, 20, 23, and 25). Congeners with 2,4-substitution on both rings, as well as 2,4,4'-tri-CB and 2,4,5,4'-tetra-CB, had partition values greater than 200 (peaks 1, 4, 6, 12, 22, and 33).

The linear relationship of adipose and plasma concentrations of PCB congeners within these structural groups (Table 3) was investigated by linear regression techniques. The results of the analysis (Table 3) were consistent with a common slope within each structural group, which was each different from the other. A slope similar to those for individual peak concentrations was found when the total PCB concentrations for compounds with 2,5-, 3,4-, and 2,4-substitution patterns were used for adipose-plasma partition.

Of the 37 PCB congeners identified, nine had a 2,3,5- or 3,5-chlorine substitution on one ring. These congeners were not a substantial proportion of the Aroclors or human samples. In terms of relative concentrations (tissue vs Aroclor) and adipose-plasma partition, the 2,3,5-substituted congeners were similar to 2,4,5-substituted congeners.

DISCUSSION

The preferential bioaccumulation of PCB congeners with nominal 4-, 2,4-, or 3,4-chlorine substitution on both rings (but not 4,4'-di-CB) was suggested by

(1) The occurrence of only such LPCB peaks in many cases, e.g., peaks 1, 4, 6, 8, 12, and 13.

(2) The higher adipose-plasma partition ratios of these peaks. The adipose-plasma partition of tri- and tetra-CBs with appro-

TABLE 2

COMPARISON OF TOTAL PCB CONCENTRATION IN ADIPOSE AND PLASMA DETERMINED BY PACKED COLUMN AND HIGH-RESOLUTION GAS CHROMATOGRAPHY (HRGC) (n = 26)

	Packed column				HRGC	
	Sum of peak areas		Webb and McCall		Sum of individual peaks	
Method*	Median	Range	Median	Range	Median	Range
Plasma LPCB, ppb	145	18-1362	95	2-2360	83	2-1412
Plasma HPCB, ppb	33	1-180	19	1-89	19	1-60
Adipose LPCB, ppm	36	5-234	23	4-157	24	2-271
Adipose HPCB, ppm	14	2-54	3	0.4-12	6	0.2-19

* LPCB, Total of GC peaks with retention time less than *p,p'*-DDE; HPCB, total of GC peaks with retention time greater than *p,p'*-DDE.

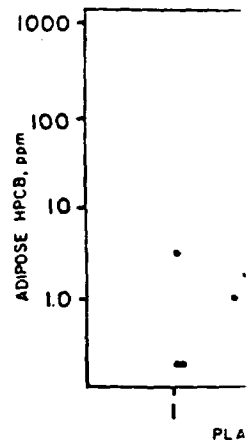

FIG. 5. Correlation of concentrations, as the sur as logarithmic values. T was adipose = 2.4 + 0.1

priate substitution patterns was comparable to those for higher chlorinated biphenyls, which have been clearly shown, in human and animal studies, to bioaccumulate.

(3) The absence or much lower concentration of 2,5-chlorine substituted PCB congeners (i.e., those with vicinal unsubstituted carbons at the 3,4-positions of the biphenyl ring) in human samples, compared with concentrations of these congeners in Aroclor mixtures.

(4) The lower adipose-plasma partition of 2,5-substituted PCB congeners.

The occurrence, in many cases, of only certain LPCB peaks (1, 6, and 8; e.g., Fig. 4) suggested that other LPCB components had been eliminated. Even where the LPCB pattern resembled that of Aroclor 1016 (e.g., Fig. 2), relatively higher concentrations of peaks 1, 6, and 8 were seen. The simplest prototype for bioaccumulation, 2,4,4'-trichlorobiphenyl, was invariably detected in these workers, and in 20 of 26 cases was the PCB congener with the highest concentration. 2,4,4'-Trichlorobiphenyl is the only major component of Aroclor 1016 (14%; Albro *et al.*, 1977) which contains the substitution pattern consistent with accumulation. Also, peaks 6 and 8 (2,4,5,4'- and 2,4,3',4'-tetrachlorobiphenyls) were always present in these workers. These components may be derived from Aroclor 1016, 1242, or 1254. In 6 cases, peak 6 (2,4,5,4'-tetrachlorobiphenyl) was greater in concentration than peak 1 (2,4,4'-trichlorobiphenyl). This could be due to greater persistence of peak 6 or to accumulation from Aroclors 1242 and 1254, since it was observed in low exposure jobs. The higher concentrations of LPCB congeners in these workers may be attributed to their current exposure to Aroclor 1016 (air levels as high as 1 mg/m^3), whereas HPCB reflected past exposure, more than 5 years before.

Further evidence of the bioaccumulative tendency of these peaks (1, 6, and 8) comes from the adipose-plasma partition ratios, although the partitions cannot be used, in the absence of observations over time, to construe long-term persistence. Peaks 1, 4, 6, 12, and 19 are a homologous series of 2,4-substituted biphenyls, all with similar adipose-plasma partitions. However, peak 1 has three unsubstituted 5,6-carbons available for metabolism, while peak 19 has none. 4,4'-Dichlorobiphenyl, with no *ortho* chlorines and four unsubstituted 5,6-positions, had a partition similar to that for 2,5-substituted

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(n = 26)

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Sum of individual peaks	
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19	1-60
24	2-271
6	0.2-19

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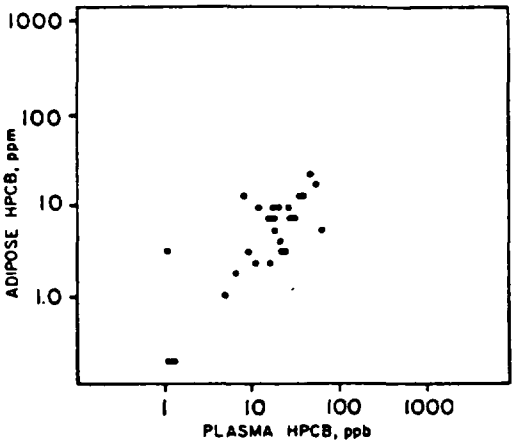

FIG. 5. Correlation of adipose with plasma HPCB concentrations, as the sum of individual peaks, depicted as logarithmic values. The linear regression equation was adipose = 2.4 + 0.19 plasma, r = 0.70.

congeners (Table 1). Thus, in addition to the electronic influence of two 4-chlorines, the presence of a chlorine substituent on at least one 2-position may determine disposition of PCBs.

The correlation of the concentrations of HPCB peaks 12, 13, and 20 of 25 with total duration of employment, and the similarity of relative concentrations in tissue to that in Aroclor 1254, suggested accumulation of these PCB congeners during the period of use of Aroclors 1242 and 1254.

By extrapolation from the Webb and McCall retention times (see Methods), the major peaks which were reported in workers exposed to Aroclor 1242 (Ouw *et al.*, 1976) corresponded to those which have been observed in this study. Chromatograms of PCBs in blood of Japanese workers exposed to different PCB mixtures also showed the major PCB congeners we observed (Watanabe *et al.*, 1977). Peaks 1, 6, and 8 were the major components observed in two workers exposed to Kanechlors 300 or 400, and peaks 6 and 8 were observed as minor components in a worker exposed to Kaneclor 500. We have also observed peaks 6 and 8 in Aroclor 1254-exposed workers (unpublished data).

No animal studies have provided comprehensive experimental data for disposition of individual PCBs, but the available data corroborate our findings. Hansell *et al.*(1977) determined hepatic concentrations of 4,4'-dichlorobiphenyl, 2,4,2',4'-tetrachlorobiphenyl, and 2,4,5,2',4',5'-hexachlorobiphenyl in the rat. 4,4'-Dichlorobiphenyl was rapidly eliminated. Although the elimination rate was apparently similar for 2,-4,2',4'- and 2,4,5,2',4',5'-chlorobiphenyls, a greater initial decrease of 2,4,2',4'-tetrachlorobiphenyl, possibly through a nonmetabolic route, provided a faster clearance rate for the tetra-CB congener. Thus distribution of PCB homologs may be similar, but elimination via metabolism may differ over long periods of time. Lutz *et al.* (1977) reported fat–blood ratios of 70 (4,4'-DCB), 70 (peak 11), and 400 (peak 22) in the rat, which are quite similar to the values observed in this work for humans (Table 3; 80, 50, and 270, respectively).

In a study of the pharmacokinetics of symmetrical hexachlorobiphenyl isomers in the rat, the pattern of chlorine substitution affected disposition (Matthews and Tuey, 1980). 2,3,6,2,3',6'-Hexachlorobiphenyl, with

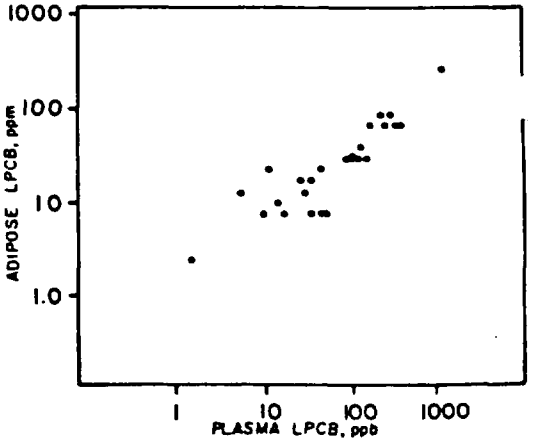

FIG. 6. Correlation of adipose with plasma LPCB concentrations, as the sum of individual peaks, depicted as logarithmic values. The linear regression equation was adipose = 6.3 + 0.18 plasma, r = 0.98.

TABLE 3

ADIPOSE-PLASMA PARTITION OF PCB CONGENERS AS INDICATED BY THE LINEAR REGRESSION COEFFICIENT (SLOPE) OF ADIPOSE VS PLASMA CONCENTRATIONS FOR 26 PCB-EXPOSED WORKERS

Chlorine substitution group:	Chlorine substitution pattern and adipose-plasma partition of individual PCB congeners within appropriately substituted groups					
	2,5-		3,4-		2,4-	
	2,5,2',5'-	80	2,4,3',4'-	160	2,4,4'-	200
	2,4,2',5'-	90	2,4,5,3',4'-	180	2,4,2',4'-	310
	2,3,2',5'-	80	2,3,4,3',4'-	130	2,4,5,4'-	200
	2,5,3',4'-	70	2,3,4,2',4',5'-	110	2,4,5,2',4'-	240
	2,4,5,2',5'-	50			2,4,5,2',4',5'-	270
Reduced model						
Common slope						
n		81				
Total PCBs		52		149		
Slope				79		204
Mean adipose		84				108
concentration, μg/g				137		
		2				205
				7		
						34

two vicinal unsubstituted carbons, was rapidly eliminated and had an adipose-blood partition less than 100. 2,3,5,2',3',5'-Hexachlorobiphenyl was persistent, with an adipose-blood partition less than 100. Hexachlorobiphenyls with 2,4,5,2',4',5- and 2,4,6,2',4',6'-substitution were also persistent with adipose-blood partition > 200 14 to 21 days after exposure. A partition value of 290 to 300 has been observed for the brominated homolog of peak 22, 2,4,5,2',4',5'-hexabromobiphenyl, in humans (Wolff *et al.*, 1979a, b), comparable with the value of 340 reported for the rat (Tuey and Matthews, 1980).

The difference in distribution of 3,4- versus 2,4-substituted PCB congeners, suggested by adipose-plasma partitions, is of interest due to their potential toxicological differences (Goldstein, 1979; Parkinson *et al.*, 1980a,b; Yoshimura *et al.*, 1973; Yamamoto *et al.*, 1976; Goldstein *et al.*, 1977). 3,4,3',4'-Tetrachlorobiphenyl, the only such isomer reported in actual PCB mixtures, could not be differentiated from other PCBs in these samples by the analytical methods used. The relative concentration of this peak (Table 1) suggested that it was not bioaccumulated; whereas other 3,4-substituted isomers, apparently bioaccumulated, were major components of the PCB body burden. However, 3,4,3',4'-tetra CB is a minor component (0.34%) of Aroclor 1242 (Albro and Parker, 1979).

The adipose-plasma partition of congeners with 3,4-substituted chlorines was somewhat lower than homologous 2,4-substituted congeners. This observation, by comparison with the low adipose-plasma partition of 2,5-substituted congeners, could be construed as evidence of more rapid metabolism. However, the concentrations and correlation with duration of exposure were not less for 2,4-substituted congeners. Indeed, in terms of availability of 5,6-positions for metabolism, the 3,4- and 2,4-substituted isomers are identical, although orientation of electrophilic substitution during metabolism may differ.

Many factors may affect persistence and disposition, and lack of adequate observations over time precludes definitive evaluation of persistence. One factor salient to adipose-plasma partition, however, is solu-

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30	2,4,2',4'-	310
30	2,4,5,4'-	200
0	2,4,5,2',4'-	240
	2,4,5,2',4',5'-	270
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9		108
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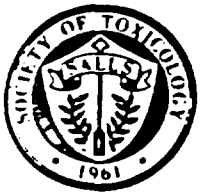

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ATTACHMENT 3

BY JOSEPH HIGHLAND

THE FIRST EVIDENCE that food supplies had become contaminated with polychlorinated biphenyls (PCBs) was reported in 1966, when fish from various Swedish waters were shown to contain these chemicals.[1] Since then, PCBs have been identified as contaminants in food supplies around the world. They have been found in pike taken from a lake in Finland, in mollusks and marine fish from Scottish waters, in a wide variety of fish caught in the St. John River system, in wildlife in California, and in trout and salmon taken from Lake Michigan.[2,3] More recent findings indicate that contamination by PCBs continues to be widespread. Within the last few months, striped bass caught in the Hudson River have been found to contain as much as 90 parts per million (ppm) PCBs, while pike caught in the Baltic Sea were shown to contain 0.31 ppm PCBs in their muscle and up to 190 ppm in their ovaries.[4,5] Moreover, plankton from the Baltic Sea, upon which many fish feed, contained levels of 25 ppm PCBs in their fat.[6]

The federal government currently conducts three separate programs in which the contamination of foods by PCBs is measured: the FDA (Food and Drug Administration) Pesticide Surveillance Program, the FDA Total Diet Program, and the USDA (U.S. Department of Agriculture) Surveillance Program.

The FDA Pesticide Surveillance Program, which began in 1968, w developed to gather information on the levels of pesticides, as well as of PCBs, in foods and animal feeds based on a statistical sampling program designed to cover specific geographical regions of the U.S. The program is intended to assure the safety of the human food supply by screening and removing shipments of foods containing PCBs or pesticides in excess of established tolerances. Commodities covered under this testing program include dairy products, eggs, fish, animal feeds, fruits, vegetables, and processed foods.

In the FDA Total Diet Program, the levels of pesticides, PCBs, and trace heavy metals in sample diets are measured. Twice a month, a diet approximating that which would be consumed in a two-week period by a hypothetical fifteen- to twenty-year-old male is collected at retail stores in five regions around the U.S. The foods are prepared in the appropriate manner and then divided into twelve basic food composite classes, including meat, fish, poultry, and leafy vegetables. Each com-

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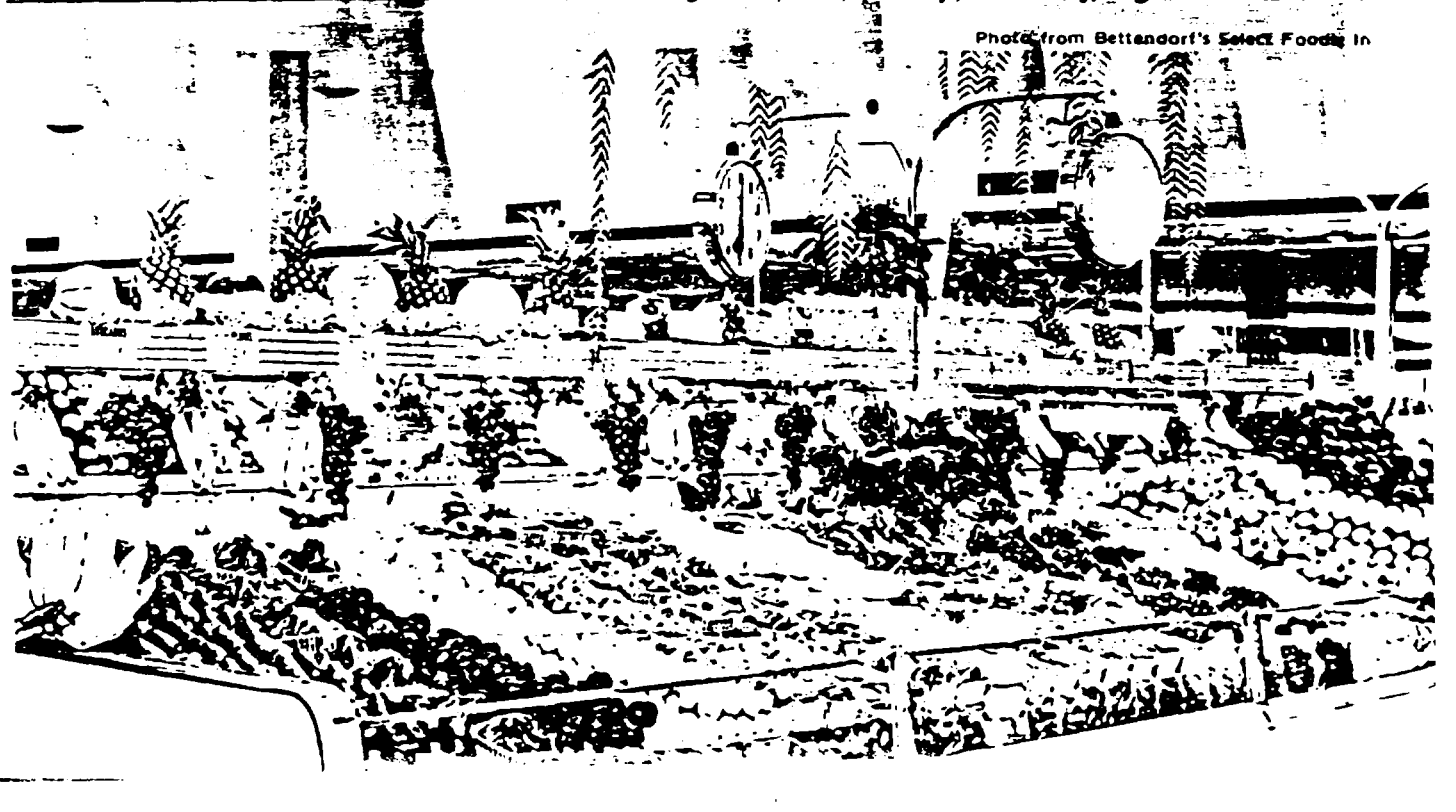

Photo from Bettendorf's Select Foods in

PCBs IN FOOD

What IS acceptable?

posite class is then analyzed for contaminants.

The USDA Surveillance Program consists of periodic sampling and analysis of meats and poultry for chemical contaminants, including PCBs. Samples are taken from federally inspected slaughterhouses.

Levels of PCBs in Food

A summary of the findings regarding PCBs in the three survey and surveillance programs are listed in Table 1.[1,2,7] Although the data are incomplete, it is evident from this table that PCBs continue to contaminate our food. Results of the FDA Pesticide Surveillance Program appear to indicate an increase in the extent of contamination, while those of the USDA Surveillance Program indicate a decrease; this apparent conflict may be explained by the fact that different foods are sampled in these programs, and it is quite possible that contamination of some kinds of foods has decreased while it has increased in others.

The apparent decrease in the levels of PCBs reported in the FDA Total Diet Program Survey between fiscal year 1973 and the first half of fiscal year 1974 must be viewed with caution. During the first half of fiscal year 1974, the average PCB levels for milk and other dairy products, for eggs and egg products, and for animal feed ingredients were equal to or greater than those reported for fiscal year 1973.

TABLE 1

COMPILATION OF DATA FROM FEDERAL SOURCES

FDA Pesticide Surveillance Program

Year	Number of Samples	Percent Contaminated	Levels of Contamination
1968-71	15,000	3.2	"detectable" (fish, specifically: 1-10 ppm)
Fiscal 1973	7,882	4.0	"detectable"
Fiscal 1974	1,723	5.4	"detectable"

FDA Total Diet Program Survey

Year	Number of Composite Food Samples	Percent Contaminated	Levels of Contamination
1968-71	900	6	trace — 0.36 ppm
Fiscal 1973	360	6	trace — 6.0 ppm
Fiscal 1974	360	4	trace — 0.05 ppm

USDA Surveillance Program

Year	Number of Samples	Percent Contaminated	Levels of Contamination
1971	4,175	4.0	trace — 15 ppm
Fiscal 1973 (Poultry only)	1,037	2.6	trace — 4 ppm
Fiscal 1974 (Poultry only)	574	1.0	1.25 ppm

Then why is there an apparent decrease between 1973 and 1974? In large part, this reflects an apparent decrease in the number of *fish* that were found to be contaminated with PCBs. In fiscal year 1973, 62.2 percent of the fish samples examined contained PCBs ranging from trace amounts to 123 ppm. In the first half of fiscal year 1974, only 35.3 percent of the samples checked were contaminated, and the levels found ranged from trace amounts to 9.7 ppm. However, the fish data reported for fiscal year 1973 included mostly freshwater fish, in which PCB contamination has been shown to be greatest, while during the first half of fiscal year 1974 the samples consisted mainly of marine fish. Since the samples are not comparable, the apparent decrease is probably a reflection of the difference in levels of PCB contamination between freshwater and marine fish rather than an indication

Tests are being conducted by the FDA Pesticide Surveillance Program on food items such as fruits and vegetables to determine the amounts of PCBs in excess of established acceptable levels.

of a general decline in PCBs in fish.

In addition to these observations, numerous specific incidents of PCB contamination of food have been reported by the FDA and the USDA. In 1970, the Campbell Soup Company detected high levels of PCBs (26.8 ppm on a fat basis) in chickens grown in New York state. As a result, 140,450 chickens were destroyed. In July 1971, the Monsanto Company informed the FDA that large amounts of fish meal might have been contaminated with PCBs during the pasteurization process, due to a leak in the heating system at the company's East Coast Terminal in Wilmington, North Carolina. An investigation by the FDA revealed that the leak had begun in April 1971 and had continued through July 1971. Contamination of the fish meal was verified, and, as a result, over 123,000 pounds of egg products and 88,000 chickens were destroyed. That same year, the FDA was notified that high levels of PCBs (20 ppm) had been found in Swift and Company turkeys. As a result, approximately 1,000,000 birds were kept from market.[1] These incidents and the results of the federal surveillance programs demonstrate further that contamination of food by PCBs is not a new phenomenon: it is a continuing problem which has resulted in part from the continued indiscriminate discharge of PCBs into the environment, and it is one which must be eliminated because of the severe health hazards posed by these compounds.

"Acceptable Dietary Intake"

The FDA has calculated what is termed an "acceptable daily dietary intake" for PCBs in the human diet – or a level of PCBs which, if ingested for an extended period of time, would be toxicologically insignificant. Even if the validity of such a concept is assumed, the basis for establishing such a level must be evaluated. In this connection, the FDA's final environmental impact statement for rulemaking on PCBs[8] indicated that both animal and human toxicological data were used to establish an allowable dietary intake for human beings.

According to data then available (1973), long-term studies of dogs and rats showed "no-effect levels" (the dose levels below which the effects looked for were not observed) of 10 ppm for three commercial types of PCBs (Aroclors 1242, 1254, and 1260) fed in the diet. Employing the standard 100-to-1 safety factor used in applying results of animal experiments to human health standards, a no-effect level for human beings, based on data derived from the dog experiments, was

It appears that the metabolism and excretion of PCBs, especially of Aroclors with high chlorine content, are even slower than for some of the most persistent pesticides, such as dieldrin and DDT.

calculated to be an intake of 0.0025 milligram per kilogram of body weight per day. From the data on rats, a figure of 0.003 milligram per kilogram of body weight was obtained. On the basis of the tests in dogs, the allowable level of PCB intake in a man weighing 70 kilograms (about 155 pounds) was figured to be 0.175 milligram per day (70 kilograms x 0.0025 milligram).

The human toxicological data used in determining the allowable daily dose were derived from investigations of human PCB poisonings in Japan. In 1968, over 1,000 Japanese persons experienced PCB poisoning after consuming rice oil contaminated with Kanechlor 400.[9,10] In addition to causing headaches, swelling of eyelids, temporary loss of vision, and many other symptoms, PCBs stored in the adipose tissue (connective tissue containing stored cellular fat) of pregnant women were passed through the placental wall to the fetuses. As a result, nine of the ten live-born babies had unusually greyish, darkened skin, and most were born underweight.

The outbreak was interpreted by the FDA to have the following implications for PCB intake standards:[8]

"Since 2,000 milligrams was reported to be the average total dose causing an effect in the Japanese, it is possible that 200 milligrams total dosage PCBs (applying a safety factor of 10 to 1) may be tolerated over a much more protracted period of time without overt adverse effect if daily exposure is held to minimal levels. This would permit ingestion of 4 micrograms [0.004 milligram] per kilogram body weight per day in a 70-kilogram man. Since the lowest total dose producing an effect in man in the Japanese incident was 500 milligrams, a similar analysis leads to an allowable protracted ingestion of 1 microgram [0.001 milligram] per kilogram body weight per day as derived from a 70-kilogram man."

On the basis of these data, the FDA arrived at an allowable dose of PCBs (for a 70-kilogram man) of 70 to 280 micrograms per day – or 0.07 to 0.28 milligram per day – compared to the 0.175 milligram-per-day permissible level established on the basis of the dog experiments. The validity of these calculations is very questionable, however. Particularly suspect are the estimates that had to be made of doses received by individual Japanese, as well as the arbitrary assumption that a safety factor of 10 to 1 is sufficient to determine a safe level of intake for a "protracted period of time." In addition, the calculations fail to consider the potential long-term effects caused by stored PCB residues.

Furthermore, according to H. Blumenthal (acting director, Division of Toxicology, Bureau of Foods, FDA) in the time since these calculations were made, there have been two important experimental observations concerning PCBs which make the previously calculated allowable daily intake level obsolete:[2] First, recent studies by J. R. Allen and his colleagues on the effects of low-level exposure of nonhuman primates to PCBs must be considered.[11,12,13,14] The effects seen in these studies of monkeys are similar in appearance and persistence to the symptoms of the human poisoning in Japan. Moreover, these symptoms were documented at dietary intake levels as low as 2.5 ppm (equivalent to about 2.5 milligrams per kilogram of body weight). To date, a no-effect level for PCBs in monkeys has not been established. This would indicate that dogs and rats are not particularly reliable subjects for tests relating to primates, since they are not as sensitive as are primates to many of the toxic effects of PCBs.

Second, Allen's work indicates that the use of gross observations of toxicity are not reliable in the study of PCBs.[11] Allen observed that routine toxicological evaluations of rats which were fed 100 ppm Aroclor 1248 for one year revealed no abnormalities in growth, mortality, or hematology. However, more sophisticated tests showed that these rats experienced drastically altered fat and cholesterol metabolism as well as changes in liver size and structure. Therefore, even if an allowable human daily dosage for PCBs calculated from animal toxicological data were acceptable, the basis for the FDA's 1972 calculations would be seriously questionable.

The chemical nature of PCBs may help to explain in part why they are subacutely and chronically toxic. The

chemicals have a low solubility in water but are highly soluble in fat.[15] Therefore, they tend to accumulate and concentrate in the body's fat stores rather than being rapidly metabolized and excreted. It appears that the metabolism and excretion of PCBs, especially of Aroclors with high chlorine content, are even slower than for some of the most persistent pesticides, such as dieldrin and DDT.[16] Thus, after ingestion, PCBs persist in the body for long periods of time and may thereby elicit chronic effects.

Excessive Intake Is Common

The FDA's acceptable daily PCB intake level of 75 to 210 micrograms per day for a 70-kilogram man seems debatable at best. As J. Wessel, scientific coordinator of the FDA, has pointed out,[1] "It is quite clear that the acceptable daily PCB intake of 75-210 micrograms per day could easily be exceeded by an adult consuming some of the heavily contaminated foods in his diet." Table 2, taken directly from Wessel's testimony,[1] illustrates this point clearly: Consuming a steady diet of combined foods from the list would produce consistent over-exposure to PCBs if each food had the maximum allowed concentration of PCBs (listed under the heading "Temporary Tolerance [ppm]").

As Wessel concludes, "Even when PCBs are present at the [permissible] tolerance level, it would be possible for an adult, with normal dietary habits, to exceed 175 micrograms [0.175 milligram] of PCBs per day." Furthermore, adults with "normal dietary habits" are not the only people who are vulnerable. Perhaps at even greater risk are those large groups within the population (such as sport fishermen or people who require special diets) who consume above-average quantities of PCB-contaminated foods, or nursing babies who ingest PCBs in their mothers' contaminated milk and who, for natural biochemical reasons, have a very limited capacity for metabolizing foreign substances such as PCBs. A. Kolbye, Jr., associate director for science, Bureau of Foods, FDA, and H. Blumenthal are clearly correct when they say, respectively:[17,2]

"Infants or young children could also readily exceed the tolerable daily exposure," and, "Thus, the best evidence indicates that children would be more susceptible to the poisoning caused by unmetabolized PCBs, since

TABLE 2

RELATIVE CONTRIBUTION TO ACCEPTABLE DAILY INTAKE (175 mcg/g) BY FOODS SUBJECT TO PCB TEMPORARY TOLERANCES

Type of Food	Serving of Food	Temporary Tolerance (ppm)	Equivalent Amount PCBs in Food	Relative Contribution to ADI
Milk	800 ml (3.5 cups)	2.5 (fat basis) 0.1 (whole product)	80 mcg	46%
Cheese	100 g (3.5 oz)	2.5 (fat basis) 0.6 (whole basis, assuming 25% fat)	60 mcg	35%
Poultry	200 g (7 oz)	5.0 (fat basis) 0.5 (whole basis, assuming 10% fat)	100 mcg	58%
Eggs	100 g (2 eggs)	0.5	50 mcg	29%
Fish	200 g (7 oz)	5.0	1,000 mcg	580%
Packaged Food	100-200 g (3.5-7.0 oz)	10.0 (Packaging)	10-60 mcg*	6-35%

*Based on 1971 FDA Survey that 10 ppm PCBs in packaging can result in migration of 0.1 - 0.6 ppm PCBs to packaged food.

Howard Dyckoff

Japanese doctor explaining the effects of PCB poisoning of rice oil victims in 1968.

PCBs can escape from food packaging materials into food itself.

Photo by Ed Carlin

they have not developed effective ways of eliminating them from the body."

Need for Reevaluation

The FDA's temporary tolerances for PCBs in food are both inadequate and inappropriate: the acceptable daily dose calculated in 1972 is now obsolete in light of more recent findings. More importantly, however, the appropriateness of any "acceptable daily dose" for PCBs is highly questionable in light of new evidence demonstrating the carcinogenicity of these compounds in experimental animals.[18] Since there is at present no scientific evidence that *any* level of intake of a carcinogen is safe, the entire concept of an *acceptable* daily dose for carcinogenic substances such as PCBs is contradictory and scientifically unsupportable.

Our growing concern about the continued contamination of food supplies by PCBs reflects an ever-increasing awareness of the extreme health hazards posed by these compounds. Federal sampling and surveillance programs have continually demonstrated the presence of PCBs in our food supply, and the existing temporary tolerance levels for PCBs established by the FDA have been insufficient to protect the public health. A new regulatory posture is needed which takes into account recent evidence of chronic effects caused by low-level dietary intakes of PCBs and, even more importantly, the new evidence substantiating the carcinogenicity of these compounds in laboratory animals.[18] □

The USDA Surveillance Program consists of periodic sampling and analysis of meats and poultry for chemical contaminants, including PCBs.

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NOTES

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2. Blumenthal, H., testimony on "PCBs in Paper Food Packaging Materials," Food and Drug Admin., Washington, D.C. (available from Hearing Clerk's Office, Docket No. 75N-0013).

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17. Kolbye, A.C., Jr., testimony on "PCBs in Paper Food Packaging Materials," Food and Drug Admin., Washington, D.C. (available from Hearing Clerk's Office, Docket No. 75N-0013).

18. Kimbrough, R.D., et al., "Induction of Liver Tumors in Sherman Strain Female Rats by Polychlorinated Biphenyl Aroclor 1260," Ctr. for Disease Contr., Atlanta, Ga., in press.

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Flood Tide of PCBs

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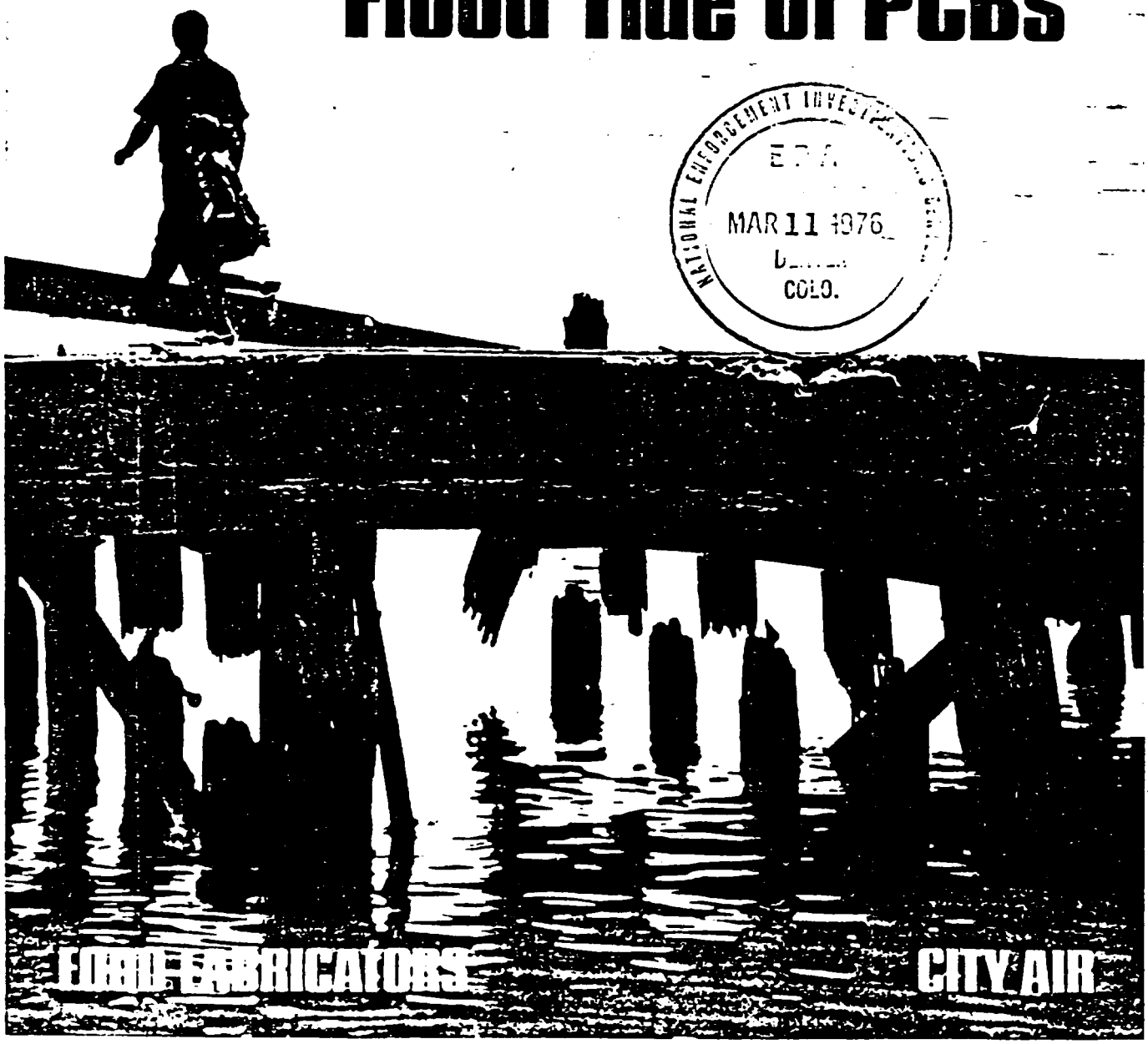

PHOTO BY WENDY HOLMES

ATTACHMENT 2

SECONDARY SOURCE ID	NIOSH/TX1350000
CAS TYPE 1 NAME	POLYCHLORINATED BIPHENYLS
CAS REGISTRY NUMBER	1336-36-3
CLASSIFICATION CODE	TUMORIGEN
TOXICOLOGY/CANCER REVIEW	CARCINOGENIC DETERMINATION:HUMAN SUSPECTED IARC◆◆ IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man.C 18;43;78
TOXICOLOGY/CANCER REVIEW	TOXICOLOGY REVIEW EVHPAZ Environmental Health Perspectives, DHEW Publication No. 1;105;72//JOCMA7 Journal of Occupational Medicine. 18;109;76//FEPRA7 Federation Proceedings, Federation of American Societies for Experimental Biology. 34;1675;75//ARVPAX Annual Review of Pharmacology. 14;139;74//ARPAAQ Archives of Pathology. 94;125;72//CHRYAQ Chemistry. 49(4);14;76//STEVA8 Science of the Total Environment. 2(4);305;74//BISNAS BioScience. 20;958;70//272T Gleason, et al., 3rd Ed., Baltimore, Williams, and Wilkins, 1969. 3;34;69
NIOSH CRITERIA DOCUMENT	CRIT DOC. OCCUPATIONAL EXPOSURE TO POLYCHLORINATED BIPHENYLS recm std-air:TWA 1.0 ug/m3 NTIS◆◆ National Technical Information Service.
STATUS	"NIOSH MANUAL OF ANALYTICAL METHODS" VOL 1 244;253, VOL 2 S121, VOL 4 S120◆ NIMHM◆
STATUS	NIOSH CURRENT INTELLIGENCE BULLETIN 7, 1975
STATUS	REPORTED IN EPA TSCA INVENTORY, 1980
STATUS	EPA TSCA 8E NO:07780209-FOLLOWUP REPLY RECEIVED AS OF APRIL, 1979
SYNONYMS	AROCLOR
SYNONYMS	AROCLOR 1221
SYNONYMS	AROCLOR 1232
SYNONYMS	AROCLOR 1242
SYNONYMS	AROCLOR 1248
SYNONYMS	AROCLOR 1254
SYNONYMS	AROCLOR 1260
SYNONYMS	AROCLOR 1262
SYNONYMS	AROCLOR 1268
SYNONYMS	AROCLOR 2565
SYNONYMS	AROCLOR 4465
SYNONYMS	BIPHENYL, POLYCHLORO-
SYNONYMS	CHLOPHEN
SYNONYMS	CHLORINATED BIPHENYL
SYNONYMS	CHLORINATED DIPHENYL
SYNONYMS	CHLORINATED DIPHENYLENE
SYNONYMS	CHLOREXTOL
SYNONYMS	CHLORO BIPHENYL
SYNONYMS	CHLORO 1,1-BIPHENYL

SYNONYMS	CLOPHEN
SYNONYMS	DYKANOL
SYNONYMS	FENCLOR
SYNONYMS	INERTEEN
SYNONYMS	KANECHLOR
SYNONYMS	KANECHLOR 300
SYNONYMS	KANECHLOR 400
SYNONYMS	KANECHLOR 500
SYNONYMS	MONTAR
SYNONYMS	NOFLAMOL
SYNONYMS	PCB
SYNONYMS	PCBs
SYNONYMS	PHENOCHLOR
SYNONYMS	PHENOCLOR
SYNONYMS	POLYCHLORINATED BIPHENYL
SYNONYMS	POLYCHLORODIPHENYL
SYNONYMS	PYRALENE
SYNONYMS	PYRANOL
SYNONYMS	SANTOTHERM
SYNONYMS	SANTOTHERM FR
SYNONYMS	SOVOL
SYNONYMS	THERMINOL FR-1
CHEMICAL DEFINITION	For toxicity information, see individual mixtures below. A series of technical mixtures, consisting of many isomers and compounds that vary from mobile oily liquids to white crystalline solids and hard non-crystalline resins. Technical products vary in composition, in the degree of chlorination and possibly according to batch IARC◆◆ IARC Monograms on the Evaluation of Carcinogenic Risk of Chemicals to Man. 7,262,74

SYNONYMS	KANECHLOR C
SYNONYMS	PCT
SYNONYMS	POLYCHLORINATED TERPHENYL
CHEMICAL DEFINITION	KANECHLOR C consists of 35% POLYCHLORINATED TERPHENYL and 5% PCB CALEDO Cancer Letters. 4:271:78
ENTRY MONTH	3106
TOXIC DATA SOURCE	CALEDO Cancer Letters. 4:271:78
TOXIDATA KEYWORDS	ORAL:MOUSE:RODENTS:TDLo:11 gm/kg/24W-C :TOXIC EFFECTS:CARCINOGENIC
TOXIC DATA SOURCE	JTSCDR Journal of Toxicological Sciences. 3:359:78
TOXIDATA KEYWORDS	ORAL:MOUSE:RODENTS:TD :10080 mg/kg/24W-C TOXIC EFFECTS:EQUIVOCAL TUMORIGENIC AGENT EQUIVOCAL TUMORIGENIC AGENT:LIVER:TUMOR
SECONDARY SOURCE ID	NIOSH/KC4550000
CAS TYPE 1 NAME	e-TOLUAMIDE, N-ISOPROPYL-alpha-(2-METHYLHYDRAZINO)-
CAS REGISTRY NUMBER	671-16-9
CLASSIFICATION CODE	TUMORIGEN
CLASSIFICATION CODE	DRUG
CLASSIFICATION CODE	MUTAGEN
CLASSIFICATION CODE	TERATOGEN
TOXICOLOGY/CANCER REVIEW	TOXICOLOGY REVIEW MIMDAL Minnesota Medicine. 57(1):19:74//32XPAD "Teratology," C.L. Berry, ed., New York, Springer, 1975 -:49:75//MUREAV Mutation Research. 39:111:77//MUREAV Mutation Research. 55:1:78//312NAA Kimbrough, R.D., Critical Reviews in Toxicology," Cleveland, Ohio 44128 2:159:73
SYNONYMS	BENZAMIDE, N-(1-METHYLETHYL)-4-((2-METHYLHYDRAZINO)METHYL)- (9CI)
SYNONYMS	IBENZMETHYZINE
SYNONYMS	2-(p-ISOPROPYLCARBAMOYLBENZYL)-1-METHYLHYDRAZINE
SYNONYMS	N-ISOPROPYL-alpha-(2-METHYLHYDRAZINO)-p-TOLUAMIDE
SYNONYMS	MATULANE
SYNONYMS	4-((2-METHYLHYDRAZINO)METHYL)-N-ISOPROPYLBENZAMIDE
SYNONYMS	1-METHYL-2-(p-(ISOPROPYLCARBAMOYL)BENZYL)HYDRAZINE
SYNONYMS	MIH
SYNONYMS	NATULAN
SYNONYMS	NSC-77213
SYNONYMS	PCB
SYNONYMS	PROCARBAZIN (German)
SYNONYMS	PROCARBAZINE
SYNONYMS	SD 4-6467
MOLECULAR FORMULA	C12-H19-N3-O
MOLECULAR WEIGHT	221.34
WISWESSER LINE NOTATION	1V1&MVR D1MM1
ENTRY MONTH	8207
TOXIC DATA SOURCE	MUREAV Mutation Research. 67:189:79
TOXIDATA KEYWORDS	MUTATION:MICROORGANISMS:S. TYPHIMURIUM BACTERIA:30 gm/L

TOXIC DATA SOURCE	JUINDS JNCI, Journal of the National Cancer Institute. 62,873,79
TOXDATA KEYWORDS	MUTATION;DNA REPAIR;E. COLI;BACTERIA;250 mg/L
TOXIC DATA SOURCE	BBALAB Biochimica et Biophysica Acta. 281,507,72
TOXDATA KEYWORDS	MUTATION;DNA DAMAGE;E. COLI;BACTERIA;2500 mg/L
TOXIC DATA SOURCE	MUREAV Mutation Research. 74,77,80
TOXDATA KEYWORDS	MUTATION;CYTOGENETICS;D. MELANOGASTER;INSECTS UNKNOWN;20 mmol/L
	MUTATION;NONDISJUNCTION;D. MELANOGASTER INSECTS;UNKNOWN;500 umol/L
TOXIC DATA SOURCE	ENMUDM Environmental Mutagenesis. 2,515,80
TOXDATA KEYWORDS	MUTATION;NONDISJUNCTION;D. MELANOGASTER INSECTS;ORAL;10 mmol/L
TOXIC DATA SOURCE	MUREAV Mutation Research. 74,77,80
TOXDATA KEYWORDS	MUTATION;DOMINANT LETHAL;D. MELANOGASTER INSECTS;UNKNOWN;20 mmol/L
TOXIC DATA SOURCE	BSIBAC Bolletino della Societa Italiana di Biologia Sperimentale. 56,1222,80
TOXDATA KEYWORDS	MUTATION;MICROORGANISMS;S. CEREVISIAE;YEASTS 12 gm/L
TOXIC DATA SOURCE	JUINDS JNCI, Journal of the National Cancer Institute. 62,901,79
TOXDATA KEYWORDS	MUTATION;MITOTIC RECOMBINATION;S. CEREVISIAE YEASTS;1 ppb
TOXIC DATA SOURCE	MUREAV Mutation Research. 80,265,81
TOXDATA KEYWORDS	MUTATION;MICROORGANISMS;A. NIDULANS;MOLDS;10 mg/plate
TOXIC DATA SOURCE	SCIEAS Science. 209,293,80
TOXDATA KEYWORDS	MUTATION;SPECIFIC LOCUS;MOUSE;RODENTS INTRAPERITONEAL;20 mg/kg
TOXIC DATA SOURCE	AIPUAN Archivio Italiano di Patologia e Clinica dei Tumori. 11,23,68
TOXDATA KEYWORDS	MUTATION;CYTOGENETICS;RAT;RODENTS INTRAPERITONEAL;300 mg/kg
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TOXIC DATA SOURCE	MUREAV Mutation Research. 59,61,79
TOXDATA KEYWORDS	MUTATION;METABOLIC ACTIVATION;MOUSE;RODENTS LYMPHOCYTE;SOMATIC CELLS;300 ug/L/4H
TOXIC DATA SOURCE	MUREAV Mutation Research. 46,218,77
TOXDATA KEYWORDS	MUTATION;SPECIFIC LOCUS;MOUSE;RODENTS INTRAPERITONEAL;600 mg/kg
TOXIC DATA SOURCE	ARTODN Archives of Toxicology. 45,123,80
TOXDATA KEYWORDS	MUTATION;SPECIFIC LOCUS;MOUSE;RODENTS PARENTERAL;600 mg/kg
TOXIC DATA SOURCE	SCIEAS Science. 209,293,80

TOXDATA KEYWORDS: MUTATION;SPECIFIC LOCUS;MOUSE;RODENTS;ORAL;20 mg/kg
MUTATION;SPECIFIC LOCUS;MOUSE;RODENTS
INTRAVENOUS;20 mg/kg

TOXIC DATA SOURCE: EBIADV Ecotoxicology and Environmental Safety. 3,122,79

TOXDATA KEYWORDS: MUTATION;SPECIFIC LOCUS;MOUSE;RODENTS;UNKNOWN 10 mg/kg

TOXIC DATA SOURCE: ARZNAD Arzneimittel-Forschung. 29,1871,79

TOXDATA KEYWORDS: MUTATION;CYTOGENETICS;MOUSE;RODENTS
INTRAPERITONEAL;100 mg/kg

TOXIC DATA SOURCE: MUREAV Mutation Research. 74,77,80

TOXDATA KEYWORDS: MUTATION;CYTOGENETICS;MOUSE;RODENTS;UNKNOWN 50 mg/kg
MUTATION;SISTER CHROMATID EXCHANGE;MOUSE
RODENTS;UNKNOWN;50 mg/kg

TOXIC DATA SOURCE: JTEHD6 Journal of Toxicology and Environmental Health. 3,287,82

TOXDATA KEYWORDS: MUTATION;SISTER CHROMATID EXCHANGE;MOUSE
RODENTS;INTRAPERITONEAL;300 mg/kg

TOXIC DATA SOURCE: MUREAV Mutation Research. 26,285,74

TOXDATA KEYWORDS: MUTATION;DOMINANT LETHAL;MOUSE;RODENTS
INTRAPERITONEAL;200 mg/kg

TOXIC DATA SOURCE: EBIADV Ecotoxicology and Environmental Safety. 3,123,79

TOXDATA KEYWORDS: MUTATION;DOMINANT LETHAL;MOUSE;RODENTS
UNKNOWN;100 mg/kg

TOXIC DATA SOURCE: MUREAV Mutation Research. 59,61,79

TOXDATA KEYWORDS: MUTATION;MAMMALIAN CELL;MOUSE;RODENTS
LYMPHOCYTE;SOMATIC CELLS;700 mg/L/4H

TOXIC DATA SOURCE: JJIND8 JNCI, Journal of the National Cancer Institute. 62,911,79

TOXDATA KEYWORDS: MUTATION;HOST MEDIATED;MOUSE;RODENTS;S. TYPHIMURIUM;BACTERIA;125 mg/kg

TOXIC DATA SOURCE: MUREAV Mutation Research. 55,1,78

TOXDATA KEYWORDS: MUTATION;SPERM MORPHOLOGY;MOUSE;RODENTS
UNKNOWN;200 mg/kg

TOXIC DATA SOURCE: CNREA8 Cancer Research. 42,122,82

TOXDATA KEYWORDS: MUTATION;SPERM MORPHOLOGY;MOUSE;RODENTS
INTRAPERITONEAL;120 mg/kg

TOXIC DATA SOURCE: MUREAV Mutation Research. 74,77,80

TOXDATA KEYWORDS: MUTATION;METABOLIC ACTIVATION;HAMSTER;RODENTS
LUNG;SOMATIC CELLS;500 umol/L

TOXIC DATA SOURCE: BBACAQ Biochimica et Biophysica Acta. 281,507,72

TOXDATA KEYWORDS: MUTATION;DNA DAMAGE;HAMSTER;RODENTS;KIDNEY
SOMATIC CELLS;25 am/L

TOXIC DATA SOURCE: MUREAV Mutation Research. 74,77,80

TOXDATA KEYWORDS: MUTATION;CYTOGENETICS;HAMSTER;RODENTS;OVARY
SOMATIC CELLS;25 mmol/L

TOXIC DATA SOURCE: ARZNAD Arzneimittel-Forschung. 29,1871,79

TOXDATA KEYWORDS: MUTATION;SISTER CHROMATID EXCHANGE;HAMSTER
RODENTS;INTRAPERITONEAL;10 mg/kg

TOXIC DATA SOURCE	MMREAV Mutation Research. 74,77,80
TOXDATA KEYWORDS	MUTATION;SISTER CHROMATID EXCHANGE;HAMSTER RODENTS;OVARY;SOMATIC CELLS;50 umol/L
	MUTATION;SISTER CHROMATID EXCHANGE;HAMSTER RODENTS;UNKNOWN;25 mg/kg
TOXIC DATA SOURCE	ARTODN Archives of Toxicology. 45,139,80
TOXDATA KEYWORDS	MUTATION;DNA SYNTHESIS;RABBIT;RODENTS INTRAVENOUS;5 mg/kg
TOXIC DATA SOURCE	ADTEH8 Advances in Teratology. 3,181,68
TOXDATA KEYWORDS	INTRAPERITONEAL;RAT;RODENTS;TDLo;25 mg/kg/(preg) ;TOXIC EFFECTS;TERATOGENIC
TOXIC DATA SOURCE	CNREA8 Cancer Research. 42,122,82
TOXDATA KEYWORDS	INTRAPERITONEAL;MOUSE;RODENTS;TDLo;53 mg/kg (1D male);TOXIC EFFECTS;REPRODUCTIVE;PATERNAL
TOXIC DATA SOURCE	FEPRA7 Federation Proceedings, Federation of American Societies for Experimental Biology. 32,745,73
TOXDATA KEYWORDS	SUBCUTANEOUS;MOUSE;RODENTS;TDLo;20 mg/kg (12D preg);TOXIC EFFECTS;REPRODUCTIVE;NEWBORN EFFECTS
TOXIC DATA SOURCE	ARZNAD Arzneimittel-Forschung. 20,1461,70
TOXDATA KEYWORDS	INTRAVENOUS;RAT;RODENTS;TDLo;1250 mg/kg/1Y-I TOXIC EFFECTS;CARCINOGENIC
TOXIC DATA SOURCE	IARCCD IARC Scientific Publications. 4,93,73
TOXDATA KEYWORDS	INTRAVENOUS;RAT;RODENTS;TDLo;125 mg/kg (22D preg) ;TOXIC EFFECTS;EQUIVOCAL TUMORIGENIC AGENT;EQUIVOCAL TUMORIGENIC AGENT REPRODUCTIVE;TUMOR;CENTRAL NERVOUS SYSTEM TUMOR
TOXIC DATA SOURCE	JNCIAM Journal of the National Cancer Institute. 59,423,77
TOXDATA KEYWORDS	INTRAPERITONEAL;MOUSE;RODENTS;TDLo;1200 mg/kg/4W-I ;TOXIC EFFECTS;NEOPLASTIC
TOXIC DATA SOURCE	CANCAR Cancer. 40,1950,77
TOXDATA KEYWORDS	ORAL;MONKEY;;TDLo;7 gm/kg/7Y-I ;TOXIC EFFECTS EQUIVOCAL TUMORIGENIC AGENT
TOXIC DATA SOURCE	ARZNAD Arzneimittel-Forschung. 20,1467,70
TOXDATA KEYWORDS	INTRAVENOUS;RAT;RODENTS;LD50;350 mg/kg
TOXIC DATA SOURCE	CNREA8 Cancer Research. 42,122,82
TOXDATA KEYWORDS	INTRAPERITONEAL;MOUSE;RODENTS;LD50;800 mg/kg TOXIC EFFECTS;BEHAVIORAL SYMPTOMS

33 2 /07
USER:

RTECS 9015-12

1	SECONDARY SOURCE ID	NIOSH/TQ1360000
	CAS TYPE 1 NAME	POLYCHLORINATED BIPHENYL (AROCLOR 1254)
	CAS REGISTRY NUMBER	11097-69-1
	CLASSIFICATION CODE	TUMORIGEN
	CLASSIFICATION CODE	TERATOGEN
	TOXICOLOGY/CANCER REVIEW	CARCINOGENIC DETERMINATION:HUMAN SUSPECTED IARC** IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. 18,43,78
	TOXICOLOGY/CANCER REVIEW	TLV-TWA 500 ug/m3; STEL 1 mg/m3 (skin) DTLVS* "Documentation of Threshold Limit Values for Substances in Workroom Air," Cincinnati, Ohio, American Conference of Governmental Industrial Hygienists, 1971 4,89,80
	TOXICOLOGY/CANCER REVIEW	TOXICOLOGY REVIEW EVHPAZ Environmental Health Perspectives, DHEW Publication No. 1,105,72//ARVPAX Annual Review of Pharmacology. 14,139,74//RREVAH Residue Reviews. 44,1,73//STEVA8 Science of the Total Environment. 2(4),305,74//BISNAS BioScience. 20,958,70
	STANDARDS/REGULATIONS	OSHA STANDARD-air:TWA 500 ug/m3 (skin) (SCP-I) FEREAC Federal Register. 39,23540,74
	NIOSH CRITERIA DOCUMENT	CRIT DOC. OCCUPATIONAL EXPOSURE TO POLYCHLORINATED BIPHENYLS recm std-air:TWA 1.0 ug/m3 NTIS** National Technical Information Service.
	STATUS	NCI CARCINOGENESIS BIOASSAY COMPLETED;RESULTS INDEFINITE:RAT (NCITR* NCI-CG-TR-38,78)
	STATUS	"NIOSH MANUAL OF ANALYTICAL METHODS" VOL 2 S121 NIMAM*
	SYNONYMS	AROCHLOR 1254
	SYNONYMS	AROCLOR 1254
	SYNONYMS	CHLORIERTE BIPHENYLE, CHLORGEHALT 54% (German)
	SYNONYMS	CHLORODIPHENYL (54% Cl)
	SYNONYMS	CLORODIFENILI, CLORO 54% (Italian)
	SYNONYMS	DIPHENYLE CHLORE, 54% DE CHLORE (French)
	SYNONYMS	NCI-C02664
	CHEMICAL DEFINITION	Composed of 11% TETRA-, 49% PENTA-, 34% HEXA- and 6% HEPTACHLOROBIPHENYLS FCTXAV Food & Cosmetics Toxicology. 12,63,74
	ENTRY MONTH	8201
	TOXIC DATA SOURCE	FCTXAV Food & Cosmetics Toxicology. 12,63,74
	TOXDATA KEYWORDS	ORAL;RAT;RODENTS;TDLo;188 mg/kg (MGN);TOXIC EFFECTS;REPRODUCTIVE;NEWBORN EFFECTS ORAL;RAT;RODENTS;TDLo;645 mg/kg (MGN);TOXIC EFFECTS;REPRODUCTIVE;NEWBORN EFFECTS
	TOXIC DATA SOURCE	FCTXAV Food & Cosmetics Toxicology. 11,471,73
	TOXDATA KEYWORDS	ORAL;RAT;RODENTS;TDLo;90 mg/kg (7-15D preg)

	TOXIC EFFECTS;REPRODUCTIVE;DEVELOPMENTAL
TOXIC DATA SOURCE	EVPHBI Environmental Physiology. 1,67,71
TOXDATA KEYWORDS	ORAL;RABBIT;RODENTS;TDLo;350 mg/kg (1-28D preg);TOXIC EFFECTS;REPRODUCTIVE;FERTILITY EMBRYOTOXICITY
TOXIC DATA SOURCE	NCITR* National Cancer Institute Carcinogenesis Technical Report Series. NCI-CG-TR-38,78
TOXDATA KEYWORDS	ORAL;RAT;RODENTS;TDLo;4 gm/kg/2Y-I ;TOXIC EFFECTS;EQUIVOCAL TUMORIGENIC AGENT
TOXIC DATA SOURCE	JNCIAM Journal of the National Cancer Institute. 53,547,74
TOXDATA KEYWORDS	ORAL;MOUSE;RODENTS;TDLo;17 gm/kg/48W-C ;TOXIC EFFECTS;NEOPLASTIC
TOXIC DATA SOURCE	BECTA6 Bulletin of Environmental Contamination & Toxicology. 18,552,77
TOXDATA KEYWORDS	SKIN;MOUSE;RODENTS;TDLo;4 mg/kg ;TOXIC EFFECTS;EQUIVOCAL TUMORIGENIC AGENT
TOXIC DATA SOURCE	FCTXAV Food & Cosmetics Toxicology. 12,63,74
TOXDATA KEYWORDS	ORAL;RAT;RODENTS;LD50;1295 mg/kg INTRAVENOUS;RAT;RODENTS;LD50;358 mg/kg
TOXIC DATA SOURCE	BECTA6 Bulletin of Environmental Contamination & Toxicology. 8,245,72
TOXDATA KEYWORDS	INTRAPERITONEAL;MOUSE;RODENTS;LD50;2840 mg/kg

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1	NAME OF SUBSTANCE	AROCLOR 1260
	MOLECULAR FORMULA	UNKNOWN
	CAS REGISTRY NUMBER	11096-82-5
	TDB NUMBER	1822
	SYNONYMS	AROCHLOR 1260
	SYNONYMS	PCB 1260
	SYNONYMS	CHLORODIPHENYL (60% CL)
	SYNONYMS	KANECLOR
	MAJOR USE	SRI DIELECTRIC FLUID IN CLOSED ELECTRICAL SYSTEMS
	MAJOR USE	IARC MONOGRAPHS V18 56 CURRENT END USE: TRANSFORMERS; FORMER END USE: HYDRAULIC FLUIDS, SYNTHETIC RESINS & DEDUSTING AGENTS
	MAJOR USE	HAWLEY. CONDENSED CHEM DICTNRY 9TH ED 74 IMPREGNATING AGENTS FOR ELECTRICAL APPARATUS; THERMOSTAT FLUIDS /AROCLOR/
	MAJOR USE	HAWLEY. CONDENSED CHEM DICTNRY 9TH ED 74 SWELLING AGENTS FOR TRANSMISSION SEALS; ADDITIVES OR BASE FOR LUBRICANTS, OILS & GREASES /AROCLOR/
	MAJOR USE	HAWLEY. CONDENSED CHEM DICTNRY 9TH ED 74 PLASTICIZERS FOR CELLULOSICS, VINYLS, & CHLORINATED RUBBER /AROCLOR/
	MAJOR USE	HAMILTON. INDUS TOX 3RD ED 289 CABLE INSULATION; SURFACE COATINGS FOR PAINTS, PLASTICS, & VARNISHES /PCB/
	MAJOR USE	HAMILTON. INDUS TOX 3RD ED 289 IN CUTTING FLUID ADDITIVES, & CARBONLESS COPY PAPER SYSTEMS /PCB/
	MAJOR USE	HAWLEY. CONDENSED CHEM DICTNRY 9TH ED 696 THEIR CHIEF USE IS IN HEAT-EXCHANGE & INSULATING FLUIDS IN CLOSED SYSTEMS /PCB/
	MAJOR USE	GOSSELIN. CTCP 4TH ED II-115 /FORMERLY/ AS FIRE RETARDANTS & VAPOR SUPPRESSORS TO PROLONG RESIDUAL LIFE OF SOME PESTICIDES /PCB/
	ANTIDOTE & TREATMENT	RUMACK. POISINDEX MGMNT 1 INITIAL PT CONTACT--GENERAL: 1) ESTABLISHMENT OF RESPIRATIONS & CREATION OF ARTIFICIAL AIRWAY IF NECESSARY. CHECK ADEQUACY OF TIDAL VOL... 2) TREAT HYPOTENSION WITH FLUIDS OR PLASMA-TRY TO AVOID VASOPRESSORS IF POSSIBLE. /GENERAL POISONING/
	ANTIDOTE & TREATMENT	RUMACK. POISINDEX MGMNT 1 SPECIFIC THERAPY: EMESIS: 1) ...ALTHOUGH VOMITING WITH SYRUP OF IPECAC PRODUCES ONLY MEAN RECOVERY OF 30% OF INGESTED DOSE, IT IS STILL PROBABLY A USEFUL PROCEDURE. SAVE INITIAL EMESIS FOR ANALYSIS. /GENERAL POISONING/
	ANTIDOTE & TREATMENT	RUMACK. POISINDEX MGMNT 1 LAVAGE.../PRC: TAP WATER IN CHILDREN SALINE IN ADULTS/. CHARCOAL: 3) MANY AGENTS ARE ADSORBED BY ACTIVATED CHARCOAL. ...DOSE: .. .5-10 TIMES

	EST WT OF...CHEM INGESTED. /GENERAL POISONING/
ANTIDOTE & TREATMENT	RUMACK. POISINDEX MGMNT 1 CATHARTICS: 4) ...SODIUM OR MAGNESIUM SULFATE 250 MG/KG ORALLY... ADULT: 20-30 G/DOSE TO 100 G TOTAL. DECONTAMINATION: 5) SAFEST DECONTAMINANT IS WATER WITH SOAP. ... SKIN DECONTAMINATION SHOULD BE THOROUGH. EYE EXPOSURE: 6) .../IRRIGATION OF EYES/ SHOULD OCCUR AT HOME OR AT WORK WITH PLAIN TAP WATER. /GENERAL POISONING/
ANTIDOTE & TREATMENT	RUMACK. POISINDEX MGMNT 1 DIALYSIS: 8) ...MAY BE CONSIDERED IN THOSE PT NOT RESPONDING TO STD THERAPEUTIC MEASURES TO TREAT A DIALYZABLE DRUG. /GENERAL POISONING/
COLOR/FORM	WATER-WHITE MOBILE LIQUIDS & PALE YELLOW VISCOUS OILS TO LIGHT AMBER RESINS & OPAQUE CRYSTALLINE SOLIDS /AROCLOR/
SOLUBILITY	SOL IN OILS & ORG SOLVENTS /PCB/
SOLUBILITY	SOL IN WATER @ 0.04-0.2 PPM /PCB/
SPECTRAL & OTHER PROPS	IARC MONOGRAPHS V18 49 /HIGH/ THERMAL STABILITY; RESISTANCE TO OXIDATION, ACIDS, & OTHER CHEM AGENTS /PCB/
SPECTRAL & OTHER PROPS	IARC MONOGRAPHS V18 49 EXCELLENT DIELECTRIC (INSULATING) PROPERTIES /PCB/
TOXICITY VALUES	NIOSH. REG TOX EFFECT CHEM SUB 1978 1002 LD50 RATS ORAL 1315 MG/KG
TOXICITY VALUES	NIOSH. REG TOX EFFECT CHEM SUB 1978 1002 TDLO RATS ORAL 1220 MG/KG/35 WEEKS CONTINUOUS; TOXIC EFFECTS: NEOPLASTIC EFFECTS
TOXICITY VALUES	IARC MONOGRAPHS V18 69 LD50 RATS ORAL 10,000 MG/KG BODY WEIGHT, ADMIN OF 50% SOLN IN CORN OIL
TOXICITY VALUES	IARC MONOGRAPHS V18 69 LD50 RATS ORAL 4000-10,000 MG/KG BODY WEIGHT, ADULT RATS
TOXICITY VALUES	IARC MONOGRAPHS V18 69 LD50 RATS ORAL 1300 MG/KG BODY WEIGHT, WEANLING RATS
TOXICITY VALUES	IARC MONOGRAPHS V18 69 LD50 RABBITS PERCUTANEOUS 1300-2000 MG/KG BODY WEIGHT, ADMIN OF 50% SOLN IN CORN OIL
TOXICITY VALUES	NIOSH. REG TOX EFFECT CHEM SUB 1978 1002 LDLO RABBITS PERCUTANEOUS 2000 MG/KG
TOXICITY VALUES	HDBK OF TOX OF PESTICIDES TO WILDLIFE 1970 20 LD50 MALLARDS ORAL GREATER THAN 2000 MG/KG, (SEX: MALE; AGE: 10 MONTHS)
MANUFACTURING INFO	NIOSH. REG TOX EFFECT CHEM SUB 1978 1002 COMPOSED OF 12% PENTA-, 38% HEXA-, 41% HEPTA-, 8% OCTA- & 1% NONACHLOROBIPHENYLS.
MANUFACTURING INFO	IARC MONOGRAPHS V18 48 AROCLOR NUMBER DESIGNATIONS CONSIST OF TWO PARTS: THE FIRST TWO DIGITS, 12, INDICATE THAT THE MOLECULE IS A BIPHENYL... THE SECOND TWO DIGITS.../60/ REPRESENT APPROX WEIGHT PERCENT OF CHLORINE IN THE PRODUCT. /AROCLOR/

MANUFACTURING INFO	IARC MONOGRAPHS V18 54 TOTAL US SALES IN 1970 COUNTED TO 33.2 MILLION KG & COMPRISED...2.2 MILLION KG AROCLOR 1260...
MANUFACTURING INFO	IARC MONOGRAPHS V18 74 APPLICATIONS: PCBS ARE WIDELY USED AS ENZYME INDUCERS IN RESEARCH LABORATORIES THROUGHOUT THE WORLD. THERE ARE INDICATIONS THAT CHLORINE CONTENT OF PCB MIXTURES IS RELATED TO LEVEL OF INCR ENZYMATIC ACTIVITY: MIXTURES CONTAINING LOWER PERCENTAGE OF CHLORINES WERE LESS ACTIVE THAN THOSE CONTAINING HIGHER PERCENTAGE. /PCB/
MANUFACTURING INFO	IARC MONOGRAPHS V18 84 ALMOST WITHOUT EXCEPTION, POLYCHLORINATED BIPHENYLS CONTAIN VARIOUS LEVELS OF POLYCHLORINATED DIBENZOFURANS AS CONTAMINANTS... IT IS NOT KNOWN IF & TO WHAT EXTENT POLYCHLORINATED DIBENZOFURANS PLAY A ROLE IN THE OBSERVED CARCINOGENIC EFFECTS OF PCBS. /PCB/
MANUFACTURING INFO	GOSSELIN. CTCP 4TH ED II-115 ...THE AVERAGE MOLECULE IN AROCLOR 1260 HAS 6 SUBSTITUENT CHLORINES, BUT VARYING PROPORTIONS OF SPECIES WITH GREATER & LESSER HALOGEN CONTENT ARE FOUND IN THE MIXTURE DEPENDING ON THE MANUFACTURING PROCESS.
MANUFACTURING INFO	HAWLEY. CONDENSED CHEM DICTNRY 9TH ED 696 BECAUSE OF THEIR PERSISTANCE & ECOLOGICAL DAMAGE FROM WATER POLLUTION THEIR MANUFACTURE HAS BEEN DISCONTINUED IN THE US (1976). /PCB/
MANUFACTURING INFO	HAMILTON. INDUS TOX 3RD ED 289 PREPN: ...FROM DIPHENYL & NAPHTHALENE WHICH MAY BE REACTED TO VARYING DEGREES WITH CHLORINE TO PRODUCE A NUMBER OF COMPD DESIGNATED BY VARIOUS TRADE NAMES SUCH AS AROCLOR... /PCB/
MANUFACTURING INFO	FRC AROCLOR 1260 IS THE FORMULATION USED MOSTLY IN LARGE CAPACITORS & TRANSFORMERS. FEDERAL REGISTER, AUG 1979.
POISONING POTENTIAL	IARC MONOGRAPHS V18 84 POLYCHLORINATED BIPHENYLS SHOULD BE REGARDED AS IF THEY WERE CARCINOGENIC TO HUMANS. /PCB/
POISONING POTENTIAL	GOSSELIN. CTCP 4TH ED II-115 TOXICITY RATING: 3(?). 3= MODERATELY TOXIC: PROBABLE ORAL LETHAL DOSE 0.5-5.0 G/KG, BETWEEN 1 OUNCE & 1 PINT (OR 1 LB) FOR 70 KG PERSON (150 LB). /PCB/
POISONING POTENTIAL	HAWLEY. CONDENSED CHEM DICTNRY 9TH ED 74 HAZARD: MAY BE TOXIC BY INGESTION & INHALATION... /AROCLOR/
POISONING POTENTIAL	NRC. DRINKING WATER & HEALTH 1977 760 FOR THE PCB AROCHLOR 1260 @ CONCN OF 1 UG/L (Q= 1) THE PROJECTED RISK FOR MAN IS 2.2X10-6 Q. THE UPPER 95% CONFIDENCE ESTIMATE OF RISK @ THE SAME CONCN IS 3.1X10-6 Q. IT SHOULD BE NOTED THAT THIS EXTRAPOLATION IS BASED ON A ONE-HIT

	MATHEMATICAL MODEL WHICH MAY BE INVALID FOR THIS CHEMICAL.
POISONING POTENTIAL	IARC MONOGRAPHS V18 70 SUSCEPTIBILITY TO TOXICITY IN MAN: HIGH. /PCB, FROM TABLE/
POISONING POTENTIAL	NRC. DRINKING WATER & HEALTH 1977 794 CATEGORIES OF KNOWN OR SUSPECTED ORG CHEM CARCINOGENS FOUND IN DRINKING WATER: HIGHEST OBSERVED CONCN IN FINISHED WATER: 3 UG/L; UPPER 95% CONFIDENCE ESTIMATE OF LIFETIME CANCER RISK: 3.1X10-6 UG/L. /FROM TABLE/
ENV/OCC EXP & LIMITS	IARC MONOGRAPHS V18 58 OSHA HEALTH STD REQUIRE...EMPLOYEE'S EXPOSURE TO PCBS CONTAINING 42% CHLORINE SHALL @ NO TIME EXCEED AN 8-HR TIME-WEIGHTED AVERAGE OF 1 MG/CU M, & EXPOSURE TO PCBS CONTAINING 54% CHLORINE SHALL NOT EXCEED AN 8-HR TIME-WEIGHTED AVERAGE OF 0.5 MG/CU M OVER ANY 8-HR SHIFT OF A 40-HR WORK WEEK. /PCB/
ENV/OCC EXP & LIMITS	IARC MONOGRAPHS V18 59 /1 APRIL 1977, USDA TOLERANCE LEVELS/...IN MILK & DAIRY PRODUCTS (FAT BASIS)...1.5 UG/G; IN POULTRY (FAT BASIS)...3 UG/G; IN EGGS...0.3 UG/G; & IN FISH & SHELLFISH...FINISHED ANIMAL FEED...ANIMAL FEED COMPONENTS.../&/...INFANT & JUNIOR FOODS: 0.2 UG/G...PAPER FOOD-PACKAGING MATERIALS WITHOUT PCB...BARRIER, 10 UG/G. /PCB/
ENV/OCC EXP & LIMITS	IARC MONOGRAPHS V18 60 IT HAS BEEN ESTIMATED THAT 12,000 PERSONS IN THE US ARE OCCUPATIONALLY EXPOSED TO PCBS. /PCB/
ENV/OCC EXP & LIMITS	IARC MONOGRAPHS V18 62 /IN 1972/...SURVEY OF AGRICULTURAL SOILS THROUGHOUT US...ONLY 0.1% SAMPLES CONTAINED DETECTABLE LEVELS OF PCB'S. IN URBAN AREAS, FREQUENCY & LEVELS...WERE HIGHER: 12 OF 19...SAMPLES FROM METROPOLITAN AREAS (63%) SHOWED DETECTABLE LEVELS. /PCB/
ENV/OCC EXP & LIMITS	IARC MONOGRAPHS V18 62 ...FISH, CHEESE & BY-PRODUCTS USED IN ANIMAL FEED WERE THE MAIN COMMODITIES IN US THAT WERE CONTAMINATED WITH PCB'S. THE PCB CONTENT OF ALL FOOD ITEMS EXCEPT FISH DECR BETWEEN 1971-1975: IT WAS ESTIMATED THAT THE PCB CONTENT OF A TEENAGE BOY'S DIET WAS 15 UG/DAY IN 1971 & 8.1 UG/DAY ON 1975. /PCB/
ENV/OCC EXP & LIMITS	IARC MONOGRAPHS V18 61 A NATIONAL SURVEY CONDUCTED IN US SHOWED THAT PCB LEVELS IN UNFILTERED WATER SAMPLES DURING 1971-1974 RANGED BETWEEN 0.1 & 3.0 UG/L. /PCB/
ENV/OCC EXP & LIMITS	IARC MONOGRAPHS V18 64 HUMAN EXPOSURE TO SMALL AMT OF PCB'S IS WIDESPREAD AS A RESULT OF ENVIRONMENTAL CONTAMINATION & HIGH STABILITY OF THESE COMPD. /PCB/
POLLUTION POTENTIAL	IARC MONOGRAPHS V18 52 PHOTOCHEMICAL

	DEGRADATION MAY BE ONE ROUTE OF THEIR BREAKDOWN IN THE ENVIRONMENT: PHOTOCHEMICAL EXPERIMENTS CONDUCTED UNDER SIMULATED NATURAL CONDITIONS ON...COMMERCIAL PCB PREPARATIONS HAVE INDICATED SEVERAL DEGRADATIVE REACTIONS, SUCH AS DECHLORINATION, POLYMERIZATION & SOLVOLYSIS. /PCB/
CLASS. BY MAJOR USE	SRI DIELECTRIC FLUID
U.S. PRODUCTION	SRI (1972) 1.1-1.4X10+10 GRAMS
U.S. PRODUCTION	SRI (1974) 1.83X10+10 GRAMS
U.S. IMPORTS	SRI (1972) 1.6X10+6 GRAMS
U.S. IMPORTS	SRI (1976) 1.32X10+8 GRAMS
U.S. EXPORTS	SRI (1971) 4.4X10+9 GRAMS
U.S. EXPORTS	SRI (1974) 2.4X10+9 GRAMS
U.S. METHOD OF MANUFACT	SRI CHLORINATION OF BIPHENYL WITH AN IRON OR FERRIC CHLORIDE CATALYST
CONSUMPTION PATTERN	SRI 100% AS A DIELECTRIC FLUID IN CLOSED ELECTRICAL SYSTEMS (1973)
MANUFACTURERS	SRI MONSANTO CO, MONSANTO INDUST CHEMS CO, SAUGET, ILL
ABSORP/DISTRIB/EXCRETE	HAWLEY. CONDENSED CHEM DICTNRY 9TH ED 74 ...ABSORBED BY SKIN. /AROCLOR/
ABSORP/DISTRIB/EXCRETE	IARC MONOGRAPHS V18 63 DURING MARCH 1964 AND FEBRUARY 1970, 48 OF 169 GOLDEN EAGLES FROM 22 STATES WERE FOUND TO CONTAIN PCBS IN EITHER BRAIN, HEART, KIDNEY, LIVER, MUSCLE OR FAT, IN CONCN RANGING FROM LESS THAN 1 TO 19 UG/G ON A WET BASIS. /PCB/
ABSORP/DISTRIB/EXCRETE	IARC MONOGRAPHS V18 63 /IN 1970/...200 & 600 UG/G PCBS /WERE REPORTED/ IN SAMPLES OF HUMAN FAT. /IN 1972/...115-180 UG/G IN ADIPOSE TISSUE, 250 UG/G IN LIVER & 125 UG/G IN KIDNEY (ALL ON FAT BASIS) /WERE REPORTED/ IN ONE AUTOPSY CASE. /PCB/
ABSORP/DISTRIB/EXCRETE	IARC MONOGRAPHS V18 64 /IN 1970/...THE MEAN PCB LEVEL IN HUMAN MILK IN TWO CALIFORNIA CITIES WAS ABOUT 0.06 UG/ML OF WHOLE MILK. ... ANALYSIS OF LIPID FRACTION OF 80 SAMPLES OF HUMAN MILK FROM VARIOUS AREAS OF US SHOWED THAT ALL EXCEPT 2 HAD CONCN...FROM 0.4-10.6 UG/G. THE AVERAGE CONCN IN ALL SAMPLES WAS APPROX 1.7 UG/G. /PCB/
ABSORP/DISTRIB/EXCRETE	IARC MONOGRAPHS V18 65 FORTY-THREE PERCENT OF 723 PLASMA SAMPLES FROM PERSONS IN SOUTH CAROLINA NOT OCCUPATIONALLY EXPOSED TO PESTICIDES WERE FOUND TO CONTAIN PCBS, WITH A MAXIMUM OF 0.029 UG/ML. /PCB/
ABSORP/DISTRIB/EXCRETE	ARENA. POISONING 4TH ED 115 PCB CONTAMINATION IS FOUND TO BE ALMOST UNIVERSAL, INCLUDING HUMAN MILK, HUMAN ADIPOSE TISSUE, & BRAIN & LIVER OF SMALL CHILDREN. /PCB/
METABOLISM	NRC. DRINKING WATER & HEALTH 1977 757 THE DEGRADATION & ELIMINATION OF PCB CONGENERS

	APPEAR TO TAKE PLACE VIA HEPATIC MICROSOMAL ENZYME SYSTEM. TWO POSSIBLE MECHANISMS FOR BIOTRANSFORMATION HAVE BEEN SUGGESTED... THE FIRST & MOST RAPID...INVOLVES FORMATION OF AN ARENE OXIDE INTERMEDIATE... THE SECOND & MUCH SLOWER USES A DIFFERENT HYDROXYLATION SYSTEM... /PCB/
STATUS	100180COMPLETE WITH PEER REVIEW COMMENTS INCORPORATED
ANIMAL/NON-HUMAN TOX EX	IARC MONOGRAPHS V18 67 GROUPS OF 10 MALE & 10 FEMALE 3-4-WEEK-OLD SHERMAN RATS WERE FED 0, 20, 100, 500 OR 1000 MG AROCLOR 1260/KG DIET... SEVERAL...GIVEN THE TWO HIGHEST DOSE LEVELS DIED BEFORE 6 MO. ...LESIONS...DESCRIBED AS ADENOFIBROSIS OF LIVER OCCURRED IN 2 MALES FED 1000 MG/KG & IN 1, 1 AND 4 FEMALES FED 100, 500 AND 1000 MG/KG...
ANIMAL TOXICITY KEYWORDS	CHRONIC;RATS;SHERMAN;ORAL;LIVER;LIVER NEOPLASMS;ADENOFIBROMA;CARCINOGENESIS CHRONIC;RATS;SHERMAN;ORAL;ND;DEATH
ANIMAL/NON-HUMAN TOX EX	IARC MONOGRAPHS V18 67 ...200 3-4-WEEK-OLD FEMALE SHERMAN RATS WERE FED...100 MG AROCLOR 1260/KG...FOR 21-22 MO; 200 FEMALE RATS...AS CONTROLS. ... 26 OF 184 EXPOSED...HAD HEPATOCELLULAR CARCINOMAS, & 144/184 HAD LIVER LESIONS DESCRIBED AS NEOPLASTIC NODULES. 1 HEPATOCELLULAR CARCINOMA & NO NEOPLASTIC NODULES WERE OBSERVED AMONG 173 CONTROL RATS.
ANIMAL TOXICITY KEYWORDS	CHRONIC;RATS;SHERMAN;ORAL;LIVER;LIVER NEOPLASMS;HEPATOMA;CARCINOGENESIS
ANIMAL/NON-HUMAN TOX EX	IARC MONOGRAPHS V18 68 RATS FED AROCLOR...1260 FOR 8 MO...SHOWED LIVER-CELL HYPERTROPHY, WITH CYTOPLASMIC INCLUSIONS & BROWN PIGMENT IN KUPFFER CELLS.
ANIMAL TOXICITY KEYWORDS	CHRONIC;RATS;ND;ORAL;LIVER;HYPERTROPHY;LIVER DISEASES CHRONIC;RATS;ND;ORAL;HEMIC AND LYMPHATIC SYSTEMS;KUPFFER CELLS;PIGMENTATION;CELLULAR INCLUSIONS
ANIMAL/NON-HUMAN TOX EX	IARC MONOGRAPHS V18 68 /IN RABBITS/ SKIN APPLICATION...PRODUCED LOSS OF WEIGHT, HYPERPLASIA & HYPERKERATOSIS OF EPIDERMAL & FOLLICULAR EPITHELIUM; CENTRILOBULAR DEGENERATION, CENTRILOBULAR LIVER-CELL ATROPHY, FOCAL NECROSIS & CYTOPLASMIC HYALINE DEGENERATION; HYDROPIC DEGENERATION OF CONVOLUTED TUBULES & TUBULAR DILATATION WITH

	CAST FORMATION...
ANIMAL TOXICITY KEYWORDS	ND;RABBITS;ND;PERCUTANEOUS;ND;EMACIATION ND;RABBITS;ND;PERCUTANEOUS;SKIN;KERATOSIS HYPERPLASIA;SKIN DISEASES ND;RABBITS;ND;PERCUTANEOUS;LIVER;LIVER DISEASES;ATROPHY;NECROSIS ND;RABBITS;ND;PERCUTANEOUS;KIDNEY;KIDNEY TUBULAR NECROSIS, ACUTE;KIDNEY DISEASES DILATATION, PATHOLOGIC;NECROSIS
ANIMAL/NON-HUMAN TOX EX	IARC MONOGRAPHS V18 68 /IN RABBITS/ SKIN APPLICATION...PRODUCED...ATROPHY OF THYMUS, LYMPHOPENIA & ELEVATED EXCRETION OF FECAL COPROPORPHYRIN & PROTOPORPHYRIN.
ANIMAL TOXICITY KEYWORDS	ND;RABBITS;ND;PERCUTANEOUS;HEMIC AND LYMPHATIC SYSTEMS;THYMUS GLAND;LYMPHOPENIA LYMPHATIC DISEASES ND;RABBITS;ND;PERCUTANEOUS;METABOLISM AND NUTRITION;PORPHYRINS
ANIMAL/NON-HUMAN TOX EX	IARC MONOGRAPHS V18 71 MALE AND FEMALE SHERMAN RATS WERE FED ON A DIET CONTAINING...500 MG AROCLOR 1260/KG...OF DIET FOR 67 OR 186 DAYS PRIOR TO MATING, REDUCED LITTER SIZE & DECREASED SURVIVAL OF OFFSPRING WERE OBSERVED.
ANIMAL TOXICITY KEYWORDS	CHRONIC;RATS;SHERMAN;ORAL;PREGNANCY;LITTER SIZE;DEATH;ANIMAL, NEWBORN
ANIMAL/NON-HUMAN TOX EX	IARC MONOGRAPHS V18 74 PCB ADMIN HAS BEEN FOUND TO RESULT IN INCREASED SYNTHESIS, HEPATIC CONTENT & EXCRETION OF PORPHYRINS IN RATS, QUAILS & CHICKENS; & THIS HAS BEEN ASSOCIATED WITH AN INCR IN LIVER MITOCHONDRIAL GAMMA-AMINOLEVULINIC ACID SYNTHETASE. /PCB/
ANIMAL TOXICITY KEYWORDS	ND;RATS;ND;ND;METABOLISM AND NUTRITION PORPHYRINS;ENZYME INDUCTION;AMINOLEVULINIC ACID SYNTHETASE ND;QUAIL;ND;ND;METABOLISM AND NUTRITION PORPHYRINS;ENZYME INDUCTION;AMINOLEVULINIC ACID SYNTHETASE ND;CHICKENS;ND;ND;METABOLISM AND NUTRITION PORPHYRINS;ENZYME INDUCTION;AMINOLEVULINIC ACID SYNTHETASE
ANIMAL/NON-HUMAN TOX EX	IARC MONOGRAPHS V18 74 /PCB/ EXPOSURE OF RATS, RABBITS, MONKEYS, CHICKS & RAINBOW TROUT...RESULTED IN INCR ACTIVITY OF HEPATIC MICROSOMAL MIXED-FUNCTION OXIDASES & SOME OTHER ENZYMES, INCL ANILINE HYDROXYLASE, O- & N-DEMETHYLASES, ESTERASE, ARYL HYDROCARBON HYDROXYLASE, NADPH, CYTOCHROME C REDUCTASE,

ANIMAL TOXICITY KEYWORDS	P-NITROPHENOL GLUCURONYL TRANSFERASE... /PCB/ ND;RATS;ND;ND;METABOLISM AND NUTRITION;ENZYME INDUCTION;NADH DEHYDROGENASE;ESTERASES ANILINE HYDROXYLASE;OXIDOREDUCTASES;ARYL HYDROCARBON HYDROXYLASES;MICROSOMES, LIVER MIXED FUNCTION OXIDASES ND;RABBITS;ND;ND;METABOLISM AND NUTRITION ENZYME INDUCTION;NADH DEHYDROGENASE;ESTERASES ANILINE HYDROXYLASE;OXIDOREDUCTASES;ARYL HYDROCARBON HYDROXYLASES;MIXED FUNCTION OXIDASES;MICROSOMES, LIVER ND;MONKEYS;ND;ND;METABOLISM AND NUTRITION ENZYME INDUCTION;NADH DEHYDROGENASE;ESTERASES ANILINE HYDROXYLASES;OXIDOREDUCTASES;ARYL HYDROCARBON HYDROXYLASES;MIXED FUNCTION OXIDASES;MICROSOMES, LIVER ND;CHICKENS;ND;ND;METABOLISM AND NUTRITION ENZYME INDUCTION;NADH DEHYDROGENASE;ANILINE HYDROXYLASE;OXIDOREDUCTASES;ARYL HYDROCARBON HYDROXYLASES;MICROSOMES, LIVER;MIXED FUNCTION OXIDASES ND;TROUT;ND;ND;METABOLISM AND NUTRITION ENZYME INDUCTION;NADH DEHYDROGENASE;ESTERASES MICROSOMES, LIVER;MIXED FUNCTION OXIDASES ANILINE HYDROXYLASE;OXIDOREDUCTASES;ARYL HYDROCARBON HYDROXYLASES
ANIMAL/NON-HUMAN TOX EX	IARC MONOGRAPHS V18 74 EXPOSURE OF RATS, RABBITS, MONKEYS, CHICKS AND RAINBOW TROUT TO PCBS RESULTED IN INCREASED ACTIVITY OF...URIDINE DIPHOSPHOGLUCURONOSYLTRANSFERASE & NITROREDUCTASE, OR A SIGNIFICANT INCR IN THE LEVEL OF CYTOCHROME P-450. /PCB/
ANIMAL TOXICITY KEYWORDS	ND;RATS;ND;ND;METABOLISM AND NUTRITION;ENZYME INDUCTION;CYTOCHROME P-450;UDP GUCURONOSYLTRANSFERASE ND;RABBITS;ND;ND;METABOLISM AND NUTRITION ENZYME INDUCTION;CYTOCHROME P-450;UDP GLUCURONOSYLTRANSFERASE ND;MONKEYS;ND;ND;METABOLISM AND NUTRITION ENZYME INDUCTION;CYTOCHROME P-450;UDP GLUCURONOSYLTRANSFERASE ND;CHICKENS;ND;ND;METABOLISM AND NUTRITION ENZYME INDUCTION;CYTOCHROME P-450;UDP GLUCURONOSYLTRANSFERASE ND;TROUT;ND;ND;METABOLISM AND NUTRITION ENZYME INDUCTION;CYTOCHROME P-450;UDP GLUCURONOSYLTRANSFERASE
ANIMAL/NON-HUMAN TOX EX	HAYES. TOX OF PESTICIDES 1975 498 EGGSHELL THINNING, LOSS OF REPRODUCTIVE ABILITY, OR BOTH HAVE BEEN ATTRIBUTED...TO PCBS. /PCB/
ANIMAL TOXICITY KEYWORDS	ND;ND;ND;ND;UROGENITAL SYSTEM;EGGS

	REPRODUCTION ND;ND;ND;ND;ND;EGG SHELL
ANIMAL/NON-HUMAN TOX EX	PRC RD KIMBROUGH ET AL, J NATL CANCER INST, VOL 55 (6), 1975. 184 FEMALE RATS WERE FED 100 PPM AROCLOR 1260 FOR APPROX 21 MO. ALL WERE KILLED WHEN 23 MO OLD. 26 OF 184 EXPTL RATS & 1 OF 173 CONTROLS HAD HEPATOCELLULAR CARCINOMAS. NONE OF THE CONTROLS BUT 146 OF 184 EXPTL RATS HAD NEOPLASTIC NODULES IN THEIR LIVERS.
ANIMAL TOXICITY KEYWORDS	CHRONIC;RATS;ND;ORAL;LIVER;LIVER NEOPLASMS HEPATOMA;CARCINOGENESIS
ANIMAL/NON-HUMAN TOX EX	PRC JA GOLDSTEIN ET AL, CHEM-BIOL INTERACT, VOL 17 (1), 1977. ISOMERICALLY PURE PCBS WERE TESTED AS INDUCERS OF HEPATIC DRUG-METABOLIZING ENZYMES IN THE RAT. THE CHLORINATED BIPHENYL ISOMERS CAN BE CATEGORIZED INTO 2 DISTINCT GROUPS OF INDUCERS OF CYTOCHROMES P-450 WHILE COMMERCIAL PCB MIXT HAVE CHARACTERISTICS OF BOTH.
ANIMAL TOXICITY KEYWORDS	ND;RATS;ND;ND;METABOLISM & NUTRITION;ENZYME INDUCTION;CYTOCHROMES;CYTOCHROME P-450
ANIMAL/NON-HUMAN TOX EX	PRC GELLERT, RJ & C WILSON, ENVIRON RES, VOL 18 (2), 1979. AROCLOR 1260 WAS FED TO FEMALE RATS DURING PREGNANCY TO STUDY REPRODUCTIVE FUNCTION IN THE MALE & FEMALE OFFSPRING. REPRODUCTIVE FUNCTION IN MALE & FEMALE OFFSPRING WAS NOT ALTERED.
ANIMAL TOXICITY KEYWORDS	CHRONIC;RATS;ND;ORAL;PREGNANCY;TERATOGENESIS NO EFFECT
ANIMAL/NON-HUMAN TOX EX	PRC DEFOE DL ET AL, J FISH RES BOARD CAN, 35 (7), 1978. THE 30 DAY ACUTE LIFE CYCLE OF NEWLY HATCHED FATHEAD MINNOW LARVAE (LESS THAN 8 HR OLD) LC50 WAS 3.3 MUG/L FOR AROCLOR 1260. REPRODUCTION IN MINNOWS OCCURRED @ CONCN AS HIGH AS 3 MUG/L WHICH SIGNIFICANTLY AFFECTED LARVAL SURVIVAL.
ANIMAL TOXICITY KEYWORDS	CHRONIC;FISHES;ND;ND;ND;LETHAL DOSE 50;DEATH MORTALITY
ANIMAL/NON-HUMAN TOX EX	PRC STOTT, WT & RO SINNHUBER, TROUT HEPATIC POSTMITOCHONDRIAL ENZYME ACTIVATION OF AFLATOXIN B1 IN A MUTAGEN ASSAY SYSTEM & THE INHIBITORY EFFECT OF PCBS. BULL ENVIRON CONTAM TOXICOL, VOL 19 (1), 1978. PRETREATMENT OF FISH WITH AROCLOR 1260 DECR MUTAGEN ASSAY RESPONSE TO AFLATOXIN B1 IN SALMONELLA TYPHIMURIUM.

ANIMAL TOXICITY KEYWORDS	ND;TROUT;ND;ND;METABOLISM AND NUTRITION ENZYMES;MITOCHONDRIA, LIVER
HUMAN TOXICITY EXCERPT	IARC MONOGRAPHS V18 70 /RESPONSES TO PCBS/: ACNE; HYPERPIGMENTATION OF SKIN; HYPERACTIVE MEIBOMIAN GLANDS; CONJUNCTIVITIS; EDEMA OF EYELIDS; SUBCUTANEOUS EDEMA; KERATIN CYSTS IN HAIR FOLLICLES; HYPERPLASIA OF HAIR FOLLICLE EPITHELIUM; HEPATIC HYPERTROPHY; DECR NUMBER OF RED BLOOD CELLS; DECR HEMOGLOBIN; SERUM HYPERLIPIDEMIA; LEUCOCYTOSIS. /PCB/
HUMAN TOXICITY KEYWORDS	ND;ND;ND;ND;ND;SKIN;ACNE;PIGMENTATION DISORDERS;EDEMA;HAIR;KERATIN;HYPERPLASIA;SKIN DISEASES ND;ND;ND;ND;ND;EYE;MEIBOMIAN GLANDS CONJUNCTIVITIS;EYELID DISEASES;EDEMA ND;ND;ND;ND;ND;LIVER;HYPERTROPHY;LIVER DISEASES ND;ND;ND;ND;ND;HEMIC AND LYMPHATIC SYSTEMS ANEMIA;LEUKOCYTOSIS;HEMOGLOBINS ND;ND;ND;ND;ND;METABOLISM AND NUTRITION HYPERLIPEMIA
HUMAN TOXICITY EXCERPT	NRC. DRINKING WATER & HEALTH 1977 757 PCBS ARE LIVER TOXINS & CAUSE CHLORACNE & POSSIBLY PERIPHERAL NEUROPATHY IN MAN. /PCB/
HUMAN TOXICITY KEYWORDS	ND;ND;ND;ND;ND;LIVER;LIVER DISEASES ND;ND;ND;ND;ND;SKIN;ACNE ND;ND;ND;ND;ND;NERVOUS SYSTEM;PERIPHERAL NERVE DISEASES
INTERACTION EXCERPT	GOSSELIN. CTCP 4TH ED II-116 THE TOXICITY OF PCB IS POTENTIATED BY CARBON TETRACHLORIDE. /PCB/
INTERACTANTS CAS NOS	ND;POLYCHLOROBIPHENYL COMPOUNDS ND;CARBON TETRACHLORIDE
INTERACTION EXCERPT	PRC MJ MURPHY ET AL, TOXICOL APPL PHARMACOL, 48 (1 PART 1), 1979. AROCLOR 1260 INDUCTION OF CYTOCHROME P-450 PRODUCED DEATH IN 11/13 RATS FOLLOWING IP ADMIN OF FLUROXENE (2.5 G/KG BODY WEIGHT). FLUROXENE POTENTIATED DESTRUCTION OF CYTOCHROME P-450 IN VIVO & THIS DESTRUCTION WAS ENHANCED BY AROCLOR INDUCTION.
INTERACTANTS CAS NOS	011096825;AROCLOR 1260 ND;FLUROXENE
LAB METHODS EXCERPT	AOAC. 10TH ED 1965 AND FOLLOWING ED 12/518 29.001-29.018. DETERMINATION OF POLYCHLORINATED BIPHENYLS IN POULTRY FAT, FISH, DAIRY PRODUCTS, PAPER & PAPERBOARD INCLUDING PROCEDURES FOR EXTRACTION, CLEAN-UP

USING FLORISIL CHROMATOGRAPHY & ANALYSIS BY GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION. /PCB/

LAB METHODS KEYWORDS GENERAL SAMPLE;CHROMATOGRAPHY, GAS

LAB METHODS EXCERPT SUNSHINE, METHOD ANALY TOX 1975 311 POLYCHLORINATED BIPHENYLS CAN BE EXTRACTED FROM A BIOLOGICAL SPECIMEN WITH METHYLENE CHLORIDE. AFTER A CLEAN-UP PROCEDURE, THE PCB CAN BE ANALYZED BY ELECTRON-CAPTURE GAS CHROMATOGRAPHY. /PCB/

LAB METHODS KEYWORDS ND;CHROMATOGRAPHY, GAS

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RTECS

1	SECONDARY SOURCE ID	NIOSH/TQ1362000
	CAS TYPE 1 NAME	POLYCHLORINATED BIPHENYL (AROCLOR 1260)
	CAS REGISTRY NUMBER	11096-82-5
	CLASSIFICATION CODE	TUMORIGEN
	CLASSIFICATION CODE	MUTAGEN
	CLASSIFICATION CODE	TERATOGEN
	TOXICOLOGY/CANCER REVIEW	CARCINOGENIC DETERMINATION:HUMAN SUSPECTED IARC** IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. 18,43,78
	TOXICOLOGY/CANCER REVIEW	TOXICOLOGY REVIEW EVHPAZ Environmental Health Perspectives, DHEW Publication No. 1,105,72//ARVPAX Annual Review of Pharmacology. 14,139,74//STEVA8 Science of the Total Environment. 2(4),305,74//BISNAS BioScience. 20,958,70
	NIOSH CRITERIA DOCUMENT	CRIT DOC. OCCUPATIONAL EXPOSURE TO POLYCHLORINATED BIPHENYLS recm std-air:TWA 1.0 ug/m3 NTIS** National Technical Information Service.
	SYNONYMS	AROCHLOR 1260
	SYNONYMS	AROCLOR 1260
	SYNONYMS	CLOPHEN A60
	SYNONYMS	CHLORODIPHENYL (60% Cl)
	SYNONYMS	PHENOCLOR DP6
	CHEMICAL DEFINITION	Composed of 12% PENTA-, 38% HEXA-, 41% HEPTA-, 8% OCTA- and 1% NONACHLOROBIPHENYLS
	ENTRY MONTH	FCTXAV Food & Cosmetics Toxicology. 12,63,74 8201
	TOXIC DATA SOURCE	APTOD9 Abstracts of Papers, Society of Toxicology. Annual Meetings. 19,A16,80
	TOXDATA KEYWORDS	MUTATION;CYTOGENETICS;RAT;RODENTS;ORAL;1080 mg/kg/26W-C
	TOXIC DATA SOURCE	FCTXAV Food & Cosmetics Toxicology. 12,63,74
	TOXDATA KEYWORDS	ORAL;RAT;RODENTS;TDLo;1675 mg/kg (MGN);TOXIC EFFECTS;REPRODUCTIVE;NEWBORN EFFECTS
	TOXIC DATA SOURCE	ENVRAL Environmental Research. 6,176,73
	TOXDATA KEYWORDS	ORAL;MOUSE;RODENTS;TDLo;74 mg/kg (62D pre/1-10D preg);TOXIC EFFECTS;REPRODUCTIVE MATERNAL;FERTILITY
	TOXIC DATA SOURCE	ENPBBC Environmental Physiology and Biochemistry. 5,54,75
	TOXDATA KEYWORDS	SUBCUTANEOUS;MOUSE;RODENTS;TDLo;143 mg/kg (21D post);TOXIC EFFECTS;REPRODUCTIVE;NEWBORN EFFECTS
	TOXIC DATA SOURCE	JNCIAM Journal of the National Cancer Institute. 55,1453,75

TOXDATA KEYWORDS	ORAL;RAT;RODENTS;TDLo;4380 mg/kg/83W-C ;TOXIC EFFECTS;CARCINOGENIC
TOXIC DATA SOURCE	FCTXAV Food & Cosmetics Toxicology. 12,63,74
TOXDATA KEYWORDS	ORAL;RAT;RODENTS;LD50;1315 mg/kg
TOXIC DATA SOURCE	ARVPAX Annual Review of Pharmacology. 14,139,74
TOXDATA KEYWORDS	SKIN;RABBIT;RODENTS;LDLo;2000 mg/kg;TOXIC EFFECTS;

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1	SECONDARY SOURCE ID	NIOSH/TQ1356000
	CAS TYPE 1 NAME	POLYCHLORINATED BIPHENYL (AROCLOR 1242)
	CAS REGISTRY NUMBER	53469-21-9
	CLASSIFICATION CODE	TUMORIGEN
	CLASSIFICATION CODE	TERATOGEN
	TOXICOLOGY/CANCER REVIEW	CARCINOGENIC DETERMINATION:HUMAN SUSPECTED IARC** IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. 18,43,78
	TOXICOLOGY/CANCER REVIEW	TLV-TWA 1 mg/m3; STEL 2 mg/m3 (skin) DTLVS* "Documentation of Threshold Limit Values for Substances in Workroom Air," Cincinnati, Ohio, American Conference of Governmental Industrial Hygienists, 1971 4,88,80
	TOXICOLOGY/CANCER REVIEW	TOXICOLOGY REVIEW EVHPAZ Environmental Health Perspectives, DHEW Publication No. 1,105,72//ARVPAX Annual Review of Pharmacology. 14,139,74//RREVAH Residue Reviews. 44,1,73//STEVA8 Science of the Total Environment. 2(4),305,74//BISNAS BioScience. 20,958,70
	STANDARDS/REGULATIONS	OSHA STANDARD-air:TWA 1 mg/m3 (skin) (SCP-I) FEREAC Federal Register. 39,23540,74
	NIOSH CRITERIA DOCUMENT	CRIT DOC. OCCUPATIONAL EXPOSURE TO POLYCHLORINATED BIPHENYLS recm std-air:TWA 1.0 ug/m3 NTIS** National Technical Information Service.
	STATUS	"NIOSH MANUAL OF ANALYTICAL METHODS" VOL 4 S120* NIMAM*
	SYNONYMS	AROCHLOR 1242
	SYNONYMS	AROCLOR 1242
	SYNONYMS	CHLORIERTE BIPHENYLE, CHLORGEHALT 42% (German)
	SYNONYMS	CHLORODIPHENYL (42% Cl)
	SYNONYMS	CLORODIFENILI, CLORO 42% (Italian)
	SYNONYMS	DIFHENYLE CHLORE, 42% DE CHLORE (French)
	SYNONYMS	GECHLOREERDEDIFENYL (Dutch)
	ENTRY MONTH	8201
	TOXIC DATA SOURCE	AECTCV Archives of Environmental Contamination and Toxicology. 3,479,75/76
	TOXDATA KEYWORDS	ORAL;RAT;RODENTS;TDLo;945 mg/kg (36W pre) TOXIC EFFECTS;REPRODUCTIVE;MATERNAL;ENDOCRINE SYSTEM ORAL;RAT;RODENTS;TDLo;1890 mg/kg (36W pre) TOXIC EFFECTS;REPRODUCTIVE;FERTILITY
	TOXIC DATA SOURCE	85CYAB "Chemistry of Industrial Toxicology," H.B. Elkins, 2nd Ed., New York, J. Wiley, 1959 2,153,59
	TOXDATA KEYWORDS	INHALATION;HUMAN;HUMANS;TCLo;10 mg/m3 ;TOXIC EFFECTS;IRRITANT

TOXIC DATA SOURCE	TXAPA9 Toxicology and Applied Pharmacology. 24,434,73
TOXDATA KEYWORDS	ORAL;RAT;RODENTS;LD50;4250 mg/kg
TOXIC DATA SOURCE	PHRPA6 Public Health Reports. 59,1085,44
TOXDATA KEYWORDS	SUBCUTANEOUS;GUINEAPIG;RODENTS;LDLo;345 mg/kg

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12672-29-6(AROCLOR 1248)

1	SECONDARY SOURCE ID	NIOSH/TQ1358000
	CAS TYPE 1 NAME	POLYCHLORINATED BIPHENYL (AROCLOR 1248)
	CAS REGISTRY NUMBER	12672-29-6
	CLASSIFICATION CODE	TUMORIGEN
	CLASSIFICATION CODE	TERATOGEN
	TOXICOLOGY/CANCER REVIEW	CARCINOGENIC DETERMINATION:HUMAN SUSPECTED IARC** IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. 18,43,73
	TOXICOLOGY/CANCER REVIEW	TOXICOLOGY REVIEW ARVPAX Annual Review of Pharmacology. 14,139,74//STEVA8 Science of the Total Environment. 2(4),305,74
	NIOSH CRITERIA DOCUMENT	CRIT DOC. OCCUPATIONAL EXPOSURE TO POLYCHLORINATED BIPHENYLS recm std-air:TWA 1.0 ug/m3 NTIS** National Technical Information Service.
	SYNONYMS	AROCLOR 1248
	SYNONYMS	CHLORODIPHENYL (48% Cl)
	ENTRY MONTH	7911
	TOXIC DATA SOURCE	ARVPAX Annual Review of Pharmacology. 14,139,74
	TOXDATA KEYWORDS	ORAL;RAT;RODENTS;LD50;11 gm/kg
	TOXIC DATA SOURCE	TXCYAC Toxicology. 6,331,76
	TOXDATA KEYWORDS	ORAL;MONKEY;;TDLo;33 mg/kg/(1-1660 preg) TOXIC EFFECTS;TERATOGENIC
	TOXIC DATA SOURCE	ARVPAX Annual Review of Pharmacology. 14,139,74
	TOXDATA KEYWORDS	SKIN;RABBIT;RODENTS;LDLo;1269 mg/kg

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1	SECONDARY SOURCE ID	NIOSH/TQ1358000
	CAS TYPE 1 NAME	POLYCHLORINATED BIPHENYL (AROCLOR 1248)
	CAS REGISTRY NUMBER	12672-29-6
	CLASSIFICATION CODE	TUMORIGEN
	CLASSIFICATION CODE	TERATOGEN
	TOXICOLOGY/CANCER REVIEW	CARCINOGENIC DETERMINATION:HUMAN SUSPECTED IARC** IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. 18,43,78
	TOXICOLOGY/CANCER REVIEW	TOXICOLOGY REVIEW ARVPAX Annual Review of Pharmacology. 14,139,74//STEVA8 Science of the Total Environment. 2(4),305,74
	NIOSH CRITERIA DOCUMENT	CRIT DOC. OCCUPATIONAL EXPOSURE TO POLYCHLORINATED BIPHENYLS recm std-air:TWA 1.0 ug/m3 NTIS** National Technical Information Service.
	SYNONYMS	AROCLOR 1248
	SYNONYMS	CHLORODIPHENYL (48% Cl)
	ENTRY MONTH	8201
	TOXIC DATA SOURCE	FEPRA7 Federation Proceedings, Federation of American Societies for Experimental Biology. 34,338,75
	TOXDATA KEYWORDS	ORAL;MONKEY;;TDLo;455 mg/kg (26W pre);TOXIC EFFECTS;REPRODUCTIVE;FERTILITY
	TOXIC DATA SOURCE	DABBBA Dissertation Abstracts International, B. The Sciences and Engineering. 40,2061,79
	TOXDATA KEYWORDS	ORAL;RABBIT;RODENTS;TDLo;165 mg/kg (1-31D preg);TOXIC EFFECTS;REPRODUCTIVE;DEVELOPMENTAL
	TOXIC DATA SOURCE	ARVPAX Annual Review of Pharmacology. 14,139,74
	TOXDATA KEYWORDS	ORAL;RAT;RODENTS;LD50;11 gm/kg;TOXIC EFFECTS
		SKIN;RABBIT;RODENTS;LDLo;1269 mg/kg;TOXIC EFFECTS;

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11141-16-5(AROCLOR 1232)

1	SECONDARY SOURCE ID	NIOSH/TQ1354000
	CAS TYPE 1 NAME	POLYCHLORINATED BIPHENYL (AROCLOR 1232)
	CAS REGISTRY NUMBER	11141-16-5
	CLASSIFICATION CODE	TUMORIGEN
	TOXICOLOGY/CANCER REVIEW	CARCINOGENIC DETERMINATION:HUMAN SUSPECTED IARC** IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. 18,43,78
	TOXICOLOGY/CANCER REVIEW	TOXICOLOGY REVIEW ARVPAX Annual Review of Pharmacology. 14,139,74//STEVA8 Science of the Total Environment. 2(4),305,74
	NIOSH CRITERIA DOCUMENT	CRIT DOC. OCCUPATIONAL EXPOSURE TO POLYCHLORINATED BIPHENYLS recm std-air:TWA 1.0 ug/m3 NTIS** National Technical Information Service.
	SYNONYMS	AROCLOR 1232
	SYNONYMS	CHLORODIPHENYL (32% Cl)
	ENTRY MONTH	7903
	TOXIC DATA SOURCE	ARVPAX Annual Review of Pharmacology. 14,139,74
	TOXDATA KEYWORDS	ORAL;RAT;RODENTS;LD50;4470 mg/kg SKIN;RABBIT;RODENTS;LDLo;2000 mg/kg

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1	SECONDARY SOURCE ID	NIOSH/TQ1352000
	CAS TYPE 1 NAME	POLYCHLORINATED BIPHENYL (AROCLOR 1221)
	CAS REGISTRY NUMBER	11104-28-2
	CLASSIFICATION CODE	TUMORIGEN
	TOXICOLOGY/CANCER REVIEW	CARCINOGENIC DETERMINATION:HUMAN SUSPECTED IARC** IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. 18,43,78
	TOXICOLOGY/CANCER REVIEW	TOXICOLOGY REVIEW ARVPAX Annual Review of Pharmacology. 14,139,74//PREVAH Residue Reviews. 44,1,73//STEVA8 Science of the Total Environment. 2(4),305,74//BISNAS Bioscience. 20,958,70
	NIOSH CRITERIA DOCUMENT	CRIT DOC. OCCUPATIONAL EXPOSURE TO POLYCHLORINATED BIPHENYLS recm std-air:TWA 1.0 ug/m3 NTIS** National Technical Information Service.
	SYNONYMS	AROCHLOR 1221
	SYNONYMS	CHLORODIPHENYL (21% Cl)
	ENTRY MONTH	7903
	TOXIC DATA SOURCE	ARVPAX Annual Review of Pharmacology. 14,139,74
	TOXDATA KEYWORDS	ORAL;RAT;RODENTS;LD50;3980 mg/kg SKIN;RABBIT;RODENTS;LDLo;3169 mg/kg

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Bull. Environm. Contam. Toxicol. 26, 489-495 (1981)

Polychlorinated Biphenyls and Polychlorinated Dibenzofurans in the Toxic Rice-Bran Oil that Caused PCB Poisoning in Taichung

P. H. Chen, K. T. Chang, and Y. D. Lu

Department of Biochemistry, National Yang Ming Medical College, Taipei, Taiwan, R.O.C.

In March 1979, an epidemic of a peculiar skin disease broke out in Taichung and Changhwa in Central Taiwan. The cause of the disease was later identified to be the ingestion of rice-bran oil contaminated with polychlorinated biphenyls (CHEN et al. 1980). At the end of 1980, the total number of reported cases was about 2000.

A similar mass outbreak of Yusho (oil disease) occurred in Western Japan in 1968 (KURATSUNE et al. 1972). In 1969, Japanese scientists reported that the toxic rice oil which caused Yusho was contaminated with polychlorinated biphenyls (TSUKAMOTO et al. 1969). A few years later, the oil was also found to be contaminated with a smaller quantity of polychlorinated dibenzofurans (NAGAYAMA et al. 1975) and a relatively large amount of polychlorinated quaterphenyls (MIYATA et al. 1978, KAMPS et al. 1978, MASUDA et al. 1979). In this study, the levels of polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs) in the toxic rice oils which caused PCB poisoning in Taichung were determined by ECD-Gas Chromatography and Gas Chromatography/Mass Spectrometry. The presence of polychlorinated quaterphenyls (PCQs) and polychlorinated terphenyls (PCTs) in the Chinese toxic rice oil has been published elsewhere (CHEN et al. 1981).

MATERIALS AND METHODS

Toxic rice-bran oil samples were obtained from six different sources in Taichung. One of these samples was collected at the rice oil retail store by the Food and Drug Bureau of the Republic of China. The other five samples were obtained from school and factory cafeterias and the families of the intoxicated patients in Taichung. The cafeterias and families all bought the toxic rice oils from the oil retailer mentioned above.

The alkali decomposition method similar to the one used by the Japanese workers (NAGAYAMA et al. 1976) was employed for the isolation of PCB and PCDF from the rice oil. This method includes the saponification of the oil with an alkali followed by extraction of PCB and PCDF with n-hexane. The condensed extract was cleaned up

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by silica gel column chromatography using n-hexane as an eluting solvent. The purified extract was analyzed by ECD-Gas Chromatography for PCB. The gas chromatograph used was a Shimadzu GC-6AM equipped with ^{63}Ni Electron Capture Detector. The column used was a 2.5 m x 2.6 mm i.d. glass column packed with 5% SE-30 on 80/100 mesh Chromosorb WAW-DMCS. Quantification of PCB was made by comparing the respective area of PCB peaks in the sample with the area of the corresponding peak in the chromatogram of a PCB standard (Kanechlor-500). The method presented by UGAWA et al. (1973) was used for the calculation of PCB quantity in each peak of Kanechlor-500 (KC-500).

The procedure for the separation of PCDF from PCB is as follows: The condensed eluate from the silica gel column chromatographic clean-up was placed on a column (1.2 x 22 cm) packed with 12 g of basic alumina. The column was first eluted with 115 mL of n-hexane containing 2% methylene chloride, then with 100 mL of n-hexane containing 25% methylene chloride. PCDF was expected to be in the eluate containing 100 mL of 25% methylene chloride in n-hexane. The eluate of PCDF fraction was concentrated in a Kuderna-Danish evaporator to about 5 mL, then was blown carefully with a very mild stream of nitrogen to below 0.5 mL. The concentrated eluate was analyzed for PCDF with a computerized GC/MS (HP 5985A) operating in the selected ion monitoring mode to enhance the instrument's sensitivity. The column used for GC/MS analysis was a 6 ft x 2 mm i.d. glass column packed with 3% OV-17 on 100/120 Supelcoport.

RESULTS AND DISCUSSION

Gas chromatograms of a mixture of KC-400 and KC-500 in a 1 to 1 ratio and PCBs in the contaminated rice-bran oil are shown in Fig. 1. As shown in this figure, in the portion of the chromatogram from peak 9 to peak 25, GC pattern of PCBs in the toxic rice

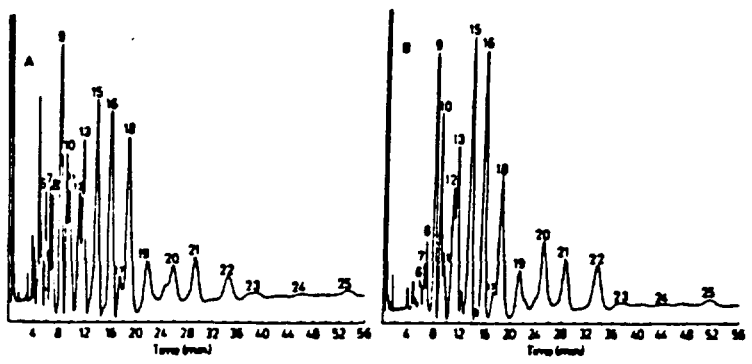

Fig. 1. Gas chromatograms of PCB. A: KC-400/KC-500 (1:1), B: Toxic rice-bran oil.

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il is similar to that of KC-400/KC-500 (1:1); however, in the front
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his decrease in intensity of early eluting peaks in the rice oil
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ss of more volatile components is based on the presumption that
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ransfer medium during the manufacturing process in which PCB was
resumably used as a heat transfer medium. The similarity of GC
attern of PCBs in the rice oil with that of KC-400/KC-500 (1:1)
es not mean that the toxic rice oil was actually contaminated
ith KC-400 and KC-500, it might be contaminated with other PCB
reparations which give a similar GC pattern. It should be pointed
ut that GC/MS analysis showed a significant difference in the
mposition of peak 9 in Fig. 1 between PCBs in the toxic rice oil
nd KC-400/KC-500 (1:1). In the rice oil, peak 9 is predominantly
ue to a tetrachlorobiphenyl with a minor amount (5-10%) of a
entachlorobiphenyl (see Fig. 2), whereas in KC-400/KC-500 (1:1)
he percentage of the pentachlorobiphenyl in peak 9 is substantial-
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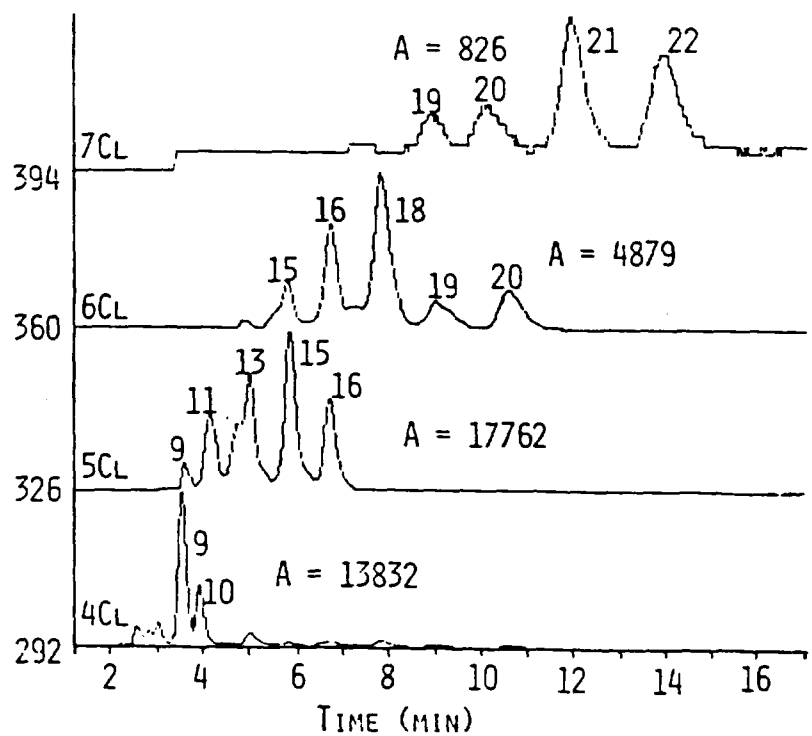


Fig. 2. Selected ion chromatogram of PCBs in the toxic rice-bran oil.

GC/MS analysis showed that the percentage distribution of PCB components in the toxic rice oil according to numbers of chlorine atoms is about 47%, 30%, 16%, 6%, and 1% for pentachloro-, tetrachloro-, hexachloro-, heptachloro-, and trichloro-biphenyls, respectively. This indicates that the toxic rice oil which caused PCB poisoning in Taichung contained larger percentages of high numbers of chlorine atoms than the rice oil which caused Yusho in Japan, for the Japanese rice oil was known to be contaminated with KC-400 which contained mainly tetrachlorobiphenyls. Since it is difficult to excrete PCBs with five or more chlorine atoms from human body, it is reasonable to expect that PCB components ingested by the Chinese patients in Taichung will be retained in the body longer than that of the Japanese Yusho patients.

GC/MS operating in selected ion monitoring (SIM) mode was used for the identification and quantification of PCDFs in the rice-bran oil. As shown in Fig. 3, the ions at m/e's 270, 306, 340, and 374, which are the molecular ions for PCDFs with 3, 4, 5, and 6 chlorine atoms, respectively, were chosen for monitoring PCDFs. Quantification of PCDFs in the rice oil was made by comparing the area of PCDF peaks in each selected ion tracing for the sample with the area of the peak(s) in the same selected ion tracing for the PCDF standard. Fig. 3 and Fig. 4 show the selected ion chromatograms of PCDFs in the rice oil and PCDFs standard, respectively. The results of GC/MS analysis showed, as is evident in Fig. 3, that PCDFs in the toxic rice-bran oil contained mostly 5 and 4 chlorine atoms with a minor amount of 6 and 3 chlorine atoms.

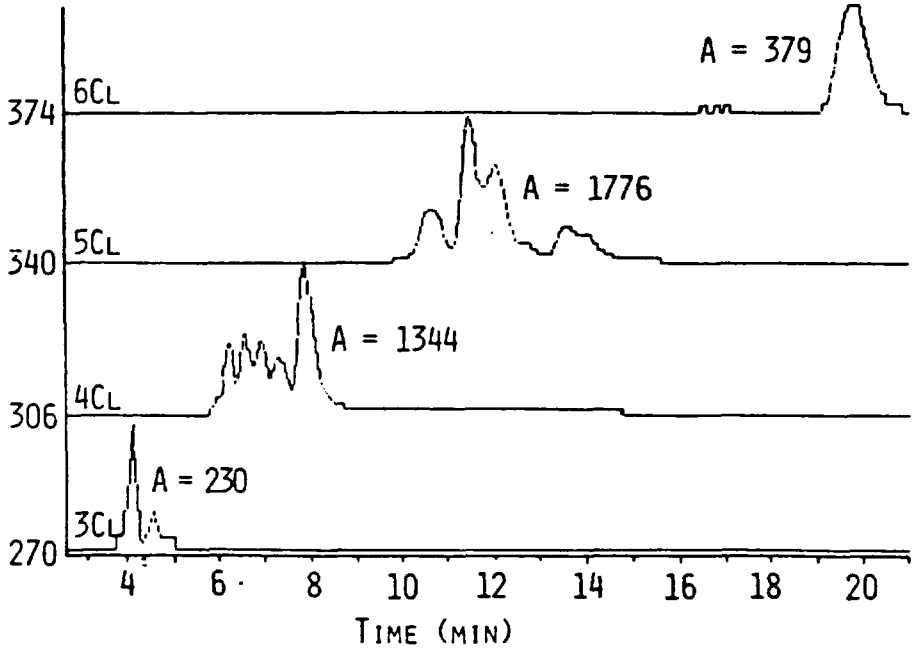
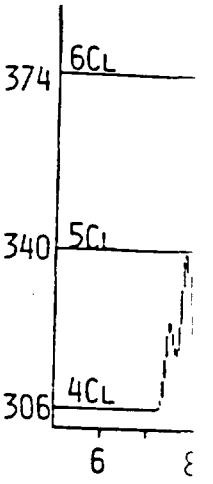


Fig. 3. Selected ion chromatogram of PCDFs in the toxic rice oil.



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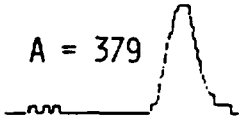

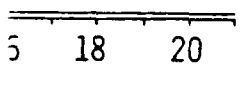

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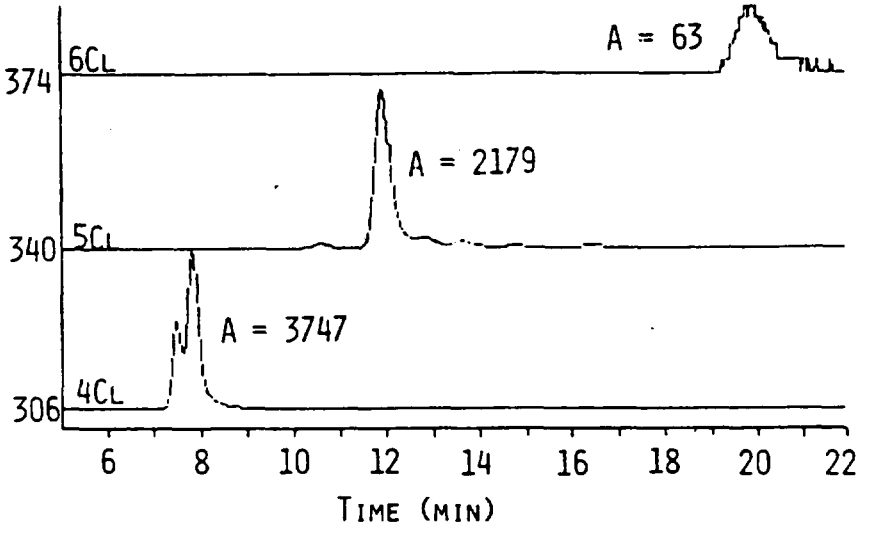

Fig. 4. Selected ion chromatogram of PCDFs standard.

The concentrations of PCB and PCDF in the toxic rice-bran oils which caused PCB poisoning in Taichung are tabulated in TABLE 1. This table shows that with the exception of sample A which has a high PCB level of 405 ppm, the other five samples contain PCB in a range of 53 to 99 ppm. Sample A was given to us by the Food and Drug Bureau (FDB) of the National Health Administration of the Republic of China. According to the FDB's report, this sample contained the highest level of PCB among the thirty-nine samples collected by them at the rice oil retail store where the intoxicated patients in Taichung bought the toxic rice oil. The other five oil

TABLE 1. The Concentrations of PCB and PCDF in the Toxic Rice-Bran Oils Which Caused PCB Poisoning in Taichung

Sample	PCB, ppm	PCDF, ppm	PCB/PCDF
A	405	1.68	241
B	53	0.180	294
C	99	0.399	248
D	78	0.250	312
E	77	0.209	368
F	65	0.297	219

samples obtained from factory and school cafeterias and the families of PCB-intoxicated patients in Taichung contained much lower levels of PCB than sample A. If PCB levels of these five samples are representative of the toxic rice oils ingested by the patients in Taichung, then PCB levels in the toxic rice oils consumed by the patients in Taichung ought to be much lower than that of the Japanese toxic rice oil which in general contained about 830-1030 ppm of PCB (NAGAYAMA et al. 1975). This does not necessarily mean that the Chinese patients in Taichung ingested less PCB than the Japanese Yusho patients because the Chinese patients consumed much more toxic rice oil than the Japanese patients. The estimated amount of PCB ingested by the Chinese patients in Taichung is published in a Special Issue of PCB Poisoning in Taiwan (LAN et al. 1981). The level of PCB in the toxic oil consumed by the patients in Changhwa is not known because no toxic oil sample has ever been available for analysis. However, based on the severity of clinical symtoms for the patients in Changhwa and the blood PCB analysis which showed that the patients in Changhwa had in general higher PCB levels than those in Taichung, one can presume that the toxic oil ingested by the patients in Changhwa contained higher levels of PCB than that consumed by the patients in Taichung. Fortunately, the number of patients in Changhwa is about one half of that in Taichung.

The levels of PCDF in the toxic rice-bran oils determined with GC/MS are mostly below 0.5 ppm with an exception of sample A which is 1.7 ppm (see TABLE 1). These PCDF levels are also lower than that of the Japanese toxic rice oil which is about 5 ppm (NAGAYAMA et al. 1975).

ACKNOWLEDGEMENTS

The authors wish to express their sincere thanks to Prof. Y. Masuda of Daiichi College of Pharmaceutical Sciences, Japan, for the donation of a PCDF standard. The authors are also grateful to the Food and Drug Bureau of the National Health Administration of the Republic of China and the Department of Dermatology of the Veterans General Hospital of the Republic of China for their assistance in obtaining the rice oil samples.

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Hydrogeology-groundwater-well logs

Boring logs-soil sampling

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Premeability tests-or field tests

Sheet pile design: istallation

Earthwork construction

Cross-section-profiles of contamination

Soil and groundwater sampling-testing

HYDROLOGICAL DATA

Surface waters-ditches, harbor

Surface runoff quantities, direction

Tidal information

Sediment sampling and testing

AIR QUALITY DATA

PCB volatilization

Air quality sampling and testing

ECOLOGICAL DATA

Plant life

Wildlife

Aquatic life

Public health

TREATMENT ALTERNATIVES

Water

Soil/sediment

DISPOSAL ALTERNATIVES

Water

Soil/sediment

BACKGROUND

Site history

Hazardous Material Characterization

Site description

Maps

Photos

OTHERS

Date

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As Requested	For Correction	Prepare Reply
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Coordination	Justify	

REMARKS

Continue to anticipate a Feasibility Study that will look very similar to an EIS when it is completed. Please consider using the Enclosed EIS as a template for the F.S. We can discuss this further, later.

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REMARKS

Per Mike Harris,

① Enclosed Find the Final Version of the Old EIS on Waukegan Harbor, including the Appendices. Also enclosed FYI is a bunch of articles on PCB's from a literature search we had done.

② As discussed, the Functional Equivalency argument is going to be a big issue on this project. I

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EMIR propylene Glycol & Sodium Powder

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NEARSHORE CURRENTS IN LAKE MICHIGAN BETWEEN MILWAUKEE AND CHICAGO, 1977-1978: IMPLICATIONS FOR TRANSPORT

DOCUMENT NO. 81/31

ENVIRONMENTAL MANAGEMENT DIVISION

Illinois Institute of Natural Resources

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DOC. NO. 81/31
October, 1981

NEARSHORE CURRENTS IN LAKE MICHIGAN BETWEEN MILWAUKEE AND CHICAGO, 1977-1978: IMPLICATIONS FOR TRANSPORT

by

K. D. Saunders

and

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Project No. 20.097

Frank Beal, Director
State of Illinois
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309 West Washington Street
Chicago, IL 60606

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ATTACHMENT 1

EXPERTS EXPERIENCED IN PARTICULAR AREAS
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ENVIRONMENT
Environment: Pesticides, Chemicals & Toxicants

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512 Brandon Rd.
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Specialties: Liver chemical toxicity; Industrial carcinogenesis; Vinyl chloride; Hepatic cancer. Affiliation: Univ. of Louisville School of Medicine (Prof. & Head, Medical Surveillance Team, & Dir, Vinyl Chloride Project Chief, Digestive Diseases & Nutrition Section). Degrees & Licenses: M.D.

GENERAL PROFESSIONAL DISCIPLINES
CONSULTING SCIENTISTS
LIFE SCIENCES
TOXICOLOGY
Environmental Toxicology:

Kay, Kingsley
16 Sky Ridge Rd.
Greenwich, CT 06830
(203) 661-6577

Specialties: Environmental medicine; Occupational health; Air pollution; Environmental toxicology in industry & agriculture; Environmental genesis of cancer in relation to occupation. Affiliation: Mount Sinai School of Medicine (Assoc. Prof, Environmental Medicine). Degrees & Licenses: Ph.D.

Born 1912 Toronto, Canada; Ph.D. 1938 (Physical Chemistry), Univ of Toronto. Assoc Prof, Environmental Medicine, Mt. Sinai School of Medicine, NY. Member, Expert Advisory Panel, Occupational Health, World Health Organization; Former Chair, Toxicity Subcommittee of Chemical Industries Committee, Int'l Labour Office. Research on environmental genesis of cancer in relation to occupation. Numerous publications: toxicology & industrial hygiene.

PRELIMINARY INJUNCTION AND ON DEFENDANTS' MOTION TO DISMISS THE PRAYER FOR DECLARATORY JUDGMENT AND IN THE ALTERNATIVE FOR SUMMARY JUDGMENT.

BOTH PLAINTIFFS ARE ENGAGED IN THE BUSINESS, INTER ALIA, OF MANUFACTURING PAPER PRODUCTS FROM RECYCLED WASTE PAPER. INCLUDED AMONG THE PRODUCTS MANUFACTURED BY PLAINTIFFS IS A PAPER FOOD-PACKAGING MATERIAL SOLD BY PLAINTIFFS IN INTERSTATE COMMERCE FOR USE IN THE PACKAGING OF FOODS. IT APPEARS THAT THE PACKAGING MATERIALS CONTAIN A CERTAIN AMOUNT OF POLYCHLORINATED BIPHENYLS (HEREINAFTER PCB'S). IT IS THE POSITION OF DEFENDANTS THAT PCB'S ARE TOXIC SUBSTANCES WHICH ABOVE A CERTAIN LEVEL SHOULD NOT BE FOUND IN FOODS FOR HUMAN CONSUMPTION. THE COMMISSIONER OF FOOD AND

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The CITY OF NEW YORK, Plaintiff,

v.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, Douglas M. Costle as Administrator, United States Environmental Protection Agency, and Charles Warren as Regional Administrator, United States Environmental Protection Agency, Region II, Defendants.

No. 80 Civ. 1677 (ADS).

United States District Court, S. D. New York.

Aug. 26, 1981.

The city of New York brought action to compel the Environmental Protection Agency to consider its evidence in support of its application for renewal of interim permit authorizing it to dump sewage sludge into the ocean. The District Court, Sofaer, J., held that, under the Act, and its 1977 amendment, the EPA was required to consider, in connection with each application for dumping, whether that particular dumping would unreasonably degrade marine environment in light of factors listed under the Act, and the EPA could not lawfully adopt a policy of denying all permits without such an examination. So ordered.

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that renders its treatment of the City's permit application arbitrary and capricious. Even if EPA's interpretation of the Act as allowing environmental effects to be dispositive were correct, the Agency would still be required to consider actual and likely dumpsite characteristics in gauging those environmental effects. Accordingly, Subpart B's conclusive presumption of unreasonable degradation is arbitrary and capricious, and it may not lawfully form the basis for an order that the City terminate its sludge dumping at the end of 1981.

FN21. With respect to the adverse effects of sewage sludge on the Bight, EPA has submitted the assessment of Dr. R. Lawrence Swanson, Director of NOAA's Office of Marine Pollution Assessment. See Declaration of Peter W. Anderson (Chief, Marine and Wetlands Protection Branch, EPA Region II), App. Swanson presented his views at an October 1979 EPA hearing on the City's application for permit renewal. He noted that the sludge contains several types of potentially harmful material, including organic matter, petroleum hydrocarbons, halogenated hydrocarbons (particularly PCB's), toxic metals, and pathogens. Swanson described the effects of those substances as including the following: alteration of benthic habitats; possible reduction of fish stocks; reduced dissolved-oxygen values; development in benthic bacteria of resistance to heavy metals and antibiotics; bacterial contamination, resulting in a ban on shellfishing within the area and the isolation of one particularly dangerous

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UNITED STATES of America, Petitioner,

and

Dow Chemical Company, Intervening Petitioner

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significant immediate improvement. These findings are at odds with those that implicitly underlie EPA's use of the Subpart B criteria to presume significant adverse effects. Although the Agency might be able—in the face of contrary evidence—to justify treating the results of its presumptions as conclusive, it has offered no such justification. Second, despite the availability of an alternative site well-suited for dumping, EPA decided to allow dumping to continue at the same site in the Bight, "until sludge dumping could be replaced by environmentally, technically, and economically viable land-based disposal methods." 43 Fed.Reg. 56,061 (1978). If EPA were properly to conclude that the City's dumping in the Bight caused unreasonable degradation, the Agency would need to explain why the alternative site could not safely be used. Yet the Agency has not suggested why dumping of the alternative site would result in unreasonable degradation. Instead, EPA has chosen to evaluate the sludge's environmental impact in a manner that makes largely irrelevant the characteristics of the proposed disposal site.

EPA contests many of the City's assertions as to the actual effects of sludge dumping on the New York Bight; for example, the Agency insists that continued dumping is causing further deterioration, while other sources of pollution in the area are diminishing.[21] The validity of the City's claims as to the dumping's actual effects on the Bight is for EPA to consider in the first instance, and this opinion does not endeavor to resolve those factual disputes. But it is precisely EPA's avowed refusal to consider these claims that renders its treatment of the City's permit application arbitrary and capricious. Even if EPA's interpretation of the Act as allowing environmental effects to be dispositive were correct, the Agency would still be required to consider actual and likely dumpsite characteristics in gauging those environmental effects. Accord-

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Swanson's catalogue is disturbing. One instinctively recoils from the mention of "fecal coliform," "pathogens," "viral particles," "fungi," "fin rot," "shell disease," and "bioaccumulation" of toxic substances. The City's sludge no doubt contains unpleasant and dangerous substances. Nevertheless, EPA must not be permitted to distort Swanson's careful statements. First, Swanson's catalogue includes many potential—not actual—dangers, some of which are not definitely linked to municipal sludge. Furthermore, the effects that Swanson discussed are caused by particular substances found in sludge; those substances are also found in other materials dumped or drained into the Bight. Swanson appeared to accept a recent estimate "that sewage sludge dumping presently accounts for 5 to 15 percent of the total pollution load in the Bight...." Swanson stated that continued dumping of sludge will contribute additional pollutants and thereby "almost certainly retard any future recovery," but he recognized that, without significant reduction in the disposal of other waste and dredged materials, "any recovery will almost certainly be masked by the effects of those other discharges." Even if significant reductions were made in major contaminant inputs, no rapid recovery of the Bight Apex would occur. *Id.* at 4, 17.

Although Swanson recommended "that the 1981 deadline be adhered to," he based that conclusion upon the desirability—not on any imminent necessity—of an end to dumping. His only definitive conclusion was that "the ocean dumping of municipal sewage sludge is not viewed as an environmentally viable disposal alternative over the long run, meaning over twenty, thirty, or fifty years." *Id.* at 22. Swanson anticipated that some municipalities might fail to adopt land-based alternatives by 1981; in that event, he recommended that "the sewage sludge dumping activity should be moved to Deepwater Dumpsite 106 as an interim measure."

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2. *Evaluation of the Need to Dump Sludge*

The 1972 Act lists "need for the proposed dumping" as the very first factor upon which EPA's criteria must be based. 33 S.C. § 1412(a)(A). In addition, in formulating its criteria, EPA must consider "[a]ppropriate locations and methods of disposal or recycling, including land-based alternatives...." *Id.* § 1412(a)(G).

EPA's interpretation of the Act, prior to 1977, emphasized the Agency's obligation to consider all the statutory factors in evaluating permit applications. In explaining its proposed regulations in 1976, EPA stated:

> Subpart A of the revised criteria states the terms of reference which the Regional Administrator or Administrator will use for making a final determination on a permit application. Each of the Subparts B, C, D, and E [of Part 227] addresses a separate consideration which is required by the statute in section 102. No subpart .n and of itself is dispositive of the issue, which the Agency believes is consistent with the broad balancing required by the statute.

41 Fed.Reg. 26,645 (1976). In particular, the Agency promised to consider precisely the type of evidence that the City seeks to present:

> One of the major criticisms of the existing regulations has been the alleged inadequate attention to the demonstration of the need for dumping.... The Agency anticipates that the Regional Administrators will place greater reliance on demonstrati[on] by the applicant that alternatives to ocean disposal are infeasible or are less environmentally acceptable.

Id. at 26,646.

EPA offered similar assurances in issuing its final regulations. In contrast to its current position—that it must consider the need for dumping only in promulgating the regulations, not in evaluating applications—EPA then acknowledged: "The Act lists need for ocean dumping as one factor to be considered in issuing permits." 42 Fed.Reg. 2462 (1977). The statement concerning available alternatives reflected a degree of reasonableness now entirely absent from the Agency's position:

> The Agency also must consider its credibility as the regulating agency. If it demands that a small community or an industry cease dumping within a period of time, it should be confident that there are feasible alternatives which may be implemented within the time period. Increasingly, this is true; and this is the reason all interim permit holders have been given firm phase-out dates. It would be improper to adopt a cessation date which has little factual foundation. Increasingly, EPA has become aware that the alternatives to ocean dumping require careful evaluation; they may not always be better.

Id. at 2464.

Similarly, EPA recognized that one of the components of the need factor was the cost of replacing ocean disposal. In response to public comment on the 1976 regulations, the Agency stated:

> [T]he regulation requires the balancing of the impacts of all forms of alternatives available, including the economic impact. The purpose of this is to make sure that the most cost-effective alternative is used whether it is ocean disposal or another form of disposal, thus, acquiring the highest degree of environmental protection consistent with reasonable economic cost.... The intent of the Ocean Dumping Act is to maintain a balanced consideration of the environmental impact and the relative costs of all alternatives, including ocean dumping. It is not environmentally responsible to single out or emphasize only one factor as a basis for determination.

Final Environmental Impact Statement: Proposed Revisions to Ocean Dumping Criteria, at H-399-400 (1977) [hereinafter cit-

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So ordered.

1. Health and Environment ⇐25.7(13)

Under the Marine Protection, Research and Sanctuaries Act of 1972, the Environmental Protection Agency is required to consider, in connection with each application for ocean dumping of sewage sludge, whether that particular dumping would necessarily degrade the marine environment in light of factors listed in the Act; the EPA could not unlawfully adopt a policy of denying all permits without examining and weighing an applicant's evidence that ocean dumping is the most reasonable alternative. Marine Protection, Research and Sanctuaries Act of 1972, §§ 2–204, 102(a) as amended 33 U.S.C.A. §§ 1401–1444, 1412(a).

2. Health and Environment ⇐25.15(1)

Doctrines of primary jurisdiction and exhaustion of administrative remedies did not bar New York City's lawsuit arising from the Environmental Protection Agency's refusal to consider city's evidence offered to renew interim ocean-dumping permit where the EPA sought no delay in the litigation to enable it to pass upon city's petition nor sought to defer a judicial ruling on its interpretation to deny city's application.

3. Estoppel ⇐62.4

No basis existed to estop city from bringing action against the Environmental Protection Agency to compel the EPA to consider city's evidence in support of its application to renew an interim ocean-dumping permit where city had made no misrepresentations, failed to attack the EPA's regulations prior to the lawsuit because the city had annually received interim permits, and genuinely expected to be able to develop acceptable alternatives before 1981 dumping deadline, and where the EPA could not claim to have detrimentally relied upon city's forbearance from suit.

4. Health and Environment ⇐25.7(13)

The Marine Protection, Research and Sanctuaries Act of 1972 delegated broad discretion to the Environmental Protection Agency, and its determinations of policy, law, and fact are all entitled to deference; thus, the EPA may lawfully adopt criteria, instead of relying directly upon factors described in the Act, and the criteria may permit the EPA reasonably to treat some factors inapplicable in specified situations; nothing in the Act requires that the EPA engage in a comprehensive balancing of listed factors in deciding every ocean-dumping permit application. Marine Protection, Research, and Sanctuaries Act of 1972, §§ 2–204, 102(a)(G) as amended 33 U.S.C.A. §§ 1401–1444, 1412(a)(G).

5. Administrative Law and Procedure ⇐763

Deference accorded an administrative agency is not limitless; the agency may not