

AMBIENT WATER QUALITY CRITERIA FOR  
POLYCHLORINATED BIPHENYLS

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## FOREWORD

Section 304 (a)(1) of the Clean Water Act of 1977 (P.L. 95-217), requires the Administrator of the Environmental Protection Agency to publish criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare which may be expected from the presence of pollutants in any body of water, including ground water. Proposed water quality criteria for the 65 toxic pollutants listed under section 307 (a)(1) of the Clean Water Act were developed and a notice of their availability was published for public comment on March 15, 1979 (44 FR 15926), July 25, 1979 (44 FR 43660), and October 1, 1979 (44 FR 56628). This document is a revision of those proposed criteria based upon a consideration of comments received from other Federal Agencies, State agencies, special interest groups, and individual scientists. The criteria contained in this document replace any previously published EPA criteria for the 65 pollutants. This criterion document is also published in satisfaction of paragraph 11 of the Settlement Agreement in Natural Resources Defense Council, et. al. vs. Train, 8 ERC 2120 (D.D.C. 1976), modified, 12 ERC 1833 (D.D.C. 1979).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304 (a)(1) and section 303 (c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criteria presented in this publication are such scientific assessments. Such water quality criteria associated with specific stream uses when adopted as State water quality standards under section 303 become enforceable maximum acceptable levels of a pollutant in ambient waters. The water quality criteria adopted in the State water quality standards could have the same numerical limits as the criteria developed under section 304. However, in many situations States may want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. It is not until their adoption as part of the State water quality standards that the criteria become regulatory.

Guidelines to assist the States in the modification of criteria presented in this document, in the development of water quality standards, and in other water-related programs of this Agency, are being developed by EPA.

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CRITERIA DOCUMENT  
POLYCHLORINATED BIPHENYLS

CRITERIA

Aquatic Life

For polychlorinated biphenyls the criterion to protect freshwater aquatic life as derived using the Guidelines is 0.014  $\mu\text{g}/\text{l}$  as a 24-hour average. The concentration of 0.014  $\mu\text{g}/\text{l}$  is probably too high because it is based on bioconcentration factors measured in laboratory studies, but field studies apparently produce factors at least ten times higher for fishes. The available data indicate that acute toxicity to freshwater aquatic life probably will only occur at concentrations above 2.0  $\mu\text{g}/\text{l}$  and that the 24-hour average should provide adequate protection against acute toxicity.

For polychlorinated biphenyls the criterion to protect saltwater aquatic life as derived using the Guidelines is 0.030  $\mu\text{g}/\text{l}$  as a 24-hour average. The concentration of 0.030  $\mu\text{g}/\text{l}$  is probably too high because it is based on bioconcentration factors measured in laboratory studies, but field studies apparently produce factors at least ten times higher for fishes. The available data indicate that acute toxicity to saltwater aquatic life probably will only occur at concentrations above 10  $\mu\text{g}/\text{l}$  and that the 24-hour average criterion should provide adequate protection against acute toxicity.

### Human Health

For the maximum protection of human health from the potential carcinogenic effects due to exposure of polychlorinated biphenyls through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at  $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$ . The corresponding recommended criteria are 0.79 ng/l, 0.079 ng/l, and 0.0079 ng/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 0.79 ng/l, 0.079 ng/l, and 0.0079 ng/l, respectively.

## INTRODUCTION

Polychlorinated biphenyls (PCBs) are the chlorinated derivatives of a class of aromatic organic compounds called biphenyls and are manufactured by the direct chlorination of the biphenyl ring system. The commercial products are complex mixtures of chlorobiphenyls and are marketed for various uses according to the percentage of chlorine in the mixture. Currently there is no production of PCBs in the United States but the sole producer of PCBs in the United States previously marketed four mixtures containing 21 percent, 41 percent, 42 percent, and 54 percent chlorine for use only in closed electrical systems under the registered trademark Aroclor. Prior to 1971, mixtures containing up to 68 percent chlorine were used in a number of other applications, including plasticizers, heat transfer fluids, hydraulic fluids, fluids in vacuum pumps and compressors, lubricants, and wax extenders.

In 1974 approximately 65 to 70 percent of domestic sales were to manufacturers of capacitors and the remainder to manufacturers of transformers while approximately 450,000 pounds of PCBs were imported primarily for use in non-closed systems. Production in the United States appeared to be one-half of the world total.

As a result of the long life of many products containing PCBs, it is believed that a substantial portion of the PCBs manufactured before 1971 are still in service and thus represent potential pollution through possible future discharge into the environment.

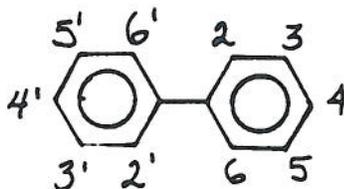
During the period 1972 to 1974, domestic production of PCBs averaged approximately 40 million pounds per year with 33 million

pounds representing the annual domestic marketed consumption during that period.

Although the environmental behavior and biological activity of a number of individual chlorobiphenyl isomers have been studied in recent years, it is still difficult to evaluate the potential toxicity of the complex mixtures actually found in the environment since their composition often changes. In making this evaluation it is necessary to weigh carefully the results of studies of individual compounds, and to compare critically the environmental and toxicological properties of the commercial mixtures.

A further complication is that several commercial PCB mixtures have been reported to contain small quantities of highly toxic contaminants, polychlorinated dibenzofurans (PCDFs). Certain of the toxic effects observed in animals and humans exposed to PCBs appear to be attributable to PCDFs, while others appear to be caused by PCBs themselves. There is also some evidence that small quantities of PCDFs may be formed from PCBs while in service or as a result of metabolic changes in certain organisms.

PCBs consist of a mixture of chlorinated biphenyls which contain a varying number of substituted chlorine atoms on the aromatic rings. The biphenyl molecule has a total of ten sites where chlorine substitution can be accommodated as shown in the following structure:



The potential positions for chlorine substitution are numbered according to the American Chemical Society standard notation. Chlorinated biphenyls having the same number of chlorine atoms per molecule are referred to as a specific class of chlorobiphenyls, with a suitable numerical prefix to define the number of substituted chlorines. Hence, there are classes varying from monochlorobiphenyls to decachlorobiphenyls. All compounds within the same class have the same molecular weight and are structural isomers of each other. They differ only in terms of the location of the chlorine atoms in the biphenyl ring. The ten classes of chlorobiphenyls, comprising 209 possible isomers, are summarized in Table 1.

Chlorobiphenyls with five or more chlorine atoms are referred to as "higher chlorobiphenyls." This distinction is made in recognition of the fact that the former group of compounds is much more persistent in the environment than the latter group. The tetrachlorobiphenyls are intermediate in persistence.

The physical properties of individual chlorinated biphenyls are known (Cook, 1972). The physical properties of the Aroclor mixtures are summarized in Table 2. Lower chlorinated Aroclors (1221, 1232, 1016, 1242, and 1248) are colorless mobile oils. Increasing chlorine content results in mixtures taking on the consistency of viscous liquids (Aroclor 1254) or sticky resins (Aroclors 1260 and 1262). Aroclors 1268 and 1270 are of white powders. With the exception of Aroclors 1221 and 1268, Aroclors do not crystallize upon heating or cooling but at a specific temperature, defined as a "pour point," change into a resinous state.

TABLE 1  
Empirical Formulation, Molecular Weights,  
and Chlorine Percentage in PCBs<sup>a</sup>

Empirical Formula Chlorobiphenyls	Molecular Weight <sup>b</sup>	Percent Chlorine <sup>b</sup>	No. of Isomers
C <sub>12</sub> H <sub>10</sub>	154	0	1
C <sub>12</sub> H <sub>9</sub> Cl	188	18.6	3
C <sub>12</sub> H <sub>8</sub> Cl <sub>2</sub>	222	31.5	12
C <sub>12</sub> H <sub>7</sub> Cl <sub>3</sub>	256	41.0	24
C <sub>12</sub> H <sub>6</sub> Cl <sub>4</sub>	290	48.3	42
C <sub>12</sub> H <sub>5</sub> Cl <sub>5</sub>	324	54.0	46
C <sub>12</sub> H <sub>4</sub> Cl <sub>6</sub>	358	58.7	42
C <sub>12</sub> H <sub>3</sub> Cl <sub>7</sub>	392	62.5	24
C <sub>12</sub> H <sub>2</sub> Cl <sub>8</sub>	426	65.7	12
C <sub>12</sub> H <sub>1</sub> Cl <sub>9</sub>	460	68.5	3
C <sub>12</sub> Cl <sub>10</sub>	490	79.9	1

<sup>a</sup>Source: Hutzinger, et al. 1974

<sup>b</sup>Based on Cl

TABLE 2

## Physical Properties of Commercial PCBs (Aroclors)\*

Property	1221	1232	1016	1242	1248
Chlorine, percent	20.5-21.5	31.4-32.5	41	42	48
Specific Gravity	1.182-1.192 (25°/15.5°C)	1.270-1.280 (25°/15.5°C)	1.362-1.372 (25°/15.5°C)	1.391-1.392 (25°/15.5°C)	1.405-1.415 (65°/15.5°C)
Distillation Range °C Corrected	275-320	290-325	323-356	325-366	340-375
Vapor Pressure (mm/HS)				4.06x10 <sup>-4</sup>	4.94x10 <sup>-4</sup>
Evaporation loss (%) 100°C 6 hr.	1.0-1.5	1.0-1.5		0-0.4	0-0.3
µSTA D-6 Mod. 160°C, 5 hr.				3.0-3.6	3.0-4.0
Pour Point°C (WTM E97) F	1 (Crystal) 34 (Crystal)	-35.5 -32		-19 2	-7 19.4
Water Solubility at 25°C(µg/l)	200		225-250	240	54

TABLE 2 (cont.)

Physical Properties of Commercial PCBs (Aroclors)\*

Property	1254	1260	1262	1268	1270
Chlorine, percent	54	60	61.5-62.5	68	71
Specific Gravity	1.495-1.555 (65°/15.5°C)	1.555-1.566 (90°/15.5°C)	1.572-1.583 (90°/15.5°C)	1.604-1.611 (25°/25°C)	1.944-1.960 (25°/25°C)
Distillation Range °C Corrected	365-390	385-420	390-425	435-450	450-460
Vapor Pressure (mm/HS)	7.71x10 <sup>-5</sup>	4.05x10 <sup>-5</sup>			
Evaporation loss (%) 100°C 6 hr.	0-0.2	0-0.1	0-0.1	0-0.6	
WATA D-6 Mod. 60°C, 5 hr.	1.1-1.3	0.5-0.8	0.5-0.2	0.1-0.2	
Boiling Point°C (WTM E97) F	10 50	31 88	35-38 99		
Water Solubility at 25°C (µg/l)	12	2.7			

\*Source: Versar, Inc., 1976  
 Hutzinger, et al. 1974  
 Mieure, et al. 1976  
 Tucker, et al. 1975  
 Mackay and Wolkoff, 1973

It is known from the studies of pesticides that soil moisture and evaporation of water have a strong influence on the rate of chlorinated hydrocarbon volatilization from soils and sand. Haque, et al. (1974) demonstrated that the periodic evaporation of water from Ottawa sand enhanced the total volatilization of Aroclor 1254 but reduced the degree of differentiation in the volatility of the higher chlorinated biphenyls (7, 6, and 5 chlorine atoms) from the tetrachlorobiphenyls. However, when Aroclor 1254 was heated in water at 100°C the total volatilization of this Aroclor was reduced compared to equivalent dry isothermal conditions, but the differentiation in volatility between the higher and lower chlorinated biphenyls was increased (Bowes, et al. 1975).

Mackay and Wolkoff (1973) calculated theoretical evaporation rates for various Aroclors from water and predicted very rapid volatilization rates. Under laboratory conditions, PCBs appear to volatilize fairly rapidly from water in aquaria (Uhlken, et al. 1973) and even from flasks plugged with glass wool (Oloffs, et al. 1972). Under the same conditions, volatilization was markedly reduced in the presence of sediments (Oloffs, et al. 1973). Hence in natural waters, it would seem likely that absorption to sediments would limit the rate of volatilization.

Solubilities of the individual chlorinated biphenyls in water have been studied by several workers and an inverse correlation between solubility and degree of chlorination has been reported (Wollnofer, et al. 1973; Haque and Schmedding, 1975; Metcalf, et al. 1975). The problem in obtaining true solution equilibria data for PCBs in water has been explained by Schoor (1975) who has given

evidence that solutions of PCBs in water are in fact stable emulsions of PCB aggregates and that the true solubility of Aroclor 1254 is less than 0.1  $\mu\text{g}/\text{l}$  in fresh water and 0.04  $\mu\text{g}/\text{l}$  in marine water.

Chlorobiphenyls are freely soluble in relatively nonpolar organic solvents (Hutzinger, et al. 1974) and lipids in biological systems (Metcalf, et al. 1975). Metcalf, et al. have reported octanol/water partition coefficients in the range of 10,000 to 20,000 for representative tri-, tetra-, and pentachlorobiphenyls. Partition coefficients with this biphasic solvent system have been found to correlate well with ecological magnification factors in aquatic organisms (Metcalf, et al. 1975).

PCBs are strongly adsorbed on solid surfaces, including glass and metal surfaces in laboratory apparatus (Schoor, 1975) and soils, sediments, and particulates in the environment (Haque, et al. 1974; Oloffs, et al. 1973; Crump-Wiesner, et al. 1974; Dennis, 1976; Munson, et al. 1976; Pfister, et al. 1969).

In aquatic environments, PCBs are associated with sediments and are usually found at much higher concentrations in sediments than in water in contact with them (Young, et al. 1976; Crump-Weisner, et al. 1974; Dennis, 1976). As with other chlorinated hydrocarbons, PCBs are probably associated particularly strongly with micro-particulates of 0.15  $\mu\text{m}$  diameter or less (Pfister, et al. 1969).

PCBs are commercially produced by the chlorination of the biphenyl ring with anhydrous chlorine in the presence of iron filings or ferric chloride as the catalyst. The crude product is

purified to remove the color and traces of the by-product hydrogen chloride, and the catalyst by treatment with alkali and subsequent distillation. The purified product is a complex mixture of the chlorobiphenyls, the precise composition depending on the conditions under which the chlorination occurred.

It has been reported that foreign PCB mixtures are similar in composition to one of the 10 Aroclor products previously manufactured in the U.S. Gas liquid chromatograms of Phenoclor DP6 (France), Clophen A60 (Germany), and Aroclor 1260 (U.S.), all mixtures containing 60 percent chlorine, show that these mixtures are virtually identical (Tas and de Vos, 1971). Jensen and Sundstron (1974) have shown that Clophen A60 and A50 (Germany) are very similar in isomer composition to Aroclors 1260 and 1254 (U.S.), respectively. Table 3 lists the distribution of the various classes of chlorobiphenyls in seven major Aroclor mixtures as reported by Mieure, et al. (1976), Webb and McCall (1973), and Hirwe, et al. (1974). The small differences in analytical results reported for Aroclors 1242 and 1254 may reflect either differences in analytical methods or variations in sample constitution.

Certain substitution patterns are believed to influence the biological activities of chlorobiphenyls. The presence of two adjacent carbon atoms without chlorine substitution in one or both rings is believed to facilitate metabolism because it permits the formation of arene oxide intermediates (Safe, et al. 1975). Essentially all chlorobiphenyls with five or fewer chlorine atoms have at least one pair of adjacent unsubstituted carbon atoms because of the rarity of 3,5-substitution in the natural mixtures.

TABLE 3

Approximate Molecular Composition of Aroclors (%)

Chlorobiphenyl	1221		1232		1016		1242		1248		1254		1260	
	1	2	2	6	1	Tr	1	2	3	2	1	2	3	2
C <sub>12</sub> H <sub>10</sub>	11	7	6	6	Tr	Tr	Tr	-	-	-	Tr	-	-	-
C <sub>12</sub> H <sub>9</sub> Cl	51	51	26	26	1	1	1	Tr	-	Tr	-	-	-	-
C <sub>12</sub> H <sub>8</sub> Cl <sub>2</sub>	32	38	29	29	20	20	16	17	4	1	0.5	-	-	-
C <sub>12</sub> H <sub>7</sub> Cl <sub>3</sub>	4	3	24	24	57	57	49	40	39	23	1	-	0.5	-
C <sub>12</sub> H <sub>6</sub> Cl <sub>4</sub>	2	-	15	15	21	21	25	32	42	50	21	16	36	-
C <sub>12</sub> H <sub>5</sub> Cl <sub>5</sub>	0.5	-	0.5	0.5	1	1	8	10	14	20	48	60	45	12
C <sub>12</sub> H <sub>4</sub> Cl <sub>6</sub>	-	-	-	-	Tr	Tr	1	0.5	-	1	23	23	18	46
C <sub>12</sub> H <sub>3</sub> Cl <sub>7</sub>	-	-	-	-	-	-	Tr	-	-	-	6	1	1	36
C <sub>12</sub> H <sub>2</sub> Cl <sub>8</sub>	-	-	-	-	-	-	-	-	-	-	-	-	-	6
C <sub>12</sub> H <sub>1</sub> Cl <sub>9</sub>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C <sub>12</sub> Cl <sub>10</sub>	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Tr - Trace (&lt;0.1 percent)

Source: <sup>1</sup>Mieure, et al. 1976<sup>2</sup>Webb and McCall, 1973<sup>3</sup>Hirwe, et al. 1974

Jensen and Sundstrom (1974) presented evidence that chlorobiphenyls with three or four chlorine atoms in the ortho- positions (2- and 6- positions) are more easily metabolized by humans than those with only one or two ortho-chlorines. Compounds with three or four ortho- substituted chlorines are virtually absent from Aroclors 1016 and 1242 but are fairly well represented in Aroclors 1254 and 1260 (Clopens A50 and A60, respectively).

McKinney (1976) has suggested that chlorobiphenyl isomers with chlorine substitution in both the 4- and 4' positions tend to be biologically active and well retained in tissues. The number and proportion of these isomers increase with increasing chlorination.

McKinney, et al. (1976) have shown an association between biological activity and substitutions in the 3,4- or 3,4,5- positions on one or both rings. The first pattern is frequently found in PCB mixtures but the second is found only as part of the 2,3,4,5-pattern which is found in only trace amounts in PCBs.

Toxic materials other than chlorinated biphenyls have been found in commercial PCB mixtures. Vos, et al. (1970), Bowes, et al. (1975), Roach and Pomerantz (1974), Nagayama, et al. (1976), and Kuratsune, et al. (1976) have detected polychlorinated dibenzofurans (PCDFs) in a number of domestic and foreign PCB mixtures at levels of 0.8 to 33 mg/kg. While 119 structurally different PCDF isomers are possible, only two have been precisely identified to date, the 2,3,7,8-tetrachloro- and the 2,3,4,7,8-pentachlorodibenzofurans (Bowes, et al. 1975).

Polychlorinated naphthalenes (PCNs) have also been identified in small quantities in Clopen A60 and Phenochlor DP 6 (both corresponding to Aroclor 1260), Aroclor 1254, and KC-400 (corresponding to Aroclor 1248) (Vos, et al. 1970; Roach and Pomerantz, 1974; Bowes, et al. 1975).

There appear to be no authenticated reports of polychlorinated dibenzo-p-dioxins (PCDDs) in commercial PCBs (Bowes, et al. 1975). The presence of potentially toxic compounds other than polychlorinated biphenyls in commercial PCB mixtures complicates both analytical and toxicological evaluation of such mixtures.

PCBs are considered to be inert to almost all of the typical chemical reactions. PCBs do not undergo oxidation, reduction, addition, elimination, or electrophilic substitution reactions except under extreme conditions. Chlorines can be replaced by reductive dechlorination with any metal hydride such as lithium aluminum hydride but temperatures of 245°C or greater are required to effect chlorine displacement.

The reactions of environmental importance that PCBs appear to undergo include alkali- and photochemically-catalyzed nucleophilic substitutions and photochemical free radical substitutions, all of which occur with alkali and water.

Photolysis generally has been found to give one type of product under environmental conditions (Hutzinger, et al. 1972, 1974; Ruzo, et al. 1972, 1974; Ruzo and Zabik, 1975; Herring, et al. 1972). Chlorine is replaced by hydroxy groups in aqueous systems.

A marked increase in rate of PCB photolysis was observed when solvents were degassed prior to irradiation (Ruzo, et al. 1974).

Oxygen is known to act as a free radical quencher by accepting energy from free radicals before any chemical change can occur. This increase in rate therefore implies that a free radical process is occurring and in the environment these photochemical transformations will be enhanced under anaerobic conditions.

The photochemical behavior of higher chlorobiphenyls appears similar to that of the tetrachlorobiphenyls (Hutzinger, et al. 1972; Herring, et al. 1972). Irradiation of Aroclor 1254 in aqueous solution gave rise to dechlorinated and hydroxylated products (Hutzinger, et al. 1972). Hexa- and octachlorobiphenyls are more photochemically reactive than tetrachlorobiphenyls, so that under irradiation the higher components of Aroclor 1254 are selectively degraded (Hutzinger, et al. 1972; Herring, et al. 1972).

The creation of free radicals by sunlight allows the environmental replacement of chlorines by hydroxy groups from water without the intervention of alkali. When this occurs at the ortho-position (found to be the most preferred for chlorine loss) the resulting 2-hydroxychlorobiphenyl is perfectly positioned to allow oxygen to bond to an ortho-position of the other ring. This results in the creation of potentially the most important class of contaminant in commercial mixtures of PCBs, the chlorodibenzofurans (CDFs).

Irradiation studies on either Aroclor 1254 or 2,5,2',5'-tetrachlorobiphenyl (Hutzinger, et al. 1972) in hydroxylic solvents have shown the formation of phenolic compounds, carboxylic compounds, and polymers along with dechlorination. Activation of the phenyl rings by metals or metallic salts make them more susceptible to

hydroxylation. Thus, in the environment, either heat, light, or metals and metal salts in water could theoretically accelerate the transformation of PCBs to PCDFs. The ultraviolet component of sunlight is sufficiently energetic to generate free radicals from both phenols and PCBs. The energies required to break the Ar-Cl bond to form hydroxy-PCBs in a hydroxylic solvent and ArO-H bond to form CDFs correspond to wavelengths near 360 to 320 nm, respectively. These wave lengths are clearly within the sunlight region.

Irradiation experiments with five pure 2-chlorinated biphenyls as 5 mg/l aqueous suspensions, showed that traces of 2-chlorodibenzofuran were detectable although only the 2,5-dichloro- and the 2,5,2',5'-tetrachlorobiphenyls provided identifiable amounts or approximately a 0.2 percent yield during a seven-day irradiation (Crosby, et al. 1973; Crosby and Moilanen, 1973). The environmental significance of this is fourfold: (1) ortho-chlorobiphenyls can be hydroxylated by radiation similar to sunlight when they are suspended in aqueous media; (2) the product(s) are converted to CDFs; (3) rates of CDF formation by this process are approximately the same as their rates of degradation, leading to an approximately steady concentration; and (4) decomposition of 2,8-dichlorobenzofuran was found to be very slow in aqueous suspension but dehalogenation did not take place to form the relatively photolytically stable 2-chlorodibenzofuran (Crosby and Moilanen, 1973).

In addition to photochemical and metallic salt formations of PCDFs from PCBs, a third route of formation has been suggested. Kanechlor KC-400 (analogous to Aroclor 1248) having an initial

PCDF content of 20 mg/kg, was shown to undergo conversion as the heat transfer fluid in a heat exchanger to give PCBs with a PCDF content of 4,975 to 11,765 mg/kg (Nagayma, et al. 1976; Kuratsune, et al. 1976). This material was identified as the agent which poisoned a large number of Japanese in 1968. A general disadvantage of PCBs in many of their applications including electrical capacitor and transformer uses as well as heat transfer uses is their tendency to decompose under the action of heat or electrical arcing to form potentially more toxic products (Broadhurst, 1972).

## REFERENCES

- Bowes, G.W., et al. 1975. Identification of chlorinated dibenzofurans in American polychlorinated biphenyls. *Nature*. 256: 305.
- Broadhurst, M.G. 1972. Use and replaceability of polychlorinated biphenyls. *Environ. Health Perspect.* 2: 81.
- Cook, J.W. 1972. Some chemical aspects of polychlorinated biphenyls (PCBs). *Environ. Health Perspect.* 1: 1.
- Crosby, D.G., et al. 1973. Environmental generation and degradation of dibenzodioxins and dibenzofurans. *Environ. Health Perspect.* 5: 259.
- Crosby, D.H. and K.W. Moilanen. 1973. Photodecomposition of chlorinated biphenyls and dibenzofurans. *Bull. Environ. Contam. Toxicol.* 10: 372.
- Crump-Wiesner, H.J., et al. 1974. Pesticides in water: A study of the distribution of polychlorinated biphenyls in the aquatic environment. *Pestic. Monitor. Jour.* 8: 157.
- Dennis, D.S. 1976. Polychlorinated biphenyls in the surface waters and bottom sediments of the major basins of the United States. *Proc. Natl. Conf. on Polychlorinated Biphenyls.* p. 193.

Haque, R. and D. Schmedding. 1975. A method of measuring the water solubility of hydrophobic chemicals: Solubility of five polychlorinated biphenyls. Bull. Environ. Contam. Toxicol. 14: 13.

Haque, R., et al. 1974. Aqueous solubility, adsorption, and vapor behavior of polychlorinated biphenyl Aroclor 1254. Environ. Sci. Technol. 8: 139.

Herring, J.L., et al. 1972. UV irradiation of Aroclor 1254. Bull. Environ. Contam. Toxicol. 8: 153.

Hirwe, S.N., et al. 1974. Gas-liquid chromatography-mass spectrometric characterization of Aroclor 1242 and 1254 components. Bull. Environ. Contam. Toxicol. 12: 135.

Hutzinger, O., et al. 1972. Photochemical degradation of chlorobiphenyls (PCBs). Environ. Health Perspect. 1: 15.

Hutzinger, O., et al. 1974. The Chemistry of PCBs. CRC Press, Cleveland, Ohio.

Jensen, S. and G. Sundstrom. 1974. Structures and levels of most chlorobiphenyls in two technical PCB products and in human adipose tissue. Ambio. 3: 70.

Kuratsune, M., et al. 1976. Some of the recent findings concerning Yusho. Proc. Natl. Conf. on Polychlorinated Biphenyls. p. 15.

- Mackay, D. and A.W. Wolkoff. 1973. Rate of evaporation on low-solubility contaminants from water bodies to atmosphere. *Environ. Sci. Technol.* 7: 611.
- McKinney, J.D. 1976. Toxicology of selected symmetrical hexachlorobiphenyl isomers: Correlating biological effects with chemical structure. *Proc. Natl. Conf. on Polychlorinated Biphenyls.* p. 99.
- McKinney, J.D., et al. 1976. Toxicology of hexachlorobiphenyl isomers and 2,3,7,8-tetrachlorodibenzofuran in chicks. I. Relationship of chemical parameters. *Toxicol. Appl. Pharmacol.* (In press)
- Metcalf, R.L., et al. 1975. Laboratory model ecosystem studies of the degradation and fate of radiolabeled tri-, tetra-, and pentachlorobiphenyl compared with DDE. *Arch. Environ. Contam. Toxicol.* 3: 151.
- Mieure, J.P., et al. 1976. Characterization of polychlorinated biphenyls. *Proc. Natl. Conf. on Polychlorinated Biphenyls.* p. 112.
- Munson, T.O., et al. 1976. Transport of chlorinated hydrocarbons in the Upper Chesapeake Bay. *Proc. Natl. Conf. on Polychlorinated Biphenyls.* p. 223.
- 

Nagayama, J., et al. 1976. Determination of chlorinated dibenzofurans in Kanechlors and "Yusho Oil". Bull. Environ. Contam. Toxicol. 15: 9.

Oloffs, P.C., et al. 1972. Fate and behavior of five chlorinated hydrocarbons in three natural waters. Can. Jour. Microbiol. 18: 1393.

Oloffs, P.C., et al. 1973. Factors affecting the behavior of five chlorinated hydrocarbons in the two natural waters and their sediments. Jour. Fish. Res. Board Can. 30: 1619.

Pfister, R.M., et al. 1969. Microparticulates: Isolation from water and identification of associated chlorinated pesticides. Science. 166: 878.

Roach, J.A.G. and I.H. Pomerantz. 1974. The finding of chlorinated dibenzofurans in a Japanese polychlorinated biphenyl sample. Bull. Environ. Contam. Toxicol. 12: 338.

Ruzo, L.O. and M.J. Zabik. 1975. Polyhalogenated biphenyls: Photolysis of hexabromo and hexachlorobiphenyls in methanol solution. Bull. Environ. Contam. Toxicol. 13: 181.

Ruzo, L.O., et al. 1972. Polychlorinated biphenyls: Photolysis of 3,4,3',4'-tetrachlorobiphenyl and 4,4'-dichlorobiphenyl in solution. Bull. Environ. Contam. Toxicol. 8: 217.

- Ruzo, L.O., et al. 1974. Photochemistry of bioactive compounds: Photo-products and kinetics of polychlorinated biphenyls. Jour. Agric. Food Chem. 22: 199.
- Safe, S., et al. 1975. The mechanism of chlorobiphenyl metabolism. Jour. Agric. Food Chem. 28: 851.
- Schoor, W.P. 1975. Problems associated with low-solubility compounds in aquatic toxicity tests: theoretical model and solubility characteristics of Aroclor 1254 in water. Water Res. 9: 937.
- Tas, A.C. and R.H. deVos. 1971. Characterization of four major components in a technical polychlorinated biphenyl mixture. Environ. Sci. Technol. 5: 1216.
- Tucker, E.S., et al. 1975. Migration of polychlorinated biphenyls in soil induced by percolating water. Bull. Environ. Contam. Toxicol. 13: 86.
- Uhlken, L.D., et al. 1973. Apparent volatility of PCBs as used in continuous flow bioassays. PCB Newsletter. 5: 4.
- Versar, Inc. 1976. Final Report. PCBs in the United States: Industrial use and environmental distribution. Report to U.S. Environmental Protection Agency. Task I. Contract No. 68-01-3259.

Vos, J.G., et al. 1970. Identification and toxicological evaluation of chlorinated benzofuran and chlorinated naphthalene in two commercial polychlorinated biphenyls. Food Cosmet. Toxicol. 8: 625.

Webb, R.G. and A.C. McCall. 1973. Quantitative PCB standards for electron capture gas chromatography. Jour. Chromatog. Sci. 11: 366.

Wollnofer, P.R., et al. 1973. The solubilities of twenty-one chlorobiphenyls in water. Analabs Research Notes. 13: 14.

Young, D.R., et al. 1976. Marine inputs of polychlorinated biphenyls off southern California. Proc. Natl. Conf. on Polychlorinated Biphenyls. p. 197.

INTRODUCTION

Most data for polychlorinated biphenyls (PCBs) found in the literature are from studies concerned with tissue levels in fish, mammals, and birds, without correlation with source or exposure concentrations. Many studies dealing with various physiological parameters are also available but, again, are such that they are of little use here. Also, PCBs often do not appear to be very acutely toxic to juvenile and adult freshwater fish and invertebrate species in static tests due to low solubility, and this can lead to erroneous judgments as to the actual toxicity of the compounds.

PCBs occur as mixtures of chemical isomers that differ in the amount of chlorination of the biphenyl structure; they have been treated herein as a single entity. Polychlorinated biphenyls were manufactured by the direct chlorination of biphenyl; production in the United States has now ceased. These mixtures were identified under the trade names Aroclor<sup>®</sup> and capactor<sup>®</sup>, and sold on the basis of percentage chlorine (e.g., 21, 42, 54, and 60 percent). Because each component of the mixtures differs slightly in its physical, chemical, and biological properties, and because a possible 209 different chlorobiphenyls may be produced, the evaluation of the potential impact of the various mixtures on the environment is complicated.

PCBs are highly lipophilic and bioconcentrate to high concentrations in tissue from concentrations in water that are often below the usual

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\*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are calculations for deriving various measures of toxicity as described in the Guidelines.

detection limits. When an evaluation of the impact of PCBs on the environment is performed, it is necessary to relate the data gathered in laboratory experiments with relatively pure mixtures to what happens to the mixtures in nature. There is evidence that percentages of chlorine change with time and location as the mixtures are transported through the environment. For example, the proportion of major peaks of Aroclor<sup>®</sup> 1254 in shrimp and fish captured from Escambia Bay, Florida, differed from each other (Nimmo, et al. 1971). The major peaks in these organisms and in organisms from laboratory studies (Hansen, et al. 1971) also differed from the standard used to calculate the amounts of the chemical in tissues. Results of environmental monitoring by Butler and Schultzmann (1978) showed that PCBs identified in fishes, Pacific staghorn sculpin and English sole from the Duwamish River in the state of Washington, during the period of fall 1972 to spring 1976, changed from those resembling Aroclor<sup>®</sup> 1254 to those resembling Aroclor<sup>®</sup> 1260 and, later, Aroclor<sup>®</sup> 1242.

## EFFECTS

### Acute Toxicity

The acute toxicity data base for freshwater invertebrate species contains 12 values for three species. These values were from both static and flow-through tests; the flow-through tests showed an LC<sub>50</sub> range from 10 µg/l for scud, Gammarus fasciatus, to 400 µg/l for the damselfly, Ischnura verticalis.

Six 96-hour LC<sub>50</sub> values (Table 1) are available for four freshwater fish species; all of these are from flow-through tests with measured concentrations. Newly hatched rainbow trout were the most sensitive species tested, with a 96-hour LC<sub>50</sub> of 2.0 µg/l for Capacitor<sup>®</sup> 21 (21 percent chlorine); largemouth bass were almost equally sensitive with a 96-hour

LC<sub>50</sub> of 2.3 µg/l (Birge, et al. 1979). The fathead minnow had a similar LC<sub>50</sub> of 7.7 µg/l for Aroclor<sup>®</sup> 1254 (Nebeker, et al. 1974). All of the acute values for fish species are for newly hatched fishes, reflecting their much greater sensitivity as compared to the other fish life stages.

The toxicity of PCBs appears to be similar for both fish and invertebrate freshwater species if test methods are considered. The lowest species mean acute value is 2.0 µg/l for rainbow trout (Table 3).

The LC<sub>50</sub> or EC<sub>50</sub> values for saltwater invertebrate species range from 10.2 to 60 µg/l (Table 1). The available data show little difference in the acute toxicity of different Aroclors<sup>®</sup>. This low variability in species sensitivity and small difference in acute toxicity of the Aroclors<sup>®</sup> tested could be real. However, it is likely that this is a function of the small number of species tested and that the solubilities of PCBs are less than their acute toxicities.

Acute toxicity tests of PCB mixtures to saltwater fish species have not produced data that can be used to obtain 96-hour LC<sub>50</sub> values because concentrations tested were not sufficiently high (Table 6). Pinfish were not affected in 48 hours by 100 µg/l Aroclor<sup>®</sup> 1254 (Duke, et al. 1970). Eighteen percent of the pinfish died after 96 hours in water to which 100 µg/l Aroclor<sup>®</sup> 1016 was added, compared to 2 percent of the control fish, (Hansen, et al. 1974a). Additional tests with saltwater fish species at slightly higher concentrations might have given data sufficient to calculate 96-hour LC<sub>50</sub> values. However, possible problems could exist in validity of acute tests with PCBs because of their low solubility in water (Schoor, 1975; Wiese and Griffin, 1978).

There are too few data for PCBs and freshwater or saltwater species to calculate a Freshwater or Saltwater Final Acute Value according to the pro-

cedures described in the Guidelines. Species mean acute values are summarized in Table 3.

### Chronic Toxicity

Results from six chronic tests with three freshwater invertebrate species, Daphnia magna, Gammarus pseudolimnaeus, and the midge, Tanytarsus dissimilis, are shown in Table 2. The chronic values for Daphnia magna of 4.3  $\mu\text{g/l}$  for Aroclor<sup>®</sup> 1248 and 2.1  $\mu\text{g/l}$  for Aroclor<sup>®</sup> 1254, were from flow-through tests with measured concentrations (Nebeker and Puglisi, 1974). The value of 0.8  $\mu\text{g/l}$  for the midge with Aroclor<sup>®</sup> 1254, and the two chronic values of 4.9  $\mu\text{g/l}$  for Aroclor<sup>®</sup> 1242 and 3.3  $\mu\text{g/l}$  for Aroclor<sup>®</sup> 1248 for Gammarus pseudolimnaeus, were also from flow-through tests with measured concentrations.

Five freshwater flow-through tests with measured concentrations have been conducted with two fish species, four with fathead minnows and one with brook trout (Table 2). The most toxic Aroclor<sup>®</sup> to fathead minnows was Aroclor<sup>®</sup> 1248 which gave a chronic value of 0.2  $\mu\text{g/l}$  (Defoe, et al. 1978); chronic values for Aroclor<sup>®</sup> 1242, Aroclor<sup>®</sup> 1254, and Aroclor<sup>®</sup> 1260 were 9.0, 2.9, and 2.3  $\mu\text{g/l}$ , respectively (Nebeker, et al. 1974; DeFoe, et al. 1978). A chronic value of 1.0  $\mu\text{g/l}$  for Aroclor<sup>®</sup> 1254 was obtained by Mauck, et al. (1978) for the brook trout.

Two geometric mean acute-chronic ratios are calculable; these are 6.4 for the fathead minnow and 11 for the scud, Gammarus pseudolimnaeus (Table 2).

No chronic tests have been reported in which saltwater invertebrate species were exposed to PCBs.

In an early-life-stage test (Table 2) with the sheepshead minnow, fertilization was not affected by Aroclor<sup>®</sup> 1254, but significantly fewer em-

bryos survived to hatching in a measured concentration of 3.48  $\mu\text{g/l}$  (Schimmel, et al. 1974). Survival of fish during the two weeks following hatching was significantly less in 0.16  $\mu\text{g/l}$ , but not different from controls in 0.06  $\mu\text{g/l}$ .

In a study to determine the effect of PCBs in fish embryos on survival, Hansen, et al. (1973) exposed adult sheepshead minnows for four weeks to Aroclor<sup>®</sup> 1254 (Table 6). Adult fish exposed to 5.6  $\mu\text{g/l}$  died, but those in 1.1  $\mu\text{g/l}$  or lower apparently were not affected. Embryos from adult fish exposed to concentrations as low as 0.14  $\mu\text{g/l}$  were placed in PCB-free flowing saltwater and observed for four weeks. Fertilization success was not affected by PCBs in embryos, but survival of embryos and the resulting fry was reduced (Table 6). Fry from embryos containing 7.0  $\mu\text{g/g}$  or more of PCB began dying a few hours after hatching. The concentration in embryos calculated to be lethal to 50 percent of the fish was 6.1  $\mu\text{g/g}$ . If PCB affects other species similarly, then other fish species with equally high concentrations of Aroclor<sup>®</sup> 1254 in their embryos may be endangered.

The effect of another PCB, Aroclor<sup>®</sup> 1016, in water on fry, juvenile, or adult sheepshead minnows was determined in a 4-week exposure (Hansen, et al. 1975)(Table 2). Survival of all three life stages was reduced in 15  $\mu\text{g/l}$  but not in 3.4  $\mu\text{g/l}$  or less. Unlike Aroclor<sup>®</sup> 1254, as much as 77  $\mu\text{g/g}$  of Aroclor<sup>®</sup> 1016 in embryos apparently did not affect survival of embryos and fry in water free of this PCB.

Concentrations of Aroclor<sup>®</sup> 1016 and 1254 affecting sheepshead minnows in chronic exposures differed markedly (7.14 and 0.098  $\mu\text{g/l}$ ); similarly, life-cycle tests with the fathead minnow and Aroclors<sup>®</sup> 1242, 1248, 1254, and 1260 yielded chronic values of 0.2 to 9.0  $\mu\text{g/l}$  (Table 2). Degree of chlorination in these tests using a freshwater fish species appears unrelated to extent of chronic toxicity and suggests that additional chronic

data on saltwater species for other Aroclors<sup>®</sup> may be needed to demonstrate adequately the presence of a relationship between degree of chlorination and chronic toxicity.

Chronic exposure of saltwater fish species to Aroclors<sup>®</sup> produced pathological effects not observed in acute tests. Hansen, et al. (1971) reported signs of poisoning in pinfish exposed to 5 µg/l Aroclor<sup>®</sup> 1254, such as fungus-like lesions on the body, hemorrhagic areas around the mouth, ragged fins etc.; and 41 to 66 percent mortality occurred. Signs of poisoning in adult sheepshead minnows exposed to 10 µg/l Aroclor<sup>®</sup> 1254 and juvenile sheepshead minnows exposed to 0.16 µg/l or greater included lethargy, fin rot, and reduced feeding (Hansen, et al. 1973; Schimmel, et al. 1974); decreased survival occurred at concentrations where these signs of poisoning were observed (Table 6).

Spot exposed to 5 µg/l Aroclor<sup>®</sup> 1254 for two weeks or longer showed fatty changes in their livers (Nimmo, et al. 1975). In intermediate stages of liver pathogenesis in fish species exposed to Aroclor<sup>®</sup> 1254, there were extreme fatty changes characterized by the presence of large vacuoles within hepatocytes and disorientation of liver cord distribution. In advanced stages of pathogenesis in moribund fish, there were intracellular PAS-positive bodies (ceroid), congestion of blood sinuses, and severe vacuolation (Table 6).

Chronic toxicity tests, including early-life-stage tests with fishes, demonstrate that the toxicity of PCBs increases with increased duration of exposure. Because data on the acute toxicity of PCBs to saltwater organisms are limited, the relationship between acute and chronic toxicity is poorly understood. Available data (Tables 2 and 6) from chronic tests demonstrate

that Aroclor<sup>®</sup> 1254 affects saltwater organisms at concentrations as low as 0.14 µg/l and Aroclor<sup>®</sup> 1016 affects pinfish at 15 µg/l. No effects have been observed at 0.06 µg/l for Aroclor<sup>®</sup> 1254 and at 3.4 µg/l for Aroclor<sup>®</sup> 1016.

#### Plant Effects

No appropriate freshwater plant effects data are available, but information which has been found for plants is given in Table 6. Information concerning the sensitivity of saltwater plant species is restricted to unicellular algae (Table 4). Fisher and Wurster (1973) found that the growth of the diatom, Rhizosolenia setigera, was reduced in a medium to which 0.1 µg/l Aroclor<sup>®</sup> 1254 was added. Likewise, Fisher, et al. (1974) demonstrated that 0.1 µg/l Aroclor<sup>®</sup> 1254 added per liter of water changed the species ratio of the alga, Dunaliella tertiolecta, and the diatom, Thalassiosira pseudonana. Fisher, et al. (1974) also showed a decrease in species diversity and species ratio change in natural phytoplankton communities at 0.1 µg/l Aroclor<sup>®</sup> 1254. In summary, some data suggest that unicellular plants are affected by concentrations of PCBs similar to concentrations that are chronically toxic to animals. Unfortunately, no data using measured concentrations were presented, and it is difficult to interpret the ecological significance of these studies.

#### Residues

Table 5 contains the results of 21 appropriate freshwater residue studies as defined by the Guidelines. The studies include only laboratory data for invertebrate and fish species and show a wide range of bioconcentration factors (BCF). Freshwater field studies were placed in Table 6 rather than Table 5 because it could not be shown that the PCB concentration in water was constant for a long period of time over the range of territory

inhabited by the organism. Freshwater invertebrate BCF values in Table 5 range from 2,700 for the phantom midge exposed for 14 days to 108,000 for the scud, Gammarus pseudolimnaeus, exposed for 60 days. BCF values for exposures of fish species (Table 5) range from 3,000 for brook trout (fillets) exposed to Aroclor<sup>®</sup> 1254 for 500 days to 274,000 for fathead minnows (whole body) exposed to Aroclor<sup>®</sup> 1242 for 255 days.

The BCF values of PCBs in saltwater species in laboratory tests are also shown in Table 5. The diatom, Cylindrotheca closterium, had a BCF of 1,000 (Keil, et al. 1971); Eastern oyster, up to 101,000 (Lowe, et al. 1972; Parrish, et al. 1974); grass shrimp, Palaemonetes pugio, 27,000 (Nimmo, et al. 1974); and in the three fish species listed, Leiostomus xanthurus, Cyprinodon variegatus, and Lagodon rhomboides, as high as 43,100 (Hansen, et al. 1971, 1973, 1974a, 1975). Bioconcentration factors for PCBs in five of six species of freshwater fishes in laboratory tests were generally similar to BCF values for saltwater species. Variation in BCF values among species is greater than the variation in BCF values when one species is exposed to various Aroclors<sup>®</sup>. For example, BCF values in adult sheepshead minnows exposed under similar conditions averaged 25,000 for Aroclor<sup>®</sup> 1016 and 30,000 for Aroclor<sup>®</sup> 1254.

Bioconcentration factors calculated from data from Escambia Bay, Florida, were greater than 230,000 for blue crab, greater than 100,000 for oysters, and greater than 670,000 for speckled trout (Duke, et al. 1970; Nimmo, et al. 1975). These data, and field data on freshwater fish species, suggest that either BCF from laboratory studies underestimate bioconcentration potentials of PCBs in the environment or that water samples from field studies inadequately characterized ambient concentrations of PCBs (Hansen, 1975).

The bioaccumulation of PCBs into aquatic organisms from PCBs in food and in water and the effects of PCBs on mammals that feed on fish and shellfish are important. The lowest maximum permissible tissue concentration (0.64  $\mu\text{g/l}$ ) is based on the effect of dietary PCBs on mink (Platonow and Karstad, 1973). Significant effects on reproduction of mink were observed at this concentration but a safe concentration was not determined.

Dividing a BCF value by the percent lipid value for the same species provides a BCF value based on 1 percent lipid content; this resultant BCF value is referred to as the normalized BCF. Each of the BCF values for which percent lipid data are available was normalized by dividing the BCF value by its corresponding percent lipid value. The geometric mean of the normalized BCF values was then calculated to be 10,400 (Table 5). The action level for marketability for human consumption established by the U.S. Food and Drug Administration (FDA) for PCBs in edible fish and shellfish is 5.0 mg/kg. Dividing the FDA action level of 5.0 mg/kg by the geometric mean of normalized BCF values (10,400) and by a percent lipid value of 15 for freshwater species (see Guidelines) gives a freshwater residue value of 0.032  $\mu\text{g/l}$ . Similarly, dividing the FDA action level of 5.0 mg/kg by the geometric mean of normalized BCF values (10,400), and by a percent lipid value of 16 for saltwater species (see Guidelines) gives a saltwater residue value of 0.030  $\mu\text{g/l}$ . The highest BCF value for edible portion of a consumed freshwater species is 9,550 for rainbow trout (Branson, et al. 1975). Dividing this value into the FDA action level of 5.0 mg/kg gives a freshwater residue value of 0.52  $\mu\text{g/l}$ . The highest BCF value for edible portion of a consumed saltwater species is the value of 101,000 for Eastern oyster (Lowe, et al. 1972). Dividing this value into the FDA action level of 5.0 mg/kg gives a saltwater residue value of 0.050  $\mu\text{g/l}$ . These concentrations

are probably too high because the average concentration in some edible species would be at the FDA action level.

For wildlife protection, the lowest maximum permissible tissue concentration is 0.64 mg/kg for mink (Plantonow and Karstad, 1973), but this level adversely affected mink. Dividing this value by the geometric mean (45,000) of whole-body BCF values for salmonids (rainbow trout, 46,000; brook trout, 42,000 and 47,000) gives a residual value for freshwater of 0.014  $\mu\text{g/l}$ . The mean BCF of 45,000 for salmonids is based only on laboratory data. Eleven BCF values for salmonids are available from field studies (Table 6). The highest is for the siscowet, but the other 10 range from 119,000 to 2,333,000 with a geometric mean of 456,000. Even if the concentrations of PCBs in water in these field studies are not documented as well as desired, the total available information strongly indicates that field BCF values for PCBs are probably a factor at 10 higher than the available laboratory BCF values. The data from Escambia Bay indicate that similar effects occur with saltwater fishes (Table 5). The model developed by Weiniger (1978) provides a possible explanation for this difference between laboratory and field data. Thus the freshwater and saltwater Final Residue Values of 0.014 and 0.030  $\mu\text{g/l}$ , respectively, are probably at least a factor of 10 too high.

#### Miscellaneous

Table 6 contains data for other effects not listed in Tables 1 through 5. The tests conducted by Birge, et al. (1979) with Capacitor<sup>®</sup> 21 are flow-through early-life-stage tests with measured concentrations, where embryos were tested from just after fertilization until 4 days post-hatch (Table 6). Test  $\text{LC}_{50}$  values for redear sunfish were 8  $\mu\text{g/l}$ ; for large-

mouth bass 1.5  $\mu\text{g/l}$ ; and for rainbow trout 2.0  $\mu\text{g/l}$ . These low values are very close to the data of Nebeker, et al. (1974) and Defoe, et al. (1978) for fathead minnows (Table 2).

Several studies have shown that tests for PCBs lasting longer than 96 hours (Table 6) provide a better estimate of long-term adverse effects (mortality, growth, pathology) than lethality in 96-hour tests. Aroclor<sup>®</sup> 1254 killed pink shrimp at a concentration of 0.94  $\mu\text{g/l}$  within 15 days (Nimmo, et al. 1971). Pink shrimp exposed to 3.0  $\mu\text{g/l}$  for 7 days were sensitive changes in salinity (Nimmo and Bahner, 1974). This species also appeared more susceptible to a viral infection after exposure to Aroclor<sup>®</sup> 1254 (Couch and Nimmo, 1974a,b).

The growth rate (height and in-water weight) of Eastern oysters was significantly reduced by exposure to 5.0  $\mu\text{g/l}$  Aroclor<sup>®</sup> 1254 for 24 weeks (Lowe, et al. 1972). These oysters also displayed general tissue alterations in the vesicular connective tissue (parenchyma) around the digestive diverticula of the hepatopancreas.

Aroclor<sup>®</sup> 1254 was toxic to the saltwater amphipod, Gammarus oceanicus, at a nominal concentration of 10.0  $\mu\text{g/l}$  (Table 6). Molting animals were particularly vulnerable to the PCB. Necrotic branchia were found in some animals exposed for about 6 days to nominal concentration of 1.0  $\mu\text{g/l}$ .

Aroclor<sup>®</sup> 1254 affected the species composition of communities of estuarine animals that developed from planktonic larvae in saltwater that flowed for four months through small aquaria (Table 6; Hansen, 1974). The number of arthropods decreased while the number of chordates increased in aquaria receiving 0.6  $\mu\text{g/l}$  of the PCB. Numbers of phyla, species and individuals were decreased by this PCB, but there was no apparent effect on the abundance of annelids, brachiopods, coelenterates, echinoderms, or

nemerteans. This study showed that a PCB can have marked effects on community structure at concentrations not much different from those that produced chronic effects on single species.

### Summary

The acute toxicity of polychlorinated biphenyls (PCBs) to freshwater animals has been measured with three invertebrate and four fish species, and the species mean acute values range from 2.0 to 283  $\mu\text{g/l}$ . The data from flow-through tests with measured concentrations are similar for fish and invertebrate species, and probably accurately reflect the toxicity of the compounds. The data from static tests are more variable, and many may not reflect actual toxicity, due to volatility, solubility, bioconcentration, and adsorption characteristics of the various PCB compounds. Eleven life-cycle or partial life-cycle tests were completed with three invertebrate and two fish species; the chronic values range from 0.2 to 15  $\mu\text{g/l}$ .

Species mean acute values for PCBs and saltwater animals range from 10.5 to 20  $\mu\text{g/l}$  from six tests on three invertebrate species. Two chronic tests have been conducted on the sheepshead minnow, providing chronic values for this species of 7.14 and 0.098  $\mu\text{g/l}$ .

The freshwater residue data show that PCBs accumulate to relatively high levels in fish and invertebrate tissues, and that for most species PCBs are not rapidly depurated when exposure is discontinued. Bioconcentration factors for invertebrate species range from 2,700 to 108,000. Bioconcentration factors for PCB exposures of fish species range from 3,000 to 274,000.

Bioconcentration data for PCBs in saltwater fish and invertebrate species show bioconcentration factors ranging from 800 to >230,000 for invertebrate species and from 14,400 to >670,000 for fish species.

The BCF values obtained from field data are generally appreciably higher than laboratory-derived BCF values, so Final Residue Values based on laboratory-derived BCF values are probably at least a factor of 10 too high.

Data available for freshwater plant species generally indicate that they are less sensitive to PCBs than are invertebrates or fish species. Data available for saltwater plant species indicate that unicellular plants are affected by concentrations of PCBs similar to concentrations that are chronically toxic to animals.

#### CRITERIA

For polychlorinated biphenyls the criterion to protect freshwater aquatic life as derived using the Guidelines is 0.014  $\mu\text{g/l}$  as a 24-hour average. The concentration of 0.014  $\mu\text{g/l}$  is probably too high because it is based on bioconcentration factors measured in laboratory studies, but field studies apparently produce factors at least 10 times higher for fishes. The available data indicate that acute toxicity to freshwater aquatic life probably will only occur at concentrations above 2.0  $\mu\text{g/l}$  and that the 24-hour average, should provide adequate protection against acute toxicity.

For polychlorinated biphenyls the criterion to protect saltwater aquatic life as derived using the Guidelines is 0.030  $\mu\text{g/l}$  as a 24-hour average. The concentration at 0.030  $\mu\text{g/l}$  is probably too high because it is based on bioconcentration factors measured in laboratory studies, but field studies apparently produce factors at least 10 times higher for fishes. The available data indicate that acute toxicity to saltwater aquatic life probably will only occur at concentrations above 10  $\mu\text{g/l}$  and that the 24-hour average should provide adequate protection against acute toxicity.

Table 1. Acute values for polychlorinated biphenyls

Species	Method <sup>a</sup>	Chemical	LC50/EC50 (µg/l)	Species Mean Acute Value (µg/l)	Reference
Scud, <u>Gammarus fasciatus</u>	FT, M	Aroclor <sup>®</sup> 1242	10	-	Mayer, et al. 1977
Scud, <u>Gammarus fasciatus</u>	S, U	Aroclor <sup>®</sup> 1248	52	-	Mayer, et al. 1977
Scud, <u>Gammarus fasciatus</u>	S, U	Aroclor <sup>®</sup> 1254	2,400	10	Mayer, et al. 1977
Scud, <u>Gammarus pseudolimnaeus</u>	FT, M	Aroclor <sup>®</sup> 1242	73	-	Nebeker & Puglisi, 1974
Scud, <u>Gammarus pseudolimnaeus</u>	FT, M	Aroclor <sup>®</sup> 1248	29	-	Nebeker & Puglisi, 1974
Scud, <u>Gammarus pseudolimnaeus</u>	S, U	2,3,4'-trichloro- biphenyl	70	-	Mayer, et al. 1977
Scud, <u>Gammarus pseudolimnaeus</u>	S, U	4,4'-dichloro- biphenyl	100	-	Mayer, et al. 1977
Scud, <u>Gammarus pseudolimnaeus</u>	S, U	2,4'-dichloro- biphenyl	120	-	Mayer, et al. 1977
Scud, <u>Gammarus pseudolimnaeus</u>	S, U	2,4,6,2',4',6'- hexachlorobiphenyl	150	-	Mayer, et al. 1977
Scud, <u>Gammarus pseudolimnaeus</u>	S, U	2,4,5,2',5'- pentachlorobiphenyl	210	46	Mayer, et al. 1977
Damselfly, <u>Ischnura verticalis</u>	FT, M	Aroclor <sup>®</sup> 1254	200	-	Mayer, et al. 1977
Damselfly, <u>Ischnura verticalis</u>	FT, M	Aroclor <sup>®</sup> 1242	400	283	Mayer, et al. 1977
Rainbow trout, <u>Salmo gairdneri</u>	FT, M	Capaclor <sup>®</sup> 21	2.0	2	Birge, et al. 1979
Fathead minnow, <u>Pimephales promelas</u>	FT, M	Aroclor <sup>®</sup> 1242	15	-	Nebeker, et al. 1974

Table 1. (Continued)

Species	Method*	Chemical	LC50/EC50 (µg/l)	Species Mean Acute Value (µg/l)	Reference
<u>Fathead minnow (juvenile), Pimephales promelas</u>	FT, M	Aroclor® 1242	300	-	Nebeker, et al. 1974
<u>Fathead minnow, Pimephales promelas</u>	FT, M	Aroclor® 1254	7.7	33	Nebeker, et al. 1974
<u>Redear sunfish, Lepomis microlophus</u>	FT, M	Capacitor® 21	19	19	Birge, et al. 1979
<u>Largemouth bass, Micropterus salmoides</u>	FT, M	Capacitor® 21	2.3	2.3	Birge, et al. 1979
<u>SALTWATER SPECIES</u>					
<u>Eastern oyster, Crassostrea virginica</u>	FT, U	Aroclor® 1016	10.2**	-	Hansen, et al. 1974a
<u>Eastern oyster, Crassostrea virginica</u>	FT, U	Aroclor® 1248	17**	-	Lowe, undated
<u>Eastern oyster, Crassostrea virginica</u>	FT, U	Aroclor® 1254	14**	-	Lowe, undated
<u>Eastern oyster, Crassostrea virginica</u>	FT, U	Aroclor® 1260	60**	20	Lowe, undated
<u>Brown shrimp, Penaeus aztecus</u>	FT, U	Aroclor® 1016	10.5	10.5	Hansen, et al. 1974a
<u>Grass shrimp, Palaeomonetes pugio</u>	FT, U	Aroclor® 1016	12.5	12.5	Hansen, et al. 1974a

\* S = static, FT = flow-through, U = unmeasured, M = measured

\*\*EC50 based on decreased growth of oysters

Table 2. Chronic values for polychlorinated biphenyls

<u>Species</u>	<u>Test*</u>	<u>Chemical</u>	<u>Limits (µg/l)</u>	<u>Chronic Value (µg/l)</u>	<u>Reference</u>
<u>FRESHWATER SPECIES</u>					
<u>Cladoceran, Daphnia magna</u>	LC	Aroclor® 1254	10-24	15	Maki & Johnson, 1975
<u>Cladoceran, Daphnia magna</u>	LC	Aroclor® 1248	2.5-7.5	4.3	Nebeker & Puglisi, 1974
<u>Cladoceran, Daphnia magna</u>	LC	Aroclor® 1254	1.2-3.5	2.1	Nebeker & Puglisi, 1974
<u>Scud, Gammarus pseudolimnaeus</u>	LC	Aroclor® 1242	2.8-8.7	4.9	Nebeker & Puglisi, 1974
<u>Scud, Gammarus pseudolimnaeus</u>	LC	Aroclor® 1248	2.2-5.1	3.3	Nebeker & Puglisi, 1974
<u>Midge, Tanytarsus dissimilis</u>	LC	Aroclor® 1254	0.5-1.2	0.8	Nebeker & Puglisi, 1974
<u>Brook trout, Salvelinus fontinalis</u>	LC	Aroclor® 1254	0.7-1.5	1.0	Mauck, et al. 1978
<u>Fathead minnow, Pimephales promelas</u>	LC	Aroclor® 1248	0.1-0.4	0.2	DeFoe, et al. 1978
<u>Fathead minnow, Pimephales promelas</u>	LC	Aroclor® 1260	1.3-4.0	2.3	DeFoe, et al. 1978
<u>Fathead minnow, Pimephales promelas</u>	LC	Aroclor® 1242	5.4-15.0	9.0	Nebeker, et al. 1974
<u>Fathead minnow, Pimephales promelas</u>	LC	Aroclor® 1254	1.8-4.6	2.9	Nebeker, et al. 1974
<u>SALTWATER SPECIES</u>					
<u>Sheepshead minnow, Cyprinodon variegatus</u>	ELS	Aroclor® 1016	3.4-15.0	7.14	Hansen, et al. 1975

Table 5. (Continued)

<u>Species</u>	<u>Tissue</u>	<u>Lipid (%)</u>	<u>Chemical</u>	<u>Bioconcentration Factor</u>	<u>Duration (days)</u>	<u>Reference</u>
<u>Spot, <i>Leiostomus xanthurus</i></u>	Whole body	1.1*	Aroclor® 1254	37,000	28	Hansen, et al. 1971
<u>Sheepshead minnow (adult), <i>Cyprinodon variegatus</i></u>	Whole body	3.6*	Aroclor® 1016	25,000	28	Hansen, et al. 1975
<u>Sheepshead minnow (juvenile), <i>Cyprinodon variegatus</i></u>	Whole body	-	Aroclor® 1016	43,100	28	Hansen, et al. 1975
<u>Sheepshead minnow (fry), <i>Cyprinodon variegatus</i></u>	Whole body	-	Aroclor® 1016	14,400	28	Hansen, et al. 1975
<u>Sheepshead minnow (adult), <i>Cyprinodon variegatus</i></u>	Whole body	3.6*	Aroclor® 1254	30,000	28	Hansen, et al. 1973
<u>Pinfish, <i>Lagodon rhomboides</i></u>	Whole body	-	Aroclor® 1016	17,000	21-28	Hansen, et al. 1974a
<u>Speckled trout, <i>Cynoscion nebulosus</i></u>	Whole body	-	Aroclor® 1254	>670,000	Field data	Duke, et al. 1970; Nimmo, et al. 1975
<u>Fishes</u>	Whole body	-	Aroclor® 1254	>133,000**	Field data	Nimmo, et al. 1975
<u>Invertebrates</u>	Whole body	-	Aroclor® 1254	>27,000**	Field data	Nimmo, et al. 1975

\* Percent lipid data from Hansen, 1980

\*\* Greatest bioconcentration factor of Aroclor® 1254 in fishes and invertebrates, respectively from Escambia Bay, Florida

Maximum Permissible Tissue Concentration

<u>Action Level or Effect</u>	<u>Concentration (mg/kg)</u>	<u>Reference</u>
Fish and shellfish	5.0	U.S. FDA, 21 CFR Part 109.30
No reproduction and mortality in mink, <u>Mustela vison</u>	0.64	Platonow & Karstad, 1973

Table 5. (Continued)

<u>Maximum Permissible Tissue Concentration</u>		
<u>Action Level or Effect</u>	<u>Concentration (mg/kg)</u>	<u>Reference</u>
Reduced survival of sheephead minnow, <i>Cyprinodon variegatus</i> , from embryos containing >7.0 mg/kg	7.0	Hansen, et al. 1973
Geometric mean of normalized BCF values (see text) = 10,400		
Marketability for human consumption: FDA action level for fish and shellfish = 5.0 mg/kg		
Percent lipid values for freshwater species (see Guidelines) = 15		
Percent lipid value for saltwater species (see Guidelines) = 16		
Freshwater:	$\frac{5.0}{10,400 \times 15} = 0.000032 \text{ mg/kg} = 0.032 \text{ } \mu\text{g/l}$	
Saltwater:	$\frac{5.0}{10,400 \times 16} = 0.000030 \text{ mg/kg} = 0.030 \text{ } \mu\text{g/l}$	
Using highest BCF for edible portion of a consumed species		
Freshwater:	Rainbow trout = 9,550	(Branson, et al. 1975)
	$\frac{5.0}{9,550} = 0.00052 \text{ mg/kg} = 0.52 \text{ } \mu\text{g/l}$	
Saltwater:	Eastern oyster = 101,000	(Lowe, et al. 1972)
	$\frac{5.0}{101,000} = 0.000050 \text{ mg/kg} = 0.050 \text{ } \mu\text{g/l}$	

Table 5. (Continued)

Wildlife protection: Lowest maximum permissible tissue concentration = 0.64 mg/kg (Platonow and Karstad, 1973)  
Geometric mean of whole body BCF values for salmonid species = 45,000

$$\text{Freshwater: } \frac{0.64}{45,000} = 0.000014 \text{ mg/kg} = 0.014 \text{ } \mu\text{g/l}$$

Freshwater Final Residue Value = 0.014  $\mu\text{g/l}$

Saltwater Final Residue Value = 0.030  $\mu\text{g/l}$

Table 6. Other data for polychlorinated biphenyls

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result</u> ( $\mu\text{g/l}$ )	<u>Reference</u>
Yeast, <u>Saccharomyces cerevisiae</u>	Aroclor® 1232 to Aroclor® 1260	160 hrs	Reduced growth	25,000	Tejedor, et al. 1979
Alga, <u>Euglena gracilis</u>	Aroclor® 1221	48 hrs	ID50	4,400	Ewald, et al. 1976
Alga, <u>Euglena gracilis</u>	Aroclor® 1242	8 days	Reduced growth	10,000	Bryan & Olafsson, 1978
Alga, <u>Scenedesmus obtusiusculus</u>	Aroclor® 1242	24 hrs	Growth inhibition	300	Larsson & Tillberg, 1975
Alga, <u>Scenedesmus quadricauda</u>	Aroclor® 1254	24 hrs	Reduction in rate of carbon fixation	0.1	Laird, 1973
Alga, <u>Chlorella pyrenoidosa</u>	Aroclor® 1268	191 hrs	Depressed cell productivity	1,000	Hawes, et al. 1976b
Alga, <u>Chlorella pyrenoidosa</u>	Aroclor® 1254	73 hrs	Reduced population growth	1,000	Hawes, et al. 1976a
Alga, <u>Chlamydomonas reinhardtii</u>	Aroclor® 1242	22 days	Reduced growth	2,000	Morgan, 1972
Alga, <u>Phormidium</u> sp.	Dichloro- biphenyl	3 hrs	Reduced motility	50	Zittel & Benecke, 1978
Cladoceran, <u>Daphnia magna</u>	Aroclor® 1248	2 wks	LC50	2.6	Nebeker & Puglisi, 1974
Cladoceran, <u>Daphnia magna</u>	Aroclor® 1254	2 wks	LC50	1.8	Nebeker & Puglisi, 1974
Cladoceran, <u>Daphnia magna</u>	Aroclor® 1254	3 wks	LC50	1.3	Nebeker & Puglisi, 1974
Cladoceran, <u>Daphnia magna</u>	Aroclor® 1254	2 wks	LC50	24	Maki & Johnson, 1975
Cladoceran, <u>Daphnia magna</u>	Aroclor®1221 to Aroclor® 1260	2-3 wks	LC50	19-182	Nebeker & Puglisi, 1974

Table 6. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result</u> ( $\mu\text{g/l}$ )	<u>Reference</u>
<u>Amphipod,</u> <u>Pontiporela affinis</u>	Field data	-	Bloconcentration factor = 1,709	-	Halle, et al. 1975
<u>Stonefly,</u> <u>Pteronarcys dorsata</u>	Aroclor® 1254	21 days	Bloconcentration factor = 740	-	Mayer, et al. 1977
<u>Dobsonfly,</u> <u>Corydalis cornutus</u>	Aroclor® 1254	7 days	Bloconcentration factor = 1,500	-	Mayer, et al. 1977
<u>Mosquito,</u> <u>Culex tarsalis</u>	Aroclor® 1254	7 days	No adult emergence	1.5	Sanders & Chandler, 1972
<u>Glass shrimp,</u> <u>Palaeomonetes kadlakensis</u>	Aroclor® 1254	7 days	LC50	3	Mayer, et al. 1977
<u>Glass shrimp,</u> <u>Palaeomonetes kadlakensis</u>	Field data	21 days	Bloconcentration factor = 2,600	-	Mayer, et al. 1977
<u>Mysid,</u> <u>Mysis relicta</u>	Field data	-	Bloconcentration factor = 125,000	-	Veith, et al. 1977
<u>Snails</u>	Field data	-	Bloconcentration factor = 45,000	-	Nadeau & Davis, 1976
<u>Crayfish,</u> <u>Orconectes nalis</u>	Aroclor® 1242	7 days	LC50	30	Mayer, et al. 1977
<u>Crayfish,</u> <u>Orconectes nalis</u>	Aroclor® 1254	21 days	Bloconcentration factor = 750	-	Mayer, et al. 1977
<u>Rainbow trout,</u> <u>Salmo gairdneri</u>	Aroclor® 1242 and Aroclor 1254	-	Inhibit ATPase activity	4 $\mu\text{g/g}$	Davis, et al. 1972
<u>Rainbow trout,</u> <u>Salmo gairdneri</u>	Aroclor® 1242	25 days	LC50	12	Mayer, et al. 1977

Table 6. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result (µg/l)</u>	<u>Reference</u>
<u>Rainbow trout, Salmo gairdneri</u>	Aroclor® 1248	25 days	LC50	3.4	Mayer, et al. 1977
<u>Rainbow trout, Salmo gairdneri</u>	Aroclor® 1254	25 days	LC50	27	Mayer, et al. 1977
<u>Rainbow trout, Salmo gairdneri</u>	Capacitor® 21	8 day early life stage	LC50	2	Birge, et al. 1979
<u>Rainbow trout, Salmo gairdneri</u>	Aroclor® 1260	25 days	LC50	49	Mayer, et al. 1977
<u>Rainbow trout, Salmo gairdneri</u>	Clophen® A-50	21 days	Induce fish hepatic microsomal enzymes	31 µg/g	Lidman, et al. 1976
<u>Rainbow trout, Salmo gairdneri</u>	Aroclor® 1242	30 days	75% mortality, 70% deformed fry	0.39 µg/g	Hogan & Brauhn, 1975
<u>Rainbow trout, Salmo gairdneri</u>	Aroclor® 1254	330 days	Kidney pathology	10 µg/g	Nestel & Budd, 1974
<u>Rainbow trout, Salmo gairdneri</u>	Aroclor® 1242	5 days	LC50	67	Mayer, et al. 1977
<u>Rainbow trout, Salmo gairdneri</u>	Aroclor® 1254	5 days	LC50	54	Mayer, et al. 1977
<u>Rainbow trout, Salmo gairdneri</u>	Field data	-	Bioconcentration factor = 120,000	-	Veith, 1975
<u>Steelhead trout, Salmo gairdneri</u>	Aroclor® 1254	24 days	Bioconcentration factor = 38,000	-	Halter, 1974
<u>Steelhead trout, Salmo gairdneri</u>	Field data	-	Bioconcentration factor = 600,000	-	Hesse, 1973
<u>Brown trout, Salmo trutta</u>	Clophen® A-50	43 days	Anemia, hypergly- cemia, altered cholesterol metabolism	10 µg/g in food	Johansson, et al. 1972

Table 6. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result (µg/l)</u>	<u>Reference</u>
Brown trout, <u>Salmo trutta</u>	Field data	-	Bioconcentration factor = 119,000	-	Velth, 1975
Atlantic salmon, <u>Salmo salar</u>	Aroclor® 1242	96 hrs	Bioconcentration factor = 600	-	Zitko & Carson, 1977
Atlantic salmon, <u>Salmo salar</u>	Aroclor® 1254	192 hrs	Mortality	>2 µg/g	Zitko, 1970
Coho salmon, <u>Oncorhynchus kislutch</u>	Aroclor® 1254	Early life stage	Chronic limit	<4.4	Halter & Johnson, 1974
Coho salmon, <u>Oncorhynchus kislutch</u>	Aroclor® 1254	72 hrs	Stimulated thyroid activity	0.48 µg/g	Mayer, et al. 1977
Coho salmon, <u>Oncorhynchus kislutch</u>	Aroclor® 1242	68 days	Induced fish hepatic AAH microsomal enzymes	1 µg/g	Gruger, et al. 1977
Coho salmon, <u>Oncorhynchus kislutch</u>	Pentachloro- biphenyl	72 days	Induction of aryl hydrocarbon hydroxylase	12 µg/g	Gruger, et al. 1976
Coho salmon, <u>Oncorhynchus kislutch</u>	Field data	-	Bioconcentration factor = 173,000	-	Velth, 1975
Chinook salmon, <u>Oncorhynchus tshawytscha</u>	Field data	-	Bioconcentration factor = 1,240,000	-	Hesse, 1973
Chinook salmon, <u>Oncorhynchus tshawytscha</u>	Field data	-	Bioconcentration factor = 240,000	-	Velth, 1975
Brook trout, <u>Salvelinus fontinalis</u>	Aroclor® 1254	18 days	Induced fish MFO system	39 µg/g	Addison, et al. 1978
Brook trout, <u>Salvelinus fontinalis</u>	Aroclor® 1254	71 wks	No effect on sur- vival, growth or reproduction	0.94	Snarski & Puglisi, 1976
Brook trout, <u>Salvelinus fontinalis</u>	Aroclor® 1254	fert. to hatch	No embryo hatch	200	Freeman & Idler, 1975

Table 6. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result (<math>\mu\text{g/l}</math>)</u>	<u>Reference</u>
<u>Lake trout, Salvelinus namaycush</u>	Field data	-	Bloconcentration factor = 1,110,000	-	Hesse, 1973
<u>Lake trout, Salvelinus namaycush</u>	Field data	-	Bloconcentration factor = 212,000	-	Veith, 1975
<u>Lake trout, Salvelinus namaycush</u>	Field data	-	Bloconcentration factor = 2,333,000	-	Parejko, et al. 1975
<u>Lake trout, Salvelinus namaycush</u>	Field data	-	Bloconcentration factor = 1,625,000	-	Veith, et al. 1977
<u>Siscowet, Salvelinus namaycush siscowet</u>	Field data	-	Bloconcentration factor = 4,125,000	-	Veith, et al. 1977
<u>Lake whitefish, Coregonus clupeaformis</u>	Field data	-	Bloconcentration factor = 110,000	-	Hesse, 1973
<u>Lake whitefish, Coregonus clupeaformis</u>	Field data	-	Bloconcentration factor = 875,000	-	Veith, et al. 1977
<u>Fathead minnow, Pimephales promelas</u>	Aroclor® 1242	30 days	LC50	28	Veith, 1976
<u>Fathead minnow, Pimephales promelas</u>	Aroclor® 1016	30 days	LC50	28	Veith, 1976
<u>Fathead minnow, Pimephales promelas</u>	Aroclor® 1016	30 days	Reduced growth	23	Veith, 1976
<u>Fathead minnow, Pimephales promelas</u>	Aroclor® 1248	30 days	LC50	4.7	DeFoe, et al. 1978
<u>Fathead minnow, Pimephales promelas</u>	Aroclor® 1260	30 days	LC50	3.3	DeFoe, et al. 1978
<u>Fathead minnow, Pimephales promelas</u>	Aroclor® 1242	30 days	Significant mortality	23	Hermanutz & Puglisi, 1976
<u>Fathead minnow, Pimephales promelas</u>	Aroclor® 1016	30 days	Significant mortality	44	Hermanutz & Puglisi, 1976

Table 6. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result (µg/l)</u>	<u>Reference</u>
<u>Fathead minnow, Pimephales promelas</u>	Aroclor® 1242	4 mos	Inhibition of ATPase activity	0.31	Cutkomp, et al. 1972
<u>Fathead minnow, Pimephales promelas</u>	Aroclor® 1254	4 mos	Inhibition of ATPase activity	0.31	Koch, et al. 1972
<u>Bluegill, Lepomis macrochirus</u>	Aroclor® 1248	5 days	LC50	136	Mayer, et al. 1977
<u>Bluegill, Lepomis macrochirus</u>	Aroclor® 1242	-	Inhibit (150) ATPase	0.6 µg/g	Desalah, et al. 1972
<u>Bluegill, Lepomis macrochirus</u>	Aroclor® 1254	-	Inhibition of ATPase	30	Yap, et al. 1971
<u>Bluegill, Lepomis macrochirus</u>	Aroclor® 1242	30 days	LC50	84	Mayer, et al. 1977
<u>Bluegill, Lepomis macrochirus</u>	Aroclor® 1254	30 days	LC50	78	Mayer, et al. 1977
<u>Bluegill, Lepomis macrochirus</u>	Aroclor® 1254	30 days	LC50	177	Mayer, et al. 1977
<u>Bluegill, Lepomis macrochirus</u>	Aroclor® 1260	30 days	LC50	400	Mayer, et al. 1977
<u>Mosquitofish, Gambusia affinis</u>	Tri-, tetra-, & pentachloro- biphenyl	6 days	Bioconcentration factor = 12,000	-	Sanborn, 1974
<u>Mosquitofish, Gambusia affinis</u>	Aroclor® 1254	1.5 hr	Avol dance	0.1	Hansen, et al. 1974
<u>Guppy, Poecilia formosa</u>	Aroclor® 1242	1 day	Significant mortality	200	Morgan, 1972
<u>Carp, Cyprinus carpio</u>	Aroclor® 1248	20 days	Altered plasma -glucoronidase activity	5 µg/g	Ito, 1973

Table 6. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result</u> ( $\mu\text{g/l}$ )	<u>Reference</u>
Carp, <u>Cyprinus carpio</u>	Aroclor® 1248	21 days	Metabolic changes	250 $\mu\text{g/g}$	Ito & Murata, 1974
Carp, <u>Cyprinus carpio</u>	Field data	-	Bioconcentration factor = 110,000	-	Velth, 1975
Carp, <u>Cyprinus carpio</u>	Field data	-	Bioconcentration factor = 43,600	-	Hesse, 1973
Carp, <u>Cyprinus carpio</u>	Field data	-	Bioconcentration factor = 390,000	-	Hesse, 1973
Channel catfish, <u>Ictalurus punctatus</u>	Aroclor® 1248	30 days	LC50	75	Mayer, et al. 1977
Channel catfish, <u>Ictalurus punctatus</u>	Aroclor® 1254	30 days	LC50	139	Mayer, et al. 1977
Channel catfish, <u>Ictalurus punctatus</u>	Aroclor® 1254	72 hrs	Stimulated thyroid activity	2.4 $\mu\text{g/g}$	Mayer, et al. 1977
Channel catfish, <u>Ictalurus punctatus</u>	Aroclor® 1242	30 days	LC50	8.7	Mayer, et al. 1977
Channel catfish, <u>Ictalurus punctatus</u>	Aroclor® 1242	20 wks	Weight loss and liver hypertrophy	20 $\mu\text{g/g}$	Hansen, et al. 1976
Channel catfish, <u>Ictalurus punctatus</u>	Aroclor® 1254	2 wks	Increased trans- aminase, lower cortisol	8	Camp, et al. 1974
Flagfish, <u>Jordanella floridae</u>	Aroclor® 1242	30 days	Fin erosion	37	Hermanutz & Puglisi, 1976
Redear sunfish, <u>Lepomis microlophus</u>	Capacitor® 21	8 day early life stage	LC50	8	Birge, et al. 1979
Largemouth bass, <u>Micropterus salmoides</u>	Capacitor® 21	8 day early life stage	LC50	1.5	Birge, et al. 1979

Table 6. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result (µg/l)</u>	<u>Reference</u>
<u>Bloater, Coregonus hoyi</u>	Field data	-	Bloconcentration factor = 81,000	-	Velth, et al. 1977
<u>Lake herring, Coregonus artedii</u>	Field data	-	Bloconcentration factor = 250,000	-	Velth, et al. 1977
<u>Rainbow smelt, Osmerus mordax</u>	Field data	-	Bloconcentration factor = 462,500	-	Velth, et al. 1977
<u>Rainbow smelt, Osmerus mordax</u>	Field data	-	Bloconcentration factor = 32,000	-	Velth, 1975
<u>Rainbow smelt, Osmerus mordax</u>	Field data	-	Bloconcentration factor = 48,000	-	Halle, et al. 1975
<u>Rock bass, Ambloplites rupestris</u>	Field data	-	Bloconcentration factor = 117,000	-	Nadeau & Davis, 1976
<u>Pike, Esox lucius</u>	Field data	-	Bloconcentration factor = 15,000	-	Hesse, 1973
<u>Yellow perch, Perca flavescens</u>	Field data	-	Bloconcentration factor = 50,000	-	Hesse, 1973
<u>Yellow perch, Perca flavescens</u>	Field data	-	Bloconcentration factor = 109,000	-	Velth, 1975
<u>Yellow perch, Perca flavescens</u>	Field data	-	Bloconcentration factor = 154,000	-	Norstrom, et al. 1976
<u>Slimy sculpin, Cottus cognatus</u>	Field data	-	Bloconcentration factor = 300,000	-	Velth, et al. 1977
<u>Slimy sculpin, Cottus cognatus</u>	Field data	-	Bloconcentration factor = 84,000	-	Halle, et al. 1975
<u>Fourhorn sculpin, Myoxocephalus quadricornis</u>	Field data	-	Bloconcentration factor = 337,500	-	Velth, et al. 1977
<u>Mink, Mustela vison</u>	Aroclor® 1254	-	Reduced reproduction	2 µg/g	Aulerich & Ringer, 1977

Table 6. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result</u> ( $\mu\text{g/l}$ )	<u>Reference</u>
Mink, <u>Mustela vison</u>	PCB residues	1 yr	Depressed growth	10 $\mu\text{g/g}$	Aulerich, et al. 1973
Mink, <u>Mustela vison</u>	Aroclor® 1254	4 mos	Reduced reproduction	1.0 $\mu\text{g/g}$	Ringer, et al. 1972
<u>SALTWATER SPECIES</u>					
Chlorophyceae, <u>Dunaliella sp.</u>	Aroclor® 1254	45 days	Bloconcentration factor = 477,000 in lipid and 30,000 in dry tissue	-	Scura & Thellacker, 1977
Ciliate protozoans, <u>Tetrahymena pyriformis</u>	Aroclor® 1254	7 days	Bloconcentration factor = 60	-	Cooley, et al. 1972
Ciliate protozoans, <u>Tetrahymena pyriformis</u>	Aroclor® 1248	96 hrs	Reduced growth	1,000	Cooley, et al. 1973
Ciliate protozoans, <u>Tetrahymena pyriformis</u>	Aroclor® 1254	96 hrs	Reduced growth	1.0	Cooley, et al. 1972
Ciliate protozoans, <u>Tetrahymena pyriformis</u>	Aroclor® 1260	96 hrs	Reduced growth	1,000	Cooley, et al. 1973
Polychaete, <u>Arenicola marina</u>	Aroclor® 1254	5 days	Bloconcentration factor = 236	-	Courtney & Langston, 1978
Polychaete, <u>Nereis diversicolor</u>	Aroclor® 1254	5 days	Bloconcentration factor = 373	-	Courtney & Langston, 1978
Rotifer, <u>Brachionus plicatilis</u>	Aroclor® 1254	45 days	Bloconcentration factor = 340,000 in lipid and 51,000 in dry tissue	-	Scura & Thellacker, 1977
Eastern oyster, <u>Crassostrea virginica</u>	Aroclor® 1254	2 days	Bloconcentration factor = 8,100	-	Duke, et al. 1970
Eastern oyster, <u>Crassostrea virginica</u>	Aroclor® 1254	24 wks	Reduced growth	5.0	Lowe, et al. 1972

Table 6. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result</u> ( $\mu\text{g/l}$ )	<u>Reference</u>
Horseshoe crab, <u>Limulus polyphemus</u>	Aroclor® 1016	96 days	Bioconcentration factor = 1,298	-	Neff & Giam, 1977
Amphipod, <u>Gammarus oceanicus</u>	Aroclor® 1254	30 days	Mortality	>10	Wildish, 1970
Grass shrimp, <u>Palaemonetes pugio</u>	Aroclor® 1254	1 hr	Avoidance	10	Hansen, et al. 1974b
Grass shrimp, <u>Palaemonetes pugio</u>	Aroclor® 1254	4 days	Water efflux affected and altered metabolic state	25-45	Roesljadi, et al. 1976a,b
Pink shrimp, <u>Penaeus duorarum</u>	Aroclor® 1248	48 hrs	LC50	32	Lowe, undated
Pink shrimp, <u>Penaeus duorarum</u>	Aroclor® 1254	48 hrs	LC50	32	Lowe, undated
Pink shrimp, <u>Penaeus duorarum</u>	Aroclor® 1254	15 days	51% mortality	0.94	Nimmo, et al. 1971
Pink shrimp, <u>Penaeus duorarum</u>	Aroclor® 1254	15 days	LC50	1.0	Nimmo & Bahner, 1976
Pink shrimp, <u>Penaeus duorarum</u>	Aroclor® 1254	2 days	Bioconcentration factor = 140	-	Duke, et al. 1970
Fiddler crab, <u>Uca pugilator</u>	Aroclor® 1242	4 days	Greater dispersion of melanin	2,000	Fingerman & Fingerman, 1978
Fiddler crab, <u>Uca pugilator</u>	Aroclor® 1254	38 days	Inhibited molting	8.0	Fingerman & Fingerman, 1977
Communities of organisms	Aroclor® 1254	4 mos	Affected composition	0.6	Hansen, 1974
Northern anchovy, <u>Engraulis mordax</u>	Aroclor® 1254	45 days	Bioconcentration factor = 13,000,000 in lipid and 1,030,000 in dry tissue	-	Scura & Thelacker, 1977

Table 6. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result (<math>\mu\text{g/l}</math>)</u>	<u>Reference</u>
<u>Spot, Leiostomus xanthurus</u>	Aroclor® 1254	-	Liver pathogenesis	5.0	Nimmo, et al. 1975
<u>Spot, Leiostomus xanthurus</u>	Aroclor® 1254	20-45 days	51 to 62% mortality	5.0	Hansen, et al. 1971
<u>Pinfish, Lagodon rhomboides</u>	Aroclor® 1254	1 hr	Avoidance	10.0	Hansen, et al. 1974b
<u>Pinfish, Lagodon rhomboides</u>	Aroclor® 1254	14-35 days	41 to 66% mortality	5.0	Hansen, et al. 1971
<u>Pinfish, Lagodon rhomboides</u>	Aroclor® 1254	2 days	Bioconcentration factor = 980	-	Duke, et al. 1970
<u>Pinfish, Lagodon rhomboides</u>	Aroclor® 1016	42 days	50% mortality	21.0	Hansen, et al. 1974a
<u>Sheepshead minnow (adult), Cyprinodon variegatus</u>	Aroclor® 1254	28 days	Lethargy, reduced feeding, fin rot, mortality	10	Hansen, et al. 1973
<u>Sheepshead minnow (juvenile), Cyprinodon variegatus</u>	Aroclor® 1254	21 days	Mortality	10	Schimmel, et al. 1974
<u>Sheepshead minnow (embryos and fry), Cyprinodon variegatus</u>	Aroclor® 1254	21 days	LC50	0.93	Schimmel, et al. 1974
<u>Sheepshead minnow, Cyprinodon variegatus</u>	Aroclor® 1254	28 days	Affected repro- duction*	0.14	Hansen, et al. 1973

\*Significantly affected hatching of embryos or the survival of fry from exposed adults.

## REFERENCES

- Addison, R.F., et al. 1978. Induction of hepatic mixed-function oxidase (MFO) enzymes in trout (Salvelinus fontinalis) by feeding Aroclor<sup>®</sup> 1254 or 3-methylcholanthrene. *Comp. Biochem. Physiol.* 61C: 323.
- Aulerich, R.J. and R.K. Ringer. 1977. Current status of PCB toxicity to mink, and effect on their reproduction. *Arch. Environ. Contam. Toxicol.* 6: 279.
- Aulerich, R.J., et al. 1973. Reproductive failure and mortality in mink fed on Great Lakes fish. *Jour. Repro. Fert. Suppl.* 19: 365.
- Bills, T.D. and L.L. Marking. 1977. Effects of residues of the polychlorinated biphenyl Aroclor<sup>®</sup> 1254 on the sensitivity of rainbow trout to selected environmental contaminants. *Prog. Fish-Cult.* 39: 150.
- Birge, W.J., et al. 1979. Toxicity of organic chemicals to embryo-larval stages of fish. *Ecol. Res. Ser. EPA 560/11-79-007.* U.S. Environ. Prot. Agency, Washington, D.C.
- Branson, D.R., et al. 1975. Bioconcentration of 2, 2', 4, 4'-tetrachlorobiphenyl in rainbow trout as measured by an accelerated test. *Trans. Am. Fish. Soc.* 104: 785.

- Bryan, A.M. and P.G. Olafsson. 1978. The effect of polychlorobiphenyls (Aroclor<sup>®</sup> 1242) on bicarbonate-14 uptake by Euglena gracilis. Bull. Environ. Contam. Toxicol. 19: 374.
- Butler, P.A. and R.L. Schultzman. 1978. Residues of pesticides and PCBs in estuarine fish, 1972-1976. Nat. Pestic. Monit. Prog. Pestic. Monit. Jour. 12: 51.
- Camp, B.J., et al. 1974. Acute effects of Aroclor<sup>®</sup> 1254 (PCB) on Ictalurus punctatus (catfish). Bull. Environ. Contam. Toxicol. 12: 204.
- Cooley, N.R., et al. 1972. Mirex and Aroclor<sup>®</sup> 1254: Effect on and accumulation by Tetrahymena pyriformis Strain W. Jour. Protozool. 19: 636.
- Cooley, N.R., et al. 1973. The polychlorinated biphenyls, Aroclor<sup>®</sup> 1248 and 1260: Effect on and accumulation by Tetrahymena pyriformis. Jour. Protozool. 20: 443.
- Couch, J.A. and D.R. Nimmo. 1974a. Ultrastructural studies by shrimp exposed to the pollutant chemical, polychlorinated biphenyl (Aroclor<sup>®</sup> 1254). Bull. Soc. Pharmacol. Environ. Pathol. 11: 17.
- Couch, J.A. and D.R. Nimmo. 1974b. Detection of Interaction Between Natural Pathogens and Pollutant Chemicals in Aquatic Animals. In: Proceedings of Gulf Coast Regional Symposium on Diseases of Aquatic Animals. LSU-SG-74-05. p. 261.

- Courtney, W.A. and W.J. Langston. 1978. Uptake of polychlorinated biphenyl (Aroclor<sup>®</sup> 1254) from sediment and from seawater in two intertidal polychaetes. Environ. Pollut. 15: 303.
- Cutkomp, L.K., et al. 1972. The sensitivity of fish ATPase to polychlorinated biphenyls. Environ. Health Perspect. April: 165.
- Davis, P.W., et al. 1972. Organochlorine insecticide, herbicide and polychlorinated biphenyl (PCB) inhibition of NaK-ATPase in rainbow trout. Bull. Environ. Contam. Toxicol. 8: 69.
- DeFoe, D.L., et al. 1978. Effects of Aroclor<sup>®</sup> 1248 and 1260 on the fathead minnow (Pimephalis promelas). Jour. Fish. Res. Board Can. 35: 997.
- Desaiah, D., et al. 1972. Inhibition of oligomycin-sensitive and insensitive magnesium adenosine triphosphatase activity in fish by polychlorinated biphenyls. Biochem. Pharmacol. 21: 857.
- Duke, T.W., et al. 1970. A polychlorinated biphenyl (Aroclor<sup>®</sup> 1254) in the water, sediment and biota of Escambia Bay, Florida. Bull. Environ. Contam. Toxicol. 5: 171.
- Ewald, W.G., et al. 1976. Toxicity of polychlorinated biphenyls (PCBs) to Euglena gracilis: Cell population, growth, carbon fixation, chlorophyll level, oxygen consumption, and protein and nucleic acid synthesis. Bull. Environ. Contam. Toxicol. 16: 71.

Fingerman, S.W. and M. Fingerman. 1977. Effects of a polychlorinated dibenzofuran on molting of the fiddler crab, Uca pugilator. Bull. Environ. Contam. Toxicol. 18: 138.

Fingerman, S.W. and M. Fingerman. 1978. Influences of the polychlorinated biphenyl preparation Aroclor<sup>®</sup> 1254 on color changes of the fiddler crab Uca pugilator. Mar. Biol. 50: 37.

Fisher, K.S., et al. 1974. Effects of PCB on interspecific competition in natural and anotobiotic phytoplankton communities in continuous and batch cultures. Microbiol. Ecology. 1: 39.

Fisher, N.S. 1975. Chlorinated hydrocarbon pollutants and photosynthesis of marine phytoplankton: A reassessment. Science. 189: 463.

Fisher, N.S. and C.F. Wurster. 1973. Individual and combined effects of temperature and polychlorinated biphenyls on the growth of three species of phytoplankton. Environ. Pollut. 5: 105.

Fowler, S.U., et al. 1978. Polychlorinated biphenyls: Accumulation from contaminated sediments and water by the polychaete Nereis diversicolor. Mar. Biol. 48: 303.

Frederick, L.L. 1975. Comparative uptake of a polychlorinated biphenyl and dieldrin by the white sucker (Catostomus commersoni). Jour. Fish. Res. Board Can. 32: 1705.

- Harding, L.W., Jr. and J.H. Phillips, Jr. 1978. Polychlorinated biphenyl (PCB) effects on marine phytoplankton photosynthesis and cell division. *Mar. Biol.* 49: 93.
- Hawes, M.L., et al. 1976a. The effects of various Aroclor<sup>®</sup> fractions on the population growth of Chlorella pyrenoidosa. *Bull. Environ. Contam. Toxicol.* 15: 14.
- Hawes, M.L., et al. 1976b. The effects of various Aroclor<sup>®</sup> fractions on the productivity of Chlorella pyrenoidosa. *Bull. Environ. Contam. Toxicol.* 15: 588.
- Hermanutz, R. and F. Puglisi. 1976. Comparative toxicity of Aroclor<sup>®</sup> 1016 and 1242 to flagfish (Jordanella floridae) and fathead minnow (Pimephales promelas). Duluth, Minnesota. (Manuscript)
- Hesse, J.L. 1973. Status report on polychlorinated biphenyls in Michigan waters. *Mich. Water Res. Comm.* June, 1973.
- Hogan, J.W. and J.E. Brauhn. 1975. Abnormal rainbow trout fry from eggs containing high residues of a PCB (Aroclor<sup>®</sup> 1242). *Prog. Fish. Cult.* 37: 229.
- Ito, Y. 1973. Studies on the influence of PCB on aquatic organisms-II. Changes in blood characteristics and plasma enzyme activities of carp administered orally with PCB. *Bull. Jap. Soc. Sci. Fish.* 39: 1135.

Ito, Y. and T. Murata. 1974. Studies on the influence of PCB on aquatic organisms-IV. Changes in serum lipid contents and formation of lipid peroxide in the tissues of carp administered with PCB orally. Bull. Jap. Soc. Sci. Fish. 40: 261.

Johansson, N., et al. 1972. Metabolic effects of PCB (polychlorinated biphenyls) on the brown trout (Salmo trutta). Comp. Gen. Pharmacol. 3: 310.

Keil, J.E., et al. 1971. Polychlorinated biphenyl (Aroclor<sup>®</sup> 1242): Effects of uptake on growth, nucleic acids, and chlorophyll of a marine diatom. Bull. Environ. Contam. Toxicol. 6: 156.

Koch, R.B., et al. 1972. Polychlorinated biphenyls: Effect of long-term exposure on ATPase activity in fish, Pimephales promelas. Bull. Environ. Contam. Toxicol. 7: 87.

Laird, E.J. 1973. Sensitivity of Dunaliella and Scenedesmus (Chlorophyceae) to chlorinated hydrocarbons. Phycologia. 12: 29.

Larsson, C.M. and J.E. Tillberg. 1975. Effects of the commercial polychlorinated biphenyl mixture Aroclor<sup>®</sup> 1242 on growth, viability, phosphate uptake, respiration and oxygen evolution, in Scenedesmus. Physiol. Plant. 33: 256.

Lidman, U., et al. 1976. Induction of the drug metabolizing system in rainbow trout (Salmo gairdneri) liver by polychlorinated biphenyls (PCBs). Acta Pharmacol. Toxicol. 39: 262.

- Lowe, J.I. Results of toxicity tests with fishes and macroinvertebrates. Data sheets available from Environ. Res. Lab. U.S. Environ. Prot. Agency, Gulf Breeze, Florida.
- Lowe, J.I., et al. 1972. Effects of the polychlorinated biphenyl Aroclor<sup>®</sup> 1254 on the American oyster, Crassostrea virginica. Mar. Biol. 17: 209.
- Maki, A.W. and H.E. Johnson. 1975. Effects of PCB (Aroclor<sup>®</sup> 1254) and p, p'-DDT on production and survival of Daphnia magna Strauss. Bull. Environ. Contam. Toxicol. 13: 412.
- Martell, J.M., et al. 1975. PCBs in suburban watershed, Reston, Va. Environ. Sci. Technol. 9: 872.
- Mauck, W.L., et al. 1978. Effects of the polychlorinated biphenyl Aroclor<sup>®</sup> 1254 on growth, survival, and bone development in brook trout (Salvelinus fontinalis). Jour. Fish. Res. Board Can. 35: 1084.
- Mayer, F.L., et al. 1977. Residues dynamics and biological effects of polychlorinated biphenyls in aquatic organisms. Arch. Environ. Contam. Toxicol. 5: 501.
- Moore, S.A. and R.C. Harriss. 1972. Effects of polychlorinated biphenyl on marine phytoplankton communities. Nature. 240: 356.

Morgan, J.R. 1972. Effects of Aroclor<sup>®</sup> 1242 (a polychlorinated biphenyl) and DDT on cultures of an alga, protozoan, daphnid, ostracod, and guppy. Bull. Environ. Contam. Toxicol. 8: 129.

Mosser, J.L., et al. 1972a. Polychlorinated biphenyls: Toxicity to certain phytoplankters. Science. 174: 191.

Mosser, J.L., et al. 1972b. Polychlorinated biphenyls and DDT alter species composition in mixed cultures of algae. Science. 175: 533.

Nadeau, R.J. and R.A. Davis. 1976. Polychlorinated biphenyls in the Hudson River (Hudson Falls - Fort Howard, New York State). Bull. Environ. Contam. Toxicol. 16: 436.

Nebeker, A.V. and F.A. Puglisi. 1974. Effect of polychlorinated biphenyls (PCBs) on survival and reproduction of Daphnia, Gammarus, and Tanytarsus. Trans. Am. Fish. Soc. 103: 722.

Nebeker, A.V., et al. 1974. Effect of polychlorinated biphenyl compounds on survival and reproduction of the fathead minnow and flagfish. Trans. Am. Fish. Soc. 103: 562.

Neff, J.M. and C.S. Giam. 1977. Effects of Aroclor<sup>®</sup> 1016 and Halowax<sup>®</sup> 1099 on Juvenile Horseshoe Crabs Limulus polyphemus. In: Physiological Responses of Marine Biota to Pollutants. Academic Press, New York. p. 21.

- Nestel, H. and J. Budd. 1974. Chronic oral exposure of rainbow trout (Salmo gairdneri) to a polychlorinated biphenyl (Aroclor<sup>®</sup> 1254): Pathological effects. *Can. Jour. Comp. Med.* 39: 208.
- Nimmo, D.R. and L.H. Bahner. 1974. Some Physiological Consequences of Polychlorinated Biphenyl- and Salinity-Stress in Penaeid Shrimp. In: *Pollution and Physiology at Marine Animals*. Academic Press, Inc., New York. p. 427.
- Nimmo, D.R. and L.H. Bahner. 1976. Metals, Pesticides and PCB: Toxicities to Shrimp Singly and in Combination. In: *Estuarine Processes. Vol. I. Uses, Stresses, and Adaptation to the Estuary*. Academic Press, Inc., New York.
- Nimmo, D.R., et al. 1971. Toxicity and distribution of Aroclor<sup>®</sup> 1254 in the pink shrimp, Penaeus duorarum. *Mar. Biol.* 11: 191.
- Nimmo, D.R., et al. 1974. Accumulation of Aroclor<sup>®</sup> 1254 in grass shrimp (Palaemonetes pugio) in the laboratory and field exposures. *Bull. Environ. Contam. Toxicol.* 11: 303.
- Nimmo, D.R., et al. 1975. Toxicity of Aroclor<sup>®</sup> 1254 and its physiological activity in several estuarine organisms. *Arch. Environ. Contam. Toxicol.* 3: 22.

Norstrom, R.J., et al. 1976. A bioenergetics-based model for pollutant accumulation by fish. Simulation of PCB and methyl-mercury residue levels in Ottawa river yellow perch (Perca flavescens). Jour. Fish. Res. Board Can. 33: 248.

O'Connors, H.B., Jr., et al. 1978. Polychlorinated biphenyls may alter marine trophic pathways by reducing phytoplankton size and production. Science. 201: 737.

Parejko, R., et al. 1975. Chlorhydrocarbons in Lake Superior lake trout (Salvelinus namaycush). Bull. Environ. Contam. Toxicol. 14: 480.

Parrish, P.R., et al. 1974. Effects of polychlorinated biphenyl, Aroclor<sup>®</sup> 1016, on estuarine animals. Assoc. S.E. Biol. Bull. 21: 74.

Platonow, N.S. and L.H. Karstad. 1973. Dietary effects of polychlorinated biphenyls on mink. Can. Jour. Comp. Med. 37: 391.

Ringer, R.K., et al. 1972. Effect of dietary polychlorinated biphenyls on growth and reproduction of mink. Am. Chem. Soc. Natl. Meet. Preprint Pap. 12: 149.

Roesijadi, G., et al. 1976a. Osmoregulation of grass shrimp Palaemonetes pugio exposed to polychlorinated biphenyls (PCBs). I. Effect on chloride and osmotic concentrations and chloride and water-exchange kinetics. Mar. Biol. 38: 343.

- Roesijadi, G., et al. 1976b. Osmoregulation of the grass shrimp Palaemonetes pugio exposed to polychlorinated biphenyls (PCBs). II. Effect on free amino acids of muscle tissue. Mar. Biol. 38: 357.
- Sanborn, J.R. 1974. The fate of select pesticides in the aquatic environment. Ecol. Res. Ser. EPA 660/3-74-025. U.S. Environ. Prot. Agency, Corvallis, Oregon.
- Sanders, H.O. and J.H. Chandler. 1972. Biological magnification of a polychlorinated biphenyl (Aroclor<sup>®</sup>) from water by aquatic invertebrates. Bull. Environ. Contam. Toxicol. 7: 257.
- Schimmel, S.C., et al. 1974. Effects of Aroclor<sup>®</sup> 1254 on laboratory-reared embryos and fry of sheepshead minnows (Cyprinodon variegatus). Trans. Am. Fish. Soc. 103: 582.
- Schoor, W.P. 1975. Problems associated with low-solubility compounds in aquatic toxicity tests: Theoretical model and solubility characteristics of Aroclor<sup>®</sup> 1254 in water. Water Res. 9: 937.
- Scura, E.O. and J.H. Theilacker. 1977. Transfer of the chlorinated hydrocarbon PCB in a laboratory marine food chain. Mar. Biol. 40: 317.
- Snarski, V.M. and F.A. Puglisi. 1976. Effects of Aroclor<sup>®</sup> 1254 on brook trout, Salvelinus fontinalis. Ecol. Res. Ser. EPA 600/3-76-112. U.S. Environ. Prot. Agency, Duluth, Minnesota.

- Stalling, D.L. 1971. PCB residues in bluegills and channel catfish exposed to Aroclor<sup>®</sup> 1248 and Aroclor<sup>®</sup> 1254 for 11 weeks. PCB Newsletter. July 28. Duluth, Minnesota.
- Tejedor, M.C. et al. 1979. Oxidative metabolism in Saccharomyces cerevisiae as affected by polychlorinated biphenyls. Bull. Environ. Contam. Toxicol. 22: 439.
- Veith, G. 1975. Baseline concentrations of polychlorinated biphenyls and DDT in Lake Michigan fish, 1971. Pestic. Monitor. Jour. 9: 21.
- Veith, G. 1976. Comparative toxicity of A-1016 and A-1242 to the fathead minnow. Environ. Res. Lab. U.S. Environ. Prot. Agency, Duluth, Minnesota. (Manuscript)
- Veith, G.D., et al. 1977. Residues of PCBs and DDT in the western Lake Superior ecosystem. Arch. Environ. Contam. Toxicol. 5: 487.
- Weininger, D. 1978. Accumulation of PCBs by lake trout in Lake Michigan. Ph.D. Thesis, Univ. of Wisconsin.
- Wiese, C.S. and D.A. Griffin. 1978. The solubility of Aroclor<sup>®</sup> 1254 in seawater. Bull. Environ. Contam. Toxicol. 20: 403.
- Wildish, D.J. 1970. The toxicity of polychlorinated biphenyls (PCB) in seawater to Gammarus oceanicus. Bull. Environ. Contam. Toxicol. 5: 202.

Yap, H.H., et al., 1971. Sensitivity of fish ATPases to polychlorinated biphenyls. *Nature*. 233: 61.

Zitko, V. 1970. Polychlorinated biphenyls (PCB) solubilized in water by nonionic surfactants for studies of toxicity to aquatic animals. *Bull. Environ. Contam. Toxicol.* 5: 279.

Zitko, V. and W.G. Carson. 1977. A comparison of the uptake of PCB and Isopropyl-PCBs (chloralkylene 12) by fish. *Chemosphere*. No. 2/3. Pergamon Press.

Zullei, H. and G. Benecke. 1978. Application of a new bioassay to screen the toxicity of polychlorinated biphenyls in blue-green algae. *Bull. Environ. Contam. Toxicol.* 20: 786.

## Mammalian Toxicology and Human Health Effects

### SUMMARY

Polychlorinated biphenyls (PCBs) have been used commercially since 1929 as dielectric and heat exchange fluids and in a variety of other applications. They have become widely disseminated in the environment worldwide. Like many organochlorine pesticides, they are highly persistent and accumulate in food webs. Human exposure to PCBs has resulted largely from the consumption of contaminated food but also from inhalation and skin absorption in work environments. PCBs accumulate in the fatty tissues and skin of man and other mammals. Metabolism occurs by hydroxylation and dihydrodiol formation with arene oxides as probable intermediates. The rate of metabolism and excretion slows dramatically as the chlorination of the biphenyl nucleus increases. Arrangement of chlorines which eliminate adjacent unsubstituted carbons greatly increase resistance to metabolism. PCBs have caused profound toxic effects in man and animals, particularly if repeated exposures occur. The skin and liver are major sites of pathology, with the gastrointestinal tract and nervous systems also being targets. Polychlorodibenzofurans which contaminate commercial PCB mixtures may contribute significantly to their toxicity. Several studies in rodents suggest strongly that some PCBs are carcinogenic and that they can enhance the carcinogenicity of other chemicals. A linear model for risk assessment has been used to estimate maximum safe levels in water and fish which will establish a level of risk for the human population from cancer. A maximum level of PCBs in water projected

to result in no more than one cancer in  $10^5$  individuals with lifetime exposure of 0.79 ng/l is suggested by the analysis.

#### EXPOSURE

The magnitude of the dispersal of these chemicals is revealed by their detection in the tissues of plants and animals in all parts of the world. PCB residues have been observed in wildlife in Sweden, North America, Great Britain, the Netherlands, and even the Arctic (Risebrough and deLappe, 1972). Because PCBs are not naturally occurring substances, their dissemination is entirely the result of human activity. Their entry into the environment has occurred by vaporization into the atmosphere, and by spilling or dumping into water or onto land. It has been estimated that of the 1970 sales of PCBs in North America, only 20 percent represented a net increase in the total amount in service. Estimated sources of loss for that year were  $1 - 2 \times 10^3$  tons for evaporation;  $4 - 5 \times 10^3$  tons for leaks and disposal of fluids; and  $22 \times 10^3$  tons for disposal by incineration and burial (Nisbet and Sarofim, 1972). The cumulative input to the environment between 1930 and 1970 was estimated to be  $3 \times 10^4$  tons to air,  $6 \times 10^4$  tons to fresh and coastal waters, and  $3 \times 10^5$  tons to dumps and landfills. In that time, up to one-third of the PCBs released to air and one-half of that released to water were probably degraded. Degradation in landfills is more difficult to estimate (Nisbet and Sarofim, 1972). PCBs have been found repeatedly to be widespread in analyses of human tissues. For example, detectable levels of PCBs have been reported in adipose tissue samples of up to 91 percent of individuals sampled in a survey of the United States population (Kutz and

Strassman, 1976; see Table 13). This finding suggests that environmental contamination may be a significant source of human exposure. Likely routes of exposure for the general population are water and particularly food, while inhalation and dermal contact are likely to be more significant routes in occupational exposure.

#### Ingestion from Water

The solubility of PCBs in water is very low, decreasing as the percent chlorination is raised. Solubilities of Aroclors in water at 20°C vary from 200 µg/l for 1242 to about 25 µg/l for 1260 (Nisbet and Sarofim, 1972). The major factors in the dynamics of PCB distribution in water are its low solubility, high specific gravity, and its high affinity for solids. Most PCBs discharged into water are found in bottom sediments near the site of discharge (Nisbet and Sarofim, 1972). Evaluation of PCB levels in surface waters and bottom sediments of the major drainage basins of the United States was conducted between 1971 and 1974 (Dennis, 1976). The data were derived from the U.S. Geological Survey (USGS) study of 1971-72 (Crump-Weisner, et al. 1974) and from additional data collected by the USGS between 1972 and 1975 (PCB data base 1972-75). It is summarized in detail in the Criteria Document for PCBs (U.S. EPA, 1976a). The highest concentrations in both water and sediment were found in the basins east of the Mississippi River. The highest levels were found in 1971 in the lower Mississippi basin, with a median concentration for the region of 3.0 µg/l and positive detections at 100 percent of stations tested. Over the time period of the study the concentrations and incidences of PCBs detected in all basins have decreased substantially. By 1974

the median level in the lower Mississippi basin had dropped to 0.1  $\mu\text{g}/\text{l}$  and the incidence of detection to 2.6 percent of stations tested. The levels in sediments, however, have persisted at much higher levels over this period of time. In 1971 median sediment levels for the Mississippi basin were 30  $\mu\text{g}/\text{kg}$  and the incidence of detection 100 percent. By 1974 the incidence had dropped to 9.9 percent, and the median level was 10.5  $\mu\text{g}/\text{kg}$ .

Although PCBs are widespread in aquatic environments (Peakall, 1975), their low solubility generally prevents them from reaching high concentrations in drinking water supplies. The persistence of PCBs and their accumulation in sediments, however, increase the significance of water as a source of human exposure by providing a reservoir of material which can continue to contaminate water long after the addition of PCBs has ceased. In combination with these factors, the lipophilicity of PCBs results in their continued introduction to, and accumulation in, the food chain. As a consequence, fish and other foods obtained from aquatic environments may become important sources of exposure even if PCB levels in the water are low.

The ability of PCBs discharged from a manufacturing facility to contaminate a drinking water system has recently been highlighted. Billings, et al. (1978) determined the levels of PCBs in the Easley-Central Water District, Pickens County, South Carolina. They observed that PCBs discharged by a capacitor manufacturing facility 12 km upstream from the water district's treatment plant were entering the water system. Finished potable water supplies were contaminated to levels as high as 818 ng/l.

## Ingestion from Food

Contamination of food with PCBs occurs primarily by three mechanisms. The first is contamination of human food as a consequence of accumulation in the food chain. The contamination of freshwater fish as a consequence of the contamination of the aquatic environment is a particularly significant route of PCB entry into the human diet which will be discussed in more detail below. The second mechanism occurs by the direct contamination of feeds or foodstuffs with PCBs. This may occur as a result of accidental spills or equipment malfunctions as was the case in the episode of rice oil contamination in Japan which led to the outbreak of Yusho or rice oil disease in 1968 (Kuratsune, et al. 1976). In this instance leaks in a heat exchanger used to process rice bran oil resulted in the contamination of the oil by the exchanger fluid (Kanechlor 400). Discovery of the contamination was made only after numerous cases of chlorinated hydrocarbon intoxication in Fukuoka prefecture, Japan. The oil was found to contain 2,000 to 3,000 ppm Kanechlor 400 which was contaminated with polychlorodibenzofurans (1.6 to 5 ppm). Average consumption of PCBs among affected individuals was estimated to be 2 g (Kuratsune, et al. 1972). By 1975 the total number of known individuals affected was 1,291. Elevated PCB levels in fat were still observed four years after the exposure, and dermatological symptoms were found in up to 89 percent of a group of 72 patients examined in 1973 or 1974. An example of accidental PCBs contamination in animal feed occurred as a result of the use of PCBs in silo coatings (Willett and Hess, 1975). The third significant source of PCBs in foodstuffs was food

packaging made from recycled paper containing PCBs (Jelinek and Corneliussen, 1976).

A special case of human exposure via food which must be considered is human breast milk. Adverse effects have been observed in breast fed infants of women with Yusho (Kuratsune, et al. 1976) and in infant Rhesus monkeys ingesting breast milk containing 7 to 16 ppm PCBs (fat basis) (Allen, 1975; Allen and Barsotti, 1976). Preliminary survey data indicate average PCB levels in human breast milk of 1.8 ppm (fat basis) (42 FR 17487), and a study of PCB exposed nursing mothers in Germany indicated average PCB levels of 3.5 ppm (Tombergs, 1972). The proximity of these values to the toxic levels in infant monkeys (7 to 16 ppm) suggests that human breast milk must be considered a significant source of PCB exposure.

The extent of contamination of the U.S. food supply has been the subject of Food and Drug Administration (FDA) and Department of Agriculture (USDA) monitoring programs since 1969. Results of these studies have been summarized by Jelinek and Corneliussen (1976). The initial analysis of 15,000 food samples between 1969 and 1971 is summarized in Table 1. The results of monitoring programs in fiscal years 1973, 1974, and 1975 are summarized in Table 2. Over the monitored period the incidence and levels of PCBs have dropped in all food classes. By 1975 the only significant food sources were fish, meat, and dairy products. Fish were by far the most significant source. The findings for the 1969-71 period led to the establishment of regulations for PCB levels in food (38 FR 18096). The temporary tolerances established at that

TABLE 1  
 Summary of PCBs in Food\*  
 Nov., 1969 - June, 1971<sup>a</sup>

Food Commodity	Positive Findings <sup>c</sup>	Avg. of Positives (ppm)	Max. Level (ppm)
Finfish	317	2.1	35.3
Oysters	12	Trace	Trace
Fish by-products	6	1.8	5.0
Cheese	44	0.3 <sup>b</sup>	1.0 <sup>b</sup>
Milk	60	2.5 <sup>b</sup>	22.8 <sup>b</sup>
Eggs	17	Trace	0.5
Potato by-products	12	1.1	4.2
Miscellaneous	11	1.9	6.5

<sup>a</sup> Approximately 15,000 samples examined

<sup>b</sup> Fat basis

<sup>c</sup> Detection limits: fish 0.5 ppm, other foods 0.05 ppm  
 (P.E. Corneliusen, personal communication)

\*Source: Jelinek and Corneliusen, 1976

TABLE 2

Summary of PCBs in Foods\*  
Fiscal Years 1973, 1974, and 1975

Food Commodity	FY 1973			FY 1974			FY 1975		
	Percent Positive <sup>b</sup>	Max. <sup>a</sup> (ppm)	Percent Positive	Max. <sup>a</sup> (ppm)	Percent Positive	Max. <sup>a</sup> (ppm)	Percent Positive	Max. <sup>a</sup> (ppm)	
Fish	60.4	123.0	44.0	16.8	17.8	9.0			
Milk	2.2	1.6	2.6	2.3	0.7	1.9			
Eggs	1.1	Trace	4.2	11.0	0.0	N.D.			
Cheese	0.9	0.5	2.6	2.8	0.0	N.D.			
Feed components	12.7	9.0	0.0	N.D.	0.3	0.9			
Animal feeds	7.2	199.5	0.0	N.D.	0.0	N.D.			
Processed fruits	4.5	19.2	0.0	N.D.	0.0	N.D.			
Infant and junior foods	1.1	Trace	0.0	N.D.	0.0	N.D.			
Meats and poultry (USDA)	1.9	0.19	1.2	0.07	0.3	0.06			
<sup>a</sup> Milk, cheese, meats and poultry reported as ppm, fat basis <sup>b</sup> Detection limits: fish 0.5 ppm, other foods 0.05 ppm (P.E. Corneliussen, personal communication)									

\*Source: Jelinek and Corneliussen, 1976

time and new tolerances recommended in 1977 (42 FR 17487) are given in Table 3. The enforcement of those tolerances and restriction of PCB use in open systems after 1970 probably account for the general decline of PCB levels in foodstuffs.

Comprehensive fish surveys conducted by the FDA in fiscal years 1973 and 1974 indicated a drop in the incidence of PCB detection in fish from less than 30 percent in 1973 to less than 20 percent in 1974. In 1973, 3 percent contained over 1 ppm and 0.5 percent contained over 5 ppm PCBs. The data from all FDA studies in the fiscal years 1973, 1974, and 1975 are summarized in Figure 1. While the incidence of PCBs in fish dropped over the period, the fraction of positive fish containing over 5 ppm PCBs increased from less than 5 percent to over 10 percent. The samples containing more than 5 ppm were from the Great Lakes. Because the study involved different sources and objectives from year to year, no conclusion as to whether a significant trend existed was drawn. It should be noted that these surveys were conducted with fish in commerce and provide no information about sport fish per se. The studies indicated that significant levels of PCBs generally do not occur in saltwater fish.

The impact of sport fish consumption was examined in a study of a group of sports fishermen who consumed an average of 24 to 25 pounds of fish annually (highest individual exposure 180 lbs/year over a two-year period). PCB residues in cooked fish ranged from 0.35 - 5.38 ppm. Plasma PCB levels ranged from a high of 0.366 ppm in the exposed group to control levels of 0.007 ppm (less than six lbs consumed per year) (42 FR 17487).

TABLE 3  
 FDA Regulations for PCBs\*

<u>I. Temporary tolerances</u>		
Commodity	PCB conc. (ppm)	Proposed Guidelines 1977
Milk (fat basis)	2.5	1.5
Dairy products (fat basis)	2.5	1.5
Poultry (fat basis)	5.0	3.0
Eggs	0.5	0.3
Finished animal feed	0.2	0.2
Animal feed components	2.0	2.0
Fish (edible portion)	5.0	2.0
Infant and junior foods	0.2	pending
Paper food-packaging material without PCB-impermeable barrier	10.0 <sup>a</sup>	

<sup>a</sup>Administrative guideline, pending hearing

\*Source: Jelinek and Corneliussen, 1976  
 42 FR 17487

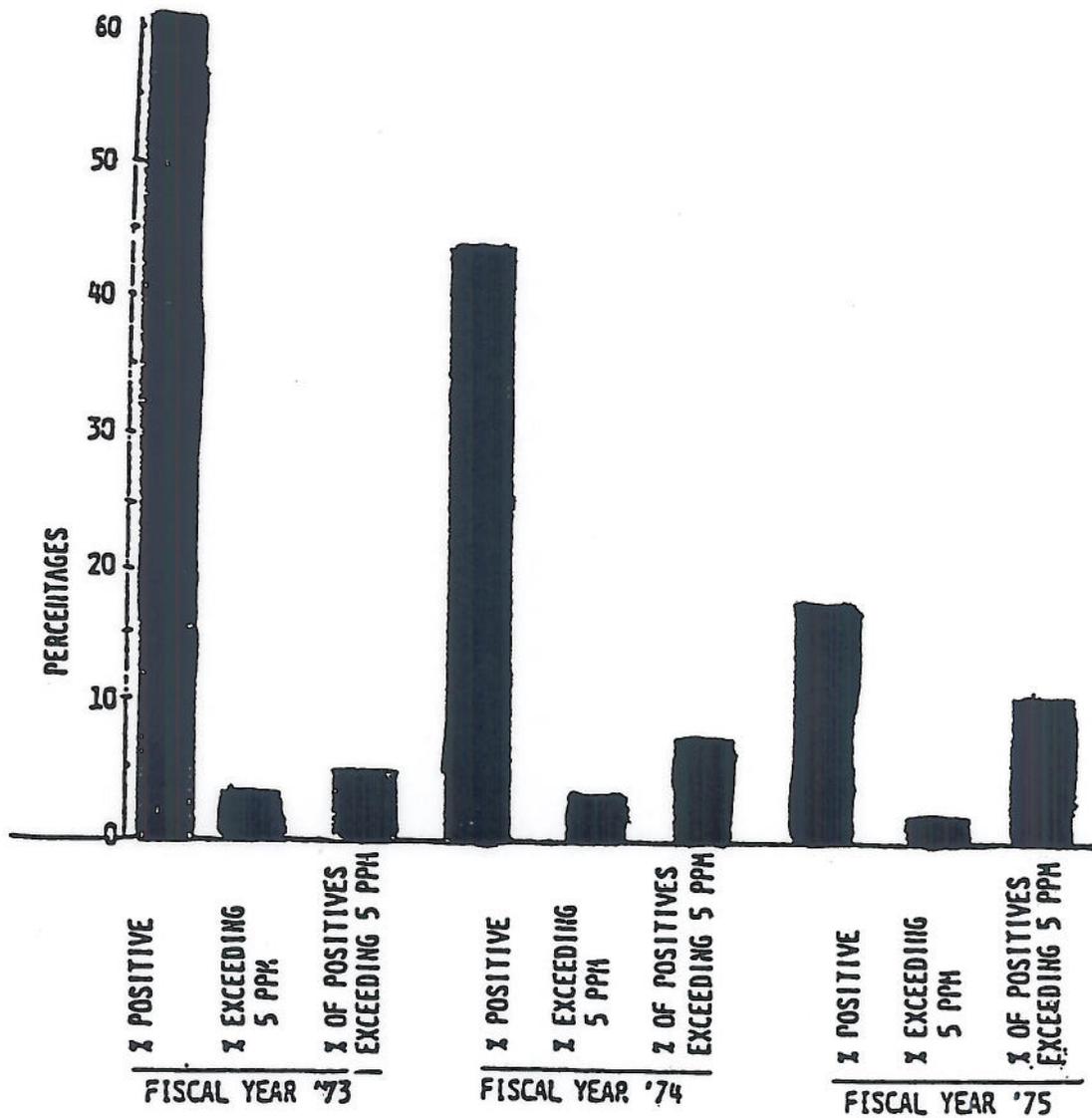


FIGURE 1

PCBs in Fish FY 73, 74, 75 (Level of detection: 0.5 ppm)

Source: Jelinek and Corneliusen, 1976

A bioconcentration factor (BCF) relates the concentration of a chemical in aquatic animals to the concentration in the water in which they live. The steady-state BCFs for a lipid-soluble compound in the tissues of various aquatic animals seem to be proportional to the percent lipid in the tissue. Thus, the per capita ingestion of a lipid-soluble chemical can be estimated from the per capita consumption of fish and shellfish, the weighted average percent lipids of consumed fish and shellfish, and a steady-state BCF for the chemical.

Data from a recent survey on fish and shellfish consumption in the United States were analyzed by SRI International (U.S. EPA, 1980). These data were used to estimate that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day (Stephan, 1980). In addition, these data were used with data on the fat content of the edible portion of the same species to estimate that the weighted average percent lipids for consumed freshwater and estuarine fish and shellfish is 3.0 percent.

Several laboratory studies, in which percent lipids and a steady-state BCF were measured, have been conducted on polychlorinated biphenyls. The mean of the BCF values, after normalization to one percent lipids, is 10,385 (see Table 5 in Aquatic Life Toxicology section). An adjustment factor of 3 can be used to adjust the mean normalized BCF to the 3.0 percent lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for polychlorinated biphenyls and the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans is calculated to be 31,200.

Higher BCF values apparently can be achieved in field exposures (Haile, et al. 1975; Norstrom, et al. 1976; Duke, et al. 1970; Nimmo, et al. 1975; Veith, 1975; Veith, et al. 1977), but those values cannot be considered quantitative because the exposure of the organism cannot be adequately documented and integrated over a long enough period of time.

In order to estimate human dietary PCB intake, the FDA conducts a continuing survey of the total diet. Composites of 12 different food categories are analyzed for PCB content. Table 4 summarizes the results of the survey from 1971 through the first half of 1975. While contamination was observed in most categories in 1972, the number of positive categories had dropped by 1973. In 1974 and 1975 only meat, fish, and poultry were observed to contain PCBs; fish was almost always the contributor of positive results in that category (Jelinek and Corneliussen, 1976). Most of the contamination noted in the other categories in earlier years was thought to result from exposure during processing or packaging because the raw foods were rarely found to contain PCBs. Total daily intake, calculated from the composite figures for a young adult male over the period 1971-75, is summarized in Table 5. Total daily intake dropped by almost 50 percent over the period, but intake in the meat-fish-poultry category changed very little. By 1974, almost all of the dietary intake resulted from the ingestion of PCB-contaminated fish. The measures taken in the early 1970's to limit the release of PCBs into the environment and to remove them from food processing environments effectively reduced direct contamination of foodstuffs to a minimum level. The persis-

TABLE 4  
 Percent of Composites Containing PCBs,  
 from the FDA Total Diet Studies\*

Fiscal Year	Dairy Products	Meat, Fish & Poultry	Grain and Cereal Prods.	Potatoes	Legume Vegetables	Root Vegetables	Garden Fruits	Oils, fats & Shortening	Sugars and Adjuncts
1971		47	13						
1972	6	46	6		6	3	3	17	6
1973	10	33	17	3				3	
1974		43							3
1975 (1st half)		40							

\*Source: Jelinek and Corneliussen, 1976

TABLE 5

Estimates of Daily PCB Intakes\*  
(Total Diet Study - Teenage Male)

Fiscal Year	Average Daily Intake of PCBs <sup>a</sup>	
	Total Diet ( $\mu\text{g}/\text{day}$ )	Meat-fish-poultry Food Class ( $\mu\text{g}/\text{day}$ )
1971	15.0	9.5
1972	12.6	9.1
1973	13.1	8.7
1974	8.8	8.8
1975 (1st half)	8.7	8.7

<sup>a</sup>Lower limit of quantitative reporting = 0.05 ppm with analytical method employed

\*Source: Jelinek and Corneliussen, 1976

tence of PCBs in aquatic environments and in fish has maintained a residual dietary exposure level. Further reduction of PCB levels in the diet will require that entry of PCBs into waterways be more tightly controlled and that monitoring of fish and other foods for PCB contamination be continued (Jelinek and Corneliussen, 1976). The recently recommended reduction of allowable PCB levels in fish to 2.0 ppm may further reduce dietary intake (42 FR 17487).

Two special situations which must be avoided to prevent unnecessary PCB ingestion should be mentioned. First, accidental contamination of foodstuffs or feeds with PCBs must be avoided. Although PCB manufacture is now stopping and distribution will cease in the near future, many PCB-containing products remain in service. Failure to exercise care in the maintenance and disposal of these units could result in the contamination of food or water. The tragic results of the episode of rice oil contamination in Japan (Kuratsune, 1972) provides ample evidence of the need for care and continued surveillance of foods. Second, although occupational exposure to PCBs will decline over the next several years, the possibility of food contamination as a consequence of transfer from workers' tools or clothing must be considered as a possible route of dietary exposure.

#### Inhalation

PCBs can enter the atmosphere by vaporization and may be found in either gaseous form or adsorbed to airborne particulates. Prior to the restriction of PCB use, substantial losses to the atmosphere resulted from evaporation of plasticizers and from improper incineration. In 1972, terrestrial input from fallout was estimated to

be 1,000 to 2,000 tons/year. Annual emission rates were estimated at 1,500 to 2,500 tons (Nisbet and Sarofim, 1972). In 1975, a study of PCB content in air in suburban areas in Florida and Colorado indicated that average atmospheric levels were approximately 100 ng/m<sup>3</sup> (Kutz and Yang, 1976). Rates of fallout along the southern California coast were estimated to average 1,800 kg/year over a 50,000 km<sup>2</sup> area (Young, et al. 1976). The distribution of PCBs in air is nonuniform, being more highly concentrated in urban areas. The aerial fallout survey in southern California indicated that sectors in the urban areas around Los Angeles had fallout rates of up to 180 kg/yr, while less industrialized sectors had rates as low as 30 kg/yr. A study of PCB levels in soil samples showed that they were rarely detectable in agricultural soils but were found in 63 percent of urban samples from 19 cities (Carey and Gowan, 1976). General human exposure to inhaled PCBs probably varies with the local conditions. In relation to the 9 µg/day intake estimated from the diet (Jelinek and Corneliussen, 1976), nonoccupational exposures by inhalation are probably small.

While inhalation of PCBs is not and most likely will not be a major route of general human exposure, it is a highly significant route of occupational exposure. Early in its commercial use an association was observed between occupational exposure to PCB vapors and chloracne (Jones and Alden, 1936; Schwartz, 1936). The benefits of controlling leaks from closed systems into work environments were noted by Meigs, et al. (1954).

A study of occupational exposure in Japan found PCB vapors at levels between 13 and 540 µg/m<sup>3</sup> and airborne particulates between 4

and  $650 \mu\text{g}/\text{m}^3$  in a survey of six industrial plants. An additional finding of  $6,270 \mu\text{g}/\text{m}^3$  PCB particulates was associated with a spill. Blood PCB levels of 99 exposed workers averaged 370 ppb as compared to levels in 32 controls of 20 ppb (Hasegawa, et al. 1972). Ouw, et al. (1976) observed Aroclor 1242 levels between 2.22 and  $0.32 \text{ mg}/\text{m}^3$  in different areas of an electrical equipment manufacturing facility in Australia. Blood Aroclor levels were analyzed by gas chromatography, and fractions with several retention times standardized against Aroclor 1242 were detected in exposed workers. Workers in an impregnation room where inhalation was a major mode of exposure had higher levels of PCBs than did workers in another area where exposure was primarily dermal. A series of 30 control individuals was not found to have detectable PCB levels. The limit of detection in this study was not reported; however, Finklea, et al. (1972) reports American control population blood levels of 0.3 to 3 ppb.

It is difficult to differentiate between industrial exposure by inhalation and dermal absorption (see Dermal section). Animal studies do indicate that animals exposed to PCB aerosols show rapid increases in liver PCB levels. Exposure to Pydranl A 200 for 15 minutes resulted in the accumulation in the liver of 50 percent of the PCBs accumulated after two hours (Benthe, et al. 1972). The lung appears to be a good site of absorption, and certain occupational environments contain significant levels of airborne PCBs. The National Institute for Occupational Safety and Health has recently proposed an occupational exposure limit of  $1.0 \mu\text{g}/\text{m}^3$  on a time weighted average 10-hour day, 40-hour week basis (NIOSH,

1977). Assuming a tidal air volume of  $10 \text{ m}^3$  in an eight-hour day and 100 percent absorption, the resulting dose at this exposure level would be  $10 \text{ ug/day}$ .

### Dermal

Dermal exposure, like inhalation exposure, is a particularly significant route in the occupational setting. With the restriction of PCB uses to sealed systems, the use of PCBs in products to which the public might be exposed has declined markedly, reducing opportunities for general exposure. Past uses of PCBs in carbonless copy paper, printer's inks, and other products probably contributed to general PCB exposures. Documented exposures are largely occupational as exemplified by the results of Ouw, et al. (1976). The authors noted that one group of employees was largely exposed through skin contact and had significantly elevated blood PCB levels.

In a variety of animal studies dermal application of several PCB-containing materials has produced both local and systemic effects including liver degeneration and death (Miller, 1944; Paribok, 1954; Vos and Beems, 1971). In neonatal rats treated by skin application with PCBs, a 5- to 10-fold increase in aryl hydrocarbonhydroxylase activity occurred in liver, skin, lung, and kidney, indicating significant distribution to these tissues after exposure by this route (Bickers, 1976; Bickers, et al. 1975).

The relative contributions of various routes of exposure can be expected to vary widely. Occupational exposures are by far the most severe with inhalation and skin contact being the major routes of absorption. A noteworthy by-product of occupational PCB expo-

sure is the elevated risk of exposure among other members of workers' families. An epidemiological study in Bloomington, Indiana revealed significantly elevated serum PCB levels among a group of 18 occupationally exposed workers (mean 71.7 ppb) and a slight elevation among 19 members of their families (near 33.6 ppb) as compared to background levels (5 to 20 ppb) (McCloskey, et al. 1978). The general public is widely exposed to PCBs but at much lower levels and primarily through the diet. Fish living in contaminated waters are by far the largest contributors to dietary PCBs (Jelinek and Corneliussen, 1976).

### PHARMACOKINETICS

#### Absorption

The efficiency of PCB absorption in the gut of rats was shown to be between 92 to 98.9 percent (Albro and Fishbein, 1972). Neither the degree of chlorination (mono-hexachlorobiphenyl) nor the dose ingested (5 to 100 mg/kg) markedly affected the efficiency of the uptake. Matthews and Anderson (1975b) observed a reduced accumulation of PCBs in adipose tissues of rats exposed orally as compared to intravenous (i.v.) injection. The differences were more pronounced with biphenyls of low chlorine content and were thought to be related to route of absorption and metabolic rates, rather than to the overall efficiency of transport across the gut. Absorption via the gut was also very efficient in adult Rhesus monkeys, 90 percent of a single dose of 1.5 or 3.0 g/kg Aroclor 1248 being absorbed from the gastrointestinal tract (Allen, et al. 1974a).

Efficient absorption via inhalation has been demonstrated in rats by Bente, et al. (1972).

In humans, absorption via the intestine has been best illustrated by the Yusho Japan incident in 1968. Among individuals ingesting less than 720 ml of contaminated rice bran oil (equivalent to 1.5 to 2.2 g Kanechlor 400), 39 percent developed severe symptoms and an additional 49 percent developed moderate symptoms of PCB intoxication. The lowest level of PCB ingestion in an affected individual was estimated to be 0.5 g (Kuratsune, et al. 1972). Absorption via the respiratory tract and skin is also efficient as indicated by occupational exposures where effects of PCB exposure can be detected even at doses too low to produce pathology (Alvares, et al. 1977).

#### Distribution

PCBs given to rats by i.v. injection are removed from the blood rapidly and stored initially in the liver and muscle. With time they are redistributed primarily to skin and adipose tissue (Matthews and Anderson, 1975b). The degree to which PCBs are stored or excreted depends on their susceptibility to metabolism and, therefore, on the degree of chlorination and availability of adjacent unsubstituted carbons. Tissue levels of mono-, di-, penta- and hexachlorobiphenyls in rats given a single injected dose at 0.6 mg/kg were determined by Matthews and Anderson (1975b). The maximum doses accumulated in each tissue increased with degree of chlorination as did the half-life in each tissue. The proportion of total PCBs present in tissues as metabolites was greatest for the mono- and dichlorobiphenyls. Hexachlorobiphenyls in tissues

were largely unmetabolized. The distribution of PCBs in adipose tissue provides a useful example of the relative accumulation of different isomers. Tissues were examined for up to 42 days; a summary of the results is presented in Table 6.

A similar pattern was observed in skin, with up to 22 percent of the hexachlorobiphenyl dose being accumulated there at 1 day and residual levels around 15 percent remaining at 42 days.

Single intravenous doses of 0.6 or 6.0 mg/kg of 2,4,5,2',5'-pentachlorobiphenyl were cleared from the blood in ten minutes and initially deposited in liver and muscle. They were subsequently translocated to adipose tissue and skin as depositories (Matthews and Anderson, 1975a).

A single administration of approximately 500 mg/kg of 2,5,2',5'-tetrachlorobiphenyl to rats resulted in a similar distribution with adipose, skin, and blood being the significant storage depots after 24 hours (Van Miller, et al. 1975).

The significance of chlorine position as well as number was addressed in a study of the pharmacokinetics of 3,5,3',5'-tetrachlorobiphenyl (TCB) by Tuey and Matthews (1977). The arrangement of chlorines on this molecule results in the absence of adjacent unsubstituted sites. The pattern of distribution of the compound following a single i.v. injection of 0.6 mg/kg was similar to that observed in earlier studies (Matthews and Anderson, 1975a,b) with adipose tissue and skin becoming the major long term storage sites. However, loss of 3,5,3',5'-TCB was slower than earlier observed for 2,4,5,2',5'-pentachlorobiphenyl (see Table 6) with the maximum adipose tissue load reaching 52.9 percent of total dose on

TABLE 6

Storage of PCBs in Adipose Tissue in Rats\*  
 (Values are Percent of Total Dose 0.6 mg/kg)

Degree of Chlorination	Maximum	Time of Maximum Stored	Amount at 7 Days
mono-	11.63 ± 5.64	1 hr	0.234 ± 0.055
di-	52.75 ± 14.99	2 hr	1.837 ± 0.213
penta-	23.54 ± 3.0	1 day	13.04 ± 2.1
hexa-	85.18 ± 21.6	42 days	56.08 ± 15.72

\*Source: Matthews and Anderson, 1975b

day 4 and the residual on day 7 remaining at 45.4 percent. The distribution of several tetrachlorobiphenyl isomers in mice was analyzed by Mizutani, et al. (1977). In all cases the accumulation of the compound was greater in the carcass than in the liver. A tendency for those isomers with adjacent unsubstituted carbons to be rapidly cleared was observed. 2,6,2',6'-TCB was very rapidly cleared from carcass and liver, and 2,3,2',3'-TCB was cleared fairly rapidly. However, 2,4,2',4'-TCB was more resistant to removal than 3,5,3',5'-TCB, which might not be anticipated on structural grounds. The half-life in the carcass of the former was 9.2 days but only 2.1 days for the latter. The degree of accumulation of the isomers was assessed by the introduction of an index referred to as a storage ratio (the daily amount entering storage/daily oral ingestion). By this measure 3,5,3',5'-TCB and 2,4,2',4'-TCB were similar with indices of 0.7 and 0.6, respectively, while the more readily metabolized 2,3,2',3'-TCB had an index of 0.06.

The distribution of 2,5,2',5'-TCB in infant Rhesus monkeys was determined after a single dose of tritiated TCB (500 mg/kg). At 72 hours the distribution differed from that in rats in that the label was more widely dispersed in the monkeys. Blood levels were lower than observed in rats, and the major storage depots were bone marrow, adrenal glands, and skin. Most of the labeled material was associated with macromolecules, although it was largely extractable and not covalently bound (Hsu, et al. 1975a).

Distribution of PCBs in the human body has not been the subject of systematic experimentation. Data available from general population surveys indicate that general patterns of distribution

are consistent with those found in other animals. When detected in the adipose tissue of the general populace, PCB levels are around 1 mg/kg (Yobs, 1972; Kutz and Strassman, 1976; Grant, et al. 1976). Plasma levels detected in the general populace are two to three orders of magnitude lower than adipose levels (Finklea, et al. 1972). Similarly, Yusho patients exhibited a 100- to 1,000-fold greater concentration in the fat of skin, liver and in adipose tissue than in plasma. Over several years both the fat and plasma levels were observed to decline to near normal levels (Kuratsune, et al. 1976). The PCBs found in human adipose tissues in the U.S. chromatographically resemble Aroclor 1254 and 1260, suggesting that less chlorinated isomers found in Aroclor 1248 are preferentially excreted (Kutz and Strassman, 1976).

#### Metabolism

The metabolism of PCBs has been studied extensively in several organisms. A detailed review of PCB metabolism was written by Sundstrom, et al. (1976a). Rather than attempt to treat the subject exhaustively, this section will summarize the major characteristics of PCB metabolism which relate to their distribution, accumulation, toxicity, and possible mechanisms of carcinogenicity.

The metabolism of PCBs depends on their chlorine content and the sites of chlorination on the biphenyl (Sundstrom, et al. 1976a; Lutz, et al. 1977). While the overall mechanisms of metabolism appear to be similar in most vertebrates examined, the capacity to metabolize PCBs declines from mammals to birds to fish (Hutzinger, et al. 1972). Elucidation of PCB metabolism has been made possible by the use of individual purified isomers. Predominantly, the pro-

ducts of PCB metabolism at all levels of chlorination are biphenylols, biphenyldiols, and dihydrodihydroxybiphenyls, although the types and proportions of specific metabolites vary in different species. A few biphenyltriols and methoxy derivatives have also been observed (Sundstrom, et al. 1976a).

The structures of several PCB metabolites support the formation of arene oxides as intermediates. The first evidence for the formation of arene oxide intermediates was obtained by Gardener, et al. (1973). They isolated trans-3,4-dihydroxy-3,4-dihydro-2,2',5,5'-tetrachlorobiphenyl as a metabolite of 2,2',5,5'-tetrachlorobiphenyl in rabbits. More direct evidence for the formation of arene oxides was obtained by Safe, et al. (1975, 1976). In rabbits and frogs the biohydroxylation of 4-chlorobiphenyl was investigated using 4'-<sup>2</sup>H-4-chlorobiphenyl. The major metabolite, 4'-chloro-4-biphenylol, retained 79 percent of the label which is consistent with arene oxide formation (Daly, et al. 1972). The subsequent isomerization of the arene oxide results in the migration of the deuterium atom from the ultimate site of hydroxylation to the adjacent carbon, an NIH shift. Daly, et al. (1972) consider the NIH shift of labeled hydrogens, halogens or alkyl substituents to be indicative of enzymatic arene oxide formation. A subsequent hydroxylation to 4'-chloro-3,4-biphenyldiol resulted in the loss of half the remaining deuterium, suggesting a direct hydroxylation rather than a second arene oxide formation (Safe, et al. 1975). 4,4'-Dichlorobiphenyl produced three metabolites in the rabbit: 4,4'-dichloro-3-biphenylol, 3,4'-dichloro-4-biphenylol, and 4'-chloro-4-biphenylol. These products are consistent with a mech-

anism involving 3,4-arene oxide formation followed by epoxide ring opening. Either a 1,2-halogen shift, with or without halogen elimination upon tautomerization, or 3-ol formation after arene ring cleavage would produce the ultimate products (Safe, et al. 1976; Sundstrom, et al. 1976a). The reactions are diagrammed in Figure 2. Other examples of PCBs for which metabolic pathways are consistent with arene oxide formation include 2,2',4,4',5,5'-hexachlorobiphenyl in rabbits (Sundstrom, et al. 1976b) and 4-chlorobiphenyl and 4,4'-dichlorobiphenyl in rats (Hass, et al. 1977). Infant Rhesus monkeys fed 2,5,2',5-tetrachlorobiphenyl excreted dihydroxy, dihydrodihydroxy, and dihydrotrihydroxy derivatives in urine (Hsu, et al. 1975b).

The K region epoxides of polyaromatic hydrocarbons are known to bind to nucleic acids in vitro (Grover and Sims, 1970) and in cultured mammalian cells (Grover, et al. 1975). Furthermore, they are capable of transforming cells in culture (Huberman, et al. 1972) although their significance in tumor induction in animals is in doubt (Grover, et al. 1975). It has been suggested that arene oxide metabolites of PCBs may react with nucleophilic sites in DNA and other macromolecules and that alkylation of critical sites may be involved in the induction of tumors (Allen and Norback, 1977).

#### Excretion

The primary routes of PCB excretion are bile (observed in feces) and urine. Excretion is closely coupled to metabolism. In rats less than ten percent of excreted PCBs were unmetabolized (Matthews and Anderson, 1975b). The rate and efficiency of excretion were highly dependent upon the degree of chlorination and

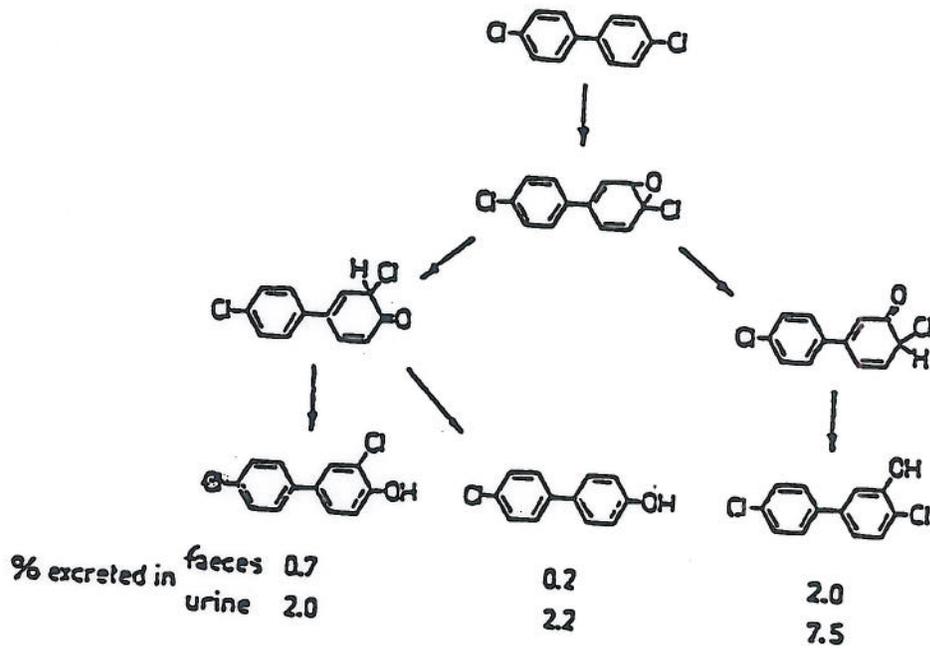


FIGURE 2  
 Metabolic Pathways for 4,4'-dichlorobiphenyl in the Rabbit  
 Source: Sundstrom, et al. 1976a

structure. Urinary excretion of PCBs accounted for the removal of 59.8, 33.9, 7.6, and 0.7 percent of total dose of mono-, di-, penta-, and hexachlorobiphenyl, respectively. Over 60 percent of urinary excretion occurred within the first 24 hours and all urinary excretion ceased by the ninth and fourth days, respectively, for penta- and hexachlorobiphenyl (Matthews and Anderson, 1975b). All the 2,4,5,2',5'-pentachlorobiphenyl excreted in urine by rats was in the form of a glucuronide conjugate of a metabolite (Chen and Matthews, 1974). While urinary excretion usually ceases within a few days, biliary excretion continues for an extended period. The relative contribution of biliary excretion to the elimination of PCBs increases with chlorination. The kinetics of excretion of mono- and dichlorobiphenyl are monophasic while the elimination of penta- and hexachlorobiphenyl is biphasic. While 90 percent of PCBs up to pentachlorobiphenyl were excreted in 42 days or less, hexachlorobiphenyl was largely retained in the tissues of the animal. Extrapolation of the excretion data indicated that only 20 percent of 2,4,5,2',4',5'-hexachlorobiphenyl would ever be excreted (Matthews and Anderson, 1975b). The absence of adjacent unsubstituted carbons greatly decreased excretion as would be expected from the effects of structure on storage and metabolism. 3,5,3',5'-Tetrachlorobiphenyl (TCB) is excreted at about the same rate as 2,4,5,2',5'-pentachlorobiphenyl (Tuey and Matthews, 1977; Matthews and Anderson, 1975a). While the half-life in fat for 2,5,2',5'-TCB was about 33 hours at 500 mg/kg dose in rats (Van Miller, et al. 1975), the half-life for 3,5,3',5'-TCB was 12 to 15 days at dose levels of 0.6 mg/kg in rats (Tuey and Matthews, 1977).

The half-lives of the individual PCB isomers in the rat may be approximated by the fecal half-lives, which are 15.7 and 22.2 hours for mono- and dichlorobiphenyl, respectively. Penta- and hexachlorobiphenyls elimination is biphasic, with first and second component half-lives of 39.2 and 211 hours for pentachlorobiphenyl and 49 and 642 hours for hexachlorobiphenyl (Anderson, et al. 1977). Because only 20 percent of the hexachlorobiphenyl is ultimately excreted, its half-life is indefinite.

Rates of elimination of a series of tetrachlorobiphenyls (TCB) in mice were determined by Mizutani, et al. (1977). Half-lives for TCB isomers in liver and the carcass ranged from 0.9 days for 2,3,2',3'-TCB to 9.2 and 7.8 days for the loss of 2,4,2',4' from carcass and liver, respectively. Structure did not influence elimination as markedly as in the rat. 3,5,3',5'-TCB had half-lives of 2.1 and 2.2 days in carcass and liver. However, stimulation of metabolism by the addition of phenobarbital did increase the rate of elimination of 2,4,2',4'-TCB more than 3,5,3',5'-TCB. The authors concluded that the rate-limiting step in the elimination of the isomers was release from storage in the tissues of the mouse rather than metabolism.

Two differences between the elimination of 2,5,2',5'-TCB in infant Rhesus monkeys and rats may be of interest in evaluating human metabolism. Single doses of 500 mg/kg to rats resulted in total elimination of about 76 percent (66 percent feces, 10 percent urine) in 72 hours (Van Miller, et al. 1975). In primates only one percent of the same dose was eliminated in feces and two percent in urine after 72 hours (Hsu, et al. 1975a). In addition, the major excreted metabolite in rats appeared to be 3-hydroxy TCB, while a

dihydrodiol TCB predominated in monkeys (Van Miller, et al. 1975; Hsu, et al. 1975b).

A final comment on the pharmacokinetics of PCBs must be addressed to transplacental and transmammary movement. Transplacental uptake of PCBs by a fetus has been documented in mice (Masuda, et al. 1978), rats (Curley, et al. 1973), Rhesus monkeys (Allen and Barsotti, 1976), and humans (Yoshimura, 1974). In mice, transplacental and transmammary uptake of PCBs were approximately 0.1 to 0.2 and 20 to 35 percent of total dose, respectively (Masuda, et al. 1978). Similar values were observed in rats (Mizunoya, et al. 1974). Female monkeys consuming 2.5 ppm Aroclor 1254 transferred enough via breast milk to produce severe hyperplastic gastritis in nursing infants (Allen and Barsotti, 1976). Recently, a preliminary mathematical model of PCB distribution in rats has been proposed (Lutz, et al. 1977; Anderson, et al. 1977).

It should be noted that most of the laboratory studies discussed above have been performed with pure isomers, while toxicity studies and environmental exposures involve commercial mixtures with possible dibenzofuran contamination. In addition, commercial mixtures tend to contain asymmetrical polychlorinated biphenyls (NIOSH, 1977).

The pharmacokinetics of PCBs can be summarized with the following points:

1. They are readily absorbed through the gut, respiratory system, and skin.
2. They may initially concentrate in the liver, blood, and muscle mass; but long-term storage in mammals is primarily in adipose tissue and skin.

3. The major metabolic products of PCBs are phenolic derivatives or dihydrodiols which may be formed through pathways with arene oxide intermediates or by direct hydroxylation. The susceptibility of individual PCB isomers to metabolism is a function of the number of chlorines present on the biphenyl and their arrangement. Biphenyls which have one or more pairs of adjacent unsubstituted carbons are more rapidly metabolized than those which do not.
4. PCBs which are readily metabolized are also rapidly excreted in the urine and bile. Excretion in urine is most prominent for the least chlorinated, while bile becomes the more significant route of excretion for more highly chlorinated isomers.
5. Those isomers which are most refractory to metabolism accumulate for increasing periods of time in fatty tissues. Highly chlorinated isomers are accumulated almost indefinitely.
6. PCBs can be transferred either transplacentally or in breast milk.
7. Nonhuman primates may retain PCBs more efficiently than rodents.

#### EFFECTS

##### Acute, Subacute, and Chronic Toxicity

Several reviews of the toxic effects of PCBs in animals and man have appeared in recent years [Kimbrough, 1974; Fishbein, 1974; Peakall, 1975; Kimbrough, et al. 1978; Cordle, et al. 1978; NIOSH, 1977 (which is particularly recommended for human effects)]. This section will attempt to highlight the most significant toxic effects observed in animals and man, but will not seek to be comprehensive.

The acute oral and dermal LD<sub>50</sub>s for PCBs in rats, mice, and rabbits are given in Tables 7, 8, and 9. In the classification by the American Industrial Hygiene Association, the PCBs are slightly toxic or almost nontoxic (Hodge and Sterner, 1949). In rats, Bruckner, et al. (1973) observed a 14-day LD<sub>50</sub> of 4.25 g/kg. Toxic effects of high doses of Aroclor 1242 included diarrhea, chromoacryorrhea, loss of body weight, unusual stance and gait, lack of response to pain stimuli, and terminal ataxia. CNS deterioration and dehydration were thought to be contributing factors. Histo-pathologic changes were observed only in liver and kidney. Miller (1944) found the guinea pig most sensitive to Aroclor 1242 followed by the rabbit and rat. In the rat, toxicity decreased with increasing degree of chlorination; however, the effect was not observed with rabbits (Fishbein, 1972).

The more significant toxic effects of PCBs are observed on repeated exposure over a period of time. Aroclor 1254 at 1,000 ppm in the diet was fatal to 75 percent of male rats in 43 days with total intakes of 500 to 2,000 mg/kg being lethal (Tucker and Crabtree, 1970). Phenoclor DP6 fed at 2,000 ppm to rats resulted in marked weight loss and death between 12 and 56 days after the initiation of treatment (Vos and Koeman, 1970). Guinea pigs treated dermally for 11 days with a total of 379.5 mg of a PCB with 42 percent average chlorine content died at intervals up to 21 days following the first application (Miller, 1944). Aroclor 1254 at 1,000 ppm in the diet killed 5/10 male rats and 8/10 female rats. At 500 ppm over eight months two males and one female died while no lethality was observed at 100 or 20 ppm. Aroclor 1260 was less

TABLE 7  
Acute Toxicity of PCBs in Several Strains of Rats and Mice\*

Compound Tested	Species and Sex	Route	LD g/kg Body Weight	Reference <sup>a</sup>
Aroclor 1254	Rat (adult, Sherman strain)	Oral	4 - 10	(5)
Aroclor 1260	Rat (adult, Sherman strain)	Oral	4 - 10	(5)
Aroclor 1254	Rat (weanling, Sherman strain)	Oral	1.295	(5)
Aroclor 1260	Rat (weanling, Sherman strain)	Oral	1.315	(5)
Aroclor 1254	Rat (female, Sherman strain)	Intravenous	0.358	(5)
Aroclor 1221	Rat (female, Sherman strain)	Oral	4.00	(6)
Aroclor 1262	Rat (female, Sherman strain)	Oral	11.3	(6)
Aroclor 1240	Rat	Oral	4.25	(7)
Aroclor 1254	Rat (Wistar, 30-day-old, M-F)	Oral	1.3	(8)
Aroclor 1254	Rat (Wistar, 60-day-old, M-F)	Oral	1.4	(8)
Aroclor 1254	Rat (Wistar, 120-day-old, M-F)	Oral	2.0	(8)
Aroclor 1254	Rat (Wistar, 120-day-old, F)	Oral	2.5	(8)
Kaneclor-400	Rat (Wistar, M)	Oral	1.30 (ml kg)	(9)
Kaneclor-400	Mice (CFI strain, M)	Oral	1.14 (ml kg)	(9)
Kaneclor-400	Mice (CFI strain, F)	Oral	1.875 (ml kg)	(9)
Kaneclor-300	Rat (Wistar strain, M)	Oral	1.57 (ml kg)	(9)
Kaneclor-300	Rat (Wistar strain, F)	Oral	1.15	(9)
BP-200 biphenyls of dichloride and below	Mice (dd strain, F)	Oral	1.05	(9)
2,4'-Dichlorobiphenyl	Mice (dd strain, F)	Oral	6.36	(10)
Trichlorobiphenyl	Mice (dd strain, F)	Oral	7.86	(10)
Biphenyl of trichloride and below	Mice (dd strain, F)	Oral	3.06 - 4.25	(10)
2,4,3',4'-Tetrachlorobiphenyl	Mice (DVI strain)	Oral	9.27	(10)
5-OH derivative of 2,4,3',4'- tetrachlorobiphenyl	Mice (CFI strain)	Intraperitoneal	2.15	(11)
2,3,4,3',4'-Pentachlorobiphenyl	Mice (CFI strain)	Intraperitoneal	0.43	(11)
	Mice (CFI strain)	Intraperitoneal	0.65	(11)

<sup>a</sup>Reference numbers from source

<sup>†</sup>Source: Kimbrough, et al. 1978

TABLE 8  
 Oral LD<sub>50</sub> (rat)<sup>a,b</sup>

Compound Tested	LD <sub>50</sub> g/kg body weight
Aroclor 1221 (Undiluted)	2.000 - 3.169
Aroclor 1232 (Undiluted)	1.26 - 2.0
Aroclor 1242 (Undiluted)	0.794 - 1.269
Aroclor 1248 (Undiluted)	0.794 - 1.269
Aroclor 1260 (50% soln in corn oil)	1.26 - 2.0
Aroclor 1262 (50% soln in corn oil)	1.26 - 3.16
Aroclor 1268 (33.3% soln in corn oil)	2.5

<sup>a</sup>Data of Panel on Hazardous Substances (6)

<sup>b</sup>Source: Kimbrough, et al. 1978

TABLE 9  
Skin LD<sub>50</sub> (rabbits)<sup>a,b</sup>

Compound Tested	LD <sub>50</sub> g/kg body weight
Aroclor 1221 (Undiluted)	3.98
Aroclor 1232 (Undiluted)	4.47
Aroclor 1242 (Undiluted)	8.65
Aroclor 1248 (Undiluted)	11.0
Aroclor 1260 (50% soln in corn oil)	10.0
Aroclor 1262 (50% soln in corn oil)	11.3
Aroclor 1268 (50% soln in corn oil)	10.9

<sup>a</sup>Data of Panel on Hazardous Substances (6)

<sup>b</sup>Source: Kimbrough, et al. 1978

toxic, with 8/10 females, but no males, dying at 1,000 ppm. No males died at lower doses, and 1/10 and 2/10 females died at 100 and 500 ppm, respectively. Substantial weight losses were observed at 100 and 500 ppm in both males and females (Kimbrough, et al. 1972). Mink have been shown to be unusually sensitive to PCBs. A mixture of Aroclors 1242, 1248 and 1254 at 30 ppm in the diet for 6 months was 100 percent lethal (Aulerich, et al. 1973), as was 3.6 ppm Aroclor 1254 over 105 days in another study (Plantonow and Karstad, 1975). Adult Rhesus monkeys (Macaca mulatta) were particularly sensitive to PCBs. Aroclor 1248 at 100 or 300 ppm in the diet for two to three months resulted in extreme morbidity within one month and almost 100 percent mortality within three months. Total intakes for the two groups were 0.8 to 1.0 g for 100 ppm and 3.6 to 5.4 g for 300 ppm (Allen, 1975).

The most consistent pathological changes occurring in mammals after PCB exposure are in the liver. In rats, rabbits, and guinea pigs, Miller (1944) observed fatty deposits after acute injections and similar changes in rabbits and guinea pigs after dermal application. In feeding experiments, marked fatty metamorphosis was noted in guinea pig liver with intracellular hyaline bodies being observed in rats. Less striking changes were noted in the kidneys, lungs, adrenals, and heart of guinea pigs. Rats exposed repeatedly to dietary PCBs show increased liver weights (Kimbrough, et al. 1972; Bruckner, et al. 1973). Kimbrough, et al. (1972) fed rats Aroclor 1254 or 1260 at levels between 20 and 1,000 ppm for eight months. Light microscopic changes observed included hypertrophy of liver cells, cytoplasmic inclusions, brown pigment in Kupffer

cells, lipid accumulation and, at higher doses, adenofibrosis. Ultrastructural examination revealed an increase in smooth endoplasmic reticulum. The effect of Aroclor 1254 was more pronounced than that of 1260. Porphyria was observed in the livers and, occasionally, other tissues of animals exposed to either mixture.

Rats fed 2,000 ppm Phenoclor DP6 also had enlarged livers with vacuolated foamy cells containing pycnotic nuclei (Vos and Koeman, 1970). Vacuolization of liver cells was also noted by Bruckner, et al. (1973) after dosing rats with 100 mg/kg Aroclor for three weeks, although no overt toxicity was manifest.

Rats fed 100 ppm Aroclor 1242 (6.6 to 3.89 mg/kg/day) or Aroclor 1016 (6.9 to 3.5 mg/kg/day) for periods of up to ten months showed no signs of overt intoxication or gross liver changes. Enlarged hepatocytes with vacuolated cytoplasm and inclusions were noted. Aroclor 1242 seemed to produce more pronounced changes than 1016. Four and six months after the discontinuation of exposure hepatocytes were still enlarged but cytoplasmic vacuoles and inclusions had diminished, suggesting a degree of reversibility of effect. Significant residual levels of PCBs remained in adipose tissue. Using electron microscopy, increased smooth endoplasmic reticulum and lipid vacuoles as well as atypical mitochondria were observed. No significant gross changes in other organs were noted (Burse, et al. 1974).

Allen and Abrahamson (1973) fed rats 1,000 ppm of either Aroclor 1248, 1254, or 1262 for 1, 3, 7, 14, 21, or 28 days or 6 weeks. Overt toxicity was not observed, although weight gain was retarded in all treated groups. The effect was inversely proportional to

percent chlorination. Increased liver size, protein, and RNA content were observed. The magnitude of changes increased with the percent chlorination. Hypertrophy was associated with proliferation of the smooth endoplasmic reticulum, formation of membranous arrays, and increased lipid droplets.

The effect of metabolism on toxicity was explored by giving rats large (1.5 g/kg) single doses of 2,5,2',5'-tetrachlorobiphenyl which produced high mortality within two to three days (Allen, et al. 1975). Pretreatment with phenobarbital to induce metabolic enzymes allowed survival without obvious ill effects following a 1.25 g/kg dose, while treatment with the microsomal enzyme inhibitor SKF 525A lead to 100 percent mortality in four days. The ability to metabolize and eliminate 2,5,2',5'-TCB appears to protect the animal. Dietary administration of 100 ppm 2,5,2',5'-TCB for three weeks produced less liver hypertrophy than Aroclor 1248.

Liver pathology in mice exposed to 1.5 mg PCB/day was essentially the same as seen in rats, including increased smooth endoplasmic reticulum and increased lipid droplets (Nishizumi, 1970).

Rabbits receiving 300 mg orally of Aroclor 1221, 1242, or 1254 for 14 weeks were examined (Koller and Zinkl, 1973). Aroclor 1221 and 1242 treated rabbits gained weight at control rates while 1254 treated rabbits did not gain as much. Livers of 1254 and 1242 treated animals were enlarged while livers of 1221 treated animals were smaller than controls. Gross liver lesions and small uteri were apparent in the 1254 treated animals but not the others. Liver pathology in 1254 treated animals included enlarged hepatocytes with foamy to granular cytoplasm and subcapsular midzonal

necrosis. Aroclor 1242 produced a liver pathology similar to 1254. Aroclor 1221 treated animals were free of histologic changes.

Dermal studies with rabbits using Clophen A60, Phenoclor DP6, and Aroclor 1260 indicated that the last was the least toxic (Vos and Beems, 1971). The former two mixtures had been shown to be contaminated with tetra- and pentachlorodibenzofuran (Vos, et al. 1970). Skin lesions produced included hyperplasia and hyperkeratosis of the epidermal and follicular epithelium and were accompanied by pathological changes in the liver and kidney. The chlorodibenzofuran impurities in the PCBs were thought to be responsible for the skin lesions. A comparison of the toxic effects of dermally applied 2,4,5,2',4',5'-hexachlorobiphenyl and Aroclor 1260 demonstrated that the skin lesions appeared sooner and were more severe after treatment with the commercial mixture. Liver changes were found in both treatment groups with the pure isomer inducing the more severe effects. From this study it was concluded that the chlorodibenzofuran contaminants in commercial mixtures probably contribute to the skin lesions (chloracne), edema formation, and liver damage. PCBs contribute in lesser degrees to chloracne and liver damage but are primarily responsible for the hepatic porphyria observed in PCB intoxication (Vos and Notenboom-Ram, 1972).

Nonhuman primates are rather sensitive to PCBs. Male Rhesus monkeys were fed 300 ppm Aroclor 1248 for three months. Effects which began to appear within a month included hair loss, subcutaneous edema, purulent discharge from the eyes, acneform eruptions, and liver hypertrophy caused by smooth endoplasmic reticulum proliferation. Marked hypertrophy of the gastric mucosa was a signif-

icant finding not usually seen in rodents. Invasion of the submucosa by the mucosal epithelium with increased cellularity and dysplasia occurred in the stomach. The dietary levels used were about 10-fold greater than the contamination levels in foods during the early 1970's, and the gastric changes observed were considered to be of particular significance to human risk (Allen and Norback, 1973). When fed low levels (2.5 and 5 ppm) of Aroclor 1248 for 52 weeks female monkeys developed periorbital edema, alopecia, erythema and acneform lesions. Effects in males were less pronounced (Barsotti and Allen, 1975). The high sensitivity of monkeys to PCBs has been confirmed and the evaluation of the toxic effects, particularly in the gastric mucosa, has been extended (McNulty, 1976; Bell, 1976). The pathologic effects of PCBs in nonhuman primates have been reviewed by Allen and coworkers (Allen, 1975; Allen and Norback, 1976).

The ability of PCBs to induce liver microsomal enzymes was demonstrated by Street, et al. (1969). Enzyme induction by commercial PCBs has been shown in rabbits (Villeneuve, et al. 1971a), rats (Litterst and VanLoon, 1972), and primates (Allen, et al. 1974b). In rats induction is observed following intraperitoneal injection (Bickers, et al. 1972) or skin application (Bickers, et al. 1975). Dietary threshold values for enzyme induction vary between 0.5 and 25 ppm (Villeneuve, et al. 1971a; Litterst, et al. 1972; Turner and Green, 1974). The induction of demethylating activity in rats by Aroclor 1254 was maximum in seven days while cytochrome P450 and nitroreductase activities continued to rise over four weeks of treatment. Activities declined slowly after

discontinuation of treatment, reaching control levels in about ten days (Litterst and VanLoon, 1974). Cutaneous exposure to PCBs resulted in a maximum induction within two to six days (Bickers, et al. 1972, 1975). Degree of induction of enzyme activities was found to correspond to increasing chlorine content of Aroclors (Litterst, et al. 1972) and of di-, tetra-, and hexachlorobiphenyl mixtures (Schmoltdt, et al. 1974). The effects of chlorine content and position of pure isomers were examined by Johnstone, et al. (1974), Ecobichon (1975), and Ecobichon and Comeau (1975). More highly chlorinated isomers and those substituted at the 4 and 4' positions were most active in inducing enzymes associated with the endoplasmic reticulum. For less localized enzymes, position was less critical, although chlorinated compounds were more effective than biphenyl.

The effects of dietary exposure to Aroclor 1254 on enzyme induction were investigated in rats by Bruckner, et al. (1977). Aroclor 1254 at 5 or 25 ppm induced dose-dependent increases in the metabolism of pentobarbitol, aminopyrine, and acetanilide after 35, 70, and 140 days of exposure. Exposure to 1 ppm had little effect on metabolism. Liver weight and serum triglyceride levels were elevated only in animals exposed to 25 ppm. In 15-day experiments induction of aminopyrine N-demethylation was observed after the first day of exposure at 5 and 25 ppm, and acetanilide hydroxylation was induced after two days. Aminopyrine N-demethylation returned to normal 15 days after the termination of exposure. Consumption of as little as 1 to 2 mg of PCBs in 24 hours was sufficient to stimulate acetanilide hydroxylation.

Commercial PCBs have been shown to induce cytochrome P450 (phenobarbital type) and cytochrome P448 (3-methylcholanthrene type) (Alvares, et al. 1973). More recent studies with purified isomers indicated that ortho-para-substituted PCBs induce P450 while meta-para-substituted PCBs induce P448. Substitution in the ortho-position dominates over meta-, and no isomers were found to induce both activities (Goldstein, et al. 1977). The induction of both systems by commercial preparations and some purified isomers has recently been shown to result from contamination with dibenzofurans. Even "99 percent pure" isomeric PCBs containing 44 ppm tetrachlorodibenzofuran effectively induces P448 while more rigorously purified material does not (Goldstein, et al. 1978). This observation serves as a reminder that the effects of trace contaminants must be kept in mind when evaluating the toxic effects of PCBs.

Enzyme inducing effects of PCBs have also been examined in vivo by the observation of shortened phenobarbital sleeping times in PCB-treated animals (Bickers, et al. 1972; Johnstone, et al. 1974; Villeneuve, et al. 1972). PCB induction of enzyme activities in other tissues has included skin (Bickers, et al. 1975), placenta and fetus (Alvares and Kappas, 1975), neonatal liver during lactation (Alvares and Kappas, 1975), and lung and kidney (Vainio, 1974).

Other systemic effects of PCBs in mammals include porphyria (Bruckner, et al. 1974), increased thyroxin metabolism (Bastomsky, 1974) and ultrastructural changes in the thyroid (Collins, et al. 1977), inhibition of ATPases (LaRocca and Carlson, 1975), and

interference with oxidative phosphorylation (Sivalingan, et al. 1973). Alterations in steroid hormone metabolism are produced by PCBs in rats (Bitman and Cecil, 1970), mice (Orberg and Kihlstrom, 1973), and other animals. Aroclor 1254 appears to reduce liver vitamin A concentrations in pregnant rabbits (Villeneuve, et al. 1971b). A more complete review of these effects can be found in Matthews, et al. (1978).

PCBs have been shown to have immunosuppressive effects in rabbits (Vos and Beems, 1971; Street and Sharma, 1975), guinea pigs (Vos and van Genderen, 1973; Vos and DeRoij, 1972), monkeys, mice (Thomas and Hinsdill, 1978), and several birds. Significant effects were observed in Rhesus monkeys exposed to dietary levels of Aroclor 1248 as low as 5.0 ppm.

Effects of Aroclor 1254 and 1260 on reproduction in Sherman strain rats were investigated (Linder, et al. 1974). Dietary levels of 5 ppm Aroclor 1254 had no effect on reproduction in rats exposed through two generations. Liver weights were increased in male and female offspring of the  $F_1$  and  $F_2$  generations. At 1 ppm, Aroclor 1254 caused increased liver weights in  $F_1$  male weanlings. With Aroclor 1254 at 20 ppm, the number of pups in the  $F_{1b}$  and  $F_2$  generations was reduced, while 100 ppm resulted in increased mortality in  $F_{1b}$  offspring and decreased the mating performance of  $F_{1b}$  adults. Aroclor 1260 produced increased liver weights in  $F_1$  offspring at 5 ppm but did not affect reproduction at 100 ppm. At 500 ppm litter sizes were reduced and survival was decreased in  $F_1$  litters. Pregnant rats given 100 mg/kg/day Aroclor 1254 on days 7 to 15 had grossly normal litters but only 30.1 percent survived to

weaning. Dosage rates of 50 mg/kg/day Aroclor 1254 or 100 mg/kg/day Aroclor 1260 did not affect reproduction or pup survival.

Rabbits fed 0.1 or 1.0 mg/kg body weight Aroclors 1221 or 1254 showed no significant decrease in number of pregnancies or number of fetuses per litter (Villeneuve, et al. 1971a). No induction of fetal liver enzymes could be detected. However, administration during gestation of 600 to 2,500 ppm Aroclor 1254 in the diet resulted in resorptions, abortions, maternal death, and asymmetric skulls in two fetuses (Villeneuve, et al. 1971b).

Reproductive effects in mice were investigated in animals treated for ten weeks with 0.025 mg/day Clophen A60 (Orberg and Kihlstrom, 1973). The length of the estrus cycle was increased from 6.6 days in controls to 8.7 days in experimental animals. Also, the percentage of implanted ova was reduced from 87.0 to 79.5. In a second study the reproductive effects of neonatal exposure to PCBs in milk were examined by injecting lactating female mice with Clophen A60. On the day of parturition and at weekly intervals for three weeks, the females were injected with 50 mg of PCB. When treated male and female offspring were mated with each other, the percent implantation dropped from a control level of 94 percent to 75 percent (Kihlstrom, et al. 1975).

In female Rhesus monkeys exposure to 25 ppm Aroclor 1248 in the diet for two months lead to the typical effects of PCB intoxication for monkeys including edema, alopecia, and acne. One animal ingesting a total of 450 mg PCB died two months after exposure ended and was found to have hyperplastic gastritis and bone marrow hypoplasia. The remaining five animals were bred three months

after treatment. Three were thought to have conceived but resorbed or aborted the embryos in the first two months of pregnancy. One delivered a fully developed but small infant (Allen, et al. 1974b).

In a more developed study both male and female Rhesus monkeys were fed either 2.5 or 5.0 ppm Aroclor 1248 in the diet (Barsotti and Allen, 1975; Barsotti, et al. 1976). The total intake in the first six months for the females was 180 and 364 mg for the 2.5 and 5.0 ppm diets, respectively. Untreated females bred to treated males had normal rates of conception (Barsotti and Allen, 1975). Treated females bred to normal males produced the following rates of conception: control, 12/12; 2.5 ppm, 8/8; 5.0 ppm, 6/8. Live births resulting from the conceptions were: control, 12/12; 2.5 ppm, 5/8; 5.0 ppm, 1/6. In the 2.5 ppm group, three fetuses were resorbed shortly after conception. In the 5.0 ppm group, three pregnancies aborted at 46, 67, and 107 days of gestation, one fetus was resorbed, one was stillborn, and one normal birth occurred. The two females who failed to conceive were subsequently bred five times without conception. The live born infants were of low birth weight and showed signs of PCB intoxication after nursing their mothers for less than two months. Three infants died 44 to 112 days after birth (Barsotti, et al. 1976). The mothers' breast milk contained 0.154 to 0.397 ppm PCBs and one contained 16.44 ppm (fat basis) (Allen and Barsotti, 1976). It should be noted that the dose levels producing these rather striking effects are within the range of contamination of the human diet observed until the mid-1970's.

Recently, adipose tissue levels of PCBs in infant Rhesus monkeys exposed in utero and via breast milk have been correlated with behavioral effects (Bowman, et al. 1978). Three of five infants born to mothers exposed to 2.5 ppm Aroclor 1248 in the diet during pregnancy and lactation survived over four months. PCB levels in fat tissue in the infants declined with a first order rate constant over a period of 8 to 23 months of age. Extrapolated maximum PCB levels were 21, 114, and 123  $\mu\text{g/g}$  fat. A battery of 11 behavioral tests was conducted with the three exposed animals and four controls over this time period and a positive correlation between reduced performance and PCB body burden was observed for seven tests.

Minks have been found to be exceedingly sensitive to PCB-induced reproductive failure. A marked increase in kid mortality was observed in commercial mink in the mid-1960's after fish meal derived from spawning Great Lakes Coho salmon was incorporated into the diet. Laboratory studies confirmed that the reproductive losses were related to the ingestion of Great Lakes fish (Aulerich, et al. 1971), and subsequent investigation showed that PCBs contaminating the fish meal were the probable toxic agents (Ringer, et al. 1972). When fed 10 ppm each of Aroclors 1242, 1248, and 1254 (30 ppm total), all 11 adult female mink died prior to the end of the normal whelping (delivery) period (Ringer, et al. 1972). Aroclor 1254 fed at 10 ppm resulted in no offspring among six females. At 5 ppm, Aroclor 1254 fed for four months prior to whelping depressed reproduction with only 3 of 12 females whelping and 3 of 9 kits born alive. At 1 ppm Aroclor 1254, 8 of 10 females whelped and

35 of 43 kits were born alive. Among control animals all 11 whelped and 56 of 66 pups were alive at birth. The reproductive toxicity of Aroclor 1254 becomes pronounced between 1 and 5 ppm in the diet (Ringer, et al. 1972). At 2 ppm in a nine month feeding trial, Aroclor 1254 significantly reduced reproduction while Aroclors 1016, 1221, and 1242 did not (Aulerich and Ringer, 1977). Assuming a food intake of 150 gm/day (Schaible, 1970), the total PCB intake in the two trials would have been 90 mg at 5 ppm for four months or 61 mg at 2 ppm for nine months (Aulerich and Ringer, 1977).

Human exposures to PCBs resulting in toxic effects have almost all resulted from the ingestion of rice oil contaminated with Kanechlor 400 in Japan or from industrial exposure. While absorption through the gut was the route of exposure in the former case, occupational exposures occur largely by inhalation or absorption through the skin.

Yusho, the disease resulting from the ingestion of contaminated rice oil in Japan, has been the subject of continuing study since the episode of exposure in 1968. Periodically, special reports on these continuing studies have been published in Fukuoka Acta Medica. These results, largely published in Japanese, have been reviewed in English by the Japanese investigators both early in the study (Kuratsune, et al. 1972; Kuratsune, 1972) and more recently (Kuratsune, et al. 1976). The cause and scope of the exposure of the Japanese public has been described above (see Ingestion from Food section). The initial symptoms of Yusho included increased eye discharge and swelling of upper eyelids, acneform eruptions and follicular accentuation, and pigmentation of the

skin. Other symptoms including dermatologic problems, swelling, jaundice, numbness of limbs, spasms, hearing and vision problems, and gastrointestinal disturbances were prominent among the complaints of patients seen within the first eight months after exposure (Kuratsune, et al. 1972). The first patients were seen almost immediately after the release of the contaminated oil in February 1968. Of a group of patients seen between October 1968 and January 1969, 55 percent became ill between June and August. It was ultimately determined that as many as 63.9 percent of those who consumed contaminated oil became ill. Among a group of 146 known users of the oil, 80 consumed less than 720 ml, and 88 percent of these users were affected. Among those who used more than 720 ml, 100 percent were affected. The clinical severity of symptoms did not differ by sex, but the age group 13 to 29 was more affected than others (Kuratsune, et al. 1972).

The analysis of the oil indicated that it contained between 2 and 3 mg/kg of Kanechlor 400 (Kuratsune, et al. 1972). It was later discovered that Kanechlor 400 contained 18 ppm of polychlorinated dibenzofurans (PCDFs) and that the PCDF concentration in "Yusho Oil" was about 5 ppm (Nagayama, et al. 1975). The PCDF level in the oil was 250 times greater than would be expected based on the level in fresh Kanechlor 400, leading Kuratsune, et al. (1976) to suggest that the concentration increased with PCB use as a heat transfer medium.

The amounts of Kanechlor 400 ingested were estimated for the original 146 person study group. The average amount ingested was estimated to be 2 g while the minimum amount ingested by a patient was about 0.5 g (Kuratsune, et al. 1972).

Laboratory evaluations of patients during the early period were summarized by Kuratsune (1972). Several changes in blood were noted, including decrease in erythrocyte count, increase in leukocyte count, and increase in serum lipids, particularly triglycerides. Blood proteins, electrolytes, and enzyme activities were normal in most instances. Some increases in urinary ketosteroid excretion were observed. The "cheesy" material from Yusho acne contained more steric and oleic acids than did "normal acne," but less myristic palmitic and palmitoleic acid. Linoleic acid was present in Yusho acne but not "normal acne." Liver biopsy indicated hypertrophy of the smooth endoplasmic reticulum, reduction of the rough endoplasmic reticulum, filamentous inclusions, and mitochondrial abnormalities. Skin changes included hyperkeratosis, cystic dilatation of the hair follicles, and marked increase of melanine in basal cells of the epidermis. Decreased sensory nerve conduction velocities were observed in 9 of 23 patients. Abnormalities of the eyes included hypersecretion of the meibomian gland and abnormal pigmentation of the conjunctiva.

Thirteen women, 11 with Yusho and 2 without, but married to men with Yusho, delivered 10 live and 2 stillborn infants between February 15 and December 31, 1968. Nine of the 10 had grayish-dark stained skin, and 5 had similar pigmentation of the gingiva and nails. Eye discharge was common. A stillborn fetus had marked hyperkeratosis, atrophy of the epidermis, and cystic dilatation of the hair follicle. Increased melanin pigment in the blood cells and the epidermis was also noted. Twelve of the 13 fetuses were small for date of birth. The growth of children affected by Yusho

was significantly lower than Japanese national standards. A detailed clinical study of four Yusho babies showed that they were small for their age, had dark pigmentation on skin and mucous membranes, and gingival hyperplasia. Teeth were erupted at birth; spotted calcification of the parieto-occipital skull, wide fontanelles, and saggital suture were present, along with facial edema and exophthalmic eyes (Yamashita, 1977).

By three years after the episode about half the patients were improving while 40 percent were essentially unchanged and 10 percent were becoming more severely affected. Even among those said to be improving, many still complained of persistant headaches, general fatigue, weakness and numbness of limbs, weight loss, and other problems (Kuratsune, et al. 1972).

An evaluation of the longer term effects of Yusho has been summarized by Kuratsune, et al. (1976). In 1972 Masuda noted a peculiar gas chromatographic pattern of PCB fractions which was common to blood, tissues and breast milk of Yusho patients (Koda and Masuda, 1975). A pattern seen in about 60 percent of Yusho patients contained a larger amount of a late eluting peak than PCB-containing tissues resulting from other types of exposures. This pattern was referred to as type A. A similar pattern seen in about 37 percent of Yusho patients was referred to as type B. These two patterns (types A and B) have never been observed in individuals (human or animal) exposed to PCBs in other situations. These types appear unique to Yusho. Tissue levels of PCBs in patients undergoing surgery or who died and were autopsied were followed over several years. Adipose tissue levels were high (13 to 76 ppm) shortly

after the end of exposure but were substantially lower by the next year. By 1970 and beyond, tissue levels were within the normal range in the cases studied. Blood levels were not determined until 1972 by which time they were in the normal range. Patients whose plasma PCB pattern was type A had higher levels than those with type B.

The discovery of substantial levels of PCDF in Yusho oil has been discussed. Levels of PCDFs in control individuals and Yusho patients were determined. No detectable (0.1 ppb) PCDFs were found in controls while tissues of patients who died in 1969 and 1972 contained 0.009 and 0.013 ppm in adipose and liver respectively. Ratios of PCB/PCDF were 144 and 4 for adipose tissue and liver, respectively. PCDF levels were higher in liver than adipose on a fat basis. Although the sample was small, the levels in whole adipose tissue appeared to have dropped to about one-third of the 1969 level by 1972.

By 1972, the dermal and mucosal signs which were most marked in the initial stages of toxicity were gradually improving. Symptoms considered to be due to internal disturbances, such as fatigue, poor appetite, abdominal pain, headache, pain and numbness in the limbs, and cough and expectoration of sputum, have become more prominent. Between March 1973 and April 1974, 79 patients were examined and blood PCBs evaluated (Koda and Masuda, 1975). Of patients with type A or B plasma PCB chromatographic patterns, a majority exhibited some or all of the typical spectrum of dermatological symptoms, with frequencies in type A patients being higher than in type B patients. Because PCB levels in type A patients were

higher than in type B, the severity of symptoms was correlated with blood PCB levels.

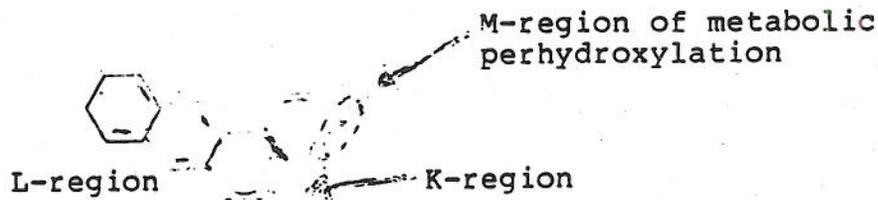
Serum triglyceride levels in males did not decline significantly between 1969 and 1974 (Okumura, et al. 1974). Levels in female patients declined but were still above normal. The elevation of triglycerides correlated with increased blood PCB levels and the type A pattern.

Serum bilirubin in patients was lower in 121 patients than in 257 controls, indicating an accelerated rate of disposal (Hirayama, et al. 1974).

Long-term effects continued to be observed in children born to Yusho mothers. Nine infants with dark brown skin pigmentation were born to Yusho mothers between 1969 and 1972, three of them to a patient between 1969 and 1971 (Yoshimura, 1974). The plasma PCB levels of 30 children born to 18 Yusho mothers were significantly above control levels but lower than maternal levels (Abe, et al. 1975). Children nursed by their mothers had higher levels than children who were not breast fed. One case was reported by Yoshimura (1974) in which a baby was thought to have acquired Yusho solely as a result of breast milk intake.

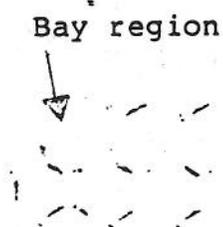
Masuda, et al. (1974) found PCB levels in breast milk of five Yusho women between 0.03 and 0.06 ppm, which was just within the normal range. A recent study of PCB levels in the breast milk in 400 Japanese women detected average levels of 0.033, 0.026, and 0.029 ppm in three measurements made at two month intervals (Yakushiji, et al. 1977). Based on these levels, they calculated that daily intake by a nursing infant would be 24  $\mu\text{g}/\text{day}$ . This can be

perhydroxylation in the M-region. The three regions of reactivity are readily distinguished in the benz(a)anthracene skeleton:



The electronic K-L theory of carcinogenic reactivity has encountered numerous inconsistencies, primarily because these relationships were derived from physico-chemical properties of the parent hydrocarbon and gave no consideration to the biological effects of activated metabolites.

Advances in recent years have focused attention on the potential reactivity of diol epoxide metabolites of PAH, and their ease of conversion to triol carbonium ions. Under the assumption that diol epoxides, which are more readily converted to carbonium ions, will be better alkylating agents to produce carcinogenesis and mutagenesis, the "bay region" theory has been proposed (Lehr, et al. 1978; Wood, et al. 1977b). Examples of a "bay region" in a polycyclic hydrocarbon are the regions between the 10 and 11 positions of BaP and the 1 and 12 positions of benz(a)anthracene:



Benzo(a)pyrene



Benz(a)anthracene

The theory predicts that diol epoxides in which the oxirane oxygen forms part of a "bay region" (e.g., BaP 7,8-diol-9,10-epoxide) will be more reactive and hence more carcinogenic than diol epoxides in which the oxirane oxygen is not situated in a "bay region."

Experimentally, the "bay region" diol epoxides of benz(a)anthracene, BaP, and chrysene were more mutagenic in vitro and/or tumorigenic than other diol epoxide metabolites, their precursor dihydrodiols, the parent hydrocarbons, or other oxidative metabolites. Moreover, quantum mechanical calculations were in accord with the concept that reactivity at the "bay region" is highest for all the diol epoxides derived from polycyclic hydrocarbons.

The bay region concept has received enough confirmation to lead to suggestions that an analysis of theoretical reactivity in this manner may be useful in screening PAH as potential carcinogens (Smith, et al. 1978). Among several indices of theoretical reactivity examined, the presence of a bay region for a series of PAH displayed a high degree of correlation with positive carcinogenic activity (Table 19).

The carcinogenic activity of BaP has been studied extensively in various animal model systems. In recent years, research on BaP has been expanded to include an examination of the tumorigenic activity of various BaP metabolites. These efforts were directed at the objective of identifying a BaP derivative which acts as the principal ultimate carcinogen resulting from metabolic activation (Levin, et al. 1976a,b, 1977a,b; Slaga, et al. 1976, 1977; Kapitulnik, et al. 1976a,b; Wislocki, et al. 1977; Conney, et al. 1977a,b).

Studies on the activity of BaP and its derivatives as complete carcinogens on mouse skin (Table 20) and as tumor initiators (Table 21) revealed that marked differences in tumorigenic potency exist. The apparent lack of activity for the BaP 7,8-diol-9,10-epoxides,

TABLE 19

## Reactivity Indices for Polycyclic Hydrocarbons\*

Compound	K- region?	L- region?	Bay region	Carcinogenicity Index	
				Arcos and Argus (1974)	Jerina, et al. (1972)
Naphthalene	-	-	-	0	-
Anthracene	-	+	-	0	-
Tetracene	-	+	-	0	-
Pentacene	-	+	-	0	-
Hexacene	-	+	-	5	?
BA	+	+	+	0	+
Benzo(a) tetracene	+	+	+	0	-
Phenanthrene	+	-	+	4	+
Benzo(c) phenanthrene	+	-	+	3	+
Chrysene	+	+	+	0	-
Benzo(b) chrysene	+	+	+	0	-
Picene	+	-	+	17	-
Triphenylene	-	-	+	3	++
Benzo(g) chrysene	+	-	+	4	+
Dibenz(a,c) anthracene	-	+	+	26	++
Dibenz(a,j) anthracene	+	+	+	27	++
Dibenz(a,h) anthracene	+	+	+	73	++++
Naphtho(2,3-b) pyrene	+	-	a	2	++ <sup>b</sup>
Benzo(a) pyrene	+	-	+	33	++
Benzo(e) pyrene	+	-	+	74	++++
Dibenzo(a,l) pyrene	+	-	+	50	+++
Dibenzo(a,i) pyrene	+	-	+	70	++++
Dibenzo(a,e) pyrene	+	-	+	16	++
Dibenzo(a,h) pyrene	+	-	+		
Tribenzo(a,e,i) pyrene	-	-	+		

\*Source: Smith, et al. 1978

<sup>a</sup>This compound does not strictly possess a bay region but does contain a "pseudo" bay region.<sup>b</sup>Jerina, et al. (1972) have assigned this as ++++.

TABLE 20  
 Skin Tumors in Mice Treated with Benzo(a)pyrene and Derivatives

Treatment <sup>a</sup>	Total No. Animals	Dose, $\mu$ moles	Mice with Tumors, %	Total No. Skin Tumors <sup>b</sup>	Reference
BaP	25	0.4	100	32	Wislocki, et al. 1977
BaP	30	0.4	100	34	Wislocki, et al. 1977
BaP	26	0.4	92	34	Albert, et al. 1978
BaP	30	0.15	100	40	Levin, et al. 1976a,b
BaP	27	0.1	96	28	Wislocki, et al. 1977
BaP	30	0.1	38	13	Levin, et al. 1977a,b
BaP	30	0.1	50	15	Levin, et al. 1977a,b
BaP	30	0.1	91	24	Levin, et al. 1977a,b
BaP	30	0.05	59	20	Levin, et al. 1977a,b
BaP	30	0.025	7	2	Levin, et al. 1977a,b
BaP	30	0.02	4	1	Levin, et al. 1977a,b
BaP	30	0.02	0	0	Levin, et al. 1977a,b
1-HOBaP	25	0.4	0	0	Wislocki, et al. 1977
2-HOBaP	29	0.4	100	37	Wislocki, et al. 1977
3-HOBaP	29	0.4	0	0	Wislocki, et al. 1977
4-HOBaP <sup>c</sup>	26	0.4	0	0	Albert, et al. 1978
5-HOBaP <sup>c</sup>	26	0.4	0	0	Albert, et al. 1978
6-HOBaP <sup>c</sup>	28	0.4	0	0	Albert, et al. 1978
7-HOBaP <sup>c</sup>	30	0.4	0	0	Albert, et al. 1978
8-HOBaP <sup>c</sup>	27	0.4	0	0	Albert, et al. 1978
9-HOBaP <sup>c</sup>	26	0.4	0	0	Albert, et al. 1978
10-HOBaP <sup>c</sup>	28	0.4	0	0	Albert, et al. 1978
11-HOBaP	28	0.4	14	4	Wislocki, et al. 1977
12-HOBaP	23	0.4	0	0	Wislocki, et al. 1977
BaP 4,5-oxide	30-39	0.4	4	1	Levin, et al. 1976a
BaP 4,5-oxide	30-39	0.1	6	2	Levin, et al. 1976a
BaP 7,8-oxide	30-39	0.4	94	37	Levin, et al. 1976a
BaP 7,8-oxide	30	0.3	53	16	Levin, et al. 1976a
BaP 7,8-oxide	30	0.15	18	5	Levin, et al. 1976a
BaP 7,8-oxide	30-39	0.1	9	3	Levin, et al. 1976a
BaP 9,10-oxide	30-39	0.4	0	0	Levin, et al. 1976a
BaP 11,12-oxide	28	0.4	0	0	Wislocki, et al. 1977
BaP 11,12-oxide	17	0.1	0	0	Wislocki, et al. 1977

TABLE 20 (cont.)

Treatment <sup>a</sup>	Total No. Animals	Dose, $\mu$ moles	Mice with Tumors, %	Total No. Skin Tumors <sup>b</sup>	Reference
BaP 7,8-dihydrodiol	30	0.3	100	42	Levin, et al. 1976b
BaP 7,8-dihydrodiol	30	0.15	100	40	Levin, et al. 1976b
BaP 7,8-dihydrodiol	30	0.1	92	28	Levin, et al. 1976a
BaP 7,8-dihydrodiol	30	0.05	76	24	Levin, et al. 1976a
BaP 7,8-dihydrodiol	30	0.025	7	2	Levin, et al. 1976a
(±)-7 $\beta$ ,8 $\alpha$ -Di-hydroxy-9 $\beta$ ,10 $\beta$ -epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (diol epoxide 1)	30	0.4	0	0	Levin, et al. 1976a
diol epoxide 1	30	0.1	0	0	Levin, et al. 1976a
diol epoxide 1	30	0.02	0	0	Levin, et al. 1976a
(±)-7 $\beta$ ,8 $\alpha$ -Di-hydroxy-9 $\alpha$ ,10 $\alpha$ -epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (diol epoxide 2)	30	0.4	13	3	Levin, et al. 1976a
diol epoxide 2	30	0.1	7	2	Levin, et al. 1976a
diol epoxide 2	30	0.02	0	0	Levin, et al. 1976a

<sup>a</sup>Female C57BL/6J mice were treated with BaP or BaP derivatives (0.02-0.4  $\mu$ mole) once every 2 weeks for 60 weeks by topical application to the shaved skin of the back.

<sup>b</sup>Skin tumors consisted mostly of squamous cell carcinomas; other skin tumors were fibro-sarcomas, papillomas, and keratocanthomas.

<sup>c</sup>Mice were treated once every 2 weeks for 56 weeks.

TABLE 21  
 Summary of the Skin Tumor Initiation Activities of Benzo(a)pyrene and its Metabolites<sup>a</sup>

Initiator	No. Mice	Dose, hmoles	Weeks of Promotion	Mice with Tumors, %	Papillomas/Mouse	Reference
BaP	30	200	23	94	4.8	Slaga, et al. 1976
BaP	30	200	30	92	5.3	Slaga, et al. 1977
BaP	30	200	21	77	2.6	Levin, et al. 1977b
BaP 4,5-epoxide	30	200	23	20	0.2	Slaga, et al. 1976
BaP 7,8-epoxide	29	200	23	81	1.9	Slaga, et al. 1976
BaP 9,10-epoxide	29	200	30	15	0.15	Slaga, et al. 1977
BaP 11,12-epoxide	30	200	30	38	0.45	Slaga, et al. 1977
BaP 7 $\beta$ ,8 $\alpha$ -diol-9 $\alpha$ ,10 $\alpha$ -epoxide	29	200	30	69	1.5	Slaga, et al. 1977
BaP 7 $\beta$ ,8 $\alpha$ -diol-9 $\beta$ ,10 $\beta$ -epoxide	28	200	30	7	0.07	Slaga, et al. 1977
BaP 7,8-dihydrodiol	29	200	30	86	5.0	Slaga, et al. 1977
(-)-BaP 7,8-dihydrodiol <sup>b</sup>	30	100	21	77	3.8	Levin, et al. 1977b
(+)-BaP 7,8-dihydrodiol	30	100	21	23	0.43	Levin, et al. 1977b

<sup>a</sup>Female CD-1 mice were treated with a single dose of initiator dissolved in acetone, acetone: NH<sub>4</sub>OH (1,000:1), or dimethyl sulfoxide:acetone (1:3) and followed 1 week later by twice-weekly applications of 10  $\mu$ g of TPA.

<sup>b</sup>Promotion was by twice-weekly applications of 16 hmoles of TPA beginning 11 days after treatment with initiator.

despite their exceptional mutagenicity, may be due to poor skin penetration of adult mouse skin because of high chemical reactivity. Indeed, as a carcinogen in newborn mice the (-) enantiomer of BaP, 7,8-dihydrodiol, and the 7,8-diol-9,10-epoxide derived therefrom are far more active than the parent hydrocarbon (Kapitulnik, et al. 1977a,b,c,d, 1978a,b). These studies on the newborn mouse clearly indicate the role of a BaP 7,8-diol-9,10-epoxide as an ultimate carcinogenic metabolite of BaP.

Further dose-response information on the sarcomagenic activity of BaP by subcutaneous injection to rats and mice is summarized in Table 22.

Temporal relationships for the development of BaP-induced skin cancers in mice have been examined by Albert, et al. (1978). Their results showed that increasing weekly doses of BaP caused a shortening of the latency period for carcinoma formation. Furthermore, it was determined that the development of papillomas as a precursor lesion to carcinoma formation occurred only at higher BaP doses (e.g., 32 and 64 µg/week). At the lower dose levels (8 and 16 µg/week), carcinomas appeared de novo without precursor papilloma formation.

The carcinogenicity of BaP by oral intake has not been studied as thoroughly as for other routes of administration. Nevertheless, tumors of various sites result when BaP is administered orally to rodents (Table 23).

With oral, intratracheal, and intravenous routes of administration, BaP is less effective than other PAH (e.g., DMBA, MCA, dibenz(a,h)anthracene) in producing carcinomas. On the other hand,

TABLE 22

## Induction of Sarcoma by Benzo(a)pyrene

Species	No. and (Sex)	Total Dose µmoles	Animals with Sarcoma, %	Average Latency, Days	Reference
Rat (Sprague-Dawley)	13 (female)	6.0 <sup>a</sup>	100	101 ± 2.7	Flesher, et al. 1976
Mouse	14 (male)	7.1 <sup>b</sup>	93	129	Duu-Hoi, 1964
Mouse	16 (female)	7.1 <sup>b</sup>	50	160	Duu-Hoi, 1964
Mouse	9 (?)	15.9 <sup>c</sup>	66.6	112	Gottschalk, 1942
Mouse	10 (?)	5.0 <sup>c</sup>	70	122	Gottschalk, 1942
Mouse	12 (?)	0.5 <sup>c</sup>	66.6	155	Gottschalk, 1942
Mouse	15 (?)	0.002 <sup>c</sup>	0	N.A. <sup>d</sup>	Gottschalk, 1942

<sup>a</sup>Administered as 0.2 µmole dissolved in 0.1 ml sesame oil by subcutaneous injection on alternate days for 30 doses beginning at 30 days of age.

<sup>b</sup>Administered as three injections of 2.4 µmoles each, given at 1 month intervals.

<sup>c</sup>Administered as a single injection under the skin of the abdomen, dissolved in 0.5 ml of neutral olive oil.

<sup>d</sup>Not applicable.

TABLE 23  
 Carcinogenicity of Benzo(a)pyrene by Oral Administration to Various Mammals\*

Compound	Species	Dose	Route of Administration	Effects
BaP	Mouse	0.2 mg in PEG <sup>a</sup>	Intragastric	14 tumors of the forestomach in 5 animals out of 11
	Mouse (age 17-116 days)	50-250 ppm	Dietary (110-197 days)	>70% incidence of stomach tumors at 50-250 ppm for 197 days; no tumors with diets containing up to 30 ppm for 110 days
	Mouse	250 ppm	Dietary	100% stomach tumor incidence when diet was fed for 30 days; 5-7 days of feeding, 30-40%; 2 to 4 days of feeding, 10 percent; 1 day of feeding, 0 percent
	Mouse (age 18-30 days)	250 ppm	Dietary (140 days)	Leukemias, lung adenomas, and stomach tumors produced
	Rat (Sprague-Dawley; age 105 days)	2.5 mg per day	Oral	Papillomas developed in the esophagus and forestomach in 3 out of 40 animals
	Hamster	2-5 mg bi-weekly	Intragastric	5 stomach papillomas in 67 animals treated for 1-5 months; 7 papillomas and 2 carcinomas in 18 animals treated for 6-9 months; 5 papillomas in 8 animals treated for 10-11 months
	Hamster	500 ppm	Dietary (4 days per week for up to 14 mo.)	12 tumors (2 esophagus, 8 forestomach, 2 intestinal) in 8 animals

<sup>a</sup>polyethylene glycol

\*Source: IARC, 1973

BaP has remarkable potency for the induction of skin tumors in mice. Therefore, caution must be exercised in considering the carcinogenicity of PAH as a class, and in extrapolating data derived from studies with BaP to the effects of PAH mixtures.

An examination of comparative carcinogenicities within the same tumor model system can provide valuable insight concerning relative risks of various PAH. By single intravenous injection of about 0.25 mg of aqueous dispersions of PAH to mice, a direct comparison of carcinogenic potency was possible (Table 24). In this test system, MCA displayed the greatest lung tumor-forming capability; dibenz(a,h)anthracene followed closely in activity with BaP being considerably less potent.

Intratracheal instillation of PAH to Syrian golden hamsters has been widely utilized for the conduct of studies on pulmonary carcinogenesis (Saffiotti, et al. 1968,1972; Henry, et al. 1975). Several studies are summarized in Table 25 and indicate that: (1) dose-response relationships are clearly evident, and (2) the co-administration of carrier particles such as  $Fe_2O_3$  (i.e., with BaP) can markedly increase tumor incidence, depending on the conditions of the experiment and physical characteristics of the particle. Since environmental exposures to PAH occur in conjunction with particulate material in air, this effect may be particularly relevant to human situation.

In addition to the hamster model system, respiratory tract tumors have been readily induced by PAH in rats and mice. The results of several representative studies are summarized in Table 26.

TABLE 24

Comparative Carcinogenicity of Polycyclic Hydrocarbons and Related Compounds  
 Measured by Induction of Lung Tumors (LT)<sup>a,b</sup>

Compound	Dose, μmoles/kg	Mice with LT/ No. of Mice	Mean No. LT/mouse	μMoles/kg for 1 LT Response
3-Methylcholanthrene, 0.1 mg	15	15/15	11	0.9
3-Methylcholanthrene, 0.5 mg	74	6/6	47	
Dibenz(a,h)anthracene	36	10/10	31	1.0
7H-Dibenzo(c,g)carbazole	38	12/12	5.7	6.0
Benzo(a)pyrene	40	10/10	3.7	9.5
Dibenz(a,j)aceanthrylene	33	9/10	2.7	14
Dibenz(a,h)acridine	36	11/12	2.0	18
8-Methylbenzo(c)phenanthrene	42	6/11	0.7	--
7-Methylbenzo(a)pyrene	38	5/10	0.6	--
5-Methoxy-7-propylbenz(a)anthracene	33	1/10	0.1	--
Benz(a)anthracene	44	2/11	0.2	--
Untreated controls	--	4/19	0.2	--

<sup>a</sup>Source: Shimkin and Stoner, 1975

<sup>b</sup>Strain A mice, 8-12 weeks old, received single intravenous injection of 0.24 mg of methylcholanthrene in aqueous dispersion and were killed 20 weeks later.

TABLE 25

## Induction of Respiratory Tract Tumors in Syrian Golden Hamsters by Intratracheal Instillation of PNH

Compound	No. Animals	Total Dose, mg	Respiratory Tumor Incidence, Percent	Reference
BaP	30	3.25 <sup>a</sup>	10	Feron, et al. 1973
BaP	30	6.5 <sup>a</sup>	13	Feron, et al. 1973
BaP	30	13	30	Feron, et al. 1973
BaP	29	26 <sup>a</sup>	86	Feron, et al. 1973
BaP	28	52 <sup>a</sup>	93	Feron, et al. 1973
BaP	48	30 <sup>b</sup>	15	Sellakumar, et al. 1976
BaP and Fe <sub>2</sub> O <sub>3</sub>	48	30 <sup>b</sup>	71	Sellakumar, et al. 1976
BaP and Fe <sub>2</sub> O <sub>3</sub> , coated	49	26.1 <sup>c</sup>	73	Henry, et al. 1975
BaP and Fe <sub>2</sub> O <sub>3</sub> , ground	49	27.4 <sup>c</sup>	84	Henry, et al. 1975
BaP and Fe <sub>2</sub> O <sub>3</sub> , mixed	43	26.3 <sup>c</sup>	12	Henry, et al. 1975
BaP and gelatin	46	26.4 <sup>c</sup>	17	Henry, et al. 1975
BaP and Fe <sub>2</sub> O <sub>3</sub>	28 (male), 29 (female)	60 <sup>d</sup>	60.7 (male), 58.6 (female)	Saffioti, et al. 1972
BaP and Fe <sub>2</sub> O <sub>3</sub>	33 (male), 34 (female)	30 <sup>d</sup>	66.7 (male), 58.8 (female)	Saffioti, et al. 1972
BaP and Fe <sub>2</sub> O <sub>3</sub>	33 (male), 30 (female)	15 <sup>d</sup>	30.3 (male), 30.0 (female)	Saffioti, et al. 1972
BaP and Fe <sub>2</sub> O <sub>3</sub>	47 (male), 41 (female)	7.5 <sup>d</sup>	12.8 (male), 9.8 (female)	Saffioti, et al. 1972

TABLE 25 (cont.)

Compound	No. Animals	Total Dose, mg	Respiratory Tumor Incidence, Percent	Reference
BaP	32 (male)	30 <sup>e</sup>	42.3	Kobayashi, 1975
BaP	28 (female)	30 <sup>e</sup>	57.7	Kobayashi, 1975
DB(a,i)P	48	12 <sup>f</sup>	75	Stenback and Sellakumar, 1974a
DB(a,i)P	48	8.5 <sup>g</sup>	64.6	Stenback and Sellakumar, 1974a
DMBA and Fe <sub>2</sub> O <sub>3</sub>	46	1.2 <sup>h</sup>	43.5	Stenback and Sellakumar, 1974b
DMBA and Fe <sub>2</sub> O <sub>3</sub>	28	0.85 <sup>i</sup>	46.4	Stenback and Sellakumar, 1974b

<sup>a</sup>Animals treated once weekly for 52 weeks with BaP suspended in 0.9% NaCl solution.

<sup>b</sup>3 mg BaP administered once weekly for 10 weeks.

<sup>c</sup>Animals received 30 weekly intratracheal instillations.

<sup>d</sup>Animals received 30 weekly instillations of BaP mixed with equal amounts of Fe<sub>2</sub>O<sub>3</sub> and suspended in 0.2 ml saline.

<sup>e</sup>Animals received 30 weekly intratracheal instillations of BaP suspended in 0.9% NaCl.

<sup>f</sup>Animals received 12 weekly intratracheal instillations of 1 mg DB(a,i)P suspended in distilled water.

<sup>g</sup>Animals received 17 weekly intratracheal instillations of 0.5 mg DB(a,i)P suspended in distilled water.

<sup>h</sup>Animals received 100 µg DMBA and 100 µg Fe<sub>2</sub>O<sub>3</sub> intratracheally once a week for 12 weeks in saline suspensions.

<sup>i</sup>Animals received 50 µg DMBA and 50 µg Fe<sub>2</sub>O<sub>3</sub> intratracheally once a week for 17 weeks in saline suspensions.

TABLE 26  
Induction of Respiratory Tract Tumors in Rats and Mice

Compound	Organism	No. Animals	Total Dose, mg	Route of Administration	Tumor Incidence, %	Reference
DMBA and Indian ink	Rat (Wistar and random-bred)	34	2.5 <sup>a</sup>	Intratracheal instillation	17.6	Pylev, 1962
DMBA and Indian ink	Rat (Wistar and random-bred)	56	6 <sup>b</sup>	Intratracheal instillation	35.7	Pylev, 1962
DMBA and Indian ink	Rat (Wistar and random-bred)	61	10 <sup>c</sup>	Intratracheal instillation	26.2	Pylev, 1962
DB(a,h)A	Mouse (DBA/2)	14 (male) 13 (female)	236 (male) <sup>d</sup> 179 (female) <sup>d</sup>	Oral	100 (male) <sup>e</sup> 77 (female) <sup>e</sup>	Snell and Stewart, 1962
MCA	Rat (Osborne-Mendel)	100	0.005 <sup>f</sup>	Pulmonary injection	19	Hirano, et al. 1974
MCA	Rat (Osborne-Mendel)	100	0.05 <sup>f</sup>	Pulmonary injection	139	Hirano, et al. 1974
MCA	Rat (Osborne-Mendel)	100	0.10 <sup>f</sup>	Pulmonary injection	279	Hirano, et al. 1974

TABLE 26 (cont.)

Compound	Organism	No. Animals	Total Dose, mg	Route of Administration	Tumor Incidence, %	Reference
MCA	Rat (Osborne-Mendel)	100	0.20 <sup>f</sup>	Pulmonary injection	47 <sup>g</sup>	Mirano, et al. 1974
MCA	Rat (Osborne-Mendel)	100	0.30 <sup>f</sup>	Pulmonary injection	40 <sup>g</sup>	Mirano, et al. 1974
MCA	Rat (Osborne-Mendel)	100	0.40 <sup>f</sup>	Pulmonary injection	51 <sup>g</sup>	Mirano, et al. 1974
MCA	Rat (Osborne-Mendel)	100	0.50 <sup>f</sup>	Pulmonary injection	45 <sup>g</sup>	Mirano, et al. 1974

<sup>a</sup>Administered as a single dose with 0.2 mg of Indian ink in 0.2 ml of a colloid protein solution.

<sup>b</sup>Administered as three 2 mg doses at monthly intervals with 0.2 mg of Indian ink in 0.2 ml of a colloid protein solution.

<sup>c</sup>Administered as five 2 mg doses at monthly intervals with 0.2 mg of Indian ink in 0.2 ml of a colloid protein solution.

<sup>d</sup>Administered as an aqueous-olive oil emulsion of DB(a,h)A given in place of drinking water for 237 to 279 days.

<sup>e</sup>Tumors were alveolegenic carcinomas, a 100% incidence of pulmonary adenomatosis was also observed.

<sup>f</sup>Administered as a single MCA-containing beeswax pellet placed directly into the lower peripheral segment of the left lung.

<sup>g</sup>Overt squamous cell carcinoma.

The published literature regarding chemical carcinogenesis in cell cultures is vast, despite the fact that systematic studies were not begun until the early 1960's due to the lack of a reproducible transformation assay. Berwald and Sachs (1963) first demonstrated that polycyclic hydrocarbons (MCA, BaP) could cause the direct malignant transformation of hamster embryo cells in culture. Transformed colonies have growth characteristics visually distinct from normal colonies and are readily seen above a background of normal cells. This assay can therefore be easily used as a screen to compare carcinogenic activity of suspect compounds. A common feature of these, and nearly all, transformed cells is that they give rise to fibrosarcomas upon inoculation into immunosuppressed animals. In addition to hamster embryo cells, malignant transformation has been demonstrated in organ cultures, liver cell cultures, fibroblastic cells derived from mouse ventral prostate, 3TC cell lines derived from mouse embryo cells, and various types of epithelial cells from humans and other animals (Heidelberger, 1973, 1975; Heidelberger and Boshell, 1975).

Early reports by Berwald and Sachs (1965) and Dipaolo and Donovan (1967) described alterations in hamster embryo cells induced by BaP, DMBA, and MCA which could be used as indicators of a change from normal to neoplastic state. The compounds were applied to cells in culture either dissolved in paraffin and impregnated on filter disks or as a colloidal suspension in growth medium. Following marked cytotoxicity, foci of transformed cells developed which displayed chromosomal abnormalities and the ability to grow indefinitely in culture. In addition, these transformed mass cul-

tures, when transplanted to four- to six-week-old hamsters, continued to grow and form tumors. A good correlation was obtained between in vitro carcinogenicity of a polycyclic hydrocarbon and the number of transformed clones they produced. The maximum rate of cell transformation in these studies was 25.6 percent in surviving cells, obtained by treatment with 10 ug/ml of BaP for six days. BaP treatment at 1 ug/ml for six days produced 19.9 percent transformation in surviving cells. Further data indicating the activity of several polycyclic carcinogens and their derivatives are summarized in Table 27. The K-region epoxides of DBahA and MCA are more active in the production of malignant transformation in hamster embryo cells than the parent hydrocarbons or the corresponding K-region phenols (Grover, et al. 1971; Huberman, et al. 1972). Although these results confirm the view that metabolism is necessary for carcinogenic activity, they conflict with data generated in vivo which indicate that K-region epoxides of polycyclic carcinogens are less active than the parent compound in various species. A possible reason for the lack of correlation is the relative instability of K-region epoxides as compared to the parent hydrocarbon when applied to the skin. It is likely that in vivo far less of the reactive K-region epoxide can survive passage through the skin to reach the basal cell layer. Furthermore, it has become apparent that the non-K-region diol-epoxide is likely to be the ultimate carcinogenic metabolite for most PAH. Several investigators have also made it evident that the toxicity and transforming activity of PAH are dissociable and occur by different processes (Landolph, et al. 1976; DiPaolo, et al. 1971a,b), with the toxicity being due to

TABLE 27

## Hamster Embryo Cell Transformation Produced by Several Polycyclic Hydrocarbons and Their Derivatives

	Concentration, µg/ml	Total No. Colonies	Cloning Efficiency, %	No. Transformed Colonies	Transformation, %	Reference
DB(a,h)A <sup>a</sup>	2.5	760	4.2	4	0.5	Huberman, et al. 1972
	5	690	3.8	4	0.7	Huberman, et al. 1972
	10	790	4.4	7	0.9	Huberman, et al. 1972
DB(a,h)A <sup>b</sup>	2.5	1,341	13.4	3	0.2	Grover, et al. 1971
	5.0	1,363	14.0	11	0.8	Grover, et al. 1971
	10	1,365	14.5	7	0.5	Grover, et al. 1971
DB(a,h)A5,6-epoxide <sup>a</sup>	2.5	598	3.3	3	0.5	Huberman, et al. 1972
	5	601	3.3	12	2.0	Huberman, et al. 1972
	7.5	395	2.5	31	7.8	Huberman, et al. 1972
	10	350	1.9	14	4.0	Huberman, et al. 1972
DB(a,h)A5,6-epoxide <sup>b</sup>	2.5	895	10.1	7	0.8	Grover, et al. 1971
	5.0	866	9.3	20	2.3	Grover, et al. 1971
	7.5	817	9.3	22	2.7	Grover, et al. 1971
	10	707	7.7	30	4.2	Grover, et al. 1971
MCA <sup>c</sup>	2.5	404	10.1	9	2.2	Huberman, et al. 1972
	5	370	9.2	10	2.7	Huberman, et al. 1972
	7.5	349	8.7	15	4.3	Huberman, et al. 1972
MCA <sup>d</sup>	2.5	664	9.6	20	3.46	DiPaolo, et al. 1971a,b
MCA epoxide <sup>c</sup>	3.5	364	2.4	13	3.6	Huberman, et al. 1972
	5	245	1.5	8	3.3	Huberman, et al. 1972
	7	103	0.7	17	16.5	Huberman, et al. 1972
BaP <sup>d</sup>	1	1,016	8.46	25	2.46	DiPaolo, et al. 1971a
	5	394	7.17	21	5.33	DiPaolo, et al. 1971a

<sup>a</sup>7-day treatment of cells seeded on a feeder layer.

<sup>b</sup>7-8 day treatment of cells.

<sup>c</sup>4-hour treatment of cells seeded in conditioned medium.

<sup>d</sup>8-day treatment of cells.

random alkylation of nucleophilic regions within the cell. However, when hamster embryo cells are pretreated with weak chemical carcinogens which can induce microsomal enzyme activity [e.g., benz(a)anthracene, methyl methanesulfonate, ethyl methanesulfonate] before the addition of a potent carcinogen (e.g., MCA, BaP, DMBA), transformation may be considerably enhanced (DiPaolo, et al. 1971a,b, 1974).

As a prescreen for chemical carcinogens, cell transformation in vitro may be one of the most sensitive techniques available. Pienta and coworkers (1977) reported that 90 percent (54/60) of the carcinogens they tested transformed hamster embryo cells in vitro, whereas none of the noncarcinogens tested showed any activity. Moreover, many of the carcinogens which have not been shown to be mutagenic toward S. typhimurium in vitro (e.g., chrysene) were capable of transforming the hamster cells. It is noteworthy, however, that large differences exist in dosage requirements for transformation among those various test systems. Calculations have been made which show that a battery of tests using S. typhimurium (Ames assay), polymerase A-deficient E. coli, and hamster embryo cell transformation is capable of detecting nearly all carcinogens tested, both PAH and non-PAH types.

The alteration of microsomal enzyme activity either in vitro or in vivo is known to have a marked effect on the carcinogenic response to PAH. Nesnow and Heidelberger (1976) reported that in 10T<sub>1/2</sub>CL8 cells, a line of contact-sensitive C3H mouse embryo fibroblasts, transformation in culture was altered by chemical modifiers of microsomal enzymes. Pretreatment of 10T<sub>1/2</sub>CL8 cells with

benz(a)anthracene, a microsomal enzyme inducer, caused a doubling in MCA-mediated transformation. Similarly, treatment with inhibitors of epoxide hydrase (e.g., cyclohexene oxide; styrene oxide; 1,2,3,4-tetra-hydronaphthalene-1,2-oxide) caused an increase in transformation over that obtained with MCA treatment alone. Thus, treatments which can induce epoxide-forming enzymes and/or lower the activity of epoxide-degrading enzymes seemed to enhance the degree of transformation in cultured cells by altering steady-state levels of oncogenic epoxides.

Chen and Heidelberger (1969a,b) developed a system using C3H mouse ventral prostate cells to examine transformation by carcinogenic hydrocarbons under conditions in which no spontaneous malignant transformation occurred. Cells treated with MCA (1  $\mu\text{g}/\text{ml}$ ) for six days in culture produced fibrosarcomas in 100 percent of mice into which they were subcutaneously injected. When treated for only one day with MCA at the single cell stage, transformed foci were found in all clones grown to confluency. A good quantitative correlation was obtained between the in vivo oncogenic activity of eight hydrocarbons (including BaP, MCA, DMBA, and DBahA) and the number of transformed colonies produced in this system. In contrast to the enhanced transforming ability of K-region epoxides relative to the parent hydrocarbon in hamster embryo cells, the K-region epoxide derived from DMBA was less active and the K-region epoxides from MCA, DBahA, and benz(a)anthracene were more active than the parent compound in mouse prostate cells (Marquardt, et al. 1972, 1974). Moreover, the epoxide derived from DMBA was more toxic than DMBA itself. The anomalous behavior of DMBA may have

been due, however, to a decreased intracellular half-life of the epoxide because of its greater chemical reactivity.

Attempts to transform human cells in culture with PAH (e.g., BaP, MCA, DMBA) have generally met with failure (Leith and Hayflick, 1974). However, Rhim and coworkers (1975) reported that a human osteosarcoma clonal cell line could be further transformed in vitro with DMBA. Morphologic alterations and abnormal growth patterns became evident in cells treated with DMBA at 2.5 and 1.0  $\mu\text{g/ml}$  in the fifth subculture 52 to 57 days after exposure. One of the altered cell lines obtained from the 1  $\mu\text{g/ml}$  treatment was tumorigenic in nude mice by subcutaneous and intracerebral injection. Interpretation of the significance of these results is made difficult by the fact that an aneuploid sarcomatous cell line had to be employed in order to demonstrate successful transformation.

The use of organ cultures for the assessment of chemical carcinogenicity suffers from the lack of reliable biochemical and morphological parameters for measuring early neoplastic changes. Nevertheless, pioneering work in the application of organ culture to chemical carcinogenesis was performed by Lasnitzki (1963). Microgram quantities of MCA added to organ cultures of rat and mouse prostate fragments caused extensive hyperplasia and squamous metaplasia. However, these preneoplastic morphological effects are generally not associated with subsequent tumor development when carcinogen-treated pieces of tissue are implanted into host animals (Heidelberger, 1973). Limited success has been achieved with organ cultures of rat tracheas, which showed characteristic morphologic alterations when treated with DMBA, BaP, and MCA (Heidelberger,

1973). In addition, Crocker (1970) has exposed respiratory epithelia from the hamster, rat, dog, and monkey to BaP at 7 to 15  $\mu\text{g/ml}$  and observed occasional squamous metaplasia. More commonly, pleomorphic cells in a dysplastic epithelium were evident as a result of the treatment. Rat tracheas maintained in organ culture have been suggested as a useful system for the predictive screening of potential carcinogens (Lindsay, et al. 1974).

A unique organ culture technique has recently been reported in which BaP (4 or 12 mg) was administered to pregnant mice (strain A and C57B1), and lung tissue of their 19- to 20-day-old embryos was subsequently explanted in culture (Shabad, et al. 1974). A transplacental influence of BaP was manifested as a proliferative stimulus in embryonic lung tissue. Hyperplasia arising in the bronchial epithelium led to the development of adenomas in a large percentage of the explants.

In the environment, man is unlikely to come in contact with only a single PAH, regardless of the route of exposure. Instead, PAH occur as complex mixtures in all environmental media. Despite this generally accepted fact, very few studies have been conducted on the carcinogenicity of defined PAH mixtures.

Among the most relevant studies conducted on the effects of PAH mixtures were those concerned with the carcinogenic components of automotive engine exhaust. Pfeiffer (1973, 1977) treated groups of 100 female NMRI mice with single subcutaneous injections of a mixture containing 10 noncarcinogenic PAH, in addition to BaP and/or dibenz(a,h)anthracene. The treatment combinations and dosages are summarized in Table 28. As the results depicted in

TABLE 28

Classification of Test Groups\*

A	Dose (µg)	Substance	B	Dose (µg)	Substance
A1	3.12	benzo(a)pyrene	B1	2.35	dibenz(a,h)anthracene
A2	6.25		B2	4.7	
A3	12.5		B3	9.3	
A4	25.0		B4	18.7	
A5	50.0		B5	37.5	
A6	100.0		B6	75.0	

C	Substance	C1 dose (µg)	C2 dose (µg)	C3 dose (µg)	C4 dose (µg)	C5 dose (µg)	C6 dose (µg)
	benzo(e)pyrene	2.15	4.3	8.75	17.5	35.5	70.0
	benzo(a)anthracene	3.125	6.25	12.5	25.0	50.0	100.0
	phenanthrene	125.0	250.0	500.0	1,000.0	2,000.0	4,000.0
	anthracene	31.25	62.5	125.0	250.0	500.0	1,000.0
	pyrene	65.1	131.2	262.5	525.0	1,050.0	2,100.0
	fluoranthene	28.1	56.25	112.5	225.0	450.0	900.0
	chrysene	3.125	6.25	12.5	25.0	50.0	100.0
	perylene	0.2	0.4	0.87	1.75	3.5	7.0
	benzo(ghi)perylene	12.8	25.6	51.25	102.5	205.0	410.0
	coronene	3.125	6.25	12.5	25.0	50.0	100.0

D	Substance	E1	E2	E3	E4	E5	E6
D1	A1 + B1	C1	+ D1				
D2	A2 + B2	C2	+ D2				
D3	A3 + B3	C3	+ D3				
D4	A4 + B4	C4	+ D4				
D5	A5 + B5	C5	+ D5				
D6	A6 + B6	C6	+ D6				

\*Source: Pfeiffer, 1977

Table 29 indicate, increases in tumor incidence could be attributed to the presence of increased amounts of BaP and of dibenz(a,h)anthracene. It is noteworthy that, at the lower dosages, dibenz(a,h)anthracene was more effective in producing tumors at the injection site than was BaP. Moreover, no effect of the 10 noncarcinogens on tumorigenic response was evident. Probit analysis of tumor incidence data indicated that the tumorigenic response from application of all 12 PAH was attributable solely to dibenz(a,h)anthracene.

Similar studies intended to reveal carcinogenic interactions among PAH found in automobile exhaust were conducted by Schmahl, et al. (1977). Eleven PAH were selected for their experiments, and various combinations were applied to the skin of NMRI mice in a proportion based on their respective weights in automobile exhaust (Table 30). Animals received twice weekly treatments for life (or until a carcinoma developed). Their results (Table 31) indicated that a mixture of carcinogenic PAH was more effective than BaP alone, and that the whole mixture (carcinogenic plus noncarcinogenic PAH) was not significantly more effective than the carcinogenic PAH group alone. Thus, the carcinogenic effects observed were solely attributable to the carcinogenic components of the mixture.

Human data: Although exposure to PAH occurs predominantly by direct ingestion (i.e., in food and in drinking water) there are no studies to document the possible carcinogenic risk to humans by this route of exposure. It is known only that significant quantities of PAH can be ingested by humans, and that in animals such exposures are known to cause cancers at various sites in the body.

TABLE 29

Tumor Incidence Resulting, by the End of the 114th Week,  
from a Single Subcutaneous Application of Test Substances\*

BaP Group (A)		DBA Group (B)		BaP + DAB Group (D)		10 PAH Group (C)		12 PAH Group (E)	
Dose ( $\mu$ g)	No. of Tumors	Dose ( $\mu$ g)	No. of Tumors	No. of Tumors	No. of Tumors	No. of Tumors	No. of Tumors	No. of Tumors	No. of Tumors
3.12	9	2.35	37	48	6	41			
6.25	35	4.7	39	44	8	55			
12.5	51	9.3	44	61	6	61			
25.0	57	18.7	56	68	4	72			
50.0	77	37.5	65	69	13	68			
100.0	83	75.0	69	79	5	82			

\*Source: Pfeiffer, 1977

TABLE 30

Doses ( $\mu\text{g}$ ) Applied in Dermal Administration  
Experiments, in Relation to Benzo(a)pyrene\*

Controls

Acetone	as solvent			
Benzo(a)pyrene	1.0	1.7	3.0	

C PAH

Benzo(a)pyrene	1.0	1.7	3.0	
-----				
Dibenz(a,h)anthracene	0.7	1.2	2.1	
Benzo(a)anthracene	1.4	2.4	4.2	
Benzo(b)fluoranthene	0.9	1.5	2.7	
total	4.0	6.8	12.0	

NC PAH

(Benzo(a)pyrene	1.0	3.0	9.0	27.0)
-----				
Phenanthrene	27.0	81.0	243.0	729.0
Anthracene	8.5	25.5	76.5	229.5
Fluoranthene	10.8	32.4	97.2	291.6
Pyrene	13.8	41.4	124.2	372.6
Chrysene	1.2	3.6	10.8	32.4
Benzo(e)pyrene	0.6	1.8	5.4	16.2
Benzo(ghi)perylene	3.1	9.3	27.9	83.7
total	65.0	195.0	585.0	1,755.0

C PAH + NC PAH

(Benzo(a)pyrene	1.0	1.7	3.0)
-----			
Total C PAH	4.0	6.8	12.0
Total NC PAH	<u>65.0</u>	<u>110.5</u>	<u>195.0</u>
Total C PAH + NC PAH	69.0	117.3	207.0

Relation of C PAH:NC PAH is constantly 1:16.25

\*Source: Schmahl, et al. 1977

TABLE 31

## Findings at the Site of Application of PAH to Mouse Skin\*

Application	Single Dose µg	Initial No. of Animals	Effective No. of Animals	Histological Diagnosis at the Site of Application				Sarcoma Abs. %	
				Negative Abs. %	Papilloma Abs. %	Carcinoma Abs. %			
Solvent	-	100	81	80	99	-	-	1	1
BaP	1.0	100	77	66	86	1	1	10	13
BaP	1.7	100	88	63	72	-	-	25	28
BaP	3.0	100	81	36	44	2	3	43	53
C PAH	4.0	100	81	52	64	4	5	25	31
C PAH	6.8	100	88	31	35	3	3	53	60
C PAH	12.0	100	90	25	28	1	1	63	70
NC PAH	65.0	100	85	84	99	-	-	1	1
NC PAH	195.0	100	84	84	100	-	-	-	-
NC PAH	585.0	100	88	87	99	-	-	1	1
NC PAH	1,755.0	100	86	70	81	-	-	15	17
C PAH + NC PAH	69.0	100	89	43	48	1	1	44	49
C PAH + NC PAH	117.3	100	93	36	39	2	2	54	58
C PAH + NC PAH	207.0	100	93	28	30	1	1	64	69

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<sup>a</sup>The decimal points have been rounded off; therefore, the sum of % values will not always be equivalent to 100%.

\*Source: Schmahl, et al. 1977

Convincing evidence from air pollution studies indicates an excess of lung cancer mortality among workers exposed to large amounts of PAH-containing materials such as coal gas, tars, soot, and coke-oven emissions (Kennaway, 1925; Kennaway and Kennaway, 1936, 1947; Henry, et al. 1931; Kuroda, 1937; Reid and Buck, 1956; Doll, 1952; Doll, et al. 1965, 1972; Redmond, et al. 1972, 1976; Mazumdar, et al. 1975; Hammond, et al. 1976; Kawai, et al. 1967). However, no definite proof exists that the PAH present in these materials are responsible for the cancers observed. Nevertheless, our understanding of the characteristics of PAH-induced tumors in animals, and their close resemblance to human carcinomas of the same target organs, strongly suggests that PAH pose a carcinogenic threat to man, regardless of the route of exposure (Santodonato, et al. 1980).

The magnitude of the carcinogenic risk of PAH to man remains obscure in the community setting. Ambient levels of PAH in air are much lower than are encountered in occupational situations, and populations exposed are much more heterogeneous with regard to age, sex, and health status. However, the current state of knowledge regarding chemical carcinogenesis would lead to the conclusion that the number of cancers produced is directly proportional to the dose received by any route. One must assume, therefore, that the small amounts of PAH present in the environment (air, food, and water) under ambient conditions contribute in some degree to the observed incidence of lung cancer in most populations.

## CRITERION FORMULATION

### Existing Guidelines and Standards

There have been few attempts to develop exposure standards for PAHs, either individually or as a class. In the occupational setting, a Federal standard has been promulgated for coke oven emissions, based primarily on the presumed effects of the carcinogenic PAH contained in the mixture as measured by the benzene soluble fraction of total particulate matter. Similarly, the American Conference of Governmental Industrial Hygienists recommends a workplace exposure limit for coal tar pitch volatiles, based on the benzene-soluble fraction containing carcinogenic PAH. The National Institute for Occupational Safety and Health has also recommended a workplace standard for coal tar products (coal tar, creosote, and coal tar pitch), based on measurements of the cyclohexane extractable fraction. These standards are summarized below:

<u>Substance</u>	<u>Exposure Limit</u>	<u>Agency</u>
Coke Oven Emissions	150 $\mu\text{g}/\text{m}^3$ , 8-hr. time-weighted average	U.S. Occupational Safety and Health Administration
Coal Tar Products	0.1 $\text{mg}/\text{m}^3$ , 10-hr. time-weighted average	U.S. National Institute for Occupational Safety and Health
Coal Tar Pitch of Volatiles	0.2 $\text{mg}/\text{m}^3$ (benzene soluble fraction) 8-hr. time-weighted average	American Conference of Governmental Industrial Hygienists

A drinking water standard for PAH as a class has been developed. The 1970 World Health Organization European Standards for Drinking Water recommends a concentration of PAH not to exceed 0.2  $\mu\text{g}/\text{l}$ . This recommended standard is based on the composite analysis

of six PAH in drinking water: 1) fluoranthene, (2) benzo(a)pyrene, (3) benzo(g,h,i) perylene, (4) benzo(b)fluoranthene, (5) benzo(k)-fluoranthene, and (6) indeno(1,3,-cd)pyrene.

The designation of these six PAH for analytical monitoring of drinking water was not made on the basis of potential health effects or bioassay data on these compounds (Borneff and Kunte, 1969). Thus, it should not be assumed that these six compounds have special significance in determining the likelihood of adverse health effects resulting from absorption of any particular PAH. They are, instead, considered to be useful indicators for the presence of PAH pollutants. Borneff and Kunte (1969) found that PAH were present in ground water at concentrations up to 50 ng/l, and in drinking water at concentrations up to 100 ng/l. Based on these data they suggested that water containing more than 200 ng/l should be rejected. However, as data from a number of U.S. cities indicate (see Exposure section), levels of PAH in raw and finished waters are typically much less than the 0.2 µg/l criterion.

#### Current Levels of Exposure

This report presents considerable data which may be used to calculate an estimate of human exposure to PAH by all routes of entry to the body. However, quantitative estimates of human exposure to PAH require numerous assumptions concerning principal routes of exposure, extent of absorption, conformity of human lifestyle, and lack of geographic-, sex-, and age-specific variables. Nevertheless, by working with estimates developed for PAH as a class, it is possible through certain extrapolations to arrive at an admittedly crude estimate of PAH exposure.

Unfortunately, there are no environmental monitoring data available for most of the PAH which are specified under the Consent Decree in NRDC v. Train. By far the most widely monitored PAH in the environment is BaP; data on BaP levels in food, air, and water are often used as a measure of total PAH. Among the PAH routinely monitored in water, four compounds are included in the Consent Decree list: BaP, IP, BbFL, and BjFL. In addition, levels of FL and BPR have been routinely determined in water, as recommended by the World Health Organization.

The reported estimated average concentrations of BaP, carcinogenic PAH (BaP, BjFL, and IP), and total PAH in drinking water are 0.55 ng/l, 2.1 ng/l, and 13.5 ng/l, respectively (see Exposure section; Basu and Saxena, 1977). Thus, assuming that a human consumes 2 liters of water per day, the daily intake of PAH via drinking water would be:

$$0.55 \text{ ng/l} \times 2 \text{ liters/day} = 1.1 \text{ ng/day (BaP)}$$

$$2.1 \text{ ng/l} \times 2 \text{ liters/day} = 4.2 \text{ ng/day (carcinogenic PAH)}$$

$$13.5 \text{ ng/l} \times 2 \text{ liters/day} = 27.0 \text{ ng/day (total PAH)}$$

Borneff (1977) estimates that the daily dietary intake of PAH is about 8 to 11  $\mu\text{g/day}$ . As a check on this estimate, PAH intake may be calculated based on reported concentrations in various foods (see Exposure section) and the per capita estimates of food consumption by the International Commission on Radiological Protection (1974). Taking a range of 1.0 to 10.0 ppb as a typical concentration for PAH in various foods, and 1,600 g/day as the total daily food consumption by man from all types of foods (i.e., fruits, vegetables, cereals, dairy products, etc.), the intake of PAH from

TABLE 32

Estimate of Human Exposure to PAH from Various Media

Source	Estimated Exposure		
	BaP	Carcinogenic PAH <sup>a</sup>	Total PAH
Water	0.0011 µg/day	0.0042 µg/day	0.027 µg/day
Food	0.160-1.6 µg/day		1.600-0.251 µg/day
Air	0.005-0.0115 µg/day	0.03-0.046 µg/day	0.164-0.251 µg/day
Total	0.166-1.6 µg/day		1.6-16 µg/day

<sup>a</sup>Total of BaP, BbFL, and IP; no data are available for food.

the diet would be in the range of 1.6 to 16.0  $\mu\text{g}/\text{day}$ . An estimate of BaP ingestion from the diet may be similarly derived. Using 0.1 to 1.0 ppb as the range of BaP concentration in various foods, total daily BaP intake would be 0.16 to 1.6  $\mu\text{g}/\text{day}$ .

Ambient air is reported to contain average levels of 0.5  $\text{ng}/\text{m}^3$ , 2.0  $\text{ng}/\text{m}^3$ , and 10.9  $\text{ng}/\text{m}^3$  for BaP, carcinogenic PAH, and total PAH, respectively (see Exposure section, Table 15). Taking the range of 15  $\text{m}^3$  to 23  $\text{m}^3$  as the average amount of air inhaled by a human each day results in an estimated intake of 0.005 to 0.0115  $\text{ng}/\text{day}$ , 0.03 to 0.046  $\text{ng}/\text{day}$ , and 0.164 to 0.251  $\text{ng}/\text{day}$  for BaP, carcinogenic PAH, and total PAH, respectively.

In summary, a crude estimate of total daily exposure to PAH would be as shown in Table 32.

Two important factors are not taken into account in this estimate. First, it is known that tobacco smoking can contribute greatly to PAH exposure in man. Exposure to BaP from smoking one pack of cigarettes per day was shown to be 0.4  $\mu\text{g}/\text{day}$  (NAS, 1972). Second, the possibility for dermal absorption of PAH is assumed to contribute only a negligible amount to the total exposure. Only in certain occupational situations is dermal exposure expected to be quantitatively important.

#### Special Groups at Risk

An area of considerable uncertainty with regard to the carcinogenic hazard of PAH to man involves the relationship between aryl hydrocarbon hydroxylase (AHH) activity and cancer risk. Genetic variation in AHH inducibility has been implicated as a determining factor for susceptibility to lung and laryngeal cancer (Kellerman,

et al. 1973a,b). It was suggested that the extent of AHH inducibility in lymphocytes was correlated with increasing susceptibility to lung cancer formation.

Paigen, et al. (1978) have examined the question of genetic susceptibility to cancer, and concluded that epidemiologic evidence supports this hypothesis. Moreover, they were able to show that AHH inducibility in lymphocytes segregates in the human population as a genetic trait. However, their studies failed to find a correlation between this inducibility and presumed cancer susceptibility, either among healthy relatives of cancer patients or in patients who had their cancer surgically removed. It is noteworthy that previous investigations on AHH inducibility were conducted in persons with active cancer.

Recent studies with other human tissues (liver and placenta) have provided important new data concerning the carcinogen-metabolizing capacity of man and its implications for cancer susceptibility. Conney, et al. (1976) examined individual differences in the metabolism of drugs and carcinogens in human tissues, and have identified drugs which may serve as model substrates to provide an indirect index of carcinogen metabolism for man. The rates for antiprene, hexobarbital, and zoxazolamine hydroxylation in human autopsy livers were highly, but not perfectly, correlated with the rates of BaP metabolism. In human placenta, an almost perfect correlation was found between zoxazolamine hydroxylase activity and BaP hydroxylase activity (Kapitulnik, et al. 1976a). Thus, metabolism of BaP and zoxazolamine by human placenta occurs by the same enzyme system(s) or by different enzyme systems under the same reg-

ulatory control (Kapitulnik, et al. 1977a). BaP and zoxazolamine hydroxylase activities were also shown to be significantly enhanced in placentas obtained from women who smoked cigarettes.

The lack of perfect correlations for the hepatic metabolism of BaP and certain drugs in many subjects indicated the presence of several monooxygenases in human liver which catalyze the oxidative metabolism of these compounds. Furthermore, large inter-individual differences exist in the capacity of humans to metabolize foreign chemicals both in vitro and in vivo. Further studies showed that 7,8-benzoflavone markedly stimulated the hydroxylation of BaP, antiprene, and zoxazolamine in human liver samples, but with a wide variation in magnitude among different samples. These results suggested the presence of multiple monooxygenases or cytochrome P-450 in the different liver samples (Kapitulnik, et al. 1977b). Moreover, 7,8-benzoflavone did not affect the hydroxylation of coumarin or hexobarbital, thereby indicating the existence of different monooxygenases for metabolism of these substrates.

Multiple forms of cytochrome P-450 have been shown in the livers of rats, rabbits, and mice, but not thus far in humans (Kapitulnik, et al. 1977a). More important, however, MCA is a potent inducer of BaP hydroxylase activity in rats but does not stimulate antiprene hydroxylase, clearly suggesting that metabolism of PAH in rodents may be regulated by different enzyme systems than in humans (Kapitulnik, et al. 1977a).

In contrast to the apparent multiplicity of cytochrome P-450 dependent enzyme systems for the oxidative metabolism of PAH in man, a single epoxide hydrase with broad substrate specificity may

be present in human liver (Conney, et al. 1976; Kapitulnik, et al. 1977c). Because the hydration of arene oxides may lead to the formation of dihydrodiol carcinogen precursors, the capacity of different humans to metabolize epoxides may affect cancer susceptibility. It is not known, however, if enhanced dihydrodiol formation would increase cancer risk or decrease cancer risk.

Thomson and Slaga (1976) did not obtain a correlation of AHH induction with skin-tumor-inducing ability in mice for a series of unsubstituted hydrocarbons. Nevertheless, the highest AHH enzyme activity was found in the epidermal layer of the skin, which is the major point of contact with many environmental chemicals. These results may be interpreted to indicate that a chemical carcinogen may not necessarily induce its own bioactivation, but instead can be transformed into a reactive intermediate by virtue of increased AHH activity stimulated by other noncarcinogenic compounds.

Due consideration must also be given to the fact that, in addition to the initiation of resting cells by a chemical carcinogen, a promotion phase involving cell proliferation is also involved in skin carcinogenesis (Yuspa, et al. 1976). Therefore, although certain aromatic hydrocarbons are effective enzyme inducers, their bioactivated metabolites may function only as initiators having no promoting ability. A potent complete carcinogen, however, will be transformed not only into a powerful tumor initiator but will also be able to interact with cellular membranes, alter genetic expression, and ultimately cause irreversible cell proliferation. These observations raise certain doubts concerning the validity and/or reliability of equating enzyme inducibility with

carcinogenic potential for chemical agents. Further reinforcement of this opinion has been provided by Shulte-Hermann (1977) who showed that cell proliferation is not a direct result of enzyme induction, even though both processes are normally coupled.

The further possibility that the genetics of AHH inducibility is organ-dependent rather than strain-dependent in animals has important implications for evaluating susceptibility to PAH-induced cancers (Kouri, et al. 1976). Most significant is the demonstration that pulmonary AHH may be inducible in all strains of mice, regardless of the inducibility of hepatic AHH. Since the respiratory epithelium represents a primary portal of entry for PAH, AHH activity which is induced in this tissue may bear importantly on susceptibility to malignancy.

Enzyme induction by PAH is not limited to AHH. Owens (1977) recently demonstrated that MCA can induce hepatic UDP-glucuronosyltransferase activity in certain inbred strains of mice. This enzyme catalyzes the conjugation and excretion of PAH substrates after they have first been oxygenated by AHH. The induction of this transferase activity and that of AHH was apparently regulated by a single genetic locus. However, transferase inducibility does not depend on AHH levels, but rather is stoichiometrically related to the concentration of a specific and common cytosolic receptor regulating both enzyme induction processes. Owens further demonstrated that AHH activity can be fully induced in certain mouse strains (e.g., by 2,3,7,8-tetrachlorodibenzo-p-dioxin) without greatly enhancing the transferase activity. Earlier studies had established that chrysene and chlorpromazine were potent inducers

of AHH activity while having little effect on transferase activity (Aitio, 1974a,b). Subsequent exposure to carcinogenic PAH (i.e., MCA) could lead to maximal oxidative metabolism but little transferase-catalyzed removal of metabolites by glucuronic acid conjugation. This situation would be exacerbated by the fact that metabolites of MCA are incapable of further inducing the transferase activity. This effect may have considerable toxicologic significance in that the highly reactive epoxides of PAH formed by the action of AHH under these circumstances may not be adequately removed by glucuronidation. Thus, one must consider the total exposure of all environmental agents and their possible effect on critical enzymatic processes before attempting to assess the toxicologic impact of exposure to a specific PAH. In summary, there is a need to further explore the relative effects of enzyme induction on the metabolic activation of chemicals to toxic products, versus metabolism of chemicals via detoxification pathways, when considering the possibility of special groups at risk.

#### Basis and Derivation of Criterion

The presently available data base is inadequate to support the derivation of individual criteria for each of the PAH as specified under the Consent Decree. This problem arises primarily from the diversity of test systems and bioassay conditions employed for determining carcinogenic potential of individual PAH in experimental animals. Furthermore, it is not possible to estimate the intake via water of individual PAH, except for those compounds which have been selected by the World Health Organization for environmental monitoring. Therefore, an approach to criterion development is

adopted in this report with the objective of deriving criteria for individual carcinogenic PAH, which will lead to effective control of PAH as a class. This approach is attractive in that it recognizes the fact that environmental exposures to PAH invariably occur by contact with complex, undefined, PAH mixtures.

The attempt to develop a drinking water criterion for PAH as a class is hindered by several gaps in the scientific data base:

- (1) The PAH class is composed of numerous compounds having diverse biological effects and varying carcinogenic potential. A "representative" PAH mixture, has not been defined.
- (2) The common practice of using data derived from studies with BaP to make generalizations concerning the effects of environmental PAH may not be scientifically sound.
- (3) No chronic animal toxicity studies involving oral exposure to PAH mixtures exist.
- (4) No direct human data concerning the effects of exposure to defined PAH mixtures exist.

However, assuming that the development of a criterion must proceed despite these obstacles, certain approaches may be taken to circumvent deficiencies in the data base. The choice of an appropriate animal bioassay from which to derive data for application to the human cancer risk assessment should be guided by several considerations. Primary emphasis must be placed on appropriate animal studies which: (1) include sufficient numbers of animals for statistically reliable results; (2) involve long-term low-level exposures to PAH; (3) include a proper control group; and (4) achieve positive dose-related carcinogenic response.

Because there are no studies available regarding chronic oral exposure to PAH mixtures, it is necessary to derive a criterion based upon data involving exposure to a single compound. Two studies can be selected, one involving BaP ingestion (Neal and Rigdon, 1967) and one involving DBA ingestion (Snell and Stewart, 1962). Both compounds are recognized as animal carcinogens, and both are known to be environmental contaminants to which humans are exposed.

Presently, there is no way to quantitate the potential human health risks incurred by the interaction of PAH, either among themselves or with other agents (e.g., tumor initiators, promoters, inhibitors) in the environment. In addition, it is known that PAH commonly produce tumors at the site of contact (i.e., forestomach tumors by oral exposure to BaP; lung tumors by intratracheal administration; skin tumors by dermal application). Thus, consideration of the extent of absorption may not always be necessary in the case of carcinogenic PAH, and will in fact result in underestimation of actual risk if only distant target sites are considered. Calculation of the water quality criterion based upon bioassay data for BaP is presented in the Appendix.

The water quality criterion for BaP derived using the linearized multistage model, as described in the Human Health Methodology Appendices to the October 1980 Federal Register notice which announced the availability of this document, is 28 ng/l. For the sake of comparison, a water quality criterion for DBA was calculated using the procedure developed by Mantel and Bryan (1961). As opposed to the linearized multistage model, which is logistic and defines acceptable risk as 1/100,000, the Mantel and Bryan (1961)

model is probabilistic and defines acceptable risk as 1/100,000,000. Furthermore, the Mantel and Bryan model (1961) is concerned with the maximum tumor incidence in treated animals at the 99 percent confidence level versus the 95 percent confidence level in the linearized multistage model. Using the Mantel and Bryan (1961) approach with DBA, the resultant water quality criterion is 13.3 ng/l.

Under the Consent Decree in NRDC v. Train, criteria are to state "recommended maximum permissible concentrations (including where appropriate, zero) consistent with the protection of aquatic organisms, human health, and recreational activities." BaP is a known animal carcinogen. Because there is no recognized safe concentration for a human carcinogen, the recommended concentration in water for maximum protection of human health is zero.

Because attaining a zero concentration level may be infeasible in some cases and in order to assist the Agency and states in the possible future development of water quality regulations, the concentrations of BaP corresponding to several incremental lifetime cancer risk levels have been estimated. A cancer risk level provides an estimate of the additional incidence of cancer that may be expected in an exposed population. A risk of  $10^{-5}$  for example, indicates a probability of one additional case of cancer for every 100,000 people exposed, a risk of  $10^{-6}$  indicates one additional case of cancer for every million people exposed, and so forth.

PAH are widely distributed in the environment as evidenced by their detection in sediments, soils, air, surface waters, and plant and animal tissues. The ecological impact of these chemicals, how-

ever, is uncertain. Numerous studies show that despite their high lipid solubility, PAH show little tendency for bioconcentration in the fatty tissues of animals or man. This observation is not unexpected, in light of convincing evidence to show that PAH are rapidly and extensively metabolized.

Lu, et al. (1977) have published the only available study regarding the bioconcentration and biomagnification of a PAH in model ecosystem environments. They reported that the bioconcentration of BaP, expressed as concentration in mosquitofish/concentration in water was zero. This was apparently due to the fact that the fish metabolized the BaP about as rapidly as it was absorbed. On the other hand, in a 33-day terrestrial-aquatic model ecosystem study, BaP showed a small degree of biomagnification which probably resulted from food chain transfer. In this case the biomagnification factor for mosquitofish was 30. Based on the results of Lu, et al. (1977) a bioconcentration (BCF) factor of 30 was employed for the purpose of calculating a water quality criterion.

In the Federal Register notice of availability of draft ambient water quality criteria, EPA stated that it is considering setting criteria for BaP at an interim target risk level of  $10^{-5}$ ,  $10^{-6}$ , or  $10^{-7}$  as shown in the following table.

<u>Exposure Assumptions</u> (per day)	<u>BaP</u> <u>Risk Levels and Corresponding Criteria (1)</u>			
		ng/l		
	<u>0</u>	<u>10<sup>-7</sup></u>	<u>10<sup>-6</sup></u>	<u>10<sup>-5</sup></u>
2 liters of drinking water and consumption of 6.5 grams fish and shellfish (2)	0	0.28	2.8	28.0
Consumption of fish and shellfish only.		3.11	31.1	311.0

- (1) Calculated by applying a linearized multistage model as previously discussed. Appropriate bioassay data used in the calculation of the model are presented in the Appendix. Since the extrapolation model is linear at low doses, the additional lifetime risk is directly proportional to the water concentration. Therefore, water concentrations corresponding to other risk levels can be derived by multiplying or dividing one of the risk levels and corresponding water concentrations shown in the table by factors such as 10, 100, 1,000, and so forth.
- (2) Approximately 9 percent of the PAH exposure, assumed to be BaP, results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of 30-fold based on the work of Lu, et al. (1977). The remaining 91 percent of PAH exposure results from drinking water.

Concentration levels were derived assuming a lifetime exposure to various amounts of PAH (1) occurring from the consumption of both drinking water and aquatic life grown in water containing the corresponding PAH concentrations and, (2) occurring solely from the consumption of aquatic life grown in the waters containing the cor-

responding PAH concentrations. Because data indicating other sources of exposure and the concentration to total body burden are inadequate for quantitative use, the criterion reflects the increment to risks associated with ambient water exposure only.

## REFERENCES

Abe, S. and M. Sasaki. 1977. Studies on chromosomal aberrations and sister chromatid exchanges induced by chemicals. Proc. Japan Acad. 53: 46.

Ahlstrom, U. 1974. Chromosomes of primary carcinomas induced by 7,12-dimethylbenz(a)-anthracene in the rat. Hereditas. 78: 235.

Ahokas, J.T., et al. 1975. Metabolism of polycyclic hydrocarbons by a highly active aryl hydrocarbon hydroxylase system in the liver of a trout species. Biochem. Biophys. Res. Comm. 63: 635.

Aitio, A. 1974a. Different elimination and effect on mixed function oxidase of 20-methylcholanthrene after intragastric and intraperitoneal administration. Res. Comm. Chem. Pathol. Pharmacol. 9: 701.

Aitio, A. 1974b. Effect of chrysene and carbon tetrachloride administration on rat hepatic microsomal monooxygenase and UDP glucuronosyltransferase activity. FEBS Lett. 42: 46.

Akin, F.J. 1976. Anti-tumorigenic effect of maleic hydrazide on mouse skin. Jour. Agric. Food Chem. 24: 672.

Albert, R., et al. 1978. Temporal aspects of tumorigenic response to individual and mixed carcinogens. Comprehensive Prog. Rep. Inst. Environ. Med., New York Univ. Med. Center, New York.

Andelman, J.B. and J.E. Snodgrass. 1974. Incidence and Significance of Polynuclear Aromatic Hydrocarbons in the Water Environment. In: CR, Critical Reviews in Environmental Control. p. 69.

Andelman, J.B. and M.J. Suess. 1970. Polynuclear aromatic hydrocarbons in the water environment. Bull. World Health Organ. 43: 479.

Andrews, L.S., et al. 1976. Characterization and induction of aryl hydrocarbon [benzo(a)pyrene] hydroxylase in rabbit bone marrow. Res. Comm. Chem. Pathol. Pharmacol. 15: 319.

Arcos, J.S. and M.F. Argus. 1974. Chemical Induction of Cancer. Vol. IIA. Academic Press, New York.

Autrup, H., et al. 1978. Metabolism of (<sup>3</sup>H)benzo(a)pyrene by cultured human bronchus and cultured human pulmonary alveolar macrophages. Lab. Inv. 38: 217.

Bailey, E.J. and N. Dungal. 1958. Polycyclic hydrocarbons in Iceland smoked food. Br. Jour. Cancer. 12: 348.

Baldwin, R.W. 1973. Immunological Aspects of Chemical Carcinogenesis. In: G. Klein and S. Weinhouse (eds.), Advances in Cancer Research. Academic Press, New York, London. 18: 1.

Bartle, K.D., et al. 1974. High-resolution GLC profiles of urban air pollutant polynuclear aromatic hydrocarbons. Int. Jour. Environ. Anal. Chem. 3: 349.

Bast, R.C., Jr., et al. 1976. Development of an assay for aryl hydrocarbon [benzo(a)pyrene] hydroxylase in human peripheral blood monocytes. Cancer Res. 36: 1967.

Basu, D.K. and J. Saxena. 1977. Analysis of raw and drinking water samples for polynuclear aromatic hydrocarbons. EPA P.O. No. CA-7-2999-A, and CA-8-2275-B. U.S. Environ. Prot. Agency, HERL, Cincinnati, Ohio.

Basu, D.K. and J. Saxena. 1978. Polynuclear aromatic hydrocarbons in selected U.S. drinking waters and their raw water sources. Environ. Sci. Technol. 12: 795.

Bayer, U. 1978. In vivo Induction of Sister Chromatid Exchanges by Three Polyaromatic Hydrocarbons. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: 2nd. Int. Symp. on Analysis, Chemistry, and Biology (Carcinogenesis - A Comprehensive Survey: Vol. 3). Raven Press, New York. 3: 101.

- Bend, J.R., et al. 1976. Hepatic and Extrahepatic Glutathione S-transferase Activity Toward Several Arene Oxides and Epoxides in the Rat. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis (Carcinogenesis - A Comprehensive Survey: Vol. 1). Raven Press, New York. 1: 63.
- Berneblum, I. 1941. The cocarcinogenic action of croton resin. Cancer Res. 1: 44.
- Berwald, Y. and L. Sachs. 1963. In vitro transformation with chemical carcinogens. Nature. 200: 1182.
- Berwald, Y. and L. Sachs. 1965. In vitro transformation of normal cells to tumor cells by carcinogenic hydrocarbons. Jour. Natl. Cancer Inst. 35: 641.
- Biedler, J.L., et al. 1961. Chromosome lesions associated with carcinogen-induced tumors in mice. Nature. 192: 286.
- Biernoth, G. and H.E. Rost. 1967. The occurrence of PAH in coconut oil and their removal. Chem. Ind. 45: 2002.
- Biernoth, G. and H.E. Rost. 1968. The occurrence of PAH in edible oils and their removal. Arch. Hyg. 152: 238. (Berl.)

Binet, L. and L. Mallet. 1964. Diffusion of PAH in the living environment. Gaz. Hop. (Paris) 135: 1142. (Included in Chem. Abstr. 60: 2282c).

Bird, C.C., et al. 1970. Protection from the embryopathic effects of 7-hydroxymethyl-12-methylbenz(a)anthracene by 2-methyl-1,2-bis-(3-pyridyl)-1-propanone (metopirone ciba) and -diethylamino-ethyl-diphenyl-n-propyl acetate (SKR 525-A). Br. Jour. Cancer 24: 548.

Bjørseth, A. 1978. Analysis of Polycyclic Aromatic Hydrocarbons in Environmental Samples by Glass Capillary Gas Chromatography. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: 2nd. Int. Symp. on Analysis, Chemistry, and Biology (Carcinogenesis - A Comprehensive Survey: Vol. 3). Raven Press, New York. 3: 75.

Bock, F.G. 1964. Early effects of hydrocarbons on mammalian skin. Progr. Exp. Tumor Res. 4: 126.

Bock, F.G. and T.L. Dao. 1961. Factors affecting the polynuclear hydrocarbon level in rat mammary glands. Cancer Res. 21: 1024.

Booth, J., et al. 1974. The metabolism of polycyclic hydrocarbons by cultured human lymphocytes. FEBS Lett. 43: 341.

Borneff, J. 1964. Carcinogenic substances in water and soil. Part XV: Interim results of the former investigations. Arch. Hyg. 148: 1. (Berl.)

Borneff, J. 1977. Fate of carcinogens in aquatic environment. Prepublication copy received from author.

Borneff, J. and H. Kunte. 1964. Carcinogenic substances in water and soil. XVI: Evidence of PAH in water samples through direct extraction. Arch. Hyg. Bakt. 148: 585.

Borneff, J. and H. Kunte. 1965. Carcinogenic substances in water and soil. XVII: About the origin and evaluation of PAH in water. Arch. Hyg. Bakt. 149: 226.

Borneff, J. and H. Kunte. 1969. Carcinogenic substances in water and soil. XXVI: A routine method for the determination of PAH in water. Arch. Hyg. (Berl) 153: 220.

Boyland, E. and P. Sims. 1967. The carcinogenic activities in mice of compounds related to benz(a)anthracene. Int. Jour. Cancer. 2: 500.

Boyland, E., et al. 1965. Induction of adrenal damage and cancer with metabolites of 7,12-dimethylbenz(a)anthracene. Nature. 207: 816.

- Brookes, P. 1977. Mutagenicity of polycyclic aromatic hydrocarbons. *Mutat. Res.* 39: 257.
- Butenandt, A. and H. Dannenberg. 1956. The Biochemistry of Tumors. In: F. Buchner, et al. (ed.), Springer-Verlag, Handbuch der Allgemeinen Pathologie. 6: 107. (Berlin)
- Buu-Hoi, N.P. 1959. Carcinogenic Materials. In: K.F. Bauer (ed.), Georg Thieme Verlag. Medizinische Grundlagen Forschung. Stuttgart. 2: 465.
- Buu-Hoi, N.P. 1964. New developments in chemical carcinogenesis by polycyclic hydrocarbons and related heterocycles: A review. *Cancer Res.* 24: 1511.
- Cahnmann, H.J. and M. Kuratsune. 1957. Determination of polycyclic aromatic hydrocarbons in oysters collected in polluted water. *Anal. Chem.* 29: 1312.
- Cawein, M.J. and K.L. Sydnor. 1968. Suppression of cellular activity in the reticuloendothelial system of the rat by 7,12-dimethylbenz(a)anthracene. *Cancer Res.* 28: 320.
- Chalmers, J.G. and A.H.M. Kirby. 1940. The elimination of 3,4-benzpyrene from the animal body after subcutaneous injection. I. Unchanged benzpyrene. *Biochem. Jour.* 34: 1191.

- Chen, T.T. and C. Heidelberger. 1969a. In vitro malignant transformation of cells derived from mouse prostate in the presence of 3-methylcholanthrene. Jour. Natl. Cancer Inst. 42: 915.
- Chen, T.T. and C. Heidelberger. 1969b. Quantitative studies on the malignant transformation of mouse prostate cells by carcinogenic hydrocarbons in vitro. Int. Jour. Cancer. 4: 166.
- Chu, E.W. and R.A. Malmgren. 1965. An inhibitory effect of vitamin A on the induction of tumors in the forestomach and cervix in the Syrian hamster by carcinogenic polycyclic hydrocarbons. Cancer Res. 25: 885.
- Chuang, A.H.L., et al. 1977. Aryl hydrocarbon hydroxylase in mouse mammary gland: in vitro study using mammary cell lines. Chem. Biol. Inter. 17: 9.
- Cohn, J.A., et al. 1977. On the occurrence of cytochrome P-450 and aryl hydrocarbon hydroxylase activity in rat brain. Jour. Exp. Med. 145: 1607.
- Colucci, J.M. and C.R. Begeman. 1971. Polynuclear Aromatic Hydrocarbons and Other Pollutants in Los Angeles Air. In: Proc. Int. Clean Air Cong. Academic Press. 2: 28.

Cone, M.V. and P. Nettesheim. 1973. Effects of vitamin A on 3-methylcholanthrene-induced squamous metaplasia and early tumors in the respiratory tract of rats. Jour. Natl. Cancer Inst. 50: 1599.

Conney, A.H. 1967. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. 19: 317.

Conney, A.H., et al. 1976. Use of Drugs in the Evaluation of Carcinogen Metabolism in Man. In: R. Montesano and L. Tomatis (eds.), Screening Tests in Chemical Carcinogenesis. IARC Publ. No. 12. Lyon, France. p. 319.

Conney, A.H., et al. 1977a. Metabolism and Biological Activity of Benzo(a)pyrene and its Metabolic Products. In: D.J. Jallow, et al. (eds.), Biological Reactive Intermediates. Plenum Press.

Conney, A.H., et al. 1977b. Regulation of drug metabolism in man by environmental chemicals and diet. Fed. Proc. 36: 1647.

Cordle, F., et al. 1978. Human exposure to polychlorinated biphenyls and polybrominated biphenyls. Environ. Health Perspect. 24: 157.

Cornfield, J. 1977. Carcinogenic risk assessment. Science. 198: 693.

Crocker, T.T. 1970. Effect of Benzo(a)pyrene on Hamster, Rat, Dog, and Monkey Respiratory Epithelia in Organ Culture. In: Proc. Biol. Div., Oak Ridge Natl. Lab. Conf. in Gatlinburg, Tenn. Oct. 8-11, 1968. AEC Symp. Series 18, Oak Ridge, Tennessee. Div. Tech. Info., U.S. Atomic Energy Comm.

Currie, A.R., et al. 1970. Embryopathic effects of 7,12-dimethylbenz(a)anthracene and its hydroxymethyl derivatives in the Sprague-Dawley rat. Nature. 226: 911.

Czygan, P., et al. 1974. The effect of dietary protein deficiency on the ability of isolated hepatic microsomes to alter the mutagenicity of a primary and a secondary carcinogen. Cancer Res. 34: 119.

Dao, T.L., et al. 1959. Level of 3-methylcholanthrene in mammary glands of rats after intragastric instillation of carcinogen. Proc. Soc. Exptl. Biol. Med. 102: 635.

Davies, R.I. and G. Wynne-Griffith. 1954. Cancer and soils in the country of Anglesey. Br. Jour. Cancer. 8: 56.

Diehl, J.S. and S.W. Tromp. 1953. First report on the geographical and geological distribution of carcinogens in the Netherlands. Leiden Foundation for the Study of Psychophysics.

Dikun, P.P. and A.I. Makhinenko. 1963. Detection of BP in the schistose plant resins, in its effluents and in water basins after discharge of effluents. Gig. i. Sanit. 28: 10.

DiPaolo, J.A. and P.J. Donovan. 1967. Properties of Syrian hamster cells transformed in the presence of carcinogenic hydrocarbons. Experi. Cell Res. 48: 261.

DiPaolo, J.A., et al. 1971a. Transformation of hamster cells in vitro by polycyclic hydrocarbons without cytotoxicity. Proc. Natl. Acad. Sci. 68: 2958.

DiPaolo, J.A., et al. 1971b. Characteristics of primary tumors induced by carcinogenic polycyclic hydrocarbons in Syrian hamsters. Jour. Natl. Cancer Inst. 46: 171.

DiPaolo, J.A., et al. 1974. Enhancement by alkylating agents of chemical carcinogen transformation of hamster cells in culture. Chem. Biol. Inter. 9: 351.

Doll, R. 1952. The causes of death among gas workers with special reference to cancer of the lung. Br. Jour. Ind. Med. 9: 180.

Doll, R., et al. 1965. Mortality of gas workers with special reference to cancers of the lung and bladder, chronic bronchitis, and pneumoconiosis. Br. Jour. Ind. Med. 22: 1.

Doll, R., et al. 1972. Mortality of gas workers - final report of a prospective study. Br. Jour. Ind. Med. 29: 394.

Draudt, H.N. 1963. The meat smoking process: A review. Food Technol. 17: 85.

Dungal, N. 1961. Can smoked food be carcinogenic? Acta Unio Intern. Contra. Cancrum. 17: 365.

Dunn, B.P. and H.F. Stich. 1976. Release of the carcinogen benzo(a)pyrene from environmentally contaminated mussels. Bull. Environ. Contam. Toxicol. 15: 398.

Fabian, B. 1965. Carcinogenic substances in edible fat and oil. Part VI: Further investigations on margarine and chocolate. Arch. Hyg. 153: 21. (Berl.)

Falk, H.L., et al. 1964. Inhibition of carcinogenesis. The effect of polycyclic hydrocarbons and related compounds. Arch. Environ. Health. 9: 169.

Faoro, R.B. and J.A. Manning. 1978. Trends in benzo(a)pyrene.

Fedorenko, Z.P. 1964. The effect of biochemical treatment of wastewater of a by-product coke plant on the BP content. Gig. i. Sanit. 29: 17.

Feron, V.J., et al. 1973. Dose-response correlation for the induction of respiratory-tract tumors in Syrian golden hamsters by intratracheal instillations of benzo(a)pyrene. Eur. Jour. Cancer 9: 387.

Filipovic, J. and L. Toth. 1971. Polycyclische Kohlenwasserstoffe in Geraeucherten Jugoslawischen Fleischwaren. Fleischwirtschaft. 51: 1323.

Flesher, J.S. 1967. Distribution of radioactivity in the tissues of rats after oral administration of 7,12-dimethylbenz(a)anthracene-<sup>3</sup>H. Biochem. Pharmacol. 16: 1821.

Flesher, J.S., et al. 1976. Oncogenicity of K-region epoxides of benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene. Int. Jour. Cancer. 18: 351.

Ford, E. and C. Huggins. 1963. Selective destruction in testis induced by 7,12-dimethylbenz(a)anthracene. Jour. Exp. Med. 118: 27.

Fox, M.A. and S.W. Staley. 1976. Determination of polycyclic aromatic hydrocarbons in atmospheric particulate matter by high pressure liquid chromatography coupled with fluorescence techniques. Anal. Chem. 48: 992.

- Frethein, K. 1976. Carcinogenic polycyclic aromatic hydrocarbons in Norwegian smoked meat. *Jour. Agric. Food Chem.* 24: 976.
- Freudenthal, R.I., et al. 1978. A Comparison of the Metabolites of Benzo(a)pyrene by Lung Mixed Function Oxidase from Rat, Rhesus, and Humans. In: R.I. Freudenthal and P.W. Jones (eds.), *Polynuclear Aromatic Hydrocarbons: 2nd. Int. Symp. on Analysis, Chemistry, and Biology (Carcinogenesis - A Comprehensive Survey: Vol. 3)*. Raven Press, New York. 3: 313.
- Gelboin, H.V., et al. 1972. Microsomal Hydroxylases: Studies on the Mechanism of Induction and their Role in Polycyclic Hydrocarbon Action. In: *Collection of Papers Presented at the Ann. Symp. on Fundamental Cancer Res., Ser. 24.* p. 214.
- Gordon, R.J. 1976. Distribution of airborne polycyclic aromatic hydrocarbons throughout Los Angeles. *Environ. Sci. Technol.* 10: 370.
- Gordon, R.J. and R.J. Bryan. 1973. Patterns of airborne polynuclear hydrocarbon concentrations at four Los Angeles sites. *Environ. Sci. Technol.* 7: 1050.
- Gottschalk, R.G. 1942. Quantitative studies on tumor production in mice by benzpyrene. *Proc. Soc. Exp. Biol. Med.* 50: 369.

Graf, W. and W. Nowak. 1966. Promotion of growth in lower and higher plants by carcinogenic polycyclic aromatics. Arch. Hyg. Bakt. 150: 513.

Graf, W., et al. 1975. Levels of carcinogenic polycyclic aromatic hydrocarbons in human and animal tissues. 3rd communication. Zbl. Bakt. Hyg. I. Abst. Orig. B. 161: 85.

Greinke, R.A. and I.C. Lewis. 1975. Development of a gas chromatographic - Ultraviolet absorption spectrometric method for monitoring petroleum pitch volatiles in the environment. Anal. Chem. 47: 2151.

Grimmer, G. 1974. Detection and occurrence of polycyclic hydrocarbons in yeast cultured on mineral oils. Dtsch. Lebensm. Rundsch. 70: 394.

Grimmer, G. and A. Hildebrandt. 1967. Content of polycyclic hydrocarbons in crude vegetable oils. Chem. Ind. p. 2,000.

Grover, P.L., et al. 1971. In vitro transformation of rodent cells by K-region derivatives on polycyclic hydrocarbons. Proc. Natl. Acad. Sci. 68: 1098.

Grundin, R., et al. 1973. Induction of microsomal aryl hydrocarbon (3,4-benzo(a)pyrene) hydroxylase and cytochrome P-450 in rat cortex. I. Characteristics of the hydroxylase system. Arch. Biochem. Biophys. 158: 544.

Guerrero, H., et al. 1976. High-pressure liquid chromatography of benzo(a)pyrene and benzo(g,h,i)perylene in oil-contaminated shellfish. Jour. Assoc. Off. Anal. Chem. 59: 989.

Haber, S.L. and R.W. Wissler. 1962. Effects of vitamin E on carcinogenicity of methylcholanthrene. Proc. Soc. Exp. Biol. Med. 111: 774.

Haddow, A., et al. 1937. The influence of certain carcinogenic and other hydrocarbons on body growth in the rat. Proc. R. Soc. B. 122: 477.

Hammond, E.C., et al. 1976. Inhalation of benzpyrene and cancer in man. Ann. N.Y. Acad. Sci. 271: 116.

Hansch, C. and A.J. Leo. 1979. Substituent Constants for Correlation Analysis in Chemistry and Biology. Wiley-Interscience, New York.

Harrison, R.M., et al. 1975. Polynuclear aromatic hydrocarbons in raw, potable, and waste waters. Water Res. 9: 331.

Harrison, R.M., et al. 1976. Effect of water chlorination upon levels of some polynuclear aromatic hydrocarbons in water. *Environ. Sci. Technol.* 12: 1151.

Hartwell, J.L. and P. Shubik. 1951. Survey of compounds which have been treated for carcinogenic activity. *Pub. Health Ser. Publ.* 149 (2nd ed.). U.S. Govern: Print. Off., Washington, D.C.

Hecht, S.S., et al. 1976. On the Structure and Carcinogenicity of the Methylchrysenes. In: R.I. Freudentahl and P.W. Jones (eds.), *Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis (Carcinogenesis - A Comprehensive Survey: Vol. 1)*. Raven Press, New York 1: 325.

Heidelberger, C. 1973. Chemical oncogenesis in culture. *Adv. Cancer Res.* 18: 317.

Heidelberger, C. 1975. Chemical carcinogenesis. *Ann. Rev. Biochem.* 44: 79.

Heidelberger, C. and P.R. Boshell. 1975. Chemical oncogenesis in cultures. *Gann Monogr. Cancer Res.* 17: 39.

Heidelberger, C. and S.M. Weiss. 1951. The distribution of radioactivity in mice following administration of 3,4-benzpyrene-5C<sup>14</sup> and 1,2,5,6-dibenzanthracene-9,10-C<sup>14</sup>. *Cancer Res.* 11: 885.

Hellstrom, K.E. 1959. Chromosome studies on primary methylcholanthrene-induced sarcomas in the mouse. Jour. Natl. Cancer Inst. 23: 1019.

Henry, M.C., et al. 1973. Respiratory tract tumors in hamsters induced by benzo(a)pyrene. Cancer Res. 33: 1585.

Henry, M.C., et al. 1975. Importance of physical properties of benzo(a)pyrene-ferric oxide mixtures in lung tumor induction. Cancer Res. 35: 207.

Henry, S.A., et al. 1931. The incidence of cancer of the bladder and prostate in certain occupations. Jour. Hyg. 31: 125.

Hetteche, H.O. 1971. Plant waxes as collectors of PCAH in the air of polluted areas. Staub. 31: 72.

Hirano, T., et al. 1974. Measurement of epidermoid carcinoma development induced in the lungs of rats by 3-methylcholanthrene containing beeswax pellets. Jour. Natl. Cancer Inst. 53: 1209.

Hoch-Ligeti, C. 1941. Studies on the changes in the lymphoid tissues of mice treated with carcinogenic and non-carcinogenic hydrocarbons. Cancer Res. 1: 484.

Hoffman, D. and E. Wynder. 1976. Respiratory Carcinogenesis. In: C.E. Searle (ed.), Chemical Carcinogens. ACS Monogr. 173. Am. Chem. Soc. Washington, D.C.

Hoffman, D. and E.L. Wynder. 1977. Organic Particulate Pollutants - Chemical Analysis and Bioassays for Carcinogenicity. In: Stern (ed.), Air Pollution, 3rd ed. Academic Press, New York. 2: 361.

Howard, J.W. and T. Fazio. 1969. A review of polycyclic aromatic hydrocarbons in foods. Jour. Agric. Food Chem. 17: 527.

Howard, J.W., et al. 1966a. Extraction and estimation of PAH in smoked foods. Part I. General Method. Jour. Assoc. Off. Anal. Chem. 49: 595.

Howard, J.W., et al. 1966b. Extraction and estimation of polycyclic aromatic hydrocarbons in smoked foods. II. Benzo(a)pyrene. Jour. Assoc. Off. Anal. Chem. 49: 611.

Howard, J.W., et al. 1966c. Extraction and estimation of polycyclic aromatic hydrocarbons in vegetable oils. Jour. Assoc. Off. Anal. Chem. 49: 1236.

Howard, J.W., et al. 1968. Extraction and estimation of polycyclic aromatic hydrocarbons in total diet composites. Jour. Assoc. Off. Anal. Chem. 51: 122.

Huberman, E. and L. Sachs. 1974. Cell-mediated mutagenesis of mammalian cells with chemical carcinogens. Int. Jour. Cancer. 13: 326.

Huberman, E. and L. Sachs. 1976. Mutability of different genetic loci in mammalian cells by metabolically activated carcinogenic polycyclic hydrocarbons. Proc. Natl. Acad. Sci. 73: 188.

Huberman, E., et al. 1972. Transformation of hamster embryo cells by epoxides and other derivatives of polycyclic hydrocarbons. Cancer Res. 32: 1391.

Huberman, E., et al. 1976a. Identification of mutagenic metabolites of benzo(a)pyrene in mammalian cells. Proc. Natl. Acad. Sci. 73: 607.

Huberman, E., et al. 1976b. Mutagenesis and transformation of normal cells by chemical carcinogens. Nature. 264: 360.

Huberman, E., et al. 1977. Mutagenicity to mammalian cells in culture by (+) and (-) trans-7,8-dihydroxy-7,8-dihydro-benzo(a)pyrenes and the hydrolysis and reduction products of two stereo-isomeric benzo(a)pyrene 7,8-diol-9,10-epoxides. Cancer Lett. 4: 35.

Hueper, W.C. 1963. Chemically induced skin cancers in man. Natl. Cancer Inst. Monogr. 10: 377.

Hueper, W.C., et al. 1962. Carcinogenic bioassays on air pollutants. Arch. Pathol. 74: 89.

Iball, J. 1939. The relative potency of carcinogenic compounds. Am. Jour. Cancer. 35: 188.

International Agency for Research on Cancer. 1973. Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds. Monographs on the evaluation of carcinogenic risk of the chemical to man. Vol. 3. Lyon, France.

International Commission on Radiological Protection. 1974. No. 23, Report of the Task Group on Reference Man. Pergamon Press, New York.

Jaffe, W. 1946. The influence of wheat germ oil on the production of tumors in rats by methylcholanthrene. *Exp. Med. Surg.* 4: 278.

Jerina, D.M. and J.W. Daly. 1974. Arene oxides: A new aspect of drug metabolism. *Science.* 185: 573.

Jerina, D.M., et al. 1972. Bay Region Epoxides of Dihydrodiols. A Concept which Explains the Mutagenic and Carcinogenic Activity of Benzo(a)pyrene and Benzo(a)anthracene. In: *Origins of Human Cancer.* Cold Spring Harbor Lab., Cold Spring Harbor, New York.

Jerina, D.M., et al. 1976. Mutagenicity of Benzo(a)pyrene Derivatives and the Description of a Quantum Mechanical Model which Predicts the Ease of Carbonium Ion Formation from Diol Epoxides. In vitro Metabolic Activation in Mutagenesis Testing. In: F.J. de Serres, et al. (eds.), Elsevier/North Holland Biomedical Press, Amsterdam. p. 159.

- Joneja, M.G. and D.B. Coulson. 1973. Histopathology and cytogenetics of tumors induced by the application of 7,12-dimethylbenz(a)anthracene (DMBA) in mouse cervix. *Eur. Jour. Cancer.* 9: 367.
- Joneja, M.G., et al. 1971. Cytogenetic studies on two types of 7,12-dimethylbenz(a)anthracene (DMBA) induced malignant tumors of mice. *Anat. Rec.* 969: 350.
- Kapitulnik, J., et al. 1976a. Comparison of the hydroxylation of zoxazolamine and benzo(a)pyrene in human placenta: Effect of cigarette smoking. *Clin. Pharmacol. Therap.* 20: 557.
- Kapitulnik, J., et al. 1976b. Lack of carcinogenicity of 4-,5-,6-, 7-,8-,9-, and 10-hydroxybenzo(a)pyrene on mouse skin. *Cancer Res.* 36: 3625.
- Kapitulnik, J., et al. 1977a. Hydration of arene and alkene oxides by epoxide hydrase in human liver microsomes. *Clin. Pharmacol. Therap.* 21: 158.
- Kapitulnik, J., et al. 1977b. Benzo(a)pyrene 7,8-dihydrodiol is more carcinogenic than benzo(a)pyrene in newborn mice. *Nature.* 266: 378.
- Kapitulnik, J., et al. 1977c. Activation of monooxygenases in human liver by 7,8-benzoflavone. *Clin. Pharmacol. Therap.* 22: 475.

Kapitulnik, J., et al. 1977d. Comparative metabolism of benzo(a)-pyrene and drugs in human liver. Clin. Pharmacol. Therap. 21: 166.

Kapitulnik, J., et al. 1978a. Marked differences in the carcinogenic activity of optically pure (+) and (-)-trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene in newborn mice. Cancer Res. 38: 2661.

Kapitulnik, J., et al. 1978b. Tumorigenicity studies with diol-epoxides of benzo(a)pyrene which indicate that (+)-trans-7 $\beta$ ,8 $\alpha$ -dihydroxy-9 $\alpha$ ,10 $\alpha$ -epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene is an ultimate carcinogen in newborn mice. Cancer Res. 38: 354.

Kato, T., et al. 1975. Studies on experimental formation of ovarian tumors - especially, the discussion of the developing process of ovarian tumors following an application of DMBA. Kurume Med. Jour. 22: 169.

Kawai, M., et al. 1967. Epidemiologic study of occupational lung cancer. Arch. Environ. Health. 14: 859.

Keegan, R.E. 1971. The trace fluorometric determination of polynuclear aromatic hydrocarbons in natural water, Ph.D. Thesis, University of New Hampshire. Available from University Microfilms, Ann Arbor, Michigan.

Kellerman, G., et al. 1973a. Aryl hydroxylase inducibility and bronchogenic carcinoma. *New England Jour. Med.* 289: 934.

Kellerman, G., et al. 1973b. Genetic variation of aryl hydrocarbon hydroxylase in human lymphocytes. *Am. Jour. Human Genet.* 25: 327.

Kennaway, E.L. 1925. The anatomical distribution of the occupational cancers. *Jour. Ind. Hyg.* 7: 69.

Kennaway, E.L. and N.M. Kennaway. 1936. A study of the incidence of cancer of the lung and larynx. *Jour. Hyg.* 36: 236.

Kennaway, E.L. and N.M. Kennaway. 1947. A further study of the incidence of cancer of the lung and larynx. *Br. Jour. Cancer.* 1: 260.

Kertesz-Saringer, M. and Z. Morlin. 1975. On the occurrence of polycyclic aromatic hydrocarbons in the urban area of Budapest. *Atmos. Environ.* 9: 831.

Kimura, T., et al. 1977. Differences in benzo(a)pyrene metabolism between lung and liver homogenates. *Biochem. Pharmacol.* 26: 671.

Kobayashi, N. 1975. Production of respiratory tract tumors in hamsters by benzo(a)pyrene. *Gann.* 66: 311.

Kolar, L.R., et al. 1975. Contamination of soil, agricultural crops, and vegetables by 3,4-benzopyrene in the vicinity of CESKA BUDE JOVICE. Cesu Hyg. 20: 135.

Kotin, P., et al. 1954. Aromatic hydrocarbons. I. Presence in the Los Angeles atmosphere and the carcinogenicity of atmospheric extracts. Arch. Ind. Hyg. 9: 153.

Kotin, P., et al. 1969. Distribution, retention, and elimination of C<sup>14</sup>-3,4-benzopyrene after administration to mice and rats. Jour. Natl. Cancer Inst. 23: 541.

Kouri, R.E., et al. 1976. Studies on pulmonary aryl hydrocarbon hydroxylase activity in inbred strains of mice. Chem. Biol. Inter. 13: 317.

Krahn, D.B. and C. Heidelberger. 1977. Liver homosgenate-mediated mutagenesis in Chinese hamster V79 cells by polycyclic aromatic hydrocarbons and aflatoxins. Mutat. Res. 46: 27.

Kraup, T. 1970. Oocyte survival in the mouse ovary after treatment with 9,10-dimethyl-1,2-benz(a)anthracene. Jour. Endocrinol. 46: 483.

Krstulovic, A.M., et al. 1977. Distribution of some atmospheric polynuclear aromatic hydrocarbons. Am. Lab. p. 11.

Kuratsune, M. 1956. Benzo(a)pyrene content in certain pyrogenic materials. Jour. Natl. Cancer Inst. 16: 1485.

Kuratsune, M. and W.C. Hueper. 1958. Polycyclic aromatic hydrocarbons in coffee soots. Jour. Natl. Cancer Inst. 20: 37.

Kuratsune, M. and W.C. Hueper. 1960. Polycyclic aromatic hydrocarbons in roasted coffee. Jour. Natl. Cancer Inst. 24: 463.

Kuroda, S. 1937. Occupational pulmonary cancer of generator gas workers. Ind. Med. Surg. 6: 304.

Landolph, J.R., et al. 1976. Quantitative studies of the toxicity of benzo(a)pyrene to a mouse liver epithelial cell strain in culture. Cancer Res. 26: 4143.

Lasnitzki, A. and D.L. Woodhouse. 1944. The effect of 1,2,5,6-dibenzanthracene on the lymph nodes of the rat. Jour. Anat. 78: 121.

Lasnitzki, I. 1963. Growth pattern of the mouse prostate gland in organ culture and its response to sex hormones, vitamin A, and 3-methylcholanthrene. Natl. Cancer Inst. Monogr. 12: 318.

Leber, P., et al. 1976. A Comparison of Benzo(a)pyrene Metabolism by Primates, Rats, and Miniature Swine. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism and Carcinogenesis (Carcinogenesis - A Comprehensive Survey: Vol. 1). Raven Press, New York. 1: 35.

Lee, R.F., et al. 1972. Uptake, metabolism and discharge of polycyclic aromatic hydrocarbons by marine fish. *Mar. Biol.* 17: 201.

Lee, R.F., et al. 1976. Fate of petroleum hydrocarbons taken up from food and water by the blue crab, Callinectes Sapidus. *Marine Biol.* 37: 363.

Lehr, R.E., et al. 1978. The Bay Region Theory of Polycyclic Aromatic Hydrocarbon-induced Carcinogenicity. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: 2nd. Int. Symp. on Analysis, Chemistry, and Biology (Carcinogenesis - A Comprehensive Survey: Vol. 3). Raven Press, New York. 3: 231.

Leith, R.S. and L. Hayflick. 1974. Efforts to transform cultured normal human cells with polycyclic aromatic hydrocarbons. *Proc. Am. Assoc. Cancer Res.* 15: 86.

Levan, G. and A. Levan. 1975. Specific chromosome changes in malignancy: Studies in rat sarcomas induced by two polycyclic hydrocarbons. *Hereditas.* 79: 161.

Levin, W., et al. 1976a. Carcinogenicity of benzo(a)pyrene 4,5-, 7,8-, and 9,10-oxides on mouse skin. Proc. Natl. Acad. Sci. 73: 243.

Levin, W., et al. 1976b. (+)-Trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene: A potent skin carcinogen when applied topically to mice. Proc. Natl. Acad. Sci. 73: 3867.

Levin, W., et al. 1977a. Role of Purified Cytochrome P-448 and Epoxide Hydrase in the Activation and Detoxification of Benzo(a)pyrene. In: D.M. Jerina (ed.), ACS Symp. Ser. No. 44, Drug Metabolism Concepts. p. 99.

Levin, W., et al. 1977b. Marked differences in the tumor-initiating activity of optically pure (+)-and (-)-trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene on mouse skin. Cancer Res. 37: 2721.

Lijinsky, W. and A.E. Ross. 1967. Production of carcinogenic polynuclear hydrocarbons in the cooking of food. Food Cosmet. Toxicol. 5: 343.

Lijinsky, W. and P. Shubik. 1965a. The detection of polycyclic aromatic hydrocarbons in liquid smoke and some foods. Toxicol. Appl. Pharmacol. 7: 337.

Lijinsky, W. and P. Shubik. 1965b. PH carcinogens in cooked meat and smoked food. Ind. Med. Surg. 34: 152.

Lindsay, D.W., et al. 1974. The Bioassay of Carcinogenesis: Effects on the Epithelial Cell Compliment of Rat Trachea Maintained in vitro. In: Experimental Lung Cancer: Carcinogenesis and Bioassays. Int. Symp. p. 521.

Lo, M. and E. Sandi. 1978. Polycyclic Aromatic Hydrocarbons (polynuclears) in Foods. In: F.A. Gunther and J.D. Gunther (eds.), Residue Reviews. Springer-Verlag. 69: 34.

Lu, A.Y.H., et al. 1976. Metabolism of Benzo(a)pyridine by Purified Liver Microsomal Cytochrome P-448 and Epoxide Hydrase. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis (Carcinogenesis - A Comprehensive Survey: Vol. 1). Raven Press, New York. 1: 115.

Lu, A.Y.H., et al. 1978. Enzymological Properties of Purified Liver Microsomal Cytochrome P-450 System and Epoxide Hydrase. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: 2nd. Int. Symp. on Analysis, Chemistry, and Biology (Carcinogenesis - A Comprehensive Survey: Vol. 3). Raven Press, New York. 3: 243.

Lu, P.Y., et al. 1977. The environmental fate of three carcinogens: Benzo(a)pyrene, benzidine, and vinyl chloride evaluated in laboratory model ecosystems. Arch. Environ. Contam. Toxicol. 6: 129.

Lunde, G. and Bjørseth. 1977. Polycyclic aromatic hydrocarbons in long-range transported aerosols. *Nature*. 268: 518.

Maher, V.M., et al. 1977. Effect of DNA repair on the cytotoxicity and mutagenicity of polycyclic hydrocarbon derivatives in normal and xeroderma pigmentosum human fibroblasts. *Mutat. Res.* 43: 117.

Malanoski, A.J., et al. 1968. Survey of polycyclic aromatic hydrocarbons in smoked foods. *Jour. Assoc. Off. Anal. Chem.* 51: 114.

Malaveille, C., et al. 1975. Mutagenicity of non-K-region diols and diol-epoxides of benz(a)anthracene and benzo(a)pyrene in S. typhimurium TA 100. *Biochem. Biophys. Res. Comm.* 66: 693.

Malmgren, R.A., et al. 1952. Reduced antibody titres in mice treated with carcinogenic and cancer chemotherapeutic agents. *Proc. Soc. Exp. Biol. Med.* 79: 484.

Mantel, N. and R.W. Bryan. 1961. Safety testing of carcinogenic agents. *Jour. Natl. Cancer Inst.* 27: 455.

Marquardt, H. 1976. Microsomal Metabolism of Chemical Carcinogens in Animals and Man. In: R. Montesano and L. Tomatis (eds.), *Screening Tests in Chemical Carcinogenesis*. Intl. Agency Res. Cancer. IARC Publ. No 12. Lyon, France. p. 309.

Marquardt, H., et al. 1972. Malignant transformation of cells derived from mouse prostate by epoxides and other derivatives of polycyclic hydrocarbons. *Cancer Res.* 32: 716.

Marquardt, H., et al. 1974. Malignant transformation in vitro of mouse fibroblasts by 7,12-dimethylbenz(a)anthracene and 7-hydroxymethylbenz(a)anthracene and by their K-region derivatives. *Int. Jour. Cancer.* 13: 304.

Martin, R.J. and R.E. Duggan. 1968. Pesticide residues in total diet samples (III). *Pestic. Monitor. Jour.* 1: 111.

Masuda, Y. and M. Kuratsune. 1971. Polycyclic aromatic hydrocarbons in smoked fish. *Katsuobuski, GANN.* 62: 27.

Mattison, D.R. and S.S. Thorgeirsson. 1977. Ovarian metabolism of polycyclic aromatic hydrocarbons and associated ovotoxicity in the mouse. *Gynecol. Invest.* 8: 11.

Mazumdar, S., et al. 1975. An epidemiological study of exposure to coal tar pitch volatiles among coke oven workers. *Air Pollut. Control Assoc. Jour.* 25: 382.

McCann, J. and B.N. Ames. 1976. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals: Discussion. *Proc. Natl. Acad. Sci.* 73: 950.

- McCann, J., et al. 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Natl. Acad. Sci. 72: 5135.
- Miller, E.C. 1978. Some current perspectives on chemical carcinogenesis in humans and experimental animals: Presidential address. Cancer Res. 38: 1479.
- Mitelman, F. and G. Levin. 1972. The chromosomes of primary 7,12-dimethyl(a)anthracene-induced rat sarcomas. Hereditas. 71: 325.
- Mitelman, F., et al. 1972. Chromosomes of six primary sarcomas induced in the Chinese hamster by 7,12-dimethylbenz(a)anthracene. Hereditas. 72: 311.
- National Academy of Sciences. 1972. Biological effects of atmospheric pollutants: Particulate polycyclic organic matter. Washington, D.C.
- Neal, J. and R.H. Rigdon. 1967. Gastric tumors in mice fed benzo(a)pyrene: A quantitative study. Texas Rep. Biol. Med. 25: 553.
- Nebert, D.W. and J.S. Felton. 1976. Importance of genetic factors influencing the metabolism of foreign compounds. Fed. Proc. 35: 1133.

Nery, R. 1976. Carcinogenic mechanisms: A critical review and a suggestion that oncogenesis may be adaptive ontogenesis. Chem. Biol. Inter. 12: 145.

Nesnow, S. and C. Heidelberger. 1976. The effect of modifiers of microsomal enzymes on chemical oncogenesis in cultures of C3H mouse cell lines. Cancer Res. 36: 1801.

Nettesheim, P. and M.L. Williams. 1976. The influence of vitamin A on the susceptibility of the rat lung to 3-methylcholanthrene. Int. Jour. Cancer. 17: 351.

Nettesheim, P., et al. 1975. Effect of vitamin A on lung tumor induction in rats. Proc. Am. Assoc. Cancer Res. 16: 54.

Newbold, R.F. and P. Brooks. 1976. Exceptional mutagenicity of a benzo(a)pyrene diol epoxide in cultured mammalian cells. Nature. 261: 52.

Newbold, R.F., et al. 1977. Cell-mediated mutagenesis in cultured Chinese hamster cells by carcinogenic polycyclic hydrocarbons: Nature and extent of the associated hydrocarbon-DNA reaction. Mutat. Res. 43: 101.

Nishimura, K. and M. Masuda. 1971. Minor constituents of whisky fusel oils. I. Basic, phenolic and lactonic compounds. Jour. Food Sci. 36: 819.

Nowell, P.C. and D.A. Hungerford. 1960. Chromosome studies in normal and leukemic human leucocytes. Jour. Natl. Cancer Inst. 25: 85.

Ottonen, P.O. and J.K. Ball. 1973. Lack of correlation between gross chromosome abnormalities and carcinogenesis with 7,12-dimethylbenz(a)anthracene. Jour. Natl. Cancer Inst. 50: 497.

Owens, I.S. 1977. Genetic regulation of UDP-glucuronosyltransferase induction by polycyclic aromatic compounds in mice. Jour. Biol. Chem. 252: 2827.

Paigen, B., et al. 1978. Human Aryl Hydrocarbon Hydroxylase and Cancer Risk. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: 2nd. Int. Symp. on Analysis, Chemistry, and Biology (Carcinogenesis - A Comprehensive Survey: Vol. 3). Raven Press, New York. 3: 429.

Panalaks, T. 1976. Determination and identification of polycyclic aromatic hydrocarbons in smoked and charcoal-broiled food products by high pressure liquid chromatography. Jour. Environ. Sci. Health. 11: 299.

Payer, H.D., et al. 1975. Accumulation of polycyclic aromatic hydrocarbons in cultivated microalge. Naturwiss. 62: 536.

Payne, W.W. and W.C. Hueper. 1960. The carcinogenic effects of single and repeated doses of BP. Am. Ind. Hyg. Assoc. Jour. 21: 350.

Peacock, P.R. 1936. Evidence regarding the mechanism of elimination of 1,2-benzpyrene, 1,2,5,6-dibenzanthracene, and anthracene from the blood-stream of injected animals. Br. Jour. Exptl. Pathol. 17: 164.

Pelkonen, O. 1976. Metabolism of Benzo(a)pyrene in Human Adult and Fetal Tissues. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis (Carcinogenesis - A Comprehensive Survey: Vol. 1). Raven Press, New York. 1: 9.

Pfeiffer, E.H. 1973. Investigations on the carcinogenic burden by air pollution in man. VII. Studies on the oncogenetic interaction of polycyclic aromatic hydrocarbons. Abl. Bakt. Hyg., I. Abt. Orig. B. 158: 69.

Pfeiffer, E.H. 1977. Oncogenic Interaction of Carcinogenic and Noncarcinogenic Polycyclic Aromatic Hydrocarbons in Mice. In: V. Mohr, et al. (eds.), Air Pollution and Cancer in Man. Intl. Agency Res. Cancer. Sci. Publ. No. 16. p. 69.

Philips, F.S., et al. 1973. In vivo Cytotoxicity of Polycyclic Hydrocarbons. In: Pharmacology and the Future of Man. Proc. 5th Intl. Congr. Pharmacol., 1972, San Francisco. 2: 75.

Pienta, R.J., et al. 1977. III. Morphological transformation of early passage golden Syrian hamster embryo cells derived from cryopreserved primary cultures as a reliable in vitro bioassay for identifying diverse carcinogens. Intl. Jour. Cancer. 19: 642.

Popescu, N.C., et al. 1976. Chromosome patterns (G and C bands) of in vitro chemical carcinogen-transformed guinea pig cells. Cancer Res. 36: 1404.

Pullman, A. and B. Pullman. 1955. Electronic structure and carcinogenic activity of aromatic molecules - new developments. Adv. Cancer Res. 3: 117.

Pylev, L.N. 1962. Induction of lung cancer in rats by intratracheal insufflation of cancerogenic hydrocarbons. Acta Un. Int. Cancer. 19: 688.

Radding, S.G., et al. 1976. The environmental fate of selected polynuclear aromatic hydrocarbons. Prepared by Stanford Research Institute, Menlo Park, California, under Contract No. 68-01-2681. Publ. No. EPA-560/5-750-009. U.S. Environ. Prot. Agency, Washington, D.C.

Rahimtula, A.D., et al. 1977. The effects of antioxidants of the metabolism and mutagenicity of benzo(a)pyrene in vitro. Biochem. Jour. 164: 473.

Redmond, C.K., et al. 1972. Long term mortality study of steelworkers. Jour. Occup. Med. 14: 621.

Redmond, C.K., et al. 1976. Cancer experience among coke by-product workers. Ann. N.Y. Acad. Sci. p. 102.

Rees, E.O., et al. 1971. A study of the mechanism of intestinal absorption of benzo(a)pyrene. Biochem. Biophys. Act. 225: 96.

Regan, J.D., et al. 1978. Repair of DNA damage by mutagenic metabolites of benzo(a)pyrene in human cells. Chem. Biol. Inter. 20: 279.

Reid, D.D. and C. Buck. 1956. Cancer in coking plant workers. Br. Jour. Ind. Med. 13: 265.

Reznik-Schuller, H. and U. Mohr. 1974. Investigations on the carcinogenic burden by air pollution in man. IX. Early pathological alterations of the bronchial epithelium in Syrian golden hamsters after intratracheal instillation of benzo(a)pyrene. Zbl. Bakt. Hyg., I. Abt. Orig. B. 159: 493.

Rhee, K.S. and L.J. Bratzler. 1970. Benzo(a)pyrene in smoked meat products. Jour. Food Sci. 35: 146.

Rhim, J.S., et al. 1975. Transformation of human osteosarcoma cells by a chemical carcinogen. Jour. Natl. Cancer Inst. 55: 1291.

Rigdon, R.H. and J. Neal. 1965. Effects of feeding benzo(a)pyrene on fertility, embryos, and young mice. Jour. Natl. Cancer Inst. 34: 297.

Rigdon, R.H. and E.G. Rennels. 1964. Effect of feeding benzpyrene on reproduction in the rat. Experientia. 20: 1291.

Riley, J.F. 1969. Mast cells. Co-carcinogenesis and anti-carcinogenesis in the skin of mice. Experientia. 4: 1237.

Rüdiger, H., et al. 1976. Benzpyrene induces sister chromatid exchanges in cultured human lymphocytes. Nature. 262: 290.

Saffiotti, U., et al. 1968. A method for the experimental induction of bronchogenic carcinoma. Cancer Res. 28: 104.

Saffiotti, U., et al. 1972. Respiratory tract carcinogenesis induced in hamsters by different dose levels of benzo(a)pyrene and ferric oxide. Jour. Natl. Cancer Inst. 49: 1199.

San, R.H.C. and H.F. Stich. 1975. DNA repair synthesis of cultured human cells as a rapid bioassay for chemical carcinogens. Int. Jour. Cancer. 16: 284.

Santodonato, J., et al. 1978. Health assessment document for polycyclic organic matters. U.S. Environ. Prot. Agency, Washington, D.C.

- Santodonato, J., et al. 1980. Multimedia health assessment document for polycyclic organic matter. Jour. Environ. Pathol. Toxicol. (In press).
- Sawicki, E. 1962. Analysis of airborne particulate hydrocarbons: Their relative proportions as affected by different types of pollution. Natl. Cancer Inst. Monogr. No. 9: 201.
- Sawicki, E., et al. 1962. Polynuclear aromatic hydrocarbon composition of the atmosphere in some large American cities. Am. Ind. Hyg. Assoc. Jour. 23: 137.
- Schlede, E., et al. 1970a. Effect of enzyme induction on the metabolism and tissue distribution of benzo(a)pyrene. Cancer Res. 30: 2893.
- Schlede, E., et al. 1970b. Stimulatory effect of benzo(a)pyrene and phenobarbital pretreatment on the biliary excretion of benzo(a)pyrene metabolites in the rat. Cancer Res. 30: 2898.
- Schmahl, D., et al. 1977. Syncarcinogenic Action of Polycyclic Hydrocarbons in Automobile Exhaust Gas Condensates. In: V. Mohr, et al. (eds.), Air Pollution and Cancer in Man. Intl. Agency Res. Cancer Sci. Publ. No. 16. p. 53.

Schmeltz, I., et al. 1978. Bioassays of Naphthalene and Lkyl-naphthalenes for Co-carcinogenic Activity. Relation to Tobacco Carcinogenesis. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: 2nd. Int. Symp. on Analysis, Chemistry, and Biology (Carcinogenesis - A Comprehensive Survey: Vol. 3). Raven Press, New York. 3: 47.

Schönwald, A.D., et al. 1977. Benzpyrene-induced sister chromatid exchanges in lymphocytes of patients with lung cancer. Human Genet. 36: 361.

Selkirk, J.K., et al. 1971. An epoxide is an intermediate in the microsomal metabolism of the chemical carcinogen, dibenz(a,h)-anthracene. Biochem. Biophys. Res. Comm. 43: 1010.

Selkirk, J.K., et al. 1974. High-pressure liquid chromatographic analysis of benzo(a)pyrene metabolism and covalent binding and the mechanism of action of 7,8-benzoflavone and 1,2-epoxy-3,3,3-trichloropropane. Cancer Res. 34: 3474.

Selkirk, J.K., et al. 1975a. Isolation by high-pressure liquid chromatography and characterization of benzo(a)pyrene-4,5-epoxide as a metabolite of benzo(a)pyrene. Arch. Biochem. Biophys. 168: 322.

Selkirk, J.K., et al. 1975b. In vitro metabolism of benzo(a)pyrene by human liver microsomes and lymphocytes. Cancer Res. 35: 3651.

Selkirk, J.K., et al. 1976. Analysis of Benzo(a)pyrene Metabolism in Human Liver and Lymphocytes and Kinetic Analysis of Benzo(a)pyrene in Rat Liver Microsomes. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis (Carcinogenesis - A Comprehensive Survey: Vol. 1). Raven Press, New York. 1: 153.

Sellakumar, A., et al. 1976. Effects of different dusts of respiratory carcinogenesis in hamsters induced by benzo(a)pyrene and diethylnitrosamine. Eur. Jour. Cancer. 12: 313.

Shabad, L.M. and A.P. Il'nitskii. 1970. Perspective on the problem of carcinogenic pollution in water bodies. Gig. Sanit. 35: 84; Hyg. Sanit. 35: 268. (Russian, English Transt.).

Shabad, L.M. and G.A. Smirnov. 1972. Aircraft engines as a source of carcinogenic pollution of the environment (benzo(a)pyrene studies). Atmos. Environ. 6: 153.

Shabad, L.M., et al. 1974. Transplacental and direct action of benzo(a)pyrene studied in organ cultures of embryonic lung tissue. Neoplasma. 22: 113.

Shamberger, R.J. 1970. Relationship of selenium to cancer. I. Inhibitory effect of selenium on carcinogenesis. Jour. Natl. Cancer Inst. 44: 931.

Shamberger, R.J. 1972. Increase of peroxidation in carcinogenesis. Jour. Natl. Cancer Inst. 48: 1491.

Shamberger, R.J. and G. Rudolph. 1966. Protection against cocarcinogenesis by antioxidants. Experientia. 22: 116.

Shendrikova, I.A. and V.A. Aleksandrov. 1974. Comparative characteristics of penetration of polycyclic hydrocarbons through the placenta into the fetus in rats. Byull. Eksperiment. Biol. i Medit. 77: 169.

Shimkin, M.B. and G.D. Stoner. 1975. Lung Tumors in Mice: Application to Carcinogenesis Bioassay. In: G. Klein and S. Weinhouse (eds.), Advances in Cancer Research. Raven Press, New York. 12: 1.

Shiraishi, Y., et al. 1973. Determination of polycyclic aromatic hydrocarbons in foods. II. 3,4-Benzopyrene in Japanese foods. Jour. Food Hyg. Soc. Japan, Shokuhin Eiseigaku Zasshi. 14: 173.

Shiraishi, Y., et al. 1974. Determination of polycyclic aromatic hydrocarbons in foods. III. 3,4-benzopyrene in vegetables. Jour. Food Hyg. Soc. Japan. 15: 18.

Shiraishi, Y., et al. 1975. Determination of polycyclic aromatic hydrocarbons in foods. IV. 3,4-benzopyrene in fish and shellfish. Jour. Food Hyg. Soc. Japan, Shokuhin Eiseigaku Zasshi. 16: 178.

Shubik, P. and J.L. Hartwell. 1957. Survey of compounds which have been tested for carcinogenic activity. Supplement 1. Pub. Health Serv. Publ. 149-1. U.S. Govern. Print. Off., Washington, D.C. p. 388.

Shubik, P. and J.L. Hartwell. 1969. Survey of compounds which have been tested for carcinogenic activity. Supplement 2. Pub. Health Serv. Publ. 149-2. U.S. Govern. Print. Off., Washington, D.C. p. 655.

Shulte-Herman, R. 1977. Stimulation of Liver Growth and Mixed-function Oxidase by alpha-Hexachlorocyclohexane: Separation of Inductive Pathways. In: V. Ulrich (ed.), Microsomes and drug oxidations. Pergamon Press, New York.

Siddiqui, I. and K.H. Wagner. 1972. Determination of 3,4-benzopyrene and 3,4-benzo-fluoranthene in rain water, ground water, and wheat. Chemosphere. 1: 83.

Simon, S., et al. 1969. Effect of cellulose casing on absorption of polycyclic hydrocarbons in wood smoke by absorbents. Jour. Agric. Food Chem. 17: 1128.

Sims, P. 1970. Qualitative and quantitative studies on the metabolism of a series of aromatic hydrocarbons by rat-liver preparations. Biochem. Pharmacol. 19: 795.

Sims, P. 1976. The Metabolism of Polycyclic Hydrocarbons to Dihydrodiols and Diol Epoxides by Human and Animal Tissues. In: R. Montesano, et al. (eds.), Screening Tests in Chemical Carcinogenesis. IARC Publ. No. 12. Lyon, France. p. 211.

Sims, P. and P.L. Grover. 1974. Epoxides in polycyclic aromatic hydrocarbon metabolism and carcinogenesis. Adv. Cancer Res. 20: 165.

Slaga, T.J., et al. 1976. Skin tumor initiating ability of benzo(a)pyrene 4,5-7,8- and 7,8-diol-9,10-epoxides and 7,8-diol. Cancer Lett. 2: 115.

Slaga, T.J., et al. 1977. Comparison of the tumor-initiating activities of benzo(a)pyrene arene oxides and dio-epoxides. Cancer Res. 37: 4130.

Smith, D.M., et al. 1975. Vitamin A and benzo(a)pyrene carcinogenesis in the respiratory tract of hamsters fed a semi-synthetic diet. Cancer Res. 35: 1483.

Smith, I.A., et al. 1978. Relationships between carcinogenicity and theoretical reactivity indices in polycyclic aromatic hydrocarbons. Cancer Res. 38: 2968.

Smyth, H.F., et al. 1962. Range-finding toxicity data: List VI. Am. Ind. Hyg. Assoc. Jour. 23: 95.

Snell, K.C. and H.L. Stewart. 1962. Induction of pulmonary adenomatosis in DBA/2 mice by the oral administration of dibenz(a,h)-anthracene. Acta. Un. Int. Canc. 19: 692.

Stenbäck, F. and A. Sellakumar. 1974a. Lung tumor induction by dibenzo(a,i)pyrene in the Syrian golden hamster. Z. Krebsforsch. 82: 175.

Stenbäck, F. and A. Sellakumar. 1974b. Squamous metaplasia and respiratory tumors induced by intratracheal instillations of 7,12-dimethylbenz(a)anthracene in Syrian golden hamsters. Eur. Jour. Cancer. 10: 483.

Stephan, C.E. 1980. Memorandum to J. Stara. U.S. EPA. July 3.

Stich, H.F. and B.A. Laishes. 1973. DNA repair and chemical carcinogens. In: H.L. Ioachim, (ed.). Pathobiology Ann. 3: 341.

Stich, H.F., et al. 1975. The search for relevant short term bioassays for chemical carcinogens: The tribulation of modern Sisyphus. Can. Jour. Genet. Cytol. 17: 471.

Stich, H.F., et al. 1976. DNA Fragmentation and DNA Repair as on in vitro and in vivo Assay for Chemical Procarcinogens, Carcinogens, and Carcinogenic Nitrosation Products. In: R. Montesano, et al. (eds.), IARC Scien. Publ. No. 12, Screening Tests in Chemical Carcinogenesis. Lyon, France. p. 15.

- Stjernsward, J. 1966. The effect of non-carcinogenic and carcinogenic hydrocarbons on antibody-forming cells measured at the cellular level in vitro. Jour. Natl. Cancer Inst. 36: 1189.
- Stjernsward, J. 1969. Immunosuppression by carcinogens. Antibiot. Chemother. 15: 213.
- Stocks, P. 1947. Regional and local differences in cancer death rates. Studies on medical and population subjects, No. 1. Gen. Regis. Off., London.
- Stoming, T.A., et al. 1977. The metabolism of 3-methyl-cholanthrene by rat liver microsomes - A reinvestigation. Biochem. Biophys. Res. Comm. 79: 461.
- Sugimura, T., et al. 1976. Overlapping of Carcinogens and Mutagens. In: P.N. Magee (ed.), Fundamentals in Cancer Prevention. Univ. of Tokyo Press, Tokyo/Univ. Park Press, Baltimore. p. 191.
- Sullivan, P.D., et al. 1978. Effect of Antioxidants on Benzo(a)-pyrene Free Radicals. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: 2nd Int. Symp. on Analysis, Chemistry, and Biology (Carcinogenesis - A Comprehensive Survey: Vol. 3). Raven Press, New York. 3: 1
- Swallow, W.H. 1976. Survey of polycyclic aromatic hydrocarbons in selected foods and food additives available in New Zealand. New Zealand Jour. Sci. 19: 407.

- Swenberg, J.A., et al. 1976. In vitro DNA damage/alkaline elution assay for predicting carcinogenic potential. Biochem. Biophys. Res. Comm. 72: 738.
- Teranishi, K., et al. 1975. Quantitative relationship between carcinogenicity and mutagenicity of polyaromatic hydrocarbons in Salmonella typhimurium mutants. Mutat. Res. 31: 97.
- Thakker, D.R., et al. 1976. Metabolism of benzo(a)pyrene: Conversion of (+)-trans-7,9-dihydroxy-7,8-dihydrobenzo(a)pyrene to highly mutagenic 7,8-diol-9,10-epoxides. Proc. Natl. Acad. Sci. 73: 3381.
- Thakker, D.R., et al. 1977. Metabolism of benzo(a)pyrene. VI. Stereo-selective metabolism of benzo(a)pyrene and benzo(a)pyrene 7,8-dihydrodiol to diol epoxides. Chem. Biol. Inter. 16: 281.
- Thakker, D.R., et al. 1978. Metabolism of 3-methylcholanthrene by Rat Liver Microsomes and a Highly Purified Monooxygenase System with and without Epoxide Hydrase. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: 2nd. Int. Symp. on Analysis, Chemistry, and Biology (Carcinogenesis - A Comprehensive Survey: Vol. 3). Raven Press, New York. 3: 253.
- Thomson, S. and T.J. Slaga. 1976. Mouse epidermal aryl hydrocarbon hydroxylase. Jour. Invest. Dermatol. 66: 108.

- Thorsteinsson, T. 1969. Polycyclic hydrocarbons in commercially and home-smoked food in Iceland. *Cancer*. 23: 455.
- Tokiwa, H., et al. 1977. Detection of mutagenic activity in particulate air pollutants. *Mutat. Res.* 48: 237.
- Toth, L. and W. Blass. 1972. Einfluss der Raeuchertechnologie auf den Gehalt von Geraeucherten Fleischwaren an Cancerogenen Kohlenwasserstoffen. *Fleischwirt.* 21: 1121.
- Tracor Jitco, Inc. 1974. Survey of compounds which have been tested for carcinogenic activity (1970-1971). *Pub. Health Serv. Publ.* 149. U.S. Govern. Print. Off., Washington, D.C. p. 1667.
- Tracor Jitco, Inc. 1976. Survey of compounds which have been tested for carcinogenic activity (1972-1973). *Pub. Health Serv. Publ.* 149. U.S. Govern. Print. Off., Washington, D.C. p. 1638.
- Tromp, S.W. 1955. Possible effects of geophysical and geochemical factors on development and geographic distribution of cancer. *Schweiz. Z. Path.* 18: 929.
- U.S. EPA. 1974. Special report: Trends in concentrations of benzene-soluble suspended particulate fraction and benzo(a)pyrene. *Publ. No.* EPA-450/2-74-022, Res. Triangle Park, North Carolina.

U.S. EPA. 1975. Scientific and technical assessment report on particulate polycyclic organic matter (PPOM), Publ. No. EPA-600/6-75-001, Washington, D.C.

U.S. EPA. 1977. National Organic Monitoring Survey, Tech. Support Div., Off. Water Supply, Cincinnati, Ohio.

U.S. EPA. 1980. Seafood consumption data analysis. Stanford Research Institute International, Menlo Park, California. Final report, Task 11, Contract No. 68-01-3887.

Vainio, H., et al. 1976. The fate of intratracheally installed benzo(a)pyrene in the isolated perfused rat lung of both control and 20-methylcholanthrene pretreated rats. Res. Comm. Chem. Pathol. Pharmacol. 13: 259.

Van Duuren, B.L. 1969. Tumor-promoting agents in two-stage carcinogenesis. Prog. Exp. Tumor Res. 11: 31.

Van Duuren, B.L. 1976. Tumor-promoting and Co-carcinogenic Agents in Chemical Carcinogenesis. In: C.E. Searle (ed.), Chemical Carcinogens. ACS Monogr. 172. Am. Chem. Soc., Washington, D.C. p. 24.

Van Duuren, B.L. and B.M. Goldschmidt. 1976. Co-carcinogenic and tumor-promoting agents in tobacco carcinogenesis. Jour. Natl. Cancer Inst. 56: 1237.

- Van Duuren, B.L., et al. 1973. Brief communications: Co-carcinogenic agents in tobacco carcinogenesis. Jour. Natl. Cancer Inst. 51: 703.
- Veith, G.D. 1980. Memorandum to C.E. Stephan. U.S. EPA. April 14.
- Veith, G.D., et al. 1979. Measuring and estimating the bioconcentration factors of chemicals in fish. Jour. Fish. Res. Board Can. 36: 1040.
- Vitzthum, O.G., et al. 1975. New volatile constituents of black tea aroma. Jour. Agric. Food Chem. 23: 999.
- Wang, I.Y., et al. 1976. Enzyme Induction and the Difference in the Metabolite Patterns of Benzo(a)pyrene Produced by Various Strains of Mice. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis (Carcinogenesis - A Comprehensive Survey: Vol. 1). Raven Press, New York. 1: 77.
- Wattenberg, L.W. 1972. Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants. Jour. Natl. Cancer Inst. 48: 1425.
- Wattenberg, L.W. 1973. Inhibition of chemical carcinogen-induced pulmonary neoplasia by butylated hydroxyanisole. Jour. Natl. Cancer Inst. 50: 1541.

Wattenberg, L.W. 1974. Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by several sulfur-containing compounds. Jour. Natl. Cancer. Inst. 52: 1583.

Wattenberg, L.W. 1977. Inhibition of carcinogenic effects of polycyclic hydrocarbons by benzyl isothiocyanate and related compounds. Jour. Natl. Cancer Inst. 58: 395.

Wattenberg, L.W. and J.L. Leong. 1970. Inhibition of the carcinogenic action of benzo(a)pyrene by flavones. Cancer Res. 30: 1922.

Wattenberg, L.W., et al. 1976. Effects of antioxidants on metabolism of aromatic polycyclic hydrocarbons. Adv. Enzyme Regul. 14: 313.

Weber, R.P., et al. 1974. Effect of the organophosphate insecticide parathion and its active metabolite paraoxon on the metabolism of benzo(a)pyrene in the rat. Cancer Res. 34: 947.

Welch, R.M., et al. 1972. Effect of enzyme induction on the metabolism of benzo(a)pyrene and 3'-methyl-4-monomethyl-amino-azobenzene in the pregnant and fetal rat. Cancer Res. 32: 973.

White, R.H., et al. 1971. Determination of polycyclic aromatic hydrocarbons in liquid smoke flavors. Jour. Agric. Food Chem. 19: 143.

- Wiebel, F.J., et al. 1973. Aryl hydrocarbon [benzo(a)pyrene] hydroxylase: Inducible in extrahepatic tissues of mouse strains not inducible in liver. Arch. Biochem. Biophys. 154: 292.
- Wiebel, F.J., et al. 1975. Aryl hydrocarbon [benzo(a)pyrene] hydroxylase: A mixed-function oxygenase in mouse skin. Jour. Invest. Dermatol. 64: 184.
- Williams, G.M. 1976. Carcinogen-induced DNA repair in primary rat liver cells cultures; a possible screen for chemical carcinogens. Cancer Lett. 1: 231.
- Wislocki, P.G., et al. 1976a. High mutagenicity and toxicity of a diol epoxide derived from benzo(a)pyrene. Biochem. Biophys. Res. Comm. 68: 1006.
- Wislocki, P.G., et al. 1976b. Mutagenicity and cytotoxicity of benzo(a)pyrene arene oxides, phenols, quinones, and dihydrodiols in bacterial and mammalian cells. Cancer Res. 36: 3350.
- Wislocki, P.G., et al. 1977. High carcinogenicity of 2-hydroxybenzo(a)pyrene on mouse skin. Cancer Res. 37: 2608.
- World Health Organization. 1970. European Standards for Drinking Water, 2nd ed., Revised, Geneva.

Wood, A.W., et al. 1976a. Mutagenicity and cytotoxicity of benzo(a)pyrene benzo-ring epoxides. *Cancer Res.* 36: 3358.

Wood, A.W., et al. 1976b. Metabolism of benzo(a)pyrene and benzo(a)pyrene derivatives to mutagenic products by highly purified hepatic microsomal enzymes. *Jour. Biol. Chem.* 251916: 4882.

Wood, A.W., et al. 1977a. Differences in mutagenicity of the optical enantiomers of the diastereomeric benzo(a)pyrene 7,8-diol-9,10-epoxides. *Biochem. Biophys. Res. Comm.* 77: 1389.

Wood, A.W., et al. 1977b. High mutagenicity of metabolically activated chrysene 1,2-dihydrodiol: Evidence for bay region activation of chrysene. *Biochem. Biophys. Res. Comm.* 78: 847.

Wynder, E. and D. Hoffman. 1965. Some laboratory and epidemiological aspects of air pollution carcinogenesis. *Jour. Air Pollut. Contr. Assoc.* 15: 155.

Wynne-Griffith, G. and R.I. Davies. 1954. Cancer and soils in the County of Anglesey - A revised method of comparison. *Br. Jour. Cancer.* 8: 594.

Yang, S.K., et al. 1977. Metabolic activation of benzo(a)pyrene and binding to DNA in cultured human bronchus. *Cancer Res.* 37: 1210.

Yang, S.K., et al. 1978. Benzo(a)pyrene Metabolism: Mechanism in the Formation of Epoxides, Phenols, Dihydrodiols, and the 7,8-diol-9,10-epoxides. In: R.I. Freudenthal and P.W. Jones (eds.), Polynuclear Aromatic Hydrocarbons: 2nd. Int. Symp. on Analysis, Chemistry, and Biology (Carcinogenesis - A Comprehensive Survey: Vol. 3). Raven Press, New York. 3: 285.

Yasuhira, K. 1964. Damage to the thymus and other lymphoid tissues from 3-methyl-cholanthrene, and subsequent thymoma production, in mice. *Cancer Res.* 24: 558.

Youngblood, W.W. and M. Blumer. 1975. Polycyclic aromatic hydrocarbons in the environment: Homologous series in soils and recent marine sediments. *Geochem. Cosmochim. Acta.* 39: 1303.

Yuspa, S.H., et al. 1976. Cutaneous carcinogenesis: Past, present, and future. *Jour. Invest. Dermatol.* 67: 199.

Zampaglione, N.G. and G.J. Mannering. 1973. Properties of benzopyrene hydroxylase in the liver, intestinal mucosa and adrenal of untreated and 3-methyl-cholanthrene treated rats. *Jour. Pharmacol. Exp. Ther.* 185: 676.

Zitko, V. 1975. Aromatic hydrocarbons in aquatic fauna. *Bull. Environ. Contam. Toxicol.* 14: 621.

## APPENDIX

### Summary and Conclusion Regarding the Carcinogenicity of Polynuclear Aromatic Hydrocarbons (PAH)

Polynuclear aromatic hydrocarbons (PAH) comprise a diverse class of compounds consisting of substituted and unsubstituted polycyclic and heterocyclic aromatic rings. They are formed as a result of incomplete combustion of organic compounds and appear in food as well as ambient air and water.

Numerous studies of workers exposed to coal gas, coal tars, and coke oven emissions, all of which have large amounts of PAH, have demonstrated a positive association between the exposures and lung cancer.

Several PAH are well-known animal carcinogens, others are not carcinogenic alone but can enhance or inhibit the response of the carcinogenic PAH and some induce no tumors in experimental animals. Most of the information about the combined carcinogenic effects of several PAH come from skin painting and subcutaneous injection experiments in mice whereas oral administration, intratracheal instillation, and inhalation have been shown to induce carcinogenic responses to single compounds. In one subcutaneous injection study in mice it was shown that a combination of several noncarcinogenic PAH compounds, mixed according to the proportion occurring in auto exhaust, does not enhance or inhibit the action of two potent PAH carcinogens, benzo(a)pyrene (BaP) and dibenz(a,h)anthracene.

The mutagenicity of PAH in the Salmonella typhimurium assay correlates well with their carcinogenicity in animal systems. PAH compounds have damaged chromosomes in cytogenetic tests, have

induced mutations in mammalian cell culture systems and have induced DNA repair synthesis in human fibroblast cultures.

The water quality criterion for carcinogenic PAH compounds is based on the assumption that each compound is as potent as BaP and that the carcinogenic effect of the compounds is proportional to the sum of their concentrations. Based on an oral feeding study of BaP in mice, the concentration of BaP estimated to result in a lifetime risk of  $10^{-5}$  is 28 ng/l. Therefore, with the assumption above, the sum of the concentrations of all carcinogenic PAH compounds should be less than 28 ng/l in order to keep the lifetime cancer risk below  $10^{-5}$ .

### Summary of Pertinent Data

The water quality criterion for BaP is based on the experiment reported by Neal and Rigdon (1967), in which benzo(a)pyrene at doses ranging between 1 and 250 ppm in the diet was fed to strain CFW mice for approximately 110 days. Stomach tumors, which were mostly squamous cell papillomas but some carcinomas, appeared with an incidence statistically higher than controls at several doses. The extrapolation was based on the following parameters:

<u>Dose</u> (mg/kg/day)	<u>Incidence<sup>a</sup></u> (No. responding/No. tested)
0.0	0/289
0.13	0/25
1.3	0/24
2.6	1/23
3.9	0/37
5.2	1/40
5.85	4/40
6.5	24/34
13.0	19/23
32.5	66/73
le = 110 days	w = 0.034 kg
Le = 183 days	R = 30 l/kg
L = 630 days	

With these parameters, the carcinogenic potency factor for humans,  $q_1^*$ , is  $11.53 \text{ (mg/kg/day)}^{-1}$ . The result is that the water concentration of BaP should be less than 28 ng/l in order to keep the individual lifetime risk below  $10^{-5}$ . It is recognized that numerous carcinogenic PAH other than BaP are found in water. However, there is probably little need to derive criteria for all such PAH, since efforts to reduce BaP levels to within acceptable limits will result in the reduction of all PAH.

<sup>a</sup>The incidences at the highest three doses were not used in the extrapolation due to lack of fit of the multistage model. See the Human Health Methodology Appendices to the October 1980 Federal Register notice which announced the availability of this document for a discussion on the fit of data to the multistage model.