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Subject: THE TOXICITY OF PCBs, THEIR CONTAMINANTS AND COMBUSTION PRODUCTS IN HUMANS, AND THEIR PERSISTENCE IN THE ENVIRONMENT

Gentlemen,

I am pleased to send you the English version of the above mentioned report of which I am the author. It has now been published.

If you need any further information, do not hesitate to contact me at the following telephone number (514) 289-2970.

Good reading!

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THE TOXICITY OF PCBs, THEIR CONTAMINANTS AND COMBUSTION PRODUCTS IN HUMANS, AND THEIR PERSISTENCE IN THE ENVIRONMENT

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INTRODUCTION

Polychlorinated biphenyls (PCBs) pollute land and water ecosystems and are found in almost every part of the world. They have been involved in two large-scale poisonings caused by food contamination, the first in Japan in 1968 and the second in Taiwan in 1979. In the early 1970s, at a time when polychlorinated biphenyls were quite common, a number of studies carried out in the United States showed that they appreciably contaminated the food chain, both directly and indirectly. Several health problems among exposed populations in industrial environments have been attributed to PCBs or their contaminants.

More recently, a new problem has been brought to light, i.e. the combustion of polychlorinated biphenyls. Under the effect of heat and given certain conditions, a portion of PCBs in combustion are converted into polychlorodibenzodioxins (PCDDs) and polychlorodibenzofurans (PCDFs), of which a number of congeners are deemed to be extremely toxic.

The principal source of the polychlorinated biphenyls still in use is electrical equipment insulated with these products, commonly called askarels. The generic term askarels designates fire-proof dielectric liquids, composed of polychlorinated biphenyls, to which polychlorobenzenes (tri- and tetrachlorobenzenes) have been added.

Moreover, we have been aware for several years that mineral oils used to insulate electrical equipment have often been contaminated by polychlorinated biphenyls; between 10 per cent and 15 per cent of such oils appear to be contaminated with PCBs in concentrations above 50 ppm (parts per million). These oils are covered by regulations governing hazardous wastes, designed to prevent discharging of PCBs in the environment and to eliminate these substances in Canada.

For the general public, the expression polychlorinated biphenyls is synonymous with danger. Given their persistence in the environment and in living organisms, people fear their effects on health and, more specifically, their consequences for reproduction and breast-fed babies, and their carcinogenic effects.

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The considerable number of quality publications devoted to PCBs bears witness to the extensive broadening of knowledge of the potential effects of PCBs on humans and the environment. For more than 10 years, scientists have closely followed poisoning victims in Japan and Taiwan and individuals subjected to long-term occupational exposure. Thanks to such follow-up and experiments on animals, it is possible to derive a clearer idea of the possible pernicious effects of these products on humans.

In light of documentation available on polychlorinated biphenyls, their contaminants and combustion products, we will attempt in this study to:

- assess the overall behavior of PCBs in the environment;

- assess the potential effects of these products on the health of humans and animals;

- assess, as far as possible, the relationship between exposure dose and effects;

- estimate, bearing in mind legislation currently in force, current and future risks of the appearance of these effects in the population and among the most heavily exposed workers;

- ascertain future prospects concerning these products.

We hope to answer a number of questions which many people are asking themselves with regard to the persistence of these products in the environment and their toxicity for humans.

1. HISTORICAL OVERVIEW

1881 (Germany)	PCBs were synthesized for the first time.
1929 (United States)	PCBs were marketed in the United States by Universal Manufacturing Equipment Co., under a licence from F.M. Clark for the use of PCBs as a dielectric, under the brand name Askarel.
1932 (United States)	Monsanto manufactured PCBs under the brand names Aroclor and Pyroclor.
1941 to 1947	Use of PCBs became more widespread in Europe and Japan.
1966 (Sweden)	Dr. Jensen discovered the presence of PCBs in the tissues of fish and piscivorous birds. To study residues of pesticides such as DDT and p,p'-DDE in these animals, he analysed samples by chromatography and discovered new contaminants which did not correspond to any known chlorinated pesticide (see Figure 1). This discovery showed that PCBs accumulate in organic tissues.
1967 (Sweden)	Through mass spectrometry, Wilmark identified the chemical nature of these contaminants, which proved to be PCBs. Subsequently, it was discovered that, because of their solubility in lipids and their resistance to degradation, PCBs accumulate in the food chain and are dispersed in the environment in a manner similar to polychlorinated pesticides such as DDT.

Since 1966, researchers around the world have focused their attention on the possible toxic effects of PCBs. Numerous publications have appeared concerning acute and chronic toxicity in several animal species.

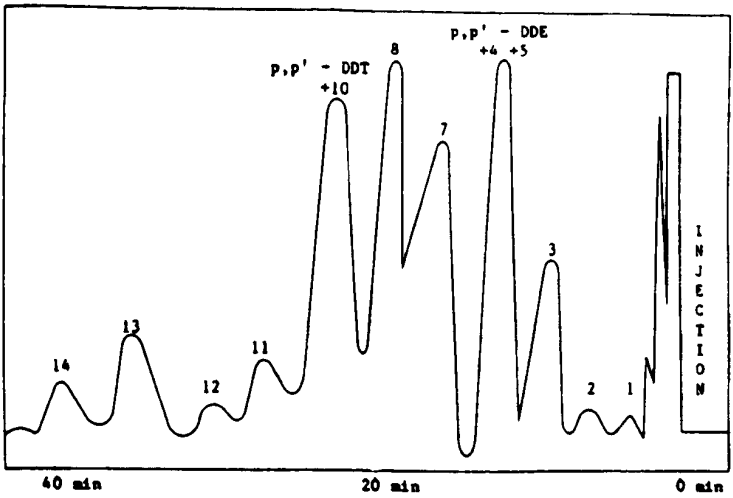

FIGURE 1: Chromatogram of a Tissue Sample from a Dead Eagle Found in the Stockholm Archipelago. Except for DDT and DDE Peaks, All Peaks Appearing on the Graph Were Unknown (courtesy of Dr. Jensen)

1968 (Japan)

The Yusho incident, a mass PCB poisoning, occurred. For more than eight months, Japanese consumed rice oil contaminated with PCBs and furans from a heat exchanger used on the production line. More than 1800 people displayed clinical symptoms (chloracne, edema of the eyelids, meibomian gland disorders, hepatic lesions, lethargy and so on).

Today, it is estimated that victims consumed between 0.5 g and 2 g of PCBs and between 2.5 mg and 7 mg of furans.

The Yusho incidant led to measures around the world to regulate the use and production of PCBs.

1969 (United States)	Contaminated milk was found in West Virginia, attributable to pastureland contaminated by herbicides containing PCBs.
1970 (United States)	The Environmental Protection Agency (EPA) established a group responsible for evaluating the extent of PCB pollution and coordinating the efforts of U.S. agencies working on the problem.
1970 to 1971 (United States)	A series of incidents occurred involving the contamination of food products by PCBs. Contaminated milk was found in Ohio and other states. At the Campbell Soup Co. in New York, chicken was contimated by wrapping paper containing PCBs. In Illinois, flour made from meat by-products was contaminated because of a leak in heat transfer equipment. In North Carolina, fish flour was contaminated by a heat exchanger during pasteurization. Prior to discovery of the problem, the flour had contaminated many chickens and eggs.
1971 (United States)	Monsanto, manufacturer of Aroclor, limited its products to applications in closed systems such as transformers and capacitors and introduced a new product, AR 1016, that is more biodegradable than previous Aroclors.
1972 (United States)	The Council of Environmental Quality (CEQ) published a raport which concluded that PCBs undeniably caused pollution. It recommendad that PCBs be used only in applications where they were truly essential and could not be replaced by other products. It also recommended limiting contact with PCBs.
1972 (Japan)	Manufacturing and uas of PCBs were prohibited.

1973 (OECD)	The council of the Organization for Economic Cooperation and Development (OECD) decided to regulate the use of PCBs in member countries. The notion of open systems (in which PCBs were henceforth banned) and closed systems was introduced.
1973 (United States)	The Food and Drug Administration (FDA) introduced a new regulation limiting the use of PCBs, in any form, in food production chains. The FDA also established the maximum concentrations of PCBs tolerated in food and materials coming into contact with food.
1975 (Canada)	Environmental Contaminants Act, Ch. 72.
1976 (United States)	The Toxic Substances Control Act (TSCA) stated that PCBs are extremely toxic and carcinogenic and requested that Congress regulate their use.
1977 (United States)	The EPA prohibited all discharges of PCBs in waterways. The TSCA asked the EPA: (1) to prohibit the manufacture of PCBs; and (2) to regulate the identification and elimination of material containing, or having contained, PCBs.
1977 (United States)	Monsanto ceased manufacturing Aroclors in the United States and Pyroclors in England.

1977 (United States)	It was noted that mineral oil in many transformers was contaminated by PCBs. A campaign to systematically determine the PCB content of such oil was launched.
1977 (Canada) August	Chlorobiphenyls Regulation No. 1 respecting the use of PCBs was adopted pursuant to the Environmental Contaminants Act (ECA).
1977 (Canada) December, Adelaide	A fire occurred in a transformer containing 380 L of askarel, part of which evaporated. Soot samples collected after the fire contained approximately 5 ppm of polychlorodibenzofurans (PCDFs). Following a study of reports of analyses effected during and after the fire, the Department of the Environment concluded that there was no longer a public health risk.
1978 (Sweden) September, Stockholm	Fire broke out in a substation, destroying 18 capacitors containing askarel; no poisonings were reported.
1971 (United States)	The EPA established a classification of material according to its PCB content. Category 1: 0-50 ppm - Fluids and apparatus with this content are deemed to be free of PCBs. Category 2: 51-500 ppm - Fluids and apparatus with this content are deemed to be contaminated by PCBs. Category 3: ≥ 500 ppm - Fluids are considered to be "pure" PCBs and apparatus to contain "pure" PCBs.
1979 (Taiwan)	Eleven years after Japan, Taiwan experienced a poisoning through PCBs and furans, also from contaminated rice oil. More than 2000 people displayed symptoms similar to those observed among victims of Yusho disease.

1980 (Canada)	Amendment of Regulation No. 1 on the use of PCBs.
1980 to 1982 (United States)	The Electric Power Research Institute (EPRI) and producers and distributors of electricity in the United States launched extensive studies to ascertain the toxicity of PCBs and develop accurate methods for identifying them in insulating oils.
1981 (United States) February, Binghamton, NY	A fire occurred in a transformer containing 680 L of insulating fluid, comprising 60 per cent PCBs and 40 percent tetrachlorobenzene. Soot containing PCBs and products such as TCDDs and TCDFs contaminated the entire 18-storey building.
1981 (United States)	A new EPA regulation was introduced respecting the use of PCBs and material containing them. However, the 0-50 ppm, 51-500 ppm and ≥ 500 ppm categories were maintained.
1983 (United States) San Francisco	A fire occurred in a transformer, contaminating the building with PCBs and breakdown products.
1984 (Canada)	Proposed regulations respecting environmental contaminants: Regulation No. 2: standards setting the maximum concentration of PCBs in certain products. Regulation No. 3: standards setting the maximum concentration and quantity of PCBs which can be discharged in the environment.
1984 (Canada) November, Québec	A fire occurred in the high-voltage laboratory of the Institut de recherche d'Hydro-Québec (IREQ); 670 L of PCBs and 2560 L of mineral oil burned. The entire building was contaminated by smoke and soot containing PCBs and its breakdown products, especially PCDFs.
1985 (Canada) January, Québec	A fire inside Hydro-Québec's Wellington substation destroyed 6 L of PCBs in a capacitor.

1985 (Canada) April, Kenora, Ontario	PCBs were spilled from a leaking transformer being shipped from Québec to Alberta.
1985 (Canada)	Several small spills of oil contaminated with PCBs occured in various locations in Canada during the shipment of transformers.
1985 (Canada) July	Chlorobiphenyls Regulations No. 2 (Product) and No. 3 (Release) were adopted with respect to PCBs.
1985 (Québec)	The Hazardous Waste Regulation was adopted, which is much more stringent with respect to PCBs than regulations elsewhere in Canada. Maximum concentrations of PCBs: . 0.15 mg/L or 0.15 ppm in liquid residues; . 0.01 mg/L or 0.01 ppm in solid residues of lixiviation.

This brief overview of the history of PCBs indicates the concerns of the general public, workers and governments with regard to these products.

2. THE CHEMISTRY OF POLYCHLORINATED BIPHENYLS (PCBs)*

2.1 Definition of PCBs

Polychorinated biphenyls (PCBs) do not exist in a pure state in nature, but are chemicals which have been synthesized from benzene and sodium chloride. The basic unit of BPCs is the biphenyl, which is obtained by vapor treatment of benzene at high tempereturee in the presence of metal catalyzers. By replacing hydrogen molecules with chlorine (Cl), molecules of chlorinated biphenyls or chlorobiphenyls are created.

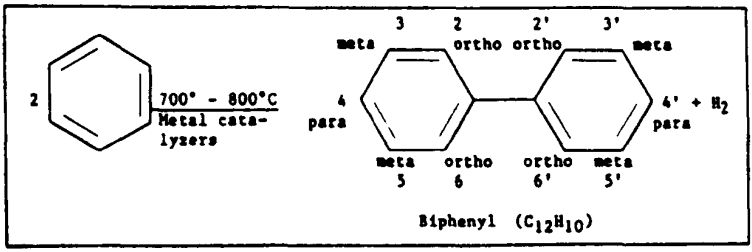

FIGURE 2: The Biphenyl Nucleus Contains Hydrogen at Positions: 2, 3, 4, 5, 6, 2', 3', 4', 5', 6'

There are 209 possible congeners corresponding to the different positions which Cl atoms can occupy on the biphenyl nucleus during their synthesis. The congeners can contain from 1 to 10 chlorine atoms, which represents 10 series of homologous compounds compriaing congeners or isomers containing a given number of chlorine atoms.**

---

* See The Chemistry of PCBs, O. Hutzinger et al., 1974.

** Congener is a general term designating the isomers of all series of homologous compounds. There are 209 congeners, encompassing all possible molecules of polychlorinated biphenyls.

A homologous compound is defined as a group made up of isomers containing a given number of chlorine atoms. All congeners containing four chlorine atoms are isomers in the tetrachlorinated homologous compound series.

In a homologous compound series, isomers are distinguished by the position of chlorine atoms.

2.2 Nomenclature of PCBs

The different molecules of polychlorinated biphenyls (congeners or isomers) are named according to the homologous compound series to which they belong and the positions of chlorine atoms on the biphenyl molecule (see Table 1).

TABLE 1: Nomenclature of 10 Groups of Isomers in the PCB Family

Number of chlorine atoms	Names of the isomers of the 10 series of homologous compounds
1	Monochlorineted biphenyl (monochlorobiphenyls)
2	Dichlorinated biphenyl (dichlorobiphenyls)
3	Trichlorinated biphenyl (trichlorobiphenyls)
4	Tetrachlorobiphenyls
5	Pentachlorobiphenyls
6	Hexachlorobiphenyls
7	Heptachlorobiphenyls
8	Octachlorobiphenyls
9	Nonachlorobiphenyls
10	Decachlorobiphenyls

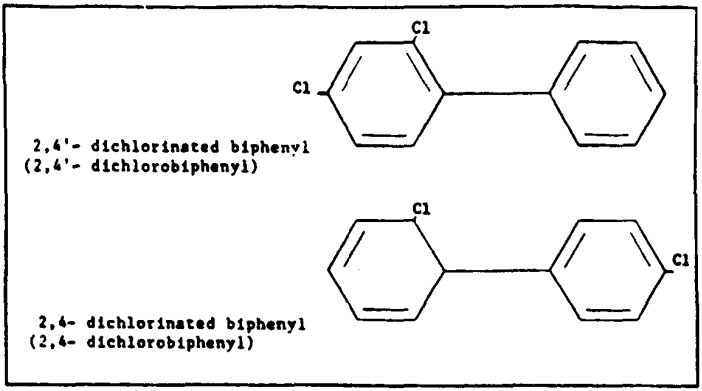

FIGURE 2.1: Two Examples of Dichlorinated Biphenyls or Dichlorobiphenyls

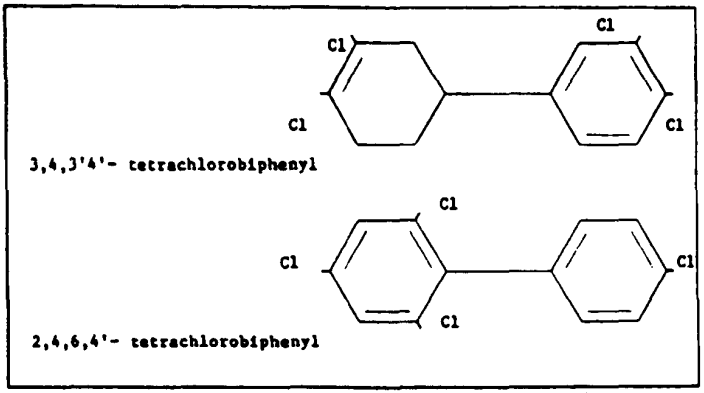

FIGURE 2.2: Two Examples of Tetrachlorobiphenyls

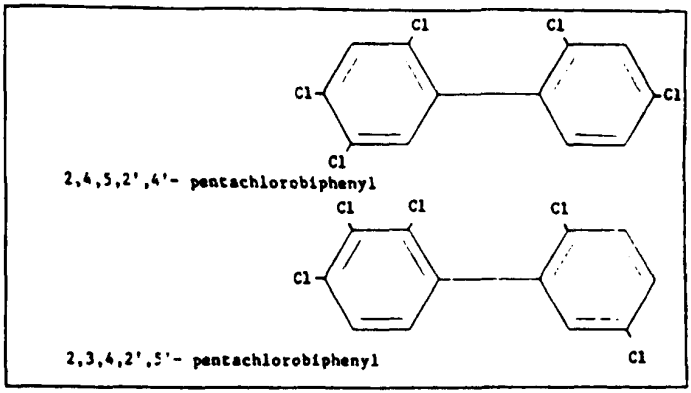

FIGURE 2.3: Two Examples of Pentachlorobiphenyls

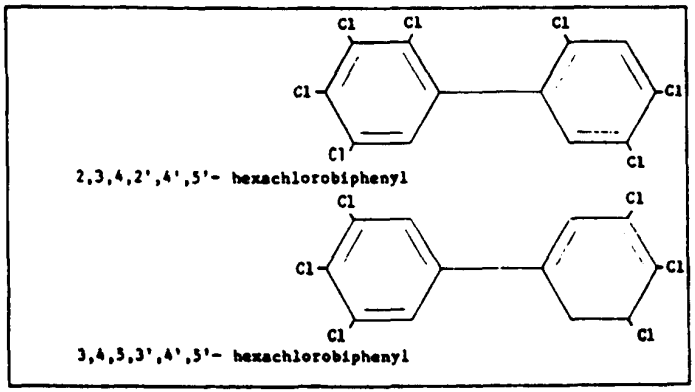

FIGURE 2.4: Two Examples of Hexachlorobiphenyls

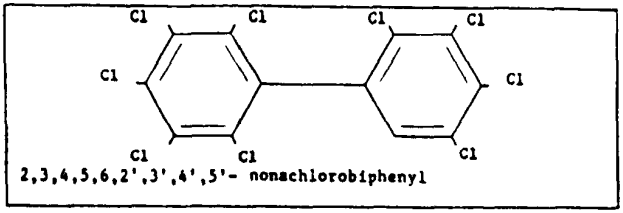

FIGURE 2.5: One Example of Nonachlorobiphenyls

2.3 Principal Properties of PCBs

PCBs have the following properties, some of which (those marked with an asterisk) are extremely important from a biological standpoint.

- Very heat stable, fire-resistant
- Very stable chemically*
- Very resistant to oxidation, acids, bases and other chemicals*
- Very high boiling points
- Very good dielectric properties (insulators)
- Only slightly soluble in water*
- Highly lipophilic*
- Very low volatility in air*
- Recognized toxicity*

2.3.1 Remarks on properties having a biological effect

2.3.1.1 Very stable chemically, very resistant to oxidation, acids, bases and other chemicals

These characteristics make PCBs in general very resistant to biological and chemical degradation. However, it is recognized that the lower the chlorine content of PCBs, the more readily biodegradable they are, and vice versa.

It must be pointed out that the position of chlorine atoms affects persist-.nce, i.e. the various isomers with the same chlorine content do not have the same effect.

2.3.1.2 Only slightly soluble in water

PCBs are not generally soluble in water and tend to settle in organic and inorganic sediments at the bottom of effluents. For this reason, concentrations of PCBs in water are always very low, usually below 1 ppb (part per billion). However, the higher the number of chlorine atoms, the less the molecules are soluble, and vice versa. As data in Table 2 indicate, the position of chlorine atoms affects the solubility of PCBs.

2.3.1.3 Highly lipophilic

PCBs are highly soluble in fats, oils and nonpolar liquids. The rate of lipidic solubility usually increases with their chlorine content. This characteristic, combined with their low solubility in water, fosters bioaccumulation of PCBs in the tissues of living organisms. Obviously, an organism's ability to metabolize a given congener also affects the bioaccumulation of PCBs. Metabolism will be examined in greater detail in Chapter 10, devoted to the pharmokinetics of PCBs.

2.3.1.4 Very low volatility in air

At room temperature, volatility is low and is affected by the number of chlorine atoms attached to the molecule (see Table 2). As the number of chlorine atoms increases, the rate of evaporation decreases. It is estimated that ambient air contains between 0.0001 and 0.01 $\mu g/m^3$ of PCBs. Of course, this concentration may be higher inside buildings polluted with PCBs, all the more so where the temperature is high, as volatility increases with temperature. The rate of air replacement also affects concentrations in buildings.

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TABLE 2: Water Solubility and Vapor Pressure of PCBs

PCB molecule	Water solubility µ mole/L, 25°C	Vapor pressure mm Hg, 25°C
2-PCB	26	$8,4 \times 10^{-3}$
4-PCB	12	$4,6 \times 10^{-3}$
2,2'-PCB	4,9	$2,1 \times 10^{-4}$
4,4'-PCB	0,3	$1,9 \times 10^{-5}$
2,5,2'-PCB	3,9	9×10^{-5}
2,3,4'-PCB	0,3	
2,5,2',5'-PCB	0,09	$3,7 \times 10^{-5}$
3,4,3',4'-PCB	0,3	
2,4,2',4'-PCB	0,23	$8,6 \times 10^{-5}$
2,3,4,5,2'-PCB	0,028	

According to the National Academy of Sciences (NAS) 1979, Mackay et al. 1980, Banerjee et al. 1980, Westcott et al. 1981, Neely 1983, McDonald (unpublished data), Smith et al. 1964 (table taken from an EPA report produced in 1983).

2.3.1.5 Recognized toxicity

Various congeners present in commercial products do not have the same toxicity. We will examine this question in greater detail in Chapter 8, which focuses on the toxicity and mechanism of action of PCBs.

However, the congeners usually recognized for their higher potential toxicity are several specific congeners belonging to groups of isomers containing between four and six chlorine atoms. The congeners of groups of isomers with three or fewer chlorine atoms, or those with seven chlorine atoms or more, are deemed to be scarcely toxic, or not toxic at all, compared with the first group.

Of all of these properties, it appears that it is the chlorine content of congeners present in various commercial products which affects the latters' persistence and toxicity. It should also be noted that considerable discrepancies are found in the persistence and toxicity of these molecules, depending on the animal species.

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3. COMMERCIAL PCB-BASED PRODUCTS

3.1 Principal Producers of PCBs

Between 1929 and 1977, PCBs were manufactured and sold by various companies throughout the world under different trade names. Table 3 lists the main producers.

TABLE 3: Principal Producers of PCBs

TRADE NAME	MANUFACTURER	COUNTRY
Apirolio	Caffaro	Italy
Aroclor[1]	Monsanto	United States
Clophen	Bayer	West Germany
Kaneclor[2]	Mitsubishi	Japan
Pyralene	Prodelec	France
Pyranol	G.E.	United States
Pyroclor	Monsanto (U.K.)	United Kingdom
Aceclor	ACEC	Belgium

[1] The most widely used product in Canada and the United States.

[2] This is the product implicated in the Yusho and Yu-Cheng poisonings.

3.2 Various Industrial Uses

3.2.1 Long-term uses (closed circuit)

- dielectric fluids called askarels in power transformers and capacitors;
- hydraulic machinery, vacuum pumps, gas turbines, heat-exchanger fluids.

3.2.2 Short-term uses (open circuit)

- lubricants
- power regulators (fluorescent light ballasts)
- rubbers and synthetic resins
- various plastics
- caulking
- paints
- glues
- waxes
- textiles
- carbonless copy paper
- inks
- dust-control agents for dirt roads
- solvents for spreading insecticides
- cutting oils (metal work)
- immersion oils (microscopy)
- military uses (electrical or electronic equipment).

3.3 Various Commercial Compounds

Commercial compounds consist of mixtures of several congeners of polychlorinated biphenyls selected for their particular properties and occasionally combined with mixtures of chlorobenzene isomers (tri- and tetrachlorobenzenes).

Each of these congeners affects the product's properties according to its chlorine content and the position of its chlorine atoms. For example, isomers with less chlorine, e.g., dichlorophenyls, are not very fire-resistant but have good dielectric (insulating) properties. On the other hand, isomers with more chlorine, e.g., pentachlorobiphenyls, are highly fire-resistant but are not as effective insulators.

Tri- and tetrachlorobenzenes added to PCBs make them less viscous.

To illustrate this diversity, let us take as an example Aroclor, manufactured by Monsanto (U.S.) for various applications (see Table 4).

TABLE 4: Use of Various Aroclor Compounds

APPLICATION	TYPES OF AROCLOR USED
Capacitors	1016, 1221, 1242, 1254
Transformers	1242, 1254, 1260
Vacuum pumps	1248, 1254
Hydraulic fluids	1232, 1242, 1248, 1254, 1260
Plastifiers (synthetic resins)	1248, 1254, 1260, 1262, 1268
Heat exchangers	1242
Waxes	1242, 1254, 1268
Dust-control agents	1254, 1260
Pesticide diluants, inks, lubricants, cutting oils	1254
Carbonless carbon paper	1242

For Aroclors, the first two digits (12 in this instance) indicate that it is a PCB-based compound. The last two digits give the percentage of chlorine in the product, e.g., AR 1242 contains 42 percent and AR 1260 contains 60 percent.

AR 1016 is an exception: it was manufactured in 1971 by distilling AR 1242. In this product, the percentage of chlorine has fallen to 41 percent and the percentage of low-chlorine isomers has increased. The resulting product is more biodegradable and less toxic than AR 1242.

Table 5 shows that the various compounds do not all contain the same isomers or the same proportions of isomers. It should be remembered that the higher the chlorine content is, the more persistent the product is in living organisms. The congeners of AR 1260 belong to groups of isomers with high chlorine content. For this reason, AR 1260 is much more persistent than AR 1242, which contains fewer high-chlorine isomers. Moreover, when a milieu has been contaminated with several Aroclor mixtures (1242, 1254, 1260), the distribution of the congeners present tends to evolve toward a chromatographic configura-

tion which increasingly resembles AR 1254 or AR 1260. This can be explained by the fact that low-chlorine isomers in AR 1242 biodegrade more quickly.

TABLE 5: Chemical Formulas of Some Aroclors

GROUPS OF ISOMERS (series of homologous compounds)	% OF ISOMERS IN THE AROCLORS			
	1242	1248	1254	1260
$C_{12}H_9Cl$	3			
$C_{12}H_8Cl_2$	13	2		
$C_{12}H_7Cl_3$	18	28		
$C_{12}H_6Cl_4$	30	40	11	
$C_{12}H_5Cl_5$	22	36	49	12
$C_{12}H_4Cl_6$	4	4	34	38
$C_{12}H_3Cl_7$			6	41
$C_{12}H_2Cl_8$				8
$C_{12}HCl_9$				1

3.4 Identification of the Chemical Structure of PCB Mixtures

It is thanks to chromatography in the gaseous stage and mass spectrometry that we are currently able to identify the chemical structures of mixtures we wish to analyse. In this way, we can determine the content of various PCB congeners in mixtures of commercial products or in contaminated milieux such as the ground, water, sediments, animal and human tissues, blood and so forth. Moreover, it is possible, using high-resolution apparatus, to measure the concentrations of contamination or combustion products of PCBs, such as polychlorodibensofurans (PCDFs), polychlorodibenzodioxins (PCDDs), quaterphanyls and so on (cf. Hutzinger et al. 1974, Albro et al. 1977, Bowes 1975, Curley et al. 1975, EPA 1983).

The high-resolution technique most frequently used to identify congeners of PCBs is the capillary column chromatography in the gaseous stage (see Figure 3, dealing with the chromatograms of Aroclors 1221, 1232, 1242, 1248 and 1254). Generally, the relative concentrations of various products found in the sample are measured, e.g., 1242, 1254 and 1260, by comparing the relative quantities of five peaks of these various products with previously known standard products. Because of overlapping of certain isomers between similar products (1242 and 1248 or 1242 and 1254), this method has its limitations. However, as little overlapping exists between 1242 and 1260, a method which makes it possible to establish this distinction is certainly valid. It should be noted that the chromatographic characteristics of PCBs are similar to those of several organochlorinated pesticides, resulting in occasional interference which may complicate identification.

Let us stress that commercial products do not contain all possible configurations of PCBs; certain congeners are absent or cannot be detected. This is important as those congeners deemed to be the most toxic (3,4,3',4' tetra- and 3,4,5,3',4',5' hexa-) are either absent or found only in trace amounts. As an example, Table 6 indicates the composition of AR 1242 and AR 1016 and reveals that they have similar compositions up to the tetra- isomers. The most polar tetra- isomers, most of the penta- and essentially all of the hexachloro- isomers are absent from AR 1016.

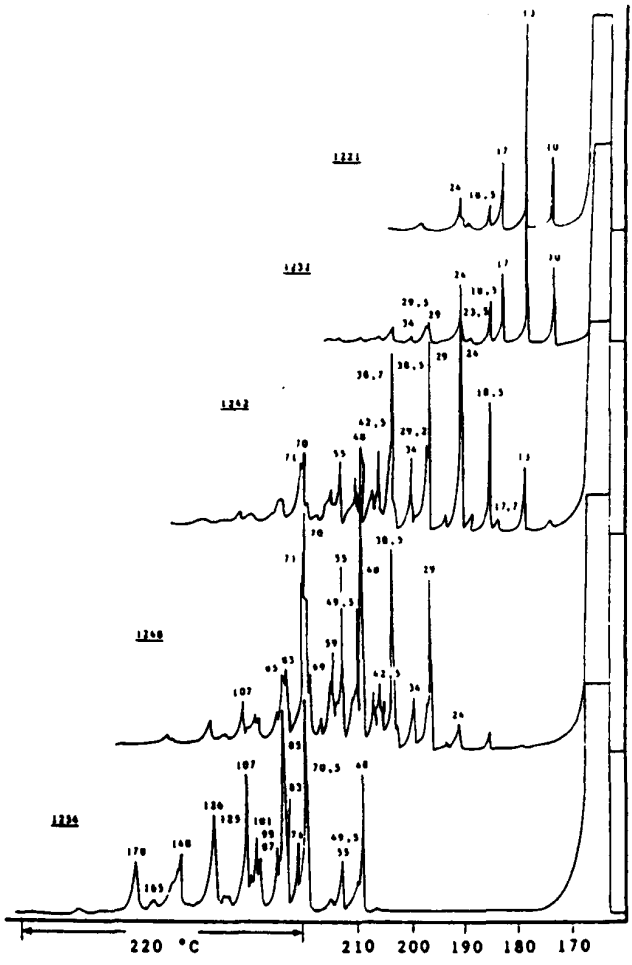

FIGURE 3: Capillary Column Chromatograms of Several Aroclors. The Number on Each Peak Corresponds to the Retention Time of Peaks in Relation to p,p' - DDT: Both Substances Were Studied Using Chromatography at 190°C (taken from J. Assoc. Offic. Anal. Chem. 55, 746, 1972, used with permission)

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TABLE 6: Chemical Composition of Aroclors 1242 and 1016
(taken from Albro and Parker, 1979)

PCB	Percentage		PCB	Percentage	
	1242	1016		1242	1016
2	0,68	0,80	2,4,2',4'	1,65	1,81
3	0,04	0,10	2,4,5,4'	2,02	1,35
4	0,22	1,00	2,5,3',4'	1,11	-
2,6	0,13	0,20	3,4,5,2'	trace	-
2,2'	3,99	4,36	3,4,5,3'	0,52	-
2,5	0,31	0,34	3,4,5,4'	0,28	-
2,4	1,04	1,16	3,4,3',4'	0,34	-
2,3'	1,24	1,37	2,5,3',5'	0,33	-
2,4'	8,97	10,30	2,4,3',4'	0,81	0,14
3,5	0,35	0,37	3,4,3',5'	0,24	-
3,4	0,09	0,11	2,3,4,4'	0,21	-
3,4'	0,12	0,12	2,4,6,3',5'	0,92	-
4,4'	0,99	1,07	2,4,6,2',3'	0,13	0,04
2,6,2'	0,97	1,08	2,3,6,2',5'	0,53	0,18
2,5,2'	9,36	10,87	2,3,6,3',5'	0,39	0,01
2,4,2'	2,92	3,14	2,3,6,2',4'	trace	-
2,3,2'	3,25	3,50	2,3,6,2',3'	0,38	0,01
2,6,3'	0,54	0,58	2,3,4,3',5'	0,46	0,16
2,6,4'	2,15	2,31	2,3,4,2',4'	0,40	-
2,5,3'	0,55	0,62	2,3,4,3',4'	0,09	-
2,5,4'	4,53	4,72	2,3,5,2',5'	0,25	-
2,4,3'	1,68	1,79	2,4,5,2',5'	0,12	-
2,4,4'	13,30	14,48	2,4,5,2',4'	0,55	-
2,3,3'	3,64	3,99	2,4,5,2',5'	0,27	-
2,3,4'	2,64	2,80	2,4,5,3',5'	0,31	-
3,4,2'	2,83	3,08	3,4,5,2',4'	0,36	-
3,4,3'	0,66	0,38	3,4,5,3',5'	0,05	-
3,4,4'	1,62	1,89	3,4,5,3',4'	0,03	-
3,5,4'	1,03	1,08	2,3,4,5,2',6'	0,07	-
2,6,2',6'	0,17	0,19	2,3,4,2',4',5'	0,08	-
2,3,6,2'	0,90	1,00	2,4,5,2',4',5'	0,02	-
2,5,2',6'	0,97	1,07			
2,5,2',5'	4,08	4,35	Monochloro-(%)	0,94	2,40
2,4,6,4'	2,18	2,40	Dichloro-(%)	17,23	19,40
2,3,5,2'	0,44	0,47	Trichloro-(%)	51,67	56,31
2,3,2',6'	0,31	0,33	Tetrachloro-(%)	24,65	21,32
2,4,5,2'	1,33	1,41	Pentachloro-(%)	5,24	0,40
2,4,2',5'	3,28	3,48	Hexachloro-(%)	0,17	-
2,3,2',5'	1,06	1,14			
2,3,4,2'	1,67	2,00			
2,3,2',3'	0,15	0,18			
2,3,3',4'	0,60	-			

4. POLYCHLORODIBENZODIOXINS (PCDDs) AND POLYCHLORODIBENZOFURANS (PCDFs)

Like polychlorinated biphenyls, both families belong to the broad family of halogenated aromatic hydrocarbons. Their members (congeners) have a structure quite similar to those of PCBs.

PCDDs and PCDFs formed during combustion of polychlorinated biphenyls result from the incomplete combustion of the latter. However, the quantity of PCDF formed is considerably more substantial than that of PCDD. PCDFs are also contaminants found in PCB-based commercial products, e.g., askarels.

A number of congeners belonging to these two families are deemed to be extremely toxic, much more so than the congeners of PCBs.

As these compounds can appear with the introduction of PCBs in the environment, it seems necessary to us to study the question of PCDFs and PCDDs to interpret the effects of PCBs on the environment and humans.

Without examining in detail their physiochemical properties, dispersal, source and levels in the environment, which would demand more extensive work than for PCBs, we will analyse certain aspects of their toxicity in Chapter 8. In this and subsequent chapters, we will make a general presentation devoted to contaminants and fires.

4.1 Polychlorodibenzodioxins (PCDDs) or Dioxins

The number of chlorine atoms can vary from 1 to 8. There are 75 congeners, including 22 tetrachlorodibenzodioxins, of which 2,3,7,8-TCDD is the best known and most toxic. This molecule is one of the best known pollutants; it is the topic of considerable research and many published articles.

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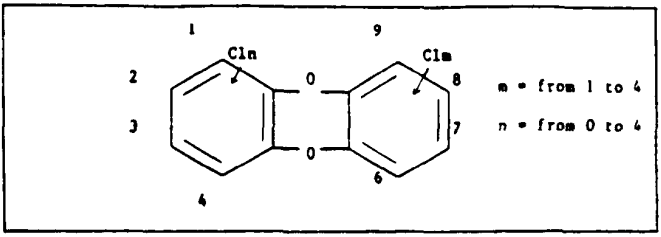
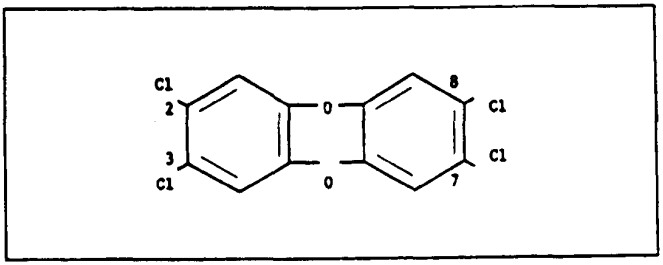


FIGURE 4: General Formula of PCDDs

The structure of PCDDs is quite similar to that of PCBs. PCDD molecules can be formed through the combustion of tetrachlorobenzene and, in limited amounts, through the pyrolysis of PCBs. They are also a by-product of the manufacture of trichlorophenol, a basic component in insecticides such as DDT.

FIGURE 5: Structure of 2,3,7,8-TCDD, the Most Toxic Molecule



4.2 Polychlorinated Dibenzofurans (PCDFs) or Furans

Furans have 135 congeners, including 38 tetrachlorodibenzofurans (TCDFs). The number of chlorine atoms can vary from 1 to 8. Their structure is also similar to that of PCBs and they can be formed by the hydrolysis or combustion of PCBs. Moreover, they are contaminants of commercial PCB-based products.

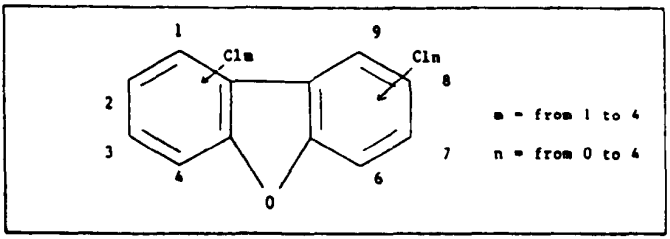


FIGURE 6: General Formula of PCDFs

The most toxic molecule is 2,3,7,8-TCDF; it has the same structure as 2,3,7,8-TCDD, except that it has one less oxygen molecule.

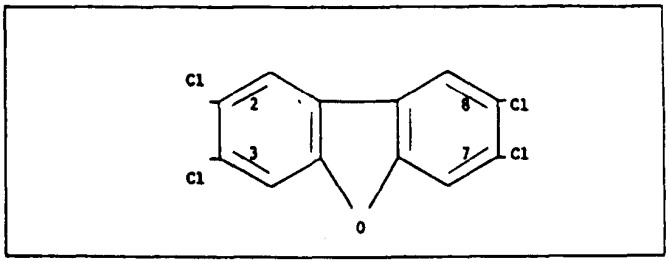


FIGURE 7: Structure of 2,3,7,8-TCDF, the Most Toxic Molecule

5. PCB CONTAMINANTS

Commercial PCB-based products are usually contaminated with small amounts of chlorinated naphthalene and traces of polychlorodibenzofurans (PCDFs).

Furans are generally considered to be highly toxic. Today, 17 years after the poisoning in Japan and six years after the incident in Taiwan, experts have concluded that symptoms displayed by the victims of both massive poisonings are caused primarily by polychlorodibenzofurans in the rice oil (Kashimoto et al., 1981 and 1985).

5.1 Presence of Polychlorodibenzofurans in Commercial Products Prior to Use

Table 7 indicates the PCDF content detected in various commercial products manufactured in the United States, Japan, Germany and France. American products contain less PCDFs, overall average concentration of which in Aroclors is 2 ppm (1242, 1248 and 1254). In products from Japan, Germany and France, this concentration varies between 8 ppm and 18 ppm .

It should be noted that Bowes (1975) and Miyata (1976, 1985) have identified 2,3,7,8-TCDF and 2,3,4,7,8-PCDF compounds in AR 1242, 1248 and 1260 and in Kaneclors 300, 400, 500 and 600. These two congeners are the most toxic in the PCDF family.

5.2 Concentration of PCDF in PCBs According to Length of Use

It has been shown (Chittim et al., 1979; Randoux, 1983) that the concentration of PCDFs in askarels increases with the length of use of transformers. Table 8 describes this phenomenon in relation to the level of T_4CDF (isomers in the tetrachlorinated homologous compound series). All measurements of TCDF were effected after 1975.

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TABLE 7: Concentration of Total PCDFs in PCB-Based Commercial Products

COUNTRY	PRODUCT AND YEAR OF PRODUCTION	TOTAL PCDF CONCENTRATION ppm	REFERENCE
United States	(Aroclors)		
	AR 1242	0,2	Albro (1977)
	(1969)	3	Miyata et Kashimoto (1978)
	AR 1248 (1969)	2	Bowes et al. (1975)
		2	Miyata et Kashimoto (1976)
	AR 1254 (1969)	1,2	Bowes et al. (1975)
	(1970)	1,9	Miyata et Kashimoto (1978)
		undetectable	
	AR 1016	< 0,005	Albro (1977)
	(1972)	< 0,001	Bowes et al. (1975)
Japan	(Kaneclors)	9	Miyata et al. (1975)
	KC 300 (before 72)	18	Miyata et al. (1975)
	KC 400 (before 68)	14	Miyata et al. (1975)
	KC 500 (before 72)	16	Miyata et al. (1975)
	KC 600 (before 72)		
Germany	Clophen A60	8,6	Bowes et al. (1975)
	Clophen A50	17	Miyata et Kashimoto (1976)
France	Phenoclor DP-4	8,0	Miyata et Kashimoto (1976)
	Phenoclor DP-5	11	Miyata et Kashimoto (1976)
	Phenoclor DP-6	11,6	Bowes et al. (1975)

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TABLE 8: Variation According to the Length of Use and the Concentration of PCDFs in Transformers* Insulated with Askarel (cf. Chittim et al., 1979)

YEAR OF INSTALLATION	AROCLOR	Chlorobenzenes/PCBs (%)	T_4CDF (mg/kg of askarel)
1957	1260	51/49	0,780
1961	1260	53,6/46,4	1,110
1963	1260	50,6/49,4	1,020
1963	1260	52,4/47,6	0,790
1963	1260	38,6/61,4	0,350
1965	1260	37,2/62,8	0,290
1970	1254	29,6/70,4	0,550
1970	1254	28,0/72,0	0,470
1971	1254	29,0/71,0	0,290
1974	1254	28,4/71,6	0,051
not used	1254	30,6/69,4	0,041

*1500-kW transformers.

5.3 Concentration of PCDFs in PCBs and Temperature (Below or Equal to Ignition Point)

Another factor which markedly affects the production of PCDFs is the increase in temperature when oxygen is present. The presence of catalyzers such as steel may accelerate the formation process. Miyata and Kashimoto demonstrated this in 1978 by heating KC 400 to 360°C for 20 days. Table 9 indicates that the original PCDFs increased by a factor of between 2.3 and 6.4, depending on the temperature and catalyzers present.

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TABLE 9: Effect of Heat on the Formation of PCDFs in KC 400 (Miyata and Kashimoto, 1978)

TEMPERATURE	DURATION IN DAYS	CATALYZER	TOTAL PCDFs mg/kg of PCBs	TOTAL TCDFs mg/kg of PCBs
Ambient	0	none	33	26.2
360°C	20	none	65	52.1
360°C	20	stainless steel	77	32.7
360°C	20	water	212.1	152.4
360°C	20	steel + water	72.1	46.6

As Table 10 indicates, Miyata (1985) noted a marked increase of PCDFs when comparing unused KC 400 with KC 400 used as a conductor in heat exchangers. One sample came from an exchanger used for 14 years at 180°C-270°C; the second came from an exchanger used for three years at 200°C-220°C. He also demonstrated the presence of polychlorinated quaterphenyls (PCQs).

TABLE 10: Concentration of PCBs, PCQs and PCDFs in Two Samples of KC 400 Used in Heat Exchangers (cf. Miyata, 1985)

KC 400 Sample	Temperature	PCBs (ppm)	PCQs (ppm)	PCDFs (ppm)
S.1 (14 years)	180 to 270°C	968 400	31 000	510
S.2 (3 years)	200 to 220°C	999 000	690	277
S.3 (not used)		999 800	209	33

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Contamination of the rice oil which caused Yusho disease in Japan and Yu-Cheng disease in Taiwan was caused by a heat exchanger. While the ratio of PCDFs to PCBs in the unused KC 400 was about 1/50 000 on the average, it was only about 1/200 and 1/400 in the contaminated oils in question. The PCQ/PCB ratio was 1/1 in Japan and 1/3 in Taiwan. The high PCDF and PCQ content can be explained by the high temperature prevailing in the heat exchanger. In Chapter II, we will discuss the role PCDFs and PCQs played among victims of these diseases.

6. PCBs AND FIRES

At present, the main problem with PCBs is that of fires in electrical equipment insulated with pure PCBs or with PCBs combined with other solvents such as tri- and tetrachlorobenzenes, which are often present in askarels. A related, but less serious problem is arousing more and more concern among the general public and workers, especially firefighters, i.e. fires in electrical equipment insulated with mineral oil contaminated by PCBs in concentrations of between several ppm and more than 500 ppm. It is estimated that about 10 percent of oil-insulated transformers currently operating are contaminated with more than 50 ppm of PCBs.

The seriousness of fires where PCBs are present is due to the formation, under intense heat, of other extremely toxic organochlorinated products, i.e. PCDFs and PCDDs. Polychlorinated quaterphenyls and methylsulfones, deemed to be less toxic than PCBs, and polychlrinated biphenyls are also formed; documentation on the latter family is limited.

When fire breaks out in equipment containing PCBs, such as a transformer or a capacitor, attention focuses on safety measures to be taken during the fire to protect firefighters combating the flames and, after the fire, to protect workers responsible for decontaminating and rebuilding the site.

To estimate risk to such workers and residents living near the site, it is important to know under what conditions and in what proportions these toxic products are formed, and the quantities of contaminants found in soot.

6.1 Formation of PCDFs, PCDDs and Hydrochloric Acid

Several factors determine the types of PCDF and PCDD congeners formed: the products used (chlorine content, presence or absence of tetrachlorobenzenes), temperature, the presence of oxygen, the presence of catalyzers in other materials burned, and so on.

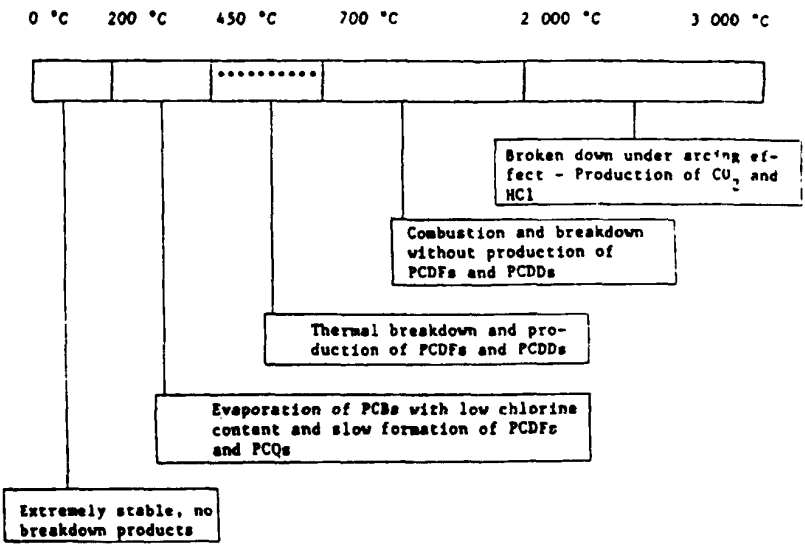

FIGURE 8: Schematic Representation of the Thermal Breakdown of PCBs and the Production of PCDFs, PCDDs and HCl

Let us analyse in greater detail the thermal breakdown of PCBs (Figure 8).

0°C to 200°C PCBs are extremely stable; no breakdown occurs.

200°C to 450°C These temperatures are below the ignition point of PCBs, although evaporation does occur and, as we noted in section 5.3, PCDFs and PCQs form slowly.

450°C to 700°C Several authors have conducted laboratory studies of incomplete combustion at these temperatures (Buser et al., Marita et al., Chittim et al.). They showed that fairly substantial quantities of PCDFs formed in a few seconds, i.e. between 10 mg/kg and 20 g/kg of PCBs depending on the temperature and the factors present. For example, Buser et al. (1978) found that by pyrolyzing hexachlorobiphenyls (H_6C8) at 550°C, 20 g of PCDFs/kg of PCBs formed, while at 700°C, only 16 mg formed. The PCDFs obtained were primarily the congeners 2,3,7,8-T_4CDF, 1,3,4,7,8-P_5CDF and 1,2,3,7,8-P_5 CDF.

When AR 1254 and AR 1260 were heated in the laboratory to about 550°C (the temperature at which optimum formation of PCDFs occurs) in a glass bubble in the presence of oxygen, about 20 g of PCDFs/kg of PCBs formed, belonging to series of homologous compounds of mono- to penta-CDF for AR 1254 and of tri- to octa-CDF for AR 1260.

700°C to 2000°C PCDFs were produced in negligible quantities, which explains why combustion ovens employed in the United States and Europe to destroy PCBs usually operate between 1000°C and 1400°C. Rappe et al. (1979) showed that no discernable PCDFs and PCDDs were formed by heating PCBs in a rotary cement oven to temperatures between 1400°C and 2000°C, which seems to indicate that this method of destroying the substances is fairly safe.

2000°C or more Under the effect of electric arcs, CO_2 and HCl, with its characteristic odor of bleach, are produced. We noted this phenomenon at the Hydro-Québec Wellington substation fire in the Maisonneuve region. Negligible quantities of PCDFs were measured in soot near the seat of combustion; the concentration was below the standard in force in Québec, i.e. below 1/500 of the Non Observed Effect Level (NOEL) in 2,3,7,8-TCDD equivalent.

6.2 Estimate of the Quantity of PCDFs Formed During a Fire

To estimate health risks in the case of fires involving PCBs, it is very important to ascertain the quantity of PCDFs formed. In this section, by extrapolating data at our disposal, we will attempt to estimate the maximum quantities of PCDFs formed.

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We know that the quantity of PCDFs formed during the combustion of PCBs depends upon temperature and the presence of oxygen. However, during a fire, all of the conditions for the optimum formation of PCBs simulated in the laboratory are not necessarily present. Temperature varies in time and space, with discrepancies of several hundred degrees Celsius between the periphery and the seat of the fire. All PCB molecules are not, therefore, subjected to the same combustion temperature. Moreover, it appears that certain PCB molecules do not obtain the oxygen needed to burn and form a PCDF molecule. These molecules spread across the ground without being pyrolyzed, as the concentrations of PCBs noted in soot testify.

Thus, it may be inferred, with little risk of error, that during a fire, the quantities of PCDFs formed may be below 20 g of PCDF/kg of PCB, but probably higher than 16 mg, i.e. at a level somewhere between the minimum and maximum values found in the laboratory.

In the following pages, we will endeavor, using data drawn from IREQ's experience, to establish in what proportion PCDFs were formed on the basis of the combustion of a known quantity of PCBs.

6.2.1 Initial hypothesis

Let us verify the following hypothesis: that under normal conditions prevailing during a fire, the quantity of PCDFs formed per kilo of PCBs does not exceed the proportional value at mid-point between 16 mg and 20 g, i.e. 565 mg of PCDFs/kg of PCBs.

$$\frac{20\,000 \text{ mg}}{565 \text{ mg}} = \frac{565 \text{ mg}}{16 \text{ mg}} = 35.35$$

To simplify calculations, let us take the value 500 mg of PCDFs/kg of PCBs.

6.2.2 Brief description of e fire at IREQ

On November 20, 1984, an explosion occurred in a capacitor bank during tests on a power transformer in the annex of the high-voltage laboratory (see Figure 9). The explosion started a fire which led to a series of explosions in other capacitors, some of which were insulated with oil, and others with PCBs. All told, 2560 L of mineral oil and 670 L of PCBs (AR 1242) were destroyed. For two hours, during which it is estimated that virtually all of the oil and PCBs were destroyed, thick, black smoke lingered inside the building. It spread to the annex, the main hall and spaces between walls and ceilings. A hole then opened up in the ceiling above the seat of the fire and the smoke escaped. However, samples taken after the fire revealed negligible contamination of the ground surrounding the building. It should be mentioned that almost all of the materials were incombustible, except, of course, the mineral oil and PCBs.

6.2.3 Verification of the initial hypothesis: that the maximum formation of PCDFs is below 500 mg/kg of PCBs

Overall, 3230 L of combustible material was destroyed, i.e. 670 L of PCBs and 2560 L of mineral oil. Taking into account densities (0.9 for oil and 1.5 for AR 1242), this meant a total of 3309 kg of fluids.

Analysis of a typical sample of soot collected near the seat of the fire indicates that it contained 39 ppm of PCDFs (mg of PCDF/kg of soot) and 2200 ppm of PCBs (mg of PCB/kg of soot). The walls of the annex were covered with a layer of oily soot between several micrometres and several millimetres thick; the walls of the main hall were covered in places with a thin film of soot at least several micrometres thick.

According to our hypothesis, the overall quantity of polychlorodibenzofurans (PCDFs) formed should be below 502 g.

(670 L of PCBs) x (1.5 kg of PCBs/L) x (500 mg PCDFs/kg of PCBs) = 502 g

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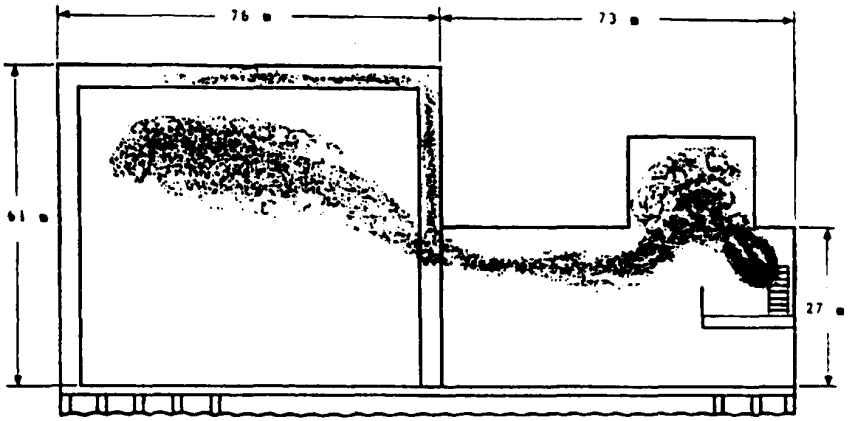

FIGURE 9: Location of the Fire in the IREQ High-voltage Laboratory (Figure Taken from a Speech by David Train and Danielle Dupont Given in September 1985 in Seattle, Washington)

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6.2.4 Is this hypothesis realistic?

Where the average concentration of PCDFs in soot is equivalent to our typical sample, i.e. 39 ppm, it would take 12 871 kg of soot to obtain 502 g of PCDFs. In this case, there were only 3309 kg of combustible materials (oils and PCBs): as there could never be four times more soot than combustible materials, it may be inferred that the formation of a maximum of about 125 mg/kg of PCDFs would be more realistic (500 ÷ 4 = 125 mg/kg).

Let us verify the hypothesis by another method. The walls, ceilings and floors of the annex and the hall are considered to be smooth and to have a total surface area of about 45 000 m^2, i.e. 30 000 m^2 in the main hall and 15 000 m^2 in the annex. According to IREQ chemist Jacques Castonguay, who supervised decontamination procedures, it would be realistic to estimate that soot in the annex was an average of 1 millimetre thick on surfaces. Taking into account the low density of soot (0.01 to 0.015), he estimates that it weighs approximately 10 g per m^2 of surface. In the worst case, where the content of soot per surface unit of the main hall was equal to that of the annex, we would arrive at a total of 450 kg of soot (45 000 m^2 x 10 g/m^2). This corresponds to a total of 17.55 g of PCDFs (450 kg of soot x 39 mg of PCDFs/kg of soot), a quantity 28.6 times lower than that in our initial hypothesis. We must, therefore, infer that the quantity of PCDFs per kilogram of PCBs is 500 mg/kg (initial hypothesis) ÷ 28.6, i.e. 17.5 mg of PCDFs/kg of PCBs.

On this basis, it can readily be inferred that the quantities of PCDFs formed at IREQ were below 500 mg/kg of PCBs. According to our reasoning, they appear to be between 17.5 mg/kg and 125 mg/kg of PCBs.

6.3 Principal Fires in Electrical Equipment Insulated with Askarels

In North America, the most serious fires involving PCBs occurred in February, 1981, in an 18-storey government building in Binghamton; in May 1983, in a commercial building in San Francisco; and in November 1984, at IREQ.

In all three instances, soot covering the surface of interior walls contained PCBs, PCDFs, PCDDs and polychlorobiphenylenes.

Table II indicates the typical quantities of PCBs, total PCDFs, total TCDFs and 2,3,7,8-TCDD from soot samples collected near the seat of fires, close to burned electrical equipment, i.e. transformers in Binghamton and San Francisco and capacitors at IREQ.

TABLE II: Typical Concentration of Total PCBs, PCDFs and TCDFs, and TCDD in Soot Samples Collected Near the Seat of Fires

FIRE	PCBs	Concentration (ppm) in soot [mg/kg] Total PCDFs	Total TCDFs	2,3,7,8-TCDF	2,3,7,8-TCDD
San Francisco, CA	86 000**	286	16.29	6.7	undetected
Binghamton, NY	7 200*	438	145	72	3
IREQ	2 500**	39	4.89	0.61	undetected***

* AR 1254 and 35% tri- and tetrachlorobenzenes
** 100% AR 1242
*** Detection limit between 10 u g/kg and 50 u g/kg

In all three cases, the quantity of PCDFs is relatively high; on the other hand, in California and at IREQ, total quantities of PCDDs are negligible and the concentration of 2,3,7,8-TCDD undetectable. The greater quantities of PCDDs and 2,3,7,8-TCDD in Binghamton can be explained by the presence of tetrachlorbenzene (35 percent) in the askarel. It has been shown that tetrachlorobensene breaks down into dioxins through incomplete combustion; PCBs tend to produce furans. The mechanism by which the combustion of PCBs produces small quantities of dioxins is not known.

It should be noted that at IREQ and in San Francisco, 2,3,7,8-TCDFs accounted for 1.5 percent and 2.3 percent, respectively, of total PCDFs, while in Binghamton they represented approximately 16 percent. This can probably be explained by the fact that AR 1254 produces more tetra-CDF isomers than AR 1242. At IREQ, more than 62 percent of PCDFs were tri-CDF isomers. From

this it must be concluded that health risks are higher with AR 1254 than with AR 1242, and that the presence of tetrachlorobenzene in askarel increases this risk. Obviously, this depends on the amounts burned.

6.4 Health Risks During an Askarel Fire and Decontamination

Without claiming to accurately estimate the risks of acute and chronic toxicity among people exposed, it appears possible to obtain approximate data by effecting comparisons with incidents where PCDFs were involved in human poisonings. As there are two thoroughly documented cases of PCDF-related poisonings (in Japan in 1968 and in Taiwan in 1979), it appears eminently worthwhile to ascertain whether risk is as considerable in the case of fires with PCBs.

As explained in section 11.2, it is estimated that victims of Yusho and Yu-Cheng diseases ingested an average of between 0.5 g and 2 g of PCBs (Kaneclor 400) and from 2.4 mg to 7 mg of PCDFs.

Moreover, we now know that PCDFs were the principal cause of these poisonings, which provides us with a useful reference point. In the following section, we will endeavor to estimate whether it is probable that a fire in electrical equipment insulated with PCBs engenders as great a risk as in the Japanese and Taiwanese incidents.

6.4.1 Risk during the fire

To estimate this risk, we will refer to the IREQ fire as we are thoroughly familiar with the circumstances surrounding it. It took firefighters approximately six hours to control the flames, which reached their peak intensity during the first two hours. It is thought that almost all of the PCBs were burned during this time. Immediately after the fire, a sample taken in the burned building revealed that the air contained less than 10 $\mu g/m^3$ of PCBs (the standard in force in Québec for AR 1242 is 1 mg/m^3 for daily exposure of eight hours). However, we do not have data on the PCDF content of the air at that time.

6.4.1.1 Exposure to PCBs

A human being inhales about 2 m^3 of air every hour. For a firefighter to inhale between 0.5 g and 2 g of PCBs, the air would have to contain between 125 mg/m^3 and 500 mg/m^3 of the substances. Given the low vapor pressure of PCBs, even at temperatures between 35°C and 45°C, it is virtually impossible to attain such concentrations.

It should be noted that, after one week, no professional or volunteer Hydro-Québec firefighter had blood PCB levels higher than concentrations found in the population at large (≤5 ppm of 1242 on average). This is an excellent indication of the low risk to which they were exposed. It should be pointed out that most of the firefighters were wearing a self-contained, positive-pressure breathing apparatus.

6.4.1.2 Exposure to polychlorodibenzofurans

In section 6.2.4 we estimated that a maximum of 125 g of PCDFs could have been formed by the combustion of PCBs (this is an overestimation). Let us take the worst hypothesis, where the 125 g of PCDFs formed gradually over two hours were evenly distributed throughout the high-voltage laboratory (a volume of about 496 219 m^3: 76 x 76 x 61 + 73 x 73 x 27 m) and remained suspended in the air: in this case, the average concentration per m^3 would be equal to the concentration existing after one hour, i.e. 0.125 mg/m^3 [½ x (125 g ÷ 496 219 m^3 of air)]. After two hours, a firefighter would have absorbed a maximum of 0.25 mg of PCDFs, which is less than the quantity ingested during the poisoning in Japan (between 2.4 mg and 7 mg). Obviously, this hypothetical case is unrealistic as the PCDFs, during their formation, were trapped in the dense, oily smoke which deposited them on the ground and walls. The PCDF content of oily soot bears witness to this fact; thus, their concentration was much lower.

Moreover, wearing a self-contained, positive-pressure breathing apparatus reduced this risk by a factor of roughly 3 000 or more. At IREQ, even in the worst hypothesis, a firefighter wearing a mask could have inhaled a maximum of 83.3 n g of total PCDFs, of which only 3 percent were tetrachloroisomers according to the analyses made at IREQ (the other isomers are less important from the standpoint of toxicity).

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These observations enable us to conclude that, during the burning of several hundreds of litres of PCBs, the risk of firefighters displaying symptoms of Yusho disease is negligible where preventive measures are taken.

As a precaution, we recommend:

- that anyone intervening in a fire in equipment insulated with askarel be sure to wear a self-contained, positive-pressure breathing apparatus and a coverall to protect the skin;

- that emergency intervention procedures be established for all areas where this type of equipment is located, and that unauthorized persons be denied access to the site during and after the fire.

6.4.2 Health risks during decontamination

This risk will be assessed in a general manner, using comparisons with the quantities of PCDFs which caused Yusho disease. It should be remembered that the total quantity of PCDFs in samples collected near the seats of fires in San Francisco, Binghamton and at IREQ were 289 mg/g, 438 mg/g and 39 mg/g of soot, respectively. In other words, to absorb a quantity of PCDFs equivalent to that in Japan, i.e. between 2.4 mg and 7 mg, workers involved in decontamination procedures would have only had to absorb several milligrams of soot (see Table 12) by inhalation, contact or ingestion.

Although PCDFs are not very volatile and soot is very oily and sticks to surfaces, it is to be noted that risks they present to health are very high. For this reason, it is important to take extraordinary precautions to prevent any contact with soot and any contamination of workers' clothing.

Risk was highest at Binghamton, because of two factors: the presence of tetrachlorobenzene, and the high chlorine content of AR 1254 as opposed to AR 1242 which was present in the other two cases. Risk in Binghamton was even

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TABLE 12: Quantities of Soot to be Absorbed to Attain the Amount of Total PCDFs Which Caused Yusho Disease

FIRE	QUANTITY OF SOOT TO BE ABSORBED (mg)
San Francisco	8.4 to 24
Binghamton	5.5 to 11
IREQ	60 to 175

higher than it appeared to be, as the relative quantities of tetra-CDF and 2,3,7,8-TCDF (the most toxic congeners of the PCDF family) were higher than in the other two cases.

6.5 Fire in Equipment Containing Mineral Oil Contaminated with PCBs

During the past decade it has been discovered that transformers insulated with oil built since the early 1950s are often contaminated with PCBs. On the basis of inventories, it is estimated that between 10 percent and 15 percent of such transformers contain concentrations of more than 50 ppm of PCBs, and less than 1 percent contain concentrations of more than 500 ppm.

For this reason, the public at large and especially workers in the electrical industry and firefighters, fear the consequences of fires involving this type of equipment. Does the presence of PCDFs during combustion of contaminated oils create a risk, and can such risk be estimated?

6.5.1 Quantities of PCBs present in contaminated oils

Oil contaminated at 50 ppm contains 50 mg of PCBs per kilogram: 1 mg of PCBs per kilogram of oil corresponds to each part per million (ppm) of PCB contamination.

A transformer containing 300 kg of mineral oil contaminated with 100 ppm of PCBs by weight contains a total of 30 g of PCBs (100 mg of PCBs/kg of oil x 300 kg of oil), i.e. a factor of 10^4 times less PCBs than in a transformer insulated with PCBs alone.

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6.5.2 Health risks posed by PCDFs formed in burning contaminated oil

Let us turn again to the example of a transformer containing 300 kg of oil contaminated at 100 ppm, i.e. a total of 30 g of PCBs. If we assume that during the fire the PCBs are destroyed under conditions similar to those of the IREQ fire, we are entitled to think that our estimate of the formation of a maximum of 500 mg of PCDFs/kg of PCBs is still valid. This would lead to a maximum total of 15 mg of PCDFs resulting from the combustion of the PCBs present (100 molecules of PCBs dispersed through 999 900 molecules of oil).

Let us imagine that the 300 kg of contaminated oil burn up in about 30 minutes, a fairly realistic estimate. This would result in the emission of an average total of 8.3 μ g/s of PCDFs over the entire surface of the burning oil at a given moment. If we take into account the chimney effect of the fire, which would tend to draw particles upward, the effect of wind dispersal and the effect of dilution over distance, it is easy to imagine that the concentration of PCDFs per unit of volume would be very small. Moreover, account must be taken of the fact that PCDFs are not very volatile (they tend to fall back to the ground).

If we combine the average human rate of inhalation (2 m^3/h) with the duration of the fire (≤ 1 hour), it appears rather unlikely that a person will absorb significant quantities of PCDFs. Through preventive measures (having firefighters use self-contained breathing apparatus and keeping bystanders away from the fire), health risks related to PCDFs become virtually nil in such a case.

A recent laboratory study (Alan et al., 1985, Southern California, Department of the Environment, International Symposium on PCBs, Seattle) showed that by burning 25 gallons (112.5 L) of oil contaminated at 9.9 ppm according to a mathematical model of dispersion of PCDFs emitted, the probability of a person in the neighbourhood of the fire absorbing a dose equivalent to that set by the EPA for lifetime exposure (risk equivalent to 1/1 000 of NOEL) was below 1 in 7 000 000.

It seems clear that these data, even based on estimates, indicate that health risks in the event of fires in contaminated oil are not nearly as pronounced as in fires involving askarels. Research must, however, be pursued to clarify

this risk. In the meantime, we advise caution: firefighters should wear appropriate protective equipment during the fire. Afterwards, even when risk of contamination by PCDFs is low, great care should be taken and the advice of experts sought before cleaning and rebuilding operations are launched.

6.6 Decontamination Criteria

IREQ's decontamination criteria comprise long-term levels of exposure to PCDDs and PCDFs currently in force in Québec. These criteria were established with the participation and consent of the Commission de la santé et de la sécurité du travail du Quebec (CSST).

TABLE 13: Toxicological Criteria on Residual Levels of PCDDs and PCDFs in the Air and on Surfaces

PCDDs AND PCDFs	CONCENTRATION IN THE AIR	SURFACE CONCENTRATION
2,3,7,8-TCDD equivalent	10 pg/m³	25 ng/m2

These criteria correspond to those established by Kim and Hawley (1984), experts attached to the New York state health department, in agreement with an advisory board set up after the Binghamton, New York fire. These experts consider safe for humans a daily exposure dose below 1/500 of the daily dose for which no toxic effects have been detected among laboratory animals, which in turn corresponds to 1/500 of the Non Observed Effect Level (NOEL).

Without outlining in detail the approach employed to establish the tolerable concentrations of PCDDs and PCDFs in the air and on surfaces to ensure that the safe exposure dose is not exceeded, it might be useful to offer a general idea of the origin of these criteria.

First, tolerable concentrations of PCDDs and PCDFs are reduced to 2,3,7,8-TCDD equivalents, with a view to adjusting the concentration of various congeners of PCDDs and PCDFs measured in relation to the concentration of 2,3,7,8-TCDD required to produce the same toxic effect in animals. A weighting factor has been established for all isomers of PCDDs and PCDFs relative to 2,3,7,8-TCDD. This concept is based on the approach suggested by Hutzinger et al. (1981, 1982).

Second, the minimum daily dose of 2,3,7,8-TCDD for which no effect is observed in laboratory animals (NOEL) was established by R.J. Kociba et al. in 1979, who stated, in agreement with the National Cancer Institute (NCI), that no toxic or carcinogenic effects or effects on reproduction were detected in rats and mice for doses below 1 ng/kg/day of 2,3,7,8-TCDD. A safety factor of 500 was proposed by the Binghamton experts and was included in Kim and Hawley's calculation.

Consequently, a lifetime daily exposure dose of 2 pg/kg/day of 2,3,7,8-TCDD equivalent was adopted for humans, corresponding to 100 pg/day for a person of average weight (50 kg). Assuming that a person breathes an average of 10 m^3 of air in the course of a working day, Kim and Hawley set the tolerable concentration in air at 10 pg/m^3 of 2,3,7,8-TCDD equivalent (100 pg/d/person ÷ 10 m^3 of air = 10 pg/m^3). They established the tolerable surface contamination criterion at 25 ng of 2,3,7,8-TCDD equivalent/m^2, using a mathematical model of absorption by the skin, bearing in mind the body surfaces exposed, i.e. arms and hands.

It should be noted that all of these criteria were complied with at IREQ once buildings had been decontaminated.

7. PCBs AND THE ENVIRONMENT

7.1 Quantities of PCBs Produced in North America

It is estimated that more than 610 million kg of PCBs were sold in the United States between 1930 and 1978; about 60 percent appear to have been used in closed-circuit systems such as transformers, capacitors and hydraulic equipment, and the remaining 40 percent for open-circuit uses such as plastics, paints, glues, waxes, inks and so on. According to a report prepared for the EPA in 1983, approximately 13.5 percent of PCBs sold, i.e. 82 million kg, have apparently been released in the environment. The remaining PCBs are either still being used (60 percent), or have been buried or put in storage centres.

Figure 10, taken from this report (EPA, 1983), indicates the annual production of PCBs in the United States between 1962 and 1978 and contains an estimate of the quantities of PCBs released in the environment every year.

An analysis of this figure reveals that:

- production of PCBs was highest between 1963 and 1970, when more than 20 million kilograms per year were manufactured;
- PCB production in the United States ceased in 1978;
- in 1973, the use of PCBs in open-circuit applications was halted on a voluntary basis, with the result that discharges in the environment ceased after that year;
- since 1962, some PCBs have been produced involunterily, especially in mineral oil used as an insulator in electrical. A study effected in 1981 by the Chemical Manufacturers Association in the United States (cited in the 1983 EPA study) revealed that prior to 1981, 6200 kg of PCBs per year came from oil contaminated at less than 50 ppm. At present, new oil used in electrical equipment in the U.S. may not contain more than 2 ppm of PCBs (2 mg/kg of oil).

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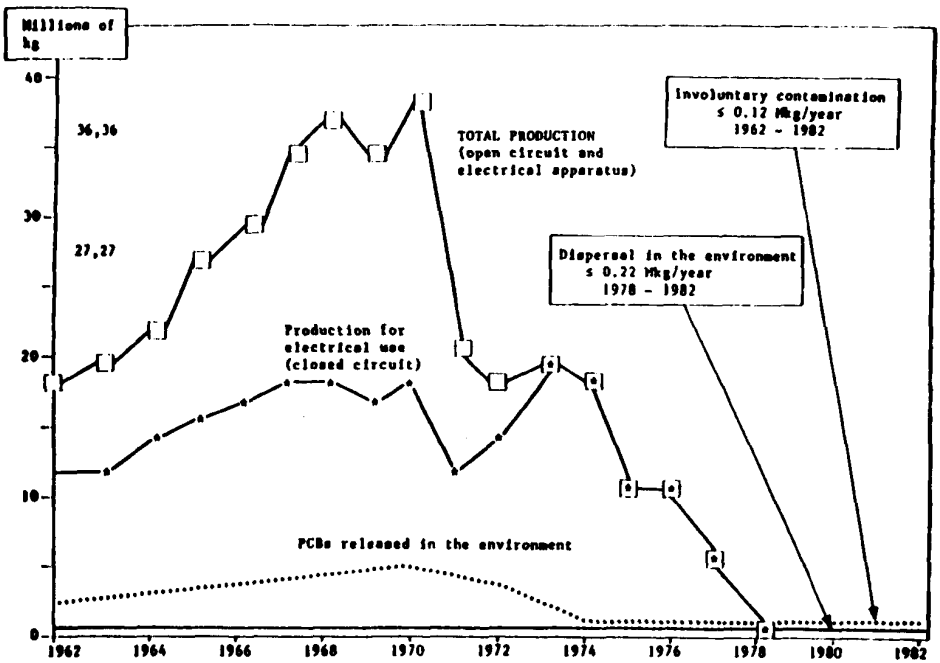

FIGURE 10: Annual Production of PCBs in the United States and Estimate of the Quantity Released in the Environment

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The most widely sold commercial products prior to the 1970s were mixtures containing less chlorine (AR 1221 and AR 1242). Figure 11 indicates total production of PCBs by family of homologous compounds between 1930 and 1978; it reveals that more than 35 percent of such PCBs were trichlorinated biphenyls, followed in descending order of importance by tetras (22 percent), pentas (16 percent) and dichloros (11.6 percent). As we will see in section 7.7, in the environment the PCBs which are degraded the fastest are the mono-, di- and trichlorinated isomers, whose half-life is several months at the most. It may, therefore, be assumed that a considerable proportion of the PCBs which were released in the environment have now disappeared.

7.2 Quantities of PCBs Still in Use in the United States and Canada*

7.2.1 Electrical equipment insulated with askarel

Of a total of 610 million kg of PCBs sold in North America, about 356 million kg are still in use, i.e. 341 million kg in the United States and 15.5 million kg in Canada (including 6.36 million in Ontario, 5 million in Québec and the remainder in the other provinces).

In Québec alone, some 5 014 900 kg appear to be in use, distributed as indicated in Table 14; of this amount, approximately 1 million kg, or roughly 20 percent, belongs to Hydro-Québec (see Table 15 for the distribution of PCBs in its transformers and capacitors insulated with askarel).

7.2.2 PCBs in contaminated transformer oil

In Canada, we do not have any information from the federal Environment department on the quantities of PCBs in transformer oil. However, at Hydro-Québec it is estimated, on the basis of an initial evaluation, that about 13 million L of transformer oil are contaminated at more than 50 ppm. The average contamination of the oil is about 200 ppm, which means that this substantial quantity of contaminated oil contains no more than 2600 kg of PCBs overall, i.e. 0.13 percent of the total quantity of PCBs found in Hydro-Québec electrical equipment. The PCB content of these thousands of transformers insulated with oil is equivalent to the quantity of PCBs found in three transformers insulated with askarel.

* According to the inventory prepared by Environment Canada (June 27, 1985).

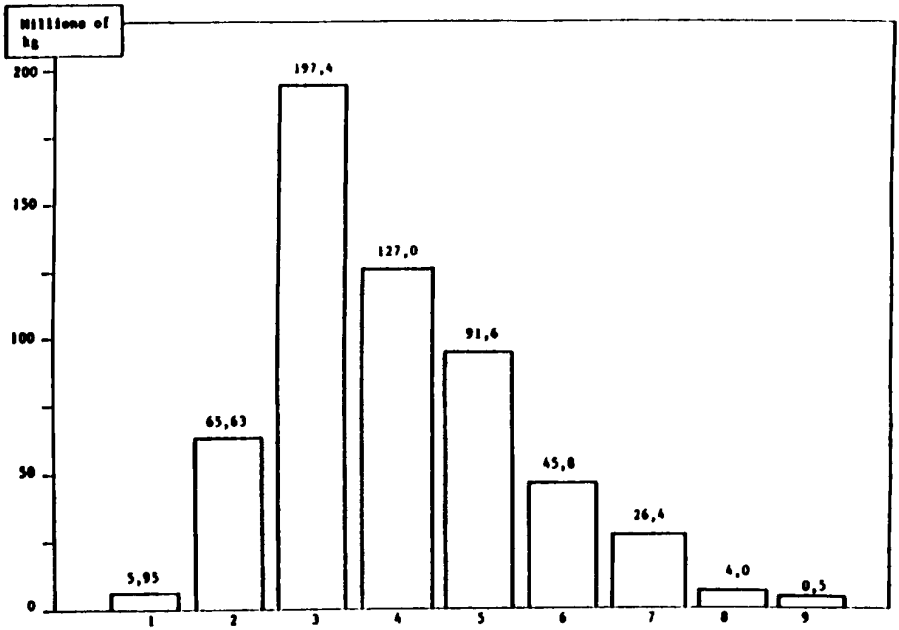

FIGURE 11: PCBs Sold in the United States between 1930 and 1978

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TABLE 14: Inventory of the Distribution of PCBs in Québec by Type of User

CATEGORY OF USER	QUANTITY (1000 kg)	(%)
Public services	1392.2	27.8
Forestry/Pulp and paper	667.0	13.3
Government facilities	509.0	10.1
Mines and smelting	459.0	9.2
Iron and steel industry	260.0	5.2
Hospitals and schools	185.0	3.7
Commercial warehousing	170.4	3.4
Textiles	107.1	2.1
Metal refining	87.1	1.8
Chemical products	83.2	1.7
Food industry/distilleries	77.6	1.5
Rubber products	46.9	0.9
Electrical equipment manufacturers	40.7	0.8
Cement industry	35.4	0.7
Oil refining	24.1	0.5
Other	869.6	17.3
TOTAL	5,014.9	100.0

TABLE 15: Distribution of PCBs at Hydro-Québec

TYPE OF EQUIPMENT	NUMBER OF APPARATUS	QUANTITY OF PCBs IN LITRES*
Transformers	115	140 000*
Capacitors	± 52 000	490 000*

* The density of PCBs is about 1.5 greater than that of water.

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From an environmental standpoint, the difference between askarels and contaminated oils is very important: the risk of contamination is much lower in the case of the latter. For example, if for contaminated oils we apply a limit of 1 g/day of PCBs (Environment Canada Chlorobiphenyls Regulations No. 3 (Release), 1985) which can be deliberately discharged in the environment in any region of Canada which is not a body of water, during the operation, repair, maintenance, breakdown, transportation or stocking of any electrical equipment, admissible dischargee can be considerable where concentrations of PCBs are low, as Table 16 indicates.

It should be stressed that, at present, in Québec any fluid containing more than 0.15 ppm of PCBs (0.15 mg/L of fluid) is deemed to be hazardous waste and, from an environmental viewpoint, must be treated as though PCBs alone were involved. On the other hand, according to Environment Canada, it is at levels of 50 ppm or more that contaminated oil is considered a hazardous waste. The Ontario Department of the Environment and the EPA in the United States also use the same criterion.

In light of the foregoing, it appears obvious that environmental protection standards, to be truly effective, should be aimed above all at askarels, then at the most contaminated oils.

TABLE 16: Ratio of the Concentration of PCBs and the Volume of Oil Containing 1 g of PCBs

CONCENTRATION OF PCBs IN OIL (ppm)	VOLUME OF OIL CONTAINING 1 g OF PCBs (L)
0.15	6 666
2	500
10	100
50	20
100	10
1 000	1

7.3 Distribution of PCBs in the Environment

The distribution of PCBs in the environment depends upon several factors:

- their entry point;
- their physical properties such as solubility and volatility (vapor tension), which determine their water-ground and water-air distribution coefficient;
- the incidence of bioaccumulation;
- their susceptibility to degradation in various environments where they are found.

According to Moolenaar (EPA, 1983), the total quantity of PCBs discharged in the environment in North America prior to 1975 is estimated at 82 x 10^6 kg, distributed in the following manner: 0.02 percent in the atmosphere, between 0.14 percent and 2.8 percent in the lithosphere (the solid crust of the globe as distinct from air and water) and the remainder in the hydrosphere, in salt water, fresh water and sediments.

The Atlantic Ocean appears to be the main reservoir of PCBs. However, the heaviest concentrations are usually found in lakes and rivers, although it is conjectured that drinking water ecosystems contain only a small proportion of the total quantity of PCBs. In Canada, the contamination of several water bodies has been studied, especially the Great Lakes in Ontario and the St. Lawrence and its principal tributaries in Québec (Eisenreich, 1982; Goulet and Laliberté, 1982).

Eisenrich estimates the total PCB content of the Great Lakes at between 0.44 x 10^6 kg and 0.5 x 10^6 kg, and that 88 percent of the substances are found in sediments (see Table 17).

We do not have data on the total quantity of PCBs in the St. Lawrence or its tributaries. Nonetheless, judging from the concentrations of PCBs measured in fish, pelecypods and sediments in the water bodies studied (Goulet, Laliberté, 1982), it may be thought that in certain milieux, the quantities of PCBs per unit of volume approach those of the Great Lakes.

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TABLE 17: Estimated Quantity of PCBs in the Great Lakes (Eisenreich, 1982)

LAKE	PCBs (10^3 kg)
Superior	38
Michigan	57
Huron	69
Onterio	122
Erie	187

7.4 Pathways of PCBs in the Environment

7.4.1 PCBs for short-term use

PCBs for short-term use, or use in open circuits, find quicker, more direct pathways to the environment than those used in closed circuits. The PCBs that were in the past used as dust-control agents on dirt roads and in solvents for spreading insecticides contaminated the surrounding environment for very brief periods. Other products containing PCBs, such as glue, plastics, paint, printing paper and so on, reached the waste stage sooner or later and ended up in dumps, incinerators, waste-water treatment plants, or were discharged directly into the environment. Left in dumps, these products eventually released their contaminants into the ground, water or air. When such waste was burned in the dump or in an incinerator, PCBs were either destroyed completely, or evaporated into the air, depending on the temperature of combustion. A certain quantity of PCBs might be degraded, forming PCDFs which spread into the environment.

7.4.2 PCBs for long-term use

Plants manufacturing PCB-based products for long-term use released waste containing PCBs, either as liquids in effluents, or solids in dumps or burial sites.

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In electrical equipment, heat exchangers and hydraulic machinery, a leak is all it takes to release PCBs in the environment. Accidental spills can also occur when such equipment is transported to service[a] or storage sites.

According to the experts who wrote a report for the EPA in 1983, leaks from electrical apparatus accounted for less than 0.25 percent of discharges in the environment. The NAS (1979) feels that the release of PCBs in the environment on sites where equipment containing PCBs is stored is negligible.

However, in light of the quantity of PCBs stored every year in Canada, it is urgent to develop technologies for destroying them.

7.5 Dispersal of PCBs in the Environment

What happens to PCBs released in the environment? Some of them spread in the ground and, even though they are hardly soluble in water, they gradually reach aquatic ecosystems. Because of their affinity for particles, they attach themselves to particles suspended in the water and ultimately settle out as sediments, which account for more than 88 percent of PCBs in aquatic ecosystems. As we will see in section 7.9.3, water rarely contains more than several parts per billion (ppb) of PCBs, while sediments contain higher concentrations of them. Through bioaccumulation, a portion of PCBs are integrated into fish and land animals. The air also contains limited quantities of PCBs; there, they are carried by winds and float until rain, snow or the fallout of atmospheric dust precipitate them. Although the atmosphere contains limited quantities of PCBs, many specialists agree that it does contribute to spreading them. As there is no direct source of PCBs in northern Québec, their presence in fish and animal species can be explained by atmospheric transmission, a fairly efficient dispersal method.

[a] The new Canadian regulation adopted in July 1985 prohibits the sale, import and export of electrical equipment containing more than 50 ppm by weight of PCBs, except when such equipment is an integral part of a building, plant or construction offered for sale.

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This hypothesis is highly plausible and, if it proves to be correct, it must be concluded that even were we to completely halt discharges of PCBs in the environment in Québec, we could not prevent releases in the United States and Ontario from reaching the province. The phenomenon is similar to the well-known problem of acid rain.

7.6 Bioaccumulation of PCBs

BPCs tend to accumulate in fish and animal species, including humans, through bioaccumulation, which is favoured by several factors, i.e. their low solubility in water, their great solubility in lipids and chemical reactions related to the catabolism and elimination of BPCs which occur in animals.

Bioaccumulation is defined as the ability of living organisms to concentrate the substances they absorb. For example, in fish, bioaccumulation is directly linked to the ratio of the concentration of a chemical substance in the fish and its concentration in water; this ratio is called the bioaccumulation factor. It depends on the species and the length of exposure to the substance, and is independent of the concentration in the milieu (in this case, water). This means that, for a fish species whose bioaccumulation factor for AR 1242 is 100 000, a concentration in the water of 0.01 ppb (0.01 µ g of PCBs/kg of water) is sufficient for the fish to carry a concentration of 1 ppm (1 mg of PCBs/kg of fish) after a certain time. Among minnows, small fish living in streams, the bioaccumulation factor has been found to be 32 000 for AR 1242 and 307 000 for AR 1254*. The maximum permanent concentration in this species is reached after about eight months of exposure. The bioaccumulation factor is different for each animal species.**

* It is normal for the components of AR 1254 to be bioaccumulated more extensively than those of AR 1242, as the former contains more molecules with high chlorine content.

** The bioaccumulation factor in certain piscivorous birds can apparently reach 10 (Goulet, Laliberté, 1982).

The bioaccumulation factor also depends on the duration of ingestion of food. It is obvious that the maximum concentration may not be achieved where exposure is sporadic or short lived. This notion is very important in helping us understand the consequences for various living species of absorbing food with high PCB content.

Take humans, for example. Let us put forward the hypothesis that their bioaccumulation factor* of PCBs in fat is equal to 100 and that the time* required to reach the maximum permanent concentration (100 x concentration in the diet), for a given concentration in their diet, is one year. Where food containing an average of 1 ppm of PCBs (1 mg/kg) is ingested every day, lipidic tissues would contain 100 ppm of PCBs after one year. However, sporadically eating food containing 1 ppm of PCBs would produce only a small concentration of this substance in human lipidic tissues.**

The total quantity of PCBs accumulated is diluted in all of the organism's fat tissues; part of the quantity ingested is ultimately eliminated. The rate of elimination depends on the number and position of chlorine atoms in the congeners present in the diet. The lipidic tissues of Canadians contain average concentrations of PCBs of about 0.1 ppm to 1 ppm, although they occasionally eat fish with concentrations of PCBs over 1 mg/kg (1 ppm).

However, among heavy fish eaters, concentrations may be much higher because of bioaccumulation and the contamination of the human trophic food chain (see Figure 12).

A considerable portion of PCBs released in the environment eventually reach aquatic ecosystems. Fish, which have a high capacity to bioconcentrate PCBs, are eaten by piscivorous birds, other fish and animals, which further bioconcentrate PCBs. Humans ultimately absorb some PCBs when they eat such fish, animals and birds.

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* The bioaccumulation factor and the maximum permanent concentration time in humans are not known.

** The Legal Marketing Allowance (LMA) of foodstuffs containing PCBs in Canada is 2 mg/kg (2 ppm).

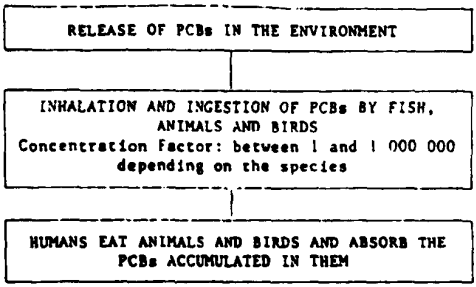

FIGURE 12: Bioaccumulation through Bioconcentration in the Food Chain

7.7 Degradation of PCBs in the Environment

7.7.1 Persistence of PCBs: the eternal question

Do PCBs remain indefinitely in the environment or are they ultimately degraded? If so, at what rate?

For a long time, it was thought that PCBs which reached the environment and living organisms could not be eliminated. This conviction was largely responsible for encouraging legislators to take measures aimed at banning the production of PCBs. Today, thanks to research on the degradation of PCBs in various ecosystems and on the presence of the substances in living organisms, including humans, it has been noted that PCBs are eventually degraded and the mechanisms which contribute to this elimination are better understood. These mechanisms comprise photolysis, aerobic and anaerobic bacterial degradation, hepatic catabolism among mammals and birds, transfer through mother's milk and

eggs among birds and fish, and so. Generally, congeners containing few chlorine atoms, i.e. three or fewer, tend to be eliminated quickly; those containing many such atoms are degraded much more slowly.

7.7.2 Process of natural elimination of PCBs in the environment

During the 1970s, the Great Lakes were the ecosystem on which researchers most frequently focussed their attention. In 1982, Eisenreich presented a summary of studies effected since 1962 on concentrations of PCBs in the environment of the lakes (International Symposium on PCBs in the Great Lakes, Michigan State University). Figure 13 summarizes data on concentrations measured in the water, the flesh of fish in Lakes Superior and Michigan, in the air above these lakes and in the eggs of seagulls nesting on islands in Lake Superior. A net reduction in the concentration of PCBs has been noted since 1974, i.e. one year after sales of the substance used in open-circuit systems were halted. It is, therefore, accurate to say that PCBs are gradually being eliminated from the environment.

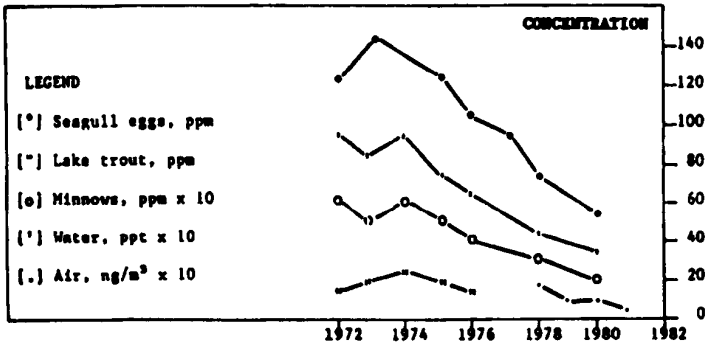

FIGURE 13: PCBs in the Environment of the Great Lakes: Changes over Time

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A more detailed analysis of this figure reveals that the concentration in:

- seagull eggs dropped from 145 ppm to 50 ppm between 1973 and 1980;

- the water fell from 7.8 ppt to 2.5 ppt (parts per trillion) between 1974 and 1980;

- minnows declined from 5.5 ppm to 2.2 ppm between 1974 and 1980;

- lake trout dropped from 20 ppm to 17 ppm between 1974 and 1976; we do not have results subsequent to 1976;

- the air fell from an average of 1.5 ng/m^3 (range of 0.9 to 3.5) in 1978 to an average of 0.3 ng/m^3 (range of 0.1 to 0.6) in 1981.

In light of these figures, it seems obvious that PCBs tend to eliminate themselves from the environment. With recent dumping control measures (July 1985) set out by the government in Regulations 2 and 3 of the Environmental Contaminants Act, it may be expected that the natural elimination process will be accelerated.

In the sections that follow, we will review various factors which affect the degradation of PCBs in different milieux and which, to some extent, enable us to predict long-term changes in the process by which PCBs are eliminated.

7.7.3 Estimated life of PCBs in different systems

7.7.3.1 Biodegradation in living organisms

In living organisms, the elimination rate of PCBs is primarily determined by the rate of hepatic catabolism, although some of the substances can be eliminated through lactation in mammals or egg laying in fish and birds. In certain species, the quantities of PCBs found in the bile and feces remain unchanged. It should also be noted that the elimination rate varies widely from one species to another.

According to studies carried out by J.F. Brown et al. (1976-1983) and Hara (1985) among workers in the transformer and capacitor manufacturing industries, and Chen (1985), among patients in Taiwan, PCBs can be divided into three groups, depending on their persistence: (1) non-persistent congeners which do not accumulate; (2) moderately persistent congeners with a half-life of between six months and five years; (3) highly persistent congeners, i.e. those which do not display a significant elimination rate during the period of observation.

We will study biochemical factors affecting the degradation rate in section 10.3. In lower mammals like rats and mice, PCBs tend to be eliminated more quickly than among humans, although the elimination of non-persistent and highly persistent congeners occurs in proportions similar to those observed in humans. (See Figure 14, taken from the 1983 EPA report, which indicates the excretion rate of PCBs in rat feces for mono-, di-, penta- and hexaphenyls).

It is important to note that the first group of non-persistent congeners accounts for approximately 90 percent of AR 1216 and 80 percent of AR 1242, while the group of highly persistent congeners, which comprises hexa-, hepta- and octachlorinated isomers with chlorine atoms in position 4-4', represent less than 50 percent of AR 1254 and more than 50 percent of AR 1260. This means that the quantity of PCBs accumulated in the organism and their persistence depend largely upon the mixture to which the subject was exposed, as the tendency for congeners with low chlorine content to be eliminated rapidly is general. It may be inferred that over time the PCBs to which we are exposed through food are, above all, congeners in the highly persistant group. Fortunately, all laboratory studies to date seem to show that these congeners have a very low rate of toxicity (Safe, 1984).

7.7.3.2 Biodegradation of PCBs in aquatic ecosystems: aerobic and anaerobic sediments

J.F. Brown et al. (General Electric Research and Development Centre, Schenectady, NY) presented a thorough review of the state of current knowledge in this field at the International Symposium on PCBs in October 1985 in Seattle. A summary follows.

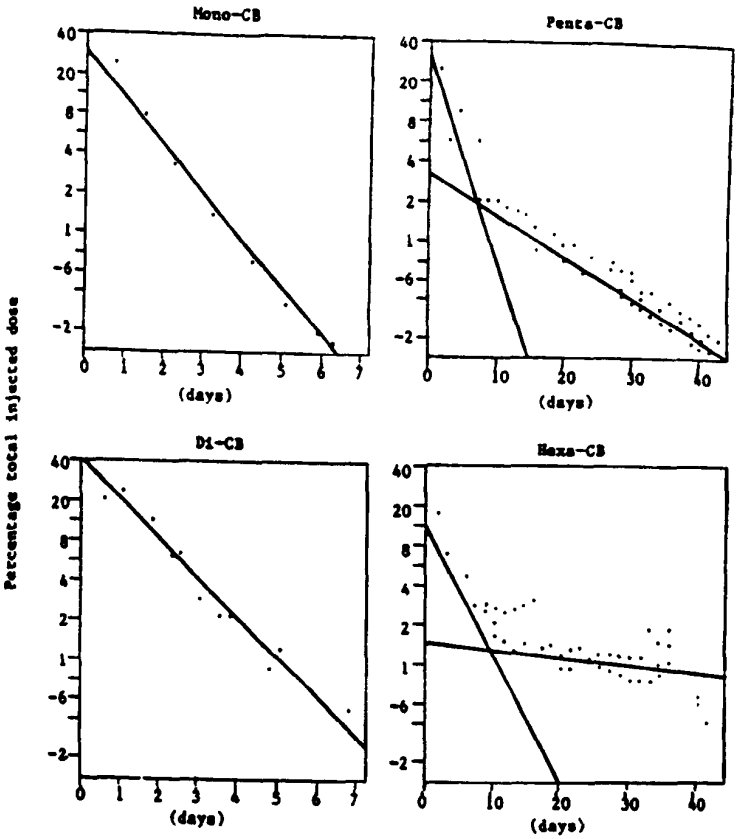

FIGURE 14: Percentage of Radioactivity in Rat Faces 7 and 42 Days after the Injection of 0.6 mg/kg of mono-CB, di-CB, penta-CB and hexa-CB

Aerobic bacterial biodegradation

In aerobic aquatic ecosystems (on the surface of sediments), large quantities of organic matter are metabolized by aerobic bacteria. This complex microbial population is able to metabolize most of the organic chemical components and natural matter present, including PCBs.

Until now, more than a dozen aerobic bacterial species have been isolated in earth and sediments, which degrade PCBs through oxidation. However, the different species identified do not all behave in the same manner in relation to the various PCB congeners. One group essentially attacks mono- and dichlorinated congeners, while others attack tetra- and pentachlorinated ones. Most of them are of the 2,3-dioxigenase type and usually attack congeners with chlorine atoms in positions 4,4', but without chlorine in positions 2,3 or 5,6. A small proportion of the types of bacteria identified are of the 3,4-dioxigenase type and generally attack congeners without chlorine in positions 3,4.

Researchers have noted that the concentration of PCBs in the flesh of fish in the Upper Hudson River fell by half in two years. Moreover, they found that the PCBs present were, above all, AR 1016 and that the bacteria isolated from sediments were of the 2,3-dioxigenase type. After two years, the residual PCBs resembled AR 1242; congeners with low chlorine content were degraded. Obviously, this group of aerobic bacteria has played an important role in the degradation of PCBs in this ecosystem.

Anaerobic bacterial biodegradation

Until very recently, it was thought that no degradation was possible in the deepest layers of sediments in aquatic ecosystems. However, in recent years, Brown et al. (1984) discovered that PCBs in such anaerobic systems are degraded, especially in the sediments of the Upper Hudson River and Silver Lake (Pittsfield, MA). Six different anaerobic bacteria were identified.

Among those found in the Hudson River, a number of them can remove chlorine atoms in the para- (4) positions and others in the meta- (3 or 5) positions, although none is able to remove the chlorine atom in the ortho- (2 or 6) positions. The net effect has been to convert an appreciable proportion of the AR 1242 deposited over the past 20 or 25 years into mono- and dichlorinated congeners in the ortho- position. On the other hand, bacteria present in Silver Lake can reduce chlorine atoms in the ortho-, meta- and para- positions, with a slight preference for position 4. However, they leave intact several tri- and tetrachlorobiphanyls. The long-term effect has been to convert the AR 1254 and AR 1260 present into a mixture of congeners whose average content is similar to AR 1242 and AR 1248, but with a different distribution of congeners.

It is interesting to note that the congeners with chlorine atoms in position 4,4', usually deemed to be more toxic than other isomers with the same number of chlorine atoms but not possessing this substitution, are those which are eliminated the most rapidly in anaerobic sediments.

7.8 Dynamics of PCBs in the Environment

During the 1950s and 1960s, considerable quantities of PCBs were released in the environment; they dispersed until a certain equilibrium was achieved in different milieux, i.e. water-ground, water-air, water-fish and so on. This balance is shifting constantly according to fluctuations of PCBs added to, or eliminated from, the environment; it depends upon the various distribution coefficients between the different milieux. In light of data studied earlier, it is also possible to predict discrepancies in the distribution of congeners over time. As we noted, since 1973, influxes of PCBs have declined considerably, and elimination has outstripped bioaccumulation. Figure 15 explains this phenomenon by describing the process.

Given that world production of PCBs has ceased and that measures have been taken to control new influxes of the substances in the environment, we can hope that, from an environmental standpoint, the PCB problem is being solved.

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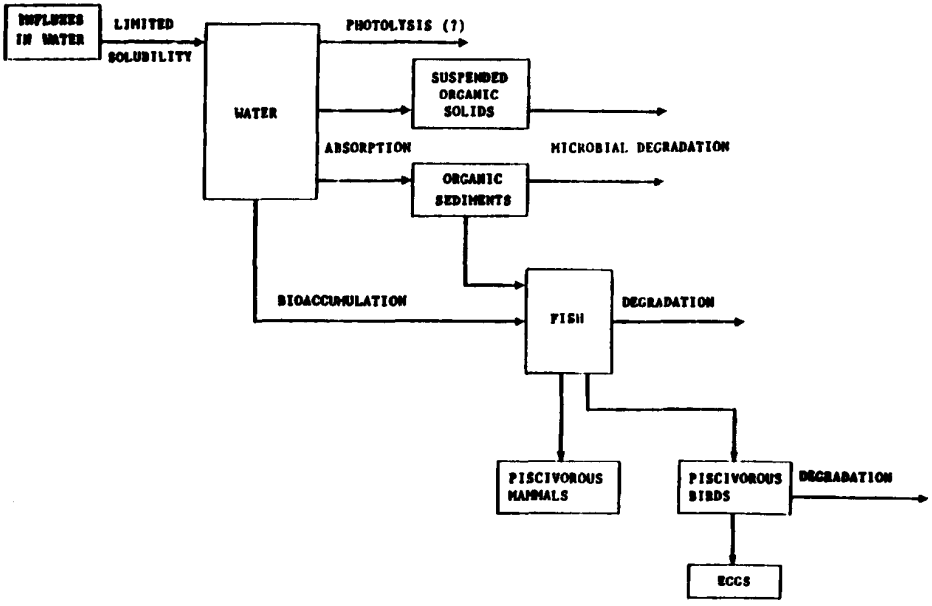

FIGURE 15: Mechanism of the Elimination and Degradation Processes (This Mechanism Prevails over Bioaccumulation)

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7.9 PCBs in the Québec Environment

It is impossible to review all of the studies published on environmental contamination through PCBs in Québec. However, readers who are interested may wish to consult syntheses of them published by J. Bélanger et al. (1977) and Goulet and Laliberte (1982).

Until now, samples have been collected throughout Québec, in the St. Lawrence, the Gulf of St. Lawrence, the principal basins of the river's tributaries, the Atlantic coast and the northwestern region of Québec. Samples have been taken in different milieux such as the water, sediments, fish, shellfish, tissues of birds (gannets, crested cormorants, seagulls, common terns, Arctic puffins, common murre and so on), seagull eggs, tissues of mammals (seals, polar bears, beluga whales and so forth), and in drinking water and milk.

An analysis of these studies reveals that the presence of PCBs in the Québec environment is fairly high, given that in certain zones direct influxes seem quite unlikely.

7.9.1 PCBs in the atmosphere

To date, it appears that the the presence of PCBs in the atmosphere in Québec has not been studied. However, as they have been found in fish and mammals in northwestern Québec, we believe that atmospheric transport is a highly efficient dispersal method.

7.9.2 PCBs in the ground

Here, too, it appears that no studies have been effected concerning the contamination of the ground in Québec. It is nonetheless plausible that the presence of PCBs in the air is an important source of ground contamination, through rainwater, snow, or simple atmospheric fallout of the substances.

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7.9.3 PCBs in water

A report published by Fisheries and Environment Canada (Québec region) mentions four especially important studies. According to the first, conducted in 1977 under the Canada-Québec agreement on the St. Lawrence, concentrations of PCBs varied between 10 ppt (10^{-12}) and 40 ppt (10^{-12}) in the water inlets and outlets of filtration plants in Montréal, Longueuil and Lévis.

The second study analyses untreated and treated water in the Montréal, Longueuil, Lévis, Sainte-Foy, Pierrefonds, Terrebonne, Pont-Viau, Saint-Eustache and Bécancour filtration plants, and was carried out by the Québec Service de protection de l'environnement between June 15 and July 25, 1977. This analysis did not reveal any PCBs (detection limit: 10 ppt).

The third study, dealing with waste water in Val-Cartier municipality, showed that concentrations of PCBs varied between 0.02 ppb (10^{-9}) and 3 ppb (10^{-9}).

Analyses of water from 26 sampling stations located in the St. Lawrence (Canada-Québec 1977) revealed concentrations below 10 ppt in most samples.

These results are hardly astonishing, given that PCBs are barely soluble in water: water is a poor indicator of the presence of the substances in the environment.

7.9.4 PCBs in sediments

An initial studied carried out by the Centre de recherchee sur l'eau de l'Université Laval (CENTREAU, 1975) centred on the St. Lawrence, from Québec City to Trois-Pistoles. In 27 sampling sites out of 169, concentrations of PCBs between 20 ppb and 220 ppb were measured in fine sediments. This is equivalent to concentrations in sediments overall of between 4 ppb and 175 ppb, with an average of 70 ppb.

The second study, carried out by the Québec Service de protection de l'environnement, focused on the rivière Richelieu, from the U.S. border to its mouth. Results ranged from undetectable to 91.2 ppb, with an average of 20 ppb.

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7.9.5 PCBs in fish and Molluscs

Samples of shellfish (pelecypods) and fish (black chub and yellow pike) were collected during the summer of 1978 in 55 sites located in 11 of the most polluted basins of tributaries of the St. Lawrence in southern Québec.

Of a total of 202 analyses carried out on fish, the concentrations of PCBs measured varied between 0.063 ppm and 5.9 ppm. Among the 11 river basins studied in southern Québec, quantities measured in one or more sites in the Assomption, Saint-François, Richelieu and Nicolet rivers were equal to or in excess of the established Canadian marketing standard, i.e. 2 mg/kg (ppm) for fish flesh. Contamination was lower in the other basins.

The concentration of PCBs in the 37 pelecypods sampled appears, on average, to be 80 times lower than in fish, with variations of between 20 and 500 times less. All of pelecypods had concentrations of PCBs far below the 2 mg/kg standard; the maximum measured was 0.116 mg/kg (ppm).

7.9.6 PCBs and wildlife

Birds

In 1973, the concentrations of PCBs in the muscles, brains and eggs of piscivorous birds in the Gulf of St. Lawrence, such as gannets, crested cormorants, long-billed gulls and common terns, varied between 2 ppm and 80 ppm (Pearse, Gruchy, Keith).

In Labrador, in Arctic puffins and common murres, concentrations of PCBs were much lower, i.e. between 0.31 ppm and 0.41 ppm.

Mammals

The amounts of PCBs measured in the fat of seals and polar bears revealed that these contaminants are present around the entire periphery of Québec from the St. Lawrence estuary to James Bay, including the Gulf and the Atlantic and Arctic Oceans. In seals, concentrations of PCBs were found to vary (Canada, 1976) between 0.16 ppm and 9.2 ppm. In the fat of polar bears, average levels

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of 7.8 ppm were measured; in beluga whales (Actualité, February 1986), concentrations were about 576 ppm in fat and as high as 1725 ppm in the whales' milk. It should be pointed out that increasingly spectacular quantities of heavy metals, DDT, Mirex and APH (polycyclical aromatic hydrocarbons) are being found in belugas.

In Chapter 11, we will study in detail the concentrations of PCBs measured in humans.

7.10 Is Environmental Contamination Alarming?

7.10.1 Consequences for humans

Until now, no study has suggested that, in the concentrations measured in the population at large, PCBs might be injurious to health. However, heavy eaters of fish have concentrations of the substances close to those of exposed workers who have experienced health problems (see sections 11.1 and 11.3).

This leads us to believe that, had the release of PCBs in the environment not been controlled, several decades from now the levels of contamination reached might have seriously threatened health. In Chapter 11, we study in detail the potential effects of PCBs on health.

7.10.2 Consequences for flora and fauna

Laboratory studies suggest that in certain species of piscivorous birds or a number of animals, the concentrations of PCBs measured might be sufficient to affect their reproduction rate. For this reason, it is important to implement control measures to avoid adverse effects on the environment.

Fortunately, studies on the persistence and degradation of PCBs in the environment (see sections 7.6 and 7.7) show that the rate of elimination now seems higher than the rate of influx.

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8. TOXICITY AND MECHANISM OF ACTION OF PCBs

8.1 Growing Knowledge

Between 1970 and 1973, a number of researchers (Nichizumi 1970, Fujita 1971, Villeneuve 1971, Kimbrough et al. 1972) clearly showed that commercial PCB compounds altered the hepatic function through ultrastructural changes and the induction of a wide variety of enzymes which affect the biotransformation of substances which are exogenous and endogenous to the organism.

In 1973, while studying the effect of Aroclors 1221, 1254 and 1260 on the induction of hepatic enzymes, Chen and Dubois demonstrated that enzymatic activity increased with the chlorine content of the various products for most of the enzymes studied.

Given the considerable heterogeneity of commercial PCB-based products, the results of these studies raise several questions:

(1) Do dichlorobiphenyls produce the same effects as tetra- or hexachlorobiphenyls?

(2) Do all isomers displaying the same chlorination have comparable toxic effects?

(3) Are the toxicological effects observed due to the biphenyl nucleus, the position of chlorine atoms or the number of chlorines present in the molecule?

(4) Does the presence of PCBs stimulate the production of enzymes other than those which have been identified?

Researchers in different parts of the world have succeeded in answering many of these questions; research is intensifying to establish potential effects on humans.

In 1974, Johnstone, Ecobichon and Hutzinger showed that the enzymatic induction of many congeners varies widely. The same year, Hansell and Ecobichon (1974) proved that the effects of congeners on hepatic alteration with respect to ultrastructure or morphology also varied considerably. The degree of alteration was directly linked to the number of chlorine atoms and the presence of chlorine in position 4,4'.

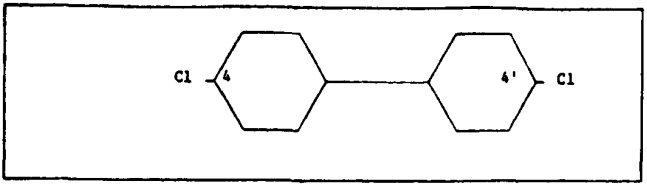

FIGURE 16: 4,4' - Dichlorobiphenyls

For example, the 2,2' dichloro- which has no chlorine in position 4,4' produces few alterations, while the 4,4' dichloro- produces more of them. Researchers have also noted that the 3,4,3',4' tetrachloro- congener is the most active of the congeners evaluated.

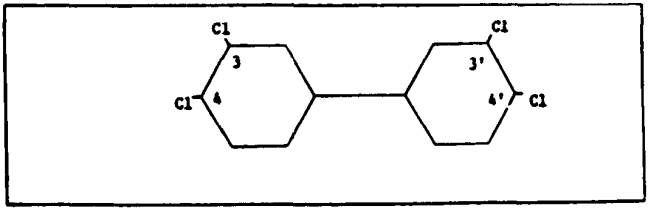

FIGURE 17: 3,4,3',4' - Tetrachlorobiphenyls

In 1975, Ecobichon and Comeau demonstrated that the results noted in the morphology of the liver are in correlation with functional effects. More precisely, PCBs carrying chlorine atoms in 4,4' have a higher enzymatic induction power than the other isomers with the same number of chlorine atoms but without this substitution.

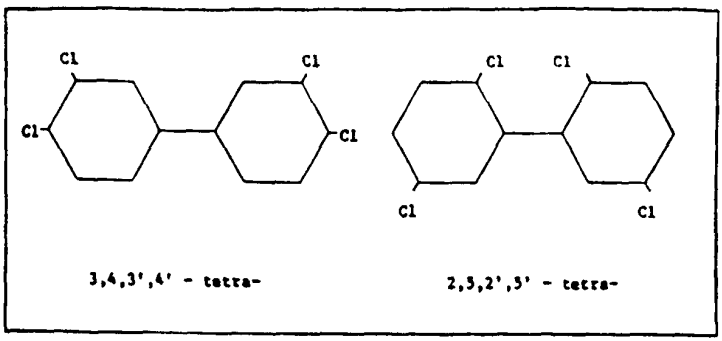

FIGURE 18: An Example of a Molecule Substituted in 4,4' (on the Left) and an Example without Substitution in These Positions

Alvarez et al. (1977) showed that AR 1016 activates the enzymatic system of drug metabolism and the microsomal mixed function oxydase (MFO) in the same way as phenobarbital in humans and in animals. AR 1016 activates the P-450 cytochrome needed for the catabolism and metabolism of endogenous and exogenous substances, such as phenobarbital and steroids.

This discovery is very important for medecine and toxicology. The induction of microsomal enzymatic activity (MFO) alters the rate of metabolism of drugs, endogenous substances such as steroids, and the degree of toxicity of a wide variety of toxic agents found in the environment. The toxic effect is usually attenuated when the metabolism of these substances is activated.

Alvarez concludes that PCBs can play a significant role in the biological response of living organisms exposed to a wide variety of chemical agents in their environment.

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Goldstein (1979) established a correlation between the induction of different hepatic enzymes of MFO by various congeners of PCBs and their toxicity. Here is his explanation: in 1977, Poland et al. showed that P-450 cytochrome could take numerous forms and that the various isomers and congeners of polychlorodibenzodioxins (PCDDs) induced these different forms with varying intensities. Cytochromes can be divided into two main groups:

(1) the phenobarbital (PB) type

(2) the 3-methylcholanthrene (3-MC) type.

The protein form of 450 cytochromes induced by PB-type substances differs from that induced by 3-MC type substances. For simplicity's sake, Goldstein called the 3-MC type cytochrome 448.

PB-type = P-450 cytochrome

MC-type = P-448 cytochrome

He concluded, as is the case with respect to dioxins (PCDDs), that congeners and isomers of PCBs which induce P-448s are much more toxic than those which induce P-450s. It has been shown that:

. some congeners, mainly those with little chlorine (mono-, di- and tri-) are inactive in the presence of inducing cytochromes (P-448 and P-450) and are scarcely or not at all toxic;

. some congeners have a PB-type effect only (or P-450) and are hardly or not at all toxic;

. others, displaying mixed induction (P-448 and P-450), are slightly more toxic than the preceding ones;

. some induce only P-448s and appear to be the most toxic. It is thought that 3,4,5,3',4' pentachlorobiphenyl displays this type of induction (MC-type).

Expanding knowledge has made it possible to demonstrate that substances belonging to the family of halogenated aromatic hydrocarbons produce a similar biological response and toxic effects, although at different doses. All of these substances, i.e. dibenzodioxin, dibenzofuran, azobenzene, naphthalene and polychlorinated biphenyls, have a similar chemical structure. Research suggests that they have a common mechanism of action: the model proposed by the scientific community appears in Figure 19 (Goldstein, 1979; Poland, 1979; Safe, 1984; Vickers, 1985; and so on).

Enzymatic induction occurs through the intermediary of one or more receptors (Ah receptor in the cytoplasm and the nucleus) genetically controlled by a simple locus called the Ah locus. This locus controls and modulates the expression of a battery of genes, including the P-448 cytochrome and aryl hydrocarbon hydroxydase (AHH), an enzyme associated with it which participates in the metabolism of halogenated hydrocarbons.

Affinity for the Ah receptor is a function of the structure of toxic substances.

8.2 Properties of the Ah Receptor

The Ah receptor appears to be an anomaly of adaptation in the evolution of living organisms (Vickers et al., 1985). Its structure and mechanism are analogous to those of steroid receptors; however, no steroid or other known endogenous compound can bind with this receptor.

Wide variations exist in the number and binding affinity of receptors between various tissues from the same animal and between various species. This may at least partially explain the appreciable differences in the toxicity of the same product in different tissues of the same animal or different species. For example, the greater susceptibility of guinea pigs and rats to thymus atrophy compared with mice and hamsters may be explained by the existence of eight times more receptors in the first two species.

The most powerful inducer of P-448 cytochrome (MC-type) currently known is 2,3,7,8-tetrachlorodibenzodioxin (TCDD) (see Figure 20). Its power of induction is 30 000 times greater than that of 3-MC. This substance is the most toxic in the family of polychlorinated aromatic hydrocarbons (dioxins, furans, PCBs, azo(oxy)benzenes, naphthalenes, quaterphenyls and so on).

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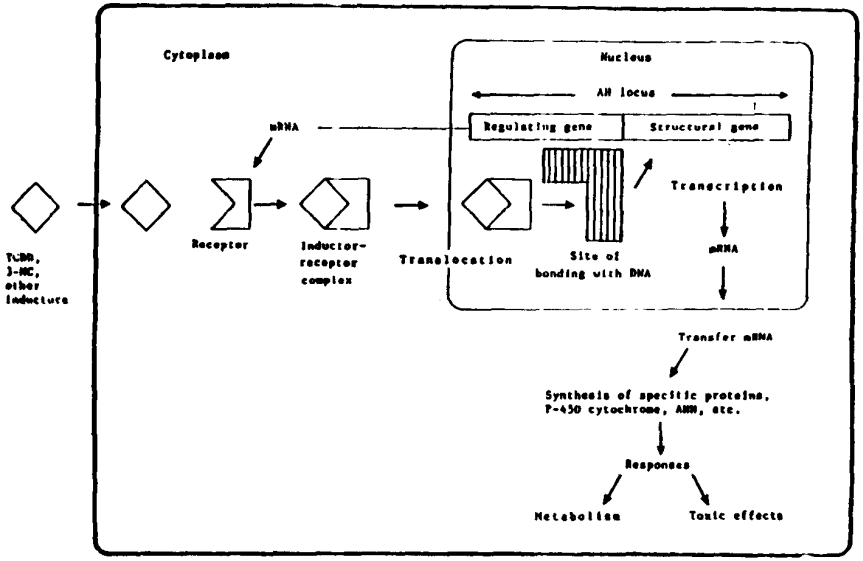

FIGURE 19: Model of the Mechanism of Action of Aromatic Halogenated Hydrocarbons at Cell Level

MONS 026779

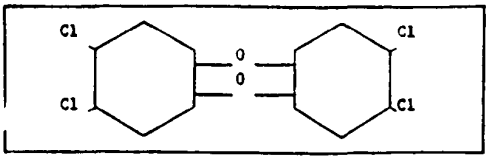

FIGURE 20: 2,3,7,8-(TCDD)

A series of studies (summarized by Safe et al. for the EPA in 1983 and for the Centre for Disease Control (CDC) in 1984) demonstrated that MC-type but not PB-type inducing substances had serious effects on animals, in particular:

. weight loss;

. atrophy of the thymus and spleen;

. lipid accumulation in the liver and hepatomegaly;

. porphyria.

It was also shown that:

(1) Different substances have widely varying effects: those of dioxins are greater than those of furans and much greater than those of PCBs.

(2) For the same substance, there are major variations (in the order of 3000) in toxic effects from one species to another.

(3) Congeners of dibenzodioxins other than 2,3,7,8-(TCDD) are all between 1 and over 30 000 times less toxic than it is.

(4) Congeners of dibenzofurans other than 2,3,7,8-tetrachlorodibenzofuran (TCDF) are between 1 and 30 000 times less toxic than it is.

(5) The biological effects of PCBs vary widely according to the different congeners. Researchers all seem to conclude that the most toxic of the polychlorinated biphenyls are:

3,3',4,4'-tetra;

3,3',4,4',5-penta-; and

3,3',4,4',5,5'-hexa.

These three substances are all inducers of MC-type cytochrome, like 2,3,7,8-(TCDD), but to a much less degree, about 20 times less. It has been shown that their coplanar structure resembles 2,3,7,8-(TCDD) and 2,3,7,8-(TCDF). Fortunately, these three PCBs are absent from commercial products, except for 3,3',4,4'-tetra-, traces of which are occasionally present. Chen found that even though 3,3',4,4' accounted for approximately 1 percent of the total PCBs in the rice oil responsible for the poisoning in Taiwan, this substance was not found in the blood of most of the patients, nor in the intestinal lipids of a patient who died in an accident two months after the poisoning. This suggests, in accordance with animal experiments, that the organism metabolizes and excretes this type of PCB fairly rapidly.

The three PCBs (tetra, hexa and penta) in Figure 21 have the following traits in common:

(1) cholorinated substitutes in position 4 - 4' (para- position);

(2) at least one substitution for each benzene nucleus in meta- positions (3,3' or 5,5');

(3) no substitution in ortho- positions (2,2' or 6,6').

If we modify these structures, either by removing chlorine atoms from para- (4,4') or meta- (3,3',5,5') positions, or both, or by adding chlorine atoms in ortho- (2,2',6,6') positions, toxicity declines. A number of these new congeners are inactive, while others are of the phenobarbital (PB) type, and the remainder are mixed (PB + MC) with toxicity higher than the PB type but lower than the MC type alone.

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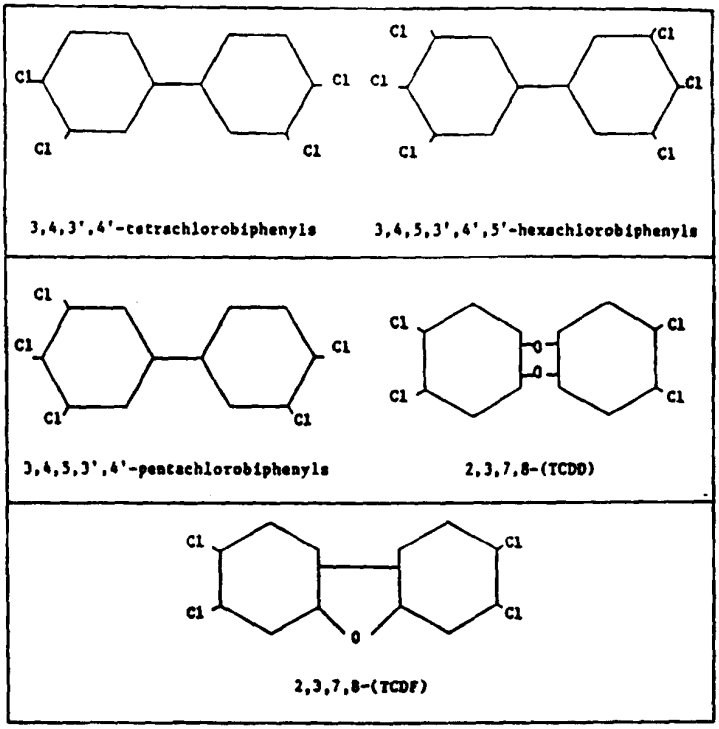

FIGURE 21: Related Structure of Several Molecules Belonging to the PCB, Dioxin and Furan Families

At present, for most of the 209 congeners of PCBs, it is possible to establish a correlation between the structure (number and position of chlorine atoms) and relative biological and toxicological effects in rats (Safe, 1984).

8.3 Other Mechanisms of Action

We know that there are other mechanisms of action of toxicity. Sono et al. (1985) have shown that the metabolism of porphyrine, in addition to being affected by the P-448 cytochrome and AHH, is also affected by another mechanism which interferes with the catabolism of uroporphyrines.

It appears that the fact that responses vary according to the animal species cannot be explained by the variation in the number of Ah receptors alone. It is currently difficult to show that such responses are attributable to greater cellular reaction to the activation of the receptor in a number of species.

8.4 Enzymatic Activity in Certain Organs and Tissues

Dioxins, furans and several PCBs induce enzymatic activity in tissues other than the liver, i.e. the lungs, kidneys and skin, although to a lesser extent. This may partially explain toxic effects on these organs. Once again, it must be stressed that response varies from one species to another.

8.5 PCBs, an Antidote for 2,3,7,8-TCDD

The preliminary findings of recent research conducted by Stephen Safe at the University of Texas are now available (interview with the author, April 1986). The researcher and his team tried to find an antidote for 2,3,7,8-TCDD in the event of acute poisoning. The best known antidote is AR 1254; it is about 800 times less toxic than 2,3,7,8-TCDD in rats. When AR 1254 is given to an animal into which 2,3,7,8-TCDD had been injected, the Aroclor acts competitively on the same receptors as those affected by the 2,3,7,8-TCDD, resulting in the inhibition of the latter's toxic effects.

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8.6 Mechanism of Action and Future Prospects

More extensive knowledge of the mechanism of the toxicity of TCDDs will enable us to better evaluate the effects on health of toxic halogenated aromatic hydrocarbons and will help determine whether humans are sensitive or resistant to these substances.

To accurately assess the risk of toxic effects to which we would be exposed by accidentally absorbing substantial quantities of PCBs, current research must, in addition to qualitatively and quantitatively evaluating the different congeners present in the organism, make it possible to estimate the toxic potential of the dose of PCBs absorbed, bearing in mind the relative toxic potential of each congener, and without overlooking differences in their degree of persistence.

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9. PCBs AND ANIMAL TOXICITY

With respect to the effect of halogenated hydrocarbons on animals, the enormous difference in toxicity noted between the species remains an enigma. Guinea pigs are the most sensitive, followed by monkeys, rats, rabbits, mice, dogs and hamsters. The sensitivity of guinea pigs to TCDD, the most widely studied substance, was 5000 times greater than that of rats. The discrepancy in toxicity is even greater still between the different isomers of dibensodioxins ($\geq 10^4$). Studies of the toxicity of furans and PCB MC-type (P-448) enzyme inducers suggest that their effects on various animal species are similar to those of dioxins, provided, however, that the dose administered is adequate. It should also be noted that the impact on each organ varies from one species to another.

9.1 Acute Toxicity

The LD50 (lethal dose to 50 percent) in mice and rats of different PCB-based commercial products varies from 2 g/kg to 20 g/kg on average.

Bruckner (1973) showed that the oral LD50 for rats, with doses spread out over 14 days, was on average 4.25 g/kg, with a confidence interval of 95 percent between 2.80 g/kg and 6.38 g/kg. It should be noted that for doses between 200 mg/kg and 1 g/kg, no sign of acute toxicity was noted.

Signs observed four hours after absorption of the dose:

- diarrhea;
- sluggishness;
- reduced response to pain;
- slight chromodacryorrhea;
- unusual behaviour and stance, characterized by arching the back and raising the rear part of the trunk.

During the next 24 hours, the rats' condition deteriorated:

- profuse diarrhea, chromodacryorrhea, adipsia, oliguria and anorexia;
- absence of response to pain;
- weakness;
- ataxia, followed by coma and death.

9.2 Chronic Toxicity

A considerable number of studies have been conducted on this subject, although it is impossible to cite all of them. We might note in passing that when doses are adequate, PCBs can produce multiple toxic responses in animals, summarized below.

1. Functional and structural damage to the liver 2. Weight loss 3. Atrophy of the thymus and spleen	Most widespread toxic effects in various species.
4. Suppression of the humoral immunological system	Not found in all species.
5. Cutaneous lesions on hair follicles and meibomian glands	Dilation of hair follicles and keratinization. Atrophy of the sebaceous glands. Syndrome resembling chloracne, in monkeys.
6. Porphyrias	Observed in monkeys and rats; probably found in all species.
7. Damage to intestinal mucosa (metaplasia)	Found in rats and monkeys.
8. Effects on reproduction	In rats, monkeys and mice: PCBs are not teratogenic but fetotoxic. Drop in fertility. Increase in the number of abortions and stillbirths. Reduction in post-weaning survival rate.

9. Effects on lipid metabolism (peroxidation)

10. Some studies suggest possible alterations:
    - in thyroid function (Barsano, 1981)
    - of the hematopoietic system (Baran, 1983)
    - of the pituitary and adrenal glands (Dunn, 1983)

11. Carcinogenic effects

As the carcinogenic effects of PCBs and their effects on reproduction are by far the problems which create the most anxiety in the public at large, we will pursue these questions further.

9.3 Animal Experimentation and Cancer

Through experimentation, it has been shown that hundreds of products can cause cancer in one or several animal species. However, some products are so common that it is impossible to keep them out of the environment. Moreover, no carcinogenic effect on humans has been demonstrated for products such as isoniazid and saccharine.

We must display considerable caution before accepting as valid for humans positive or negative results observed with respect to animals. Experimentation and epidemiology must complement each other.

Furthermore, we must not overlook the fact that humans are never exposed to a single carcinogenic agent, but to many potentially carcinogenic physical or chemical agents. It is likely that most human cancers result from the repeated action of a single factor, such as cigarette smoke, asbestos and so on.

All chemical or physical carcinogenic agents have a common property, i.e. they can alter DNA molecules; this alteration is a key factor in carcinogenesis.

At present, in the realm of animal experimentation, the following theory is usually accepted to explain the onset of cancer: the birth of a cancer is the result of a long process, requiring several modifications of the cell. At least two phases can be discerned in animals:

- Initiation, a phase during which the cell undergoes one or more mutations capable of causing cancer. The mutated cell retains its ability to reproduce, despite the functional change.

- Promotion, which can occur much later. It takes place when the cells of the tissue containing the ''initiated'' cell are subjected to the action of an agent which, among other possible effects, provokes a proliferation of initiated cells, forming clones of mutated cells. The mechanisms regulating pro-

motion are not well-known; we are aware of products with an inhibiting effect on the sequence of phases necessary for the onset of a cancer.

However, the distinction between initiation and promotion is somewhat artificial, as we know that some allogenic factors can be both initiators and promoters of cancer, e.g., ionizing rays. This distinction helps us understand how some substances can intervene in the process of carcinogenesis.

9.3.1 Do PCBs have initiator effects?

9.3.1.1 Mutagenicity

Three types of tests designed to detect the mutagenicity of PCBs on DNA have been effected, i.e. the Ames Test, Micronucleus Test and Unscheduled DNA Synthesis Test. Results indicate that PCBs do not appear to be mutagenic (G.S. Probst et al., 1981; Watanabe et al., 1981; EPA, 1982, p. 216).

9.3.1.2 Ability of PCBs to induce cancer in laboratory animals

(a) In rodents:

Two studies of male mice (Nagasaki et al., 1972; Ito et al., 1973) have shown a higher incidence of hepatocarcinoma in animals which received Kaneclor 500 in concentrations of 500 ppm in their diet for 32 weeks.

Another study (Kimbrough et al., 1975) has shown a significant increase in hyperplastic nodules (benign lesions) and hepatocarcinoma in female rats given AR 1260 at a concentration of 100 ppm in their diet for 84 weeks.

In 1978, the National Cancer Institute published a study on the possible carcinogenesis of AR 1254 in female and male rats receiving three different doses in their diets (25 ppm, 50 ppm and 100 ppm) for between 104 and 105 weeks. Researchers found:

- hepatomas and hepatic hyperplastic nodules. The incidence of hepatomas is not significant, but the incidence of hyperplastic nodules appears to be related to the administration of PCBs; and

- metaplasias and carcinomas of the gastrointestinal tract, but the incidence of carcinomas is not significant. This was confirmed by Morgan et al. in 1981.

No research has resulted in a significant increase in hepatocarcinomas in rodents fed compounds with lower chlorine content, such as Kaneclor 500 (55 percent) or AR 1260 (60 percent).

(b) Dogs:

No carcinogenic effect has been shown in dogs which absorbed high doses, i.e. more than 100 ppm, of PCBs for two years and were destroyed at the end of the period.

9.3.2 Promoter and inhibitor effects

By exposing animals to carcinogens with known initiation potential and to PCBs, research conducted since 1974 has confirmed that the effect of PCBs on cancer depends on the time they are administered in relation to the time when cancer-inducing substances are administered.

9.3.2.1 Makiura et al. (1974)

This study indicates a high incidence of hepatomas with the administration of three known carcinogens: 3'-Me-DAB (incidence: 65 percent), N-2 FAA (53 percent), and DENA (92 percent). However, if these compounds are administered simultaneously with PCBs, cancer is inhibited (incidence: 0 percent).

9.3.2.2 Kimura et al. (1976)

These researchers reassessed the effect of PCBs on rats which had been given 3'-Me-DAB. No cancer was obtained with PCBs, but a 13 percent incidence of it with 3'-Me-DAB was noted. When PCBs were given before the administration of 3'-Me-DAB, the incidence of cancer remained 13 percent, but rose to 64 percent when they were given afterwards.

9.3.2.3 Nishizumi (1976)

According to Nishizumi, Peraino had shown in 1971 that phenobarbital (PB) inhibited the carcinogenic effect of 2-FAA when given before, and enhanced its effect if given after. Knowing that PCBs and PB have similar effects on the enzymatic activity of the liver, he wanted to compare their carcinogenic effect when combined with DENA, a known carcinogen. His research reveals that the effects of PCBs and PB are similar: when either product is administered after treatment with DENA for 5 or 10 weeks, a significant increase in cancer is noted.

9.3.2.4 J. Hendricks et al. (1977)

This team showed that when PCBs are administered to rainbow trout at the same time as aflatoxin, a powerful initiator of cancer, the incidence of cancer is reduced significantly compared with the onset rate when aflatoxin alone is administered.

9.3.2.5 Ito et al. (1978)

Like Nishizumi (1976), Ito et al. once again proved the similar effect of PCBs and phenobarbital on promoting cancer once the latter is initiated with 2-FAA. Moreover, they showed that when a partial hepatectomy is performed on rats given 2-FAA, there is also a slight cancer-promoting effect. The promoting effect is further accentuated by adding PCBs or phenobarbital.

9.3.2.6 Preston et al. (1981)

Preston et al. wished to verify a hypothesis put forward by several researchers, who thought that the promotion of cancer by PCBs might be due more to its contaminants, especially furans (PCDFs). They compared the affect of purified AR 1254 with that of AR 1254 containing PCDFs. Their research shows that:

- administered alone, AR 1254 and PCDFs do not initiate cancer;

administering AR 1254 or AR 1254 with PCDFs after the administration of DENA had a cancer-promoting effect but the effect of the second compound was more pronounced.

They concluded that PCDF impurities were not the only factors in the promoter effect attributed to AR 1254.

9.4 Remarks on Cancer and PCBs

9.4.1 The results obtained by Kimbrough in rats and Nagazaki and Ito in mice suggest that PCBs have a cancer initiating effect. However, some researchers feel that account must be taken of the fact the cumulative doses of PCBs (1.2 g to 3 g) administered to laboratory animals are sufficient to largely affect their immunological systems (atrophy of the thymus and spleen, suppression of the humoral immunologic system) and cause weight loss, thereby appreciably altering the animals' resistance to cancer. The incidence of hyperplastic nodules and hepatomas in animals in control groups was not zero; we cannot, therefore, exclude the possibility of the promoter effect of PCBs in predisposed animals.

9.4.2 When lower doses are given, gastrointestinal metaplasias and hepatic hyperplastic nodules appear, creating serious doubt among researchers, as a number of them believe that such lesions could be pre-cancerous.

9.4.3 PCBs seem to be both a promoter and an inhibitor of liver cancer, depending on when they are administered in relation to the administration of carcinogens known to initiate cancers. Moreover, this effect is comparable to that of other known enzymatic inducers, such as phenobarbital.

9.4.4 For an animal study on cancer to be valid, an adequate number of animals must be evaluated over their entire livee. For example, dogs live for between 10 and 14 years; it is, therefore, hardly surprising that no cancers have been found in them after two years.

9.4.5 It seems extremely hard to draw conclusions on the impact of these results on humans. Research should be pursued to further clarify this question.

9.5 Effects on Animal Reproduction

9.5.1 Estrogenic potential of PCBs and consequences

It is increasingly apparent that PCBs alter the metabolism of sexual hormones, through 450-type cytochromes, enzymes which play a role in the cellular metabolism of steroid hormones such as estrogens and progesterone. We know that certain congeners activate the MFO system through P-450 cytochrome, which suggests they have an estrogenic potential.

It has been shown (Allen et al., 1979; Barr et al., 1979; Sager et al., 1983; Barlov 1982 and so on) that by administering certain doses of PCBs to laboratory animals, effects are produced on the reproductive function, especially an alteration of the menstrual cycle (irregularities, lengthening of the cycle, increase in the duration of menstruation, alteration of the rate of bleeding and, occasionally, inhibition of ovulation), leading to a reduction in the fertility rate, an increase in the number of abortions and stillbirths, a drop in birth weight and an increase in the rate of post-natal deaths. However, these studies were unable to show that PCBs have a teratogenic effect, i.e. no effects were demonstrated on morphology.

9.5.2 Dose-effect relationship in certain species

A Non Observed Effect Level (NOEL) has not been defined for animals. The wide variation in response from one species to another does not simplify the task of researchers who want to define one.

In rhesus monkeys, Allen et al. (1979) noted that nursing babies whose mother had ingested 5 mg of AR 1248/kg of food during her eight-month pregnancy displayed important signs, i.e. a drop in weight and retarded growth at birth, acne, edema of the eyelids and skin pigmentation, and neonatal edema. The total quantity the mother ingested during the eight months of pregnancy corresponded to 210 mg of PCBs; the babies consumed PCBs during the first eight months of their lives through their mother's milk, which contained between 154 u g of PCBs/L and 397 u g of PCBs/L. However, for a diet containing between

0.5 mg and 1 mg of AR 1248/kg of food (total consumption during the eight months of pregnancy: 60 mg), Allen noted very few effects, i.e. a slight drop in birth weight, and poor pigmentation during the lactation period, with subsequently normal development.

According to a study carried out by Spencer (1982), AR 1254 had harmful effects on reproduction in rats which consumed between 25 ppm and 900 ppm of PCBs (mg of PCBs/kg of food). A rat with an average weight of 250 g which consumed 20 g of food per day ingested, on average, 2.5 mg of PCBs/kg of body weight per day. At 25 ppm, no effects on the attachment and embedding of the fertilized ovum in the endometrium were noted on day 12, but the survival rate fell as the dose increased above this level. At 100 ppm, birth weight was significantly reduced ($P \leq 0.05$).

Thus, in rats, the NOEL for reproduction is less than 2.5 mg of PCBs/kg of body weight per day, which is clearly higher than the NOEL which can be assumed in rhesus monkeys, as we just noted, because a diet for the latter of 1 mg of AR 1248/kg of food (1 ppm) over eight months corresponds to about 20 µ g of PCBs/kg of body weight ingested, on average, per day. Therefore, it seems that the NOEL in the rhesus monkey is below 20 µ g of PCBs/kg of body weight per day.

An analysis of studies conducted on mammals and birds reveals the effects on reproduction potential in some species when their diet contains between 1 ppm and 100 ppm of PCBs. As we know that piscivorous birds and fish-eating animals can concentrate PCBs by a factor of between 1 and 10 times greater than the concentration absorbed, it is readily apparent that had we continued to release PCBs in the environment at the same rate as in the 1960s and 1970s, they would have become a serious threat to some species. It is suspected that these substances are seriously threatening beluga whales.

It is acknowledged that PCBs in birds are inert as long as they remain in body fat. However, the situation changes markedly when food consumption decreases, for example, during reproduction and migration seasons and unfavourable periods. When body fat is consumed to provide energy, considerable quantities of organochlorinated compounds are released in the blood and reach other tissues, the liver, brain, gonads and so forth. Toxic effects could appear during these periods in the most sensitive species.

Although these data are incomplete, we believe they are sufficient to justify the implementation of efficient but rational control measures to prevent the dispersal of PCBs in the environment.

10. PHARMOKINETICS OF PCBs

10.1 Pathways of PCBs

PCBs can enter the organism in three ways:

by ingestion: intestinal absorption ~ 100%

by inhalation: absorption ~ 100%

by skin contact: rapid, intense absorption (Wester et al., 1983)

The properties of skin absorption on the abdomen in rhesus monkeys and guinea pigs are similar to those in humans, which enables us to study the phenomenon in the laboratory. Wester et al. (1983) studied the rate of skin absorption, the elimination rate in urine and the efficacy of washing skin for Aroclors marked at ^{14}C in rhesus monkeys and guinea pigs. Washing was effected with water and acetone. The following conclusions were reached:

. Without washing the skin appears to have a fairly high capacity for absorption. In guinea pigs, 33 percent of the AR 1242 and 56.75 percent of the AR 1254 applied were absorbed. In rhesus monkeys, between 15 percent and 34 percent of the AR 1242 was absorbed. This difference is a function of the quantity of PCBs per unit of surface.

. Washing the skin immediately after the application removed 59 percent of the AR 1242.

. Washing the skin 24 hours after the application had virtually no effect: it removed about 1 percent of the AR 1242 and 20 percent of the AR 1254.

These observations reveal the importance of preventive measures to avoid absorption by contact. This applies to almost pure PCB compounds. However, it should be mentioned that the absorption of PCBs by contact with oil contaminated with PCBs will certainly not be as extensive. The affinity of PCBs for oil will limit such absorption except when the oil itself is absorbed; however, because of its chemical structure (chains of saturated hydrocarbons), oil can hardly be absorbed by the skin.

10.2 PCB Distribution

Once absorbed, PCBs enter the blood and are distributed in the most vascularized tissues, i.e. the liver, heart and muscles. They are subsequently metabolized and eliminated by the organism or redistributed in tissues, especially in lipids. Generally, PCBs with low chlorine content are quickly metabolized and eliminated, while those with high chlorine content (tetra- to octa-) tend to persist longer in the organism. This has been confirmed by animal studies (Matthews et al., 1979) and clinical studies (Chen 1982 and 1985; Hara, 1985, and so on). Shortly after occupational exposure, mono-, di- and tri-CBs are eliminated, while hexa- and hepta-CBs persist for a long time.

The following table shows the distribution of PCBs in the tissues of a Taiwan patient who died in an accident two months after he was diagnosed as having Yu-Cheng disease. The highest concentrations were found in lipids in the intestines, bronchia and heart, while the lowest were in the brain and spleen.

TABLE 18: Concentration of PCBs in the Tissues of a Patient Suffering from Yu-Cheng Disease

Tissue	PCBs (ppm)
Intestinal lipids	10.81
Bronchi	5.56
Large intestine	3.28
Heart	2.48
Stomach	0.66
Liver	0.65
Jejunum	0.27
Kidney	0.25
Lung	0.18
Brain	0.08
Spleen	0.07

10.3 Elimination of PCBs in the Blood

Hara (1985) followed the PCB levels in the blood of workers exposed to the substances in a capacitor factory in Japan. He took annual blood samples for seven years after the plant closed. One group of workers had been exposed to Kaneclor 300 (43 percent chlorine), while another had been exposed to both Kaneclor 300 and Kaneclor 500 (55 percent chlorine). Kaneclor 300 contains, above all, isomers of tri- and tetra-CB, while Kaneclor 500 mainly contains isomers of penta- and hexa-CB. The results are outlined below.

In workers exposed to Kaneclor 300 and 500, the PCB level in the blood decreased an average of 27.5 percent over seven years; during the same period, it fell 61.1 percent among those exposed to Kaneclor 300 alone.

Table 19, taken from a study by Chen et al. in 1985 to assess the rate of elimination of PCBs in the Taiwan patients, indicates the differences in the rate of elimination of various congeners identified in patients' blood.

An analysis of this table reveals that not only do PCBs with high chlorine content (hexa-CB) tend to persist longer, but that the position of the chlorine atoms plays an important role. It is even possible to identify the structural properties which promote or delay elimination. For example, the molecules without chlorine in the adjacent meta- and ortho- positions (positions 2,3 or 2',3' or 5,6 or 5',6'), but with chlorine atoms in positions 4 and 4', are eliminated more slowly.

In the same study, using preliminary data, Chen was able to extrapolate the half-life of the two penta-CBs which had the highest concentrations in the poisoned patients, as Table 20 indicates. He also noted that the elimination time for the same congener varied greatly from one person to another.

TABLE 19: Comperative Rate of Elimination of the Principal PCBs in the Blood of Taiwanese Patients (cf. Chen et al., 1985)

Peak No	Chlorine Substitution	Elimination Rate	Adjacent Unsubstituted Carbon Atoms
9-4-a	2,4,5,4' (?)	+++	2',3';5',6'
9-4-b	2,5,3',4'	+++++	3,4;5',6'
9-4-c	2,4,3',4'	++++	5,6;5',5'
10-4	2,3,3',4' (?)	+++++	4,5,6;5',5'
11-5-a	2,5,2',3',5'	++++	3,4
11-5-b	2,5,2',4',5'	++++	3,4
11-5-c	2,4,2',4',5'	++	5,6
12-5-a	2,3,2',4',5'	+++++	4,5,6
12-5-b	2,5,2',3',4'	+++++	3,4;5',6'
12-5-c	2,4,2',3',4'	++++ (?)	5,6;5',6'
13-5	2,3,6,3',4'	+++++	4,5;5',6'
15-5	2,4,5,3',4'	+++	5',6'
16-5	2,3,4,3',4'	+++	5,6;5',6'
16-6	2,4,5,2',4',5'	+	none
18-6	2,3,4,2',4',5'	+	5,6
19-6	2,3,4,2',3',4'	++	5,6;5',6'
20-6	2,3,4,5,3',4'	+	5',6'
21-7	2,3,4,5,2',4',5'	+	none
22-7	2,3,4,5,2',3',4'	+	5',6'

+: extremely slow ++: very slow +++: slow ++++: less rapid
+++++: rapid

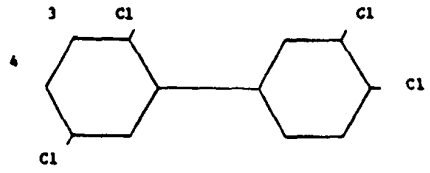
10.4 PCBs in Breast Milk and the Placenta

Kuwabara et al. (1978), Yakushiji et al. (1978, 1984), and Kodoma et al. (1980) have shown that mothers eliminate some of the PCBs in their tissues by breastfeeding; the PCBs are thus transferred to their babies. PCB elements in mother's milk are the same as those in the blood and adipose tissues. Hara (1985) showed that there is a linear relationship between the level of PCBs in blood and milk. The concentration in the milk was about 10 times that in the blood (for example, if the blood concentration was 10 ppb, it was 100 ppb in the mother's milk), although it was below the concentration in adipose tissues as mother's milk contains only 1 percent to 4 percent lipids (see sections 9.5.2 and 11.1.2).

The placental concentration of PCBs is about 5 to 10 times less than the blood concentration. The placental barrier appears to be effective.

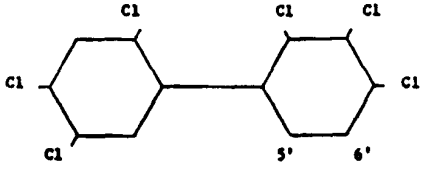
TABLE 20: Average Half-life of Two Penta-CBs

Average half-life (in months)	Penta-CB congeners
9.8 ± 5	2,4,5,3',4'
6.7 ± 2.5	2,3,4,3',4'



2,5,3',4'

Rapid Elimination
(positions 3,4 free)



2,4,5,2',3',4'

Slow Elimination
(positions 5',6' free)

FIGURE 22: Examples of Rapidly and Slowly Eliminated PCBs

11. PCBs AND HUMAN TOXICITY

At present, fish is the principal source of PCBs absorbed by the population at large. However, as PCBs were used in open systems, such as paints, solvents, inks and so on, until 1976, it is not an exaggeration to state that we have all been exposed to the substances to a greater or lesser degree.

Exposure can occur in three ways:

. environmental exposure through surroundings, air, water and food;

. accidental exposure in the event of specific contamination accidents, as was the case in Japan, or accidental dispersal in drinking water supplies and so on;

. occupational exposure occurs among workers who handle equipment containing PCB-based compounds.

11.1 PCB Levels in the Population and Effects on Health

11.1.1 PCB levels in the blood

According to data from the Centre for Disease Control, the average level of PCBs in the blood in the United States is about 5 ng/mL to 7 ng/mL (ppb). However, some people have higher PCB levels without having experienced occupational exposure to the substances.

Kreiss et al. (1982) evaluated the PCB level in the blood of 458 residents of Triana, Alabama, a fishing village. The average level varied between 3.2 ppb and 157.9 ppb, with an average of 17.2 ppb. Within the group:

98 people (21.4 percent) had a level greater than 30 ppb;
60 people (13.1 percent) had a level greater than 40 ppb;
41 people (9.0 percent) had a level greater than 50 ppb; and
 7 people (1.5 percent) had a level greater than 100 ppb.

Medical tests have shown an association between PCB levels and the following parameters: γ GT, cholesterol rate, and diastolic blood pressure. However, it should be noted that some of the people had low blood pressure and high PCB levels and vice versa.

In Canada, we have no statistical data about the level of PCBs in human blood. A limited study suggests that PCB levels in the blood of Hydro-Québec office employees and manual workers are more or less the same as those found in the general population throughout Canada and the United States.

11.1.2 PCB levels in mother's milk and effects on health

Wickizer et al. (United States, 1981) examined samples of mother's milk collected from 1057 breastfeeding mothers.* All of the samples contained PCB residues, varying from traces to concentrations of 5.1 mg of PCBs/kg of lipids found in mother's milk, with an average of 1.5 mg of PCBs/kg of lipids (1.5 ppm).

Of this number, 49 percent had concentrations between 1 mg/kg and 2 mg/kg of lipids; 17.4 percent, between 2 mg/kg and 3 mg/kg of lipids; and 6.4 percent, more than 3 mg/kg of lipids. The average lipidic content of the milk was 34.8 g/L (3.48 percent); consequently, an average of 1.5 mg of PCBs/kg of lipids is equivalent to 52.5 μ g of PCBs per litre of milk. In the first eight months of their lives, the children consumed an average of 9.4 mg of PCBs, of which 8 mg remained in the organism. Wickizer et al. concluded that in 1981, the milk of about 50 percent of mothers in Michigan had a PCB concentration higher than the limit allowed by the Food and Drug Administration for cow's milk intended for drinking (1.5 mg/kg based on lipidic content). However, a group of experts under the direction of B.W. Weil recommended to Michigan women who had not experienced occupational exposure to PCBs to continue breastfeeding. A study conducted by Jacobson et al. (1983) among 313 newborn babies and their

* This study was carried out in Michigan. Samples were collected in three Grand Rapids hospitals and in one in Muskegon (located on Lake Michigan). Grand Rapids is the most important metropolitan region bordering on Lake Michigan and is located near the Grand River, which is very popular for salmon and trout fishing.

mothers in the region studied by Wickizer (1981) did not reveal any effects among the babies or their mothers.

In Canada (Atkinson, 1979), a reduction in the average PCB level in mother's milk among Ontario women was noted: it fell by a factor of 2, from 40 u g/L between 1970 and 1974 to 20 u g/L in 1978. No toxic effect resulting from PCBs among newborn babies has been reported in Ontario in the past.

Both studies show that in 1978 the PCB level in mother's milk in Ontario (20 u g/L) was 2.5 times lower than the average rate observed in 1981 in Michigan women (52.5 u g/L).

In light of the analysis on the degradation of PCBs (see section 7.7), it may be expected that the average PCB level among Canadians will be lower still in 1986.

11.1.3 PCBs and lipids

According to studies conducted by the Centre for Disease Control in the United States and Environment Canada (1976), the average PCB level in the lipids of Americans and Canadians is between 0.1 ppm and 1 ppm (1 mg/kg of fat). Some people, especially heavy fish eaters living in the most contaminated regions, may have lipidic concentrations of PCBs exceeding 10 ppm. Generally, it is thought that this concentration is, on average, between 150 and 200 times the concentration of PCBs in the blood.

11.1.4 Effects on health in the population

The PCB levels of people who have not experienced accidental or occupational exposure to the substances does not seem to cause serious health problems, even in those groups most likely to be contaminated, such as fishermen.

11.2 The Yusho and Yu-Cheng Poisonings

11.2.1 Description

The first massive PCB poisoning occured in 1968 in Japan, where more than 1780 people, mainly residents of Fukuoka and Nagasaki, were poisoned after consuming rice oil contaminated by PCBs, furans and quaterphenyls for six months. The Japanese called the resulting illness Yusho (''rice oil'') disease. The morbidity rate is not known. The oil contained more than 2000 ppm of Kaneclor 400; it is thought that the consumption of PCBs varied between 0.5 g and 2 g, and that of furans, from 2.4 mg to 7 mg.

Eleven years later, the same type of poisoning occurred in Taiwan, where the epidemic affected the cities of Tai-Chung, Hsincho, Miaoli and Chang-Hua. According to the records of the Taiwan provincial health department, 2061 cases were reported. Although victims displayed the same symptoms as those suffering from Yusho disease, the Taiwanese called it Yu-Cheng disease. The rice oil in Taiwan contained Kaneclor 400 and Kaneclor 500, furans and quaterphenyls; more or less the same quantities of PCBs and furans were consumed in Taiwan and Japan. In the cities mentioned, the morbidity rate per 100 000 inhabitants varied between 56.56 and 1348.81. Symptoms usually appeared between 1.5 months and more than 6 months after ingestion began, with an average of 3 or 4 months.

With respect to human toxicity, these two events have contributed the most to furthering understanding of the effects of PCBs and their contaminants, mainly dibenzofurans and quaterphenyls.

Between 1968 and 1973, the official diagnosis of Yusho disease focused almost exclusively on symptoms involving the mucous membranes and skin. In addition to such symptoms (by far the most common), patients complained of fatigue, loss of appetite, headaches, weakness and edemas of the extremities. Nausea and vomiting were also reported; at the time, they were considered minor complications. Laboratory findings also showed such anomalies as increased triglycerides, anemia, lymphocytosis, hypoalbuminemia and reduced neuro-sensorial conducting speed.

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It was only five years later, in 1973, that PCBs were identified in the blood samples of patients suffering from Yusho disease, at very low concentrations (5.9 ± 4.5 ppb and 6.7 ± 5.3 ppb on average). These concentrations in the blood were much lower than those measured later in workers exposed to PCBs in transformer and capacitor factories. Indeed, we now know that such concentrations were very high, reaching as much as 1319 ppb (Maroni, 1981, Italy, concentration between 88 ppb and 1319 ppb; Wolff, 1982, United States, 1 ppb to 546 ppb; Hara, 1985, Japan, 121 ± 76 ppb). However, these symptoms of Yusho disease were much more serious than those displayed by industry workers.

Later, with more sophisticated analysis methods, it was possible to measure furans and quaterphenyls in the contaminated oil; concentrations of these substances were by no means negligible, which raised a number of questions.

. Were the symptoms of Yusho disease actually caused by PCBs? Might not dibenzofurans be the principal cause?

. What are the real hazards of PCBs?

The Taiwan poisoning provided the answers to these questions, as both accidents are similar: the oils were contaminated with PCBs, furans and quaterphenyls in both instances and the quantity of these contaminants ingested was also the same.

Less than a year after the symptoms appeared, the PCB level in the blood of Yu-Cheng patients varied from 3 ppb to 1156 ppb, with an average of 99 ppb. Although these levels are equivalent to those in workers experiencing occupational exposure, the severity and persistence of the symptoms of Yu-Cheng disease were considerably more pronounced.

After 1979, polychlorinated dibenzofurans were identified with certainty in the blood of patients suffering from Yu-Cheng disease and in the tissues of those with Yusho and Yu-Cheng diseases. Some of these substances, such as 2,3,4,7,8-pentachloro-DF, are deemed to be highly toxic. It has been shown that this furan accumulates in the livers of humans, monkeys and rats and is very toxic to animals (for example, it causes atrophy of the thymus, weight loss and hypertrophy of the liver with marked enzymatic induction at low doses, i.e. between 1 µ g/kg and 10 µ g/kg). On the basis of epidemiological studies conducted in Japan and Taiwan, the average consumption of PCDFs has been estimated at about 3.4 ng, which corresponds to 68 µ g/kg of body weight for a person weighing 50 kg.

These data, supported by animal experiments, have led the scientific community to conclude that most of the effects observed at the time of both poisonings were attributable primarily to the ingestion of polychlorinated dibenzofurans.

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11.2.2 Symptoms observed in patients with Yusho and Yu-Cheng diseases

Peri-ocular signs (earliest symptoms of Yu-Cheng disease, at an average plasmatic concentration of 41 ppb of PCBs).

- accentuated conjunctive pigmentation;
- abnormal cyst formations in meibomian glands;
- secretions;
- eyelid edema.

Cutaneous lesions (mild to severe)

- grade I: circumscribed pigmentation;
- grade II: comedones;
- grade III: mild acne eruptions;
- grade IV: extensive distribution of acne eruptions with purulent infections.

These symptoms regressed in time, but in some patients suffering from Yusho disease, they persisted after more than 10 years.

Hepatic lesions

These lesions ranged from the disturbance of liver parameters (such as γ GT, SGOT and SGPT) with no clinical signs, to hepatomegaly and jaundice.

General impairment

Weakness and fatigue.

Rise in triglyceride and cholescerol rates

Neurological lesions

At the onset of the disease, some patients displayed decreased peripheral sensitivity.

Effects on reproduction

- no teratological effect;
- lower average birth weight;
- some babies born with hyperpigmentation and chloracne.

These symptoms disappeared after two or three years.

Effects in nursing babies

Some nursing babies whose mothers were poisoned displayed symptoms of Yusho disease, with the appearance of dermatological problems such as chloracne. The symptoms disappeared after two or three years.

Immunological lesions (studied in Taiwan)

- increased IgM and IgA;
- normal IgG;
- reduction of T cells, active T cells and helper T cells;
- increased cutaneous and respiratory infections.

Three years later, a marked improvement in these immunological parameters and a drop in the infection rate were noted.

11.3 Occupational Exposure and Effects on Health

11.3.1 Fischbein et al. (1979)

This clinical study of 326 workers exposed to PCBs showed a correlation between the level of exposure, dermatological problems and SGPT level. Other objective and subjective symptoms and current biological blood parameters were not linked to PCBs.

11.3.2 Maroni (1981)

The study of 80 workers in a capacitor factory revealed that the PCB level in their blood varied between 41 ppb and 1319 ppb. Fifteen of them had cutaneous diseases, including four cases of chloracne. An association was also noted between PCB levels and the increase of γ GT, SGOT and SGPT.

11.3.3 Smith (1982)

This study, conducted for the National Institute of Occupational Safety and Health (NIOSH) in three groups of Monsanto workers, revealed:

- a positive correlation between exposure to PCBs and subjective symptoms of skin and mucous membranes irritations and systemic illnesses. No clinical anomaly attributable to PCBs was observed;
- a positive correlation between exposure to PCBs and SGOT, γ GT and plasma triglycerides;
- an inversely proportional correlation with HDL-cholesterol.

11.3.4 Colombi et al. (1982)

This study showed a slight prophyrinogenic effect in workers at a capacitor factory by measuring an increase in total urinary porphyrine. However, the porphyria did not reach the stage of clinical signs.

11.3.5 Ruwsbars et al. (1978)

The study of PCB levels in the blood of women exposed to the substances in the workplace and in their children from 0 to 13 years old made it possible to evaluate the effects of such exposure on their health. It was observed that the PCB level in the blood of these women and their children was higher than among patients suffering from Yusho disease and that levels measured in the children increased with the duration of the lactation period. None of the children displayed signs of PCB poisoning.

TABLE 21: Concentration in the Blood in u g/L (ppb) in Women Exposed in the Workplace, Their Children, Japanese Patients and Non-exposed Women

	EXPOSED WOMEN	CHILDREN OF EXPOSED WOMEN	JAPANESE PATIENTS	NON-EXPOSED WOMEN
SAMPLE	n = 20	n = 39	n = 12	n = 28
RANGE	8.3 to 84.5	0.8 to 93.2	1.8 to 8.6	0.8 to 5.7
AVERAGE ± STANDARD DEVIATION	38.6 ± 21.5	14.8 ± 18.1	4.2 ± 1.9	2.6 ± 1.2

11.3.6 Sahl (1985)

Only one study has been published on the potential effects of PCBs among electrical company workers. It was conducted recently at Southern California Edison (Sahl, October 1985, International Symposium on PCBs, Seattle). It centred on 220 employees in positions with high potential for exposure between 1979 and 1984 (maintenance workers and installers), who were compared with a group of office workers not subject to risks of exposure and a group of new employees who underwent a pre-hiring examination.

The length of exposure in the group of high-risk workers varied from 1 to 37 years, with an average of 14 years. The PCB level in their blood varied from 1 ppb to 15 ppb, with an average of 3 ppb and a standard deviation of about 4 ppb. These levels correspond to those measured among 738 new employees and the 1058 employees already working for the utility, whose PCB levels displayed the same proportions (average: 5 ppb; standard deviation: 4 ppb). Thus, there was no difference in the PCB level in the blood of the three groups studied.

Sahl concluded that working for this firm did not result in greater accumulation of PCBs than in the population at large. According to him, at such levels, nothing indicated that there were health risks for workers as other studies in the industrial milieu had not showed significant effects on the health of workers whose blood contained much higher PCB levels, i.e. between 50 ppb and 1300 ppb.

11.4 Remarks on Health Risks in the Workplace

11.4.1 No study in the workplace has dealt with the effects of PCBs on the reproductive system; consequently, we cannot conclude that no such effects exist. On the other hand, Kuwabara did not note any health problems in the children of women who gave birth after exposure in the workplace.

11.4.2 Although the concentration of PCBs in the blood of workers is higher than the rates observed in Japan and Taiwan, it has been noted that the health problems of workers are much less pronounced. With the exception of mild cutaneous disorders and variations in hepatic parameters without symptoms, no clinical signs have been associated with PCBs found in workers.

11.4.3 Researchers have noted that, once exposure to PCBs was halted, signs and symptoms were quickly reversible (after less than one year), while symptoms persisted in victims of Yusho disease even after 10 years. They explain this phenomenon by the presence of PCDFs in the tissues of Japanese patients; this shows that PCDFs continue to produce toxic effects beyond a 10-year period.

11.4.4 As Yusho and Yu-Cheng diseases are attributable primarily to polychlorodibenzofurans, we now have a reference point on the quantities of furans which can produce toxic effects in humans. Between 2.4 mg and 7 mg of total PCDFs were sufficient to produce the signs and symptoms observed at the time of both massive poisonings.

11.4.5 According to the NIOSH study (Criteria for a recommended standard occupational exposure to PCB's, 1977), chloracne usually appears in exposed workers having blood PCB levels above 200 ppb. Chloracne seems to be the first symptom to appear in workers in the electrical equipment industry.

11.5 PCBs and Cancer in Humans

Only three epidemiological studies have been conducted until now to determine whether PCBs cause cancer.

11.5.1 Brown (1981)

In 1977, at the request of NIOSH, Brown (1981) carried out a retrospective study of mortality among 2567 workers in two PCB manufacturing plants in the United States.

There were relatively few deaths (163), which seriously limits the study's statistical value. Moreover, follow-up lasted only 15 years. A latency period of between 20 and 30 years is often necessary to observe a fatal cancer. More than 50 percent of the sample had been exposed for two years or less.

Greater mortality levels than anticipated were noted for rectal cancer (4 cases observed for 1.19 expected) and liver cancer (3 cases observed for 1.07 expected) was noted. However, if both populations are combined, no excess is statistically significant (probability less than 0.05). The study continues, although no other results have been published since 1981.

11.5.2 Bahn et al. (1976)

In the course of this study of a group of 72 workers in a petrochemical plant in the northeastern United States, three cases of melanomae were found, two in a group of 31 men who had handled AR 1254 in the past and were extensively exposed, and the third in a group of 41 R&D employees, less exposed to PCBs. In the first group, the incidence was significantly higher than expected: 0.06 cases observed for 0.04 expected, with probability less than 0.001.

However, the sample studied was relatively small and the workers had also been exposed to other chemical products and to the sun. We know that prolonged exposure to the sun can cause melanomas.

11.5.3 Bertazzi et al. (1981)

This retrospective study of mortality covered 1310 employees (290 men and 1020 women) who had worked for at least six months in a capacitor factory in Italy between 1946 and 1970. The researchers found 8 cancer deaths among the men (4558 person-years) for 3.3 expected with p = 0.04. The excess was attributable primarily to cancer of the digestive tract and the lymphatic and hematopoietic system (ganglions and blood). Six cancers (for 2.3 expected, p not significant) were observed in the women (16 088 person-years), including two cancers of the lymphatic and hematopoietic system. Bertazzi concluded that the limited number of deaths and the fairly low average age of the population made it difficult to draw a valid conclusion. This study continues; to our knowledge, no other data have been published.

11.6 Remarks on PCBs and Cancer in Humans

11.6.1 An epidemiological study of cancer can be defined as a statistical study of the health of populations which consists of evaluating and comparing the frequency of various cancers in populations with different characteristics such, for example, as exposure or non exposure to PCBs. Doel (1981) and the International Agency for Research on Cancer (IARC, 1980) have defined criteria to determine the validity of the results obtained. The main criteria are:

- The excess of cancer observed in the exposed group must be higher than what is statistically probable;
- it must be possible to establish a relationship with dose and duration of exposure;
- there must be no biases or confusing factors;
- the association between exposure and cancer must be observed several times under different circumstances.

By applying these criteria to the studies mentioned, we find that data available until now and the results obtained do not allow us to conclude that PCBs have a carcinogenic potential in humans.

11.6.2 Statistically, all three studies are limited and do not enable us to establish a cause-and-effect relationship between the cancers observed and exposure to PCBs. Moreover, a number of methodological weaknesses may have led to mistakes, i.e. considerable variability in exposure time and the age of exposed persons, exposure to other products, occasionally very short latency period, information drawn from death certificates and subject to error, and so on.

11.6.3 A study which does not show a statistically significant increase in cancer is called a negative study or a test with a negative result. In light of such studies, one is tempted to conclude that the substance studied is not carcinogenic, although this conclusion is not necessarily fair. Many reasons can explain why a cancer study is negative. Often, this results from the poor sensitivity of the method or its lack of power: number of samples, exposure time and the relationship between exposure and dosage, and so on, as in the case of studies cited earlier, on the basis of which we can neither confirm nor deny a carcinogenic effect. However, given the rather high number of samples in studies conducted by Brown and Bertazzi, i.e. 2567 and 1310 workers, respectively, we can at least confirm that if PCBs have a carcinogenic potential, it appears to be relatively small.

12. APPLICATION OF STANDARDS AND REGULATIONS AND HEALTH AND ENVIRONMENTAL PROTECTION

12.1 Do PCBs Belong to the Category of Chemical Substances Which Threaten Health and the Environment?

First, let us clarify what we mean by a chemical substance which threatens health and the environment.

Direct hazard: direct contact with the product may produce acute or chronic effects in humans or animals, or have a destructive effect on wildlife.

Indirect hazard: products do not pose a direct threat, although once they are released in the environment they can, in the long run, be prejudicial to ecosystems and indirectly threaten health. Contamination by such products can reduce the number of animal or plant species, slow down important economic activities, cause irreversible ecological changes and, consequently, prevent humans from benefiting from nature.

Theoretically, the most hazardous chemical products have some of the following characteristics. They can:

. spread in very high concentrations in the environment;

. persist for a long time in the environment and accumulate there indefinitely;

. accumulate in living tissues and move through the food chain;

. cause biological or geochemical changes, even in small concentrations;

. be impossible to recover once dispersed in the environment;

. enter the environment in several ways, from many sources.

In light of the preceding chapters, it can be concluded, with some qualification, that PCBs have, in part, some of the following characteristics:

. relatively high concentrations of them have been found everywhere in the environment, as a result of uncontrolled dumping over three decades;

. some congeners with high chlorine content persist for a long time in the environment (half-life of more than two 2 years), but not indefinitely;

. they accumulate in living tissues and move through the food chain;

. they produce biological changes in the most sensitive species for concentrations in the order of mg/kg of body weight. According to laboratory data, some animal species could be affected at concentrations currently found in the environment;

. they cannot be recovered once they are dispersed in the environment;

. since their use in open systems was halted, sources of infiltration in the environment have been appreciably reduced; PCBs tend to be eliminated slowly from the environment, as the degradation rate seems to surpass the influx rate.

Nonetheless, we believe that all of these characteristics of the behavior of PCBs in the environment are sufficient to justify measures to control the risk of spills.

12.2 Are PCBs a Direct Threat to Health?

We have seen that PCBs have caused health problems in workers of both sexes exposed to them in industrial workplaces, especially in plants manufacturing transformers and capacitors using these substances. Such problems, affecting mainly the skin and liver, occur after the accumulation of considerable quantities of the substances in the organism when PCB concentrations in the blood and lipids reach levels generally above 200 ppb and 40 ppm, respectively. At the concentrations found in the population at large, no notable effects have been observed.

1.2.2.1 Exposure in the workplace

12.2.1.1 Exposure to askarels

PCB-based fluids called askarels, found in electrical equipment, can be absorbed by inhalation or direct contact.

By inhalation

In Québec, the following occupational exposure standards are currently in force (Regulation respecting the quality of the work environment):

AR 1260	0.5 mg/m^3	x	[8] hours
AR 1254	0.5 mg/m^3	x	[8] hours
AR 1242	1 mg/m^3	x	[8] hours

Were they stringently applied, these standards would result in an average accumulation by inhalation of 5 mg/day for AR 1260 and AR 1254, and 10 mg/day for AR 1242. Based on all of the information we have at our disposal concerning occupational exposure, we might well expect symptoms after 3 or 4 months' exposure. This standard seems ineffective to us, but as it will be impossible to experience such exposures in the future, it is now inapplicable.

By contact

As askarels contained in electrical equipment seem to be relatively absorbable by the skin, it is important to provide workers handling such equipment or fluids with effective skin protection.

12.2.1.2 Exposure to oils contaminated by PCBs

Is contact with contaminated oils hazardous for workers or the public, in the event of an accidental spill?

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This question is complex, as the risk depends upon the type of exposure, the quantity of PCBs in the oil, the frequency of exposure, the degree of absorption by the skin, the surface exposed and so on. A concentration of 50 ppm is often cited as a risk threshold. It should be pointed out that this concentration is the permissible limit of PCB content for fluids to be governed by the same environmental protection legislation as pure PCBs. We know that PCBs have an affinity for oil: for the 50 ppm contained in the oil to be absorbed by the skin, the oil would have to be completely absorbed. This means, for example, that a person must have daily contact with 10 mL of oil contaminated at 50 ppm; in this instance, a maximum of 50 µ g of PCBs would be absorbed, provided the 10 mL of oil were completely absorbed by the skin. However, as the skin absorbs very little of this type of oil (see section 10.1), the risk certainly does not exceed several micrograms of PCBs per day. If, in addition, workers adopt hygienic and protective measures, such as wearing gloves and washing thoroughly when oil is found on the skin, the quantity of PCBs absorbed becomes negligible.

This perhaps explains the results obtained by Sahl among workers at Southern California Edison, where he found that installers and maintenance workers exposed to PCBs for several years did not have blood levels of the substances higher than those in the population at large.

12.2.2 Exposure through food

The Legal Marketing Allowance (LMA) for food in Canada is 2 mg/kg (2 ppm).

As we noted earlier, consumers of fish are the most exposed. In Canada, those at highest risk appear to be consumers of fish caught in the Great Lakes and certain tributaries of the St. Lawrence. The average consumption of fish in Canada is 125 g per week; consequently, a person eating this quantity of fish contaminated at 2 ppm ingests an average of 250 µ g of PCBs per week.

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12.3 Indirect Threat to Health, Flora and Fauna

See Chapter 7.

12.4 Are Measures to Combat PCB Pollution Effective?

There is no doubt that measures taken in the early 1970s to prohibit the use in food processing plants of equipment containing PCBs and to halt the use of the substances in open circuits led to a substantial reduction in the threat to health and the environment.

In the late 1970s and in early 1985, legislation prohibiting the production of new equipment containing PCBs and improved measures designed to prevent accidental PCB spills while equipment is being transported or handled have certainly enhanced the efficacy of earlier measures.

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CONCLUSION

Our analysis of available data respecting, on the one hand, the toxicity of PCBs, their contaminants and combustion products in humans and, on the other, their persistence in the environment, leads us to make the following observations.

PCBs can produce toxic effects in animals and humans; these effects are closely related to the quantity of the substances absorbed by the organism. However, because of widely varying responses to PCBs and related products from one animal species to another, it seems very hard to apply the data obtained with laboratory animals to humans in order to quantify toxicological risks and risks of cancer in humans.

However, the data obtained using laboratory animals are very useful. They provide very precise information on the type of response which PCBs produce in mammals, and indications of the potential risk which some species in the environment are facing. They also explain the differences in toxicity and persistence of various commercial PCB-based compounds in humans, and shed new light on the mechanism of action and the metabolism of PCBs in living organisms.

For a long time, it was thought that PCBs were highly toxic, because of the severe clinical signs displayed in 1968 by Japanese poisoned by rice oil contaminated with PCBs and other substances which were unknown at that time (furans, quaterphenyls and methylsulphones). Through intensive follow-up among victims and studies conducted among workers exposed to PCBs in the workplace, and others carried out following the Taiwan poisoning in 1979, the scientific community concluded that the seriousness of the symptoms of Yusho disease were attributable primarily to furans found in the rice oil and not to PCBs. This conclusion is supported by a considerable body of research on humans and laboratory animals.

When compering the symptoms of industry workers among whom plasma and lipid concentrations of PCBs were equal to or higher than those measured in victims of Yusho and Yu-Cheng diseases, researchers noted marked differences. In populations exposed to PCBs in the workplace, the substances have caused a number of cases of chloracne, other types of dermatosis and a slight alteration of hepatic and lipidic functions, although these signs disappeared several months after exposure ceased. Among patients suffering from Yusho and Yu-Cheng diseases, the symptoms were generally much more severe and persisted in some of them 15 years later. Recent research has shown that these effects are due to the presence of furans in the patients' tissues.

At the concentrations usually observed, no toxic effect has been noted in the population at large. Even in populations of fishermen who consume substsntial quantities of contaminated fish, no important effects due to PCBs were observed. It should be noted that in some fishermen, plasma concentrations of PCBs were higher than the average concentration measured in patients in Japan and Taiwan.

With regard to potential effects on reproduction, no laboratory study has shown that PCBs have a teratogenic effect, i.e. they do not appear to cause deformities. However, in laboratory animals there is a threshold beyond which the substances are fetotoxic (increased abortions, reduced birth weight) and probably have a contraceptive effect (reduced fertility rate).

It may be thought that such effects might occur in all living organisms. However, the absence of documentation on women exposed in the workplace to high doses of PCBs during the 1950s, 1960s and 1970s prevents us from estimating the exact dose at which such problems would occur.

At present, one source of anxiety in the population at large is the potential effect of PCBs in mother's milk. Studies have been conducted in Japan among children between 0 and 13, who were born to mothers exposed to PCBs in the industry and breast fed, and in the United States and Ontario in the Great Lakes region, among women and their nursing babies. These studies did not

reveal any effects among these babies during breast feeding and childhood. American specialists have concluded that women should not avoid breast feeding despite the presence of traces of PCBs in mother's milk.

Data on the incidence of neoplastic diseases in populations exposed to PCBs do not enable us to establish a cause-and-effect relationship. Although the data do not entirely exclude the possibility that PCBs are potentially carcinogenic, we can conclude that this potential is certainly very limited. In our opinion, to significantly confirm or deny a cause-and-effect relationship, studies would have to deal with much broader population samples that those in studies conducted until now, for example, by grouping together several exposed populations. However, such studies seem hard to conduct as there are no longer any highly exposed populations in the industry since the manufacture of PCBs has been prohibited.

The greatest health risk is fire in equipment insulated with PCBs (askarels), incomplete combustion of which leads to the formation of substances deemed to be extremely toxic, primarily furans and, where there is an associated solvent (polychlorobenzenes), dioxins. It is, therefore, essential that preventive measures be taken to reduce these risks to a minimum in the event of a fire. We feel that risk is higher in workers responsible for decontaminating and rebuilding sites than it is in firefighters. For the latter, a positive-pressure breathing apparatus, gloves and a coverall make health risks negligible.

Exceptional protective measures must be taken among workers responsible for decontaminating and rebuilding sites, to prevent effects similar to those produced by Yusho disease. After the fire, care must be taken to ensure that only authorized personnel enter the site of the fire and only after experts have advised them on protective measures. Obviously, high priority should be accorded to replacing electrical equipment insulated with askarel in zones where contamination risks are high.

We have also shown that in the event of a fire in equipment insulated with oil contaminated by small concentrations of PCBs (several hundred ppm), firefighters face very limited risks, even without protective equipment; if they wear positive-pressure breathing apparatus and coveralls, risk is virtually eliminated. Of course, for greater safety, we recommend that such measures be taken.

Before the site is cleaned up, an assessment must be carried out to determine the concentrations of toxic products present and establish what measures are needed to decontaminate it.

Protective measures must be adopted for industry workers called upon to handle, maintain or transport equipment containing askarels, to avoid skin contact with PCBs. Where temperatures are high, for example, or ventilation is poor, it may be necessary to wear a self-contained, positive-pressure breathing apparatus. To avoid absorption of PCBs through the skin, splashes must be immediately and thoroughly washed with soap and water. The risk of absorbing contaminated oil by contact is much more limited than with askarel, because of the small concentration of PCBs in the oil and the low absorption rate by the skin of the type of oil used in such applications.

The environment is widely contaminated and such contamination reaches the trophic food chain of humans. All living organisms carry traces of PCBs. In some ecosystems, contamination is such that over time, some species could very well have disappeared had not the uncontrolled dumping of PCBs in the environment been halted.

Studies carried out in the Great Lakes since 1970 tend to show that, since the production and sale of PCB-based compounds for open-circuit applications were halted in 1973, the quantity of PCBs which are eliminated in the environment exceeds new influxes. Researchers have noted a significant reduction in concentrations of PCBs in sediments, water, the air and in fish and birds.

Although production of PCBs has been halted throughout the world, because there are still large quantities of the substances in use in electrical equipment, it remains essential to maintain measures governing dumping, through legislation and regulations. Such measures will accelerate the process of eliminating PCBs from the environment, which has already begun.

Current knowledge on the risks of toxicity and the general behavior of PCBs in the environment allows us to adopt a rational approach to effectively combat PCB pollution, by reducing health and environmental risks, while taking into account the socio-economic impact of the measures adopted, and their applicability.

In our view, to fully eliminate PCBs from the Canadian environment, we will have to install PCB destruction units like those in operation in the United States and Europe. Storing electrical equipment containing PCBs eliminates some risks, such as fire, although the latter risk still exists when this type of equipment is used in an electrical system. On the other hand, storage does not protect us from catastrophes such as earthquakes, which would cause tons of PCBs to be released in one place.

We can find a solution to any pervasive problem which threatens health and the environment. As world-wide production of PCBs has stopped, and as the substances are gradually biodegrading and governments are taking measures to control PCB dumping in the environment, we can hope that within several decades they will no longer pose their current threat.

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Research and Development

EPA/600/2-86/090

EPA

Microbial Decomposition of Chlorinated Aromatic Compounds

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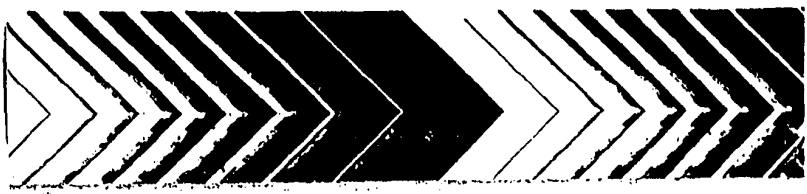

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MICROBIAL DECOMPOSITION OF CHLORINATED AROMATIC COMPOUNDS

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FOREWORD

Today's rapidly developing and changing technologies and industrial products and practices frequently carry with them the increased generation of solid and hazardous wastes. These materials, if improperly dealt with, can threaten both public health and the environment. Abandoned waste sites and accidental releases of toxic and hazardous substances to the environment also have important environmental and public health implications. The Hazardous Waste Engineering Research Laboratory assists in providing an authoritative and defensible engineering basis for assessing and solving these problems. Its products support the policies, programs and regulations of the Environmental Protection Agency, the permitting and other responsibilities of State and local governments and the needs of both large and small business in handling their wastes responsibly and economically.

This report is a compendium describing the current level of understanding of chlorinated aromatic compound decomposition by microbiological pathways. The halogenated aromatic compounds are one of the most persistent collections of chemicals contaminating the environment. The persistent nature of these chemicals is attributable to the inability of the environment to cleanse itself of these contaminants. Since microbiological communities are fundamental participants in the detoxification chain, the environment generally does not have microorganisms capable of degrading the halogenated aromatic compounds. This report specifically identifies microorganisms capable of degrading many of the halogenated organic species. In many cases, the substrate is tracked through a decomposition pathway to end product. Many factors contribute to the biodecomposition of a given chemical; among the most important are: the chemical nature of the substrate molecule and substituents, substrate concentration, environmental parameters, nutrient and growth factor availability, and the presence of organisms capable of degrading the substrate. However, insufficient information is currently available to assess the potential biodegradability of a substrate based on information generated under different environmental conditions.

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ABSTRACT

This report was initiated because of a need to bring together a review of the literature pertaining to microbial metabolism of chlorinated aromatic compounds. The information gathered here is extensive although not exhaustive. Most attention has been given to reports of bacterial, fungal, and cyanobacterial pathways of substrate degradation where metabolites or end products have been identified. Studies which report data on metabolites arising from incubation of the substrate with mixed cultures or environmental samples and studies which show disappearance of the compound have also been evaluated and included.

In addition to separate chapters on each class of chlorinated aromatic compounds, reviews of microbial physiology, genetics, and methods of biodegradation assessment are included. One chapter reviews biodegradation of these compounds in scaled up processes.

The potential biodegradation pathways for all classes of chloroaromatic compounds have been brought together into an overview diagram.

The review indicates that many factors are involved in assessing the biodegradability of a compound including the nature of the molecule, substrate concentration, environmental parameters, availability of nutrients and growth factors, and presence of degradative microorganisms. Not enough information is presently available to permit extrapolation from one environment to another or to utilize data on a similar compound to assess the biodegradability potential of a given substrate.

This report includes an illustrated list of compounds, a glossary, a reference list, an additional bibliography, citation index, and a subject index.

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ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

BOD	– biochemical oxygen demand
COD	– chemical oxygen demand
H_c	– Henry's law constant
HRT	– hydraulic residence time
MCRT	– mean cell residence time
MLSS	– mixed liquor suspended solids
MLVSS	– mixed liquor volatile suspended solids
ppb	– parts per billion (μg/l)
ppm	– parts per million (μg/ml or mg/l)

Abbreviations of herbicides are noted at first mention and in index.

SYMBOLS

a	– empirical constant in Langmuir isotherm
b	– empirical constant in Langmuir isotherm
C_a	– concentration of substrate in the mixed liquor
C_l	– concentration of substrate in the liquid phase
C_s	– concentration of substrate in the solid phase
f_l	– weight fraction of lipid-like compounds in the biomass
K^b	– a biological disappearance rate constant, first order in substrate concentration
K_f	– empirical constant for the Freundlich isotherm equation
K_{ow}	– octanol-water partition coefficient
K_s	– Monod half-saturation constant
K^w	– first order stripping rate constant
n	– empirical constant in Freundlich isotherm equation
Q_{in}/V	– ratio of the air flow rate to the hydraulic reactor volume
REM_b	– percent removal of the substrate from the system by the biotransformation fate mechanism
REM_e	– percent removal of the substrate from the system in the effluent
REM_s	– percent removal of the substrate from the system by sorption on biomass
REM_w	– percent removal of the substrate from the system by the stripping fate mechanism
X	– concentration of biomass in the mixed liquor
Y	– yield coefficient
μ	– specific growth rate
μ_m	– maximum specific growth rate

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SECTION I
INTRODUCTION

The first synthesized organochloride compound, ethyl chloride, was prepared in about 1440, but large scale synthesis of industrially important chlorinated organics, including chlorinated aromatic compounds, occurred during only the past few decades (214a). In general, the chlorinated aromatics of industrial synthesis or byproducts thereof represent one class of xenobiotic recalcitrant compounds. These compounds have few or no naturally occurring structural analogs and are persistent or resistant to both biological and abiotic degradation. Many of the chlorinated aromatics share similar physical chemical properties of low water solubility and high K_{ow} (octanol-water partition coefficients) which suggest lipophilicity or bioaccumulation potential.

Properties such as persistence, bioaccumulation, and demonstrable chronic and acute toxicity to human and nonhuman animal populations cause immediate concern related to their environmental health effects and their potential for ecosystem perturbation on long- and short-term exposure. These concerns cause the frequent appearance of these chemicals on EPA priority pollutant lists and have led to extensive research on their fate in the environment and their potential for microbiological transformation to less hazardous molecules.

The term biodegradation has had many different meanings. The Biodegradation Task Force, Safety of Chemicals Committee, Brussels (299) has defined biodegradation as the molecular degradation of an organic substance resulting from the complex action of living organisms. A substance is said to be biodegraded to an environmentally acceptable extent when environmentally undesirable properties are lost. Loss of some characteristic function or property of the substance by biodegradation may be referred to as biological transformation. In this text, we have attempted to restrict use of the term biodegradation in favor of more specific terminology. In this respect, biotransformation refers to any alteration of an organic molecule by organisms, and mineralization means the transformation of an organic molecule to its inorganic component parts with release of halide, CO_2, and/or methane.

The potential for microbial transformation of chlorinated aromatic compounds is related to the two fundamental roles of heterotrophic microorganisms in the global ecosystem. Both roles relate to the central concept of microbial decomposition of organic matter to release stored energy in the organic molecules (whether natural or anthropogenic in origin) and to return essential nutrients, such as CO_2, to biogeospheric nutrient pools. The first has thermodynamic implications while the second relates to elemental and nutrient cycling. While these simplified generalizations apply to most naturally produced organic matter, certain aromatic polymers such as lignin are persistent in the environment. Factors contributing to persistence of organic compounds have been discussed previously (92a) and can be summarized as insolubility, large molecular size or polymeric nature, toxicity, and anthropogenic origin. Although most or all of these factors are important for various chlorinated aromatics, the anthropogenic origin of most chlorinated aromatics is critical for the

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prediction of the rate of elimination of the compound from the environment and its eventual fate.

In general, microbial decomposition or biodegradative capabilities have coevolved with the synthesis of organic matter by plants and animals over the millenia. However, in the case of many chlorinated organics of industrial synthesis, 40 to 50 years is an unlikely time frame to expect evolution of enzyme systems capable of decomposition of such compounds. Yet evidence has accumulated that some of these compounds can be biologically transformed and extensively biodegraded by a diversity of heterotrophic microorganisms.

Such evidence has arisen primarily from studies using pure and mixed microbial cultures and from lesser-controlled environmental fate experiments. This evidence has promoted much additional research on the molecular mechanism of biodegradation, which in turn has permitted studies leading to increased knowledge of more detailed aspects of biodegradation itself. Currently, major questions exist as to the rate at which biodegradation occurs in various environments and the potential for kinetic prediction of pollutant fate based on laboratory and environmental observations. Research needs directed at this major question have caused a renewed focus on individual microbial populations that may be specifically responsible for biodegradation of a narrow spectrum of chlorinated aromatic substrates, and on physical/chemical environmental parameters that may modulate both the population and their catabolic activity. Developments in these areas have led to the relatively recent detection of bacterial strains harboring extrachromosomal DNA (plasmids) that genetically encodes enzymes which mediate the biodegradation of specific groups of aromatic and chlorinated aromatic compounds. Coupled with the availability of new molecular genetic techniques, such as DNA probe technology, it has become feasible to plan research to examine catabolic gene maintenance and transfer in natural populations and to detect specific biodegradative microorganisms in the environment. Such development will lead to greater predictive capabilities on the long-term persistence of selected chlorinated aromatic pollutants, and will provide for insight in the evolution and reassortment of genes responsible for biodegradation. With these genetic techniques there is a potential therefore to enhance biodegradative capacity among natural populations.

This report begins with three sections that provide an overview of microbial physiology, genetic information transfer and processing, technologies for gene manipulation, and methods of biodegradation assessment. These principles are applicable to microorganisms in general and are specifically relevant to metabolism of chlorinated aromatic compounds. Readers wishing review of these basic microbiological concepts should read these chapters before turning to subsequent sections.

The next section includes a review of pathways of metabolism of non-chlorinated aromatic compounds from which the chlorinated pollutants are derived. The microbial metabolism of most of these compounds has been studied extensively and these data form the basis for an understanding of the biotransformation pathways of the more complex chlorinated aromatic molecules.

The following nine sections discuss the microbial metabolism of the chlorobenzoic acids, chlorobenzenes, chlorophenols, pentachlorophenol, chlorophenoxy and chlorophenyl herbicides, chlorinated biphenyls, DDT and related compounds, and chlorinated dioxins and dibenzofurans. These compound classes represent all of the major classes of chlorinated aromatic molecules with environmental pollution potential.

Each chapter details the information available on metabolism of these compounds by pure cultures or consortia of bacteria, cyanobacteria, and fungi. Emphasis has been placed on studies in which metabolites have been identified and, where possible, complete or partial pathways have been reported. Studies reporting evolution of CO_2 and chloride release have been reviewed and are discussed as well. The disappearance of these pollutants in soils, water, sewage, and other environments has also been noted, although the body of literature relating to herbicide disappearance is so extensive that it has only been summarized and has not been reviewed as completely as the other topics. The fate of these compounds in scaled-up biological treatment processes is the subject of the next section.

The final chapter summarizes the information reviewed here and draws together the pathways of metabolism of chlorinated aromatic molecules into an overview diagram indicating the potential for biodegradation of a compound under optimum conditions. This is an idealized representation, as few environmental situations would comprise all the factors necessary for complete biotransformation and/or mineralization of these recalcitrant compounds.

This report includes a list of citations and a bibliography of additional references, including several excellent reviews on the biodegradation of various classes of organic compounds. All of the compounds noted in this report, including metabolites, are registered in an illustrated alphabetical list in the appendix. A citation index and a subject index are also included. Finally, a glossary of many of the scientific terms that appear in the text is appended in order to aid the reader.

This document is intended to be a general reference for environmental decision-makers who are interested in the fate of chlorinated aromatic compounds with respect to microbial activity. It is also meant to provide a resource for scientists and engineers involved in environmental predictive fate assessments. In addition, this review is designed to be a continuing resource for environmental microbiologists interested in the areas of metabolic pathways of chlorinated aromatic compound dissimilation and biodegradative fate of these potential pollutants.

The organization of this document into specific chapters and associated review sections is intended to facilitate its use by these diverse groups of people. This is an extensive although not exhaustive review of the literature pertaining to biodegradative fate of these classes of chlorinated aromatic compounds.

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SECTION 2
CONCLUSIONS

1. The biodegradability potential of a given compound depends on many factors. Chemical determinants include the ionic state of the compound, the number, types, and position of substituents, and the general form of the molecule. Environmental parameters affecting microbial metabolism include pH, temperature, redox state, moisture, reactor configuration, kinetics, and system turbulence considerations, and interference by competitive processes such as sorption, stripping, and photodegradation. Other factors involved in microbial growth and metabolism include availability of nutrients and growth factors, concentration of substrate, competitive interference by other substrates, and formation of toxic metabolites.

2. Many microorganisms require a period of acclimation before biodegradation occurs. Once a population is acclimated, it sometimes retains its predisposition to metabolize the substrate, and subsequent additions of substrate are metabolized after a shorter lag or no lag period.

3. Some compounds are mineralized by consortia of microorganisms in cases where no single species has been shown to be capable of that process.

4. Fungi and bacteria metabolize most compounds by different biochemical pathways.

5. In general, chlorophenoxy herbicides and chlorobenzenes can be metabolized to chlorophenols and then to chlorocatechols. Phenylamide herbicides and other compounds with nitrogen containing substituents are decomposed to chloroanilines, which can be oxidized to chlorocatechols and a variety of products. Chlorocatechols may be converted by a variety of different mechanisms to ponchlorinated ring cleavage products. Chlorobenzoic acids may be transformed by three different routes to ring cleavage products: (1) through a substituted catechol, (2) through chlorosalicylic acid, or (3) anaerobically through benzoic acid to methane and carbon dioxide.

6. The above conclusions represent general pathways for substrate dissimilation. Prediction of the biodegradability of a particular compound on which no data exists, based on data about similar types of compounds, can be loosely made. For instance, the degree of chlorination affects the rate and extent of metabolism of PCBs and other compounds.

7. Experiments in the laboratory or in a particular environment cannot be readily extrapolated to other environments, because of the need to consider the parameters affecting the biodegradability potential listed above.

8. Small scale lab tests are essential in order to perform properly controlled experiments to assess the effects of many of the above-mentioned biodegradation factors. Experimental design necessary to achieve the highly controlled results desired in small-scale testing nearly always requires compromise of factors needed to apply results to larger-scale systems.

9. Scale-up testing in larger-scale systems designed to simulate the desired full scale application (wastewater treatment system, land farming, environmental scenario, etc.) is required to collect data (often kinetic data) allowing the prediction of process performance and comparison among full-scale systems.

10. Many of the available scaled-up studies focus on substrate disappearance or removal from the given feedstream without quantification of abiotic fates or adequate regard for calculation of biokinetic rate constants.

11. Additional emphasis should be given to establishing consistent scale-up methodology and to implementing additional work for generation of reliable scale-up data on the biological treatment of chlorinated aromatic compounds.

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SECTION 3
OVERVIEW OF MICROBIAL PHYSIOLOGY

A study of the specific pathway of degradation of a compound by an organism necessarily is limited to specific biochemical reactants, products, and reactions occurring within the cell. Much of the content of this document is concerned with just such features. When genetics of the pathways are discussed, the relationship to the total cell is even more remote. Therefore, it is important to begin with a firm understanding of the physiology of the microbial cell, its structure, requirements for growth and survival, and relationship to its environment. While the cell is often described as a microscopic biochemical reactor, the activities of the cell are intimately connected to and shaped by its external environment. Information presented in this chapter is based on several general references, which may be consulted for further details (4, 107, 276).

The primary physical difference between bacteria and fungi is the presence of a membrane surrounding the DNA material. The enclosed structure is called the nucleus. Cells containing a membrane-bound nucleus are referred to as eukaryotic cells. The DNA of bacteria and cyanobacteria is contained in a diffuse region without a surrounding membrane called the nucleoid and these forms are called prokaryotes (Figure 1). Although it is tempting to consider bacteria as primitive compared to eukaryotes, the complexity of their biochemical reactions, and their regulatory and adaptive mechanisms, preclude such a label.

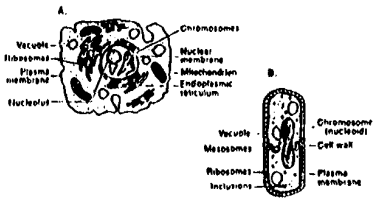

Figure 1. Cellular organization. A - Typical eukaryotic animal cell. B - Typical prokaryotic rod shaped bacterium.
Reference 4.

MICROBIAL CELL STRUCTURE

Most bacterial cells average about 1 to 2 microns in length and are rod shaped. Among all bacteria, however, cell size ranges from one tenth to 100 times the average bacteria size. Although most of the bacteria found in the environment are rod shaped, some water isolates are shaped like commas or spirals, and many bacteria, especially pathogenic species, are spherical (cocci). Other bacteria take unique shapes and forms as well.

The shape of the cell is conferred by a rigid cell wall composed mostly of peptidoglycan, lipid, lipopolysaccharide, and protein (Figure 2). There are charged polymers within the cell wall which assist in the uptake of ions and some nutrients. The wall also acts as a molecular sieve which prevents entry of some large molecules and prohibits loss of proteins, i.e., enzymes, from within the cell.

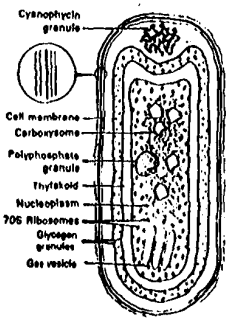

Figure 2. Schematic diagram of cyanobacterial vegetative cell (insert) Enlarged view of cell envelope, showing outer membrane and peptidoglycan wall layers and cell membrane.

Reference unknown

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Bacteria and cyanobacteria can be divided into two groups based on cell wall structure and composition. Classically the bacteria have been differentiated into Gram positive and Gram negative groups according to a staining procedure called the Gram stain. Electron microscopic techniques have shown differences in the form of the cell walls between the two types of bacteria. The Gram positive cell wall is composed of a single dense layer of peptidoglycan. Embedded in the peptidoglycan matrix are polysaccharides and teichoic acids. The cell wall is closely associated with the cytoplasmic membrane which has a double-track appearance with a central transparent layer. The Gram negative cell wall is more complex. The outermost layer is a wavy, double-track membrane which differs in chemical composition and in function from the cytoplasmic membrane. This layer is composed of lipopolysaccharides, phospholipids, and proteins. Internal to the outer membrane is a thin rigid layer of peptidoglycan. Between the cytoplasmic membrane and the outer cell wall membrane lies the periplasmic space containing enzymes (Figure 3). In contrast, the fungi have cell walls composed mainly of polysaccharides. The particular types of polysaccharides are characteristic of the taxonomic group of the fungi.

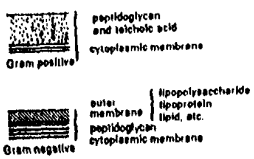

Figure 3 Cell wall structures seen in thin-section electron microscopy. A Diagrammatic representation of the Gram positive wall, B of the Gram negative wall. The location of wall components is indicated.

Reference 107.

Some bacterial cells are motile, and of these the most common mechanism is by use of flagella, hairlike helical structures several times the length of the cell. Some genera possess only 1 or 2 flagella, while in other genera the flagella are present over the entire cell surface. The flagella rotate to propel the cell through the water. Some bacteria, including some cyanobacteria, move in a characteristic gliding motion by flexing the cell wall against a surface in a manner similar to inchworm movement.

Some bacteria have the ability to attach to solid surfaces. In a few genera this may be accomplished by hairlike pili or by structures called holdfasts. In most bacteria attachment occurs by a capsule or slime layer composed of organic polymers, mostly polysaccharides. After initial contact with the surface, the cell synthesizes polymers which bridge the gap and attach firmly to the surface. It then may become impossible to remove the cell without destroying it. When the cell divides, the nonattached portion can move, but the new cell arising from the attached portion of the cell remains in place.

Other functions of the capsule or slime layer include protection of the cell from such conditions as desiccation. In many pathogenic bacteria, the presence of the capsule affords protection against white blood cells and antibodies. The capsule may also serve as storage sites for excess nutrients or wastes.

In freshwater environments, the cell contains a higher concentration of salts than the surrounding medium. The cell would expand and lyse without the protection against osmotic shock afforded by the cell wall together with the cell membrane. The cytoplasmic membrane is internal to the cell wall and has additional functions (Figure 4). It is selectively permeable and often facilitates movement of a substrate into or out of the cell against a concentration gradient. For other substrates transport is almost completely prevented. The rate of transport can be specific for the particular substrate, and two substrates very closely related structurally can have very different transport rates. Some substances enter or leave by passive diffusion. The cytoplasmic membrane also maintains the osmotic gradient, is the site of enzymes involved with cell wall synthesis, and is the site of oxidative metabolism and energy conversions. More complex constructions of the cytoplasmic membranes are found in specialized groups of bacteria such as the cyanobacteria and the methane-utilizing bacteria. The cyanobacteria contain internal membrane structures called thylakoids which contain the photosynthetic apparatus.

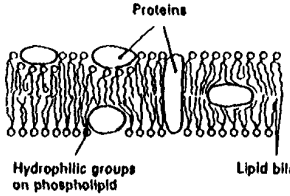

Figure 4. Structure of membranes, a diagrammatic representation. The lipid molecules are probably in constant motion.

Reference 107.

In fungi, cell growth occurs only at the tip of the hypha, and the plasma membrane below the tip contains a large number of membrane-bound vesicles which may hold the enzymes and cell wall precursors needed for cell growth. In addition, the fungal plasma membrane is involved with osmotic regulation and nutrient uptake. Functions such as oxidative metabolism are reserved to certain membrane bound organelles which are absent in bacteria.

All cells contain chromosomal DNA; in bacteria it is circular and double stranded, resembling a helical ladder. Bacteria also contain extrachromosomal DNA called plasmids which code for auxiliary functions in the cell, such as resistance to antibiotics and heavy metals and ability to metabolize some organic compounds. Fungi contain a number of linear chromosomes. The DNA contains the code which guides the structure and metabolism of the cell. Specific features in the functioning of DNA will be discussed in a later section.

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Eukaryotic cells contain mitochondria, which are organelles bounded by a double membrane. These function in ATP generation and the oxidative metabolism of substrates, activities carried out in bacteria by the cytoplasmic membrane. There are other specialized structures within some bacterial or fungal cells which function in storage of excess nutrients or gaseous products. The Gram-positive genera *Bacillus* and *Clostridium* form spores when exposed to unfavorable conditions. The spores are extremely resistant to heat, desiccation, radiation, acids, and chemical disinfectants, yet when exposed to favorable conditions will germinate and form a vegetative cell within hours.

GROWTH REQUIREMENTS

Bacteria of one type or another have been found in all environments and under all conditions with the possible exception of pure vacuum. A specific bacterial species may grow under a wide variety of conditions or it may have very exacting requirements for cell growth.

Certain nutrients are required by all cells. Carbon is most important and those cells that obtain it from organic substrates are referred to as heterotrophs. Autotrophs can fix carbon from carbon dioxide. A few specialized groups can utilize other substrates; nitrifying bacteria, for example, can oxidize methane at aerobic/anaerobic interfaces. Other essential nutrients include phosphorus, usually derived from phosphates, and nitrogen, usually obtained from nitrate or ammonia. These three elements are the most common nutrients that limit growth. Other necessary growth factors include sulfur, magnesium, potassium, calcium, and other metallic elements. While some bacteria synthesize all their required vitamins and growth factors, other bacteria must obtain some from the environment. Water is also a specific requirement in cellular metabolism. The bacterial cell is composed of about 80% water, and water is both the solvent and a specific cofactor in many biochemical reactions.

An important physical parameter for growth is temperature. An increase in temperature may inactivate enzymes or may be lethal to the cells, while a decrease in temperature may simply inhibit growth. Cellular enzyme activity is also governed partially by the ambient temperature. Upon warming, the cells may resume normal cellular function. An individual microbial species usually has a minimum, an optimum, and a maximum temperature for growth. Those that grow best at temperatures below 20°C are called psychrophiles. Mesophiles grow from about 15°C to about 45°C, and most bacteria are grouped into this category. Thermophiles grow at temperatures above 50°C. These names permit categorization of a situation which in reality represents a gradation of microbial tolerances for temperatures ranging from the arctic environment to thermal springs.

The oxygen requirements of microorganisms vary considerably. Obligate aerobes grow only in the presence of air and use aerobic respiration to obtain energy. Obligate anaerobes grow only in the absence of air. The sensitivity of anaerobes to molecular oxygen is due to lack of enzymes which render the superoxide free radical ion harmless through reduction. Facultative anaerobes will grow in the presence or absence of air using alternate chemical electron acceptors such as O_2 or NO_3. Microaerophiles have a narrow range of tolerance for their gaseous environment and require a reduced air environment or in some cases an increased proportion of carbon dioxide.

Bacteria also respond to changes in pH of the medium. Most bacteria (neutrophiles) grow best at neutral pH (pH 7). However, acid-producing bacteria (acidophiles) grow very well at lower pH values and strains adapted to alkaline environments (basophiles) grow at pH of 8 or 9. Fungi often tolerate extremes of pH better than bacteria. Halophilic bacteria require high salt concentrations, while other species are salt-tolerant although high salt concentrations are not mandatory for

survival. Organisms have been isolated from the deep ocean. Some strains survive both at the deep ocean pressure and at atmospheric pressure and are called barotolerant. Other deep ocean strains have only been kept alive by bringing them to the surface in pressurized vessels, and these bacteria are said to be barophilic. Many common strains of bacteria found at atmospheric pressure are killed when placed in a high-pressure environment.

THE CELL GROWTH CYCLE

The bacterial cell normally grows until it reaches a certain size, at which time it divides by binary fission into two identical daughter cells. During this time the bacterial DNA replicates and is partitioned to opposite sides of the growing cell. The cell wall and cytoplasmic membrane divide the cell in 1 of 2 ways depending on the particular genus.

In some genera the elongated cell pinches in equatorially until 2 cells are formed. In other genera a double cytoplasmic membrane is formed in the middle of the cell followed by synthesis of a double cell wall. When wall construction is complete the 2 daughter cells separate. Not all cells divide in synchrony, so in a culture all cells all stages of the growth cycle are represented.

Fungi grow by elongation at the tip of the hyphe. Many move into a yeast stage during which the cell divides by budding. An outgrowth appears at some point on the cell surface and grows until it is almost the size of the mother cell. The cellular organelles including DNA are replicated and a copy is partitioned into the daughter cell which eventually is walled off. The mother cell does not increase greatly in size during cell division.

POPULATION GROWTH

Laboratory studies with pure cultures of bacteria traditionally have demonstrated exponential growth in batch culture, in which all essential nutrients are present in excess and growth parameters are optimal. In this situation, population growth follows a characteristic cycle which begins with the lag phase of growth, during which the cells are adapting to the new environment (Figure 5). Enzyme synthesis induced

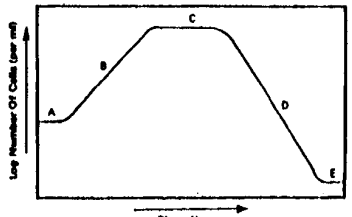

Figure 5 Bacterial growth curve. A, lag phase, B, logarithmic phase, C, stationary phase, D, decline phase, E, surviving population

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by contact with a new substrate occurs during this phase. Cells that are preadapted to the substrate or the growth conditions experience a shortened lag phase or no lag phase at all. Following the lag phase is a period of unrestricted multiplication called the log or exponential growth phase, so named because of the binary fission process of cell division. The rate of growth (cell division) depends upon the composition of the growth medium and the environmental parameters, and under optimal conditions a cell may divide every 15 minutes. When a nutrient becomes limiting or when inhibitory or toxic products accumulate, the cell enters the stationary growth phase. The individual cell is still viable although not replicating. This phase is manifested within the total culture by an equivalence between the number of cells produced by cell division and the number of cells dying, thus, there is no net change in the number of cells in the population. When the number of cells dying becomes greater than the number of cells being formed, the death phase ensues. The population eventually stabilizes at a constant low number of surviving cells. Because this cycle is characterized by an abundance of nutrients, it is rarely seen in the natural environment.

In cultures where (1) cells are able to proliferate, (2) there is an absence of inhibitors, (3) there is a homogeneous mixture of cells and nutrients, and (4) the substrate is the limiting factor in growth, cellular growth can be related mathematically with the disappearance of substrate. In this case, Monod kinetics apply as in equation 1:

(1)

$$\frac{dX}{dt} = \mu_s \frac{C_s X}{K_s + C_s}$$

The instantaneous change in cell concentration over time, dX/dt, is equal to the maximum specific growth rate, times a fraction including the substrate concentration in the mixed liquor, C_s, the cell concentration, X, and the Monod half-saturation constant, K_s.

The half-saturation constant is equal to the substrate concentration at which the specific growth rate is one half μ_s (In batch culture this situation occurs at the end of the exponential growth phase. Typically the constants μ_s and K_s are determined in batch culture tests using linearized graphical plots of the reciprocals of the measured cellular growth and substrate concentrations. Alternatively, they may be determined in a series of continuous culture tests.

CONTINUOUS CULTURE

Techniques for continuous cultures have been developed to provide a constant environment for microbial growth. The physical factors of temperature, pH, O_2 concentration, etc., are well controlled, and nutrients can be supplied at controlled rates coupled with removal of potentially toxic waste materials. Population growth, therefore, occurs at a constant rate which in some systems can be varied by changing the availability of a nutrient. Studies conducted in continuous systems such as the chemostat and the recycling fermentor have helped to establish the energy requirements of cells under growth or maintenance (survival) conditions in addition to exploring the response of cells to various types of nutrient or other growth factor limitations. Maintenance of a cell population under steady-state conditions with selective pressure, such as a nonutilizable carbon source, may enable mutants with

capability to metabolize the substrate to be generated and then grow to sufficient population levels to be recovered.

CELL DEATH

The most widespread measure of cell death is loss of reproductive capability. However, the medium used to detect survivors may not be adequate to demonstrate cell division, although the cell may be viable and capable of reproduction in another environment. The problem of defining cell death has not yet been resolved

PURE AND MIXED CULTURE METABOLISM

Populations in which all the cells are of the same species are considered to be a pure culture. Except for the ongoing process of mutation, discussed in a later section, the process of cell reproduction by binary fission with replication of genetic information ensures that the pure culture will express essentially the same properties. The population will be nearly homogeneous in its ability to metabolize a substrate.

In some cases, a substrate may be metabolized only partially by a particular species and a product may accumulate. In a parallel situation, another species may be able to metabolize that product further, although the second species may lack enzymes needed to metabolize the parent substrate. By themselves, neither species could mineralize the substrate of interest. However, a mixed culture of the two organisms might act in concert with one species, mineralizing the product resulting from metabolism of the substrate by the other species. A consortium of more than two species may be required to mineralize a substrate and the effective species may be bacteria, fungi, or a mixture of the two.

Cometabolism refers to the fortuitous metabolism of a compound while the cell obtains its carbon and energy from another source (273). Such metabolism may be partial or complete and depends upon enzymes already active in the cell.

SUBSTRATE UPTAKE AND TRANSPORT

Some motile bacteria possess the ability to move along the concentration gradient of a specific compound in its environment. This phenomenon, called chemotaxis, may permit these strains to scavenge some nutrients more efficiently or to move away from toxic or inhibitory compounds.

Some bacteria are able to utilize as carbon and energy sources substrates which are too large to enter the cell. These strains secrete hydrolytic enzymes into the culture medium which break the high molecular weight compounds (such as proteins, starch, or cellulose) into smaller components which can enter the cell.

The transport of other substrates (lower molecular weight) into cells depends on a number of interrelated factors. The substrate must be able to pass the complex cytoplasmic membrane which is composed of a hydrophobic zone surrounded on both sides by hydrophilic layers. Some lipid-soluble substances can pass across this zone by free diffusion which is dependent on the difference in substrate concentration inside and outside of the membrane. The rate of uptake by this mechanism, called passive transport, depends upon the size and charge of the substrate.

Within the cytoplasmic membranes are proteins which couple substrate transport to an energy-yielding process. Called active transport, this mechanism is the usual mode of entry for most substrates and ions, and the concentration within the cell protoplasm can be much greater than the concentration outside the cell. The proteins involved in active transport can be very specific for a particular substrate to the exclusion of structurally related analogs.

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ENZYMES

Enzymes are proteins which are the most efficient known catalysts for biochemical processes. They serve to increase the rate of a reaction, often causing a reaction to occur under physiological conditions which otherwise could only occur under extremes of pH, temperature, or concentration.

Classically, enzymes were grouped into 9 categories based on function. These names are still used frequently. (1) Dehydrogenases mediate the loss of a hydrogen ion from a substrate with the acceptor being other than molecular oxygen. (2) Oxidases catalyze loss of a hydrogen ion with molecular oxygen as the acceptor. (3) Kinases transfer a phosphate group from ATP or other nucleoside triphosphate to the substrate. (4) Phosphatases mediate the hydrolytic cleavage of phosphate esters. (5) Mutases catalyze transfer of a functional group between two positions in the same molecule. (6) Synthetases mediate condensation of two separate molecules coupled with cleavage of ATP. (7) Decarboxylases achieve decarboxylation of the substrate. (8) Thiokinases catalyze the ATP-dependent formation of thiol esters. (9) Carboxylases catalyze the ATP-dependent addition of carbon dioxide to the acceptor substrate.

These categories have been replaced by 6 classes of enzymes in a formal system developed by the International Enzyme Commission (39). (1) Oxidoreductases act on the CH-OH group of a substrate, requiring NAD^+ or $NADP^+$ as the hydrogen acceptor. This category includes dehydrogenases and oxidases. (2) Transferases catalyze the transfer of an intact group of atoms, such as methyl or phosphorus containing groups from a donor to an acceptor molecule. Kinases and mutases are included in this group. (3) Hydrolases, including phosphatases, mediate the transfer of chemical groups to water. (4) Lyases, such as decarboxylases, catalyze the addition of groups to substrates containing double bonds, or the removal of groups from substrates to yield products with double bonds. (5) Isomerases catalyze a change in the atomic configuration of a molecule without a change in the number or kind of atoms. (6) Ligases are involved in the formation of a product resulting from the condensation of 2 different molecules coupled with the breaking of a pyrophosphate linkage in ATP. This class includes synthetases, thiokinases and carboxylases.

Most enzymes are notably specific in their actions, catalyzing the reaction of a particular substrate, but having no activity against a very closely structurally related substrate. Some enzymes, however, act on many related compounds. These enzymes act on a specific structural component of different substrates. Enzyme specificity is related to two features of the substrate. First is the specific chemical structure which is attacked by the enzyme. Second, the substrate must also contain a binding group which binds to the enzyme in such a way as to permit optimal association of the susceptible structure with the enzyme. The active site on the enzyme is the area containing both the binding site and the catalytic site, and the three-dimensional configuration of the complex resembles a "lock and key" relationship.

The activity of enzymes can be inhibited either irreversibly or reversibly. Irreversible inhibitors destroy or bind to a functional group on an enzyme which is necessary for its catalytic activity. Reversible inhibitors may be either competitive or noncompetitive. A competitive inhibitor has a similar structure to that of the substrate and therefore can be bound by the enzyme. However, the enzyme has no activity against the inhibitor. Since the inhibitor and the substrate compete for the binding sites of enzymes, the action of a competitive inhibitor can be partially reversed by increasing the concentration of substrate. Noncompetitive inhibitors bind to the enzyme in an area other than the binding site, and in so doing alter the catalytic site so as to make it inactive. The affected site is often called the regulatory site of the enzyme and is reversibly occupied by the inhibitor. Lowered concentrations of the inhibitor increase the activity of the enzyme. When the inhibitor is a

direct product of a series of reactions involving the enzyme, the regulatory process is called feedback inhibition and is an important cellular mechanism for regulating metabolic processes such that energy is not wasted on production of unnecessary metabolites. Enzymes with a regulatory site, called allosteric enzymes, can be stimulated as well as inhibited by specific effector molecules which bind to the regulatory site. The effector molecule may be the substrate itself, signalling the enzyme to initiate the metabolic pathway. Often only one key enzyme in a pathway is regulated; the activity of the rest of the enzymes is limited by the availability of their specific substrate.

Enzymes may also be controlled at the level of enzyme synthesis. A reduction in the amount of enzyme would reduce the total enzymic activity. The genetic system for synthesis of enzymes consists of several parts (Figure 8). This general model shows several structural genes which code for the enzyme proteins. More than one enzyme may be part of a system. In addition, each system contains one control gene coding

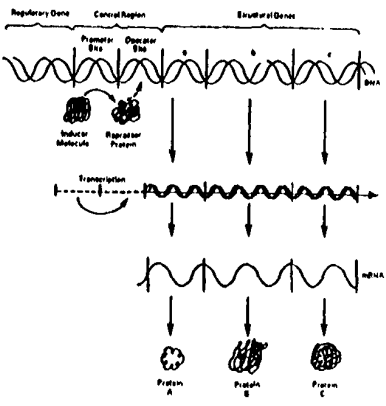

Figure 8. Regulation of enzyme synthesis
Adapted from Reference 278.

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for a protein, called the repressor, which binds specifically to a control site called the operator region. The repressor protein binds to the operator region in the absence of an inducer molecule, and the entire enzyme system is inactive. When the inducer is present it binds with the one repressor protein and the complex has reduced affinity for the operator region. The operator region then complexes with RNA polymerase at the adjacent promoter region and transcription of the structural genes is initiated. When the concentration of the inducer molecule falls below a critical point, the operator region is again blocked by the repressor protein and enzyme synthesis ceases.

Very long or branched metabolic pathways, in which an intermediate substrate may be directed to alternative pathways, are regulated by sequential induction, in which sections of the pathway are under separate regulatory control. The product of one series of steps acts as the inducer for the next several steps. This prevents the cell from wasting energy on unnecessary or unproductive metabolic processes.

Gene systems for a particular function which are grouped in one place physically on the genome are rare in eukaryotes. Genes for a particular metabolic pathway are most likely to be scattered over many chromosomes. However, regulatory genes still function in a similar fashion at separate control sites. Some bacteria (prokaryotes) also have systems in which the genes are scattered along the chromosome.

A more general type of control is called catabolite repression, in which the control protein binds to operator sites of many enzyme systems. This permits a favored substrate to be utilized preferentially before other substrates are metabolized. As long as the substrate of choice is present, other substrates are not metabolized, even though they may also be present. When the concentration of the favored substrate is reduced, enzyme systems for metabolism of the other substrates are induced. The favored pathway is more efficient and therefore costs the cell less energy.

Another consideration in the effectiveness of enzyme activity is the physical location of the enzyme with respect to the substrate. In eukaryotes the enzyme may be enclosed within membrane-bound organelles. In prokaryotes an enzyme may be enclosed within the periplastic space between the cell wall and the cytoplasmic membrane, while the substrate may be extracellular or intracellular.

All of the factors regulating enzyme activity may act in concert. Control of metabolic processes is finely tuned to the nutritional opportunities available, so that the cell acts in the most energy-efficient manner possible.

METABOLIC ENERGY PRODUCTION

All microorganisms need a source of energy for maintenance of cell viability and growth. The manner in which energy is obtained varies, and bacteria can be classified according to the source of their energy requirement. Phototrophs such as cyanobacteria use light directly in a photosynthetic process. Chemotrophs oxidize organic or inorganic compounds. A chemotroph which can derive its carbon requirements from carbon dioxide is a lithotroph, while a chemotroph which utilizes organic carbon is known as an organotroph.

The most common method of gaining energy is through oxidation reactions, which are normally coupled to the formation of ATP and other high energy molecules. Many different kinds of substrates can be oxidized, but eventually the substrates are modified to metabolites which can enter one of only a few pathways for carbon distillation. These pathways can be divided into two categories, fermentation pathways in which organic compounds serve as both the electron donor and electron acceptor, and respiration pathways in which oxygen or an inorganic compound or ion serves as the terminal electron acceptor.

Aerobes utilize a respiratory pathway known as the tricarboxylic acid cycle (Figure 7). For each mole of glucose converted to acetyl-CoA which completes the

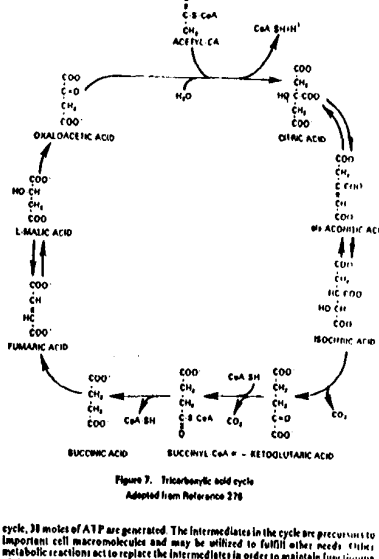

Figure 7. Tricarboxylic acid cycle
Adapted from Reference 278

cycle, 38 moles of ATP are generated. The intermediates in the cycle are precursors to important cell macromolecules and may be utilized to fulfill other needs. Other metabolic reactions act to replace the intermediates in order to maintain functioning of the cycle.

Under anaerobic conditions some facultatively anaerobic bacteria utilize an aerobic respiration. This is an oxidative process utilizing the same pathway for substrate degradation as aerobic respiration, except that nitrate or another inorganic compound is substituted for oxygen as the terminal electron acceptor.

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3 KETOADIPIC ACID PATHWAY

One of the major pathways for the degradation of aromatic compounds in bacteria and fungi is the 3-ketoadipic acid pathway (Figure 8). The primary aromatic substrate is converted to either catechol or protocatechuic acid, each of which undergoes several catabolic reactions in two separate but parallel pathways, until they converge to three common intermediates, 3-ketoadipic acid enol-lactone, 3-ketoadipic acid and 3-ketoadipyl-CoA, which is cleaved to form succinic acid and acetyl-CoA. These two end products enter the tricarboxylic acid cycle. The pathway

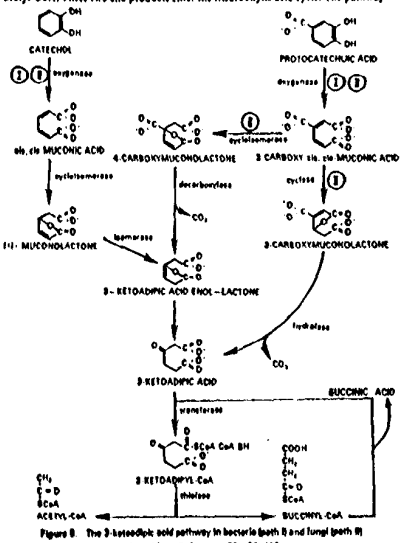

Figure 8. The 3-ketoadipic acid pathway in bacteria (path I) and fungi (path II)
Adopted from References 96a, 93, 408.

is strictly aerobic and regulated exclusively by control of enzyme synthesis. Although the chemistry of the pathway is the same in all bacteria, the pathway is regulated differently in different groups. Details of this pathway are found in the chapter on parent compounds.

The 3-ketoadipic acid pathway is one of the best studied cellular metabolic processes. The chemistry of the pathway has been elucidated (144, 238, 289, 340, 341, 342, 409), the enzymes isolated and their amino acid composition and other properties identified (123, 307, 336, 337, 345, 348, 396, 477, 478, 479, 480), and microbial regulation of the pathway described (335, 338, 339). A comprehensive review of the pathway has been published (408).

PLASMIDS

Genes coding for vital functions of the bacterial cell are located on the chromosome and are passed to every daughter cell. However, some metabolic processes, while not essential, confer considerable advantages on cells with these capabilities. The genes for these processes are coded for on plasmids, circular strands of DNA which can replicate autonomously. Plasmids can be passed from cell to cell as well as being replicated in the progeny; thus, an entire population can quickly acquire the specific characteristic. Traits which are often coded for on plasmids include the ability to metabolize unusual substrates including many aromatic compounds, resistance to antibiotics, and ability to survive in the presence of heavy metals. Presence of a specific plasmid is often a guide to the metabolic capability of that cell. Regulation of enzymes coded for on plasmids is similar to that discussed earlier, and catabolic repression may be effective across both plasmid and chromosomal DNA. Thus, a substrate which could be metabolized by two pathways, one on the plasmid and one on the chromosome, may be metabolized by one pathway preferentially while the other is repressed. A pathway for mineralization may involve some steps coded for on the plasmid and others coded for on the chromosome.

Since plasmids may be considered potential vehicles for genetic reassortment and transfer, they may also be viewed as mediators of evolution of biodegradative capabilities within microbial populations. At the population level, the development of DNA probes labeled with ^{32}P or fluorescent reagents can permit detection and monitoring of specific catabolic genes. Such applications would likely utilize colony hybridization techniques to directly probe for complementary target DNA in individual microbial colonies. Information derived from such experiments would allow measuring the selective pressure required to maintain catabolic genes in the natural population. In addition, the survival and transfer of novel catabolic genes originating from recombinant DNA technologies can also be tracked in the environment. Such information can be useful in the finer detailed prediction of the kinetics of biodegradation and the likelihood of utilizing genetically engineered microorganisms to degrade specific chlorinated aromatic pollutants.

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SECTION 4
CELLULAR GENE CODING AND GENETIC TECHNOLOGIES

STRUCTURE AND FUNCTION OF DNA

Deoxyribonucleic acid (DNA) consists of 4 kinds of deoxyribonucleotides linked together in a specific sequence. DNA is usually double-stranded. Of the four kinds of ribonucleoside bases, there are two subsets of hydrogen bonded pairings, adenine with thymine and guanine with cytosine. RNA (ribonucleic acid) is also composed of 4 types of bases, but with uracil substituted for thymine. Each nucleoside base is joined to the carbon-1 of a pentose sugar (deoxyribose in DNA, ribose in RNA). A phosphate molecule is joined through an ester linkage to the carbon-5 and the resulting molecule is known as a nucleotide. The nucleotides are joined by linking a hydroxyl group on the carbon-3 of a pentose to the phosphate group on the carbon-5 of another pentose to form a phosphodiester bridge. The pentose carbons are primed (' '') in order to distinguish them from carbons in the bases, thus the two ends of single-strand DNA are known as the 5'-phosphate end and the 3'-hydroxyl end. DNA from different species has characteristic relative amounts of the 4 nucleotides and this property has served to help identify unknown species of bacteria and establish evolutionary relationships among the species (Figure 9, Figure 10).

Double-stranded DNA consists of two strands in which the 5'-end of one strand is paired with the 3'-end of the opposite strand. The two strands are complementary — an adenine on one strand always pairs through hydrogen bonding with a thymine on the other strand, as does guanine with cytosine.

Each set of three bases along a strand is called a codon and codes for a specific message, usually formation of an amino acid. Some triplet sets of bases are stop messages while others are nonsense codons and lead to premature termination of message reading. There is some redundancy in the triplet codes. Several triplets code for the same amino acid, so a change in one base may not cause a functional change in the message. Groups of triplets code for sequences of amino acids which become proteins after some modifications. The DNA segment which codes for a single sequence of amino acids is known as a gene. The products of several genes may combine to form a protein. The total genetic material of a cell is called the genome, consisting of the chromosome and in some cases plasmids. In eukaryotic cells the chromosome includes some proteins also.

Since bases are read in groups of three, the deletion or addition of one or two bases will cause a shift in the reading frame such that the DNA is no longer read correctly. This alteration is called a frame-shift mutation and may or may not be lethal to the cell.

Hydrogen bonding as well as hydrophobic interactions of the molecules free DNA to take on a highly structured configuration resembling a double helix. Physical and chemical perturbations such as heat or acidity cause denaturation or unwinding of the DNA, eventually leading to separation of the two strands. The covalent molecules joining the bases within each strand are not broken. When the physical or

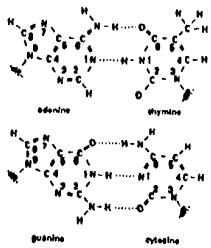

Figure 9. The pairing of adenine with thymine and guanine with cytosine by hydrogen bonding. The symbol —dR— represents the deoxyribose moieties of the sugar phosphate backbones of the double helix. Hydrogen bonds are shown as dotted lines

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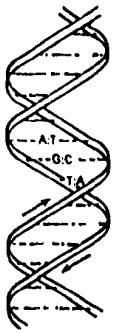

Figure 10 Schematic representation of the DNA double helix. The outer ribbons represent the two deoxyribosephosphate strands. The parallel lines between them represent the pairs of purine and pyrimidine bases held together by hydrogen bonds. Specific examples of such bonding are shown in the center section, each dot between the pairs of bases representing a single hydrogen bond. The direction of the arrows corresponds to the 3' to 5' direction of the phosphodiester bonds between adjacent molecules of 2 deoxyribose. After J. Mandelstam and K. McQuillen, Biochemistry of Bacterial Growth, 2nd ed. New York: Wiley, 1973.

Reference: Stanier, R.Y., E.A. Adelberg, and J. Ingraham, The Microbial World, Prentice Hall, Inc., Englewood Cliffs, N.J. 1976.

chemical stress is removed, the two strands will anneal spontaneously. When denatured DNA from two different sources is mixed, a certain percentage of DNA from the separate sources will anneal to form a DNA-DNA hybrid. The amount of hybridization depends on the percentage of complementary sections between the DNA. Strands of RNA can also combine with DNA to form RNA-DNA hybrids.

The chromosomal DNA of *E. coli* consists of about 4×10^6 base pairs, has a molecular weight of about 2.6×10^9, and is about 1400 μm long (the *E. coli* cell is about 2 μm long). Eukaryotic cells contain from 10 to 600 times as much DNA as *E. coli* cells. Eukaryotic DNA is organized into several linear chromosomes, each carrying a unique set of genes as well as proteins.

Segments of DNA in eukaryotic cells are repeated many times, while prokaryotes usually lack repetitive sequences. Some regions of eukaryotic DNA are characterized by inverted repetitions of base sequences which may be a few to a thousand base pairs long. Eukaryotic genes also contain segments which do not code for an amino acid and are not translated. These intervening sequences or introns have been found in all eukaryotic genes yet examined. Their function is unclear. They have been postulated to contain regulatory signals or to separate the genes into smaller units which can be readily recombined into new genes.

The definition of what constitutes a gene has modified over the years as the biochemical exploration of the cell has become more detailed. A gene classically has been taken to mean the genetic material which specifies a single trait. More recently, portions of the DNA have been classified as structural genes if they code for a single polypeptide (a portion of a protein) or for a specific type of RNA, or as regulatory sequences if they function to mark the beginning and end of structural genes or start or terminate transcription.

There are two functions of the DNA molecule. The first function is to serve as a template for its own replication. Enzymes separate the two strands, add new complementary bases to each intact strand, and ligate the bases. Since the original two strands were complementary, the two new complete DNA molecules are identical, each containing one old strand and one newly synthesized complementary strand.

TRANSCRIPTION

The process of converting the information coded by DNA into RNA is called transcription and is the second function of DNA (Figure 11). Only portions of the chromosome which code for a specific sequence or sequences of required genes is transcribed at any given time. A single strand of RNA complementary to the DNA strand is generated. Most of the RNA so formed is called messenger RNA (mRNA), which codes for the amino acids which comprise the polypeptides of the proteins. Other sections generated are transfer RNAs (tRNA), ribosomal RNAs (rRNA), and regulatory sequences.

The mRNA serves to carry the genetic message from the DNA in the nucleus of nucleolus to the ribosome, a collection of proteins and RNA units which is the site of protein synthesis in the cytoplasm. A single mRNA molecule contains the message needed to code for one or several polypeptides as well as a leader region and intergenic spacer regions which are not translated.

TRANSLATION

The process of protein biosynthesis according to the code carried by the mRNA is called translation and takes place at the ribosomes in the cytoplasm. There are specific transfer RNAs that recognize each triplet codon which codes for an amino acid. The tRNA molecule has receptor sites for both the mRNA chain and specific

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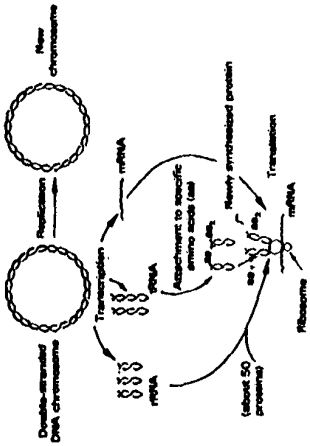

Figure 11. The general plan of synthesis of nucleic acids and proteins (Reference: Stanier, Adelberg and Ingraham, op. cit.)

amino acids, and serves as an adapter to bring the appropriate amino acid in close proximity to the developing polypeptide. Enzymes then attach the amino acid to the chain and remove the tRNA. When a termination codon is read, biosynthesis stops and the chain is released from the ribosome. The polypeptide finally is subjected to post-translational modification of some of its amino acids and undergoes folding into its characteristic three-dimensional shape, which renders the molecule biologically active.

Ribosomes are composed of ribosomal RNA and proteins. The rRNAs have a specific three-dimensional structure and serve as a framework for the binding of the polypeptide subunits. The ribosomal proteins are postulated to function in the process of synthesizing the polypeptide chain.

The process of translation is repetitive and a single mRNA molecule can be read simultaneously by several ribosomes spaced closely along the length of the molecule. In bacteria, translation of mRNA begins while the molecule is still being transcribed from the DNA. Thus, in prokaryotes these two processes are closely linked. The prokaryotic mRNA is quickly degraded by nucleases, so that efficient regulatory control over protein synthesis is maintained.

MUTAGENIC EVENTS

Most traits of bacteria are conservative and are reproduced in each generation. However, like all living organisms, bacteria may undergo mutations in which the genetic message is altered. If the alteration is lethal for the cell, the message is not passed on because the cell dies. Other mutations may not result in a change in expression of the message, since there is some redundancy built into DNA codes. In some cases, an alteration in the DNA code may lead to an alteration in the cell's metabolism. Some mutations allow the cell to survive at unfavorable temperatures or in the presence of potentially toxic compounds. In other cases, the cell acquires the ability to use previously unsuitable substrates. Most mutations are either lethal or place the cell at an environmental disadvantage.

Mutations occur randomly, on the order of approximately one in one million cells for a given characteristic. Some agents, including ultraviolet light, some kinds of radiation, and some chemical agents, cause increased mutagenesis. These mutations are characterized by being randomly distributed across the DNA. Mutations can also be selected by applying selective pressure to a population. For instance, in the presence of an unfavorable environment, only those cells which have mutated in such a way as to adapt to the environmental situation will survive.

Cells have powerful mechanisms for excising mutations from DNA. There are enzymes which recognize specific types of mutations and replace them with the correct message. Thus, the number of mutations passed to progeny cells may be a small fraction of the total number of mutagenic events sustained by the cell. In the case of massive mutations, such as radiation damage, the cell sets into motion a complex series of steps designed to foster cell replication at the expense of almost every other cellular function. The resultant cells are usually heavily damaged and do not function normally. The survival rate of these cells is very low.

Classical genetic techniques are based on manipulation of whole cells and environments to select and induce desired mutations. One common induction procedure is to expose a population of cells to a broadly acting mutagen and then place the surviving cells in the desired environment. Some mutations which have occurred in the genetic region of interest may enable those cells to grow or express the desired trait. A disadvantage of this method is that multiple mutations may have occurred in other genetic regions of the cell which may change the properties of the cell in unknown ways. Another method of obtaining mutations is to expose the

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population directly to the selective property. Only cells which can adapt will grow. However, such adaptation may take weeks, months or longer before the altered population is large enough to be observed.

Some types of genetic alterations in the cell occur as normal cellular events. Exogenous DNA can enter the cell by a variety of mechanisms and once inside can recombine with the chromosomal DNA. This process, called genetic recombination, can result in addition of new genetic information or the substitution of homologous DNA sequences. One method of genetic recombination is the transfer of exogenous DNA into a recipient bacterial cell. This process is called transformation and is the only direct evidence for DNA being the genetic material. Only a minority of recipient cells is competent at any given time to receive the DNA. Once the DNA has entered the cell, it may find its homologous region on the chromosome, recombine, and become a permanent part of the host chromosome, or it may recirculatize into an autonomous plasmid which replicates and is passed to daughter cells along with the chromosome.

Conjugation is a method which permits the entry of large segments of DNA from a donor cell into a recipient (Figure 12). Direct cell-to-cell contact is required between the donor cell, which possesses a particular plasmid-encoded mating appendage through which the DNA passes, and the recipient cell which lacks the appendage. Upon contact, the donor cell is stimulated to begin replication of the plasmid and the copy is threaded through the conjugation bridge to the recipient. The donor chromosome itself can be transferred to the recipient cell if the plasmid has integrated into the chromosome. As long as contact can be maintained, transfer of genetic material continues. The transfer of genetic material always begins at the plasmid origin of replication. Therefore, by separating the cells at specific time intervals and noting which traits have been transferred, mapping of the genes along the chromosome can be achieved.

Transduction is the term given to transfer of genetic material by bacteriophages which are viruses specific for bacteria (Figure 13). During the packaging of viral DNA into phage heads in the lytic cycle, some portions of bacterial DNA will be incorporated instead. When these particles are expelled from the cell and infect another cell, the bacterial DNA is released into the new cell to recombine with homologous host DNA.

In eukaryotic cells both parental chromosomes contribute genes to the daughter chromosome. Both parental chromosomes undergo cleavage at homologous points and segments of the chromosomes are exchanged. The new combinations of genes are spliced together and passed to the progeny cells.

Some segments of DNA are highly mobile and can leave their original position in the chromosome to be inserted elsewhere. Each end of these transposable elements or transposons contains short DNA pieces called insertion sequences. The insertion sequences are recognized by specific enzymes which catalyze their insertion into new sites on the chromosome or plasmid.

In recent years techniques have been developed which permit the direct manipulation of specific genes. Many of these techniques are now well established and are being applied to solve specific problems. Descriptions of the fundamental techniques and methods follow. Additional information can be obtained from references which served as the basis for this chapter (270, 294, 334). In particular, reference 294 contains details of the methods discussed here.

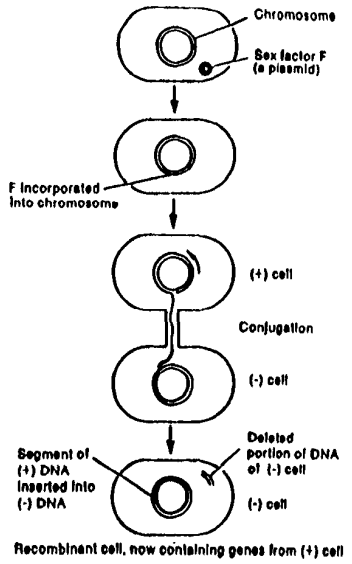

Figure 12. Transfer and recombination of genes during bacterial conjugation. The DNA of the (+) cell is replicated by the rolling-circle process, and the resulting single strand containing F is introduced into the (-) cell.
Reference 270

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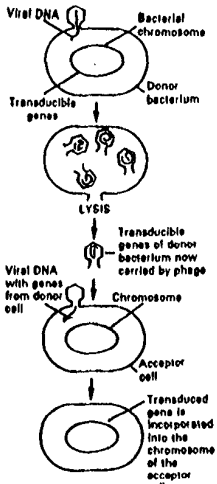

Figure 13. Genetic recombination during viral transduction of bacterial genes into a recipient cell Reference 378.

CURRENT BIOCHEMICAL TOOLS FOR GENETIC MANIPULATION

A series of prokaryotic enzymes has been isolated which can be used to cleave DNA and then splice different pieces together to form new strands. These enzymes are now being utilized in the laboratory.

Restriction Endonucleases

Restriction endonucleases are enzymes which specifically recognize certain sequences within double-stranded DNA. These sequences are usually 4 to 6 nucleotides long with a two-fold axis of symmetry. Examples of restriction endonuclease recognition sequences are shown in Table 1. A particular four nucleotide recognition site might arise once in every 46 (4096) pairs, assuming random distribution of DNA base pairs. The endonucleases cleave the DNA molecules either at the axis of symmetry, yielding blunt double stranded ends, or at positions offset from the center, giving fragments of DNA with one protruding single-stranded end known as "sticky" ends. DNA from different sources acted on by the same restriction endonuclease will produce complementary termini. In some cases, different restriction endonucleases with different recognition sequences will produce complementary termini as well. These ends can join with complementary ends on a different fragment to form new molecules.

TABLE 1. RECOGNITION SITES FOR RESTRICTION ENDONUCLEASES*

Enzyme	Recognition sequence	Termini	
	3′ 5′		
Bam HI	↓ GGATCC CCTAGG ↑	G CCTAG	GATCC G
EcoRI	↓ GAATTC CTTAAG ↑	G CTTAA	AATTC G
HaeIII	↓ GGCC CCGG ↑	GG CC	CC GG
HindIII	↓ AAGCTT TTCGAA ↑	A TTCGA	AGCTT A
MboI	↓ GATC CTAG ↑	NN NCTAG	GATCNN NN
PstI	↓ CTGCAG GACGTC ↑	CTGCA G	G ACGTC
TaqI	↓ CGCG GCGC ↑	CG GC	CG GC

*Reference 254.
†Arrows indicate site of cleavage. (A) Adenine, (C) Cytosine, (G) Guanine (T) Thymine.

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Deoxyribonuclease

Deoxyribonuclease (DNase) is an enzyme that cleaves double stranded or single-stranded DNA randomly, yielding fragments with 5'-phosphate termini. Depending on the conditions of the reaction, either the double strand of DNA is cleaved at approximately the same site or each strand is cleaved independently. Under certain conditions this enzyme creates nicks in double-stranded DNA which does not destroy the unity of the molecule. The concentration of DNase in the solution will affect the extent of nicking. Fragments of DNA containing regions of interest can be inserted into other molecules. Nicked regions on DNA permit insertion of nucleotides. The order of insertion can be controlled to permit creation of a defined DNA strand, and the nucleotides can be radiolabeled to permit tracking of the constructed strain during other manipulations.

Polymerases

Polymerases are enzymes that add nucleotides to the 3'-hydroxyl terminus created when the double-stranded DNA molecule is nicked by DNase. The enzyme can also remove nucleotides from the 5'-phosphate ends. Both of these processes acting at the same time result in movement of the nick along the intact strand of DNA (nick translation). If the nucleotides being added are radioactive, such as with ^{32}P, the labeled DNA can be prepared with a high specific activity. Normally the replacement nucleotides are distributed uniformly along the DNA molecule, since the nicks occur randomly throughout the DNA.

Reverse Transcriptase

This enzyme, also known as RNA-dependent DNA polymerase, uses mRNA as the template for transcription to form double-stranded DNA. Single-stranded DNA or RNA can also be utilized by this enzyme to make probes for use in hybridization experiments. Initiation of the action of reverse transcriptase requires a short DNA primer sequence base-paired to the template. Primers can be generated by exhaustively digesting DNA and retrieving the fragments. Since the fragments represent random portions of DNA, some fraction of them will bind to the template and can be used as primers. The discovery that eukaryotic mRNA contains multiple adenylate bases at the 3'-end allows construction of complementary polymer thymine residues at that site, which then will act as a primer.

The RNA template possessing a primer is then mixed with solutions of the 4 nucleotides. Usually only one nucleotide is radioactively labeled. Reverse transcriptase then catalyzes synthesis of the complementary DNA (cDNA). Following completion of the reaction, the RNA strand can be selectively degraded. Complementary DNA to be used as hybridization probes are retained in single-stranded form. However, the cDNA can form a hairpin loop at its 5'-end which acts as a self primer for synthesis of the complementary strand, or a second primer can be added to the cDNA to initiate synthesis, resulting in double-stranded cDNA.

Ligases

T4 DNA ligase links together complementary fragments of double-stranded DNA by forming a phosphodiester bond between adjacent 3'-hydroxy and 5'-phosphate ends. The ends may be either sticky (one strand of the double-stranded molecule extends beyond the other) or blunt (both strands end at the same place). T4 RNA ligase joins single-stranded RNA or DNA. These enzymes can also catalyze circularization of DNA molecules if the concentration of DNA in solution is low.

Transferases

The enzyme, terminal deoxynucleotidyl transferase adds deoxynucleotides to the 3'-hydroxyl end of DNA. By adding homopolymer sequences of one type of nucleotide to one set of DNA fragments, and a series of complementary homopolymer nucleotides to a second set of DNA molecules, the two populations can be joined by their newly formed complementary ends.

Methylases

These enzymes add a methyl group to particular nucleotides. Some restriction endonucleases will fail to recognize a sequence which differs only by addition of a methyl group. This is an important component of cellular defense systems, in which host DNA is methylated to protect against host restriction endonucleases which will attack foreign (nonmethylated) DNA.

MECHANICAL SHEARING OF DNA

Double-stranded DNA can be broken by the shearing forces present in solutions. Very small fragments (approximately 300 base pairs in length) can be obtained by subjecting the solution to sonication with ultrasound. Larger fragments of about 8,000 base pairs result from stirring the solution at high speed in a blender. The DNA molecule is sheared randomly along its length, producing fragments with short single stranded ends.

CLONING VEHICLES

In order for a fragment of DNA to be replicated, it must contain a specific sequence called an origin of replication. Plasmids and prokaryotic chromosomes each usually contain one origin of replication. DNA which possesses an origin of replication is called a replicon. If a fragment of DNA in a cell cannot replicate, it will be diluted out of the population after several generations. Therefore, DNA fragments of interest must be attached to replicons, called vectors or cloning vehicles, before insertion into the cell. Replicons which are not native to the host cell may not be functional after insertion. The combination of the replicon and the foreign DNA fragment creates a hybrid molecule often called a chimera. The process of constructing a hybrid DNA molecule is known by several names, including genetic engineering or gene manipulation, to acknowledge the potential for creating new combinations of genes, and gene cloning or molecular cloning because this method allows amplification of the chimera via growth of the host population of organisms, each carrying the identical piece of genetic information. The DNA can be extracted from the new population and the chimeras recovered.

Plasmids

Plasmids are stable, extrachromosomal, circular double-stranded DNA replicons which are inheritable, but are also dispensable. Under constant selective pressure the plasmids will be replicated in the daughter cells, but when not essential for cell function many plasmids are lost from the cell. Plasmids contain from 1,800 to 200,000 base pairs. Conjugative plasmids carry a set of genes that promotes bacterial conjugation; nonconjugative plasmids lack these genes. The term relaxed plasmids refers to plasmids which are present as multiple copies (10 to 200 copies) within a single cell, while stringent plasmids are limited to 1 to 3 copies per cell. Generally, plasmids of relatively high molecular weight are conjugative and stringent, while low molecular weight plasmids are nonconjugative and are present in multiple numbers.

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If cellular protein synthesis is blocked, chromosomal and stringent plasmid replication ceases, while relaxed plasmids continue to replicate and can increase their copy number to several thousand per cell. Therefore, relaxed plasmids are most useful for molecular cloning processes. Some plasmids ("promiscuous plasmids") can be transferred into a wide range of Gram-negative bacteria. These plasmids are potentially useful in transferring genetic information to diverse bacterial hosts. Some plasmids are incompatible with others and cannot coexist within the same cell. Plasmids have been grouped into incompatibility classes on the basis of mutual incompatibility.

Plasmids useful as cloning vectors generally are small and under relaxed control. They carry an easily selectable marker (such as antibiotic resistance) which allows identification of transformants which have acquired the plasmid. These plasmids also contain a single recognition sequence for a given restriction endonuclease which permits insertion of DNA into a region of the plasmid not essential for replication. A restriction site located within the marker genes will inactivate the gene when foreign DNA is inserted, providing a tracer for successful DNA insertion.

Some plasmids have been modified to include polylinkers, segments of DNA that contain closely spaced recognition sites for several restriction endonucleases. Generally, plasmids so modified are small and lack natural restriction sites. Use of small plasmids is advantageous in that they are less likely to be damaged physically during handling. Small plasmids also tend to generate higher copy numbers.

Construction of plasmid vectors involves cleaving both foreign DNA and plasmid DNA with the same restriction endonuclease to form complementary ends. Both types of DNA are mixed and are ligated. In some of the resulting molecules the foreign DNA will be ligated to the plasmid DNA, and a circular recombinant plasmid recovered. Use of a restriction site within a marker gene simplifies the process of detecting recombinant plasmids. The inserted DNA inactivates the gene. Plasmids which recircularize without insertion of foreign DNA will express the marker characteristic and can be rejected during the screening process.

Recircularization of plasmid DNA can be minimized using a procedure called directional cloning. This method takes advantage of the fact that most plasmid vectors carry single recognition sites for more than one restriction enzyme. A plasmid is digested with two such endonucleases. The larger fragment is separated and ligated with foreign DNA containing ends complementary with the two dissimilar ends generated by the two restriction enzymes. The plasmid fragment itself does not contain complementary ends and therefore will not circularize.

Another method of preventing recircularization involves treating the linear plasmid DNA with alkaline phosphatase. This enzyme removes the 5'-terminal phosphates. Ligation requires both a 3'-hydroxyl and a 5'-phosphate end. The foreign DNA combines with the treated plasmid DNA to create a circular molecule with a single nick on each strand where the phosphates have been removed. This open circular molecule can be inserted into cells much more efficiently than linear DNA and so most of the transformants will carry recombinant plasmids.

Bacteriophages

Bacteriophages (phages) are viruses which attack bacteria. The most extensively studied phage is bacteriophage l which contains a double-stranded linear DNA molecule about 50,000 base pairs long with single-stranded complementary sticky ends. The intact phage consists of the genomic material surrounded by a protein coat with a protruding tail. The tail attaches to the bacterial cell and injects only the DNA into the cell. One of two processes of replication, either lysogeny or the lytic cycle, can be initiated within the cell.

During lysogeny, the viral DNA is integrated into the host chromosome and is replicated and transmitted to progeny cells along with the host chromosome. This can happen indefinitely. At some point the lysogenic cell is triggered to begin the second process of replication, the lytic cycle. Alternatively, upon infection the viral DNA can initiate the lytic cycle immediately.

The lytic cycle begins with viral adsorption and DNA penetration. These steps require specific conditions and the phages are host specific. After entry into the host cell, the linear viral DNA circularizes via its complementary sticky ends and replicates as an independent molecule. Copies of the DNA are continuously made. Transcription of the molecule is initiated soon after replication begins. One of the earliest proteins produced is a regulatory element which acts to prevent deleterious activities of the host which might otherwise prevent further transcription. During the late phase of transcription, proteins involved in assembly of the head and tail and cell lysis are produced. As many as 200 copies of a phage can be replicated within a single host cell.

During the assembly phase of the lytic cycle, a linear copy of the DNA becomes coiled into a phage prehead. When the head is filled, an additional protein attaches and locks the DNA into the head. The head finally attaches to the preassembled tail unit to form a complete phage particle. Progeny phage particles are released in a single burst when the host cell lyses. Each phage is then able to infect another cell.

Bacteriophage l Vectors

About one-third of the phage l genome is nonessential for virus replication and can be replaced by foreign DNA so that the total length of the genome is conserved. Although phage l contains several recognition sites for each of the restriction endonucleases of interest, derivatives of phage l have been developed which no longer carry restriction sites in critical areas of the genome, but carry only 1 or 2 such sites in nonessential regions. The phage l thus has been manipulated to become a useful cloning vector while still retaining its infective and lytic properties.

Cosmid Vectors

Cosmids are constructed vectors designed for cloning large fragments of eukaryotic DNA. They consist of a drug resistance marker, a plasmid origin of replication, one or more restriction sites, and the ligated sticky end of phage l (the cos site). They are very small, so that large amounts of foreign DNA can be added to the molecule. The complete cosmid DNA is packaged into a bacteriophage coat which mediates its injection into the host cell. Inside the cell the cos site allows circularization and the plasmid origin of replication initiates replication.

Single-stranded Bacteriophage Vectors

Bacteriophages containing single-stranded DNA replicate in a different manner from phage l. After penetration, the single-stranded form is converted to double-stranded DNA which can be isolated and used as a cloning vector. The double-stranded form replicates until 100 to 200 copies are made. Then DNA replication shifts to produce large amounts of only one of the two DNA strands. Single strands are incorporated into the phage coats and the mature phage particles are continually extruded from the cell without lysing the host cell. The single-stranded DNA thus produced can be recovered and used as a template for DNA sequencing or for generating DNA probes.

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METHODS OF MANIPULATING DNA

Isolation and Purification of Plasmid DNA

Plasmids inserted into cells can be amplified by growing the bacteria to high cell yields in the presence of an antibiotic providing selective pressure from the plasmid. The cells can be harvested by centrifugation and then lysed by several methods. Lysis procedures include: boiling, treatment with alkali, and treatment with the surfactant sodium dodecyl sulfate. Lysozyme is added to help break apart the cell walls. The treated solution is centrifuged to remove DNA from other cellular material.

The DNA preparation contains chromosomal DNA as well as plasmid DNA. These two types of DNA can be separated by taking advantage of several differences in the properties of chromosomal and plasmid DNA. For some applications, the crude DNA preparation from as little as 10 ml of culture may be used successfully. After treatment by one of the lysis procedures, plasmid DNA is recovered from cells in intact circular form, while chromosomal DNA generally is extracted in short linear pieces. When plasmid DNA of high purity is required, centrifugation at very high speeds in a solution of cesium chloride and ethidium bromide will separate the DNA according to density. The linear chromosomal DNA takes up more of the intercalating agent ethidium bromide than the plasmid DNA. The chromosomal DNA is physically stretched more than the plasmid DNA, and therefore will be a less dense molecule. When subjected to ultracentrifugation, the two types of DNA form narrow bands in separate regions of the centrifuge tube. Contaminating protein will form a third band, and RNA will form a pellet. The ethidium bromide can be removed after this step or it can be retained during subsequent procedures.

Isolation and Purification of Bacteriophage λ DNA

Bacteriophages which are lysogenic can be recovered by inducing the bacterial culture to begin the lytic cycle. One method useful for plaques containing a temperature-sensitive repressor is to raise the temperature of the culture briefly.

Phages which are not lysogenic may be amplified by infecting the host bacterial culture with a low number of phages. Much of the bacterial culture will replicate for several generations, increasing the number of host cells, before successive rounds of the lytic cycle infect the entire culture.

Cell debris remaining after completion of the lytic cycle is removed by centrifugation. The remaining solution can be subjected to density gradient ultracentrifugation, after which the intact bacteriophage particles appear as a thin band. Crude bacteriophage preparations useful for many purposes can be obtained without density gradient ultracentrifugation.

DNA can be recovered from the phage particles by treatment with a solution of a protein-digesting enzyme and sodium dodecyl sulfate. The protein components can be extracted into phenol and removed by centrifugation.

Separation and Purification of DNA Fragments

Gel electrophoresis is a sensitive method for resolving mixtures of DNA. Samples containing DNA are loaded onto a slab of agarose or polyacrylamide gel. The gel is submerged into a buffer solution of nearly the same electrical resistance and a current is applied. Various types of DNA -- linear, nicked circular, and closed circular -- will migrate through the gel at different rates, depending upon the molecular size of the DNA, the concentration of agarose or polyacrylamide, the applied current, and the DNA base composition and temperature. Under some conditions single strands of DNA can be separated. The DNA is stained with the fluorescent dye ethidium bromide and can be detected directly. Bands of DNA can be cut from the gel and the DNA recovered.

The DNA is then packaged into a vector and transformed into a bacterial culture for amplification. Colonies containing plasmids are grown on nitrocellulose or nylon filters seated on agar petri plates, while bacteriophage plaques are formed in a lawn of indicator bacteria on agar media and then eluted into a liquid suspension which is stable indefinitely.

Identification of Recombinant Clones

In situ hybridization of bacterial colonies or bacteriophage plaques on agar media is rapid and can efficiently screen large numbers of potential clones. Colony hybridization involves lysing the colonies on the nitrocellulose or nylon filter and then fixing the DNA to the filter *in situ*. Bacteriophage plaques are transferred to the nitrocellulose filters following plaque formation. Filters are placed in contact with plaques on the agar and then removed. Some portion of the viruses in the plaques will be removed with the filter. The DNA is then fixed, in some cases by heat treatment. The DNA probe of choice is labeled with ^{32}P and the hybridization reaction between the probe and the fixed DNA carried out.

Hybridization of Probes to Immobilized DNA

Hybridization reactions are governed by such factors as solvent used, temperature, length of hybridization, concentration and specific activity of the ^{32}P-labeled probe or density of fluorescent probe, and washing procedures after hybridization. Prior to hybridization, the filters are treated with one of a number of compounds to saturate sites on the filter with nonspecific affinity for single- or double-stranded DNA, in order to ensure that the probe DNA will not bind directly to the filter. The ^{32}P-labeled probe DNA is denatured (double-stranded molecule separated into its single-strand components) and added to the filters. During incubation, the single strands of the probe DNA will join to complementary DNA strands on the filter. Following hybridization the filters are washed thoroughly to remove unbound DNA and dried. An autoradiograph is made by placing the filters in contact with X-ray film. Following development, radioactive signals representing positive homology of the probe with plasmid or phage DNA can be correlated with colonies or plaques on the agar plates. Those colonies can be retrieved and the recombinant DNA contained therein amplified.

Other procedures employ DNA probes with induced fluorescence and/or antigenic properties for use with fluorescent antibodies to detect positive hybrids. Both methods have the sensitivity to detect as little as 1 picogram of DNA or as few as 10,000 copies of a single gene.

MAPPING OF RESTRICTION ENDONUCLEASE RECOGNITION SITES

The order of bases on a strand of DNA can be determined using a number of techniques; usually more than one is required to obtain a detailed map. By cleaving DNA with restriction endonucleases having known recognition sites, the presence and number of such sequences can be resolved.

The relative positions of dissimilar endonuclease recognition sequences can be determined by first labeling one end of linear DNA with radioactive nucleotides to obtain a reference point. Digestion of separate aliquots of the DNA by different restriction enzymes is followed by gel electrophoresis and autoradiography. Resulting fragments of different lengths define the distance of each recognition site from the labeled end, and the relative distances between each site can be determined as well.

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Small fragments of DNA can be mapped using an exonuclease which digests the DNA from each end by single nucleotides. Samples of the digestion reaction are withdrawn at time intervals and are treated with restriction endonucleases. As the DNA digestion progresses, the recognition sites disappear in specific order related to their position along the molecule.

IDENTIFICATION OF DNA SEQUENCES WITHIN FRAGMENTS

The Southern transfer technique is an effective method for identifying particular sequences of DNA. Fragments of DNA created by one of the previously described methods are separated by size on agarose gels using electrophoresis. The DNA within the gel is stained with ethidium bromide and denatured (strands separated) *in situ*. The DNA is then eluted from the gel directly onto nitrocellulose filters by placing the gel on absorbent paper whose edges are trailing in a salt solution. The filter is placed on the gel and more absorbent paper placed above the filter. Wicking action will cause the DNA to migrate from the gel to the filter with the relative positions of the fragments intact. The filter is treated with ^{32}P-labeled probe DNA of known base composition and then washed well. Fragments containing complementary bands will hybridize to the probe and can be visualized after autoradiography.

EXPRESSION OF PROKARYOTIC GENES IN FOREIGN HOSTS

Most genetic engineering studies have used *E. coli* as the host with introduction of either *E. coli* genes or genes from other prokaryotes or eukaryotes. Other studies have involved introduction of genes from one strain to another strain of the same species. Little research has been conducted on expression of *E. coli* genes into other hosts. Some studies have noted that *E. coli* genes cloned into a *B. subtilis* host were not expressed, although the genes themselves when recovered and inserted back into *E. coli* were functional (334). This has been attributed to differences in the specificity of the RNA polymerases of the two hosts. There are differences as well between the translation mechanisms of *E. coli* and *B. subtilis*. Thus, *B. subtilis* genes function in *E. coli* but the reverse is not true.

GENE CLONING IN YEASTS

The yeast *Saccharomyces cerevisiae* has received the most attention with respect to application of genetic engineering techniques. This species contains a plasmid which replicates with high copy number although it has no known function (334). Fragments of yeast DNA as well as an *E. coli* plasmid vector have been cloned into this plasmid and the recombinant molecule transforms yeast with high frequency and replicates in both *E. coli* and yeast. Some yeast genes replicate autonomously and these can be used to construct vectors which transform yeasts with high efficiency.

EXPRESSION OF EUKARYOTIC GENES IN A PROKARYOTIC HOST

Genes from the fungi *S. cerevisiae* and *Neurospora crassa* have been cloned into and expressed in *E. coli* (334). However, many other genes from eukaryotic sources have been cloned into prokaryotic hosts but have not been expressed. This has been explained in part by the differences in mechanism of expression of the genes (protein synthesis). The steps involved in synthesis of a functional protein include transcription of the DNA, translation of the mRNA, and post-translational modification of the newly-formed polypeptide. Transcription and translation require a promoter or a binding site recognizable by the host RNA polymerase. Further, the

protein produced is often subject to rapid degradation unless it is protected by the modified amino acids or three-dimensional configuration of the native protein. Even if all of these components are present, genes which are expressed in a foreign host may not necessarily give rise to a stable gene product.

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SECTION 5

METHODS OF BIODEGRADATION ASSESSMENT

A decade ago biodegradation was measured at a worst case level by substrate disappearance. Many reports in the area of environmental microbiology and wastewater engineering documenting biodegradation were accompanied by gas chromatographic analysis showing the net loss over time of a parent compound. In some cases, even visual disappearance of insoluble crystalline organics, such as naphthalene, was used as a gross measure of biodegradation. Most often, abiotic or sterile control samples were used in such assays of biodegradation. However, little insight was developed into nonmetabolic interactions among organisms and pollutant substrates and the measured biodegradation. In the area of wastewater treatment, BOD or COD removal measured by comparing influent and effluent concentrations was used as measures of pollutant biodegradation, with the assumption that recalcitrant organics had essentially the same fate as labile organics in wastewater treatment.

Such approaches to measuring biodegradation have been replaced by more stringent parameters to give accurate estimates of microbial catabolic potential under laboratory conditions and to determine more accurately the environmental fate of chlorinated aromatic pollutants. The most stringent criteria for accurately estimating biodegradation include mass or material balance approaches and mineralization approaches. In actual practice, both approaches are frequently integrated to give the best estimate and predictive capability of determining biodegradative fate. In either instance, the use of radiolabeled (primarily ^{14}C) substrates complements the approach, especially at environmentally realistic, low concentrations. These trace concentrations may make conventional analytical approaches more difficult and/or expensive. The approaches are summarized in Table 2.

Laboratory assays for biodegradation, using labeled or unlabeled compounds, require a determination of physical processes which contribute to the overall loss of the substrate. Accurate material balances are determined by an accounting of substrate loaded onto biomass and suspended particulate(s), aqueous phase substrate, residual substrate sorbed to glassware and reaction vessels, and volatilized substrate. Assuming efficient recovery for each component phase, the difference between input and accounted-for residual and comparison to abiotic control samples should give a reasonable approximation of true biodegradation. However, even under these circumstances, biodegradation may be poorly understood if aqueous phase and cellular-associated substrate is transformed to polar oxidized products. In such cases ^{14}C analysis without conventional analysis (HPLC) may underestimate biodegradation, as conventional analysis may fail to detect transformation products that in themselves are resistant to further microbial degradation. Joint conventional and ^{14}C analysis (where available) provide excellent material balance analyses for biodegradation of parent substrate and accumulated metabolic products.

TABLE 2 MATERIALS BALANCE AND MINERALIZATION APPROACHES TO BIODEGRADATION ASSESSMENT

Biodegradation approach	Process examined
Materials balances	Recovery of parent substrate
	Recovery of radiolabeled parent substrate and metabolic products
Mineralization	Production of carbon dioxide, methane, or their carbon radiolabeled congeners from the parent substrate
	Release of substituents groups, e.g. chloride or bromide ion

Mineralization assays as a measure of total biodegradation (oxidation or reduction to terminal decomposition products) have enjoyed utility as unambiguous measures of biodegradation. In the event that non-^{14}C-labeled substrates are employed, mineralization assays must include an absolute materials balance for the system. Such approaches have been used to study anaerobic biodegradation resulting in methane (CH_4) production as the terminal mineralization product (417).

In more general practice, CO_2 production is the most common measure of pollutant mineralization. With the commercial availability or custom synthesis of ^{14}C-labeled organic pollutants, measurements of $^{14}CO_2$ production indicating both the extent and rates of substrate degradation have become common practice.

In cases involved with biodegradation of aromatic and chlorinated aromatic pollutants, the parent substrate is generally chosen with aromatic ring labeled ^{14}C atoms. Production of $^{14}CO_2$ during biodegradation is therefore the result of aromatic ring oxidation and cleavage, representing virtually total destruction of the parent aromatic molecule and associated bioactive properties that are of initial concern from environmental health and ecological perspectives. In alternative mineralization approaches where ^{14}C-parent substrate may be available, the release of halogen ions, generally Cl^- or Br^-, from the aromatic ring during ring oxidation and cleavage is a good measure of biodegradation. However, if the goal is to determine terminal decomposition, care must be taken to assure that halogen ion release follows ring cleavage rather than preliminary reductive or oxidative dehalogenation of the aromatic ring.

An integrated flow diagram (Figure 14) describes biodegradation assessment. Carbon-radiolabeled parent substrate is added to a reaction system containing the microbiological population of interest. In a time course fashion, samples are withdrawn or replicate samples are sacrificed. At each time point, material balances for the parent substrate and ^{14}C are prepared using a combination of conventional procedures (most conveniently analyzed by HPLC) and liquid scintillation analysis of radioactive decay of ^{14}C. Mineralized products such as $^{14}CO_2$ or $^{14}CH_4$ can be collected and analyzed by liquid scintillation analysis and confirmed by conventional analytical methods. Where specific identification and confirmation are required, mass spectrometry (MS) or GC/MS can be employed for isolated products.

The resulting data, when compared to appropriate abiotic controls to accommodate nonspecific sorption, volatilization or stripping, and photolytic or other abiotic degradation mechanisms, provide the information to determine the potential

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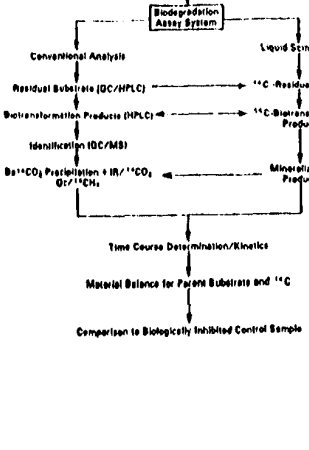

Figure 14. Integration of materials balance and mineralization approaches in biodegradation assessment

and extent of degradation. A relative rank, in terms of rates of degradation, may be assigned if multiple comparisons are being made.

Biodegradation assessment may be divided into two broad categories: one comprising studies of the environment or laboratory studies which simulate the environment, and a second category which includes pure culture studies under defined environmental conditions. Environmental studies yield information on disappearance or movement of the substrate. Pure culture systems permit studies at the molecular level, including information about specific enzyme systems and gene involvement in the manifestation of degradative capability. Studies of degradation must include both mechanisms of induction and the mode of action of the enzymes. However, results from laboratory studies are not necessarily an indication of results to be expected in the environment. These studies must be correlated with environmental conditions for an assessment of *in situ* biodegradation.

Primary degradation or biotransformation is considered to be disappearance of the substrate, without consideration of metabolite formation or mineralization. While primary degradation is important evidence to assess the potential biodegradability of the molecule, only knowledge of the metabolites formed or of complete mineralization will enable confirmation of the biodegradability of the substrate under the specific environmental conditions used.

The biodegradation of a chlorinated compound is considered complete when the chloride ion is returned to its mineral state (HCl or Cl^-) and the carbon skeleton converted to cellular products (8). Appearance of the chloride ion is most conveniently measured by using an ion-selective electrode.

Disappearance of the substrate may be due to a number of factors in addition to biodegradation. These include photolytic decomposition, volatilization, chemical degradation, and sorption and irreversible binding to soils, clays, or organic matter including cells. In addition to their separate effects, these factors may work in concert with the biota to degrade the substrate. In studies of biological degradation, these factors must be controlled, eliminated, or accounted for.

CHEMICAL ANALYTICAL TECHNIQUES

The ability to quantify the amount of substrate in a given experimental system is of prime importance. To this end, sophisticated analytical techniques have been developed which allow the unambiguous identification of the substrate or its metabolites.

Gas-liquid partition chromatography (GC) is a separation technique which combines high sensitivity, accuracy, and repeatability. Low concentrations of the sample are required. The sample may be solid, liquid, or gaseous as long as the sample can be volatilized at the operating temperature of the instrument. Samples which are insufficiently volatile can sometimes be derivatized and converted to a more volatile ether or ester compound.

The sample to be analyzed is injected along with a gas which carries the sample along a column packed with inert particles coated with a liquid. The solutes in the sample are distributed between the liquid and gas phases according to the relative solubility of the solute in the liquid. Solutes of lower solubility or high volatility move through the column at faster rates. As the bands exit from the column they are recorded as roughly symmetrical peaks with retention times related to their relative partition coefficients. These can be compared with the retention times of standard materials. This does not constitute absolute proof of identity, however, as two or more solutes can elute from the column with the same retention time.

The solutes separated by GC can be analyzed directly by a mass spectrometer (MS) for determination of the molecular structures of the compounds (GC/MS). In gas

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chromatography-mass spectrometry, the separated sample effluent from the GC is highly ionized and accelerated in a vacuum, causing it to fragment into smaller ions. These ions are separated according to their mass-to-charge ratio (m/z) and then collected according to their relative abundance in the sample. The fragmentation pattern results from the molecular structure and is characteristic of the type of compound. The mass spectrum of the compound can be used to recreate its molecular structure and often it can be compared with mass spectra in computerized libraries for quick identification of unknown compounds.

The use of GC/MS has become widespread because of the potential unequivocal identification and quantification of metabolites and residual substrate. Materials present in minute quantities can be identified within a mixture of other materials.

Additional information regarding the types of bonds between functional groups can be obtained through infrared spectroscopy. The different types of bonds absorb at specific frequencies when infrared radiation is passed through the molecule, and the resulting spectrum is unique to the particular material. Infrared spectra are most useful when used in conjunction with other methods of compound identification and when looking for specific functional groups.

Liquid chromatography can be an effective technique for the separation of materials of either lower volatility or ionic structure. The sample, in a solvent, is passed through a column containing an absorbing material dispersed on an inert support. The solutes partition between the liquid and the absorbing material according to their relative affinities and solubilities. Each solute elutes from the column at characteristic time intervals. The great variety of both solvent and stationary phases makes this a very versatile and sensitive technique for both separation and identification of compounds by comparison with known standards. Increased resolution can be obtained by decreasing the size of the particles in the stationary phase. This formerly required high pressures to achieve the desired flow rates, leading to the term high pressure liquid chromatography (HPLC). Newer developments have reduced the required pressure and this technique is now widely referred to as high performance liquid chromatography. Solutes eluted from a liquid chromatography column can be used for other applications including mass spectrometry.

A convenient way to monitor a substrate, subjected to biodegradation testing, is to use a radiolabeled compound. When the exposure of a compound to biodegrading agents is halted, the residual quantity of the compound can be measured by counting the radioactive emission of the solution in a liquid scintillation counter. If the compound is uniformly labeled (all atoms of the given element are radioactive) then the metabolites separated by liquid chromatography can be examined for radioactivity and a determination of the fate of the original substrate can be made. This identifies the metabolites arising from the substrate and eliminates any ambiguity arising from the presence of metabolites which may have arisen from sources other than the substrate of interest.

Radioactive carbon (^{14}C) labeled compounds are particularly useful in mineralization experiments in which carbon dioxide is evolved. Mineralization of the substrate will result in radioactive carbon dioxide which can be quantified. The advantage of this method is that it is extremely specific and gives unequivocal evidence of the extent of mineralization for the radiolabeled compound. However, few compounds are routinely available in labeled form and custom synthesis is expensive. Stringent regulations govern the use and disposal of radioactive compounds.

ANALYSES OF METABOLIC ACTIVITY

Determination of the fate of a substrate in a biological community gives specific evidence of metabolic activity. However, several nonspecific methods of analysis are available which measure the metabolic activity of the microorganisms. If the substrate of interest is the only available source of a required nutrient or energy-metabolic activity is considered to be directly related to the presence of the substrate. Lack of metabolic activity is considered to be evidence of inability of the microbial population to utilize the substrate as long as all other essential nutrients and growth factors are present. These methods do not give information about the metabolic products arising from substrate utilization.

The biochemical oxygen demand (BOD) is a measure of the oxygen required to biochemically oxidize all the carbonaceous and nitrogenous matter in a sample. This test is subject to many variations in the methods of sample collection and measurement of data, and results are comparable only between tests performed by the same protocol. The BOD test is most useful for sewage and other samples with high organic content. The test is not very sensitive as a measure of the bio-oxidation of recalcitrant substrates.

A more sensitive measurement of oxygen uptake, as a substrate is utilized, can be obtained with such manometric devices as a Warburg respirometer. The procedure is based on the ideal gas law which states that at constant temperature and constant gas volume a change in the amount of a gas can be measured by the change in its pressure. The utilization of a substrate by microorganisms usually involves utilization of oxygen and production of carbon dioxide. If the carbon dioxide is absorbed in alkali the only change in gas volume or pressure will be uptake of oxygen. Both the rate and amount of oxygen uptake can be determined by this method. Measurements may be taken at specified time intervals and a graph of oxygen uptake vs. time can be constructed. When the graph indicates a straight line function with time, the enzyme systems are usually considered to be saturated with respect to substrate, although exceptions exist and in some cases higher levels of substrate may result in an increased rate. Determination of the amount of oxygen in moles taken up per mole of substrate yields information on the completeness of substrate oxidation.

Alternatively, carbon dioxide evolution as measured by trapping in alkali gives a measure of the complete mineralization of a compound supplied as the sole source of carbon. If the substrate contains radioactively-labeled carbon, the liquid scintillation counter can be used to measure carbon dioxide evolution as captured in the alkali trap.

Warburg respirometry has been used to develop the technique of simultaneous adaptation (407) for determination of the involvement of specific compounds in the pathway of substrate metabolism. Simultaneous adaptation is based on the theory that cells adapted to metabolize the primary substrate will also be adapted to metabolize all the intermediates of the pathway, but will not affect other substrates. In respirometry tests, this is seen as immediate uptake of oxygen after addition of the substrate or its metabolites. When other substrates are introduced, no uptake or uptake only after a lag period is observed. The only prerequisite for this system is that the enzymes be largely adaptive, such that they are not induced until the specific substrate is present. Therefore, a limitation to this test is involvement of nonspecific enzymes.

Assays have been developed for determining the presence of specific enzyme in a solution or culture. The assays are usually designed so that a substrate which is acted on directly and specifically by the enzyme of interest is made available. Enzyme activity is monitored by measuring loss of the substrate or appearance of a product by spectrophotometric, chemical or chromatographic methods. These assays are most

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useful for enzymes which are specific for a single substrate. Some enzyme assays require that a cell free culture filtrate be prepared, while others work with intact cells.

Enrichment cultures are widely used to search for organisms with degradative ability. The substrate of interest is supplied as the growth-limiting nutrient in a culture medium to which a mixed culture of microorganisms is added. The substrate most commonly is the sole source of carbon, and the inoculum may include sewage sludge, sediment samples, or river or ocean water. An inoculum is often selected from environments thought to be contaminated with the substrate of interest. Only organisms with ability to degrade the substrate will be able to grow in the culture medium and eventually will become the dominant population. These cells then can be recovered and isolated from the other cells added in the original inoculum. Evidence of substrate utilization is obtained indirectly by measuring growth of the population of the degradative organism, indicating incorporation of the substrate into cellular material. Further tests, usually with the isolated culture, are required to determine if the substrate is completely mineralized or whether intermediate metabolites remain. As some bacteria can grow in the absence of specifically added carbon, control experiments must be performed to ensure that the substrate is necessary for growth.

PARAMETERS FOR PURE CULTURE STUDIES

Bacteria used for pure culture studies may be selected from enrichment cultures. Identification of these isolates permits the results of such studies to be analyzed in the context of other such studies. Investigations of specific enzymatic or genetic features of degradative bacteria are more easily integrated with other studies when reference bacteria are used. These reference bacteria represent isolates which have been identified and then deposited in culture libraries such as the American Type Culture Collection (ATCC) or the National Collection of Industrial Bacteria (NCIB). Bacteria registered with such libraries are always indicated by a reference number which permits other researchers to obtain the same strain for subsequent studies. However, bacteria frequently mutate during repeated subculturing in a laboratory, and a strain studied for a length of time may no longer resemble the original culture. For this reason, the conditions under which a strain is maintained should be reported. Of particular importance is the frequency with which bacteria lose their degradative capability when removed from the substrate of interest. Such cultures must be maintained on media containing the substrate.

Some general parameters for biodegradation in solution are:

- The concentration of the substrate is an important consideration in all studies of biodegradation. The capability of bacteria to degrade substrates supplied at trace levels may be very different from the response to high concentrations.
- Chemicals used in formulating culture media should be of the highest purity possible, particularly when the contaminating chemical may be implicated in the degradative strategy of the organism.
- Parameters such as pH, temperature, and dissolved oxygen should be monitored, as fluctuations may affect not only the metabolic activities of the bacteria but also the chemical nature of the substrate.
- As newer techniques of analysis become available, broad studies comprising many of the techniques discussed here become feasible. Knowledge of both the environmental degradative behavior of the bacterial culture and its genetic structure can lead to manipulation of the culture toward increased degradative capability.

SECTION 6
METABOLISM OF NONCHLORINATED AROMATIC COMPOUNDS

CHEMISTRY OF BENZENE AND SUBSTITUTED BENZENES

The basic chemical structure of most aromatic compounds is the benzene ring. Benzene (molecular formula C_6H_6) is the simplest six-membered aromatic carbocycle. The entire molecule is structurally flat, i.e., the six carbon atoms and attached hydrogens lie in the same plane. The six aromatic electrons are delocalized and thereby confer the stability which is inherent to aromatic structures. The stability of benzene refers to the availability of the aromatic electrons for bonding. When the reactivities of aromatic and aliphatic carbocycles are compared under identical conditions, the aromatic systems are found to be less reactive, hence more stable.

The naming of substituted benzenes can be quite confusing due to several completely different sets of nomenclature rules. For instance, there is a set of trivial names such as toluene, phenol, and aniline. These trivial names are enigmatic to the casual observer since there are no rules, only a historical selection. Positional isomers have at least two nomenclature systems. The terms *ortho*, *meta*, and *para* have been used to identify the position of the substituents attached to the benzene ring. Finally, the International Union for Pure and Applied Chemistry offers a numerical description for positional isomers (Figure 13). To show the interrelationships of these systems, catechol, for example, is the trivial name for 1,2-dihydroxy benzene and a more cumbersome *ortho*-hydroxy phenol.

Positional location of substituents (*ortho*, *meta*, *para* or 1,2; 1,3; 1,4) is important to the overall reactivity of an aromatic molecule. A substituent on the benzene ring substantially influences the mode of reaction for a given chemical or biochemical system, i.e., the where and how of the attack by another reactive molecule. Common substituents attached to chlorinated aromatic molecules, available common articles of commerce, are: the hydroxyl (-OH), amino ($-NH_2$), methyl ($-CH_3$), and phenyl ($-C_6H_5$) groups which can render the molecule more reactive to electrophilic ("electron-loving") reaction conditions. Additional halogen substituents serve to deactivate the aromatic ring for electrophilic attack. Each of the cited substituent groups has a directing influence on subsequent electrophilic substitution which occurs mainly at the *ortho* and *para* positions (316).

The chemical nature and structural position of a substituent on the benzene ring affects the mode and ease of microbial attack on the compounds. The biological "recalcitrance" of a molecule, i.e., the resistance of a molecule to microbial degradation, is a direct consequence of the chemical nature and structural position of a substituent on the aromatic ring (2). Mention throughout this work of the recalcitrance of a compound will refer to biological activity rather than chemical or photooxidative processes.

MICROBIAL ATTACK ON BENZENE STRUCTURES

The first step in oxidative microbial attack on benzene involves hydroxylation of the ring. This is accomplished by different mechanisms in prokaryotes (bacteria) and

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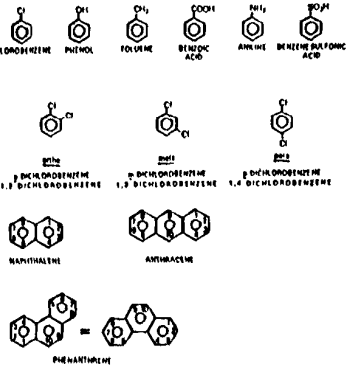

Figure 18 Common names and conventional nomenclature for substituted benzenes.

eukaryotes (fungi, mammals, etc.). Bacteria employ a dioxygenase which incorporates two atoms of oxygen from an oxygen molecule simultaneously into the ring (Figure 16) (15, 175, 205). Molecular oxygen is a required substrate for this enzyme (84). The resultant intermediate compound is a *cis*-1,2-dihydroxy 1,2 dihydrobenzene which loses two hydrogens to become catechol (1,2-dihydroxybenzene), a process mediated by the enzyme *cis*-benzene glycol dehydrogenase (14). The stereospecific nature ("cis") of the intermediate compound was identified in 1968 (181) and confirmed in 1970 (178) for *Pseudomonas putida*, and since then has been shown to be true for all bacterial species studied (14, 176, 177, 205).

Substituted benzenes similarly are oxidized to *cis*-dihydrodiols (substituted catechols) by bacteria. Some species oxidize the substituent before hydroxylation of the aromatic ring while others attack the ring yielding a substituted catechol. For example, toluene is attacked by *P. aeruginosa* with oxidation of the methyl group to benzyl alcohol, benzaldehyde and benzoic acid, followed by ring hydroxylation to form catechol (354, 331), while *Achromobacter* spp. and *Pseudomonas* spp., including *P. putida*, hydroxylate toluene directly to form 3-methylcatechol (Figure 17) (82, 175, 176, 179, 182, 332). Other alkylbenzenes are also subject to these two types of oxidative degradation, either oxidation of the aromatic ring to form an alkylcatechol or oxidation of the alkyl substituent to form an aromatic carboxylic acid which is dihydroxylated to catechol (84, 177, 177a, 181).

Benzoic acid is metabolized by a number of different pathways, depending on the bacterial strain. *Alcaligenes eutrophus* oxidizes benzoic acid to catechol. This mechanism in *A. eutrophus* is by way of the 3,5-cyclohexadiene-1,2-diol 1-carboxylic acid intermediate (74, 368) catalyzed by benzoic acid 1,2-dioxygenase, a two-protein enzyme composed of NADH-cytochrome *c* reductase and another protein (475). The intermediate is converted to catechol by a single protein (Figure 18). The fluorescent pseudomonad group also oxidizes benzoic acid to catechol (410, 467). In contrast, the acidovorans pseudomonad group monohydroxylates benzoic acid to *m*-hydroxybenzoic acid and subsequently to either gentisic acid (*P. acidovorans*) by *m*-hydroxybenzoic acid 6-hydroxylase or protocatechuic acid (*P. testosteroni*) by *m*-hydroxybenzoic acid 4-hydroxylase (467). Other *Pseudomonas* spp. monohydroxylate benzoic acid to *p*-hydroxybenzoic acid by utilizing benzoate 4-hydroxylase, and metabolize this intermediate further to protocatechuic acid by the enzyme *p*-hydroxybenzoate 3-hydroxylase (Figure 9) (82).

Pseudomonas PN-1 metabolizes benzoic acid, *p*-hydroxybenzoic acid and *m*-hydroxybenzoic acid to protocatechuic acid aerobically. However, under anaerobic conditions of nitrate respiration both protocatechuic acid and *m*-hydroxybenzoic acid are metabolized through *p*-hydroxybenzoic acid to benzoic acid. The mode of attack resulting in ring cleavage has not been elucidated (430, 431).

Members of *Streptomyces* spp. metabolize benzoic acid via catechol, *m*-hydroxybenzoic acid via gentisic acid, and *p*-hydroxybenzoic acid via protocatechuic acid (421). Two pathways have been shown in facultatively thermophilic *Bacillus* spp. In one species, benzoic acid, *m*-hydroxybenzoic acid and *p*-hydroxybenzoic acid are all metabolized via gentisic acid (64). The conversion of *p*-hydroxybenzoic acid to gentisic acid by this organism requires an "NIH Shift" of the carboxyl group. In another species, benzoic acid is metabolized through salicylic acid to 2,3-dihydroxybenzoic acid (406). The latter pathway is analogous to that shown for *Azotobacter* sp. (450).

The phototrophic bacterium *Rhodopseudomonas palustris* is unable to use benzoic acid as a substrate for aerobic growth (191), although early work postulated an aerobic pathway via protocatechuic acid and catechol (357). The organism can metabolize *p*-hydroxybenzoic acid aerobically via the protocatechuic acid path (125). Under anaerobic photosynthetic conditions, however, benzoic acid is

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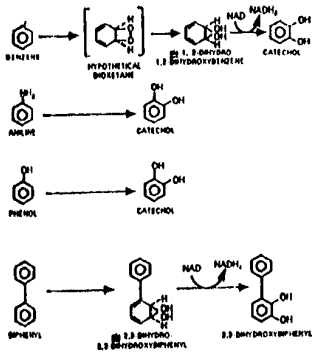

Figure 16. Oxidation of aromatic molecules by bacteria
Adapted from References 14, 16, 63, 71a, 141, 176, 178, 183, 190, 287, 328

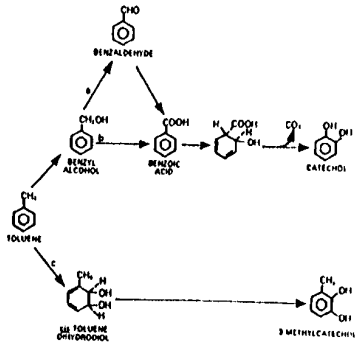

Figure 17. Pathways for the bacterial metabolism of toluene. Pathway (a) *P. putida* mt-2; (b) *P. aeruginosa*, (c) *P. putida*, *Pseudomonas* sp.; *Achromobacter* sp.
Adapted from References (a) 471a, (b) 264, (c) 92, 178

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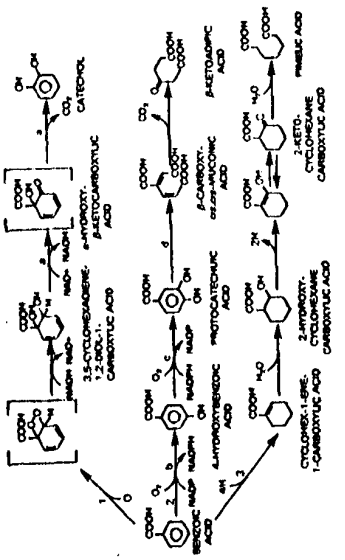

Figure 18 Pathways for the metabolism of benzoic acid (1) *A. eutrophus*, (2) soil pseudomonad, (3) *R. palustris* (anaerobic photometabolism) Enzymes: (a) 3,5-cyclohexadiene 1,2 diol 1-carboxylate dehydrogenase; (b) benzoate 4-hydroxylase; (c) p hydroxybenzoate 3 hydroxylase; (d) protocatechuate oxygenase
Adapted from References (1)368, (2)82, (3)124, 125, 181.

metabolized reductively to pimelic acid via cyclohexanol (Figure 18) (124, 125, 181). Early decarboxylation does not occur. The enzymes involved are thought to be reductases such as ferredoxin coupled to the light-induced electron transport system (125).

Rhodococcus sp. strain AN-117 utilizes aniline as a sole source of carbon and energy and metabolizes it exclusively by conversion to and *ortho* cleavage of catechol by inducible enzymes (235). In contrast, strain SB3, thought to be a pseudomonad, utilizes a constitutive *meta* cleavage pyrocatechase and hydroxymuconic semialdehyde dehydrogenase. However, aniline degradation occurs only when the cells are grown on aniline, indicating the presence of another inducible enzyme. Aniline-grown resting cells of *Frateuria* sp. ANA-18 oxidize aniline without a lag and oxidize catechol at a faster rate than aniline (9). Metabolites resulting from incubation of a cell free extract with aniline include *cis, cis*-muconic acid, beta ketoadipic acid and ammonia.

A mutant strain of a *Nocardia* sp. has been shown to convert aniline to catechol via simultaneous dioxygenation (16). This pathway is partially corroborated by data indicating that a *Pseudomonas* sp. grown on aniline oxidizes catechol rapidly, 4-aminophenol moderately quickly, 2-aminophenol slowly, and phenol not at all (449). However, only half the ammonia theoretically expected from the direct formation of catechol from aniline was recovered. This discrepancy has not been resolved. *Azotobacter* strain AN1 growing on aniline was simultaneously adapted to oxidize catechol but not phenol or 2-aminophenol (199). Transient formation of a catechol was noted indicating replacement of the amine group with a hydroxyl.

Biphenyl (a benzene-substituted benzene) is dihydroxylated by *Beijerinckia* sp., *P. putida*, and an unidentified Gram-negative bacterium to *cis*-2,3-dihydro-2,3-dihydroxybiphenyl and subsequently to 2,3-dihydroxybiphenyl (Figure 19) (183, 287). Phenol is oxidized directly to catechol via phenol hydroxylase (63, 141, 196, 326).

Polynuclear aromatic hydrocarbons are made up of fused aromatic rings. The three simplest compounds, naphthalene, anthracene, and phenanthrene, are metabolized by bacteria to form *cis*-dihydrodiols by the same mechanism as that shown for benzene (Figure 20) (72, 227). A mutant strain of *Beijerinckia* sp. (strain B836), as well as *P. putida* strain 199, forms (+)-*cis*-1,2-dihydroxy-1,2-dihydroanthracene from anthracene (226). These two organisms also convert phenanthrene to (+)-*cis*-3,4-dihydroxy-3,4-dihydrophenanthrene. A minor product formed is *cis*-1,2-dihydroxy-1,2-dihydrophenanthrene and there were no other diols recovered during these experiments. Pseudomonads also oxidize phenanthrene to 3,4-dihydroxyphenanthrene (Figure 21) and then to 1,2-dihydroxynaphthalene which is metabolized via the naphthalene pathway (Figure 22) (145, 267, 282, 373).

Naphthalene is metabolized by pseudomonads through *cis*-1,2-dihydro-1,2-dihydroxynaphthalene, 1,2-dihydroxynaphthalene, salicylaldehyde, salicylic acid, and catechol (106). The enzyme which catalyzes the conversion of the 1,2-dihydro-1,2-dihydroxynaphthalene to 1,2-dihydroxynaphthalene is *cis* naphthalene dihydrodiol dehydrogenase (224). Its proposed mechanism is stepwise, the first step bacterial-enzyme catalyzed and the second step a nonenzymatic enolization (224).

Anthracene is oxidized to 1,2-dihydroxyanthracene by a naphthalene-grown *Pseudomonas* sp. (151, 374). This intermediate is metabolized further to 2,3-dihydroxynaphthalene which follows an unknown degradative pathway through salicylic acid (Figure 23) (145).

In all of the above examples with the exception of phenol, bacterial attack on the benzene ring proceeds via a dioxygenase with formation of a *cis*-dihydroxybenzene. This mechanism is consistent with almost all bacterial oxidations of all substituted benzenes studied to date. The phenol hydroxylase is a monooxygenase but results in an *ortho* dihydroxylated molecule.

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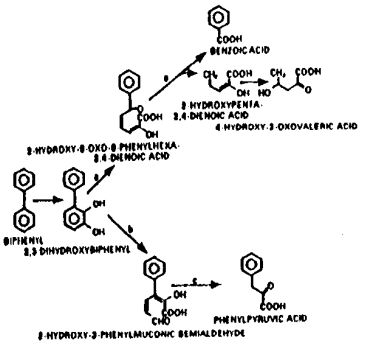

Figure 19. Metabolism of biphenyl by (a) *P. putida* and (b) *Beijerinckia* sp
Adapted from References [a]70, 71; [b]183, 287; [c]287.

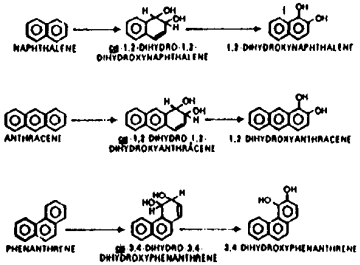

Figure 20. Mechanism of bacterial attack on naphthalene, anthracene, and phenanthrene
Adapted from References 145, 324

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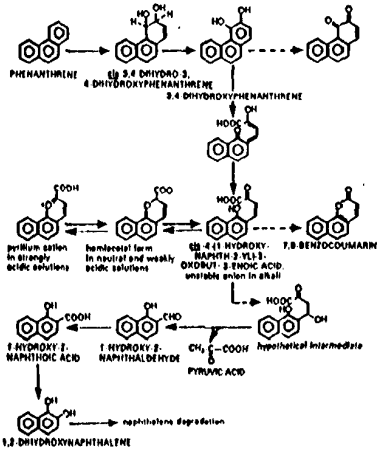

Figure 21. Pathway of phenanthrene metabolism by Pseudomonas sp. Dashed lines indicate hypothetical pathways
Adapted from Reference 149.

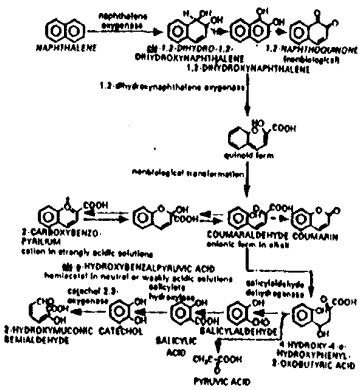

Figure 22. Pathway of naphthalene metabolism by Pseudomonas sp. Dashed lines indicate hypothetical pathways
Adapted from Reference 100.

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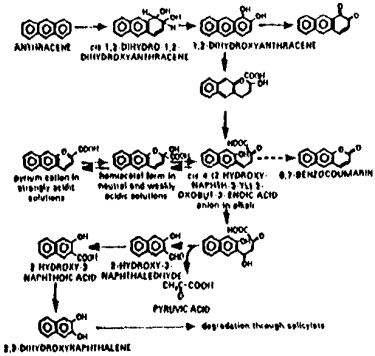

Figure 23 Pathway of anthracene metabolism by *Pseudomonas* spp. Dashed lines indicate hypothetical pathways
Adapted from Reference 148

ATTACK ON AROMATIC STRUCTURES BY CYANOBACTERIA

The cyanobacteria have not been studied as extensively with regard to aromatic degradation. An investigation of the degradation of naphthalene by the cyanobacterium *Oscillatoria* sp. revealed the presence of 1-naphthol as the major metabolite (Figure 24), 57% of the 1-naphthol formed involved the incorporation of molecular oxygen (76a, 78a). Small amounts of *cis*-1,2-dihydroxy-1,2-dihydronaphthalene were recovered which readily dehydrated to form 1-naphthol. Traces 1,2-dihydroxy-1,2-dihydronaphthalene was not recovered. There are three possible pathways which suggest a mechanism for attack of cyanobacteria on the aromatic ring: (a) similar to that for mammalian systems (path I, Figure 24), (b) unique to photosynthetic organisms (path II), or (c) similar to that for heterotrophic bacteria (path III) (78a)

RING FISSION OF DIHYDROXY AROMATIC COMPOUNDS BY BACTERIA

Bacteria can open an aromatic ring containing two hydroxyl groups if the groups are located *ortho* (adjacent) or *para* (opposite) to each other. If the groups are *ortho*, ring cleavage can occur either between the two (*ortho* cleavage) or next to one group (*meta* cleavage). The choice of which pathway is induced depends partly on the substrate and partly on the genetic constitution of the particular bacterial species. Both pathways can be induced independently of each other. An organism may utilize one pathway preferentially although it contains the enzymes for both pathways. For example, *P. putida* (*arvilla*) mt-2 metabolizes catechol via the *meta* pathway, although it contains the enzymes for both the *meta* and *ortho* pathways (322).

The ortho cleavage pathway of catechol leads to formation of 3-ketoadipic acid (Figure 25). Substituted catechols can be metabolized by an analogous path until either the substituent is expelled or the compound formed along the pathway cannot be metabolized further. Thus, the *ortho* pathway for protocatechuic acid distillation converges with that of catechol at 3-ketoadipate enol-lactone after the carboxyl group is expelled (Figure 26) (66, 84, 104). This latter compound is converted to 3-ketoadipic acid, which picks up coenzyme-A from succinyl CoA to form the intermediate 3-ketoadipyl-CoA. Cleavage of this compound results in acetyl-CoA and succinyl-CoA which in turn exchanges its coenzyme-A with 3-ketoadipyl-CoA leaving one molecule of succinic acid (406). These aliphatic molecules enter the cell's tricarboxylic acid cycle.

The *meta* cleavage pathway (Figure 25) results in the formation of pyruvic acid and acetaldehyde from catechol (102, 103, 180, 330). Substituted catechols usually form pyruvic acid and another aldehyde or an acid (Figure 26). The substrate 3-methyl catechol yields acetate while 4-methyl catechol yields formic acid (327). The non-fluorescent pseudomonad group metabolizes protocatechuate by the *meta* pathway, utilizing protocatechuate 4,5-oxygenase with subsequent production of oxaloacetic acid and pyruvic acid. *Meta* cleavage of protocatechuic acid in *Bacillus* spp. is catalyzed by protocatechuate 2,3-oxygenase and yields pyruvic acid and acetaldehyde via the 2 hydroxymuconic semialdehyde intermediate (93). *Meta* cleavage degradation of catechol results in two possible routes of metabolism as demonstrated in *Azotobacter* sp. and *P. putida* NCIB 10015 (382, 383), the major route involving an NAD$^+$ dependent dehydrogenase and resulting in formation of 4-oxalocrotonic acid and the minor route not requiring NAD$^+$ but rather employing a hydrolase to form 2-oxopent-4-enoic acid directly. The paths converge at this step. Degradation of 3-methylcatechol is constrained to follow the hydrolase pathway only, as the NAD$^+$ dependent path requires the presence of an aldehyde group on the molecule. Other

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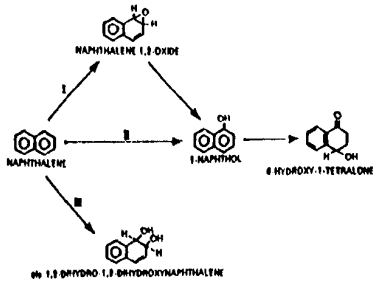

Figure 24 Pathways for naphthalene metabolism by Oscillatoria sp., strain JCM. (I) Metabolism via 1,2-oxide, (II) light-dependent direct hydroxylation of naphthalene; (III) metabolism via dihydrodiol.
Adapted from Reference 79a

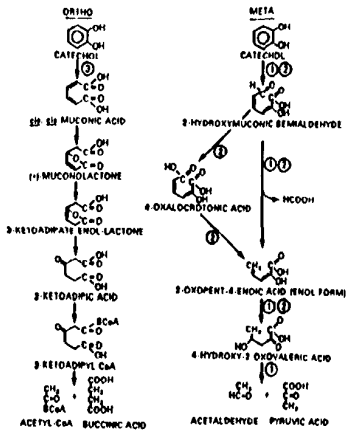

Figure 25. Ortho- and meta-cleavage pathways of catechol metabolism by bacteria
Adapted from References (1) 27, 103; (2) 302, 302a, 303, 498a; (3) 340, 408

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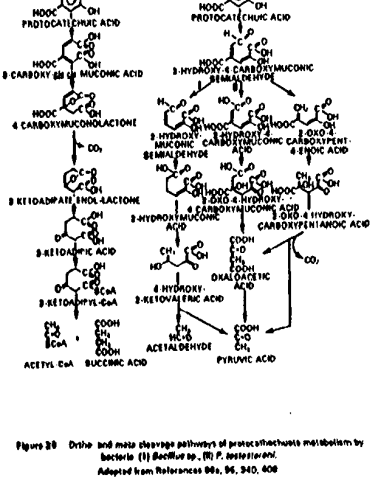

Figure 20 Ortho and meta cleavage pathways of protocatechuate metabolism by bacteria (1) *Bacillus* sp., (R) *P. testosteroni*.
Adapted from References 86a, 96, 340, 408

compounds which undergo *meta* cleavage include naphthalene, 2,3-dihydroxyphenylpropionic acid, 2,3-dihydroxybenzoic acid, and homoprotocatechuic acid (84, 106).

Compounds that contain two hydroxyl groups located opposite to each other (*para* substitution), such as homogentisic acid and gentisic acid, are usually cleaved at the bond between one hydroxyl and the adjacent side chain, leading to fumaric acid and acetoacetic acid or fumaric acid and pyruvic acid, respectively (Figure 21) (84).

Metabolism of benzoic acid by *P. testosteroni* and *P. acidovorans* follow divergent pathways with a different hydroxylase being induced in each one (Figure 20), resulting in *meta* and *para* cleavage pathways, respectively. *P. testosteroni* produces two moles of pyruvic acid and one of formic acid, while *P. acidovorans* induces the gentisic acid pathway and produces one mole each of fumaric acid and pyruvic acid (467).

Biphenyl is dioxylated at the carbon-2 and carbon-3 positions may be cleaved in the *meta* position in two ways. An unidentified Gram-negative organism and a mutant strain of *Beijerinckia* sp. metabolize this compound by cleavage between the carbon-3 and carbon-4 positions to yield 2-hydroxy-3-phenylmuconic semialdehyde and subsequently phenylpyruvic acid (183, 207). However, *P. putida* cleaves 2,3-dihydroxybiphenyl between the carbon-1 and carbon-2 positions to form 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid and then benzoic acid (70, 71).

The dihydroxy fused ring compounds are cleaved initially by *meta* cleavage between the angular carbon and the adjacent hydroxyl. Hydroxynaphthalene is cleaved to form *cis*-*o*-hydroxybenzalpyruvic acid and subsequently salicylaldehyde, salicylic acid, and catechol (Figure 13). Catechol may be degraded by *ortho* or *meta* cleavage (106, 224, 255, 267, 353). In some bacteria, phenanthrene is dihydroxylated to *cis*-4-(1-hydroxy-naphth-2-yl)-2-oxobut-3-enoic acid, 1-hydroxy-2-naphthoic-hyde, 1-hydroxy-2-naphthoic acid, and 1,2-dihydroxynaphthalene (Figure 12) (145, 151, 267, 371). Subsequent steps follow the pathway through salicylic acid. Other bacteria, including fluorescent and nonfluorescent pseudomonad groups, vibrios, and *Aeromonas* spp., metabolize phenanthrene to 1-hydroxy-2-naphthoic acid, 2-carboxybenzaldehyde, *o*-phthalic acid, and protocatechuic acid, which may undergo *ortho* or *meta* cleavage (255, 256). The first ring cleavage product of 1,2-dihydroxyanthracene is *cis*-4-(2-hydroxynaphth-3-yl)-2-oxobut-3-enoic acid which is degraded to 2-hydroxy-3-naphthaldehyde, 2-hydroxy-3-naphthoic acid and 2,3-dihydroxy-naphthalene (Figure 14) (145, 151, 267, 374). Degradation of 2,3-dihydroxynaphthalene continues through salicylic acid by an unknown pathway (145).

ATTACK OF AROMATIC STRUCTURES BY EUKARYOTES

The fate of aromatic-organic substances in mammalian systems has been studied extensively, both *in vivo* (injecting animals directly and recovering metabolites in body fluids or tissues) and using extracts of liver (or other) cells which contain the enzymes active in degradation of compounds (the microsomal enzymes). The mechanisms by which fungi and yeasts degrade aromatic compounds have been shown to be analogous to that of mammalian systems (404).

In contrast to bacteria, fungi utilize a monooxygenase which incorporates one atom of molecular oxygen into the benzene ring while converting the other to water. The resulting intermediate is an epoxide, which undergoes hydration with water to form a *trans*-1,2-dihydroxy-1,2-dihydro intermediate and subsequently a *trans*-dihydroxy compound (Figure 29) (175). Alternatively, the epoxide can isomerize to form phenols (177). Both of these mechanisms operate in the attack on naphthalene

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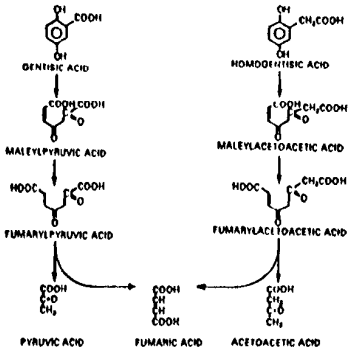

Figure 27. Pathways of gentisic acid and homogentisic acid metabolism by bacteria. Adapted from References 84, 84a, 286a

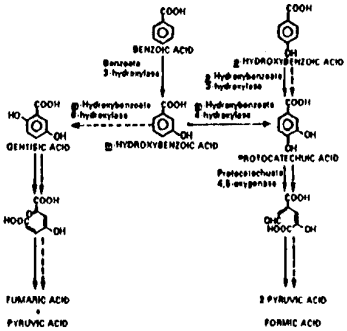

Figure 28. Divergent pathways for the metabolism of benzoic acid, p-hydroxybenzoic acid, and m-hydroxybenzoic acid by P. testosteroni (solid arrows) and P. acidovorans (broken arrows) Adapted from Reference 687.

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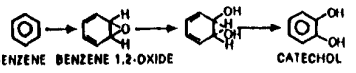

Figure 29 Formation of catechol from benzene in fungi, yeasts, and mammals

by *Cunninghamella elegans* in which the primary metabolite is 1-naphthol (Figure 30)(76). Anthracene is oxidized by *C. elegans* predominantly to trans 1,2-dihydroxy-1,2-dihydroanthracene with formation of 1-anthryl sulfate (sulfate conjugation of 1-anthrol) as well (73). Other unidentified metabolites are also produced. Other compounds which have a hydroxyl group added during fungal metabolism include acetanilide, aniline, anisole, benzene, benzoic acid, biphenyl, and toluene (404). In some cases the position of the hydroxyl is variable or more than one hydroxyl group is added to the ring. For example, *C. elegans* hydroxylates biphenyl to 2-dihydroxybiphenyl, 3-hydroxybiphenyl, 4-hydroxybiphenyl, 2,4'-dihydroxybiphenyl, and 4,4'-dihydroxybiphenyl (116).

DEGRADATION OF DIHYDROXYLATED AROMATIC COMPOUNDS BY YEASTS AND FUNGI

In general, fungi and yeasts lack many of the ring fission dioxygenases characteristic of bacteria (5, 274). In most fungi and yeasts, catechol and hydroxyquinol are cleaved only by the *ortho* mechanism, utilizing 1,2-dioxygenases only (5, 327, 395). Phenol is metabolized through catechol by the *ortho* pathway (393). *Aspergillus niger* converts benzoic acid to benzaldehyde (359). A single strain of *Penicillium* sp. only has been found to utilize the *meta*-fission pathway (67). However, certain catabolic enzymes of the yeasts have broader substrate specificities than the equivalent bacterial enzymes. In addition, a third hydroxyl group can be introduced into the aromatic ring (5). Thus, although the yeast *Trichosporon cutaneum* lacks dioxygenases for protocatechuic acid, gentisic acid, and homoprotocatechuic acid, it can metabolize these substrates by means of NAD(P)H-dependent hydroxylases (Figure 31).

Methoxylated aromatic compounds are demethylated and converted to the corresponding hydroxybenzoic acids by microfungi (42) but are reduced to their corresponding aldehydes or alcohols by the wood-rotting basidiomycete *Polystictus versicolor* (147). The metabolism of protocatechuic acid through 3-carboxymuconic acid and 3-carboxymuconolactone to 3-ketoadipate by *Neurospora crassa* is typical of many fungi (67, 188). *P*-Protocatechuic acid is formed from *p*-hydroxybenzoic acid or *p*-methoxybenzoic acid. The protocatechuic acid 3,4-oxidase of *Rhodotorula mucilaginosa* was used to identify the first metabolite of protocatechuic acid as 3-carboxy-*cis*, *cis*-muconic acid (67). Fungi contain a lactonizing enzyme which converts this compound to 3-carboxymuconolactone. This is in contrast to bacteria, which form 4-carboxymuconolactone (93). The product of 3-carboxymuconolactone degradation is 3-ketoadipic acid.

Some groups of fungi form catechol from protocatechuic acid (67) via catechol 1,2-oxygenase. Further degradation leads to *cis,cis*-muconic acid and (+)-muconolactone via a *cis,cis*-muconic acid-lactonizing enzyme and a muconolactone. Eventually 3-ketoadipic acid is formed and subsequently metabolized to succinic acid and acetyl-CoA. The catechol pathway is similar to that of bacteria (67). Fungal

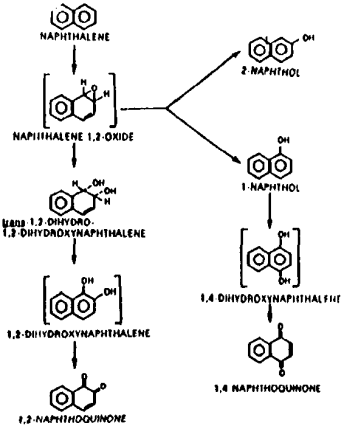

Figure 30 Pathway of naphthalene metabolism by *C. elegans*
Adapted from Reference 76

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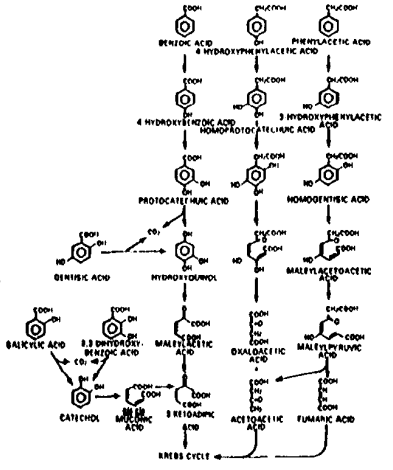

Figure 31. Metabolism of aromatic compounds by *T. cutaneum*
Adapted from Reference 8

metabolism of 1,2-dihydroxynaphthalene or 1-naphthol leads to 1,2 naphthoquinone or 1,4 naphthoquinone, respectively (75, 76, 78). These pathways are similar to those of mammalian microsomal extracts and are due to the cytochrome P-450 present in some fungi as well as in mammals (75). Monohydroxylated biphenyl compounds are hydroxylated further to various dihydroxy compounds (184a). These transformations are similar to those of mammals (184a, 404).

SUMMARY

Bacteria and eukaryotes differ fundamentally in the mechanism of primary oxidation of aromatic compounds. Bacteria usually add two atoms of molecular oxygen from the same atmospheric oxygen molecule using a dioxygenase enzyme. The mechanism of the oxidative addition results in a *cis*-1,2-dihydrodiol intermediate. In a few cases, such as when the aromatic ring is already monooxygenated (as for phenol), a hydroxylase (a monooxygenase enzyme) is utilized. The *ortho*-dihydrodiol molecules are subject to ring cleavage by either of two mechanisms. *Ortho* cleavage enzymes such as catechol 1,2-oxygenase open the ring between the adjacent hydroxyl groups. The molecule subsequently is metabolized via *cis, cis*-muconic acid and 3-ketoadipic acid to acetyl-CoA and succinic acid. In contrast, the *meta* cleavage pathway opens the ring adjacent to one hydroxyl group using enzymes such as catechol 2,3-oxygenase. The intermediate compounds of this pathway include 3-hydroxymuconic acid, and the end products include pyruvic acid and an aldehyde. Compounds such as benzoic acid initially may be monohydroxylated in the *meta* position. A second hydroxylase may then form a *para*-dihydroxylated molecule which is metabolized via the gentisic acid pathway to fumaric acid and pyruvic acid. An alternative pathway of benzoic acid distimilation attributable to bacteria is demonstrated in anaerobic reductive metabolism by *R. palustris* which forms pimelic acid via the cyclohexanol intermediate.

Fused-ring compounds are *ortho*-dihydroxylated at the positions adjacent to the angular carbon, and are cleaved by the *meta* pathway.

Cyanobacteria appear to attack aromatic structures by a mechanism similar to that of heterotrophic bacteria, which results in a *cis*-dihydrodiol intermediate. However, the exact mechanism has not been elucidated. Fungi and other eukaryotes attack benzene structures by monooxygenases which incorporate one atom of molecular oxygen, forming an epoxide. Subsequently the epoxide may undergo hydration with water to form a *trans*-1,2-dihydrodiol intermediate. Alternatively, a monohydroxylated compound may be formed.

The ortho mechanism is most commonly utilized by fungi for ring fusion. The protocatechuic acid pathway in fungi differs from that of bacteria in that 3-carboxy-*cis,cis*-muconic acid is lactonized to the 3-lactone in fungi and the 4-lactone in bacteria. Fungi lack many of the substrate-specific dioxygenases characteristic of bacteria, but some of their catabolic enzymes have broader substrate specificities than the equivalent bacterial enzymes. A third hydroxyl group may be added to the ring by fungi to facilitate metabolism of a molecule. The similarity in metabolism by fungi and mammals of many aromatic compounds has been demonstrated by comparison of the resultant metabolites (Table 3) (404). Thus, fungi may serve as models for many mammalian metabolic mechanisms.

MONS 026863

TABLE 3 COMPARISON OF METABOLITES FORMED BY EUKARYOTES AND MAMMALIAN SYSTEMS*

Substrate	Fungal metabolites	Mammalian metabolites	
		In vitro	In vivo
Aniline	Acetanilide, 2-hydroxyacetanilide, and 4-hydroxyaniline	4-Hydroxyaniline	Acetanilide, 2-, 3-, and 4-hydroxyaniline
Anisole	2- and 4-Hydroxy anisole, phenol	2- and 4-Hydroxy anisole, phenol	2- and 4-Hydroxy anisole
Benzene	Phenol	Phenol	Phenol
Benzoic acid	2- and 4-Hydroxybenzoic acid, 3,4-dihydroxybenzoic acid	3-Hydroxybenzoic acid	2-, 3-, and 4-hydroxybenzoic acid
Biphenyl	2- and 4-Hydroxybiphenyl, 4,4'-dihydroxybiphenyl	2- and 4-Hydroxybiphenyl	4-Hydroxy-, 3,4-dihydroxy- and 4,4'-dihydroxy biphenyl
Chlorobenzene	2- and 4-Hydroxychlorobenzene	2-, 3-, and 4-Hydroxychlorobenzene	2-, 3-, and 4-Hydroxybenzene
Naphthalene	1- and 2-Hydroxynaphthalene	1- and 2-Hydroxynaphthalene	1- and 2-Hydroxynaphthalene
Toluene	2- and 4-Hydroxy toluene	2- and 4-Hydroxy toluene, benzyl alcohol	Benzoic acid and conjugates

*Adapted from reference 404.

SECTION 7
CHLOROBENZOIC ACIDS

Chlorobenzoic acids are introduced into the environment as degradative products of polychlorinated biphenyls (1, 334a) and herbicides as well as in direct application as herbicides (411). For example, the multisubstituted herbicide 2,3,6-trichlorobenzoic acid is a growth regulator similar in function to 2,4-dichlorophenoxyacetic acid (208).

BACTERIAL METABOLISM AND CHLOROBENZOIC ACIDS

The degradation of 2,3,6-trichlorobenzoic acid (Figure 32) has been investigated using resting cell suspensions of *Brevibacterium* sp. grown on benzoic acid (208, 210). The major resulting product is 3,5-dichlorocatechol which appears with stoichiometric release of one mole of chloride and one mole of CO_2 per mole of herbicide metabolized (208). The initial oxidation of 2,3,6-trichlorobenzoic acid takes place at the unsubstituted carbon-4 position. This is followed by a one-step oxidative dechlorination at the adjacent chlorinated carbon. The pathway thus proceeds through 2,3,6-trichloro-4-hydroxybenzoic acid to 2,3,5-trichlorophenol and subsequently to 3,5-dichlorocatechol (Figure 33). The dichlorocatechol accumulates in the medium and is toxic to the *Brevibacterium* sp. cells. However, resting cell suspensions of *Achromobacter* sp. grown on benzoic acid will cleave 3,5-dichlorocatechol by the *meta* pathway to form 2-hydroxy-3,5-dichloromuconic semialdehyde (210). This metabolic accumulates and is toxic to the *Achromobacter* sp. cells.

Several species of bacteria have been shown to metabolize 3-chloro- and 4-chlorobenzoic acid. Resting cell suspensions of *Arthrobacter* sp. grown on benzoic acid oxidize 3-chlorobenzoic acid to 4-chlorocatechol, which is not inhibitory to growth or oxygen uptake (210, 211). *Pseudomonas aeruginosa* strain 142 accumulates 3-chlorocatechol from the metabolism of 3-chlorobenzoic acid (246). *Acinetobacter calcoaceticus* strain Bs5 grown on succinic acid or pyruvic acid will cometabolize 3-chlorobenzoic acid to both 3-chloro- and 4-chlorocatechol, which accumulate and are toxic (362). When mixtures of chlorocatechols can be formed, 3-chlorocatechol is the major metabolite (261, 362). Meta cleavage of 3-chlorocatechol results in an acylhalide which acts as an acylating agent and inactivates the *meta* pyrocatechase (catechol-cleaving enzyme) irreversibly, resulting in the lethal accumulation of catechols (261). Inefficient ortho cleavage will also result in the accumulation of chlorocatechols. *Meta* cleavage of 4-chlorocatechol yields 5-chloro-2-hydroxymuconic semialdehyde. Corresponding chlorocatechols are also formed from 3-chlorobenzoic acid and 4-chlorobenzoic acid by *Azotobacter* sp. grown on benzoic acid (314) and by *Pseudomonas* sp. WR912 (195).

Cells grown on chlorinated compounds including 3-chlorobenzoic acid are induced to produce high levels of pyrocatechase II, which has high activity against chlorocatechols as compared to catechols (118). Cells grown on nonchlorinated substrates express only pyrocatechase I, which does not function in chlorocatechol oxygenation. Pyrocatechases I and II are separate catechol 1,2-dioxygenases (118). Pyrocatechase I is involved in the degradation of catechol via the 3-ketoadipate pathway (117). Pyrocatechase II is similar to the *Brevibacterium* spp. pyrocatechase

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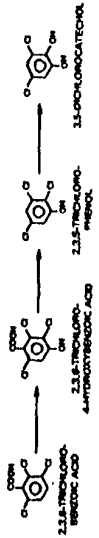

Figure 33 Formation of 3,5-dichlorocatechol from 2,3,6-trichlorobenzoic acid by *Brevibacterium* sp.
Adapted from Reference 208.

(326) and is unusual in its broad substrate specificity (119). The ability to cleave chlorocatechols, which are toxic, appears to be a crucial factor in the ability of microorganisms to degrade chloroaromatic compounds (118).

In cells of *Pseudomonas* sp. WR912, pyrocatechases I and II are both induced when the growth substrate is unsubstituted benzoic acid (195). This organism can use benzoic acid, 3-chloro-, 4-chloro-, and 3,5-dichlorobenzoic acids as sole sources of carbon and energy. Each is metabolized to the corresponding chlorocatechol, which undergoes *ortho* cleavage to form the chlorinated muconic acid. The muconic acid in each case is cycloisomerized with coincident or subsequent stoichiometric elimination of the chloride ion (Figure 33). Because of the wide range of substrates, the benzoic acid 1,2-dioxygenase of *Pseudomonas* sp. WR912 is characterized as being of low substrate specificity and also not stereospecific, similar to the corresponding enzyme of *P. putida* mt-2 (195).

In contrast, the benzoic acid 1,2-dioxygenase of *Pseudomonas* sp. B13 (Figure 34) shows narrow substrate specificity, as this organism metabolizes only 3-chlorobenzoic acid (195). The isomer 2-chlorobenzoic acid does not induce oxygen uptake, and 4-chlorobenzoic acid is oxidized only at very low reaction rates (367). Cells grown on 3-chlorobenzoic acid are adapted simultaneously to metabolize benzoic acid, but the reverse is not true (119). The enzymes of the 3-keto-adipic acid pathway are induced in cells grown on benzoic acid, and the chlorinated catechols accumulate. In contrast, resting cell suspensions of cultures grown on 3-chlorobenzoic acid produce both 3-chloro- and 3-chlorodihydroxybenzoic acid in almost equal quantities. A branched pathway thus exists for metabolism of 3-chlorobenzoic acid by *Pseudomonas* sp. B13 (Figure 34). Along one branch 3-chlorodihydroxybenzoic acid is metabolized to 3-chlorocatechol and along a parallel branch 3-chlorodihydroxybenzoic acid is converted to 4-chlorocatechol. The common enzyme involved, 3,5-cyclohexadiene-1,2-diol-1-carboxylic acid dehydrogenase, has the same relative activity in both benzoic acid-grown and 3-chlorobenzoic acid grown cells (365). The chlorocatechols are metabolized to muconic acids by pyrocatechases which are induced only in 3-chlorobenzoic acid-grown cells. The muconate cycloisomerase II enzyme which acts on the muconic acids to perform cycloisomerization has much higher activity in 3-chlorobenzoic acid-grown cells (119). Combined dechlorination and regeneration of the diene system is a spontaneous secondary reaction (386). *P. putida* strain B7 isolated from soils treated with pesticides also contains two pyrocatechases, one specific for the nonchlorinated catechol and the other specific for chlorinated catechols (187). The former is controlled by chromosomal genes and the latter is plasmid mediated. Chloromuconic acid was detected upon incubation of this strain with 3-chlorobenzoic acid.

The mutant strain *Alcaligenes eutrophus* B9 also produces 3-chloro- and 5-chlorodihydroxybenzoic acid from cooxidation of 3-chlorobenzoic acid (367). A strain of *P. putida*, harboring a plasmid termed pAC25, degrades 3-chlorobenzoic acid via 3-chlorocatechol and 3-chloromuconic acid (86). Chloride is released and maleylacetic acid rather than 3-ketoadipic acid is produced. This pathway is analogous to one path of 3-chlorobenzoic acid metabolism by *Pseudomonas* sp. B13.

Four strains of *Pseudomonas* spp. which utilize 3-chlorobenzoic acid as the sole source of carbon and energy for growth were isolated from sewage which had been enriched with the substrate (194). One isolate studied in detail, *Pseudomonas* sp. strain 111, resembles *Pseudomonas* sp. B13 in metabolism of 3-chlorobenzoic acid and benzoic acid, and therefore seems to possess both pyrocatechases. A black pigment, resulting from oxidation and polymerization of unmetabolized catechols, forms when *Pseudomonas* sp. strain 111 is incubated with both 3-chlorobenzoic acid and benzoic acid without prior adaptation to 3-chlorobenzoic acid. In contrast, another species isolated from the sewage enrichment culture metabolizes catechols and chlorocatechols rapidly enough to prevent occurrence of the black pigment.

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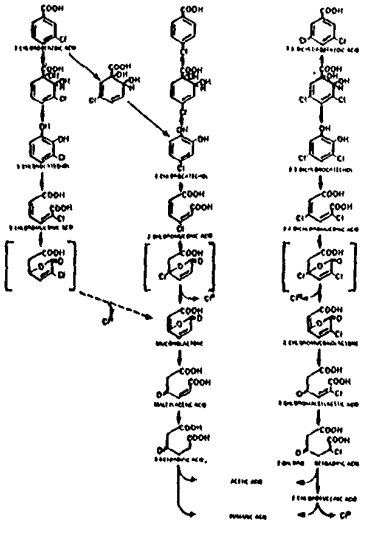

Figure 33 Pathways of metabolism of chlorobenzoic acids by *Pseudomonas* sp. WR612. Compounds in brackets are hypothetical intermediates.
Adapted from Reference 196.

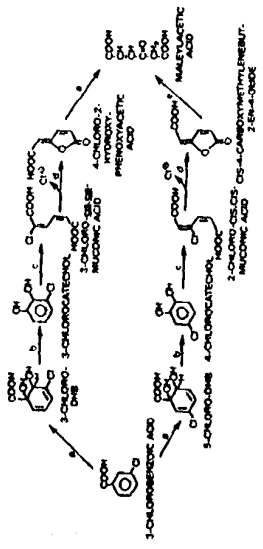

Figure 34. Metabolism of 3-chlorobenzoic acid by *Pseudomonas* sp. B13. DHB = 3,5-cyclohexadiene-1,2-diol-1-carboxylic acid. Enzymes: (a) benzoate dioxygenase, (b) DHB dehydrogenase, (c) pyrocatechase II, (d) muconate isomerase II, (e) 4-carboxymethylenebut-2-en-4-olide hydrolase
Adapted from References 110, 388, 389.

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A hybrid strain has been developed which combines the ortho-cleavage pathway of *Pseudomonas* sp. B13 with the relatively nonspecific toluate 1,2-dioxygenase of *P. putida* mt-2 (364). This derivative of *Pseudomonas* sp. B13 acquired the TOL plasmid from *P. putida* mt-2. In the resulting cells, both 4-chlorobenzoic acid and 3,4-dichlorobenzoic acid as well as 3-chlorobenzoic acid are metabolized (Figure 35). The enzyme, toluate 1,2-dioxygenase from the genes on the plasmid, is induced and slightly modified to result in increased turnover of the chlorinated compound used as the selective substrate. Dihydrodihydroxybenzoic acid dehydrogenases from both plasmid and chromosomal sources are induced. In addition, ortho pyrocatechases I and II are induced, but not the unproductive *meta* pyrocatechase.

A *Bacillus* sp. grown on benzoic acid uses a unique pathway to cometabolize 4-chlorobenzoic acid to 5-chloro-2,3-dihydroxybenzoic acid via 5-chlorosalicylic acid (406). Another unique pathway involving enzymatic rather than spontaneous elimination of chloride ion was demonstrated in *Bacillus brevis* isolated from polluted river water (96). This organism utilizes 5-chloro-2-hydroxybenzoic acid (5-chlorosalicylic acid) as a sole carbon and energy source. The first step in metabolism is cleavage between the carbon-1 and carbon-2 by a specific 5-chlorosalicylate 1,2-dioxygenase. This enzyme requires a halogen (except iodine) or a methoxyl group (but not a hydroxyl) at carbon-5 position. Only one hydroxyl group is present on the molecule. After loss of the chloride ion and formation of maleylpyruvic acid, metabolism continues along the steps of the gentisic acid pathway (Figure 36).

A novel pathway for 3-chlorobenzoic acid metabolism in which the chloride is replaced by a hydroxyl group in the first step has been demonstrated in a *Pseudomonas* sp. isolated from soil (231). This organism utilizes 3-chlorobenzoic acid as a sole source of carbon for growth and metabolizes it to 3-hydroxybenzoic acid and subsequently to 2,3-dihydroxybenzoic acid.

Similarly, an *Arthrobacter* sp. growing on 4-chlorobenzoic acid as the sole source of carbon produces 4-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid (protocatechuic acid) (380). A strain of *Arthrobacter globiformis* also utilizes 4-chlorobenzoic acid as the sole carbon source and metabolizes it via 4-hydroxybenzoic acid and protocatechuic acid, with release of chloride (486). Direct replacement of the chloride by the hydroxyl group precludes formation of the potentially toxic chlorocatechol. *Pseudomonas* sp. strain CBS 3 also utilizes 4-chlorobenzoic acid as the sole source of carbon for growth (257) by the same pathway. The enzymes induced by growth with this substrate have been identified as 4-chlorobenzoate-4-hydroxylase, 4-hydroxybenzoate-3-hydroxylase, and protocatechuate-3,4-dioxygenase. The first enzyme was not induced by growth with 4-hydroxybenzoic acid or any of several other chlorinated and nonchlorinated substrates. The mechanism of action of this enzyme has not been elucidated. Protocatechuic acid was metabolized by the 3-ketoadipic acid pathway following *ortho* cleavage.

ALGAL METABOLISM OF CHLOROBENZOIC ACIDS

The only reference to algal metabolism of chlorobenzoic acids obtained involves a unialgal culture of *Chlamydomonas* sp. strain A2 isolated from sewage (221). The nonaxenic culture (bacteria present) transforms 4-chloro-3,5-dinitrobenzoic acid to 3-hydroxymuconic semialdehyde, indicating a *meta*-cleavage pathway. Approximately 20% chloride release was reported. Since the culture contained bacteria along with the algae, dechlorination might have been due to the action of the algae or it might have been a bacterial process with the algae providing fixed carbon or growth factors.

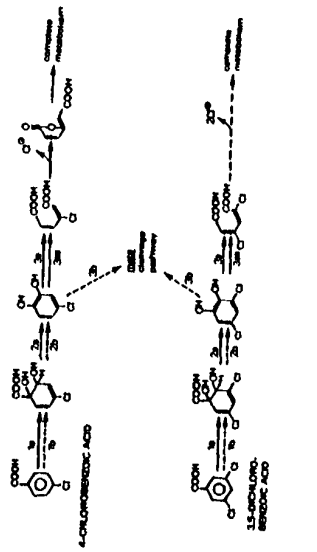

Figure 39. Metabolism of 4-chlorobenzoic acid and 3,5-dichlorobenzoic acid by *Pseudomonas* sp. B13 transconjugants. Enzymes marked (a) from *Pseudomonas* sp. B13; those marked (b) from donor *P. putida* mt-2. Enzymes: (1a) benzoate 1,2-dioxygenase; (1b) toluate 1,2-dioxygenase; (2a, 2b) dihydrodihydroxybenzoate dehydrogenase; (3a) pyrocatechase I; (3aa) pyrocatechase II; (3b) *meta* pyrocatechase. Adapted from References 484.

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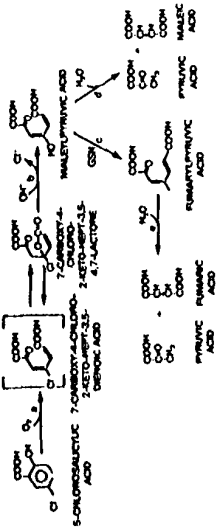

Figure 28 Metabolism of 5-chlorosalicylic acid by *B. cepacia*. Enzymes: (a) 5-chlorosalicylic acid 1,2-dioxygenase, (b) "lactone hydrolase", (c) maleylpyruvate isomerase, (d) maleylpyruvate hydrolase, (e) fumarylpyruvate hydrolase. GSH = reduced glutathione

Adapted from Reference 90

FUNGAL METABOLISM OF CHLOROBENZOIC ACIDS

Aspergillus niger cultures utilized both 2-chloro- and 3-chlorobenzoic acid as sole sources of carbon and energy (390). Protocatechuic acid and 4-hydroxybenzoic acid were isolated from both samples. Cells grown on these substrates oxidize all four compounds as well as benzoic acid more rapidly than do cells grown on glucose. Adapted cultures dechlorinate either substrate, while glucose grown cells do not have this capability. Dehalogenating activity was also noted in the cell-free extracts of cultures grown on 2-chlorobenzoic acid.

METABOLISM OF CHLOROBENZOIC ACIDS IN SOILS AND BY CONSORTIA

Under cometabolic conditions with glucose as the additional carbon source, a sewage plant effluent inoculum metabolized 3-chlorobenzoic acid with production of 3-chlorocatechol (212). Upon continued incubation this metabolite disappeared with concomitant appearance of 2-hydroxy-3-chloromuconic semialdehyde. After a 30 day period of no discernible metabolism, the semialdehyde was metabolized with appearance of stoichiometric amounts of inorganic chloride. There was no additional increase in cell numbers due to the presence of chlorobenzoic acid until degradation of the semialdehyde occurred.

Diluted wastewater sludge supernatant fluid mediated disappearance of 16 mg/l 3-chlorobenzoic acid within 14 days, although no degradation of 2- or 4-chlorobenzoic acid was seen after 23 days (193). Readaption of the sludge inocula to 3-chlorobenzoic acid greatly reduced the time required for metabolism of both 3- and 4-chlorobenzoic acid. Soil suspension also did not degrade 4-chlorobenzoic acid in 25 days, although the other 2 substrates were metabolized within 14 days. Application of 2,3,6-trichlorobenzoic acid to soil resulted in 30% chloride release within one month (113). No intermediate metabolites were detected.

A sewage microcosm enriched with chlorinated benzoic acids resulted in development of a consortium of Gram-negative motile rods and Gram positive pleomorphic rods which could utilize as sole carbon and energy sources benzoic acid, 2-chloro-, 3-chloro-, 4-chloro- and 3,4-dichlorobenzoic acids but not 2,4 dichloro or 2,3,6-trichlorobenzoic acid (114). Addition of biodegradable benzoic acids did not lead to decomposition of any of the substrates. Degradation of 3-chlorobenzoic acid led to formation of both 4-chlorocatechol and 5-chlorosalicylic acid, the latter compounds disappearing from solution. Formation of both metabolites indicates two separate pathways of metabolism within the consortium.

Pronamide [3,5-dichloro-*N*-(1,1-dimethyl-2-propynyl)benzamide] is an herbicide used for weed control on crops of lettuce and alfalfa and other legumes (153). Pronamide was metabolized in soils to $^{14}CO_2$ from both ^{14}C(carbonyl)- and ^{14}C(ring)-labeled substrate. In addition, seven other metabolites were found, none of which was dechlorinated. The potential metabolite 3,5-dichlorobenzoic acid was also metabolized with 80% of ^{14}C(carbonyl)- and 30% of ^{14}C(ring)-labeled substrate recovered as $^{14}CO_2$ within 28 days.

Dicamba [3,6-dichloro-2-methoxybenzoic acid] is used on wheat, oats, barley and flax crops for weed control. Studies with a temperature-gradient table showed that an increase in temperature from 5 to 35°C resulted in a linear increase in the disappearance rate for the herbicide in silty clay and heavy clay, while the range for a linear relationship of temperature to disappearance was 9 to 30°C for sandy loam, a soil with a lower moisture holding capacity (401). Studies with Regina heavy clay have shown that only 10% of the herbicide remains five weeks after application (399). The only radioactive metabolite recovered from application of carboxyl group labeled dicamba was 3,6-dichlorosalicylic acid. About 20% of the substrate was

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recovered as $^{14}CO_2$, and metabolites which were not radioactive were not identified. Dicamba applied at a rate of 1.1 kg/ha to a silty clay with high organic content almost entirely disappeared within two weeks (398). At the same application rate onto sandy loam and heavy clay, there was less than 10% substrate remaining after 4 weeks when the moisture content was high, although residues were still detected after 6 weeks in low-moisture soils. After 4 weeks, over 90% of the substrate applied to sterile soils was recovered. Studies with ^{14}C(carboxyl)- and ^{14}C(ring)-labeled dicamba revealed that 10% was released as $^{14}CO_2$ in 6 weeks and 45% in 17 weeks from ^{14}C(carboxyl)-dicamba, and 9% in 6 weeks from ^{14}C(ring)-dicamba (398). The only metabolite which could be recovered was 3,6-dichlorosalicylic acid.

REDUCTIVE DECHLORINATION

The chlorobenzoic acids have served as model substrates for the elucidation of anaerobic reductive dechlorination by consortia of bacteria from anaerobic sediment or sludge environments. This pathway for dechlorination of aromatic compounds involves removal of the aryl halide from the aromatic ring (Figure 37) (417). A consortium resulting from enrichment with 3-chlorobenzoic acid mineralizes this substrate through benzoic acid to methane and CO_2. The substrate 4-amino-3,5-dichlorobenzoic acid is converted to 4-amino-3-chlorobenzoic acid by replacement of one chlorine atom with a hydrogen atom. No chloride shift takes place. Neither 2-chloro- nor 4-chlorobenzoic acid was metabolized in experiments lasting for one year of incubation (206). In multi-substituted compounds, the *meta* substituent is utilized preferentially. Thus, 2,5-dichlorobenzoic acid is reduced to 2-chlorobenzoic acid, 3,4-dichlorobenzoic acid to 4-chlorobenzoic acid, and 2,3,6-trichlorobenzoic acid to 2,6-dichlorobenzoic acid (417). In these experiments, persistence was not correlated with the number of halogens present on the molecule.

Although benzoic acid was always an intermediate in anaerobic mineralization of the chlorobenzoic acids, acclimation to the chlorinated substrate did not result in acclimation to benzoic acid (206). This phenomenon was explored further with the consortium acclimated to 3-chlorobenzoic acid. Metabolism of 3,5-dichlorobenzoic acid to 3-chlorobenzoic acid proceeded with accumulation of 3-chlorobenzoic acid until the parent substrate concentration fell to a low level. Only then was 3-chlorobenzoic acid metabolized to benzoic acid followed by production of methane and CO_2. Similarly, 4-amino-3,5-dichlorobenzoic acid was metabolized to 4-amino-3-chlorobenzoic acid which accumulated until the concentration of parent substrate decreased to a low level, after which the intermediate metabolite was converted to 4-aminobenzoic acid which was not degraded further. Three events were postulated to be due to competitive substrate inhibition, in which one enzyme involved in multiple steps of a degradative pathway acts only on the parent compound until its concentration falls below a threshold level (418). Under these conditions, bacteria from environments receiving several structurally related chemicals may metabolize substrates selectively due to competitive substrate inhibition.

The consortium could be acclimated to degrade 4-amino-3,5-dichlorobenzoic acid to its metabolites, even though this substrate was not used as a sole carbon and energy source and was not mineralized (206). Aliquots of the substrate added after acclimation were metabolized without a lag. Partial dechlorination of several compounds by sewage microflora acclimated to nonchlorinated products was reported (221). Substrates attacked include 2-chlorotoluene, 3-chlorobenzoic acid, 4-chloro-2,5-dinitrobenzoic acid, 4-chloroaniline, 4-chlorobiphenyl, 4-chlororesorcinol and 4-chlorobenzonitrile. From 2-47% of the chlorine from these substrates was removed within 2 days. The substrate 4-chloro-3,5-dinitrobenzoic acid was 13 to 65% dechlorinated in sewage but was only 12 to 29% dechlorinated within 20 days by single isolates of bacteria from the sewage.

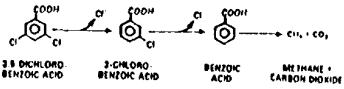
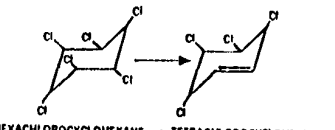


Figure 37 Representative pathway for the reductive dechlorination of chlorobenzoic acids by anaerobic microbial consortia
Adapted from References 206, 417

Reductive dechlorination of pentachlorophenol has been demonstrated in anaerobic soils (217, 266, 320). Resultant products include isomers of tetrachlorophenols, trichlorophenols, dichlorophenols and 3-chlorophenol. The methylated chloroanisole analogues of these isomers have been detected as well. These investigations led to the conclusion that the chloride ions *ortho* and *para* to the hydroxyl group are utilized preferentially (217). Pentachlorophenol metabolism is discussed further in a later section. In contrast, the reductive dechlorination of [1]-T to DDD described in another section involves the alkyl chlorides but not the chlorides attached to the ring (107, 189a, 203, 234). This has been demonstrated in yeasts as well as bacteria and in pure cultures as well as consortia. Lindane (gamma-hexachlorocyclohexane) is a nonaromatic molecule which is also reductively dechlorinated (Figure 38). The major metabolite is gamma-tetrachlorocyclohexene, followed by benzene, monochlorobenzene, and small amounts of tri- and tetrachlorobenzenes (192).



γ-HEXACHLOROCYCLOHEXANE γ-TETRACHLOROCYCLOHEXENE

Figure 38 Primary metabolic reductive dechlorination of γ-hexachlorocyclohexane by anaerobic microorganisms
Adapted from References 182, 307

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SUMMARY

The chlorobenzoic acids can be metabolized to several different intermediate products in bacteria. The most common mechanism results in the conversion of the chlorobenzoic acids to chlorocatechols. If the *meta* cleavage pathway is the only route induced in the bacteria, then chlorocatechols are metabolized usually to chloromuconic semialdehydes, which are not metabolized further. In addition, the *meta* cleavage product of 3-chlorocatechol inactivates the meta pyrocatechase, causing an accumulation of the toxic chlorocatechols. This pathway does not lead to mineralization of the chlorinated substrate, and ultimately results in cell death and release of chlorinated intermediates into the environment. In contrast, *ortho* cleavage of chlorocatechols is a successful pathway for chlorobenzoic acid mineralization. The *ortho* pyrocatechase results in the formation of chlorinated muconic acids, which are cycloisomerized with coincident or subsequent elimination of the chloride ion. After release of the chloride ion, the compound is fully metabolized by established cellular mechanisms.

One block to utilization of chlorinated aromatic compounds in organisms expressing the *ortho* pyrocatechase is specificity of the enzyme required for the first oxygenation step. The hybrid strain constructed from Pseudomonas sp. B13 and *P. putida* mt-2 incorporates the relatively nonspecific oxygenase of *P. putida* mt-2, carried on the TOL plasmid, into *Pseudomonas* sp. B13 which metabolizes chlorinated compounds via the *ortho* pathway. The hybrid strain is capable of mineralizing a wider range of chlorobenzoic acids than either parent.

Another pathway which has been discovered replaces the chloride ion by a hydroxyl group directly, resulting in a nonchlorinated hydroxybenzoic acid which can be metabolized by established cellular mechanisms. A third series of metabolic pathways results in hydroxylation of the chlorobenzoic acid without loss of prior substituents. A specific and unique enzyme opens the ring and an enzyme has been identified which specifically dechlorinated the resulting aliphatic acid molecule. Data on algal and fungal pathways for metabolism of chlorobenzoic acids are lacking. The relaxed substrate specificities of fungi suggest that these organisms may be of prime importance in metabolism of the chlorobenzoic acids.

SECTION 8
CHLOROBENZENES

MICROBIAL METABOLISM OF CHLOROBENZENES

Chlorobenzenes are used as industrial solvents and diluents for polychlorinated biphenyl compounds (PCBs) and thus have a complementary distribution as pollutants in the environment, including capacitor and transformer storage and disposal (295). They are solvents for paints and appear as byproducts in the textile dyeing industry and in other industries. Pentachloronitrobenzene is used as a fungicide for seeds and soils.

A chemostat seeded with a mixture of soil and sewage samples was used to enrich for an organism capable of utilizing chlorobenzene as a sole growth substrate (346). After nine months an unidentified bacterium, strain WR1306, was isolated which degrades chlorobenzene with stoichiometric release of chloride ion. Detection of the enzymes *cis*-1,2-dihydroxycyclohexa-3,5-diene (NAD+) oxidoreductase, catechol 1,2-dioxygenase, muconate cycloisomerase, 4-carboxymethylenebut-2-en-4-olide hydrolase and NADH-dependent maleylacetate reductase, and isolation of the metabolite 3-chlorocatechol, enabled construction of a pathway for chlorobenzene dissimilation (Figure 39). The proposed pathway is similar to that demonstrated for the metabolism of other nonphenolic benzene compounds such as 3-chlorobenzoic acid. Chlorobenzene is converted to 3-chlorocatechol which is cleaved by the *ortho* pathway. The substituted muconic acid thus formed is cycloisomerized with coincident or subsequent elimination of chloride. The nonchlorinated intermediate is metabolized to 3-oxoadipic acid which enters the cell's tricarboxylic acid cycle.

Attempts to find other strains of bacteria capable of using chlorobenzene as a sole source of carbon and energy for growth have been hampered by the hydrophobicity of the compound (346). Strain WR1306, although capable of growth on chlorobenzene, was inhibited by high concentrations of the compound. Accumulation of the toxic metabolite 3-chlorocatechol is lethal for cells which have not evolved a mechanism for efficient metabolism of this compound. The mechanism of catechol cleavage must be by the *ortho* rather than the *meta* pathway for production of metabolites useful in cell biosynthesis (discussed further in section on chlorobenzoates).

Resting cells of *P. putida* grown on toluene oxidize chlorobenzene and to a lesser extent all three isomers of chlorotoluene (182). Cometabolic growth of *P. putida* on toluene and chlorobenzene results in formation of 3-chlorocatechol. Cells grown on toluene and 4-chlorotoluene metabolize the latter through *cis*-4-chloro-2,3-dihydroxy-1-methylcyclohexa-4,6-diene to 4-chloro-2,3-dihydroxy-1-methylbenzene.

Bacteria were isolated which utilize 1-chloronaphthalene for growth (451). Chloronaphthalene diol (8-chloro-1,2-dihydro-1,2-dihydroxynaphthalene) and 1-chlorosalicylic acid were recovered as metabolites.

An alternative pathway has been developed which proposes chlorophenols as intermediates in the degradation of chlorobenzenes (19). However, the formation of phenolic products from the dihydrodiol metabolite may occur spontaneously under

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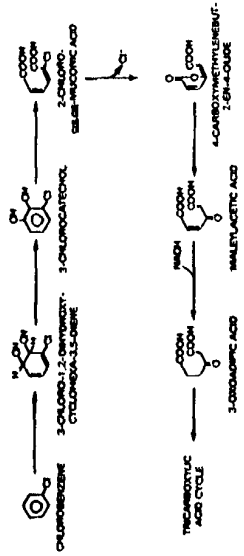

Figure 39 Pathway of chlorobenzene mineralization by bacterial strain WR1300. Adapted from Reference 306

mild acid conditions as well as enzymatically (366). Confirmation of this pathway requires isolation of the enzymes involved.

Pure cultures of bacteria isolated from pond water and pond sediment samples metabolize dichlobenil (2,6-dichlorobenzonitrile) to 2,6-dichlorobenzamide, 2,6-dichlorobenzoic acid and several other metabolites which appeared in trace quantities (131, 132). An *Arthrobacter* sp. which was grown on benzonitrile metabolized more than 70% of ^{14}C-dichlobenil to 2,6-dichlorobenzamide and an additional 20% to other metabolites within 6 days (314).

The degradation of chloronitrobenzenes by fungi has been studied and the mechanism is believed to result in detoxification of the fungicides (94). The yeast *Rhodosporidium* sp., when grown in a complex nutrient medium containing 4-chloronitrobenzene, produces several metabolites. This information enabled a branched pathway to be proposed (Figure 40) (94). The common early steps of 4-chloronitrobenzene metabolism involve sequential reduction of the substrate to form 4-chloronitrosobenzene and subsequently 4-chlorophenylhydroxylamine. This product may be metabolized by two mechanisms. The main pathway is further reduction of the hydroxylamine to 4-chloroaniline, followed by acetylation to produce 4-chloroacetanilide, the major metabolite. This product accumulates in the culture medium. An alternative mechanism involves a shift of the hydroxyl group from nitrogen to carbon (called a Bamberger rearrangement) resulting in conversion of the hydroxylamine to 4-hydroxyaniline and 2-amino-5-chlorophenol. Formation of 4-hydroxyaniline involves loss of the chloride ion and subsequent acetylation results in formation of 4-hydroxyacetanilide.

Pentachloronitrobenzene is metabolized to pentachloroaniline by *Streptomyces aureofaciens*, *Rhizoctonia solani*, *Fusarium oxysporum* and many other genera of fungi and actinomycetes (80, 322). In addition, *F. oxysporum* also metabolizes the substrate to pentachlorothioanisole (Figure 41) (322). The introduction of a methylthio group was also noted in the metabolism of 2,4-dichloro-1-nitrobenzene by *Alcaligenes faecalis* AHU6010 (Figure 41) (428). The source of the sulfur atom has not been established. The metabolite may be formed by secondary degradation of a glutathione degradation product, as proposed for rhesus monkey metabolism, or the methylthio group may be transferred from S-adenosylmethionine as demonstrated by cells of *Mycobacterium* sp. (428).

Metabolism of 1,4-dichloro-2,5-dimethoxybenzene (chloroneb) to 2,5-dichloro-4-methoxyphenol by *R. solanii* a detoxification mechanism which results in tolerance by the organism to at least twice the concentration of metabolite as product (204). This conversion occurred only at a high ratio of mycelia to growth medium. *Sclerotium rolfsii* and *Saccharomyces pastorianus* did not metabolize chloroneb. *Neurospora crassa* converts chloroneb to an unidentified product.

In a broad study of 23 species, conversion of chloroneb to 2,5-dichloro-4-methoxyphenol occurred only in cultures actively growing in nutrient medium (468). The most active species was a *Fusarium* sp. which demethylated 60 to 80% of a 5 ppm solution within 10 days. Thirteen other species demethylated the fungicide as well, although *R. solani* neither grew in the presence of nor demethylated chloroneb. *Fusarium* sp. also converted the metabolite back to chloroneb at the rate of 4% in 7 days. Eleven other species performed the same transformation. Demethylation of 2,5-dichloro-4-methoxyphenol to form 2,5-dichlorohydroquinone occurred in four species, but at concentrations of 10% or less of the applied 2,5-dichloro-4-methoxyphenol.

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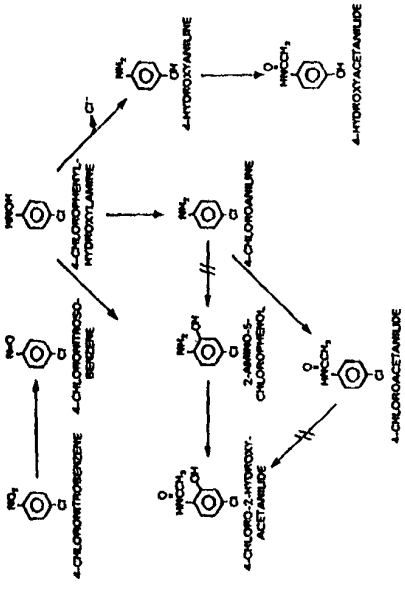

Figure 40. Pathways of metabolism of 4 chloronitrobenzene by *Rhodospordium* sp.
Adapted from Reference 94.

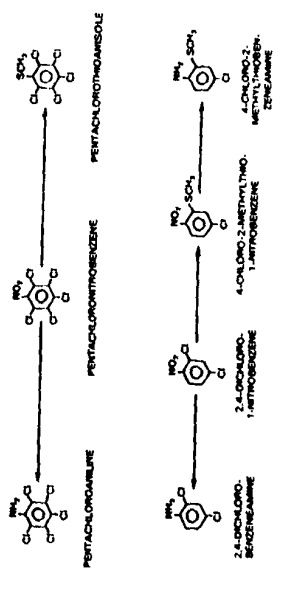

Figure 41. Metabolism of pentachloronitrobenzene by *F. oxysporum* and 2,4 dichloro 1-nitrobenzene by *M. phlei* ATCC 19070
Adapted from References 322, 428

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METABOLISM OF CHLOROBENZENES BY MICROBIAL COMMUNITIES

The chlorobenzenes are largely volatile and attempts to study their biodegradation have been hampered by disappearance of the substrates. A mesocosm experiment using tanks of seawater amended with mixtures of volatile organic compounds showed that at 3 to 7°C both 1,4-dichlorobenzene and 1,2,4-trichlorobenzene disappeared at rates explainable by volatilization (446). At warmer temperatures (20 to 23°C), the rate of disappearance was much more rapid, indicating biodegradation by the planktonic and microbial communities.

Application of 1,2,3- and 1,2,4-trichlorobenzene at a rate of 50 mg/g soil resulted in $^{14}CO_2$ evolution of more than 10% after several weeks (295). Addition of high levels of organic matter increased only 1,2,3-trichlorobenzene mineralization. Extracts of soil dosed with 1,2,3-trichlorobenzene yielded 2,3- and 2,6-dichlorophenol, and 3,4,5-trichlorophenol, while 1,2,4-trichlorobenzene samples yielded 2,4-, 2,5-, and 3,4-dichlorophenol.

A mixed population of soil bacteria precultured on benzene metabolized benzene and chlorobenzenes (20 to 200 mg/l) to chlorophenols (19). Mono through tetrachlorobenzenes were monohydroxylated at a position *ortho* to the chloride. No phenol was detected in media containing pentachlorobenzene. Diphenyls eventually were detected in the media.

A granular activated carbon column was seeded with a mixed culture of bacteria (primary sewage) and supplied with acetate as a carbon source (53). A biofilm was formed which after acclimation metabolized more than 90% of a 90 to 30 mg/l solution each of chlorobenzene, 1,2-di-, 1,4-di-, and 1,2,4-trichlorobenzene. Partial disappearance of 1,3-dichlorobenzene was noted. Studies with ^{14}C-1,4-dichlorobenzene confirmed that these substrates were mineralized to $^{14}CO_2$.

Hexachlorobenzene was applied to soybin plots at a rate resulting in 6 ppm in the upper 2 cm of the soil (28). The bulk of the material was volatilized with 24% remaining after 29 days and 3.4% after 19 months. The remaining material was unchanged substrate. The original application resulted in 9.11 ppm in the 2 to 4 cm layer which did not change during the course of the experiment.

Several experiments concerning the metabolism of dichlobenil in soils have shown that the major metabolite is 2,6-dichlorobenzamide (98, 314, 314a, 462a). Other metabolites appeared in trace quantities and could not be identified. After 81 days' incubation of the substrate ^{14}C-labeled in the nitrile group, only trace amounts of $^{14}CO_2$ or volatile ^{14}C-compounds could be recovered. More than 85% of the substrate added at 1 ppm remained unaltered.

Formation of 2,6-dichlorobenzamide in a pond water and sediment system was followed by a decrease in its concentration, indicating further transformation (314). Carbonyl-^{14}C-labeled 2,6-dichlorobenzamide was metabolized with 5.6% converted to $^{14}CO_2$ after 40 days and 28% recovered as metabolites.

Application of 1 mg/l dichlobenil to a farm pond resulted in initial sorption of the herbicide to the soil with subsequent disappearance from both water and soil (371). Less than 10% remained 90 days after treatment. Soils which had been pretreated with 2,6 dichloro-4-nitroaniline showed evolution of $^{14}CO_2$ when treated with this radiolabeled fungicide (189). No $^{14}CO_2$ evolution was noted in soils which had not been pretreated. Some unidentified metabolites were also seen. A pure culture of rod shaped bacteria was isolated which also converted the fungicide to $^{14}CO_2$.

Greenhouse soils treated with pentachloronitrobenzene were analyzed for the presence of metabolites (108). Products recovered included pentachloroaniline, pentachlorothioanisole, and tetrachloronitrobenzene. Hexachlorobenzene and pentachlorobenzene were detected but are known to be present as impurities in technical grade pentachloronitrobenzene.

Extracts of soils amended with 1,000 ppm pentachloronitrobenzene revealed the presence of pentachloroaniline but no polychloroazobenzenes (62). Soils treated periodically for 12 years still showed residual pentachloronitrobenzene and the technical grade impurities tetrachloronitrobenzene, pentachlorobenzene, hexachlorobenzene, pentachloroaniline and methylthiopentachlorobenzene when analyzed 1 to 5 years later (29).

Anaerobic flooded or moist Hagerstown silty clay loam was treated with 10 ppm pentachloronitrobenzene (319). After 40 days' incubation there was no evolution of $^{14}CO_2$ and only slight volatilization of the unchanged substrate. The main metabolite formed was pentachloroaniline, and lesser amounts of pentachlorothioanisole and pentachlorophenol were also detected.

Chloroneb (^{14}C-ring labeled) was applied to soil plots at the rate of 2 lb/acre (370). Another layer of soil was applied to the plots. After 12 months 40% of the original activity was recovered, of which 90% was unchanged substrate and 10% an unidentified metabolite.

SUMMARY

Chlorobenzenes containing less than five chlorines can be mineralized by acclimated populations under permissive conditions. High concentrations of these compounds are toxic to the bacteria. Most of the information regarding metabolism of the chlorobenzenes has come from studies with soil or mixed culture consortia. There is little information available on pathways of metabolism by pure cultures.

A single study indicates that hexachlorobenzene is not metabolized in soils. Pentachloronitrobenzene was not oxidized in a soil study, although under anaerobic conditions pentachloronitrobenzene is converted to several metabolites.

The available evidence indicates that chlorocatechols or chlorophenols are the primary degradation products of chlorobenzenes. These metabolites can be metabolized by mechanisms discussed in the appropriate sections.

MONS 026873

SECTION 9

CHLOROPHENOLS

The chlorophenols are used extensively as antifungal agents and are often applied as a preservative to freshly sawn lumber. They have found some use as herbicides and in food processing plants to control mold (99). Chlorophenols are also common degradation products of chlorophenoxy herbicides. The wood shavings from lumber processes have been used for litter in chicken houses and contain high levels of these chlorophenols, especially 2,3,4,6-tetrachlorophenol and pentachlorophenol (101). The chlorophenols degrade to volatile chloroanisoles via methylation of the oxygen atom and the resulting compounds have been implicated in the "musty taint" of chicken eggs and meat (101).

BACTERIAL METABOLISM OF CHLOROPHENOLS

Studies with *Arthrobacter* spp. have confirmed methylation as a mechanism for chlorophenol metabolism. Conversion of 2,4,6-trichlorophenol to 2,4,6-trichloroanisole has been demonstrated. Methylation is also the predominant reaction in the conversion of guaiacols (o-methoxyphenol) to veratroles (1,2-dimethoxybenzene) by *Arthrobacter* spp. (329). For example, 4-chloroguaiacol, 4,5-dichloroguaiacol, and 3,4,5-trichloroguaiacol are converted to the corresponding veratroles by dense cell suspensions of cultures grown on hydroxybenzoic acid (Figure 42). Low concentrations of catechols have also been found in the culture medium. An exception to this mechanism has been demonstrated in the metabolism by *Arthrobacter* sp. strain 1395 of 3,4,5-trichloroguaiacol to 3,4,5-trichlorosyringol, which requires hydroxylation and subsequent methylation of the previously unsubstituted carbon-6 (Figure 42). This latter compound is resistant to further degradation by this species.

An alternative mechanism results in the formation of chlorocatechols from chlorophenols. Cells of a *Nocardia* sp. grown on phenol metabolize 2-chlorophenol to 3-chlorocatechol, 3-chlorophenol to 4-chlorocatechol, and 4-chlorophenol to 4-chlorocatechol (406). Similarly, phenol-grown *Pseudomonas* sp. B13 or *Alcaligenes eutrophus* cells metabolize 2-chlorophenol to 3-chlorocatechol and 4-chlorophenol to 4-chlorocatechol (262). *Pseudomonas* sp. B13 can utilize 4-chlorophenol as the sole source of carbon and energy, and with this substrate can cometabolize 2-chlorophenol and 3-chlorophenol completely without accumulation of metabolites (262). A phenylcarbamate-degrading *Arthrobacter* sp. also metabolizes 4-chlorophenol to 4-chlorocatechol (466a). While the same initial enzyme is used for the first step in both phenol and 4-chlorophenol degradation, phenol-grown cells contain a muconate-lactonizing enzyme which has little activity for 3-chloromuconic acid, the metabolite of 4-chlorophenol (118).

Resting cell suspensions of *Achromobacter* sp. metabolize 4-chlorocatechol to 4-chloro-2-hydroxymuconic semialdehyde and 3,5-dichlorocatechol to 3,5-dichloro-2-hydroxymuconic semialdehyde via a unique catechol 1,6-oxygenase which differs from the more common catechol 2,3-oxygenase (Figure 43) (209). Neither product is metabolized further. *Pseudomonas putida* metabolizes 4-chlorophenol to 4-chlorocatechol, and then employs the *meta* cleavage enzyme catechol 2,3-dioxygenase to

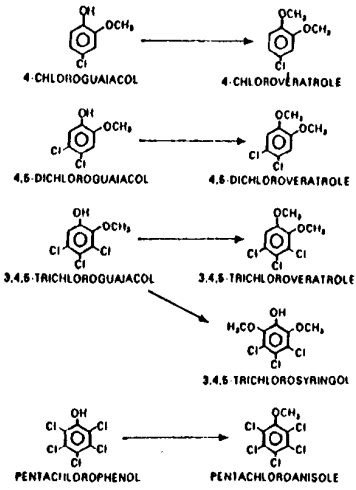

Figure 42 Methylation of chlorophenols by *Arthrobacter* spp. Adapted from Reference 329.

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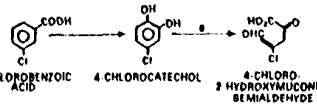
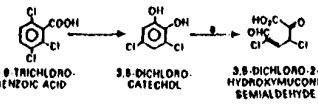


3-CHLOROBENZOIC ACID | 4-CHLOROCATECHOL | 4-CHLORO-2 HYDROXYMUCONIC SEMIALDEHYDE



2,3,6-TRICHLORO-BENZOIC ACID | 3,6-DICHLORO-CATECHOL | 3,6-DICHLORO-2-HYDROXYMUCONIC SEMIALDEHYDE

a = catechol 1,6-oxygenase

Figure 43 Cometabolism of chlorocatechols via catechol 1,6 oxygenase by resting cell suspensions of *Achromobacter* sp
Adapted from Reference 206

product 2-hydroxy-5-chloromuconic semialdehyde, which accumulates to 10% of the starting substrate. Free chloride amounting to 85% of the substrate is recovered, although a pathway for liberation of the chloride has not been elucidated (311).

Two species of bacteria were utilized to produce a genetically constructed strain with altered ability to metabolize aromatic compounds (308). *Pseudomonas* sp. B13, with ability to metabolize chlorophenols, and *Alcaligenes* sp. A7, which degrades phenol by the *meta* path and has no activity against chlorophenols, were combined to produce a mutant (designated A7-2) which utilizes phenol by the *ortho* path and also metabolizes 2-, 3-, and 4-chlorophenol as well as 3-chlorobenzoic acid. Three enzymes were isolated, pyrocatechase II and cycloisomerase III, which have high activity for chlorinated substrates, and a third enzyme which functions exclusively in the chloroaromatic pathway to perform a dehalogenating cycloisomerization of chloromuconic acids (Figure 44).

The 2,4,5-trichlorophenoxyacetic acid-degrading strain of *P. cepacia* AC1100 can dechlorinate a wide variety of chlorophenols (233). Resting cell suspensions can dechlorinate within 3 hours at 0.1 mM substrate concentration, the following chlorophenols: 2,3-, 2,4-, and 2,5-dichlorophenol, 2,3,4- and 2,4,5-trichlorophenol, 2,3,4,6- and 2,3,5,6-tetrachlorophenol and pentachlorophenol. The strain has less activity against 2,4,6-trichlorophenol and 2,3,4,5-tetrachlorophenol and metabolizes 2,6- and 3,5-dichlorophenol and 2,3,5-, 2,3,6-, and 3,4,5-trichlorophenol poorly.

METABOLISM OF CHLOROPHENOLS BY FUNGI

Fungal metabolism of chlorophenols often involves methylation in a manner analogous to that of bacteria (172). A study of 116 fungal isolates from chicken house litter revealed that 59% produce 2,3,4,6-tetrachloroanisole from 2,3,4,6-tetrachlorophenol at conversion efficiencies of from 1 to 83%. Flask cultures in this study were sealed and incubated for five days, so the transformation may have occurred under aerobic or anaerobic conditions. The fungi demonstrating this ability include *Aspergillus* spp., *Paecilomyces* spp., *Penicillium* spp., and *Scopulariopsis* spp. (100, 101, 172). Some strains metabolize 2,3,4,6-tetrachlorophenol without formation of the anisole, suggesting an alternate mechanism for chlorophenol metabolism (172). The yeast *Rhodotorula glutinis* grown on phenol converts 3-chlorophenol to 4-chlorocatechol (448).

There is little additional information available on fungal metabolism of chlorophenols, although there is evidence to indicate that a *Penicillium* sp. produces 2,4-dichlorophenol as a natural metabolite (8).

METABOLISM OF CHLOROPHENOLS BY MIXED MICROBIAL CULTURES

Soil populations exhibited enhanced rates of metabolism of 2-chlorophenol after prior acclimation by soil percolation (447). Following an initial decrease in 4-chlorophenol concentration during soil percolation, however, additional applications of that substrate were not metabolized. In other experiments, soil inocula mediated the complete disappearance of 4-chlorophenol within 25 days, although neither 2- nor 3-chlorophenol was degraded during that time (193).

Wastewater sludge supernatant liquid required 14 to 25 days for complete disappearance of 16 mg/l 2-chlorophenol and 3-chlorophenol, although 4-chlorophenol disappeared within 14 days (193). Disappearance of 1 mg/l 2- and 4-chlorophenol was faster in polluted acclimated or nonacclimated river water than in diluted sewage inocula experiments (139). The substrates were not degraded by the sewage microorganisms after 25 days, while less than 15 days was required for disappearance of the substrates from river water.

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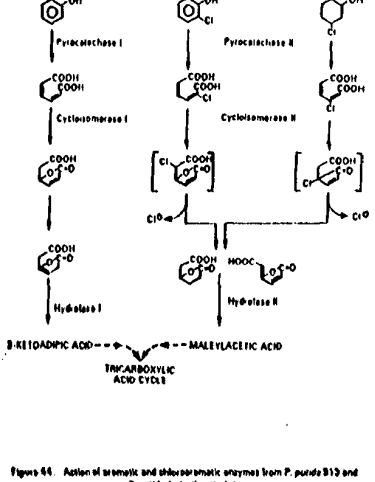

Figure 44. Action of aromatic and chloroaromatic enzymes from *P. putida* B13 and *P. putida* derivative strains
Adapted from Reference 201

An acclimated sludge culture exposed to 100 mg/l substrate was able to metabolize the following compounds within 5 days with chloride release as noted (218): 2-, 3-, or 4-chlorophenol 100%, 2,4-dichlorophenol, 100%, 2,5-dichlorophenol, 16%, 2,4,6-trichlorophenol, 75%, and sodium pentachlorophenolate, 0%.

The fate of the monochlorophenols when incubated anaerobically with a 10% municipal sewage sludge inoculum was determined (54). At a concentration of 50 mg/l, 2-chlorophenol required 3 weeks, 3-chlorophenol 7 weeks, and 4-chlorophenol 16 weeks for complete disappearance. Mineralization was monitored by measuring net methane production, and results indicated nearly complete mineralization of 2-chlorophenol. Methane was produced from 3-chlorophenol after a lag period of 5 weeks, and 4-chlorophenol was not mineralized. During the degradation of 2-chlorophenol, phenol was recovered as the initial metabolite, followed by methane production. This is consistent with other studies in which dechlorination was shown to be the initial step in the reductive metabolism of 3-chlorobenzoic acids (417).

Fresh undiluted sludge samples also reductively dechlorinated several dichlorophenols with removal of the *ortho* chloride, such that 2,6-dichlorophenol was converted to 2-chlorophenol, 2,3- and 2,5-dichlorophenol to 3-chlorophenol, and 2,4-dichlorophenol to 4-chlorophenol (53). Two isomers which lack an *ortho* substituent, 3,4- and 3,5-dichlorophenol, were not metabolized during the 6 weeks of the experiment.

Undiluted sludge samples were acclimated to the monochlorophenols by repeated inoculations with 20 mg/l substrate, until the cultures could metabolize 25 mg/l within 1 week (53). Each acclimated sludge culture was then challenged with a 20 mg/l solution of other chlorophenols. Cultures acclimated to 2-chlorophenol metabolized both 2- and 4-chlorophenol at equal rates and 2,4-dichlorophenol somewhat more slowly. However, 3-, 2,3-di-, 2,5-di-, and 2,6-dichlorophenol were not metabolized. Sludge inocula acclimated to 3-chlorophenol also metabolized 4-chlorophenol, and 3,4- and 3,5-dichlorophenol but not 2-chlorophenol or 2,3- or 2,5-dichlorophenol. Acclimation to 4-chlorophenol also permitted metabolism of 3-chlorophenol and at much slower rates 2-chlorophenol and 2,6- and 3,4-dichlorophenol as well. The population acclimated to 4-chlorophenol seemed to have a broader substrate range than the other acclimated populations. Incubation of 2- and 4-chlorophenol-acclimated sludge inocula with uniformly ring ^{14}C-labeled 2-and 4-chlorophenol and 2,4-dichlorophenol showed that in all cases nearly complete mineralization of the substrates to ^{14}C-methane and $^{14}CO_2$ occurred.

Experiments in which tainted litter was incubated with sawdust, pentachlorophenol, and 2,3,4,6-tetrachlorophenol, showed nearly quantitative conversion of the latter substrate to 2,3,4,6-tetrachloroanisole (101). There was virtually no conversion in the absence of the litter inoculum. Pentachlorophenol was 50% converted to pentachloroanisole after 29 days.

Aspergillus sydowi, *Scopulariopsis brevicaulis*, and a *Penicillium* sp. were isolated from the litter and each species was also found to be capable of the above substrate conversions.

SUMMARY

Many of the chlorophenol compounds have been shown to be metabolizable by pure cultures or mixed natural populations of microorganisms both aerobically and anerobically. In most cases complete mineralization occurs. However, the rate of disappearance of the isomers vary widely, depending upon degree of acclimation of the population and other environmental factors. Mixed cultures seem to be required for complete mineralization of the chlorophenols.

MONS 026876

SECTION 10
PENTACHLOROPHENOL

Pentachlorophenol (PCP) is widely used in a variety of agricultural and industrial applications as a fungicide, bactericide, insecticide, herbicide, and molluscicide (36, 91, 466). It is most widely used in the United States and elsewhere for wood preservation, both for newly cut timber and for slime control in pulp and paper production. PCP is usually used as a 5% solution in petroleum solvents or as the water-soluble sodium or potassium salt.

BACTERIAL METABOLISM OF PCP

In spite of its use as a fungicide and bactericide, PCP is metabolizable by a variety of microorganisms. Reports of decomposition of PCP in rice paddy soil and other soils or aquatic environments (90, 217, 253, 266, 282, 320, 354) were followed by experiments with consortia and pure cultures of bacteria which demonstrated chloride release and $^{14}CO_2$ formation from labeled PCP (89, 354, 369, 411, 423, 459). However, few studies have identified metabolites arising from pure culture metabolism of PCP.

Cultures of *Pseudomonas* spp. produce both tetrachlorocatechol and tetrachlorohydroquinone from PCP (Figure 45) (423, 424). These are metabolized rapidly soon after they are produced. There is no evidence of methylation of PCP to form pentachloroanisole. Amino acid analyses with hydrolysates of bacterial cells indicate incorporation of ^{14}C derived from PCP into the cell constituents (423). Pentachlorophenol is metabolized by *Arthrobacter* spp. to pentachloroanisole at levels of less than 0.5% conversion at approximately 44 mM substrate concentration (325).

A bacterium identified as *Mycobacterium* sp. which cannot use PCP as a growth substrate methylates PCP to pentachloroanisole (424, 425). Other methylations by washed cell suspensions of this culture result in the formation of tetrachloro-1,2-dimethoxybenzene and tetrachloro-1,4-dimethoxybenzene. Additional metabolites include tetrachlorocatechol, tetrachlorohydroquinone, tetrachloro-2-methoxyphenol and tetrachloro-4-methoxyphenol. The formation of these products indicates that the main metabolite is the methylated derivative of PCP, but in addition, PCP is hydroxylated in the *ortho* or *para* positions and subsequently methylated at these positions. As pentachloroanisole is less toxic to the bacteria than PCP, methylation is suggested as a detoxification mechanism (424). The methylation of PCP was also demonstrated in cell-free systems of *Mycobacterium* sp. (422). The mechanism appears to involve the enzymes that transfer the methyl group from S-adenosylmethionine to the hydroxyl groups of these substrates.

A naphthyric soil corynebacterium was isolated which utilizes PCP as a sole source of carbon and energy for growth (369, 89). By measuring $^{14}CO_2$ evolution, the conversion rate was calculated to be 10 mg PCP per mg of dry cell weight per hour (90). Cells of this isolate, referred to as KC3, when grown on PCP also show immediate uptake, as measured by ultraviolet spectrophotometry, of a wide variety of chlorophenol isomers, including 2,3,5-, 2,3,6-, and 2,4,6-trichlorophenol, 2,3,4,6-, and 2,3,5,6-tetrachlorophenol and pentachlorophenol (89). Uptake of 3,4,5-tri-

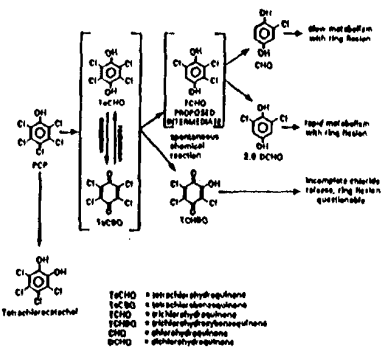

Figure 48. Proposed pathway for pentachlorophenol (PCP) metabolism by the bacterial cultures KC-3 and by *Pseudomonas* sp.
Adapted from References 369, 423

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chlorophenol and 2,3,4,5-tetrachlorophenol is delayed. While the *para* and *meta* monochlorophenols are oxidized, as measured by manometric techniques, the *ortho* isomer is oxidized poorly and phenol itself not at all (89). Chloride is not released to an appreciable degree from any of the monochlorophenols. In general, release of chloride is greatest from the 2,6-substituted di-, tri-, and tetrachlorophenols. Isomers with chloride substitutions in other positions are less well attacked by culture KC3.

Substantial investigations into the metabolism of PCP by the KC3 isolate failed to show accumulation of metabolites in the medium. However, mutants were developed which failed to grow in a PCP-minimal salts medium (369). One of these mutants, designated ER-47, converts PCP primarily to 2,6-dichlorohydroquinone. KC3 parent cells adapted to PCP release chloride from 2,6-dichlorohydroquinone rapidly and without a lag. A trace of monochlorohydroquinone also appears but its role in the pathway of biological degradation is uncertain, as it is only slowly attacked by parent KC3 cells. A second mutant, ER-7, accumulates several metabolites, including tetrachlorohydroquinone, tetrachlorobenzoquinone, and trichlorohydroxybenzoquinone (Figure 36). These three products are converted rapidly and spontaneously from the hydroquinone through the benzoquinone to the more stable hydroxybenzoquinone. The latter product is metabolized by KC3 but dechlorination is not complete and the ring is not ruptured. Tetrachlorohydroquinone is rapidly metabolized to 2,6-dichlorohydroquinone but this metabolic transformation must compete with the rapid spontaneous transformation to the trichlorohydroxybenzoquinone.

Another series of experiments explored the metabolism of sodium pentachlorophenate by a wide variety of bacteria metabolically active for phenols, chlorophenols or chlorobenzenes (379). Metabolites were identified by detecting acetyl derivatives using combined gas chromatography and mass spectrometry. With few exceptions metabolites occurred in concentrations of less than one percent of the starting material. Reported metabolites included PCP-acetate, pentachloroanisole, tetrachloroanisoles, tetrachlorophenols, and tetrachlorodihydroxybenzenes.

FUNGAL METABOLISM OF PCP

The role of fungi in detoxifying PCP has been studied to some degree. Fungi associated with PCP-treated wood reduce PCP to a less toxic metabolite (122, 208). Three *Trichoderma* spp. metabolized sodium pentachlorophenate (Na PCP) within 2 weeks in a malt extract medium as well as on wood treated with Na-PCP (98). Pentachloroanisole was detected in the culture medium of *T. virgatum* after 5 days' incubation at levels corresponding to 10-20% of the starting Na-PCP. It is unclear whether this is an integral step of the pathway or whether methylation is a side reaction (98).

During a comparison of the growth of several species of fungi on PCP, *Trichoderma* spp. were the only ones which reduced PCP levels after 12 days' incubation at 5 to 10 mg/l concentration (98). Fungi inactive against PCP were *Cryptococcus fragrans*, *C. pilifera*, *Oraphium* spp., and *Penicillium* sp. Another experiment showed that *Trichoderma viride* and *Conlophora puteana* reduced the concentration of PCP in treated wood blocks, although *C. puteana* was much more sensitive to PCP in liquid culture (441). It was postulated that the presence of an alternative substrate of wood or the binding capacity of PCP to wood reduced exposure of the fungus to below the toxic level, thus permitting metabolism of PCP.

DISAPPEARANCE OF PCP IN ENVIRONMENTAL SAMPLES

The standard procedure of applying PCP in a carrier solvent to wood products has complicated subsequent analyses of disappearance and biodegradability. A carrier

which is too volatile will carry PCP with it as it evaporates (277). A carrier which retains liquidity at ambient temperatures will bleed from the wood until an equilibrium is established. PCP in solution will be carried along, reducing the final concentration in the wood and increasing the amount reaching the surrounding environment.

Extraction and analysis procedures are also subject to error, including incomplete extraction due to poor choice of extracting solvent, and use of procedures which extract pure PCP but not polymerized molecules, which may be present in technical grade PCP at levels as high as 18 percent (277).

Degradation of PCP with release of chloride and CO_2 has been demonstrated in a number of environments. In a waste stream continuously contaminated with PCP, acclimation occurred after 3 weeks (354). The microflora, particularly the attached bacteria, metabolized up to 0.63 ppm influent concentration. Pure cultures were isolated from the waste stream which were capable of mineralizing 100 mg/l PCP in 90 hr with almost complete chloride release.

A soil perfusion apparatus using rice paddy soil effected disappearance of PCP with more than 90% liberation of chloride (459). A *Pseudomonas* sp. isolated from the enrichment culture degraded 40 mg/l PCP in 10 days with complete chloride release.

PCP added to moist garden soil at 150 to 200 mg/l soil-water concentration was 25% metabolized after 12 days when the experiment was conducted using outdoor shaded test plots (128). When a culture of *Arthrobacter* sp. ATCC 11790 was mixed into the soil, about 85% of the PCP disappeared during the same time. Under laboratory conditions addition of the bacterial culture reduced the half-life for PCP disappearance from 12 - 14 days to 1 day. This *Arthrobacter* sp. utilizes PCP as the sole source of carbon and energy with complete release of chloride (130).

Comparisons of aerobic and anaerobic metabolism of PCP have shown that aerobic metabolism is much more efficient (282). Enrichment cultures established in fermentors fed with 2 mg/l PCP revealed a half-life of 0.36 days under aerobic conditions and 192 days under anaerobic conditions. Addition of glucose or 4-chlorophenol as cometabolic substrates depressed the rate of PCP metabolism.

Soils treated with 10 mg/l PCP and incubated for 24 days under aerobic conditions revealed considerable loss of ^{14}C-labeled material from the system (330). Of 59% total recovered material, 51% was identified as pentachloroanisole. Volatile products and CO_2 were not measured. In the same system maintained under anaerobic conditions, 3% of the material was converted to metabolites and no $^{14}CO_2$ was detected. Metabolites included about 5% pentachloroanisole and lesser amounts of 2,3,6-trichlorophenol, 2,3,4,5-tetrachlorophenol and 2,3,5,6-tetrachlorophenol.

PCP applied to flooded paddy soil, simulating anaerobic conditions, was metabolized after 3 weeks to the following products: 3-chlorophenol, 2,4-, and 2,5-dichlorophenol, 2,3,5-, and 2,4,5-trichlorophenol, and 2,3,4,5-, 2,3,4,6-, and 2,3,5,6-tetrachloroanisole (217).

The rate of PCP metabolism in 11 soils was found to be related to the organic matter content of the soils (266). Degradation products included a mixture of tri- and tetrachlorophenols.

A major PCP spill on the Mississippi River Gulf Outlet left PCP levels as high as 1.60 mg/g in the sediment (109). At 18 months there was no detectable PCP in the sediment. Studies arising from the spill indicated that the degradation rates increased with increasing sediment redox potential. Maximum degradation occurred at pH 8 at +500 mV. Less degradation occurred at pH 9 and at pH less than 8.

PCP-degrading bacteria have been isolated both from polluted sites and from sites not known to be contaminated with PCP (419). An enrichment consortium established under continuous culture conditions became adapted to metabolize 500

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mg/l PCP. *Arthrobacter* sp. strain NC was isolated from the culture and metabolized 100 mg/l PCP until the pH decreased to 6.15. Upon adjustment to pH 7.1 the residual PCP was metabolized. The strain was capable of growth at pH 6.0 upon other substrates. Other experiments showed a correlation of toxicity with the acid form of PCP.

SUMMARY

In summary, there is evidence that PCP is attacked by bacteria and fungi cometabolically or as sole source for growth with release of chloride and CO_2. The pathway involves dechlorination and hydroxylation either *ortho* or *para* to the phenolic hydroxyl group, forming a catechol or a quinone, respectively. However, the mechanism of this process is not understood and the enzymes involved have not been isolated. Further, the steps of the pathway leading to carbon incorporation into cell contents and CO_2 formation have not been elucidated. In fungi, methylation has been detected as a prominent metabolic process, but its role in PCP degradation has not been established.

SECTION II
CHLOROPHENOXY AND CHLOROPHENYL HERBICIDES

Compounds with an arylcarboxylic acid parent structure have plant growth-regulating properties. They produce physiological effects such as morphogenic abnormalities, promote the rooting of cuttings, and aid in setting fruit in the absence of pollination (445).

There are three principal chlorine-substituted phenoxyacetic acids used widely as herbicides, (2,4-dichlorophenoxy)acetic acid (2,4-D), (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T), and (4-chloro-2-methylphenoxy)acetic acid (MCPA). They are selective against broadleaved weeds and woody broadleaved plants and are commonly used in lawns, grass pastures, and cereal crops (293). Other phenoxyalkanoic acids are useful in controlling weed species which are resistant to the phenoxyacetic acids. The phenoxybutyric acid herbicides have very low toxicity to plant species such as legumes which are damaged by exposure to phenoxyacetic acids. After application, they are activated by target plants (weeds) which β-oxidize them to their corresponding toxic phenoxyacetic acids. Other herbicides in this class include the phenoxyethyl esters, which are applied when deep soil penetration is required or in noncrop areas.

The phenoxyalkyl acid herbicides are detoxified in soils and aquatic environments due to microbial action (13, 60, 111, 293, 343, 376, 377). Bacteria and fungi which metabolize the herbicides have been isolated from soils (Table 6). The products of microbial metabolism may be phytotoxic or they may result in inactivation of the herbicide (290, 292). These products may be similar to those formed as a consequence of plant metabolism, such as 2,4-dichlorophenol from 2,4-D and 3,4,5-trichlorophenol from 2,4,5-T. There is evidence that 2,4,5-trichlorophenol, rather than 2,4,5-T, is the active agent which damages the plants (283). Some processes of microorganisms prevent activation of the herbicides rather than actually detoxifying them (3). For this reason the pathways by which microorganisms metabolize the phenoxy herbicides are of importance in determining the choice of herbicide for a given application.

2,4-D

The herbicide most extensively studied has been 2,4-D. Bacteria including *Pseudomonas* sp., *Arthrobacter* sp., *Achromobacter* sp., *Mycoplana* sp., and *Flavobacterium peregrinum*, cleave the molecule at the ether linkage between the oxygen and the aliphatic side chain to form glyoxylic acid and 2,4-dichlorophenol (Figure 46) (31, 32, 40, 152, 295, 296, 412, 433, 434, 451). The latter compound is metabolized to 3,5-dichlorocatechol, *cis,cis* 2,4-dichloromuconic acid, 2-chloro 4-carboxymethylene but-2-enolide and 2-chloromaleylacetic acid (46, 393). Chloromaleylacetic acid is degraded further to succinic acid via 2-chloro-4 ketoadipic acid and chlorosuccinic acid. The entire pathway has also been demonstrated using cell free extracts of *Arthrobacter* sp. (40, 44, 126, 142, 393, 434). Cleavage of the ether-oxygen bond in phenoxyacetic acid to form the phenol has been proven to

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occur between the aliphatic side chain and the ether-oxygen in experiments with *Arthrobacter* sp. using 18O2 (198).

TABLE 4. MICROORGANISMS THAT METABOLIZE PHENOXY ACIDS

Phenoxy acid	Organism	References
2,4-D	*Achromobacter* sp.	31, 32, 412, 414
	Arthrobacter sp.	45, 126, 285, 434, 44, 46, 284, 288
	Arthrobacter globiformis	283
	Corynebacterium sp.	375
	Flavobacterium peregrinum	405a, 411a, 412, 414
	Mycoplana sp.	451
	Nocardia sp.	283
	Pseudomonas sp.	148, 169, 170, 171, 142, 162, 171a
	Sporocytophaga congregata (*F. aquatile*)	226
	Streptomyces viridochromogenes	51a
2,6-D	*Achromobacter* sp.	31, 32
2-Chlorophenoxyacetic acid	*Achromobacter* sp.	31, 32, 412, 414
	Arthrobacter sp.	45, 284, 285, 286, 44, 46, 126, 434
	F. peregrinum	412, 414
4-Chlorophenoxyacetic acid	*Pseudomonas* sp.	142, 148, 162
	Achromobacter sp.	31, 32, 412, 414
	Arthrobacter sp.	45, 284, 286, 288
	F. peregrinum	412, 414
	Mycoplana sp.	451
	Nocardia sp.	283
	Pseudomonas sp.	126, 142, 143, 148, 162, 169, 170, 171, 171a

(continued)

TABLE 4. (continued)

MCPA	*Achromobacter* sp.	31, 32, 412, 414
	Arthrobacter sp.	45, 284, 285, 286, 44, 46, 126, 434
	F. peregrinum	45
	Mycoplana sp.	451
	Pseudomonas sp.	142, 148, 152, 169, 170, 171, 171a
2,4,5-T	*Achromobacter* sp.	31, 32
	Brevibacterium sp.	207
	Mycoplana sp.	451
	S. viridochromogenes	51a

The enzymes mediating the degradation of 2,4-D are relatively nonspecific. Oxygen and a reduced pyridine nucleotide (NADH or NADPH) are required, indicating that the enzyme(s) may be a mixed function oxidase (44). The broad specificity of the enzymes is reflected in the findings that *Pseudomonas* sp. cells grown on 2,4-D also metabolize 4-hydroxyphenoxyacetic acid and phenoxyacetic acid (285). *Arthrobacter* sp. cell-free extracts grown on 2,4-D also oxidize MCPA, 2,4-dichlorophenol, 4-chloro-2-methylphenol and 3,5-dichlorophenol. Neither 6-hydroxy-2,4-dichlorophenol nor 2,4-dichloroanisole is oxidized, indicating that neither is an intermediate in 2,4-D metabolism (284). The enzyme extracts also convert 2-chlorophenoxyacetic acid to 2-chlorophenol and 4-chlorophenoxyacetic acid to 4-chlorophenol which subsequently forms 4-chlorocatechol. Catechol is converted to *cis,cis*-muconic acid and 4-chlorocatechol to *cis,cis*-3-chloromuconic acid. Chloride is released from 3-chloromuconic acid to form 4-carboxymethylene-but-2-enolide, maleylacetic acid and subsequently succinic acid (Figure 47) (40, 44, 434). This pathway is analogous to that demonstrated for 3,5-dichlorocatechol during 2,4-D degradation.

A *Corynebacterium* sp. isolated from soil by enrichment culture metabolized 2,4-D with nearly complete chloride release after 48 hours (375). An application rate to soil of 3,000 ppm was metabolized but neither growth nor metabolism was noted upon application of 3,500 ppm. No metabolites were seen during the incubation period.

A bacterial strain tentatively identified as *F. peregrinum* was isolated from enrichment culture with 2,4-D in soil (414). This strain metabolized 100 ppm 2,4-D in 25 days and upon addition of 0.1% yeast extract metabolized 0.1% 2,4-D in 12 to 16 days. Chloride release was estimated at 70% of that in 2,4-D within 39 days.

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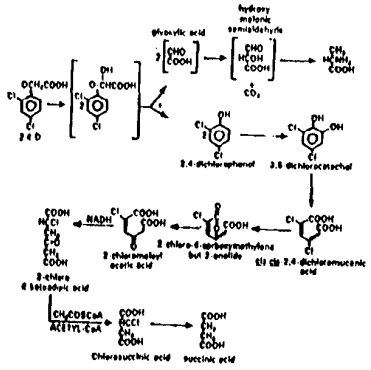

Figure 46 Bacterial metabolism of 2,4 D
Adapted from References 46, 142, 433

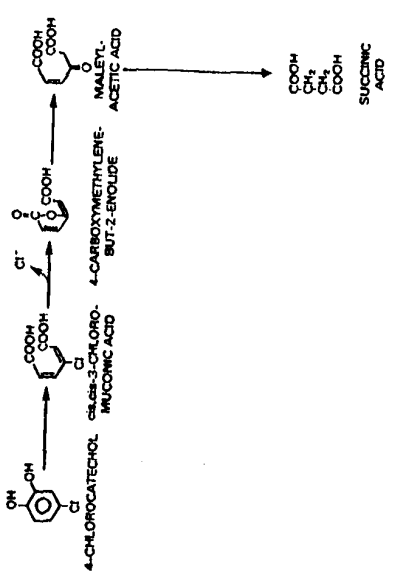

Figure 47 Metabolism of 4-chlorocatechol by Arthrobacter sp
Adapted from References 46, 434.

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Flavobacterium aquatile metabolized 0.01% 2,4-D in sterile soil, nonsterile soil, and on solid agar plates, but not in a soil extract medium or on semisolid agar (275). MCPA was not metabolized in sterile soil. In similar experiments, *Corynebacterium* sp. metabolized both herbicides in sterile soil and on solid agar.

A number of bacteria were isolated by enrichment culture from sewage or soil amended with 2,4-D or 2,4,5-T (376). None utilized either substrate as the sole source of carbon. Forty-one of 52 strains cometabolized 2,4-D only while 19 strains utilized both 2,4-D and 2,4,5-T. Experiments with these 19 isolates incubated with 2,4,5-T in nutrient medium showed that 12 isolates produced chloride ion and 8 produced a phenolic compound with or without concomitant production of free chloride.

MCPA

The metabolism of MCPA has been studied extensively in cultures of *Pseudomonas* sp. NCIB 9340 and *Arthrobacter* sp. (45, 46, 169, 170, 412) as well as cell-free extracts of *Arthrobacter* sp. (40). Initial attack on the molecule results in oxidative cleavage of the ether linkage to form a phenol and glyoxylic acid (Figure 48) (169). The phenol thus formed is 4-chloro-2-methylphenol (5-chloro-o-cresol). In *Pseudomonas* sp. NCIB 9340 this product is metabolized to 5-chloro-3-methylcatechol and then to *cis,cis*-4-chloro-3-methylmuconic acid. The chloride ion is lost upon lactonization by dehydrochlorination to form 4-carboxymethylene-2-methyl-2,3-butenolide and subsequently 4-hydroxy-3-methylmuconic acid (170). Formation of the two double bonds of the lactone is an unusual feature in the metabolism of aromatic compounds by bacteria.

The three enzymes mediating the conversion of 5-chloro-3-methylcatechol to 4-hydroxy-3-methylmuconic acid have been isolated (171). These enzymes, responsible for ring cleavage, lactonization, and delactonization, confirm that lactonization and dehalogenation is a one-step process.

The enzymes which attack MCPA are also relatively nonspecific. Oxygen as well as NADH or NADPH are required for enzymatic activity. *F. peregrinum* cells grown in the presence of MCPA are induced to oxidize 2,4-D as detected by manometric techniques, although MCPA is not metabolized (413). A strain thought to be an *Achromobacter* sp., isolated from enrichment culture with MCPA, metabolized 50 mg/l MCPA or 2,4-D with addition of 0.05% yeast extract in 4 days (414). Experiments showed a faster rate of oxygen uptake with 2,4-D than MCPA although the organism was cultured on MCPA. Nonacclimated cultures showed no oxygen uptake with either MCPA or 2,4-D.

2,4,5-T

The tri-chlorinated phenoxy herbicide, 2,4,5-T, has proven much more difficult to degrade. The utilization of 2,4,5-T by a *Pseudomonas* sp. appears not to be plasmid encoded, unlike 2,4-D and MCPA metabolism (154). The herbicide is metabolized to 2,4,5-trichlorophenol by both *P. fluorescens* and *P. cepacia* AC1100 (349, 377). *Brevibacterium* sp. when grown on benzoic acid converts 2,4,5-T to 3,5-dichlorocatechol without a lag, indicating removal of one chloride ion (207). This latter compound can be metabolized (Figure 46).

Pseudomonas cepacia AC1100 is a strain modified in the laboratory which utilizes 2,4,5-T as the sole source of carbon and energy for growth (249). More than 97% of a 1 g/l solution was degraded within 6 days with stoichiometric chloride release. In 2 hr, resting cells promoted 50% disappearance of 2,4,5-T although only 15% chloride release was evident. Within 24 hours, there was complete substrate disappearance with 94% chloride release. Resting cells also mediated release of more than 50% of the chloride from 2,4,5-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachloro-

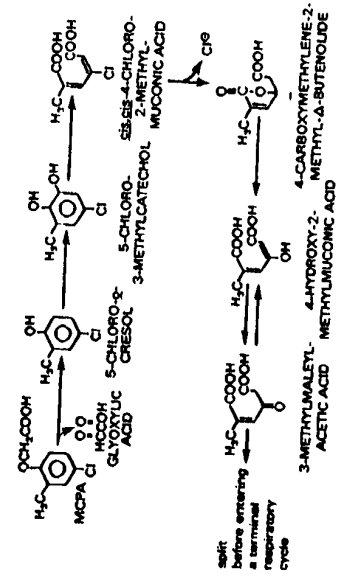

Figure 48 Pathway for MCPA metabolism by *Pseudomonas* sp. NCIB 9340

Adapted from References 169, 170.

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phenol. Additionally, resting cells were also shown by oxygen electrode determination to oxidize 2,4-D at relatively high rates but not (2,4,5-trichloro-phenoxy) propionic acid. Phenoxyacetic acid was oxidized at low rates only. When inoculated into soil *P. cepacia* AC1100 mediated 95% chloride release of a 1 mg/g application of 2,4,5-T within 1 week (85). The optimum conditions were 25% moisture content at 30°C.

4-CHLOROPHENOXYACETIC ACID

The degradation of 4-chlorophenoxyacetic acid by a soil pseudomonad proceeds through 4-chloro-2-hydroxyphenoxyacetic acid to 4-chlorocatechol (Figure 49) (143). This organism also metabolizes 4-chlorocatechol to cis-cis-3-chloromuconic acid. While direct evidence for subsequent steps in the pathway was not obtained, the culture medium did contain a lactone which is analogous to that described for *Arthrobacter* sp. The pseudomonad was not induced to grow on 4-chlorophenol and this product was not found in the culture medium, indicating that this compound is not an intermediate in 4-chlorophenoxyacetic acid metabolism.

An unidentified gram-negative organism isolated from soil also metabolizes 4-chlorophenoxyacetic acid through 4-chloro-2-hydroxyphenoxyacetic acid and 4-chlorocatechol, as determined by simultaneous adaptation experiments (146). The same technique was used to determine that *Achromobacter* sp. grown on 4-chlorophenoxyacetic acid immediately oxidizes 4-chloro-2-hydroxyphenoxyacetic acid, 4-chlorocatechol, and catechol, but not 4-chlorophenol (414). The first step of 4-chlorophenoxyacetic acid metabolism in these pathways is hydroxylation of the ring, followed by ether cleavage. This is in contrast to *Arthrobacter* sp. metabolism of several chlorophenoxy compounds in which cleavage of the ether linkage is the primary step yielding 4-chlorophenol (284).

OTHER PHENOXY HERBICIDES

The metabolism of phenoxy herbicides with longer aliphatic side chains has also been studied. Two mechanisms appear to mediate degradation of these compounds. The primary mechanism is β-oxidation, a mechanism common to plants (283). Evidence for β-oxidation comes from observations with cultures of *Flavobacterium* sp. which were grown on 4-(2,4-dichlorophenoxy)butyric acid (4-(2,4-D)B) and then tested for products arising from oxidation of higher carbon-number homologs. Phenols were detected in all cases but more so with compounds containing an odd number of carbons in the side chain (291). When the side chain contained an odd number of carbons, primarily 2,4-dichlorophenol was recovered, while 2,4-D was recovered from metabolism of compounds with an even number of carbons in the side chain. Extracts of these cultures also contained free aliphatic acids which indicates ether cleavage, a second mechanism of phenoxy acid degradation similar to that shown for other herbicides (290, 292). This organism when grown on 4-(2,4-D)B also oxidizes (as determined by manometric techniques) 3-(2,4-D)propionic acid, 4-(4-chlorophenoxy)butyric acid, 4-(4-methyl-2-chlorophenoxy)butyric acid, 2,4-dichlorophenol, and 4-chlorocatechol but not 2,4-D (60, 292). The failure to oxidize 2,4-D argues against β-oxidation as the controlling mechanism for these degradations as the product of β-oxidation of 4-(2,4-D)B would be 2,4-D (292). The enzymes involved in these oxidations are adaptive rather than constitutive. The oxidation of these substrates led to the proposal of a pathway for the degradation of 4(2,4-D)B through 2,4-dichlorophenol to 4-chlorocatechol with loss of one chloride ion and subsequent metabolism of 4-chlorocatechol by established pathways (Figure 50). The side chain is cleaved initially at the ether linkage and then is metabolized by β-oxidation (292).

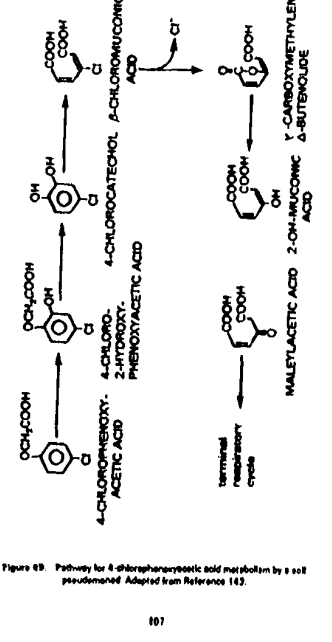

Figure 49. Pathway for 4-chlorophenoxyacetic acid metabolism by a soil pseudomonad. Adapted from Reference 143.

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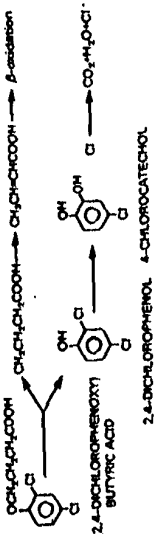

Figure 80 Pathway for the metabolism of 4-(2,4-dichlorophenoxy) butyric acid by *Flavobacterium* sp

Adapted from Reference 292

Two species of *Nocardia*, *N. opaca* strain 1b and *Nocardia* sp. strain E2 have been shown to use β-oxidation for degradation of phenoxy acid homologs above the acetic congener (461). The corresponding chlorinated phenols are generated from (3-chlorophenoxy)propionic and (4-chlorophenoxy)propionic acids. The six-carbon homolog (2-chlorophenoxy)caproic acid is metabolized to (2-chlorophenoxy)butyric acid and (4-methyl-2-chlorophenoxy)caproic acid is metabolized to (4-methyl-2-chlorophenoxy)butyric acid. Similarly, (2,4-dichlorophenoxy)caproic acid is metabolized to (2,4-dichlorophenoxy)butyric acid. Metabolism of 4-(4-chlorophenoxy)butyric acid to 3-hydroxy-4-(4-chlorophenoxy)butyric acid is followed by metabolic conversion to (4-chlorophenoxy)propionic acid. A similar pathway is followed by 4-(3-chlorophenoxy)butyric acid (462). These studies with strain 1b showed that 3-hydroxy acid intermediates appear during the metabolism of all the even-numbered butyric acids.

An alternative mechanism has been noted in *N. coeliaca* (432). Although β-oxidation is operative in this organism, α-oxidation operates in the metabolism of compounds with 10 or 11 side-chain carbons (phenoxydecanoic acid and phenoxyundecanoic acid). This process, demonstrated for monochlorinated pinderivatives, involves two enzymes: (a) a peroxidase catalyzing peroxidative decarboxylation of the fatty acid to yield CO_2 and the fatty aldehyde with one less carbon, and (b) a dehydrogenase catalyzing oxidation of the aldehyde to the corresponding acid.

The salt of phenoxy compound, sodium 2-(2,4-dichlorophenoxy)ethyl sulfate is widely used in commercial formulations and is metabolized to 2-(2,4-dichlorophenoxy)ethanol by both *P. putida* 1-LA and cell-free filtrates of *Bacillus cereus* var. mycoides (379, 445). 2,4-D eventually appears in the *B. cereus* cell-free filtrates. Metabolism by *P. putida* does not result in production of 2,4-D. The enzyme of *P. putida* which breaks the oxygen-sulfur bond does not require prior activation (279). This is a novel alkylsulfatase, as other microbial alkylsulfatases break the chain at the carbon-oxygen bond.

FUNGAL METABOLISM OF PHENOXY HERBICIDES

Studies on fungal metabolism of phenoxy compounds have included studies with *Aspergillus niger*. Hydroxylation is a major mechanism, although not all vacant ring sites are hydroxylated. Thus, (2,4-dichloro-5-hydroxyphenoxy)acetic acid is the major metabolite of 2,4-D metabolism (Figure 81). A minor metabolite (2,5-dichloro-4-hydroxyphenoxy)acetic acid, appears as the result of a novel hydroxyl chloride replacement and chloride shift (148). The latter compound also is the only compound formed from metabolism of 2,5-D (148, 149). The hydroxyl chloride shift is similar to that seen in 2,4-D metabolism by many plants in which the major metabolite is (2,5-dichloro-4-hydroxyphenoxy)acetic acid and the minor metabolite is (3,5-dichloro-4-hydroxyphenoxy)acetic acid. Both metabolites require a hydroxyl chloride shift (283).

Hendersonula toruloidea metabolizes 2,4-D with production of $^{14}CO_2$ (470). In 8 weeks, 28.8% of (carbon-1)-^{14}C 2,4-D and 2.8% of ring-^{14}C 2,4-D were released. *Stachybotrys atra* produced only 3% of (carbon-1)-^{14}C 2,4-D as $^{14}CO_2$ after 8 weeks.

Phytophthora megasperma var. *sojae* metabolized 10 mg/l 4-(2,4-D)B with 45% disappearance in 21 days, but no production of 2,4-D was noted (402). The organism also did not metabolize 2,4-D, suggesting that β-oxidation is not a primary mechanism in the metabolism of 4-(2,4-D)B.

MCPA is metabolized to (4-chloro-5-hydroxy-2-methylphenoxy)acetic acid by *A. niger* (149). The metabolism of 2- or 4-chlorophenoxyacetic acid by fungi does not result in ring cleavage, in contrast to the activity of bacteria (150). Metabolism of (4-chlorophenoxy)acetic acid yields compounds hydroxylated in the 2 or 3

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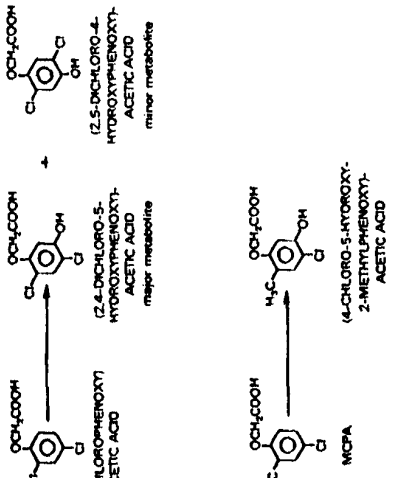

Figure 9.1 Metabolism of 2,4-D and MCPA by *A. niger*

positions, the latter a novel product. Similarly, (2-chlorophenoxy)acetic acid yields compounds hydroxylated in the 4- or 5-positions, the latter also a novel product. Minor products include 2-chloro-3-hydroxy and 2-chloro-6-hydroxy acids.

The microorganisms present in soil treated repeatedly with herbicides were isolated and identified (436). Bacteria capable of metabolizing 2,4-D include *Arthrobacter* sp., *Bacillus* sp., *Pseudomonas* sp., and *Sarcina* sp. Fungi include *Penicillium megasporum* and another *Penicillium* sp. Bacteria which can metabolize MCPA include *Arthrobacter* sp., *Corynebacterium* sp., and *Pseudomonas* sp., and fungi include *Fusarium culmorum*, *Mucor* sp., *Penicillium* sp., *Zygorhynchus moelleri* and four *Verticillium* spp. Of these, two bacteria and five fungi also metabolize 2,4-D.

METABOLISM OF PHENOXY HERBICIDES IN SOILS

A sample of Philippine soil was treated with 2,4,5-T for 4 months, after which 2,4,5-trichlorophenol was recovered (377). A mixture of microorganisms was removed and incubated with 2,4,5-T. Loss of 10% of substrate was recorded with liberation of 8% of the initial radioactivity of the uniformly ring-labeled substrate as $^{14}CO_2$ in 25 days. The major metabolite was 2,4,5-trichlorophenol, which was readily metabolized with about 75% of the chloride in this metabolite liberated as free chloride. About 40% of this compound was released as $^{14}CO_2$ in 25 days. Products arising from incubation of the mixed culture include 3,5-dichlorocatechol, cis,cis-2,4-dichloromuconic acid, 2-chloro-4-carboxymethylene but-2-enolide, chloromaleic acid, succinic acid, and 4-chlorocatechol. These products with the exception of 4-chlorocatechol, are all found in the 2,4-D degradative pathway subsequent to 3,5-dichlorocatechol (Figure 46).

The fate of uniformly ring-^{14}C-labeled 2,4-D and 2,4,5-T was explored in 6 different soils (304). Metabolites of 2,4,5-T included 2,4,5-trichlorophenol and 2,4,5-trichloroanisole, while no metabolites were detected after 2,4-D incubation. About 20 to 35% of the substrates were recovered from the humic and fulvic acids and humin fractions, indicating formation of polymeric humic substances of 2,4-D and 2,4,5-T mediated by additional hydroxyl groups on the rings. Depending on the soil, up to 83% of 2,4-D and 71% of 2,4,5-T applied at 1 ppm concentration was converted to $^{14}CO_2$ in 150 days.

Diclorfop-methyl, (±)-methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]] propionic acid, undergoes rapid hydrolysis of the ester bond in field soils (297). At 1 ppm the resultant diclorfop is rapidly metabolized in aerobic soils with isolation of two metabolites when the ^{14}C-label is the chlorinated ring and one metabolite when the label is in the nonchlorinated ring. The ubiquitous metabolite was identified as 4-(2,4-dichlorophenoxy)phenol. Other experiments indicated that intermediates include diclorfop acid with subsequent decarboxylation to form phenylether (397). In 25 weeks, 25 to 35% of each type of labeled substrate was converted to $^{14}CO_2$ (397). In anaerobic soils diclorfop persists with no evolution of CO_2 and formation of only trace amounts of a metabolite.

The herbicide 2-(2,4-dichlorophenoxy)ethanol is often applied to soils in the ineri form sodium 2-(2,4-dichlorophenoxy)ethyl sulfate (443). In sterile soils conversion to the active form occurs only at pH 3 to 4, while in nonsterile soils conversion takes place at pH 3 to 7, within 45 minutes after application. Thus, in soils with pH greater than 4, herbicide activation is thought to be biologically mediated.

The isopropyl, n-butyl, and isooctyl esters of 2,4,5-T, the n-butyl ester of (2,4-dichlorophenoxy)butyric acid, and the isooctyl ester of (2,4-dichlorophenoxy) propionic acid were applied to 4 Saskatchewan soils at 4 ppm concentration (400). In moist soils there was nearly complete conversion of the substrate to the free acids

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within 24 hours, with the exception of the isooctyl ester of (2,4-dichlorophenoxy)-butyric acid, which was completely converted in 72 hours. In air-dried soils there was very little loss of ester in the same time.

The isooctyl ester or dimethylamine salt of 2,4-D was applied to soils at a concentration of either 1.6 or 16 ppm (669). After 58 days 60 to 80% of the ring- or carboxyl-labeled substrate was released as $^{14}CO_2$, while 1% was recovered as 2,4-D. *Eighty percent of the labeled substrate in the runoff water was recovered as $^{14}CO_2$* after 3 weeks, with an additional 3% more recovered in the next 5 weeks; the remaining material was not 2,4-D.

The primary effluent of municipal sewage was added to a nutrient medium containing 2,4-D (376). *Within 7 days almost all the substrate disappeared in a similar test phenoxyacetic acid disappeared within 12 days.* Subsequent additions of either of these substrates resulted in metabolism without a lag period. No disappearance of 2,4,5-T was noted after 60 days.

Incubation of Mashat clay with medium containing these herbicides resulted in 90% disappearance of 2,4-D in 14 days on initial application, with 3 days required for 75% disappearance of additional applications of substrate (376). Phenoxyacetic acid required 16 days for *initial disappearance, and subsequent applications were* metabolized in 4 days. Evolution of $^{14}CO_2$ began from 7 to 60 days after herbicide application, and after 4 months from 5.2 to 34% was recovered depending on the soil.

After 12 weeks of incubation in sandy loam, 71 to 84% of either ring-^{14}C- or (carbon-1)-^{14}C- or (carbon-2)-^{14}C 2,4,-D was released as $^{14}CO_2$ (470). The concentration of substrate in the soil was not given.

Several South Vietnamese soil and mud samples were treated with carboxyl-^{14}C *labeled 2,4,5-T (65). Two samples* were thought to be treated previously with a 50:50 mixture of 2,4-D and 2,4,5-T, while two samples were thought to be uncontaminated. At 1 ppm concentration, almost 70% was evolved as $^{14}CO_2$ in 49 days. At 15 ppm, three soils converted 70 to 80% of the substrate to $^{14}CO_2$ in 168 days, while one sample, *thought to be previously uncontaminated, evolved more than 95% of the* material as $^{14}CO_2$.

In moist Philippine soils (upland conditions), 20 ppm 2,4-D disappeared more rapidly than when applied to flooded soils, but after 6 weeks the concentration of 2,4-D was similar in both moist and flooded soils (482). The same results were reported in the disappearance of 10 ppm 2,4,5-T from one of the soils. However, in another soil 2,4,5-T remained in flooded soils for a 4 week lag before undergoing *rapid and complete disappearance in the next 4 weeks, while in the moist soils* gradual disappearance was noted with about 40% remaining after 12 weeks. No disappearance of 2,4-D or 2,4,5-T was noted in sterile control samples after 12 weeks' incubation.

The persistence of 2,4-D and MCPA in soils following repeated applications was measured (436). Ten weeks were required for disappearance of 2,4-D upon first application. The herbicide disappeared after 7 weeks upon second application in the *second year, and required only 6 weeks for disappearance after 10 years of repeated* applications. MCPA required 20 weeks for disappearance in the first year, 10 weeks in the second year, and 7 weeks after 10 years. Soils pretreated with either herbicide showed accelerated 2,4-D disappearance after 10 years but not after one year of herbicide application. Enhancement of *MCPA disappearance was noted after either* one year or 10 years of pretreatment with either herbicide. The numbers of degradative bacteria or fungi were not significantly different after 0, 1 or 10 years of pretreatment.

A seed bioassay (mustard or cress) showed that 2,4-D disappears faster than MCPA, and 2,4,5-T much slower than either, from both a light clay soil and a sandy loam (414). The first application of 2,4-D to soil at 55 ppm concentration required 14

days for disappearance of herbicidal activity, while 7 days were required for disappearance of the second application at 120 ppm and 4 days upon third application of 200 ppm.

A soybean bioassay also indicated that 2,4,5-T lasted much longer than 2,4-D or MCPA (111). The rate of application had no effect at application rates from 5 to 20 lb/acre. The rate of disappearance of herbicide increased with increasing tempera*ture and increasing moisture. Although MCPA is* subject to degradation by photolysis, experiments with 1 mg/l MCPA incubated in ice water in the dark showed disappearance of 75% in 6 days, as opposed to 15% disappearance due to photolysis alone (405).

Application of bifenox, methyl 5-(2,4 dichlorophenoxy) 2-nitrobenzoate, to a greenhouse soil mix at a rate equivalent to 1.7 kg/ha showed that after 313 days 78% of the benzoate ring-^{14}C-labeled material and 57% of phenoxy ring-^{14}C-labeled material was bound to the soil (273). *After 7 days following* initial application very little additional bifenox disappeared, although only 20 to 26% was bound to the soils. The metabolites, which were identified by thin layer chromatography, included the acid of bifenox, 5-(2,4-dichlorophenoxy)-2-nitrobenzoic acid, nitrofen (2,4-dichlorophenoxy-4-nitrophenyl ether), 5-(2,4-dichlorophenoxy)anthranilic acid, and other unidentified compounds. These metabolites were also found as degradation products in plants grown in bifenox treated soil.

CHLOROPHENYL HERBICIDES

Pseudomonas sp. strain CBS 3 utilizes 4-chlorophenylacetic acid as the sole source of carbon and *energy (358). Initially-formed* metabolites include 4-chloro-3-hydroxyphenylacetic acid, 3-chloro-4-hydroxyphenylacetic acid and 4-chloro-3-hydroxyphenylacetic acid. This strain, however, cannot grow on 3-chloro-4-hydroxy or 4-chloro-3-hydroxyphenylacetic acid (296). Metabolism of 4-chloro-2-*hydroxy*phenylacetic acid results in formation of 4-chloro-2,3-dihydroxyphenylacetic acid. This is not the primary pathway of substrate metabolism. Upon further incubation 3,4-dihydroxyphenylacetic acid (homoprotocatechuate) appears, indicating direct removal and replacement of the *chloride before ring cleavage.* Homoprotocatechuate is metabolized to homogentisic acid (2,5-dihydroxyphenylacetic acid) and then to the *meta* cleavage product maleylacetoacetate resulting from the action of homogentisate 1,2-dioxygenase. An *Arthrobacter* sp. similarly produces 4-*chloro*-3-*hydroxy*phenylacetic acid from 4-chlorophenylacetate, along with an additional unidentified metabolite (110).

The herbicide chlorofenprop-methyl [2-chloro-3-(4-chlorophenyl) propionic acid methyl ester] is readily metabolized (364). In *mixed cultures of soil microorganisms*, 4-chlorocinnamic acid and 4-chlorobenzoic acid have been identified as metabolites. The latter product has been recovered from soil amended with the herbicide. Two strains, thought to be a *Flavobacterium* sp. and a *Brevibacterium* sp., were isolated *from the soil and found to convert* 4-chlorocinnamic acid to 4-chlorobenzoic acid. Two *Arthrobacter* spp. have been shown to grow on 4-chlorobenzoic as the sole source of carbon and energy. Thus, a consortia or a mixed soil population would be able to mineralize chlorofenprop.

A chlorophenyl insecticide known as SD 8280 [2-chloro-1-(2,4 dichlorophenyl) vinyl dimethyl phosphate] was studied with respect to its degradation in soils (372). The major products were 2,4-dichlorobenzoic acid and 1-(2,4 dichlorophenyl)-2-ethanol. Lesser amounts of 2-chloro-1-(2,4-dichlorophenyl)vinyl methyl hydrogen phosphate and 2',4'-dichloroacetophenone were also formed. Other products were noted but not identified, although they were shown not to be 2,4-dichlorophenol or

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2,4-dichlorobenzyl alcohol. None of the recovered metabolites represent alterations to the chlorinated ring of the molecule.

SUMMARY

Most of the chlorophenoxy herbicides appear to be biodegradable to CO_2 and free chloride under the right conditions. These results have been shown for both pure cultures and in soils containing mixed populations. Adaptation of the cultures to the substrate is required and results in faster disappearance of the compound. The persistence of some compounds in some environments indicates that under some conditions these herbicides could be considered to be recalcitrant compounds. Seasonal variations in herbicide degradation have also been noted (460).

Once a population has become adapted to metabolize a substrate, however, that capability persists for long periods of time. Thus, yearly applications of an herbicide are sufficient to maintain a degradative population in soil. A population adapted to metabolize a particular substrate is often also adapted to metabolize other related compounds. This has particular application where crop rotation is accompanied by usage of different herbicides.

SECTION 12
PHENYLAMIDE AND MISCELLANEOUS HERBICIDES

The phenylamide herbicides include the groups of phenyl ureas, N-phenyl carbamates, and acylanilides. Each takes the general form R-NH-CO-X where R is a halogenated or nonhalogenated aromatic hydrocarbon (239, 174). In urea herbicides, X is an amino group with methyl, alkyl, or methoxy substituents. Carbamates have the form whereby X is an alkoxy group. In acylanilide herbicides, X is an alkyl group. In many cases these herbicides are degraded to substituted anilines (305).

The urea herbicides are specific inhibitors of photosynthesis, but can have a selective effect because of their low water solubility and low mobility in soil. They can be applied to kill shallow-rooted weeds while having no effect on deeper-rooted plants of interest. In the late 1940s, a large number of substituted urea compounds were compared with 2,4-D for herbicidal activity and were found worthy of further development. Originally used as industrial weed killers, they have more recently been used in agricultural applications (174). The carbamates comprise a wide range of active compounds which, depending on the chemical substituents, are used for such purposes as herbicides, insecticides, and medicinals (186a). As insecticides, the carbamates inhibit the action of acetylcholinesterase, an enzyme required for proper neurotransmitter substance functioning. The degree of fit between the inhibitor and the enzyme lends selectivity to the activity of the carbamates. The herbicidal carbamates have also been used since the 1940s. Amide herbicides (substituted anilides) were developed in the 1950s and are used for such crops as corn and rice.

BACTERIAL METABOLISM OF CHLORINATED ANILINES

The degradation of such herbicides as monuron, diuron, linuron, and propanil results in formation of 3,4-dichloroaniline. A strain of *P. putida* has been isolated which mineralizes 3,4-dichloroaniline in the presence of aniline (483). The rate of mineralization was enhanced by increasing the concentration of aniline. In the presence of 500 mg/l propionanilide, as much as 50% of ring labeled chloroaniline (added at 10 to 60 mg/l concentration) was metabolized to $^{14}CO_2$ within 2 weeks, accompanied by some chloride release (484). A pathway for 3,4-dichloroaniline was proposed which involves *ortho* cleavage through 4,5-dichlorocatechol and further metabolism to succinic acid (Figure 52). This pathway is analogous to that shown for aniline. Dichloroaniline is also metabolized by a different mechanism to dichloroformylanilide (245, 484).

Another *Pseudomonas* sp., strain O, mineralizes 3,4-dichloroaniline to CO_2 when grown in the presence of 4-chloroaniline (488). In 9 days, 15% of 0.5 mM dichloroaniline was converted to CO_2.

Studies with 4-chloroaniline have shown that this metabolite is utilized as a sole source of carbon and nitrogen by *Pseudomonas* sp. strain O (487). After 10 days, 64% of a 2.5 ppm solution of the substrate was released as $^{14}CO_2$. Ammonium cation, rather than nitrate or nitrite anion, accumulates suggesting that the amino group is removed directly without oxidation. Other ^{14}C-labeled products accumulate but are

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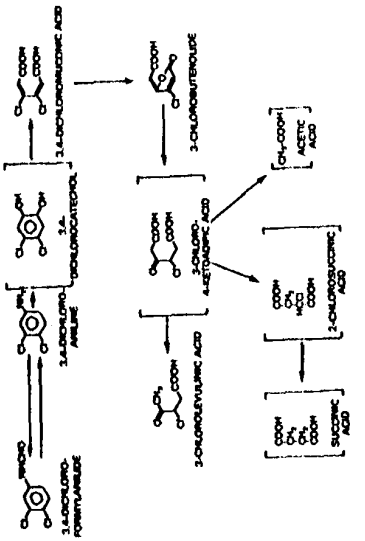

Figure 31. Pathways of 3,4 dichloroaniline metabolism by *P. putida*

Adapted from Reference 184

not incorporated into the cell biomass. Resting cells of this strain grown on 4 chloroaniline also oxidize aniline, catechol, and 4-chlorocatechol but not 4-chloronitrobenzene or 4-chlorophenol, indicating dioxygenase attack on the molecule (487). The *meta* cleavage metabolite 2-hydroxy-5-chloromuconic semialdehyde also accumulates in the medium. This strain also utilizes 2-chloroaniline and 3-chloroaniline as sole sources of carbon and nitrogen. *P. multivorans* strain A1, when grown with aniline present in the medium, converts 2-chloroaniline to 3-chlorocatechol, and 3-chloroaniline and 4-chloroaniline both to 4-chlorocatechol, which is metabolized subsequently to CO_2 and cell constituents (361).

A strain of *Alcaligenes faecalis* utilizes 3-chloroaniline or 4-chloroaniline under cooxidative conditions with sodium acetate or sodium pyruvate (419). Both chloroanilines are oxidized to 4-chlorocatechol, which undergoes *meta* cleavage to 5-chloro-2-hydroxymuconic semialdehyde and then to 2-chloro-4-oxalocrotonic acid. Similarly, an unidentified isolate which utilizes 4-chloro-aniline as the sole carbon source demonstrated, via Warburg respirometry, oxidation of 4 chlorocatechol without a lag (56).

The metabolism of 4-chloroaniline by *Paracoccus* sp. under both aerobic and anaerobic conditions was investigated (43). Transformation was faster under anaerobic conditions with 100% of a 20 mg/l solution converted within 2 days to a volatile product plus several other metabolites. The volatile product was not identified but was shown not to be CO_2. In the same period, 75% of the substrate was utilized aerobically, although the aerobic population was larger than the anaerobic population. The major product of aerobic metabolism is 4-chloroacetanilide, although several other products are formed as well. *Paracoccus* sp. also transforms 2-, 3-, 4-chloroaniline, 2,3-, 2,4-, 2,5-, and 3,4-dichloroaniline both aerobically and anaerobically, with more complete primary degradation occurring under anaerobic conditions.

A study of the anaerobic conversion of 4-chloroaniline by the same organism showed pH-dependent formation of products (310). In a medium containing nitrate, 80% of a 100 ppm solution was transformed within 48 hours to the condensation product 1,3-bis(p-chlorophenyl)triazine. However, sterile anaerobic solutions at pH of 5 to 6 also yielded this product, although no transformation took place at pH 7. It was postulated that the conversion of nitrate to nitrite and decrease in pH were the primary effects of bacterial metabolism, while triazine formation was a nonbiological secondary effect. *Paracoccus* sp. also formed small amounts of 4-chloroacetanilide during incubation with 4 chloroaniline.

A wide variety of both Gram positive and Gram-negative bacteria were isolated from soil which had been enriched with 4-chloroaniline (134). The most active species was identified as *Bacillus firmus*. This species could not use the substrate as the sole source of carbon, but when grown with ethanol transformed 4-chloroaniline to 4-chloroacetanilide as the main metabolite, with lesser amounts of 4-chloropropionanilide also produced. Two other products were identified as 7-chloro-2-amino-3H-phenoxyazine-3-one and 7-chloro-2-amino-3H-3-hydroxyphenoxyazine, and were postulated to result from spontaneous condensation of 4-chloroaniline with subsequent hydroxylation. The acylation of the substrate described here is considered to be a detoxification process in microorganisms (134).

Aniline-grown resting cells of *Rhodococcus* sp. AN 117 convert 2-chloro- and 3-chloroaniline to 3-chloro- and 4-chlorocatechol, respectively. An additional product identified as 2-chloromuconic acid results from metabolism of 2-chloroaniline. Cometabolism of 3-chloroaniline in the presence of 1802 resulted in production of another product which was identified as the g-lactone of 3-hydroxymuconic acid, formed by incorporation of two molecules of oxygen (221). Appearance of this product was associated with disappearance of 4-chloro catechol

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This suggests that dechlorination is associated with fermentation of the 3-chloromuconic acid, a mechanism shown previously in pseudomonads. When fresh cultures were supplied with benzoate plus 2- or 3-chloroaniline as the sole source of nitrogen, growth of cells and disappearance of both substrates occurred (222). The growth yield was similar to that obtained when NH_4NO_3 was utilized as the sole nitrogen source. No growth occurred when 4-chloroaniline was supplied as the nitrogen source.

The fungicide 2,6-dichloro-4-nitroaniline is metabolized by many bacteria including *F. coli* and *P. cepacia* (442). The first step is reduction of the nitro group to an amine, forming 2,6-dichloro-p-phenylenediamine. This compound is then acetylated to produce 4-amino-3,5-dichloroacetanilide. The first reductive step occurs much faster under anaerobic conditions than under aerobic conditions.

FUNGAL METABOLISM OF CHLORINATED ANILINES

Fungi as well as bacteria produce peroxidases which are responsible for the polymerization of chloroanilines. Species with this capability include *Geotrichum candidum* L-3 and *Aspergillus* sp. (47, 271). The substrate 3,4-dichloroaniline is converted to 3,3',4,4'-tetrachloroazobenzene by *G. candidum* L-3 and *Aspergillus* sp. (271) and is converted to 3,3',4,4'-tetrachloro-azoxybenzene in *Fusarium oxysporum* cultures (240). However, this latter reaction varies with the culture conditions and the azoxy condensation product has not been found in soils.

The fungal metabolism of 4-chloroaniline follows several pathways (Figure 53). A major pathway is N-hydroxylation such as is demonstrated by *F. oxysporum* (242). This species metabolizes 4-chloroaniline to 4-chlorophenylhydroxylamine which is subsequently converted to 4-chloronitrosobenzene and 4-chloronitro-benzene. Condensation products which appear include both 4,4'-dichloroazo-benzene and 4,4'-dichloroazoxybenzene. In addition, 4-chloroacetanilide appears as an acylation reaction and may undergo hydrolysis to yield 4-chloroaniline. Free chloride is produced as the result of some of these reactions.

The culture filtrate of *O. candidum*, as well as the purified fungal enzymes peroxidase and aniline oxidase, converts 4-chloroaniline to several condensation products including 4,4'-dichloroazobenzene and 4-chloro-4'-(4-chloroanilino)-azobenzene (49). These reactions have also been demonstrated with horseradish peroxidase. *Streptomyces* sp. also formylates 4-chloroaniline with resultant production of 4-chloroformylanilide as well as 4-chloroacetanilide and at least two other metabolites (301).

Ring hydroxylation is a mechanism demonstrated by *F. oxysporum* which results in metabolism of 3-chloroaniline to 3-amino-4-chlorophenol and 4-chloroaniline to 2-amino-3-chlorophenol (Figure 53) (155). These molecules are hydroxylated in the *ortho* position. The aminophenols are relatively unstable and can undergo condensation and polymerization reactions, although 2-amino-4-chlorophenol has been detected in soil as well as in the pure culture studies reported above. The *ortho*-substituted substrate 2-chloroaniline is not hydroxylated by *F. oxysporum*.

METABOLISM OF CHLORINATED ANILINES IN SOILS

There is evidence that in soils 3,4-dichloroaniline is slowly mineralized. Radiocarbon-labeled humic-bound material is mineralized in some soils ($^{14}CO_2$ production) at about the same rate as the average soil organic matter polymer (304). The addition of aniline to soils enhances mineralization of both free and humic-bound 3,4-dichloroaniline (483). This was attributed to selection by the aniline analogue for chloroaniline-degradative populations as well as the induction by aniline of the common metabolic pathway.

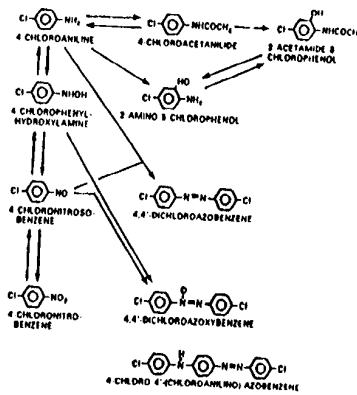

Figure 53 Metabolism of 4-chloroaniline by microorganisms
Adapted from References 49, 155, 242

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Application of 10 ppm ring-labeled ^{14}C-3,4-dichloroaniline to a rice paddy ecosystem yielded a total recovery (extractable plus nonextractable) of almost 69% (210). Less than 4% of the material applied to soil was recovered in the water or rice plants. CO_2 evolution was not determined. Extraction of the soil yielded 3.4% of the original material as dichloroaniline, 4% as tetrachloroazobenzene, and 44.7% as polar material.

The degradation of aniline, 4-chloroaniline, and 3,4 dichloroaniline in four different soils was compared (420). At 1 ppm application rate, 16 to 26% aniline was converted to CO_2 after 10 weeks, while after 16 weeks 12 to 23% chloroaniline and 4 to 12% dichloroaniline were mineralized. In soils 4-chloroaniline can be converted to 4-chlorophenylhydroxylamine via biological mechanisms (48). This compound then undergoes condensation with 4-chloroaniline to form 4,4'-dichloroazobenzene in a nonbiological reaction.

Condensation of two molecules of 3,4-dichloroaniline forms 3,3',4,4'-tetrachloroazobenzene which is relatively persistent in the environment (51). This reaction has been demonstrated in Nixon sandy loam (11, 22, 24) as well as in other soils (22, 48, 51, 245). The conversion of chloroanilines to chloroazobenzenes has been shown to occur by a peroxidase mechanism (25). A mixture of substituted anilines, hydrogen peroxide, and peroxidase resulted in the formation of chloroazobenzene (51). Bacteria including *Bacillus* sp., *Arthrobacter* sp., and *Pseudomonas* sp. exhibit peroxidase activity (271). A pathway has been proposed which includes transformation of the hypothetical 3,4-dichloroanilidyl molecules to 3,4-dichlorophenylhydroxylamine. Two of these molecules are condensed to the hypothetical 3,3',4,4'-tetrachlorohydrazobenzene which is converted to 3,3',4-4'-tetrachloroazobenzene (51). However, studies in which dichloroaniline was applied to herbicide-treated soils did not show formation of tetrachloroazobenzene, suggesting that dichloroaniline is not the prime precursor for tetrachloroazobenzene (30). Also, production of tetrachloroazobenzene in soils incubated with propanil was not correlated with the quantity of peroxidase producing microorganisms recovered from the soil (58). Cell-free peroxidase was found only rarely in the soil samples. Recovery of peroxidase increased upon amendment of the soils with nutrient sources and additionally upon sonification of the samples, which may have released cell-bound or intracellular enzymes. Addition of protease-peptone decreased recovery of peroxidase-producing organisms but increased soil peroxidase recovery. However, this peroxidase did not form tetrachloroazobenzene from dichloroaniline. Another condensation product detected as a humic-bound residue in soils treated with propanil is 4-(3,4-dichloroanilino)-3,3',4'-trichloroazobenzene (32).

Warburg respirometry studies indicated that aniline-acclimated activated sludge microflora oxidized 800 mg/l 3-chloroaniline at low rates over a 192-hour incubation period (292). Oxidation of 4-chloroaniline occurred at slightly higher rates, and after a 100-hour lag period rapid oxidation of 3-chloroaniline took place.

METABOLISM OF UREA HERBICIDES

A *Fusarium* sp. utilizes the larvacide diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] as its sole source of carbon and energy (389). The pathway was elucidated to include initial formation of 2,6-difluorobenzoic acid and 4-chlorophenylurea. The latter compound is metabolized to 4-chloroaniline, and then 4-chloroacetanilide, followed by reductive dehalogenation to acetanilide and further metabolism to cell constituents. Other fungi including *Cephalosporium* sp., *Penicillium* sp., and *Rhodotorula* sp., although unable to utilize diflubenzuron as a sole carbon source, metabolized the compound to 2,6-difluorobenzoic acid and 4-chlorophenylurea, indicating cleavage of the urea bridge.

Evidence that the urea herbicides are degraded in the environment accrues from studies which showed that such compounds as 3-(4-chlorophenyl)-1,1-dimethylurea (monuron) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron), when applied at rates of 1 to 2 lb/acre annually in the eastern part of the United States, left no residual phytotoxicity after 4 to 8 months (203). Higher application rates required longer times for disappearance of phytotoxicity.

Potential mechanisms for disappearance include biological, leaching, volatilization and chemical decomposition (203). Leaching is considered to be significant only in porous soil or if there is a great amount of rainfall. The low vapor pressure and aqueous solubilities of these herbicides make volatilization unlikely to be an important mechanism. Photodecomposition may be a factor in dry areas in which the herbicide remains on the soil surface, but these compounds are stable to chemical decomposition in aqueous solutions. Biological studies revealed that the rate of herbicide inactivation is greater in nonsterile than in sterile soils. This was shown by the amount of radiocarbon-labeled CO_2 evolved from soils amended with ^{14}C(methyl)-labeled monuron, and a *Pseudomonas* sp. was isolated which oxidized this substrate in Warburg respirometry studies (203).

The herbicide N'-(4-chlorophenoxy)phenyl-N,N-dimethylurea is metabolized when placed in contact with soils such as sandy loam or humus soil (173). Sorption of the compound to the soils has an effect on the rate of degradation. Enriched bacterial cultures derived from the soil samples also metabolized the herbicide by successive demethylation to N'-(4-chlorophenoxy)phenyl-N-methylurea and subsequently to N'-(4-chlorophenoxy)phenylurea. No appreciable amounts of CO_2 were released. Fungal isolates of *Penicillium* sp. and *Aspergillus* sp. removed less than 50% of the carbonyl group but did not transform the compound further. The metabolism of this herbicide in plants also follows successive demethylation leading to CO_2 evolution.

Diuron is used for long-term weed control in peach orchards, and has been detected as long as 3 years after the last application in a field consisting of Lucas loamy sand (248). The levels of 3,4-dichloroaniline were very low and decreased to undetectable levels in 3 years, while the potential condensation product tetrachloroazobenzene was not detected. The decomposition of diuron is enhanced by changes in environmental conditions that favor the growth of microorganisms (306). Thus, increasing the temperature of incubation from 10 to 30°C or adding organic matter to the soils both increase the rate of diuron decomposition. The rate of herbicide inactivation is much greater than the rate of CO_2 evolution, and investigations showed that the loss of one methyl group decreases herbicidal activity by half while loss of both methyl groups completely inactivates the molecule. The pathway of diuron metabolism in soils was proposed to be 3-(3,4-dichlorophenyl)-1,1-dimethylurea to 3-(3,4 dichlorophenyl)-1-methylurea, then loss of the second methyl group to form 3-(3,4-dichlorophenyl)urea, followed by hydrolysis of the urea to form the aniline derivative 3,4-dichloroaniline, which accumulates as the major product (303).

Microbial enrichment cultures from pond water and pond sediment treated with diuron revealed a similar pathway of degradation (131, 132). Three additional unidentified products were also detected. The enrichment cultures included mixtures of fungi and bacteria as well as consortia of bacteria. Some of the mixed cultures converted more than 90% of the substrate to CO_2 within 3 weeks. Of 30 single isolates, however, only 3 could partially metabolize diuron after 4 weeks' incubation.

Under anaerobic conditions reductive ring dechlorination of diuron occurs (12). Enrichment cultures from pond water and sediment incubated anaerobically rapidly degraded diuron to 3-(3-chlorophenyl)-1,1-dimethylurea in stoichiometric amounts. This product was not degraded further and no other products were detected. Repeated additions of diuron resulted in rapid metabolism of the substrate.

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Chlortoluron [N-(3-chloro-4-methylphenyl)-N'-dimethylurea] has a half-life in a variety of soils of 4 to 6 weeks (403). The major degradation product is monomethyl chlortoluron. However, the product of subsequent demethylation was never detected. This may indicate that cleavage of the molecule to form 3-chloro-4-methylaniline follows monomethyl chlortoluron formation. Although the substituted aniline was not detected, this product rapidly disappears from the soils when applied directly.

Methoxy phenyl urea herbicides have quite high herbicidal selectivity and additionally are not very persistent in soil after application (174). Metabolism of these compounds differs from that of the dimethyl phenyl urea herbicides.

Soils treated with 3-(4-chlorophenyl)-1-methoxy-1-methylurea (monolinuron) yielded a *Bacillus sphaericus* isolate which could cometabolize ^{14}C-(ureido)-monolinuron to $^{14}CO_2$ and 4-chloroaniline (452). Maximum degradation occurred after the end of logarithmic growth. The substituted aniline was not degraded by the organism but was lost from the culture through volatilization. Linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] was metabolized to stoichiometric amounts of 3,4-dichloroaniline, while the dimethyl compounds monuron and diuron were not degraded. Cell-free extracts of *B sphaericus* also transformed the methoxy herbicides to substituted anilines with the aliphatic portion of the molecule degraded to CO_2 plus another metabolite (453). The degradation product of linuron was identified as N,O-dimethylhydroxylamine (133). The cell-free extracts were less active against the dimethyl substituted monuron and diuron (453).

The soil fungus *Cunninghamella echinulata* Thaxter degrades linuron and monolinuron through stable hydroxymethyl intermediates (435). Linuron is metabolized to 3-[3,4-dichlorophenyl]-1-methoxy-1-hydroxymethylurea and subsequently 3-(3,4-dichlorophenyl)-1-methoxyurea and 3-(3,4-dichlorophenyl)-1-methylurea. Disappearance of compounds such as linuron from nonsterile, but not from sterile, soils has been noted (120). The degradation of monolinuron during waste composting was investigated (310). After three weeks of composting, N-methoxy-N'-(4-chlorophenyl)urea was present in trace amounts and was the only metabolite detected.

METABOLISM OF CHLORINATED PHENYL CARBAMATE HERBICIDES

The phenyl carbamate herbicides (also known as carbanilates) are used to kill weeds on crop plants such as rice. Substituted anilines arise from degradation of these compounds, as the primary mechanism of degradation seems to be hydrolysis of the ester linkage (239).

The cell-free enzyme extract from a *Pseudomonas* sp. isolated from a soil enrichment culture was capable of converting several chlorophenylcarbamates to corresponding chlorinated anilines (244). The compounds tested included CIPC, [isopropyl-N-(3,4-dichlorophenyl)carbamate], sec-butyl-N-(3,4-dichlorophenyl)-carbamate, α-carbolsopropoxyethyl-N-(3-chlorophenyl)carbamate, 2-chloroethyl-N-(3-chlorophenyl)carbamate, 2-(1-chloropropyl)-N-(3-chlorophenyl)carbamate, 2-ethylhexyl-N-(3-chlorophenyl)carbomate and α-carbo-(2,4-dichlorophenoxyethoxy)-ethyl-N-(3-chlorophenyl)carbomate. In contrast, the enzyme preparation had no activity against 3-(4-chlorophenyl)-1,1-dimethylurea (monuron).

Penicillium jensenl was isolated from soil which had been treated with barban [(3-chlorophenyl)carbamic acid 4-chloro-2-butyl ester] (472). The mold did not utilize barban as a carbon source, although trace amounts of 3-chloroaniline appeared after incubation. Incubation of the mycelia with barban resulted in

production of large amounts of 3-chloroaniline, which was metabolized by the mycelia suspensions without production of free chloride.

A mixture of bacteria and fungi isolated from soil enriched with the analogous chlorinated analogue isopropyl phenylcarbamate (propham) metabolized a wide range of herbicides to their corresponding chlorinated aniline products (305). These included compounds ring-substituted with 3-chloro-, 4-chloro-, 3,4-dichloro-, 3,4-dichloro-, and 2,4,5-trichloro- substituents. Microorganisms comprising the consortium included *Mycobacterium* sp., *Arthrobacter* sp., *Corynebacterium* sp., *Fusarium* sp., *Nocardia* sp., *Streptomyces* sp., *Aspergillus* sp., and *Penicillium* sp.

A wide variety of fungal isolates from treated soil metabolized swep [methyl N-(3,4-dichlorophenyl)carbamate] to 3,4-dichloroaniline with formation of trace amounts of 3,3',4,4'-tetrachloroazobenzene (240). The fungi included *Aspergillus ustus, A versicolor, Fusarium oxysporum, F. solani, Penicillium chrisogenum, P. nigulosum* and *Trichoderma viride*. The isolates were most active on CIPC and only slightly active against the phenylurea diuron and 3-(3,4-dichlorophenyl)-1-methylurea. The rate of formation of 3-chloroaniline was the same as the rate of disappearance of CIPC due to metabolism by *Aspergillus fumigatus*, indicating that hydrolysis of the ester bond is the first step in degradation of this herbicide (471).

Swep is metabolized to 3,4-dichloroaniline with formation of 3,3',4,4'-tetrachloroazobenzene in soils such as Nixon sandy loam (11). Soil microorganisms obtained from muck soil metabolized 1240 mg/l isopropyl-N-(3 chloro-phenyl)carbamate (CIPC; chlorpropham) to 3-chloroaniline with complete chloride release within 13 days (241). A similar pathway with complete dechlorination within 16 days was observed in the metabolism of 2-chloroethyl N-(3-chloro-phenyl)carbamate (CEPC). The isolates lost degradative capability if they were maintained on nutrient agar for several days but they could be readapted to use these herbicides as a sole source of carbon.

METABOLISM OF ACYL ANILIDE HERBICIDES

The substituted anilide herbicides are structurally related to the urea herbicides and the carbanilates, and like them are degraded to substituted anilines.

Strains of *Pseudomonas striata* and *Achromobacter* sp. metabolize N-(3-chloro-4-methylphenyl)-2-methylpentanamide[3'-chloro 2-methyl *p* valero-toluidide, solan] to 3-chloro-*p*-toluidine from which chloride is released quantitatively (Figure 54) (240). Azobenzene products were not detected in these experiments.

Fungi from several genera, including *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and *Trichoderma* sp. also metabolize solan to 3-chloro-*p*-toluidine with chloride release (240). Cultures of *A. niger* metabolize solan to a product identified as 3'-chloro-4'-methylacetanilide (Figure 45)(455). Cell-free extracts converted solan to the substituted aniline, but in growing cultures this product was rapidly acetylated to effect detoxification and the free aniline could not be recovered.

Strains of *P. striata* and *Achromobacter* sp. metabolize both swep and N-(3,4-dichlorophenyl)propionamide (propanil) to 3,4-dichloroaniline and small quantities of 3,3',4,4'-tetrachloroazobenzene (240). *Corynebacterium pseudodiphtheriticum* NCTC 10803 utilizes propanil as the sole source of carbon and energy for growth (186). The resulting products are 3,4-dichloroaniline which accumulates in the medium, and the propionic acid moiety which is utilized for cell growth.

A strain of *F. solani* was isolated which utilizes propanil as a sole source of carbon and energy (269). Dichloroaniline accumulates in the medium until it reaches toxic levels. The enzyme responsible for propanil hydrolysis to propionate and dichloroaniline was identified as an acylamidase (270). This enzyme is specific for molecules with a short chain, as it could not hydrolyze dicryl [N-(3,4-dichlorophenyl)metha-

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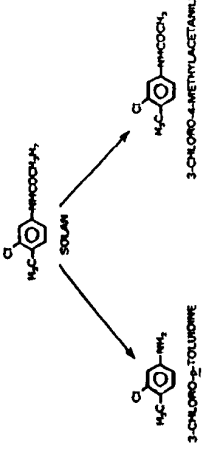

Figure 84. Metabolism of solan by microorganisms
Adapted from References 240, 466

crylamide) or the six-carbon herbicide 2-methylpentanamide. Other fungi, including *Aspergillus ustus, A. versicolor, Fusarium oxysporum, F. solani, Penicillium chrysogenum, P. nigulosum* and *Trichoderma viride*, also metabolize propanil to 3,4-dichloroaniline with formation of trace amounts of 3,3',4,4-tetrachloroazobenzene (240).

The interaction of *Penicillium piscarium* and *Geotrichum candidum* incubated with propanil results in increased growth over either alone (50). *P. piscarium* contains an acylamidase which converts propanil to dichloroaniline. *G. candidum* cannot utilize propanil but contains a peroxidase which converts dichloroaniline to the tetrachloroazobenzene. Each fungus reduces the toxic level of the other's byproduct of metabolism, demonstrating a synergistic or mutualistic interaction.

The yeast *Pullularia pullulans* and two *Penicillium* spp. were isolated and found to utilize N-(3,4 dichlorophenyl)-2-methylpentanamide as a sole source of carbon and energy, metabolizing the herbicide to dichloroaniline and 2-methyl-valeric acid (392). The enzyme was inducible and differed in level of activity and substrate specificity among the three species. Cell-free extracts of one of the *Penicillium* spp. hydrolyzed a wide variety of other acyl anilides as well, but had no activity against diuron or CIPC.

An unusual product, identified as N-(3,4-dichlorophenyl)-2-methyl-2,3-dihydroxy propionamide, was detected in the culture medium of a *Rhizopus japonicus* culture growing in the presence of dicryl (456). This product results from the double hydroxylation of the ethylene double bond of dicryl, and was the only metabolite detected. *R. japonicus* also hydroxylates the side chain of N-(3,4-dichlorophenyl)-pentanamide to produce N-(3,4-dichlorophenyl)-3-hydroxy-2-methylpentanamide (457). This mechanism results in detoxification of the herbicide.

Since propanil is an inhibitor of photosynthesis it is a potential poison to the algae as well (473). A study of the effect of propanil on cyanobacteria indicated depression of photosynthesis but also showed conversion of propanil to the less toxic 3,4-dichloroaniline in both axenic (bacteria-free) and contaminated cultures. The species studied incorporated those found in flooded rice paddy fields and soil, and included *Anabaena cylindrica, A. variabilis, Nostoc muscorum, N. entophytum, Tolypothrix tenuis*, and *Gloeocapsa alpicola*.

In soils propanil is converted to 3,4-dichloroaniline and the condensation product 3,3',4,4'-tetrachloroazobenzene (30, 87). Uniformly-labeled ^{14}C-propanil applied to soils was transformed to 3,4-dichloroaniline and the multiple condensation product 4-(3,4-dichloroanilino)-3,3',4'-trichloroazobenzene which accumulated to 2% of the substrate (22). At high concentration (500 mg/l), the dichloroaniline was volatile, while at lower concentrations (5 to 10 mg/l) dichloroaniline and its condensation product were humic-bound. The aliphatic portion is degraded to CO_2. Studies with ^{14}C(carbonyl) propanil revealed 70% conversion to $^{14}CO_2$ within 25 days, while soils amended with ^{14}C(ring)-propanil yielded only 3% $^{14}CO_2$ during the same time period (87). *Pseudomonas* sp. strain O also converts propanil to 3,4-dichloroaniline (488).

Propanil sprayed onto flooded rice plots was dissipated quickly and disappeared within 24 hours (112). The major metabolite was 3,4-dichloroaniline which sorbed to soils. Only a trace of 3,3',4,4'-tetrachloroazobenzene was detected, although dilution caused by the flooding may have precluded condensation of the dichloroaniline.

The amount of tetrachloroazobenzene formed in soils as a result of propanil or 3,4-dichloroaniline application was found to be highly variable (213). In a comparison of 9 soils, those at a pH of 4.5 to 5.5 showed the most production. Tetrachloroazobenzene formation was not correlated with the organic matter content of the soils, and air-dried soil samples showed 87 to 99% reduction in product formation. More tetrachlororobenzene was produced from direct application of

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dichloroaniline than from molar equivalent application of propanil. In a contradictory study, however, more tetrachloroazobenzene was produced from propanil than from the equivalent application of 3,4-dichloroaniline (58).

The degradation rates of herbicides in Nixon sandy loam have been correlated with the number of carbon atoms in the side chain (23). The four-carbon molecule dicryl is metabolized at a slower rate than propanil, and the six-carbon herbicide N-(3,4-dichlorophenyl)-2-methylpentanamide is the most persistent of the three. Each is converted to 3,4-dichloroaniline, 3,3',4,4'-tetrachloroazobenzene and another metabolite.

MISCELLANEOUS PESTICIDES

Chlordimeform [N'-(4-chloro-o-methylphenyl)-N,N-dimethylmethanimidamide] represents another class of formamide compounds used as insecticides. Chlordimeform is metabolized in sandy loam to several products within 90 days (220). These include 4-chloro-6-nitro-o-toluidine and 4-chloro-o-toluidine (Figure 46). The latter product is condensed to form 4,4'-dichloro-2,2'-dimethylazobenzene. Other coupling products which were detected include N-(4-chloro-o-tolyl)-2-methyl-p-benzoquinone monoimine and 2-(4-chloro-o-toluidino)-N-(1-chloro-o-tolyl)-6-methyl-p-benzoquinone monoimine. These are formed by a one-electron oxidation mediated by peroxidases, and appearance of these products is pH-dependent.

A mixed population of soil microorganisms mediated a novel conjugation of the aniline moiety of chlordimeform with malonic acid to form 4'-chloro-2'-methylmalonanilic acid (Figure 35) (378). This mechanism was previously found only in plants as a means for detoxification of certain D-amino acids.

The herbicide N-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide underwent extensive metabolism of the side chain during 90 days' incubation in soils (481). However, no alteration of the chlorinated ring structure was noted. The cyanobacterium Oscillatoria sp. metabolized N'-(4-chloro-o-tolyl)-N,N-dimethylformamidine with production of $^{14}CO_2$ from either tolyl-^{14}C- or ring-^{14}C-labeled substrate (34). Extensive nonbiological degradation of this compound occurs but no evolution of $^{14}CO_2$ in the absence of the algae was noted.

Tecloftalam [N-(2,3-dichlorophenyl)-3,4,5,6-tetrachlorophthalamic acid] is a bactericide used on rice plants (252). An analysis of its fate under flooded soil conditions analogous to those of rice paddy fields indicated that after 32 weeks most of the recoverable radiolabeled material was isolated as two or more products chlorinated in the tetrachlorophthalamic ring. Nine percent of the carboxyl-labeled material was converted to $^{14}CO_2$. Tecloftalam was recovered as a minor metabolite. No further transformations occurred during the 32 weeks of the experiments.

Metabolism of chloroethoxynil (2,4-dichlorophenyl-3'-methoxy-4'-nitrophenyl ether) in flooded soil resulted in production of a number of compounds due to alteration of the molecule without loss of chloride (328). Cleavage of the ether bond results in production of 2,4-dichlorophenol.

Production of amino derivatives from other diphenyl ether herbicides was shown to be faster in flooded than in moist soils (329). A number of bacteria including those from the genera Bacillus, Pseudomonas, Enterobacter and Escherichia mediated this conversion, although disappearance of the herbicides was noted in sterile soils as well. The herbicides tested included nitrofen (2,4-dichlorophenyl 4'-nitrophenyl ether), 2,4,6-trichlorophenyl 4'-nitrophenyl ether, 2,4-dichloro-6-fluorophenyl 4'-nitrophenyl ether, and chloroethoxynil.

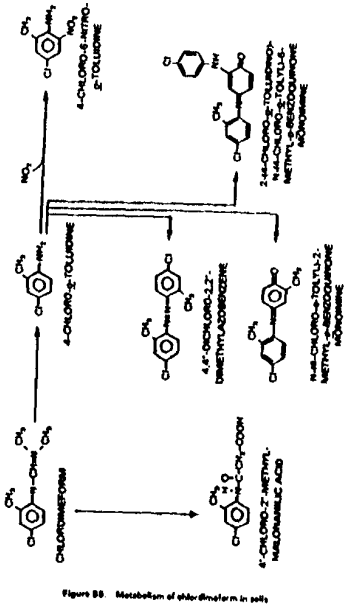

Figure 88 Metabolism of chlordimeform in soils
Adapted from References 220, 378

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SUMMARY

The herbicides described here were formulated with degree of persistence after application as a prime consideration. All of these compounds undergo primary degradation readily in soils and in a variety of microbial cultures. The resulting products are chlorinated anilines and other metabolites arising from the aliphatic side chain. When the side chain is not chlorinated, it is metabolized to cell constituents and CO_2. Little research has been published regarding the fate of chlorinated or other highly substituted side chain metabolites.

The bulk of the herbicides remain as chlorinated anilines. There is some evidence for volatilization of these compounds in arid zones if sorption to soils is delayed or if the concentration of chloroanilines is very high. However, the chlorinated anilines are readily and strongly bound to soil humic substances. Monosubstituted anilines, particularly 3-chloroaniline and 4-chloroaniline, are utilizable as sole carbon sources by some microorganisms and are metabolized in laboratory experiments. Dichloroaniline is toxic to microorganisms at low concentrations and evidence for its degradation is indirect. It is not clear how sorption to soils affects degradation of the chloroanilines in the environment.

Chlorinated anilines are metabolized by dioxygenases to chlorocatechols. Organisms which convert the chlorinated anilines to chlorocatechols and then also metabolize the chlorocatechols do so by the meta pathway. Limited evidence suggests that the amine group is removed directly without oxidation.

Under some conditions azobenzenes are formed from condensation of chloroanilines via peroxidases. The concentration of chloroanilines must be very high for this mechanism to be operative in soils.

Primary degradation of the urea herbicides occurs by successive demethylation of the side chain. The side chain is then metabolized leaving the chloroaniline moiety. Methoxy phenyl ureas are metabolized by a different mechanism, as microorganisms active against these compounds have little activity against the dimethyl herbicides.

Reductive dechlorination to a limited extent of the aromatic moiety has been reported. However, the effectiveness of this mechanism in the environment has not been investigated. Anaerobic utilization of 4-chloroaniline takes place readily, but the resultant volatile products or products have not been identified.

The primary mechanism of degradation of the phenyl carbamate herbicides is hydrolysis of the ester linkage to form chlorinated anilines. These compounds are metabolized by a wide variety of fungi and bacteria as noted above.

Hydrolysis of the acyl anilide herbicides results in formation of the chlorinated anilines with utilization of the aliphatic side chain for cell growth. An unusual mechanism employed by *R. japonicus* results in hydrolysis of the side chains of dicryl and N-3,4-dichlorophenyl-2-methyl pentanamide. This modification results in detoxification of the molecules.

Studies with various species of algae have shown that these organisms metabolize acyl anilide compounds with production of chlorinated anilines.

Metabolism of these classes of herbicides is dependent upon factors which affect microbial soil activity, including such factors as pH and soil composition which affect the chemical state of the compound as well. These compounds are relatively easily broken down to chlorinated anilines and side chain metabolites. The chlorinated anilines may be volatilized or bound to soils before or concomitantly with microbial metabolism. It is not clear what effects these competing processes have on biodegradation of these molecules. There have been few studies on the persistence or metabolism of compounds arising from the side chain, particularly those containing chloride ions.

SECTION 13
CHLORINATED BIPHENYLS

Polychlorinated biphenyls (PCBs) have been used widely in industrial applications because of their thermal stability, excellent dielectric (electrically insulating) properties, and resistance to oxidation, acids, bases, and other chemical agents. PCBs therefore have found use in capacitors and transformers as dielectric fluids, in hydraulic systems, gas turbines and vacuum pumps, and as fire retardants and plasticizers (215). In 1971, however, Monsanto Company, the sole U.S. producer, voluntarily restricted the use of PCBs to closed systems (capacitors, transformers, vacuum pumps, gas-transmission turbines) and discontinued production entirely in 1978 (281). These applications use complex mixtures of PCBs marketed under the trade names Aroclor (Monsanto Company, USA), Clophen (Germany), Phenoclor and Pyralene (France), Kanaclor and Santotherm (Japan), and Fenclor (Italy). Arkarels are synthetic mixtures of chlorinated biphenyls and trichlorobenzenes.

The Aroclor products are denoted by a four-digit number in which the first two indicate the type of molecule ("12" indicates a chlorinated biphenyl) and the last two digits indicate the weight percent chlorine. Aroclor 1254 consists of chlorinated biphenyls with 54% by weight chlorine and on average 5 chlorines per molecule, although it has been reported to contain 69 different chlorinated biphenyl molecules (162). Similarly, Aroclor 1242 is 42% chlorine by weight and averages 3 chlorines per molecule. Aroclor 1016 also consists primarily of trichlorinated compounds but contains fewer penta- and hexachlorinated molecules than Aroclor 1242. There are 210 possible PCB compounds containing 0 to 10 chlorine atoms per biphenyl molecule. However, many of these have never been found in commercial PCB mixtures.

The PCBs have been released into the environment for many years and are a worldwide contaminant. They are lipophilic and sorb strongly to the lipids and fats of animals including fish, mussels, and birds. PCBs also undergo biological magnification in such common aquatic invertebrates as daphnids, mosquito larvae, stoneflies and crayfish. The concentration of PCBs in the invertebrates can be as high as 27 500 times that in water (167). These invertebrates subsequently are eaten by fish and birds, and bioaccumulation occurs at all levels of the food chain.

MICROBIAL METABOLISM OF PCBs

Most of the studies on microbial metabolism of PCBs have explored the biodegradability of the Aroclors in natural environments or in laboratories using pure strains or mixed cultures. These studies have shown that PCBs containing fewer than 5 chlorines per molecule are extensively degraded, while heavier molecules tend to persist in the environment (26, 91, 163, 167, 220a, 281, 394, 438, 474). These studies are corroborated by environmental analyses which indicate that PCBs found in weathered samples contain 5 or more chlorine atoms per molecule (162).

Few studies have attempted to elucidate the pathways of degradation of pure compounds of a chlorinated biphenyl. Several studies have shown that 4-chlorobiphenyl can be metabolized to 4-chlorobenzoic acid, indicating hydroxylation, ring

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cleavage, and degradation of the nonchlorinated ring of the molecule. This has been demonstrated with soil bacteria, a sewage effluent isolate identified as *Achromobacter* sp. pCb, an unidentified facultative anaerobe called strain B206, *Acinetobacter* sp. P6, and *Alcaligenes* sp. Y42 (1, 163, 164, 231, 426). *Acinetobacter* sp. P6 can use 4-chlorobiphenyl as the sole source of carbon for growth (164). Growth of both *Achromobacter* sp. strain B 218 and *Bacillus brevis* strain B 257 on 4-chlorobiphenyl as the sole source of carbon generates the same metabolites (298). The pathway of degradation involves formation of a 2,3-dihydroxy intermediate with *meta* cleavage to form eventually 4-chlorobenzoic acid. Other metabolites were isolated which represent successive oxidation and utilization of the aliphatic carbons from the cleaved ring (Figure 36).

The formation of chlorinated benzoic acids from chlorinated biphenyls is the most common route of PCB degradation. Both *Alcaligenes* sp. Y42 and *Acinetobacter* sp. P6 convert a large number of biphenyl compounds to the corresponding chlorinated benzoic acids (Table 5) (163, 168). For several compounds containing multiple chlorines with one on the second ring, loss of that chlorine occurs in the formation of the chlorobenzoic acid. Studies with ^{14}C-2,5,2',5'-trichlorobiphenyl confirmed the formation of ^{14}C-2,5-dichlorobenzoic acid and a yellow intermediate by resting cell suspensions of both *Alcaligenes* sp. Y42 grown on biphenyl and *Acinetobacter* sp. P6 grown on 4-chlorobiphenyl (164). The patterns of metabolism of PCBs by *Alcaligenes* sp. Y42 and *Acinetobacter* sp. P6 are similar. The general path of degradation proceeds through *meta* cleavage compounds to chlorobenzoic acids which accumulate during the metabolism of chlorobiphenyls. Metabolism of some chlorinated biphenyls is blocked after production of the dihydroxy intermediate (precursor to ring cleavage), while for other compounds the *meta*-cleavage intermediate accumulates.

Pseudomonas sp. strain 7500 grown on biphenyl metabolizes 2,4'-dichlorobiphenyl to two different monochlorobenzoates, indicating that both rings are capable of being attacked (296). An intermediate metabolite was identified as 2-hydroxy-6-oxo-6-(chlorophenyl)chlorohexa-2,4-dienoic acid.

The degradation of 2,4,4'-trichlorobiphenyl by *Acinetobacter* sp. P6 grown on 4-chlorobiphenyl was studied in detail and a pathway was proposed involving *meta* cleavage after 2',3'-hydroxylation (Figure 37) (166). Hydroxylation occurs on the ring with the fewest chlorine substituents. The predominant metabolite is the *meta* cleavage product, although small amounts of the dichlorobenzoic acid appear. The occurrence of a yellow *meta* cleavage product and subsequent production of chlorobenzoates in the degradation of other PCBs suggests that this may be a general pathway for most PCB metabolism in bacteria.

On the basis of these studies, several generalizations were made regarding the effect of the structure of the PCBs on microbial degradation (163, 164, 165, 168). Degradation decreases as the number of chlorines per molecule increases. Two chlorines on the *ortho* positions of a single ring (i.e., 2,6-) or on both rings (i.e., 2,2'-) inhibit degradation. PCBs with one unsubstituted ring are more readily metabolized than PCBs with the same number of chlorines on both rings. On PCBs with unequally substituted rings, the ring with fewer substitutions is preferentially cleaved. PCBs with a chlorine on the 4'-position are metabolized to stable *meta*-cleavage products.

The occurrence of nitro-containing metabolites in extracts of media containing the unidentified facultative anaerobe B 206 and 4-chlorobiphenyl was investigated (427). When ammonium sulfate is added to the culture medium as the nitrogen source, 4-chlorobiphenyl is metabolized to 3-hydroxy-4'-chlorobiphenyl and 4-hydroxy-4'-chlorobiphenyl. However, when the nitrogen source is sodium nitrate, the metabolites 2-hydroxy-nitro-4'-chlorobiphenyl and 4-hydroxynitro-4'-chlorobiphenyl ap-

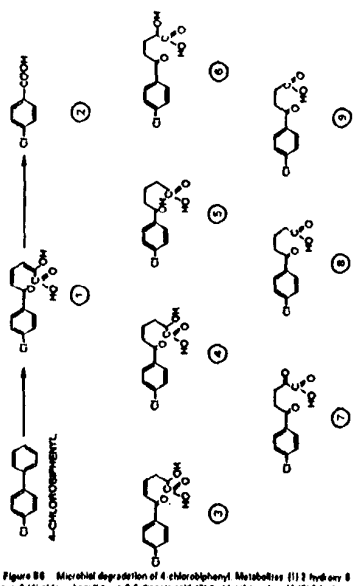

Figure 36 Microbial degradation of 4-chlorobiphenyl. Metabolites: (1) 2-hydroxy-6-oxo-6-(4'-chlorophenyl)-hexa-2,4-dienoic acid, (2) 4-chlorobenzoic acid, (3) 2-hydroxy-6-oxo-6-(4'-chlorophenyl)-4-hexenoic acid, (4) 6-oxo-6-(4'-chlorophenyl)-2-hydroxy-hexanoic acid, (5) 6-hydroxy-6-(4'-chlorophenyl)-hexanoic acid, (6) 2-hydroxy-6-oxo-6-(4'-chlorophenyl)-pentanoic acid, (7) 2,6-oxo-6-(4'-chlorophenyl)-pentanoic acid, (8) 6-oxo-6-(4'-chlorophenyl)-pentanoic acid, (9) 4-oxo-4-(4'-chlorophenyl)-butanoic acid

Adapted from Reference 298

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TABLE 5. METABOLISM OF CHLORINATED BIPHENYL COMPOUNDS BY ALCALIGENES SP. Y42 AND ACINETOBACTER SP. P6*

Substrate	Products
Substitutions on one ring:	
2-Chlorobiphenyl	2-Chlorobenzoic acid
3-Chlorobiphenyl	3-Chlorobenzoic acid
4-Chlorobiphenyl	4-Chlorobenzoic acid
2,3-Dichlorobiphenyl	2,3-Dichlorobenzoic acid
2,4-Dichlorobiphenyl	2,4-Dichlorobenzoic acid
2,5-Dichlorobiphenyl	2,5-Dichlorobenzoic acid
2,6-Dichlorobiphenyl†	2,6-Dichlorodihydroxybiphenyl, 2,6-dichlorotrihydroxybiphenyl
3,4-Dichlorobiphenyl	3,4-Dichlorobenzoic acid
3,5-Dichlorobiphenyl	3,5-Dichlorobenzoic acid
2,3,4-Trichlorobiphenyl	2,3,4-Trichlorobenzoic acid
2,3,6-Trichlorobiphenyl†	2,3,6-Trichlorodihydroxybiphenyl, 2,3,6-dichlorotrihydroxybiphenyl
2,4,5-Trichlorobiphenyl	2,4,5-Trichlorobenzoic acid
2,4,6-Trichlorobiphenyl	2,4,6-Trichlorobenzoic acid), 2,4,6-trichlorodihydroxybiphenyl,† 2,4,6-trichlorotrihydroxybiphenyl†
2,3,4,6-Tetrachlorobiphenyl	2,3,4,6-Tetrachlorobenzoic acid
2,3,5,6-Tetrachlorobiphenyl	None
2,3,4,5,6-Pentachlorobiphenyl	None
Substitutions on both rings:	
2,2'-Dichlorobiphenyl	2-Chlorobenzoic acid
2,4'-Dichlorobiphenyl	2-Chlorobenzoic acid
3,3'-Dichlorobiphenyl	3-Chlorobenzoic acid
4,4'-Dichlorobiphenyl	4-Chlorobenzoic acid
2,4,4'-Trichlorobiphenyl	2-Chlorobenzoic acid, 2,4-dichlorobenzoic acid
2,5,2'-Trichlorobiphenyl	2-Chlorobenzoic acid, 2,5-dichlorobenzoic acid
2,6,3'-Trichlorobiphenyl	3-Chlorobenzoic acid, 2,6-dichlorobenzoic acid

(continued)

TABLE 5 (continued)

2,5,4'-Trichlorobiphenyl	2-Chlorobenzoic acid, 2,5-dichlorobenzoic acid
3,4,2'-Trichlorobiphenyl	2-Chlorobenzoic acid
2,3,2',3'-Tetrachlorobiphenyl	2,3-Dichlorobenzoic acid, an unidentified dichloro compound
2,3,2',5'-Tetrachlorobiphenyl	Dichlorobenzoic acid, an unidentified dichloro compound
2,4,2',4'-Tetrachlorobiphenyl†	2,4-Dichlorobenzoic acid
2,4,2',5'-Tetrachlorobiphenyl†	Dichlorobenzoic acid
2,4,3',4'-Tetrachlorobiphenyl†	Dichlorobenzoic acid
2,5,2',5'-Tetrachlorobiphenyl†	2,5,2',5'-Tetrachlorodihydroxyl biphenyl
2,5,3',4'-Tetrachlorobiphenyl†	Dichlorobenzoic acid
2,6,2',6'-Tetrachlorobiphenyl†	None
3,4,3',4'-Tetrachlorobiphenyl†	3,4-Dichlorobenzoic acid
2,4,6,2',3'-Pentachlorobiphenyl†	2,4,6-Trichlorobenzoic acid, an unidentified trichloro compound
2,4,6,2',5'-Pentachlorobiphenyl†	2,4,6,2',3'-Tetrachlorodihydroxy benzoic acid

*Adapted from reference 183, 184, 168.

†Metabolism by *Acinetobacter* sp. P6 only.

‡Metabolism by *Alcaligenes* sp. Y42 only.

pear. These metabolites were interpreted to result from a nonenzymatic reaction between an arene oxide intermediate and nitrate or nitrite anions. This organism subsequently accumulates 4-chlorobenzoic acid in the medium (426). The appearance of the monohydroxy intermediates suggests a rare monooxygenase mechanism for PCB degradation, although the phenylphenols may also be an artifact arising during the isolation procedure (71a).

Acinetobacter sp. strain P6 resting cells were incubated for 4 hours with several Kanechlor PCB mixtures (167). Kanechlor KC100 (primarily dichlorobiphenyls) was metabolized to monochlorobenzoates. Kanechlor KC300 was metabolized to benzoates with 1 to 3 chlorines, dihydroxybiphenyls with 2 to 4 chlorines, ring meta cleavage products with 2 to 3 chlorines, and many other unidentified compounds with 2 chlorines. Kanechlor KC500 was scarcely metabolized, although some dihydroxy isomers were noted.

Studies were conducted utilizing *Alcaligenes* sp. strain UM 2 which was isolated on diphenylmethane and known to metabolize dichlorinated biphenyls (474). A mixture of di- and trichlorinated biphenyls at 0.05% concentration (32% by weight) was 80% metabolized in 1 day and completely metabolized in 3 days. At 0.25% concentration, 22% was metabolized in 1 day and 29% in 3 days. Under cometabolic conditions, a 100 mg/l PCB mixture of di-, tri-, and tetrachlorobiphenyls (41% by weight) was 70% metabolized in 2 days and 80% in 6 days. In a minimal medium with only a small quantity of carbon source, 30% was metabolized in 6 days. Most of the remaining substrate was tetrachlorobiphenyl. The metabolites included mono- di-, and trichlorobenzoates, monohydroxychlorobiphenyl, 2-hydroxy-6-oxo-chlorophenylhexa-2, 4-dienoic acid, chlorobenzoylpropionic acid, chlorophenylacetic acid, and 3-chlorophenyl-2-chloropropenoic acid (a substituted cinnamic acid).

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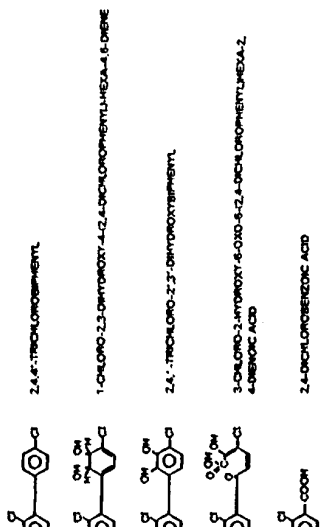

Figure 57 Pathway of 2,4,4'-trichlorobiphenyl metabolism by Acinetobacter sp. P6

Adopted from Reference 166

Pseudomonas sp. strain 7509 also formed mono- and dichlorobenzoic acids during metabolism of Aroclor 1242 (36a). A number of nonchlorinated aromatic and aliphatic compounds were isolated after 2 months' incubation of Aroclor 1242 with several strains of bacteria isolated from lake water (233). No chlorinated metabolites or oxidized derivatives of PCBs were detected.

The disappearance of Aroclor products and individual PCB isomers during incubation with *Nocardia* sp. NCIB 10605 was monitored (26). The following isomers were 60 to 100% metabolized within 2 weeks: 2,4'-di, 2,3-di, 3,4-di, 2,3,2'-tri, 2,3,4'-tri- and 3,4,3'-trichlorobiphenyl, while 2,5,4'-trichlorobiphenyl was 60% metabolized in 73 days. There was little transformation of 2,4,6-tri-, 2,4,2',4'-tetra-, or 2',4',6,2'-tetrachlorobiphenyl in 9 days, and no degradation of 4,4'-dichlorobiphenyl was detected after 121 days. However, 4,4'-dichlorobiphenyl was 50% metabolized in 2 days when present as a component of Aroclor 1242 During 52 days' incubation Aroclor 1242 was 88% metabolized and in 100 days was 95% metabolized. Aroclor 1016 was 96% metabolized in 52 days.

There are two reports in the literature concerning the degradation of PCBs by fungi. *Rhizopus japonicus* converts 4-chlorobiphenyl to 4-chloro-4'-hydroxybiphenyl and 4,4'-dichlorobiphenyl to an unidentified hydroxylated metabolite (454). *Cunninghamella echinulata* Thaxter metabolizes 2,5-dichloro-4'-isopropylbiphenyl by oxidation of the isopropyl group to form 2,5-dichloro-4'-biphenylcarboxylic acid and by hydroxylation of the chloroline-substituted phenyl group (440).

METABOLISM OF PCBs BY MIXED MICROBIAL CULTURES

A mixed microbial population in lake water metabolized 2-chlorobiphenyl to 2-chlorobenzoic acid and chlorobenzoylformic acid (394). In contrast, 2,4'-dichlorobiphenyl was not metabolized after 8 months' incubation.

A mixed microbial culture derived from river sediments was able to metabolize 4-chlorobiphenyl rapidly, with 99% removal in 30 days (265). Acclimated and nonacclimated cultures showed similar results. There was transitory formation of a metabolite thought to be 2-hydroxy-6-oxo-6-(4-chlorophenyl)hexa-2,4-dienoic acid as well as production of 4-chlorobenzoic acid. The substrate was ^{14}C-labeled on the chlorinated ring only and production of $^{14}CO_2$ was noted after 4-chlorobenzoic acid formation. The substrates 2-chloro- and 3-chlorobiphenyl were also metabolized, but 2-chlorobenzoic acid was not degraded further.

A marine mixed microbial community metabolized all three monochlorinated biphenyls (52). Metabolites were not identified.

A river water die-away test demonstrated 50% removal of 1 to 100 mg/l 2-chloro-, 3-chloro-, or 4-chlorobiphenyl within 2 to 5 days (17). The compound was uniformly ^{14}C-labeled in the chlorinated ring and up to 50% of the label appeared as monochlorobenzoic acid and subsequently as $^{14}CO_2$. No degradation of 2,2',4,4'-tetrachlorobiphenyl was noted after 98 days' incubation.

A mixture of bacteria was isolated by enrichment culture with garden soil using benzene as the substrate (18). Several PCB isomers were incubated with the mixed culture and benzene for up to 6 weeks and the medium subsequently tested for presence of chlorobenzoate metabolites. Neither 2-chloro- nor 2,7'-dichlorobiphenyl was metabolized to chlorobenzoic acids, although 4-chlorobiphenyl formed 4-chlorobenzoic acid. The substrate 2,4,4'-trichlorobiphenyl formed copious amounts of 4-chloro- and 2,4-dichlorobenzoic acid in the ratio 5:2. Loss of one chloride was noted in the formation of 4-chlorobenzoic acid from 2,4'-dichlorobiphenyl, 2,5-dichlorobenzoic acid from 2,2',5-trichlorobiphenyl, 4-chlorobenzoic acid and 3,4-dichlorobenzoic acid from 3,4,4'-trichlorobiphenyl, 4-chlorobenzoic acid and 2,4-dichlorobenzoic acid from 2,4,4'-trichlorobiphenyl, and 2,3,4,5-tetrachlorobenzoic acid from both 2,3,3',4,5-pentachlorobiphenyl and 2,3,4,,4',5-pentachlorobiphenyl.

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A soil plot was treated with 1 ppm 2,2'-dichlorobiphenyl (317). After one growing season almost half the remaining material was unchanged substrate. About 9% were soluble metabolites and almost 42% were unextractable residues. After 1 year, 74% of the remaining material were unextractable residues. The metabolites included monohydroxy derivatives of the substrate as well as other products.

Metabolism of 4,4'-dichlorobiphenyl by a mixed microbial culture obtained from the filtrate of an activated sludge sample resulted in formation of 4,4'-dichloro-2,3-hydroxybiphenyl and 4-chlorobenzoic acid (439). Metabolism of this substrate is repressed by the presence of alternative carbon sources. Under similar experimental conditions, including an incubation time of 14 days, there was no metabolism of 2,4,5-tri-, 2,2',5,5'-tetro, 2,2',3,4,5'-penta-, 2,2',3,4,5,5'-hexa- and decachlorobiphenyl.

Metabolism of 2,5,2',5'-tetrachlorobiphenyl and 2,5,2'-trichlorobiphenyl occurred in seawater with production of a compound thought to be a lactone acid (68a). No degradation was noted during the incubation of the tetrachlorobiphenyl with anaerobic marsh mud during a 45-day incubation period.

A microbial consortium obtained from activated sludge metabolized the isopropyl group of 4-chloro-4'-isopropylbiphenyl to a hydroxyl substituent, forming 4-chloro-4'-hydroxybiphenyl, followed by formation of 4-chlorobenzoic acid (440). Intermediates of the isopropyl metabolism pathway were identified. Addition of glucose as an alternate carbon source repressed metabolism of the chlorinated substrate.

Biphenyl enrichment of both uncontaminated soils and soils contaminated with PCBs resulted in isolation of mixed cultures which were incubated with Aroclor 1242 (91). Cometabolism with sodium acetate enhanced metabolism of all the PCBs including the higher chlorinated molecules, although this phenomenon may be due to the increased biomass resulting from growth on the simpler carbon source. Extensive degradation of all the lower chlorinated isomers was noted with up to 68% metabolism of the tetrachlorinated biphenyls in 15 days.

A related study was conducted to determine the influence of inoculum concentration on the aerobic bio-oxidation of 3,3'-dichlorobenzidine which is used in the manufacture of azo dyes (57). The effluent from a domestic sewage treatment plant was used as the inoculum and the substrate concentration was 20 mg/l. When present as the sole carbon source, the substrate was not metabolized. However, the presence of yeast extract in the medium promoted extensive disappearance of the substrate within 28 days. In another experiment, 2 mg/l 3,3'-dichlorobenzidine was added to lake or reservoir water and incubated 14 days (10). Neither metabolites nor $^{14}CO_2$ was recovered after incubation. Increasing disappearance of substrate with time was correlated with increasing biomass, which served as a sorbent for the substrate. The supernatant fluid from settled activated sludge material served as the inoculum for flasks containing 3,3'-dichlorobenzidine and additional carbon sources. After 4 repeated weekly subcultures to flasks of fresh media, no metabolites of the substrate were recovered.

SUMMARY

The limited number of studies on the degradation of specific chlorinated biphenyl compounds by pure strains of bacteria has served to establish some general features of PCB metabolism. A few strains of bacteria have been shown to mineralize some chlorinated biphenyls. In most cases bacteria with the capability of degrading one ring of a chlorinated biphenyl compound are unable to degrade the resulting chlorinated benzoates. These compounds accumulate in pure cultures. Evidence exists for the complete mineralization of chlorinated benzoates by other strains of bacteria (discussed in Section 7 on chlorobenzoates), and mixed cultures of bacteria

have been shown to mineralize PCBs with 4 or fewer chlorines per molecule. More heavily substituted PCBs appear to resist degradation and accumulate even in environments where lower PCBs are degraded.

The mechanism of hydroxylation of PCBs by bacteria has not yet been elucidated nor have the enzymes mediating the steps in the proposed pathways been isolated. Two pathways have been proposed, the first analogous to the pathway of degradation of biphenyl. Initial hydroxylation occurs in the 2,3 position of the less substituted ring, followed by *meta* cleavage and subsequent degradation of the aliphatic portion of the molecule to form substituted benzoic acids. Chlorines on the aliphatic carbons are lost during this process. However, this may not be the mechanism for degradation of PCBs substituted in all the *ortho* positions. A second pathway based on presence of a monooxygenase in bacteria has been proposed after discovery of 4-hydroxy-4'-chlorobiphenyl in extracts of bacterial cultures incubated with 4-chlorobiphenyl. More evidence corroborating this mechanism needs to be obtained to determine how widespread this pathway is. Limited evidence on fungal metabolism of PCBs indicates activity of a monooxygenase in a manner similar to that shown in biphenyl metabolism.

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SECTION 14
DDT AND RELATED COMPOUNDS

DDT, 1,1,1-trichloro-2,2-bis(*p* chlorophenyl)ethane, received its abbreviation from the trivial name dichlorodiphenyltrichloroethane. This chlorinated aromatic is one of the most persistent pesticides in the environment. Since it is lipophilic, it readily accumulates in microorganisms and invertebrates and undergoes biomagnification as fish and birds higher in the food chain ingest DDT-contaminated organisms (79, 229, 363, 311). Widespread effects of DDT poisoning include eggshell thinning and birth defects. Reviews of the literature pertaining to DDT metabolism in microbial systems have been published in 1976 and 1980 (138, 228).

BACTERIAL METABOLISM OF DDT

Due to the widespread incidence of toxicity demonstrated after DDT ingestion, the intestinal flora of various animals became the focus for studies of the metabolism of DDT. DDT is converted directly to DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethane, also referred to as dichlorodiphenyldichloroethane (Figure 58). The reaction involves removal of a chloride ion from the aliphatic portion of the molecule. This is a reductive dechlorination reaction requiring anaerobic conditions, in contrast to the oxidative pathways of metabolism of most other pesticides.

The involvement of intestinal bacteria in DDT metabolism by mammals has been demonstrated in a pectine mix which showed that rats converted DDT to DDD when fed by stomach tube, but not when injected intraperitoneally (308). The coliform bacteria *Escherichia coli* and *Enterobacter aerogenes* isolates from rat feces demonstrate this reaction as well. Microorganisms isolated from rat intestines which convert DDT to DDD include *Clostridium perfringens*, *Streptococcus* sp., *Bacteroides* sp., *E. coli* and other coliforms, yeasts, and to a lesser extent *Lactobacillus* sp. (35). *Klebsiella pneumoniae* also converts DDT to DDD (464). DDE (1,1-bis(*p*-chlorophenyl)-2-chloroethylene) can be detected after 20 hours at concentrations from 5 to 10% in cultures of *Streptococcus* sp., *Bacteroides* sp., *Pseudomonas* sp. and *Lactobacillus* sp. (the latter after a 72 hour incubation) (35). However, DDE has been shown to be produced nonenzymatically as well as enzymatically (464).

Proteus vulgaris, isolated from the intestines of DDT-resistant mice, converts DDT to DDD subsequently to 1,1-bis(*p*-chlorophenyl)-2-chloroethane and 1,1-bis(*p*-chlorophenyl)ethane, representing three successive reductive dechlorinations (Figure 58) (20,21). DDE also appears in the medium. The excreta of stable flies become the source of three bacteria, *E. coli*, *Serratia marcescens*, and a third unidentified strain, which convert DDT to DDD (90%) and DDE (5%) after 24 to 72 hours anaerobically but not aerobically (415). Bacteria from bovine rumen fluid convert ^{14}C-DDT to ^{14}C-DDD (311). This same reaction was noted for DDT incubated with water from Clear Lake, California and with reduced iron porphyrins (hemoglobin or hematin) (311). The isomer *o,p'*-DDT which constitutes 10-20% of

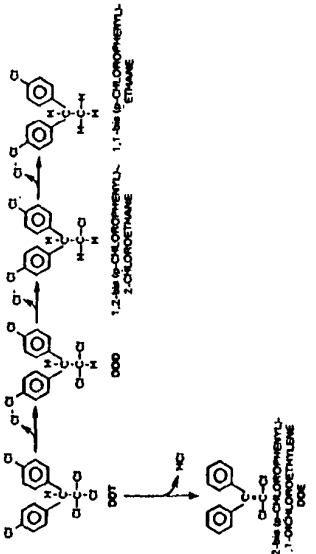

Figure 58. Reductive dechlorination of DDT and dehydrochlorination of DDT.

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technical grade DDT is converted by rumen microorganisms to *o,p'*-DDD at the same rates as *p,p'*-DDT (161). This conversion occurs as well in *E. aerogenes* both aerobically and anaerobically (309).

The direct conversion of DDT to DDD without the DDE intermediate was confirmed in *E. aerogenes* using deuterated DDT (355). The deuterium atom present at the 2-position in DDT is retained in the product, indicating that the chlorine is replaced by hydrogen (or hydride ion) directly without the intermediary species. The membranes of *E. coli* were shown to be the site of reductive dechlorination of this species. The process required flavine-adenine dinucleotide (FAD) and anaerobic conditions (140).

Cell-free extracts of *E. aerogenes* also convert DDT to DDD. This activity is due to reduced Fe(II) cytochrome oxidase (464). More complete degradation of DDT occurs in both whole-cell preparations and cell-free extracts of this organism (Figure 89)(463,465). Metabolism follows the pathway DDT → DDD → DDMU → DDMS → DDNU → DDA → DPM → DBH → DBP →, where the abbreviations represent the compounds as follows: (DDMU) 1-chloro-2,2-bis(*p*-chlorophenyl)ethylene; (DDMS) 1-chloro-2,2-bis(*p*-chlorophenylsulfonyl)ethane, (DDNU) unsym-bis(*p*-chlorophenyl)ethylene (DDA) 2,2-bis(*p*-chlorophenyl)acetate or more commonly dichlorodiphenylacetate; (DPM) dichlorodiphenylmethane; (DBH) dichlorobenzhydrol; and (DBP) dichlorobenzophenone. The enzymatic conversion of DDT to DDE is a dead-end side reaction. DDA is the end product of vertebrate metabolism. The conversion of DDA to DBP does not require anaerobic conditions. This pathway has also been demonstrated in anaerobic cultures of *E. coli* (268).

A single study reports that under aerobic conditions, cultures of *Bacillus cereus* metabolize DDT by this pathway within 7 days, although the use of screw-cap flasks in these experiments may have allowed some anaerobiosis to develop. Cultures of *E. coli* incubated aerobically for 24 hours with intermittent shaking converted DDT to DDD (75%) and DDE (25%) (247).

Environmental isolates also have the ability to convert DDT to DDD. Viable cells of *Bacillus megaterium* convert DDT to DDD (201). Three hundred bacterial strains from Lake Michigan each converted DDT to DDD and many converted ^{14}C-DDD to ^{14}C-DDMS (1-bis(*p*-chlorophenyl)ethane (302). Bacteria isolated from marine and brackish water and sediment converted ^{14}C-DDT to water-soluble metabolites (232). Forty-seven of 100 isolates effected 5 to 10% conversion while an additional 38 isolates converted less than 5% of the starting material. Twenty-five isolates did not produce water-soluble metabolites, indirectly indicating that the presence of those metabolites in the other cultures was biologically mediated. Twenty-three of 26 plant pathogenic and saprophytic strains of bacteria representing nine genera, converted DDT to DDD anaerobically (230). Eighteen bacterial strains, mostly *Pseudomonas* spp. which previously had been shown to metabolize dieldrin, also degraded DDT to DDD (349). In addition, 14 of these isolates produced DDA and 10 produced a diketol-like compound.

Pseudomonas aeruginosa 640A isolated from DDT-polluted soil of the Crimean region was used to construct 2 derivatives (185). Strain BS816 carries a plasmid encoding the genes which degrade naphthalene and salicylate by *ortho*-cleavage, and strain BS827 carries a plasmid which effects *meta* degradation. Both plasmids were obtained from strains of *P. putida*. The parent *P. aeruginosa* and the two derivatives all metabolize DDT with the formation of the same metabolites. Strain BS816, carrying the plasmid coding for *ortho* cleavage, degrades DDT most extensively, converting 89% of the DDT to DDD,1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDDE), phenylpropionic acid (PPA) and phenylacetic acid (PAA).

An organism identified as a *Pseudomonas* sp. was isolated by enrichment culture for its ability to use diphenylethane as a sole source of carbon and energy for growth

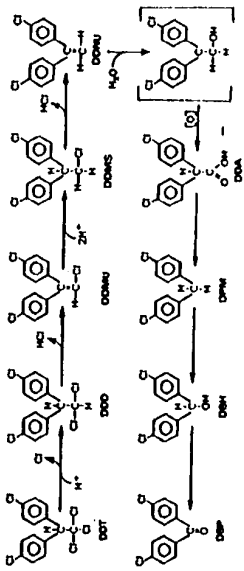

Figure 89 Metabolism of DDT by bacteria DDT = 1,1,1-trichloro 2,2 bis (p chloro phenyl) ethane; DDD = 1,1-dichloro-2,2-bis (p chlorophenyl) ethane, 2,2 bis (p chloro phenyl) ethene; DDMU = unsym-bis (p chlorophenyl) ethylene, DDOH = 2,2-bis (p chlorophenyl) ethanol (hypothetical metabolite), DDA = dichlorodiphenylacetate, DPM = dichlorodiphenylmethane; DBH = dichlorobenzhydrol, DPB = dichlorobenzophenone
Adapted from References 463, 465

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(157). The ability of this organism to grow on several other metabolites of DDT was tested. Diphenylethane is converted to 2-phenylpropionic acid and metabolized further. Diphenylmethane is metabolized with intermediate production of phenylacetic acid and 1-(*p*-chlorophenyl)-1-phenylethane is metabolized with the production of 2-(*p*-chlorophenyl)propionic acid as the only metabolite, indicating cleavage of the unsubstituted ring. The DDE analog 1-(*p*-chlorophenyl)-1-phenylethene is metabolized only to 2-(*p*-chlorophenyl)-2-propenoic acid. The substrate 1-(*p*-chlorophenyl)-1-phenylethanol is converted to 2-(*p*-chlorophenyl)-2-hydroxypropionic acid which is slowly metabolized further. During metabolism of this substrate minor amounts of the nonchlorinated metabolite 2-hydroxy-2-phenylpropionic acid are produced, indicating attack on the chlorinated ring. Each of the above substrates served as the sole source of carbon and energy for growth. The compound 2,2-diphenylethanol is not metabolized. Analogs of DDT which have chlorine substituents on both rings are not metabolized, and 1,1-diphenyl-2,2,2-trichloroethane is not metabolized. However, when diphenylethane is available in the medium, this organism cometabolizes bis(*p*-chlorophenyl)methane to *p*-chlorophenylacetic acid which accumulates (158). With the same cosubstrate, the organism cometabolizes 1,1-bis(*p*-chlorophenyl)ethane to 2-(*p*-chlorophenyl)propionic acid with transient appearance of two hydroxylated metabolites, 1-(*p*-chloro-α-hydroxyphenyl)-1-(*p*-chlorophenyl)ethane and 1-(*p*-chloro-α-hydroxyphenyl)-1-(*p*-chlorophenyl)ethanol. Accumulation of toxic chlorinated carboxylic acids may have inhibited metabolism of the substrate. Compounds which are recalcitrant to cometabolic activity have substitutions in the ethane or ethene sections of their structures which may cause sterle hindrance.

A large sampling study of oceanic and near-shore environments established that 35 of 95 isolates degraded DDT to many of the metabolic products previously identified, with DDD the major metabolite (350). The only environmental samples which failed to mediate DDT degradation were the oceanic water samples.

FUNGAL METABOLISM OF DDT

The earliest studies involving microbial metabolism of DDT were conducted using commercial yeast cakes (*Saccharomyces cerevisiae*) (234). Reductive dechlorination was demonstrated by the appearance in culture media of ^{14}C-DDD from ^{14}C-DDT labeled in the phenyl group. DDE was shown not to be a necessary intermediate metabolite.

A study of 8 fungi incubated with DDT for 6 days did not reveal degradation (80). However, in the same study 6 of 9 actinomycetes were found to convert DDT to DDD. These 6 actinomycetes are *Nocardia erythropolis*, *Streptomyces aureofaciens*, *S. viridochromogenes*, *S. cinnamomeus*, and with lesser efficiency *S. albus* and *S. antibioticus*. All cultures in this study were incubated aerobically with shaking. Another study of microbial cultures with the capability to degrade dieldrin showed that 2 *Trichoderma viride* strains could degrade DDT to DDD, a "dicofol-like" metabolite, and DDA (349). The dicofol-like compound was subsequently identified as 1-bis(*p* chlorophenyl)ethane (302). Earlier studies with several strains of *T. viride* established differences in the metabolites produced by each strain (300).

Shake cultures of *Mucor alternans* in nutrient media containing ^{14}C-DDT produced 3 hexane soluble and 3 water soluble metabolites within 2 to 4 days (7). The total activity recovered was about equally divided between the two phases. The major metabolite is water soluble. These metabolites were unidentified since the results obtained from thin layer chromatography were different from those for DDE, DDD, DDA, DBP, dicofol, or 1,1-bis(*p* chlorophenyl)ethane. *M. alternans* converted 15% of the DDT starting material to 3 unidentified water soluble products in another

experiment as well (212). A comparison of three products with 1,1 bis(*p*-chlorophenyl)acetic acid (DDA), PCPA, DBP, DBH and 2-chlorosuccinic acid failed to reveal their identities. Attempts to reproduce this metabolic activity in the natural environment were unsuccessful, as the addition of *M. alternans* spores to DDT-treated soil failed to promote any degradation after 11 weeks' incubation (7).

A sequential experiment was developed to study the interactive effects of bacteria and fungi (156). *Hydrogenomonas* sp. was grown on dichlorodiphenylmethane or *p*-chlorophenylacetic acid, both metabolic products of DDT. *Hydrogenomonas* sp. cannot liberate free chloride from metabolism of these compounds. The culture supernatant fluid was extracted and added to a basal salts solution, which became the growth medium for a culture of *Fusarium* sp. Growth occurred under anaerobic conditions, and chloride was detected in the medium, indicating that the products of *Hydrogenomonas* sp. metabolism were degraded to CO_2, H_2O, and HCl by *Fusarium* sp. The ability to perform this mineralization decreased if the 2 microbial populations were incubated together.

In another study of the interactive effect of other fungi on DDT degradation by *M. alternans*, the addition of other fungal cultures or the cell-free spent media from some cultures repressed the formation of water soluble metabolites (6). Other fungi, including *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Fusarium oxysporum*, *Penicillium notatum*, *Rhizopus arrhizus* and *Trichoderma viride*, failed to produce water soluble metabolites of DDT. However, water soluble ^{14}C-products appeared after incubation of ^{14}C-DDT with the excretory products retained in culture media after growth of all of the above fungi including *M. alternans*, with the exception of *R. arrhizus*. This discrepancy in the appearance of water soluble metabolites may be attributed to sorption of degradation products by the mycelia or to further degradation by cells to metabolites that are not water soluble.

The path of DDT metabolism by *Fusarium oxysporum* has been established and follows the route DDT → DDD → DDMU → DDHO → DDOH → DDA → DBP, with DDE formed from DDT (135, 136, 136a). DDHO is the aldehyde intermediate which is rapidly converted to DDOH and DDA. This path is similar to the described bacterial. The enzymes involved in DDT metabolism which have been isolated include DDT dehydrochlorinase and those that decompose DDMU, DDA, and DDOH (165a). DDT inhibits the fungal esterase while DDD strongly activates the same enzyme (159). The net effect is enzyme activation which results in detoxification of the molecule.

FUNGAL METABOLISM OF OTHER COMPOUNDS

The acaricide chlorobenzilate (ethyl 4,4'-dichlorobenzilate) ^{14}C-labeled in the aliphatic moiety was cometabolized by *Rhodotorula gracilis* with glucose as an additional carbon source (312, 313). Production of $^{14}CO_2$ was correlated with culture growth. Metabolites included 4,4'-dichlorobenzilic acid, dichlorobenzophenone, and other unidentified products. The same results were obtained in the metabolism of chlorobenzilate (isopropyl 4,4'-dichlorobenzilate). Alteration of the chlorinated rings was not noted.

PERSISTENCE AND DEGRADATION OF DDT IN THE ENVIRONMENT

DDT is converted to DDD by anaerobic but not aerobic sludge microorganisms (202). The same results were found using Pawnee silt loam treated with ^{14}C-DDT. Under anaerobic conditions DDD was recovered while under aerobic conditions DDE was the only metabolite (190). Another study of anaerobic soil treated with

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DDT reported formation of DDD and traces of other metabolites including DDE, DDA, dicofol, 4-chlorobenzoate, DBP, and DBM (189a). Conversion of DDT to DDD in flooded soil was faster when more organic matter was present (69).

The conversion of DDT to DDD has been demonstrated in sterile as well as nonsterile environments (59), and has been related to the redox potential (Eh) of the soil. Sewage sludge samples sterilized in a variety of ways all resulted in conversion of DDT to DDD if the Eh was sufficiently low (489). The rate of DDT degradation is highest in soils with the lowest redox potentials, in the range of -90 to -250 mV (184). Studies of DDT degradation in a variety of anaerobic and aerobic environments using various carbon sources and various soils, have shown differences in the amount of degradation and the efficiency of substrate and product recovery which preclude extrapolation from laboratory to the environment or even from one experiment to another (346, 347). Little conversion was found in moist anaerobic soil with Eh of +350 mV or in flooded anaerobic soil with an Eh that dropped from +400 to +200 mV. Flooded stirred soil (Eh = 0 m V) also showed little degradation. However, stirred anaerobic soil treated with lime (Eh = -250 m V) and glass beads inoculated with mud (Eh = -250 to -300 m V) mediated greater than 95% conversion of DDT (346).

It appears that at low Eh, DDT undergoes an irreversible redox type of reaction with transient formation of a free radical before conversion to DDD (184). The reaction is thought to be mediated by reduced iron porphyrins, with cell metabolism not being necessary (489).

Numerous studies have been conducted with regard to the persistence of DDT and its metabolites in environments treated with the insecticide (115, 131, 278, 324, 360, 429, 444, 485). All of the studies have reported residues of DDT, DDE and DDD in the environment at the time of sampling. The longest period of time between last application of DDT and sampling was 17 years, and on the basis of these data half-life numbers for DDT of 2.5 to 35 years have been reported (324). In general, the amount of these residues that remains in the soil 1 to 2 years after application is similar to the amount recovered 9 years or longer after the first sampling.

SUMMARY

Studies on metabolism of DDT by bacteria and fungi have shown that reductive dechlorination of the nonaromatic portion of the molecule is the necessary primary step. The pathway of metabolism of DDT, first described for *F. aerogenes* but subsequently confirmed in other bacteria and in fungi, describes a series of steps requiring anaerobiosis (DDT to DDA) followed by a series of steps that may require aerobic conditions (DDA to DBP). Although some studies report aerobic conversion of DDT to metabolites, the oxygen tension was not rigorously defined in these experiments. All of these metabolites retain the chloride ions on both aromatic rings of the molecule.

The effect of a concerted attack by several microbial species has been demonstrated in a two-stage experiment in which *Hydrogenomonas* sp. was grown on either of the DDT metabolites dichlorodiphenylmethane or *p*-chlorophenylacetic acid. The resulting filtered media became the growth substrate for cultures of the fungus *Fusarium* sp., which anaerobically liberated free chloride. Indicating mineralization to CO_2, H_2O, and HCl. The nature of this pathway and the enzymes involved have not yet been elucidated.

Other studies which have shown differing efficiencies of DDT metabolism between pure culture and consortia or sewage/soil studies, indicate that degradation of this compound is highly dependent on environmental factors including coexistence of other organisms. DDT persists in the environment despite the presence of organisms capable of metabolizing the compound to at least DDD. Of particular importance is

the redox potential of the environment. Only under highly reducing conditions can the necessary first step of conversion of DDT to DDD be achieved. This reaction does not require microbial mediation. Further steps in DDT degradation may require environmental conditions and microbial activities which have not yet been elucidated. DDT, DDD, and DDE are highly persistent in all environments treated with DDT.

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SECTION 15
CHLORINATED DIOXINS AND DIBENZOFURANS

The chlorinated dibenzo-*p*-dioxins and dibenzofurans are produced as byproducts during the formation of many other chemicals, including 2,4,5-T, hexachlorophene, pentachlorophenol and other chlorinated phenols, and polychlorinated biphenyls (360). Chlorinated dioxins have been found in the fly ash and flue gases from municipal generators in Switzerland, presumably due to pyrolysis of chlorophenol salts, and the formation of chlorinated furans has been tied to the pyrolysis of polychlorinated biphenyls and polychlorinated diphenyl ethers. These compounds are used as heat exchange fluids and as hydraulic liquids. From 3 to 25% of the polychlorinated biphenyls burned may be converted to chlorinated dibenzofurans (360). There is no known technical use for the chlorinated dibenzo-*p*-dioxins, of which 75 congeners can exist, and the chlorinated dibenzofurans, of which there are 135 theoretical congeners (360). The positional isomers of the dioxins vary greatly in their acute toxicity and biological activity, and the most potent isomer, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is considered the most potent low-molecular-weight toxin known (mean lethal dose in guinea pigs 0.6 mg/kg body weight) (387).

Interest in these compounds was generated after an epidemic of "chick edema factor" in 1957 due to 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin that caused the death of millions of broiler chickens, and an accident in a chemical plant in 1976 in Seveso, Italy that released a cloud of toxic materials including TCDD to the surrounding environment (259, 356). TCDD has also been shown to cause chick edema factor (387). The extreme toxicity of the compound of major interest, TCDD, has focused most research on this isomer.

MICROBIAL METABOLISM OF DIOXINS AND FURANS

To date none of the chlorinated or nonchlorinated dioxins or furans have served as a sole source of carbon or energy for growth by any microorganism in a wide range of screening and enrichment experiments (214, 259, 260, 301). *Pseudomonas* sp. NCIB 9816, which can utilize naphthalene as a sole carbon source, can cometabolize the nonchlorinated molecule dibenzo-*p*-dioxin when salicylic acid is present in the growth medium (259). Studies to determine the products of cometabolism were conducted with a mutant, *Pseudomonas* sp. NCIB 9816 strain 11, which oxidizes naphthalene only to *cis*-1,2-dihydroxy-1,2-dihydronaphthalene. Dibenzo-*p*-dioxin is cometabolized to 2 neutral products, identified as *cis*-1,2-dihydroxy-1,2-dihydrodibenzo-*p*-dioxin and 2-hydroxydibenzo-*p*-dioxin. When the first product is incubated aerobically or anaerobically with cell extracts of the parent organism in a medium containing NAD^+, a third product is formed and was identified as 1,2-dihydroxydibenzo-*p*-dioxin (Figure 60). This metabolite competely inhibits or inactivates the enzyme 1,2-dihydroxynaphthalene oxygenase, which in the parent splits the naphthalene ring in the analogous pathway.

Similarly, a *Beijerinckia* sp. grown on dibenzo-*p*-dioxin and succinic acid produces 1,2-dihydroxydibenzo-*p*-dioxin (260). Cell growth is inhibited after 4

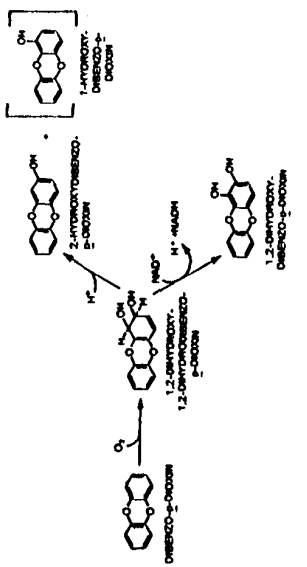

Figure 60 Oxidation of dibenzo-*p*-dioxin by *Pseudomonas* sp. NCIB 9816. Bracketed compound is hypothetical intermediate.
Adapted from Reference 259.

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hours. Cell extracts incubated with 1,2-dihydroxydibenzo-*p*-dioxin show a brief initial rate of oxidation followed by a decline to the nonenzymatic rate. The rate of oxidation was determined polarographically using an oxygen electrode to measure oxygen consumption. Two oxygenases were isolated from the cell extract, 2,3-dihydroxybiphenyl oxygenase which also oxidizes 1,2-dihydroxydibenzo-*p*-dioxin, and catechol oxygenase which has no activity against the dioxin metabolite. Both these oxygenases are inhibited when incubated with cell extracts.

This *Beijerinckia* sp. utilizes biphenyl as a sole carbon and energy source. When grown on succinic acid plus biphenyl, resting cells oxidize 1-chloro- and 2-chlorodibenzo-*p*-dioxin at a high rate. Dibenzo-*p*-dioxin and the isomers 2,3-, 2,7-, and 2,8-dichlorodibenzo-*p*-dioxin are oxidized at a lower rate, followed by 1,2,4-trichlorodibenzo-*p*-dioxin (260).

A mutant strain of this species called *Beijerinckia* sp. 8B/36 was isolated which metabolizes several aromatic hydrocarbons to *cis*-dihydrodiols (260). When grown on succinic acid plus dibenzo-*p*-dioxin, a neutral product is formed which was identified as *cis* 1,2-dihydroxy-1,2-dihydrodibenzo-*p*-dioxin and has identical characteristics to the product of *Pseudomonas* sp. NCIB 9816 metabolism. Also appearing in the medium is the metabolite 2-hydroxydibenzo-*p*-dioxin. The mutant *Beijerinckia* sp. 8B/36 converts both 1-chloro- and 2-chlorodibenzo-*p*-dioxin to neutral products which appear to be *cis*-dihydrodiols, but no products appear after cometabolism with 2,3-dichloro- or 2,7-dichlorodibenzo-*p*-dioxin.

An unidentified bacterium was isolated from contaminated Seveso soil and incubated aerobically in a complex nutrient medium containing ^{14}C-TCDD (214, 351, 352). After 34 weeks 2 polar metabolites appeared. One was isolated in very small quantities and was not identified. The other was found to be a hydroxylated derivative. This microbial metabolite also appears in a culture of *P. testosteroni* strain 0103b after incubation for 36 weeks and in a culture composed of a mix of 6 bacteria from Seveso soil. The metabolite was postulated to be 1-hydroxy-2,3,7,8-TCDD, assuming no chlorine rearrangement took place.

TCDD was metabolized by *Bacillus megaterium* to several polar metabolites (358). The most active cultures were incubated with 5 μg/l TCDD introduced in an ethyl acetate carrier, a solvent which increases cell permeability. When ethyl acetate was the carrier and the amount of soybean extract in the medium was reduced, as much as 55% of the dioxin was recovered as polar metabolites. TCDD was also converted in small amounts to a polar metabolite in farm soil which had been incubated for 2 months. The quantity of this metabolite did not increase with time after 2 months. Other soils similarly incubated failed to produce any metabolites. Two strains of bacteria which converted TCDD to polar metabolites were isolated from the farm soil samples.

A large screening study examined 100 bacterial isolates for ability to metabolize TCDD (301). These strains all had shown previous ability to metabolize persistent pesticides, but only 3 showed some ability to metabolize TCDD as determined by thin-layer chromatography. The product or products were not identified.

Degradation of TCDD by an extracellular laccase (*p*-diphenol:oxygen oxidoreductase) produced by the fungus *Polyporus versicolor* was investigated (68). Crude enzyme extracts incubated with TCDD under a variety of conditions failed to modify the substrate.

There has been one report on the microbial metabolism of dibenzofuran (77). A comparison was made of the cooxidation of this compound by *Cunninghamella elegans*, *Beijerinckia* sp. and *Beijerinckia* sp. 8B/36 discussed previously with regard to dibenzo-*p*-dioxin metabolism. The mutant strain oxidizes dibenzofuran to a mixture of 1,2-dihydroxy-1,2-dihydrodibenzofuran and the unstable 2,3-dihydroxy-2,3-dihydrodibenzofuran which under acidic conditions dehydrates to a mixture of

2-hydroxy- and 3-hydroxydibenzofuran (Figure 61). The fungal culture forms a much more stable 2,3-dihydrodiol which yields 2-hydroxydibenzofuran and 3-hydroxydibenzofuran only when heated with acid, although the ratios of the 2 products are similar after bacterial or fungal metabolism. These results are consistent with the unstable bacterial metabolite being of the *cis* configuration and the stable fungal metabolite arising from an epoxide to form a *trans* configuration. The fungal culture also forms a small amount of 2,3-dihydroxydibenzofuran, and the parent *Beijerinckia* sp. in the presence of NAD$^+$ forms 1,2-dihydroxy- and 2,3-dihydroxydibenzofuran.

DIOXIN PERSISTENCE AND DEGRADATION IN SOILS

Evaluation of the persistence of TCDD in soils is complicated by the strong sorptive properties of TCDD, making recovery for analysis difficult (301, 352). In addition, artifacts may arise during the exhaustive extraction and analytical procedures involved. TCDD incubated with lake sediment for less than 1 hour and then extracted with solvents and analyzed by thin layer chromatography and ^{14}C-radioactivity showed 6 to 7% conversion by metabolism to metabolites, indicating the generation of artifacts during the procedure or the presence of impurities (458). Analysis of a commercial ^{14}C-TCDD preparation revealed the presence of 3% contaminants, including TriCDD, some anisole isomers, and some other components (352). Upon incubation, TCDD became less easily extractable while the other components were in comparison readily extractable, leading to artificial enrichment of the isomer during the analysis. This could cause misinterpretation of experimental results. Formation of metabolites would be expected to increase with time unless precluded by a toxicity threshold or by further metabolism.

Finally, since TCDD is only present in the environment as a contaminant of other chemicals, the analytical procedure must be able to measure TCDD at levels lower than a few parts per million (197). Recovery of ^{14}C-TCDD as measured by combustion from soils receiving 1.78 ppm TCDD were 53% after 1 year from Hagerstown silty clay loam containing 2.5% organic matter, and 67% from Lakeland loamy sand containing 0.9% organic matter (246). At an application rate of 17.8 ppm, 89% was recovered from Hagerstown loam and 73% from Lakeland sand. No metabolites were detected. Little $^{14}CO_2$ was evolved from TCDD-treated soils during 10 weeks' incubation. Extracts of soil treated with 2,7-dichlorodibenzo-*p*-dioxin (DCDD) contained a major metabolite in addition to the parent substrate (246). The metabolite was not identified. About 5% of the added radioactivity in a 0.7 ppm application of DCDD to soils was evolved after 10 weeks. TCDD incubated in lake water for 589 days was not altered (458). However, a lake water and sediment system incubated for the same length of time produced metabolites amounting to 1 to 4% of the original substrate. These products were polar (water soluble) and some were extractable and some were nonextractable in chloroform. The addition of nutrients enhanced formation of metabolites.

SUMMARY

The chlorinated and nonchlorinated dioxins and furans have not yet been shown to be utilizable as a sole carbon source for growth and energy. The parent nonchlorinated dibenzo-*p*-dioxin can be hydroxylated by several bacteria. The hydroxylated products accumulate and are recalcitrant to further oxidation. Some of the mono-, di-, and trichlorinated isomers are also oxidized by some bacteria, although at lower rates. TCDD is also hydroxylated by a few species of bacteria. Not all of the metabolites arising from TCDD metabolism have been identified. The

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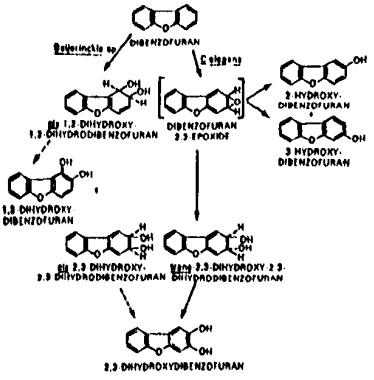

Figure 61. Oxidation of dibenzofuran by *Beijerinckia* sp. and *C. elegans*. Bracketed compound is hypothetical intermediate. Dashed lines indicate postulated reactions. Adapted from Reference 77.

dibenzofurans have been less well studied, although it has been demonstrated that the nonchlorinated substrate can be hydroxylated by both bacteria and fungi. Studies of the metabolism of these compounds have been hampered by the difficulty of extraction and product analysis, and by the extreme toxicity of TCDD, the isomer of greatest interest.

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SECTION 16
BIODEGRADATION OF CHLORINATED AROMATIC COMPOUNDS IN SCALED-UP BIOLOGICAL WATER RELATED TREATMENT PROCESSES

INTRODUCTION

The previous sections have demonstrated that a wide variety of chlorinated aromatic compounds are subject to biodegradation by a diversity of pure and mixed bacterial cultures. The significance of this information relates to the perceived potential for both environmental and wastewater biodegradation and/or detoxification of chlorinated aromatic pollutants. The extrapolation of such laboratory-derived results to environmental degradation and waste treatment is imprecise due to optimization, acclimation and high cell density cultures employed in most biochemical and physiological studies, which are rarely if ever met in real world biodegradation scenarios. In addition, real world complexity of the environmental matrix in which biodegradation occurs frequently necessitates the use of imperfect measures of biodegradation that cannot readily be correlated with those used to assess biodegradation in a laboratory environment.

Much of the work assessing biodegradation potential has been done in small-scale laboratory glassware with the hope that adequate comparisons might be drawn to full-scale environmental or treatment systems. Many factors may vary between the small-scale lab systems and the full-scale systems. A summary of factors that influence organic biodegradability is presented in Tables 6, 7, and 8 (385). Small-scale lab tests can assess the importance of many of these factors in well-designed, controlled experiments. However, many issues of importance in relating small-scale test results to full scale process performance depend on interactions of the various individual factors and the rates of material and biomass changes. These often are influenced by the physical design of the system and mass and energy transfer considerations. The turbulence and mixing potential of the system are also of major importance.

For these reasons, environmental scientists and engineers have turned to larger scale experiments to simulate the design and physical features of the full-scale system, whether a treatment or an environmental system. In these systems, material and energy kinetics can be measured, competitive abiotic processes can be studied, and these results can be linked to mathematical models to describe the system and allow scale-up to full-scale systems with more certainty.

This chapter focuses on scale-up studies to determine the biodegradability of chlorinated aromatics reported in the literature. Much information is available on continuous wastewater treatment systems. This was emphasized in this chapter over the environmental microcosm work, since the focus of this work is on treatability as

TABLE 6 CHEMICAL FACTORS INFLUENCING ORGANIC BIODEGRADABILITY IN WATER[a]

Chemical factors	Consequences
Substrate structural considerations	
Molecular weight or size	Limited active transport
Polymeric nature	Extracellular metabolism required
Aromaticity	Oxygen-requiring enzymes
Halogen substitution	Lack of dehalogenating enzymes
Solubility	Competitive partitioning
Toxicity	Enzyme inhibition, cell damage
Xenobiotic origin	Evolution of new degradative pathways
Environmental factors	
Dissolved oxygen	O_2-sensitive and O_2 requiring enzymes
Temperature	Mesophilic temperature optimum
pH	Narrow pH optimum
Dissolved carbon	Organic/pollutant complexes are concentration dependent for growth
Particulates, surfaces	Sorptive competition for substrate
Light	Photochemical enhancement
Nutrient and trace elements	Limitations on growth and enzyme synthesis

[a]Reference 385.

TABLE 7. BIOLOGICAL FACTORS INFLUENCING ORGANIC BIODEGRADABILITY IN WATER[a]

Biological factors	Consequences
Enzyme ubiquity	Low frequency of degradative species
Enzyme specificity	Analogous substrates not metabolized
Plasmid encoded enzymes	Low frequency of degradative species
Enzyme regulation	Repression of catabolic enzyme synthesis required acclimation or induction
Competition	Extinction or low density populations
Habitat selection	Lack of establishment of degradative populations
Population regulation	Low population density of degradative organisms

[a]Reference 385.

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TABLE 8 EXPERIMENTAL FACTORS INFLUENCING ORGANIC BIODEGRADABILITY*

Experimental factors	Problems encountered
Analytical method	
Substrate disappearance	Competing abiotic processes
Biotransformation	Complex analysis
Mineralization	Incomplete biochemical pathways
Scale up/down	Comparability among reactor designs and effects on kinetics
Feedstock complexity	
Chemically/biologically defined	Poor simulation and predictability
Complex waste/wastewaters	Difficult interpretation

*Reference 386

opposed to persistence in the environment. Little information was found on scale-up studies in the soil matrix.

PENTACHLOROPHENOL

Pentachlorophenol has been studied in several scaled-up systems. These include studies in aerobic "fiber-wall" reactor systems (140) as well as more conventionally-designed lab activated sludge systems. (37, 129, 315)

A lab-scale test with a continuous, aerobic, "fiber-wall" reactor was used to study the bio-oxidation of PCP in a synthetic and in an authentic wood-preserving wastewater (140). In the synthetic case, the concentration of PCP in the feed was 20 mg/l, the COD was 300 mg/l, the acclimation period was 15 days, and the operational period was 30 days. Reagent grade, commercial grade, and improved commercial grade pentachlorophenol were used. The improved commercial grade had fewer impurities, including chlorodioxins. Table 9 shows a survey of the results of these experiments. Other concentrations were also tested. These data show a general inhibition of the disappearance of PCP in the commercial preparation relative to the reagent and improved commercial grade preparations. Presumably, this is related to impurities present, possibly chlorodioxins. Actual waste rivaled the synthetic tests in PCP degradation performance. No proof of mineralization or estimation of other fate mechanisms was given in these tests.

A series of lab-scale continuous-stirred tank reactors (CSTR) was used to determine the aerobic biodegradation of PCP in wastewater treatment applications (319). The testing protocol included a phase where the inoculum was acclimated to PCP over 90 days from initial concentrations of 1 mg/l to 20 mg/l of PCP in a "fiber-wall" reactor. This sludge was then introduced into continuous-stirred tank reactors with no sludge or cell recycle. The hydraulic residence times (HRT) and the mean cell residence times (MCRT) were, therefore, equal and ranged from 3.2 to 16.3 days. Data collected included disappearance data on COD and PCP as well as reactor suspended solids concentrations. Proof of mineralization of PCP by use of ^{14}C-PCP was employed in related batch studies and fate information was collected on sorption and stripping mechanisms in adjunct batch experiments. No confirmatory

TABLE 9 THE EFFECT OF PENTACHLOROPHENOL PURITY ON DISAPPEARANCE IN CONTINUOUS SYSTEMS*

Parameter	Reagent grade	Commercial grade	Improved commercial grade†	Actual waste
Feed PCP (mg/l)	20	20	20	17.8
Feed COD (mg/l)	515	515	515	1336
Hydraulic residence time (hr)	6	6	6	6
Effluent PCP (mg/l)	0.53-0.7	1.9	0.3	0.2
Effluent COD (mg/l)	15.8-45.6	29.1	52.9	216
Activated sludge initial pentachlorophenate degrading capacity (mg PCP g cell - 1 hr -)	0.4-0.49	0.11	0.4	

*Reference 140.
98% pure PCP.
Blend from four manufacturers, 76-85% pure.
†Same PCP concentration as commercial grade, "substantially reduced" chlorodioxins. Dow Chemical Co., Improved Commercial Grade Penta (XD-8108.00L)

specific analysis of radiolabeled intermediates in the effluent or in the biomass are offered.

Results from this study include a first order kinetic rate constant, (the maximum specific growth rate, μm, divided by the Monod saturation constant, K_s) of 0.00177 $\mu g^{-1}d^{-1}$ with a minimum attainable PCP CSTR reactor concentration of 27 $\mu g/l$. Aqueous phase concentrations of PCP ranged between 51-293 $\mu g/l$ in the reactor. PCP had little effect on the removal of other COD in this study. The batch fate testing indicated that neither stripping nor sorption were significant PCP removal mechanisms and PCP was mineralized with some carbon incorporated into the cellular material.

A synthetic waste containing PCP was treated in a continuous lab-scale activated sludge system consisting of a 6.25-l mixed liquor vessel and a 1.66-l external clarifier (129). Air was added at the rate of 6.25 l/min through a sintered glass sparger. Sludge was wasted at 15 min intervals throughout the experiment. HRT's ranged from 8.9 to 10.4 hours and the MCRT was maintained at 6.2 days. Parameters measured included total solids, sludge volume index, PCP and reducing sugar concentrations in the clarifier, and clarifier effluent turbidity.

Screened wastewater treatment plant sludge was acclimated to PCP using a fill and draw reactor. An *Arthrobacter* sp. strain (ATCC 33790) was also added to another acclimation reactor with the effect of a lag period reduction to 1-3 days as opposed to over 6 days for the unamended sludge.

Steady-state operation of the activated sludge reactor achieved reductions in concentration from 40 mg/l PCP to about 1 mg/l. Unsteady-state transient conditions were studied by increasing the feed to 120 mg/l. Shock loading effects

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were analyzed with the use of kinetic models. Systems where the *Arthrobacter* sp. was added continuously showed a considerably improved transient response to the shock load than did the acclimated systems without addition of the strain.

Continuous lab-scale activated sludge units, consisting of 11-l aeration factors (air sparged in at ≈ 2 l/min) and 4-l external clarifiers with partial sludge recycle were challenged with 0.6 mg/l of PCP (37). The feed stream consisted of a pulp mill foul condensate with substantial amounts of non-PCP carbon, largely as methanol. Mean cell residence times were 4.9 and 9.3 days and hydraulic residence times were 25.4 and 34.0 hours, respectively. PCP was added as U-^{14}C-PCP and label analysis and specific PCP analysis were performed on the mixed liquor supernate as well as waste biomass and offgas. Other operating conditions are reported as well.

Unlike the other studies, the emphasis of this study was to develop information on PCP sorption on biomass and, therefore, a biomass not acclimated to PCP was used. BOD_5 and TOC removals from the aqueous phase were 63 to 69% and 86 to 96%, while PCP removals were 11.6 to 7.0%, depending on the reactor MCRT. Essentially, all of the PCP removal was either sorbed to or soluble in the aqueous component of the waste sludge. No significant biological transformation or mineralization was evident and stripping of PCP was below the detection limit. Good accountability was found for both labeled and unlabeled PCP.

Batch sorption tests were also incorporated and comparison of the batch data and the continuous runs suggests that the data fits a Langmuir-type isotherm with an apparent saturation of the biomass at an aqueous phase concentration of 2 mg/l PCP. The PCP data collected at concentrations less than the apparent saturation concentration agree well with a proposed sorption equilibrium equation based on the PCP octanol-water partition coefficient. An equation proposed for estimation of stripping based on the Henry's law constant for associated PCP was found to exaggerate the amount stripped. However, use of the Henry's law constant for the pentachlorophenate form was expected to show better agreement. No such Henry's law constant is available.

Consistent with information in earlier chapters, PCP has been found to biotransform in scaled-up systems using acclimated biomass or systems amended with known PCP degraders. No scaled-up studies have attempted to elucidate transformation products other than $^{14}CO_2$, but complete mineralization is strongly indicated in at least one study. Inhibition of other compounds (potentially chlorodienins) on the biomass has been noted and, therefore, the waste matrix in which the PCP resides may be important in determining the extent and rate of biodegradation. Conventionally-designed activated sludge systems operating with biomass not acclimated to PCP or experiencing transient shock loads either fail to achieve biotransformation of PCP or do so at greatly reduced rates. In these cases, PCP removal from the aqueous phases is poor and sorption to biomass (or other suspended solids) is expected to be a significant removal mechanism.

In summary, effective continous PCP degradation appears to require a biomass with specific PCP degradative capability (PCP degrader sub-population) as evidenced by the importance of acclimation and a steady and transient-free feed. Systems allowing longer cell residence times may also have an advantage over designs incorporating short cell residence time, although evidence here is not conclusive.

CHLORINATED BIPHENYLS

Several studies have occurred in which various mixtures and/or specific congeners of chlorinated biphenyls (PCBs) have been tested for biodegradability in larger, continuous experiments. These have included tests on consortia taken from operating wastewater treatment plants (200, 236, 438), as well as tests using specific

organisms (280). Still other research has been completed focusing on the fates of PCBs in environmental microcosms (272).

PCB commercial mixtures (Aroclors 1221, 1016, 1242, 1254, and MCS 1043) were tested for biodegradation using a lab-scale activated sludge test (438). Because of the test protocol, evaluation of the HRT and MCRT was not possible. The sludge inoculum was obtained from a municipal treatment plant and was acclimated for several weeks on a synthetic feed composed of glucose, nutrient broth, and KH_2PO_4. PCBs were not included in the acclimation feedstream. Initial MLSS concentrations were adjusted to 2500 mg/l. PCBs dissolved in ethanol were injected into the reactors. Disappearance of PCBs from the mixed liquor was measured using a hexane extraction followed by specific GC-EC or UV analysis. PCB spikes into the mixed liquor were used to measure analytical recoveries of PCBs from the liquor containing biomass and supernate.

Sorption on biomass was checked by sonic homogenization and extraction of the mixed liquor from an Aroclor 1016 run and extraction in the standard manner. Comparison with results without sonic homogenization showed similar PCB recoveries. No sorption checks were made with higher chlorinated PCBs, nor was proof of mineralization or biotransformation reported. Stripping was checked with Aroclor 1221, MCS 1043, and Aroclor 1016 by use of hexane offgas scrubbers connected to the reactors. Stripping rates of 4.2, 6.1, and 3.6%, respectively, were reported for the above PCB mixtures.

Results of these tests, equating disappearance with degradation, are presented in Table 10. A 48-hour cycle with an addition rate of 1 mg PCB over this interval was used.

TABLE 10. DISAPPEARANCE OF COMMERCIAL CHLOROBIPHENYL MIXTURES[a]

Mixture	Percent chlorine of mixture	Percent disappearance during test
Biphenyl	0	100
Aroclor 1221	21	81 ± 6
MCS 1043	30	66 ± 16
Aroclor 1016	41	33 ± 14
Aroclor 1242	42	28 ± 18
Aroclor 1254	54	15 ± 38

[a]Reference 438

A lab study of activated sludge challenged with carbon radiolabeled 2,5,4'-trichlorobiphenyl and 2,4,6,3',4'-pentachlorobiphenyl was performed to determine the fates of these compounds in biological processes (200). Municipal sewage sludge was placed in an aerated glass column and the offgas was scrubbed in hexane and toluene to recover stripped ^{14}C-PCB. No acclimation of the sludge to PCBs was reported. Initial concentrations were 0.178 mg/kg and 0.231 mg/kg trichloro biphenyl and pentachlorobiphenyl, respectively.

Metabolic byproducts of the trichlorobiphenyl were found in the sludge while none were found in the aqueous phase. One percent of the ^{14}C-trichlorobiphenyl was estimated to undergo biodegradation. The degradation products were found to be

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less volatile than the parent compound and, therefore, accumulated in the biomass. No evidence of degradation of the pentachlorobiphenyl was found in this study.

A lab-scale semi-continuous test using a "fill-and-draw" technique (236) was performed using municipal sludge acclimated over 3 months on 1, 5 and 10 μg/l of Kanechlor 500 (a PCB product with its main component being pentachlorobiphenyl). The units were aerated for 12 hours and then settled for 0.5 hours, after which time the clear supernate and sludge in excess of 25% of the reactor volume was wasted. The reactor was recharged with synthetic feed containing the PCBs, glucose, sodium glutamate, and inorganic nutrients.

The BOD of the feed was 320 mg/l. Specific PCB analysis in the sludge included centrifugation of the sludge, digestion of the solids with methanol-KOH, and subsequent hexane extraction. The aqueous phase was extracted with hexane and extracts were combined, water washed, dried with anhydrous Na_2SO_4, cleaned up with a Florisil column, concentrated, and analyzed by GC-EC. COD measurements and respirometric measurements were also taken during the study.

In batch respirometric tests, oxygen uptake of biomass with 1 and 5 μg/l PCBs was stimulated relative to the control without PCBs. Semicontinuous reactors fed up to 10 μg/l PCBs experienced high BOD removal efficiencies (98.6 to 99.1%). Major removals of PCBs in the semicontinuous reactors were found at all feed concentrations over the 12 hour aeration period. Equilibrium concentrations were achieved during the first hour of the period. Table 11 shows the distribution of PCBs in the semicontinuous reactors for sludge acclimated at 1, 5, and 10 μg/l PCB.

TABLE 11 DISTRIBUTION OF KANECLOR 500 IN ACTIVATED SLUDGE SEMICONTINUOUS SYSTEMS*

Sludge PCB acclimation concentration μg/l	Initial PCB concentration μg/l	Removal in wasted sludge (%)	Remaining in effluent (%)	Unaccounted (%)
1	0.6	45.6	31.3	23.1
5	0.48	75.9	15.0	9.1
10	0.85	81.6	12.1	6.4

*Reference 236

The majority of the PCBs charged to the system were removed with the wasted sludge. The authors tested stripping of PCBs in their semicontinuous reactors at air flow rates of 0.1 l/min of air per l of mixed liquor and found significant disappearance (65%) after 20 h aeration. They conclude that stripping could account for the PCB losses experienced in the semicontinuous runs. Finally, challenge of a municipal anaerobic digester sludge with 31 μg/l of PCBs (wet weight) incubated at 38°C for 40 days showed no disappearance of the starting PCB material. Resistance to anaerobic biotransformation was concluded.

A lab-scale continuous aerobic study of the degradation of Aroclor 1221 by a *Pseudomonas* sp. strain 7509 culture is reported (280). Inocula were acclimated on a feed in which Aroclor 1221 was the sole carbon source. A fermentation vessel was used as the reactor and raw sewage with a BOD_5 of 140 to 170 mg/l, fortified with 30 mg/l each of nitrogen (as NH_4Cl) and phosphorus (as KH_2PO_4/K_2HPO_4), was

amended with Aroclor 1221 at concentrations of 50 and 100 mg/l. The waste was fed at rates between 13 and 91 ml/hr to the 14-l reactor in which the agitator rate and the dissolved oxygen concentration was monitored. The HRT ranged from 4.6 to 32 days and was equal to the MCRT.

The broth (or mixed liquor) was sampled periodically and acidified prior to hexane extraction. GC-FID was used for disappearance analysis. No distinction can be made between the compounds in the aqueous and biomass compartments of the mixed liquor since the solids were not separated prior to analysis. Neither stripping nor sorption was measured and no proof of transformation or mineralization was reported.

At high HRTs (16 to 32 days), all of the Aroclor 1221 fed disappeared. At lower HRTs (4.6 to 10.7 days), some of the specific congeners began to appear in the mixed liquor indicating, according to the author, a preference of the organisms for certain congeners. The implication is that under nonstressed conditions, biphenyl, 2-chlorobiphenyl, and 4-chlorobiphenyl will degrade readily while 2,5-dichlorobiphenyl, 2,4'-dichlorobiphenyl, and 4,4'-dichlorobiphenyl are more recalcitrant. 2,4'-dichlorobiphenyl was found to build up as an indicator of the lower biodegradation rates. Aroclors 1016 and 1254 were also tested with resulting accumulation of all of the components. Switching from continuous to batch operation indicated disappearance of many of those congeners. Specific congeners were not identified in the Aroclor 1016 and 1254 runs.

Rates of 2,2',4,5'-tetrachloro-, 2,2',4,4',5,5'-hexachloro- and 2,2',3,3',4,4',5,6'-octachlorobiphenyls were introduced into a model system that included sediment, water, and air compartments (272). The model systems were fitted with a special gas bubbling device to enable investigation of removal by jet drop entrainment, and were operated in the dark. Anaerobic, sterile (bactericidal $HgCl_2$), and aerobic biotic systems with and without macroinvertebrates were studied. The macroinvertebrates were grown in separate vessels and then added to the model systems. The equivalent of 7500 chironomids/m² (*Chironomus plumosus* type) and 25,000 tubificids/m² (*Tubifex tubifex*) were added to the system. Gas flow to the system was estimated to be from 0.00005 to 0.003 ml/min of gas per ml of water. Thus, air flow to liquid volume ratio was from 30 to 2000 times less than that found in diffused air biological treatment systems. ^{14}C-PCB was used and no specific compound analysis was performed on the parent compound or possible metabolites or conversion products. Congeners recovered in each experiment averaged 68%, 40%, and 31% for tetra-, hexa-, and octachlorobiphenyls, respectively. Distribution of PCBs in the various tests are found in Tables 12, 13, and 14.

Although this experiment was intended to simulate a natural ecosystem and clearly differs from experiments on engineered systems, some conclusions are suggested that may relate to engineered treatment systems. First, although the vast majority of the congeners partition with the sediments (90 to 99.9%), the presence of biomass (especially the macroinvertebrates) was a determinative factor in the partitioning related to dispersion of sediment particles and jet-drop entrainment. In an engineered system, the turbulence of the system may override the turbulence from the macroinvertebrates with the result of much greater suspended material potentially being available for adhesion to walls and other surfaces. Of potentially greater importance is increased jet-drop entrainment, because of higher suspended solids concentrations and substantially higher air flow to water volume ratios in the engineered system. Truly aerosolized jet drops may be collected in scrubbers or filters and therefore may be included in measures of stripping potential, even though the substrate is actually not a vapor but a mechanically-carried liquid-solid droplet.

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Unfortunately, no literature addressing this potentially important removal mechanism in engineered systems is evident.

TABLE 12. DISTRIBUTION OF 2,3',4,5'-TETRACHLOROBIPHENYL IN A SEDIMENT-WATER-AIR MODEL SYSTEM*

Compartment	Aerobic with macro-invertebrates	Aerobic without macro-invertebrates	Sterile	An-aerobic
Sediment	97 71	98 31	98 81	99 31
Dissolved in water	0 03	0 06	0 02	0 03
Particles in water	0 06	0 03		
Macroinvertebrates	0 85			
Glass walls (particle adhesion)	0 20			0 02
Glass walls (extractable)	0 10	0 02		
Air filters	0 03	0 01		0 02
Vessel stoppers	0 06	0 06		0 04
Surface microlayers	0 03	0 01		
Jet-drop impactors	0 93	1 48	0 18	0 04

*Reference 273.
†All values as percent of recovered compound.

TABLE 13. DISTRIBUTION OF 2,2',4,4',5,5'-HEXACHLOROBIPHENYL IN A SEDIMENT-WATER-AIR MODEL SYSTEM*

Compartment	Aerobic with macro-invertebrates	Aerobic without macro-invertebrates	Sterile	An-aerobic
Sediment	96 11	99 91	99 91	99 91
Dissolved in water	0 06			0 02
Particles in water	0 14			
Macroinvertebrates	2 73			
Glass walls (particle adhesion)	0 48	0 01		0 02
Glass walls (extractable)	0 02		0 17	0 02
Air filters	0 01		0 02	
Vessel stoppers			0 02	
Surface microlayers	0 04	0 03		0 03
Jet drop impactors	0 36	0 03	0 10	0 03

*Reference 273.
†All values as percent of recovered compound

TABLE 14. DISTRIBUTION OF 2,2',3,3',4,4',5,6'-OCTACHLOROBIPHENYL IN A SEDIMENT-WATER-AIR MODEL SYSTEM*

Compartment	Aerobic with macro-invertebrates	Aerobic without macro-invertebrates	Sterile	An aerobic
Sediment	90 01	99 61	98 81	99 41
Dissolved in water	0 10	0 03	0 06	0 45
Particles in water	1 00	0 02	0 09	0 01
Macroinvertebrates	3 00			
Glass walls (particle adhesion)	4 87	0 0		
Glass walls (extractable)	0 07			0 01
Air filters	0 01			
Vessel stoppers	0 30			0 05
Surface microlayers	0 07	0 03		0 11
Jet-drop impactors	0 99	0 24		0 04

*Reference 272.
†All values as percent of recovered compound.

A single scaled-up study has been reported to date that conclusively demonstrates PCB biodegradation. In this study, metabolic byproducts accounting for about 1% of the trichloronbiphenyl were recovered. No other studies present conclusive evidence for biodegradation. This is largely due to use of parent compound disappearance data for analysis and lack of proof for biotransformation or mineralization. No work is evident using labeled congeners to support an argument for enzymatic processes. Several studies have shown major potential for PCB disappearance related to stripping and to sorption on biomass or other solids (sediments). Analytical difficulty in extractive recovery of the higher chlorinated congeners from sediments suggests that in the absence of methods to digest the biomass or to determine specific recoveries of the higher congeners in the biomass, extractive analysis of the biomass or the total mixed liquor (biomass and supernate) may not recover substantial portions of the parent compounds strongly sorbed to the cellular or solids matrix. Spiking with a PCB mixture and complete extractive recovery is not conclusive proof of recovery of PCBs from unknown biomass samples unless no saturation effect of the biomass with sorbed PCBs exists and sufficient time is allowed for the PCBs to achieve equilibrium partitioning with the biomass. Absence of either condition would lead to more of the spiked sample residing in the aqueous compartment and misleadingly high apparent recovery values. In other words, special caution must be taken when equating PCB-spiked mixed liquor or biomass recovery with actual sample PCB recovery.

The strongest evidence of biodegradation, notwithstanding the above comments, is the study by Tiu (280) in which ratios of the specific congeners in Aroclor 1221 change depending on the operating conditions. It is difficult to say how sorption or stripping mechanism would vary as widely as reported for the same congeners under different operating conditions unless enzymatic processes are at work. Also the PCB

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mixture studied is comprised of congeners that, in other microbial tests (reported in an earlier chapter), have been shown to undergo biotransformation.

In general, further scaled-up studies on PCBs are needed that include analytical protocols to offer proof of biotransformation or mineralization, quantify the sorption and stripping mechanisms, and study other potentially important mechanisms such as jet-drop entrainment in engineered systems, before engineered biological processes may be considered for PCB treatment.

DICHLOROPHENOL

A lab-scale activated sludge reactor with sludge recycle (liquid volume of aeration tank, 3 l) was used to study the simultaneous biodegradation of 2,4-dichlorophenol (DCP) and phenol (33). The phenol concentrations ranged from 14.9 to 45.7 mg/l and the DCP concentrations ranged from 52.4 to 121 mg/l. A 1:1 carbon ratio of both substrates was desired. The sludge was previously acclimated to phenol and then was acclimated to DCP by gradual replacement of the phenol in the feed with DCP. The acclimation process required 70 days to complete. The reactors were operated with HRT between 2.5 to 6.25 hr and MCRT of between 1.75 and 10.7 days.

MLVSS concentrations ranged between 46 and 299 mg/l. Analysis focused on substrate disappearance with no determination of stripping or sorption. Chloride analysis as a test of mineralization and COD determinations did not account for the phenol or DCP disappearance, implying biotransformation of the substrates.

Biokinetic rate constants for the runs yielded disappearance rate constants of 0.00098 l mg^{-1}hr^{-1} for phenol and 0.045 hr^{-1} for DCP. The Monod half-saturation constant for DCP was 63 mg/l. Yield coefficients for phenol and DCP were 0.67 mg VSS/mg phenol and 0.39 mg VSS/mg DCP, respectively. A combined biomass decay coefficient of 0.014 hr^{-1} was presented.

A mathematical rationale leading to deterministic estimates of fates from biological waste water treatment processes has been proposed (38). For continuous, complete-mix, activated sludge units where the biological disappearance of the parent compound is described by a rate equation first order in substrate concentration, and where sorption is occurring at concentrations below any potential biomass saturation concentration, the following equations are proposed for first estimates of the percent substrate stripped, sorbed, and wasted in the waste biomass, and biotransformed to another compound:

$$REM_r = \frac{A}{1 + A + S + B} \quad (2)$$

$$REM_s = \frac{S}{1 + A + S + B} \quad (3)$$

$$REM_b = \frac{B}{1 + A + S + B} \quad (4)$$

$$REM_t = \frac{1}{1 + A + S + B} \quad (5)$$

$$A = HRT \frac{X K_{ow} f_l}{1000 \rho_l MCRT} \quad (6)$$

$$S = HRT K'' = \frac{Q_{ow}}{V} 3.71 \times 10^{-3} (H_c)^{0.95} \quad (7)$$

$$B = HRT K' \quad (8)$$

where: REM_r, REM_s, REM_b, REM_t are the percent removals of the substrate from the system by the sorption, stripping, biological transformation, and effluent fate mechanisms, respectively.

HRT is the hydraulic residence time of the activated sludge system (hr).

X is the concentration of biomass as MLSS (mg/l).

K_{ow} is the substrate octanol-water partition coefficient (concentration in octanol/concentration in water).

f_l is the fraction of lipids or lipophilic compounds in the biomass (weight fraction).

ρ_l is the mean density of the lipophilic biomass compounds (g/l).

MCRT is the mean cell residence time for the biomass in the systems (hr).

Q_{ow}/V is the ratio of the air flow rate into the system to the system hydraulic volume (min^{-1}).

K' is a biological disappearance rate constant, first order in substrate concentration (hr^{-1}).

Equations 6 to 8 are discussed individually in separate papers (37, 385, 431).

If assumptions are made such that the equations described above are applicable to the DCP experiment discussed earlier, (complete-mix system, $Q_{ow}/V = 0.1$ min^{-1}, H_c of DCP = 13.4 torr l mol^{-1}, K_{ow} of DCP = 1202, etc.) then the data reported can be used to calculate K'' for each experimental series and the mechanism removals can be estimated. Table 13 presents the results of this analysis. It may be concluded from this analysis that stripping can be a significant removal mechanism, especially in the instance where K' is relatively slow. In run 4, 16% of the DCP removed from the system (other than in the effluent) was stripped. As the biotransformation rate increased, the stripping potential was vastly reduced to less than 0.1% of the total DCP removed (other than in the effluent) (Run 1). Sorption of DCP to the waste sludge taken from the system is not a significant fate mechanism for DCP. However, depending upon the level of extracellular water wasted with the biomass (waste sludge solids concentration), more DCP could be lost in the waste sludge than that shown in Table 13.

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TABLE 15 FATE ESTIMATES OF 2,4-DICHLOROPHENOL REMOVAL FROM A LAB ACTIVATED SLUDGE SYSTEM USING PROPOSED EQUATIONS*

Run	MCRT (hr)	HRT (hr)	Calculated K' (hr⁻¹)	Percent removal of DCP by: Sorption	Stripping	Biotransformation	Effluent
1†	267	25	0.075	0.008	0.034	83.0	33.6
2†	42	8.25	0.043	0.67	2.0	21.6	76.9
3†	109	8.25	0.070	0.25	1.9	29.8	68.1
4†	20	8.25	0.020	0.78	2.2	10.8	86.2

*Data taken from Reference 33
†Runs with phenol and 2,4-dichlorophenol at a ratio of 1 to 1 carbon from each substrate.
‡Runs with glucose and 2,4-dichlorophenol at a ratio of 1 to 1 carbon from each substrate.

Finally, the first order biotransformation rate constants, K', are similar to values reported by Ockrent, et al (33) derived using more conventional empirical biokinetic rate constant methods ($K' = 0.04 \pm 0.005\ hr^{-1}$). Calculated rate constants from Table 10 show wider variance and indicate strong relationships between biological rates and the MCRT.

TRICHLOROCARBANILIDE

Trichlorocarbanilide (TCC) was studied in both lab-scale batch flask tests and continuous activated sludge systems (184a). TCC with ^{14}C label on the 4-chloroaniline (p-chloroaniline or PCA) ring as well as the dichloroaniline (DCA) ring was used.

Sludge was obtained from a municipal wastewater treatment plant. In the activated sludge units, the biomass concentration and air flow rates were controlled at 4000 µg/l as MLSS and 0.03 standard ft³/hr of CO_2-free air. Oxygen was trapped in an amine solution to recover $^{14}CO_2$. The ^{14}C content of the sludge was determined by a combustion-$^{14}CO_2$ recovery method. 200 µg/l of TCC were added to the feed stream.

Batch flask tests showed that 90% of the theoretical $^{14}CO_2$ evolved from incubation with both raw sewage and activated sludge after 12 weeks. A lag of 2 weeks was noted in the raw sewage before $^{14}CO_2$ evolution. Concentrations of 200 µg/l of TCC cosubstrated in nearly 100% theoretical $^{14}CO_2$ evolution at 12 weeks while concentrations of both 20 µg/l and 2000 µg/l TCC resulted in less mineralization at this incubation time.

Sorption of TCC to activated sludge was determined by contacting activated sludge at concentrations from 0 to 2000 µg/l with 20 to 200 µg/l of TCC. Sorption was calculated based on the disappearance from the aqueous phase over 2 hr. A Freundlich isotherm was generated showing TCC-on-sludge concentrations of 2 and 0.1 µg/l at aqueous concentrations of 78 and 0 µg/l, respectively. Continuous operation of the activated sludge unit at a 10 hr HRT for 90 days showed 20 to 30% of theoretical $^{14}CO_2$ evolution for the dichloroaniline ring while from 40 to 60% of

TABLE 16 FATE OF TRICHLOROCARBANILIDE IN LAB SCALE ACTIVATED SLUDGE SYSTEMS*

Substrate	Stream	% of Feed	% Recovered
^{14}C-PCA TCC†	Effluent	3.2	93.4
	$^{14}CO_2$	66.1	
	Activated sludge	24.1	
^{14}C-DCA TCC§	Effluent	30.3	91.4
	$^{14}CO_2$	25.9	
	Activated sludge	35.2	

*Reference 184a
†Trichlorocarbanilide with a labeled 4 Chloroaniline ring
§Trichlorocarbanilide with a labeled dichloroaniline ring

theoretical $^{14}CO_2$ was evolved from the chloroaniline ring. Table 16 highlights the fate of the radioactivity in the continuous tests.

Although no specific analysis of the sludge was undertaken to determine the chemical composition of ^{14}C found in the sludge (i.e., parent compound, metabolites, cellular material), such analysis was done on the effluent. Chloroaniline, dichloroaniline, aniline condensation products, and unknowns were found in addition to the TCC parent compound.

This study convincingly supports conclusions related to the biotransformation and mineralization of TCC. These findings are consistent with those stated in an earlier chapter on chloroaniline herbicides even though this molecule's structure varies somewhat. In addition, this study is among the earliest work found that considered major fate mechanisms and offers proof of biotransformation or mineralization in a scaled-up biological wastewater system treating chloroaromatic compounds. Unfortunately, lack of information on the MCRT limits the calculation of biokinetic rate constants and the direct extrapolation of these results to other design configurations.

DICHLOROBENZENE

A pilot-scale activated sludge system was operated on a side-stream of sewage from the city of Zurich, Switzerland (303). 1,4-Dichlorobenzene was present at all times in the system feed and it was used as an indicator compound to determine the nonbiological removal mechanisms of stripping and sorption on the biomass. The major assumption made in this study was that 1,4 dichlorobenzene was conserved and was not biotransformed at the operating conditions of the study. The aeration vessel volume was 11.3 to 13m³ and the HRT ranged from 2.5 to 5 hr with MCRT's ranging from 74 to 182 hr. Sorption and stripping mechanisms were quantified but proof of biotransformation (or the absence of) was not provided.

The fates of DCB were reported to be 72% stripped, less than 3% sorbed on wasted sludge, 10% in the effluent, and 15% unaccounted. Data presented in an earlier section indicated that chlorobenzenes (except hexachlorobenzene) can be mineralized but there is a lack of knowledge on the biochemical pathways. The DCB not accounted for in this study may be undergoing biotransformation but the variance in the material balance related to analytical shortcomings precludes a definite

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statement. A study discussed later in this chapter offers evidence of DCR biotransformation.

COMBINED STUDIES ON SEVERAL CLASSES OF CHLOROAROMATICS

Lab and Pilot Studies

Several studies have occurred using a given experimental protocol on a variety of chloroaromatic compounds. Table 17 shows the classes of chloroaromatics studied for each of the these compounds.

Benzoic acid, 2-chlorobenzoic acid, 3-chlorobenzoic acid, 4-chlorobenzoic acid, 2,4-dichlorobenzoic acid, 2,5-dichlorobenzoic acid, 2,6-dichlorobenzoic acid, 3,5-dichlorobenzoic acid, phenoxyacetic acid, and 2,4-dichlorophenoxyacetic acid were studied in lab-scale continuous flow reactors resembling chemostats (391). The HRT and MCRT were equal in these reactors. The reactors were completely mixed by aeration and used suspended biomass. The lag for acclimation of municipal biomass to the specific substrates was determined. Kinetic disappearance data were collected on the specific substrates and on dissolved organic carbon (DOC) as well. Batch testing also was performed to determine kinetic rate constants for comparison with the continuous tests. Proof of mineralization of the specific substrates was determined by measurement of chloride ion release.

The lag for acclimation of the initial sludge for the monochlorobenzoic acids was in the range of 10 to 20 days. The biomass began to show acclimation to 3,5-dichlorobenzoic acid at about 20 days but the acclimation process was continued through 100 days. 2,5-dichlorobenzoic acid became acclimated abruptly at 100 days. 2,4- and 2,6-dichlorobenzoic acids did not show acclimation during this testing protocol.

The author argues that long term acclimation for some of the compounds is evidence for genetic changes in the organisms as opposed to enzyme induction or population effects. Maximum specific growth rates, μ_m, the Monod half-saturation constant, K_s, and the yield coefficient, Y, are shown in Table 18.

Good agreement was found between μ_m and K_s for the continuous flow reactors and associated batch tests. The MCRT of the systems were found to be strongly related to to the effluent concentrations of the specific substrates. MCRTs of 3 to 15 days were required to achieve a 0.5 mg/l effluent concentration of the monochlorobenzoic acids while 6 to 50 days were needed to achieve 0.25 mg/l effluent concentrations.

TABLE 17. CLASSES OF CHLOROAROMATICS STUDIED IN SEVERAL EXPERIMENTAL STUDIES

Compound	Reference					
	61	127	250	251	391	416
Pentachlorophenol	X		X	X		X
Chlorinated Biphenyls						
Aroclor 1242	X					
Aroclor 1254	X					

(continued)

TABLE 17. (continued)

Chlorophenols						
2-Chlorophenol	X					
2,4-Dichlorophenol	X	X	X	X		X
2,4,6-Trichlorophenol	X					
Chlorobenzenes						
Chlorobenzene	X					
1,2-Dichlorobenzene	X	X	X	X		X
1,3-Dichlorobenzene	X	X				
1,4-Dichlorobenzene	X	X				
1,2,4-Trichlorobenzene	X	X				
Hexachlorobenzene	X					
Chlorobenzoic acids						
2-,3- and 4-chlorobenzoic acid					X	
2,4-, 2,5-, 2,6-, and 3,5-Dichlorobenzoic acid					X	
Chlorophenoxy compounds						
2,4-Dichlorophenoxyacetic acid					X	

Benzoic acid, 2-chlorobenzoic acid, 3-chlorobenzoic acid, 4-chlorobenzoic acid, 2,4-dichlorobenzoic acid, 2,5-dichlorobenzoic acid, 2,6-dichlorobenzoic acid, 3,5-dichlorobenzoic acid, phenoxyacetic acid, and 2,4-dichlorophenoxyacetic acid were studied in lab-scale continuous flow reactors resembling chemostats (391). The HRT and MCRT were equal in these reactors. The reactors were completely mixed by aeration and used suspended biomass. The lag for acclimation of municipal biomass to the specific substrates was determined. Kinetic disappearance data were collected on the specific substrates and on dissolved organic carbon (DOC) as well. Batch testing also was performed to determine kinetic rate constants for comparison with the continuous tests. Proof of mineralization of the specific substrates was determined by measurement of chloride ion release.

The substrates were mineralized to CO_2 and cellular material but no fate measurements were made on stripping or sorption mechanisms. Performance of systems with glucose and the 2,4-dichlorophenoxyacetic acid indicated no effect of glucose on 2,4-D disappearance but lower glucose disappearance rates related to 2,4-D presence. However, no strong inhibition or toxic effects of the substrates on the biomass were seen at feed concentrations of 50 to 200 mg/l. The effluent concentration is, of course, much less than the feed concentrations.

A major study on a variety of organics was undertaken (250, 251, 416). Continuous lab-scale activated sludge units were challenged with chlorophenols, chlorobenzenes, and pentachlorophenol as well as a number of other organics. These compounds were added to a synthetic "base mix" containing ethylene glycol, ethyl alcohol, acetic acid, glutamic acid, glucose, phenol, and various inorganic nutrients. The chloroaromatics were added such that the BOD_5 achieved was ~250 mg/l. HRT was held to about 8 hours and the MCRT ranged between 43 and 146 hrs. Stripping was measured by trapping the organics from the offgas on a solid sorbent and all specific compound analysis generally followed EPA analytical protocols. Methodology for

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TABLE 18 BIOKINETIC RESULTS FOR 2,4-D AND CHLOROBENZOIC ACIDS*

Compound	μ_m** (day^{-1})	K_s‡ (mg/l)	$Y_{x/s}$†† (mg/mg)
2,4 Dichlorophenoxyacetic acid	2 3	5 4	0 14
2-Chlorobenzoic acid	1 0	2 4	0 22
3 Chlorobenzoic acid	0 6	2 0	0 14
4 Chlorobenzoic acid	1 2	1 1	0 25
2 5 Dichlorobenzoic acid	0 8	1 8	0 18
3 5 Dichlorobenzoic acid	0 06	35 3	

*Reference 381.
**Maximum specific growth rate.
‡Monod half-saturation constant.
††Yield coefficient.

TABLE 19 FATE OF SEVERAL CHLOROAROMATICS IN A LAB-SCALE ACTIVATED SLUDGE SYSTEM*

Compound	Percent removed by Stripping	Sorption	Biotransformation
2,4-Dichlorophenol			95 2
Pentachlorophenol		0 68	97 3
1,3-Dichlorobenzene	21 7		78 2

*Reference 260, 351, 418.

sorption quantitation was not clear. No proof of mineralization or biotransformation was offered. Acclimation to the compounds was allowed for 4 weeks before 2 months of continuous data collection. The reactor volume was 3 l for aeration and 3 l for an internal clarifier. Air flow to the reactors ranged from 2 to 3 l/min. Table 19 presents fate data on specific chloroaromatics. These studies utilized an air flow-to-liquid volume ratio greater than 1 min^{-1} and the resultant data probably exaggerates the stripping mechanism. Disappearance attributed to biotransformation for DCP and PCP are in agreement with earlier studies reviewed in this chapter. Biotransformation of 1,2-dichlorobenzene is in conflict with a study discussed earlier but is consistent with general predictions on the biodegradability of chlorobenzenes.

Full-Scale Studies

Many full-scale biodegradation studies on chloroaromatics are reported. However, only a few have attempted to quantify the abiotic fates of the compounds. Also, variation in the waste composition and flow and in other physical variables often makes it difficult to interpret the results. Two of the most significant examples of full-scale plant studies are discussed here.

A study to determine the fates of priority pollutants for 30 publicly owned wastewater treatment plants has generated input-output data on several chlorinated

aromatics including 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, chlorobenzene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, hexachlorobenzene, Aroclor 1242, and Aroclor 1254 (61). This work was designed to allow statistical analysis on the occurrence and fates of priority pollutants in the system feedstream, intermediate process streams, system effluent, and waste sludge streams. The material that disappeared in the process was reported but since no air sampling was undertaken, no quantitative attempt was made to distinguish between stripping and biotransformation removal mechanisms. EPA analytical protocols were used and often the compounds were grouped into the "volatile", "acid extractable", and "base neutral" groupings arising from the analytical workup. Sampling periods were for approximately 6 days with 24 hr composite samples, and compounds were often at concentrations near the analytical detection limit. Since the influent was often variable and the sampling period was of the same order of magnitude as the plant's MCRT, material balance data and conclusions must be viewed with caution.

A related study was performed on a single publicly-owned wastewater treatment plant for a 30 day period (127). Over this period, variations in influent flow and substrate concentrations could be more precisely quantified and conclusions regarding compound fate could be made. Table 20 summarizes the removal data of

TABLE 20 MASS REMOVAL OF CHLOROAROMATIC COMPOUNDS IN A FULL-SCALE WASTEWATER TREATMENT PLANT*

Compound	Percent removal in‡ Primary treatment	Secondary treatment§	Overall treatment
2,4 Dichlorophenol	2	46	47
1,3 Dichlorobenzene	14	30	40
1,4 Dichlorobenzene	0	88	88
1,2,4-Trichlorobenzene	13	79	82

*Reference 127.
‡Calculation includes (total mass accounted for in minus total mass accounted for out)/total mass accounted for in. Thus, compound in the aqueous effluent is combined with that found in the waste solids. Removal mechanisms here are biotransformation, stripping, and other abiotic mechanisms excluding sorption.
§Based on activated sludge units alone.

2,4 dichlorophenol, 1,3-dichlorobenzene, 1,4-dichlorobenzene, and 1,2,4 trichlorobenzene found in this study. The removal calculation sums the substrate entering the system from all streams and the substrate leaving in all streams. Thus, sorption and effluent removal mechanisms are not considered "removed" whereas biotransformation, stripping and other abiotic mechanisms, excluding sorption, are considered "removed".

Full-scale plant data requires special planning and careful implementation to yield satisfactory data on biotic and abiotic removal mechanisms. Conclusive material balances often are impeded because of feed variability and low compound concentrations. Use of labeled compounds is expensive at large scale and questions related to the environmental release of labeled compounds exist. Therefore, the

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capability of collecting data leading to proof of biotransformation or mineralization is limited

SUMMARY

In order to conclusively establish biodegradation of chlorinated aromatic compounds in larger scale systems and to collect data that are of use in extrapolation and system design, several factors must be included in the experimental design. These include: (1) measurement or prediction of abiotic fate processes, (2) proof of biotransformation or mineralization, and (3) suitable measurement and reporting of important process variables relating to the calculation of biokinetic rates. Failure to include these factors leads to inconclusive results on compound fates or the inability to use the data for predictions on other (even similarly designed) systems.

Calculation of biokinetic rate constants based only on "removal" leads to wide variances in the rate constants for compounds with major abiotic fate tendencies and precludes reliable scale up and more direct comparison between systems. Only a few scale-up studies are available on chlorinated aromatics in general, and only a subset of these contain data suitable for drawing conclusions relative to biodegradation and which allow comparison between systems. At times, coupling the results from several studies may allow an enlightened judgment regarding biodegradation, but there is no substitute for a single, well-designed study. In general, much additional work is needed to generate reliable scale-up data for biological treatment of chloroaromatic compounds.

SECTION 17

OVERVIEW OF MICROBIOLOGICAL DECOMPOSITION OF CHLORINATED AROMATIC COMPOUNDS

Most of the studies reviewed here have explored the metabolism of a single compound by a single organism. A few have reported the metabolites formed in soils or by contrived microbial consortia. Taken together, however, these studies indicate the potential ultimate fate of the chloroaromatic compounds.

The chlorophenoxy herbicides and the chlorobenzenes can be metabolized to chlorophenols (Figure 62). Chlorophenols may in turn be metabolized to chloroanisoles, but the most common route of biodegradation is to chlorocatechols. Phenylamide herbicides and other compounds with nitrogen-containing substituents are metabolized to chloroanilines. The chloroanilines form a variety of products including chlorocatechols (Figure 63). Other products represent alterations of the aliphatic moiety.

Chlorocatechols, in turn, may be metabolized by several different mechanisms to nonchlorinated ring cleavage products (Figure 64). There are two main pathways. One results from *meta* cleavage to form a chlorohydroxymuconic semialdehyde which, after loss of the chloride, forms pyruvate and an aldehyde. The second pathway involves *ortho* cleavage to form β ketoadipic acid via chloromuconate. The products of this pathway that are incorporated into cell constituents are succinate and acetyl-CoA.

Chlorobenzoic acids may be metabolized by 3 routes (Figure 64), the first through protocatechuic acid (a substituted catechol) to 3 ketoadipic acid. The second route metabolizes chlorobenzoic acids through chloromuconic acid to maleylpyruvic acid. Chloronaphthalenes are also metabolized through chlorosalicylic acid. Anaerobic metabolism of chlorobenzoic acids involves reductive dechlorination to benzoate followed by formation of CH_4 and CO_2. The ultimate products of each of these metabolic pathways are either available for incorporation into cellular material or represent ultimate mineralization.

These pathways all represent a general biochemical potential. Whether or not a specific compound is actually metabolized to nonchlorinated products depends on many factors. The compounds which are most readily metabolized are the lower chlorinated forms. However, some compounds with only one chlorine, for instance 2-chloroaniline, are not readily metabolized. That both the position and the number of substituents are important in determining the biodegradability of a molecule. It is not only the number of chlorines but also the general form of the molecule itself which governs biodegradability. Compounds such as chlordane/ins and DDT are not shown on these figures because they appear to be highly resistant to substantive microbial attack. The exact physicochemical features of any given molecule that govern its biodegradability remain to be elucidated.

Even chlorinated molecules for which complete pathways of mineralization have been developed are not metabolized in all systems. Although a few compounds are amenable to anaerobic biodegradation, most require the availability of molecular oxygen for ring cleavage. Other environmental parameters which may place

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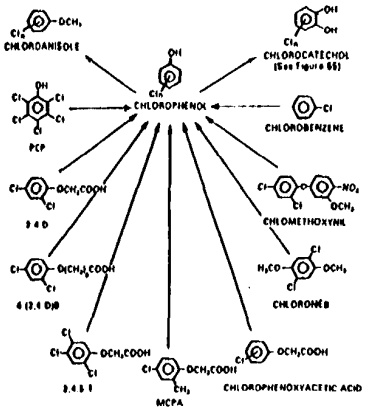

Figure 69 Chlorinated aromatic compounds metabolized to chlorophenols This figure presents possible pathways extrapolated from various studies In actual environmental systems a given transformation may be inhibited by a number of factors Terminal compounds shown may be recalcitrant or insufficient research may exist on which to base a conclusion Refer to text for further discussion

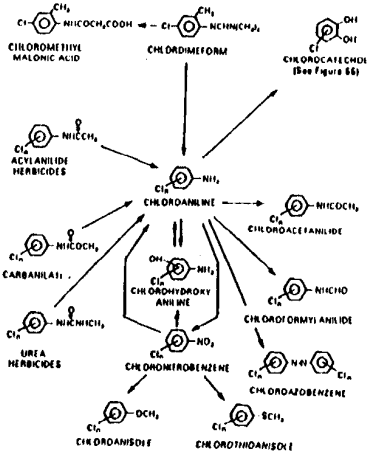

Figure 83 Chlorinated aromatic compounds metabolized to chloroanilines This figure presents possible pathways extrapolated from various studies In actual environmental systems a given transformation may be inhibited by a number of factors Terminal compounds shown may be recalcitrant or insufficient research may exist on which to base a conclusion Refer to text for further discussion

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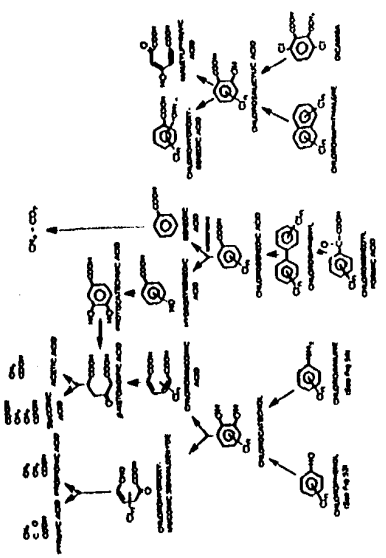

Figure 84 Metabolism of chlorocatechols, chlorobenzoic acids, and chlorosalicylic acids. This figure presents possible pathways extrapolated from various studies. In actual environmental systems a given transformation may be inhibited by a number of factors. Terminal compounds shown may be recalcitrant or insufficient research may exist on which to base a conclusion. Refer to text for further discussion.

restrictions on biological activity include pH, temperature, and moisture. Upon exposure to the environment, the chemical state of the substrate may be altered to a form resistant to microbial attack.

The chemical itself may be degradable but the system may lack other nutrients necessary for microbial activity. Alternatively, other chemicals present may be preferred substrates, preventing metabolism of the substrate of interest. Another compound may also act to repress the activity of enzymes required for substrate metabolism. Accumulation of toxic metabolites may also repress further metabolic activity.

Ample evidence exists that some chemicals require the activities of several different groups of microorganisms for complete mineralization. Such consortia may not be found in the system containing the substrate. The interactions of these microorganisms pose additional constraints regarding production and utilization of potentially toxic metabolites as well as competition for nutrients and growth factors.

Finally, the dynamics of pollutant appearance in the system is of critical importance. Most microorganisms require a period of acclimation to the substrate before metabolism occurs. During this period, the substrate level must be high enough to promote acclimation without being toxic or inhibitory. Prior exposure to the compound helps to shorten the acclimation period. Such exposure to other pollutants may also predispose the microbial population to adaptation to the substrate of interest. Or such acclimation may result in destruction of microorganisms capable of substrate utilization in favor of a population adapted to a different substrate, or in virtually complete destruction of the microbial flora.

This review of microbiological decomposition of chlorinated aromatic compounds indicates that while certain metabolic pathway generalizations exist, as reflected in Figures 82, 83, and 84, biodegradation of the compounds shown is dependent on many other variables and cannot be assumed in any given biotic system. Data on many of these variables are needed to allow prediction of the metabolic fate of chlorinated aromatic compounds.

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APPENDIX
ILLUSTRATED LIST OF COMPOUNDS

1

ACETALDEHYDE

2

ACETOACETIC ACID

3

ACETYL CoA

4

ACONITIC ACID

5

ADENINE

6

ALANINE

7

AMIBEN (chloramben, 3-amino-2,5-dichlorobenzoic acid)

8

2-AMINO-5-CHLOROPHENOL

9

ANILINE

10

ANTHRACENE

11

BARBANIL (3-chlorophenyl carbamic acid 1-chloro-2-butynyl ester)

12

BENTHIOCARB (S-4-chlorophenyl methyl diethylthiolcarbamate)

13

BENZALDEHYDE

14

BENZENE

15

BENZENE 1,2 OXIDE

16

BENZENESULFONIC ACID

17

7,8 BENZOCOUMARIN

18

BENZOIC ACID

19

BENZYL ALCOHOL

20

BITENOX (methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate)

21

BIPHENYL

22

BUTURONE (3-(4-chlorophenyl)-1-isobutyryl-5-methylurea)

23

2-CARBOXYBENZOPYRILIUM

24

7-CARBOXY-4-CHLORO-3-KETO-HEPT-5,6-DIENOIC ACID

26

7-CARBOXY-4-CHLORO-3-KETO-HEPT-4,7-LACTONE

28

4-CARBOXYMETHYLENE-BUT-3-EN-4-OLIDE

27

4-CARBOXYMETHYLENE-3-METHYL-4-BUTENOLIDE

29

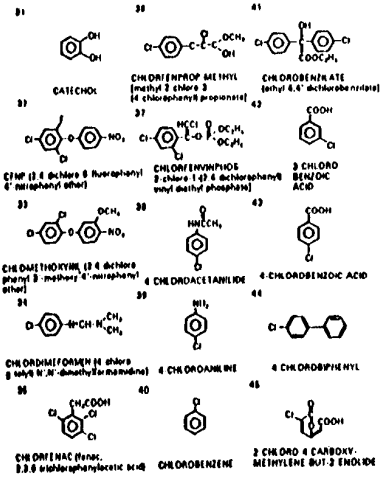
3-CARBOXY-cis,cis-MUCONIC ACID

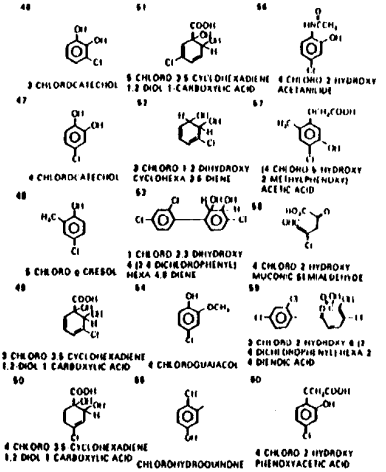
30

3-CARBOXYMUCONOLACTONE

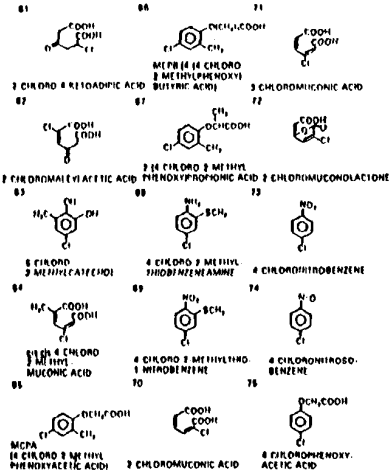

4-CARBOXYMUCONOLACTONE

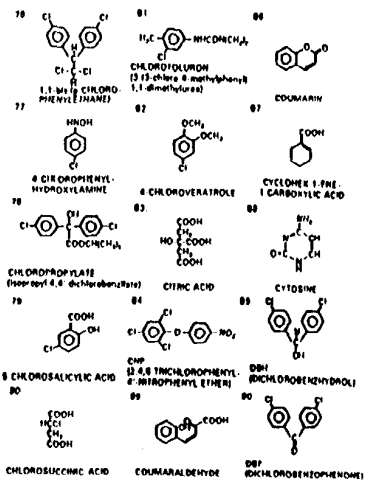
MONS 026947


31
CATECHOL
38
CHLORFENPROP METHYL
[methyl 2 chloro 3
(4 chlorophenyl propionate)]
41
CHLOROBENZILATE
[ethyl 4,4' dichlorobenzilate]
39
CFNP (2 4 dichloro 6 fluorophenyl
4' nitrophenyl ether)
37
CHLORFENVINPHOS
[3 chloro 1 (2 4 dichlorophenyl
vinyl diethyl phosphate)]
42
3 CHLORO
BENZOIC
ACID
33
CHLOMETHOXYNIL (3 4 dichloro
phenyl 3 methoxy 4' nitrophenyl
ether)
36
4 CHLORDACETANILIDE
43
4 CHLORDBENZOIC ACID
34
CHLORDIMEFORMEN (4 chloro
3 tolyl N',N' dimethylformamidine)
39
4 CHLOROANILINE
44
4 CHLORDBIPHENYL
35
CHLORFENAC (fenac,
2,3,6 trichlorophenylacetic acid)
40
CHLOROBENZENE
45
2 CHLORO 4 CARBOXY-
METHYLENE BUT-2 ENOLIDE

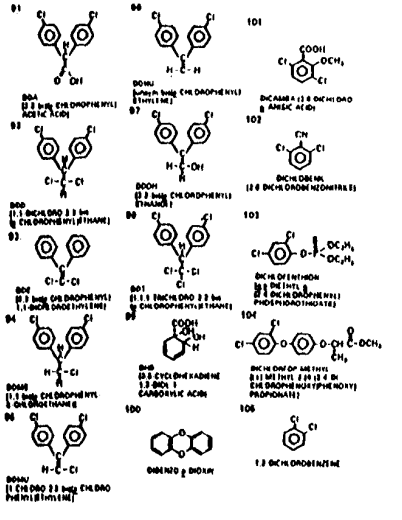

48
3 CHLOROCATECHOL
51
5 CHLORO 2 5 CYCLOHEXADIENE
1,2 DIOL 1 CARBOXYLIC ACID
56
4 CHLORO 2 HYDROXY
ACETANILIDE
47
4 CHLOROCATECHOL
52
3 CHLORO 1 2 DIHYDROXY
CYCLOHEXA 3 6 DIENE
57
[4 CHLORO 6 HYDROXY
2 METHYLPHENOXY]
ACETIC ACID
49
5 CHLORO 6 CRESOL
53
1 CHLORO 2 3 DIHYDROXY
4 (2 4 DICHLOROPHENYL)
HEXA 4,6 DIENE
58
4 CHLORO 2 HYDROXY
MUCONIC SEMIALDEHYDE
46
3 CHLORO 2 5 CYCLOHEXADIENE
1,2 DIOL 1 CARBOXYLIC ACID
54
4 CHLOROGUAIACOL
59
3 CHLORO 2 HYDROXY 6 (2
4 DICHLOROPHENYL) HEXA 2
4 DIENOIC ACID
50
4 CHLORO 2 5 CYCLOHEXADIENE
1,2 DIOL 1 CARBOXYLIC ACID
55
CHLOROHYDROQUINONE
60
4 CHLORO 2 HYDROXY
PHENOXYACETIC ACID

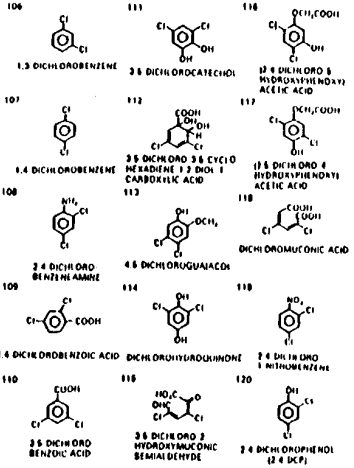
MONS 026948


65
3 CHLORO 4 KETOADIPIC ACID
66
MCPB (4 (4 CHLORO 2 METHYLPHENOXY) BUTYRIC ACID)
71
3 CHLOROMUCONIC ACID
67
3 CHLORO 4 METHYL PHENOXYACETIC ACID
68
3 (4 CHLORO 2 METHYL PHENOXY)PROPIONIC ACID
72
3 CHLOROMUCONOLACTONE
63
5 CHLORO 3 METHYLCATECHOL
69
4 CHLORO 2 METHYL THIOBENZENEAMINE
73
4 CHLORONITROBENZENE
64
5(6)h 4 CHLORO 3 METHYL MUCONIC ACID
69
4 CHLORO 3 METHYLTHIO 1 NITROBENZENE
74
4 CHLORONITROSO BENZENE
65
MCPA (4 CHLORO 2 METHYL PHENOXYACETIC ACID)
70
3 CHLOROMUCONIC ACID
75
4 CHLOROPHENOXY ACETIC ACID


76
1,1 bis (p CHLORO PHENYL) ETHANE)
81
CHLOROTOLURON (3 (3 chloro 4 methylphenyl) 1,1 dimethylurea)
84
COUMARIN
77
4 CHLOROPHENYL HYDROXYLAMINE
82
4 CHLOROVERATROLE
87
CYCLOHEX 1 ENE 1 CARBOXYLIC ACID
78
CHLORPROPYLATE (Isopropyl 4,4' dichlorobenzilate)
83
CITRIC ACID
88
CYTOSINE
79
5 CHLOROSALICYLIC ACID
85
CNP (2 4,6 TRICHLOROPHENYL 4' NITROPHENYL ETHER)
89
DDH (DICHLOROBENZHYDROL)
80
CHLOROSUCCINIC ACID
86
COUMARALDEHYDE
90
DBP (DICHLOROBENZOPHENONE)

MONS 026949





MONS 026950

121 (2,4 DICHLORO PHENOXYACETIC ACID)



122 (DICHLOBENZURON (3-(3,4 DICHLOROPHENYL) 1-METHYL UREA)



123 (4-(2,4 DICHLOROPHENOXY) BUTYRIC ACID)



124 (2,4 DICHLOROPHENOXY ETHANOL)



125 (2-(2,4 DICHLOROPHENOXY) ETHYL SULFATE SODIUM SALT)



126 (4,5 DICHLOROVERATROLE)



127 (9,10 DIHYDRO 1,3 DIHYDROXYANTHRACENE)



128 (1,2 DIHYDRO 1,3 DIHYDROXYBENZENE)



129 (1,2 DIHYDRO 1,3 DIHYDROXYBENZENE)



130 (3,3 DIHYDRO-2,2 DIHYDROXYBIPHENYL)



131 (9,10 DIHYDRO 1,2-DIHYDROXYNAPHTHALENE)



132 (1,2 DIHYDRO-1,2 DIHYDROXYNAPHTHALENE)



133 (3,4 DIHYDRO 3,4 DIHYDROXYPHENANTHRENE)



134 (1,2 DIHYDROXY ANTHRACENE)



135 (2,3 DIHYDROXYBENZOIC ACID)



138 (2,3 DIHYDROXYBIPHENYL)



141 (3,4 DIHYDROXYPHENANTHRENE)



140 (GENTISIC ACID)



137 (1,2 DIHYDROXY-DIBENZO p DIOXIN)



142 (DPM DICHLORODIPHENYLMETHANE)



147. (GLYOXYLIC ACID)



136 (1,2 DIHYDROXY-1,2 DIHYDRODIBENZO p DIOXIN)



143 (FUMARIC ACID)



148 (GUANINE)



139 (1,2 DIHYDROXYNAPHTHALENE)



144. (FUMARYLACETOACETIC ACID)



145 (HEXACHLOROBENZENE)



140 (1,4 DIHYDROXYNAPHTHALENE)



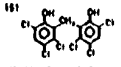
148 (FUMARYLPYRUVIC ACID)



150 (HEXACHLOROCYCLOHEXANE)



MONS 026951

151

HEXACHLOROPHENE
[2,2'-METHYLENE bis
[3,4,6-TRICHLOROPHENOL]]

150

β HYDROXYBENZALPYRUVIC ACID

161

6 HYDROXY 6 (4 CHLOROPHENYL) HEXANOIC ACID

152

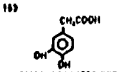
HOMOGENTISIC ACID

157

β HYDROXYBENZOIC ACID

162

2 HYDROXY CYCLOHEXANE CARBOXYLIC ACID

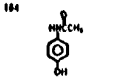
153

HOMOPROTOCATECHUIC ACID

158

p HYDROXYBENZOIC ACID

163

1 HYDROXY DIBENZO p DIOXIN

154

4 HYDROXYACETANILIDE

159

β HYDROXYBENZOIC ACID

164

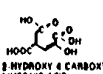
2 HYDROXY DIBENZO p DIOXIN

155

4 HYDROXYANILINE

160

2 HYDROXY 4 CARBOXY MUCONIC SEMIALDEHYDE

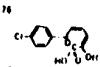
165

2 HYDROXY 4 CARBOXY MUCONIC ACID

166

4 HYDROXY 1 β HYDROXYPHENYL 2 OXOBUTYRIC ACID

171

3 HYDROXYMUCONIC ACID

176

2 HYDROXY 6 OXO 6 (4 CHLOROPHENYL) HEXA 2 4 DIENOIC ACID

167

6 HYDROXY β KETOCARBOXYLIC ACID

172

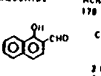
3 HYDROXYMUCONIC SEMIALDEHYDE

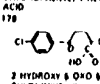
177

2 HYDROXY 6 OXO 6 (4 CHLOROPHENYL) 4 HEXENOIC ACID

168

HYDROXYMALONIC SEMIALDEHYDE

173

8 HYDROXY 2 NAPHTHALDEHYDE

178

2 HYDROXY 6 OXO 6 (4 CHLOROPHENYL) PENTANOIC ACID

169

4 HYDROXY 3 METHYL MUCONIC ACID

174

3 HYDROXY 2 NAPHTHOIC ACID

179

2 HYDROXY 6 OXO 6 PHENYLHEXA 2 4 DIENOIC ACID

170

2 HYDROXYMUCONIC ACID

175

ca 4 HYDROXY NAPHTH 2 YL 2 OXOBUT 3 ENOIC ACID

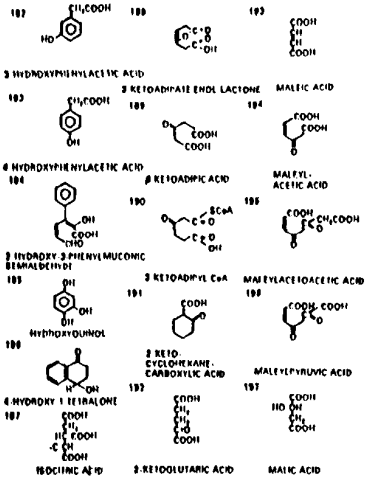
180

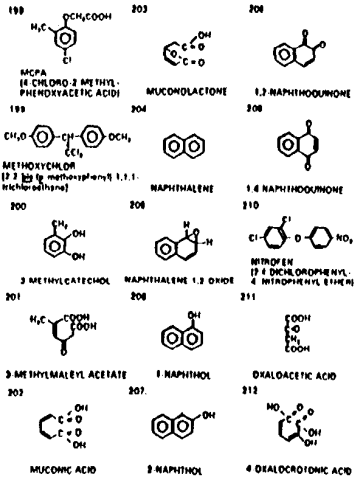
2 HYDROXYPENTA 2 4 DIENOIC ACID

181

4 HYDROXY 2 OXOVALERIC ACID

MONS 026952


187
3-HYDROXYPHENYLACETIC ACID
188
3-KETOADIPATE ENOL LACTONE
189
MALEIC ACID
193
4-HYDROXYPHENYLACETIC ACID
189
β-KETOADIPIC ACID
194
MALEYL-ACETIC ACID
194
3-HYDROXY-5-PHENYLMUCONIC SEMIALDEHYDE
190
3-KETOADIPYL CoA
195
MALEYLACETOACETIC ACID
195
HYDROXYQUINOL
191
2-KETO-CYCLOHEXANE-CARBOXYLIC ACID
198
MALEYLPYRUVIC ACID
196
4-HYDROXY-1-TETRALONE
192
3-KETOGLUTARIC ACID
197
MALIC ACID
187
ISOCITRIC ACID


199
MCPA (4-CHLORO-2 METHYL-PHENOXYACETIC ACID)
203
MUCONOLACTONE
206
1,2-NAPHTHOQUINONE
199
METHOXYCHLOR (2,2 bis (p-methoxyphenyl) 1,1,1-trichloroethane)
204
NAPHTHALENE
208
1,4-NAPHTHOQUINONE
200
3-METHYLCATECHOL
205
NAPHTHALENE 1,2 OXIDE
210
NITROFEN (2,4-DICHLOROPHENYL-4-NITROPHENYL ETHER)
201
3-METHYLMALEYL ACETATE
206
1-NAPHTHOL
211
OXALOACETIC ACID
202
MUCONIC ACID
207
2-NAPHTHOL
212
4-OXALOCROTONIC ACID

MONS 026953

313



3 OXOADIPIC ACID

316



5 OXO 5 (4' CHLOROPHENYL) PENTANOIC ACID

323



PCP (PENTACHLOROPHENOL)

314



3 OXO 4 CARBOXYPENT-4 ENOATE

318



2 OXO 4 HYDROXY 4 CARBOXYMUCONIC ACID

324



PENTACHLOROANILINE

319



4 OXO 4 (4' CHLOROPHENYL) BUTANOIC ACID

320



2-OXO 4 HYDROXY-CARBOXYPENTANOIC ACID

325



PENTACHLOROANISOLE

316



5 OXO 5 (4' CHLOROPHENYL) 2 HYDROXYHEXANOIC ACID

321



2 OXOPENT-4 ENOIC ACID

326



PENTACHLORONITROBENZENE

317



5.5 OXO 5 (4' CHLOROPHENYL) PENTANOIC ACID

322



PCMC (4 CHLOROPHENYL N METHYLCARBAMATE)

327



PENTACHLOROTHIOANISOLE

328



PHENANTHRENE

333



PROTOCATECHUIC ACID

338



SUCCINIC ACID

329



PHENOL

334



PYRUVIC ACID

339



SUCCINYL CoA

330



PHENYLACETIC ACID

335



SALICYLALDEHYDE

340



TECHLOFTHALAM (N (2,3 dichlorophenyl) 3,4, 5,6 tetrachlorophthalamic acid)

331



PHENYLPYRUVIC ACID

336



SALICYLIC ACID

341



TETRACHLORODIBENZOQUINONE

332



PIMELIC ACID

337



SD 8280 (2 chloro 1 (2 4 dichlorophenyl) vinyl dimethyl phosphate)

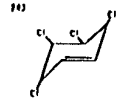
342



TETRACHLOROCATECHOL

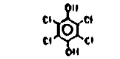
MONS 026954

243



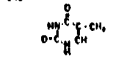
TETRACHLORDCYCLOHEXENE

244



TETRACHLORHYDROQUINONE

245



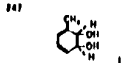
THYMINE

246



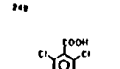
TOLUENE

247



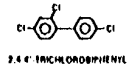
6,8 TOLUENE DIHYDRODIOL

248



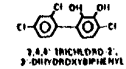
2,3,6 TRICHLORO-BENZOIC ACID

249



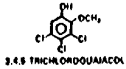
2,4,4' TRICHLORDBIPHENYL

250



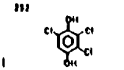
2,4,6' TRICHLORO 2',3'-DIHYDROXYBIPHENYL

251



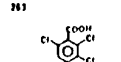
3,4,5 TRICHLORDGUAIACOL

252



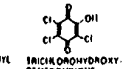
TRICHLORHYDROQUINONE

253



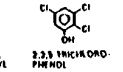
2,3,5 TRICHLORO 4 HYDROXYBENZOIC ACID

254



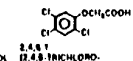
TRICHLORHYDROXY-BENZOQUINONE

255



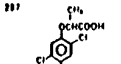
2,3,5 TRICHLORO-PHENOL

256



2,4,5 T (2,4,5-TRICHLORO-PHENOXYACETIC ACID)

257



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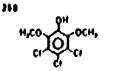

2,4,5 TRICHLORDSYRINGOL

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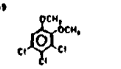

2,4,5 TRICHLORDVERATROLE

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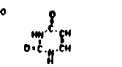

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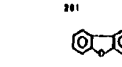

DIBENZOFURAN

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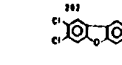

2,3,7,8 - TETRACHLORDDIBENZOFURAN

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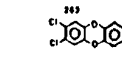

2,3,7,8 TETRACHLORDDIBENZO P DIOXIN

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GLOSSARY

Acidophile: Organism that grows very well at an acidic pH

Active transport: Process in which substrate entry into a cell is coupled to an energy-yielding process

Adenine: Purine base unit of a nucleoside.

Allosteric enzyme: Enzyme that contains a regulatory site.

Anaerobic respiration: Oxidative process similar to aerobic respiration but which utilizes nitrate or another inorganic compound as the terminal electron acceptor.

ATP (adenosine triphosphate): Ribonucleoside 5'-triphosphate that serves as a phosphate-group donor in the cell's energy cycle.

Autoradiograph: Picture formed on film resulting from exposure to radioactive particles.

Autotroph: Organism that obtains its energy from the oxidation of organic or inorganic compounds

Bacteriophage: Virus that attacks bacteria.

Barophilic: Requiring high barometric pressures for survival.

Barotolerant: Ability to survive at a wide range of barometric pressures

Basophilic: Organism that grows at alkaline pH.

Binary fission: Process of cell replication whereby a single cell divides into two.

Budding: Process of cell replication whereby a protuberance from a cell grows into another cell.

Capsule: Extracellular polysaccharide which accumulates around the cell and functions in cell attachment, defense, and protection.

Carboxylase: Enzyme that catalyzes the ATP-dependent addition of carbon dioxide to the acceptor substrate.

Catabolite repression: Inhibition of enzyme activity by binding of a control protein to the operator site.

Cell wall: Outer portion of bacterial cell that confers upon the cell its shape.

Chemostat: An apparatus used for the continuous culture of microbial populations in a steady state in which the growth rate is maintained by the substrate dilution rate.

Chemotaxis: Movement in response to a specific chemical

Chemotroph: See autotroph.

Chimera: Molecule consisting of a replicon and another fragment of DNA.

Chromosome: A single large molecule of DNA that contains many genes

Codon: A group of three adjacent nucleotides that codes for an amino acid

Cometabolism: Process by which a substrate is metabolized by a cell while the cell utilizes another substrate as its energy source

Competency: State in which a cell is able to undergo transformation

Competitive inhibitor: Chemical which has a similar structure to an enzyme substrate and therefore binds to the enzyme, but which does not activate the enzyme

Complete-mix reactor: A reactor in which, at any instant in time, the concentration of constituents is the same at any point in the reactor. The effluent concentration of a constituent is therefore also the same as the concentration in the reactor

Conjugation: Process of DNA transfer from one bacterial cell to another by direct cell to cell contact

Conjugative plasmid: Plasmid that contains genes for bacterial conjugation

Consortia: Mixtures of different populations

Continuous stirred tank reactor (CSTR): A reactor in which mechanical agitation is used to generate a complete mix condition where, at any instant in time, the concentration of constituents in the same at any point in the reactor. The effluent concentration of a constituent is therefore also the same as the concentration in the reactor

Copy number: The number of copies of a single plasmid present within a single cell

Cosmid: Constructed vector used for cloning large fragments of DNA.

Cytochrome P-450: Protein that serves as an electron carrier in enzymatic hydroxylation reactions and can also transfer electrons to oxygen

Cytoplasmic membrane: The limiting boundary of the cell protoplasm, composed of protein and phospholipid, that functions in substrate transport, osmotic regulation, cell wall synthesis, oxidative metabolism and energy production

Cytosine: Pyrimidine base unit of a nucleoside.

Death phase: Period in which reduction in population occurs due to cell death

Decarboxylase: Enzyme that catalyzes decarboxylation of the substrate.

Dehydrogenase: Enzyme that mediates the loss of a hydrogen ion from a substrate with the acceptor being other than molecular oxygen

Denaturation: Process of separating double stranded DNA into single strands

Deoxyribonuclease: Enzyme that catalyzes random cleavage of double stranded or single stranded DNA.

Deoxyribonucleic acid (DNA): A polynucleotide consisting of deoxyribonucleotide units that serves as the carrier of genetic information

Determinhibile: Use of knowledge of the causes of a process to arrive at a prediction of its performance

Dioxygenase: Enzyme that catalyzes the addition of two atoms of molecular oxygen to a molecule

Enzyme: Protein which both lowers the energy of activation of and directs the metabolic pathway taken by chemical reactions in an organism

Eukaryote: Organism characterized by having a nucleus surrounded by a membrane

Exogenous DNA: DNA molecule which is not an integral part of the cell genome

Exonuclease: Enzyme that removes single nucleotides from the end of a DNA molecule

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Exponential phase (log phase): Growth phase during which cells divide by binary fission.

Extracellular: Outside of the outermost layer of a cell (cell membrane or cell wall).

Extracellular water: The water in biomass "voids" residing between cells. Compounds residing in extracellular water are not included in measures of passive or active cellular uptake.

Facultative anaerobe: Organism that grows in the presence or absence of air.

Feedback inhibition: Inhibition of an allosteric enzyme early in a metabolic pathway by a later product of the pathway.

Fermentation pathway: Metabolic pathway in which organic compounds serve as both the electron donor and the electron acceptor.

Fiber wall reactor: A reactor in which the biomass is contained within a fibrous inner cavity. The aqueous solution and its dissolved constituent are thus permitted to pass through the fiber wall but the biomass (fixed film and suspended) is contained within the inner cavity. This type of reaction obviates the need for biomass separation by settling and recycle to the system.

Fill-and-draw reactor: A mode of operating a reactor in which reactants and products are added or removed over discrete time intervals but the reactions are allowed to proceed continuously. Since concentrations of constituents change cyclically during the test, results of this type of test may only approximate true continuous and steady state tests.

Flagellum: A hairlike organelle attached to a cell that functions in motility.

Freundlich Isotherm: An isotherm equation relating the equilibrium partitioning of a component between liquid and solid compartments (or phases). The equation is of the form

$$C_s = K_f C_l^n$$

where C_s and C_l are concentrations of the component in solid and liquid compartments, respectively, and K_f and n are empirical constants.

Gene: A DNA segment that codes for a single polypeptide chain or RNA molecule.

Genetic recombination: Process of combining DNA from different sources into a molecule.

Genome: The entire group of genes of a cell.

Gram negative: Term given to bacteria that lose the primary stain (crystal violet) of the Gram staining procedure upon exposure to alcohol or other decolorizing agent.

Gram positive: Term given to bacteria that retain the primary stain (crystal violet) of the Gram staining procedure upon exposure to alcohol or other decolorizing agent.

Gram stain: Differential staining procedure in which crystal violet, Gram's iodine, decolorizing agent such as alcohol, and safranin are sequentially applied to bacterial cells. Most bacteria can be divided into two groups based on whether they retain or lose the primary stain (crystal violet) during the procedure. The response of bacterial cells to this procedure has been linked to differences in the cell wall composition.

Guanine: Purine base unit of a nucleoside.

Halophile: Organism that requires high salt concentrations for growth.

Henry's law constant: A constant that describes the equilibrium partitioning of a compound between liquid and gas compartments (or phases) at a given condition, where the concentration of the compound in the liquid compartment is sufficiently low. Many units are possible with this constant and caution should be taken in its use.

Heterotroph: Organism that requires an organic form of carbon for energy.

Holdfast: Appendage of some bacteria consisting of a fine stalk which may possess adhesive material; functions in attachment.

Hybridization: Process of joining two nucleotides.

Hydraulic residence time (HRT): The time that the bulk aqueous phase resides in a continuous reactor volume. This may be calculated as the ratio of the system's hydraulic volume and the flow rate of the aqueous feed stream, assuming no change in fluid density during the reaction.

Hydrolase: Enzyme that mediates the transfer of a chemical group to water.

Hydrolysis: Cleavage of a molecule by reaction with water.

Hydrophilic: Water-loving; refers to polar molecules that associate with water.

Hydrophobic: Water hating; refers to nonpolar molecules that are insoluble in water.

Hypha: A fungus thread.

Inducer: A molecule that induces the activity of an enzyme.

Insertion sequence: Segment of DNA occurring on either end of a transposon.

In situ: In its original position.

Intron: An intervening sequence in a gene that is transcribed but excised before translation of the gene.

In vitro: In a test tube or beaker ("in glass").

In vivo: In a living organism.

Irreversible inhibitor: Chemical that destroys or binds to a functional group on an enzyme, thereby preventing its catalytic activity.

Isomerase: Enzyme that catalyzes a change in the atomic configuration of a molecule without a change in the number or kind of atoms.

Jet drop entrainment: A mechanism in which small particles may be transported into a gaseous phase based on the collapsing of bubbles at the gas liquid surface. Small droplets of fluid originating at the base of the bubble are formed and accelerated as the top of the bubble breaks and the bottom of the bubble merges with the gas liquid surface. These drops may be aerosolized and thus carried with the gas phase.

3-Ketoadipate pathway: Aerobic pathway of aromatic compound dissimilation in which the end products are the tricarboxylic acid cycle intermediates succinate and acetyl CoA.

Kinase: Enzyme that catalyzes transfer of a phosphate group from ATP or other nucleoside triphosphate to the substrate.

Lag phase: Growth phase during which adaptation to the environment occurs and no increase in cell number is seen.

Langmuir Isotherm: An isotherm equation relating the equilibrium partitioning of a component between liquid and solid compartments (or phases). The equation is of the form:

$$C_s = \frac{ab\,C_l}{1 + b\,C_l}$$

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where C_s and C_l are concentrations of the compound in solid and liquid compartments, respectively, and a and b are empirical constants. This isotherm may be derived from considerations of mass transport onto surfaces with limited capacity to sorb the compound.

Ligase: Enzyme that catalyzes the formation of a product resulting from the condensation of two different molecules, coupled with the cleavage of a pyrophosphate linkage in ATP.

Lipophilic: Refers to molecules that associate with lipids.

Lithotroph: Bacteria that use inorganic compounds as substrates for respiratory metabolism.

Log phase: See exponential phase.

Lyase: Enzyme that catalyzes the addition of a chemical group to the double bond of a substrate or the removal of a chemical group to form a double bond.

Lyse: Breaking apart of the cell wall.

Lysogeny: Infection of a bacterial cell by a virus during which the viral genome becomes integrated into the cell genome, is repressed, and is replicated with the cell genome.

Lytic cycle: Process by which a bacteriophage infects a cell, replicates, and is released into the environment.

Macroinvertebrates: Group of organisms that lack a backbone; in this context refers to species that live in the water.

Maximum specific growth rate (μ_m): The specific growth rate of biomass on a substrate that is limiting growth as defined by the Monod equation:

$$\mu = \mu_m \frac{C_s}{K_s + C_s}$$

where μ is the specific growth rate, μ_m is the maximum growth rate measured, K_s is the Monod half-saturation constant ($K_s = C_s$ at a growth rate of $\mu_m/2$), and C_s is the concentration of the growth-limiting substrate.

Mean cell residence time (MCRT): The mean of the cellular time distribution descriptive for the reactor configuration under consideration. This equals the hydraulic residence time in complete mix, suspended growth reactors that have no biomass separation and recycle. This is a design variable that may be determined in suspended growth reactor systems that have provision for biomass separation and recycle and is determined by biokinetic rate constants and physical design in fixed film systems.

Mesocosm: A constructed laboratory representation of an environment including atmospheric, hydrospheric, and geospheric parts with associated flora and fauna.

Mesophile: Organism that grows best at temperatures from 15°C to about 45°C.

Messenger RNA (mRNA): RNA molecule that serves to carry the genetic message from the DNA to the ribosome.

Meta position: Position on an aromatic molecule separated from the point of reference by one carbon position.

Methylase: Enzyme that adds a methyl group to particular nucleotides.

Microaerophile: Organism that has a narrow range of tolerance for its gaseous environment and requires either a reduced air environment or, in some cases, an increased proportion of carbon dioxide.

Microcosm: A small scale version of a mesocosm. See mesocosm.

Mineralize: Convert a molecule to inorganic ions and molecules.

Mitochondria: Organelles in a eukaryotic cell which are the sites of oxidative metabolism.

Mixed liquor: The mixture of the aqueous phase and suspended biomass in the aeration reactor of a biological treatment process.

Mixed liquor suspended solids (MLSS): A measure of biomass in suspended biological processes where solids are separated from the mixed liquor, are dried, and weights are determined gravimetrically. Standard methods exist for this test.

Mixed liquor volatile suspended solids (MLVSS): A measure of biomass in suspended biological processes where solids are separated from the mixed liquor, dried, heated to remove volatile organics, and weights are determined gravimetrically. Standard methods exist for the test.

Monod half-saturation constant (K_s): A constant defined in the Monod equation. See maximum specific growth rate.

Monooxygenase: Enzyme that catalyzes the addition of one atom of molecular oxygen to a molecule.

Mutant: Cell in which the genome has undergone mutation.

Mutase: Enzyme that catalyzes transfer of a functional group between two positions on the same molecule.

Mutation: Alteration of the genetic message.

NAD^+, NADH: Nicotinamide adenine dinucleotide, a coenzyme which functions in oxidation reduction reactions as hydrogen and electron carriers.

$NADP^+$, NADPH: Nicotinamide adenine dinucleotide phosphate. Same function as NAD; see NAD.

Neutrophile: Organism that grows best at a neutral pH.

NIH shift: Migration of a hydrogen atom from one carbon to the adjacent carbon on an aromatic molecule.

Noncompetitive inhibitor: Chemical that binds to an enzyme in an area other than the binding site, thereby altering and inactivating the catalytic site.

Nucleoid: The area in a prokaryotic cell that contains the chromosome and is not bounded by a membrane.

Nucleoside: A compound composed of a purine or pyrimidine base covalently linked to a pentose sugar.

Nucleotide: A nucleoside with a phosphate group attached to one of the pentose hydroxyl groups.

Nucleus: The membrane-bound organelle in a eukaryotic cell that contains the chromosome.

Nick: A breakage in one strand of a double stranded DNA molecule.

Obligate aerobe: Organism that grows in the presence of air and uses aerobic respiration to obtain energy.

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Obligate anaerobe: Organism that grows only in the absence of air.

Octanol-water partition coefficient (K_{ow}): A constant that describes the equilibrium partitioning of a compound between equal volumes of n-octanol and water at a given temperature. Partitioning between other immiscible fluids and between other compartments can be mathematically related to this constant.

Operator region: Regulatory site of a gene.

Organelle: A discrete portion of a cell, with a specific function.

Organotroph: Bacteria that use organic compounds as substrates for respiratory metabolism.

Origin of replication: Sequence of DNA required for replication of the molecule.

Ortho position: Position on an aromatic molecule adjacent to the point of reference.

Osmotic shock: Sudden change in the solute concentration of the environment surrounding a cell.

Oxidase: Enzyme that catalyzes loss of a hydrogen ion with molecular oxygen as the acceptor.

Oxidoreductase: Enzyme that mediates alterations of the C1I-OH group of a substrate and requires NAD^+ or $NADP^+$ as the hydrogen acceptor.

Para position: Position on an aromatic molecule separated from the point of reference by two carbon positions, effectively opposite to the point of reference.

Passive transport: Process by which substrates enter a cell by free diffusion dependent on the difference in substrate concentration inside and outside of the cell.

Pathogenic: Capable of causing disease.

Periplasmic space: Area between the cytoplasmic membrane and the cell wall.

Phosphatase: Enzyme that mediates the hydrolytic cleavage of phosphate esters.

Phototroph: Organism that obtains its energy from light.

Phytotoxic: Capable of inhibiting the growth of plants or algae.

Plasmid: Small circular DNA molecule that is extrachromosomal and replicates autonomously.

Pleomorphic: Capable of changing shape.

Polylinker: Segment of DNA that contains closely spaced recognition sites for several restriction endonucleases.

Polymerase: Enzyme that adds nucleotides to the 3'-hydroxyl terminus or removes nucleotides from the 5'-phosphate terminus of nicked DNA.

Primary degradation: The initial alteration of a compound.

Primer: Short section of DNA required to be attached to mRNA to initiate the activity of reverse transcriptase.

Prokaryote: Organism characterized by lacking a nuclear membrane.

Protoplasm: The cell within the cell wall.

Protoplast: A viable cell that lacks a cell wall.

Psychrophile: Organism that grows best at temperatures below 20°C.

Recycling fermenter: An apparatus for the continuous culture of microorganisms in a steady state whereby the cells are returned to the culture vessel while medium and waste materials are removed.

Regulatory sequence: A DNA segment involved in regulating a gene.

Regulatory site: Area on an enzyme reversibly occupied by a noncompetitive inhibitor.

Relaxed plasmid: Plasmid which is present in a cell as multiple copies.

Replicon: DNA molecule that contains an origin of replication.

Repressor protein: Protein that binds to the operator region of a gene and blocks its transcription.

Respiration pathway: Metabolic pathway in which oxygen or other inorganic compound or ion serves as the terminal electron acceptor.

Resting cell: A viable cell which is not actively growing or dividing.

Restriction endonuclease: Enzyme that recognizes and cleaves specific sequences of nucleotides within double stranded DNA.

Reverse transcriptase (RNA-dependent DNA polymerase): Enzyme that catalyzes the formation of double-stranded DNA from the information on mRNA.

Reversible inhibitor: Chemical that binds to an enzyme but which may be removed with resulting activation of the enzyme; see competitive inhibitor, noncompetitive inhibitor.

Ribonucleic acid (RNA): A polynucleotide composed of ribonucleotide units.

Ribosomal RNA (rRNA): RNA molecule attached to the ribosome that serves as a framework for the binding of the polypeptide subunits of a protein.

Ribosome: Site of protein biosynthesis.

RNA-dependent DNA polymerase: See reverse transcriptase.

RNA polymerase: Enzyme that catalyzes the formation of RNA from the information on DNA or RNA.

Semi-continuous reactors: Reactors that are operated by adding or removing reactant or products over discrete time intervals, but where the reactions are allowed to proceed continuously. See fill and draw reactor.

Sequential induction: Control of a long metabolic pathway such that sections of the pathway are under separate regulatory control and each section is induced by the product of a prior section.

Sludge volume index (SVI): A measure of settleability of suspended biomass that is based on the volume of solids settled from a mixed liquor over a given time interval. Standard methods exist for this test.

Stationary phase: Growth phase during which no net change in cell numbers is seen, number of cells generated and dying is equivalent.

Steady state: The condition where properties of a system of any given point in the system are the same over time.

Steric hindrance: The inability of atoms or groups on a molecule to rotate freely because of mutual repelling due to van der Waals forces.

Sticky end: Linear double stranded DNA in which one strand extends beyond the other.

Stringent plasmid: Plasmid which is present in a cell in one or, at the most, three copies.

Structural gene: A gene that codes for a protein.

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Suspended biomass: The state of biomass growth where sufficient mechanical energy is introduced by the cells or from external sources to favor free suspension of cells or flocs of biomass in the mixed liquor or broth and to avoid the formation of a fixed biofilm on reactor surfaces.

Synthetase: Enzyme that mediates condensation of two separate molecules coupled with cleavage of ATP.

T_4 DNA ligase: Enzyme that links together complementary fragments of double-stranded DNA.

T_4 RNA ligase: Enzyme that links together complementary fragments of single-stranded DNA or RNA.

Taxonomy: The science of arranging organisms into logical groups describing in detail the basic taxonomic unit, the species.

Terminal deoxynucleotidyl transferase: Enzyme that adds deoxynucleotides to the 3'-hydroxyl end of DNA.

Thermophile: Organism that grows at temperatures above 50°C.

Thiokinase: Enzyme that catalyzes the ATP-dependent formation of thiol esters.

Thylakoid: Internal membrane structure in cyanobacteria that contains the photosynthetic apparatus.

Thymine: Pyrimidine base unit of a deoxyribonucleoside.

Transcription: Process of converting information coded by DNA into RNA.

Transduction: Bacteriophage-mediated transfer of genetic material into a cell.

Transferase: Enzyme that catalyzes the transfer of an intact group of atoms from a donor to an acceptor molecule.

Transfer RNA (tRNA): RNA molecule that serves to bring a specific amino acid into proximity with the developing polypeptide.

Transformation: Process of transfer of exogenous DNA into a cell.

Translation: Process of protein biosynthesis according to the code carried by the mRNA.

Transposon: A segment of DNA that can be moved from one site on a chromosome to another.

Tricarboxylic acid cycle: Respiration pathway utilized by aerobic organisms.

Unsteady state: The condition where properties of a system at any given point in the system are changing over time.

Uracil: Pyrimidine base unit of a ribonucleoside.

Vector: A replicon to which a fragment of DNA may be attached so that the fragment may be replicated.

Vesicle: Cavity filled with liquid or gas.

Viable: Capable of growing.

Yield coefficient (Y): The ratio of the change in biomass concentration, X, and the change in substrate concentration, C_s, over an interval of time. This is a measure of biomass production for unit substrate removal.

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May 1986

DEVELOPMENT OF ADVISORY LEVELS FOR POLYCHLORINATED BIPHENYLS (PCBs) CLEANUP

Exposure Assessment Group
Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, D.C.

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16. ABSTRACT

This document presents background information used in developing advisory levels of PCBs in soil estimated to be permissible in protecting public health. The results of exposure assessment and health effects studies are combined to arrive at the permissible levels of PCBs. Health effects studies conducted using animals for the duration of 10-30 days are used to determine the 10-day advisory levels for PCB cleanup. The long-term advisory levels are based on the carcinogenic risk evaluations.

Exposure pathways considered in estimating the 10-day and long-term average daily intakes include soil ingestion, inhalation, dermal contact, ingestion of contaminated food, and ingestion of water. Exposure to drinking water contaminants is presumed to occur independently of other pathways, because water could come from a clean public water system. The exposure pathways most pertinent to the evaluation of permissible PCB levels in soil are soil ingestion, vapor inhalation, and contaminant contact with human skin.

The currently available modeling techniques considered most appropriate within the constraints of availability of input data are used to estimate exposures. PCB advisory levels are presented as ranges of values to reflect the difference in soil-air partition coefficients depending on soil type, different types of commercial Aroclors, and variations in the soil ingestion rate.

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FOREWORD

The Exposure Assessment Group (EAG) of EPA's Office of Research and Development has three main functions: 1) to conduct exposure assessments; 2) to review assessments and related documents; and 3) to develop guidelines for Agency exposure assessments. The activities under each of these functions are supported by and respond to the needs of the various EPA program offices. In relation to the third function, EAG sponsors projects aimed at developing or refining techniques used in exposure assessments, and at applying these techniques to develop health-based advisory levels for contaminant cleanup. This document is one of these projects and was done for the Office of Solid Waste and Emergency Response.

Polychlorinated biphenyls (PCBs), commercially known as Aroclors, consist of mixtures of chlorinated biphenyl compounds. Many sites contaminated by PCBs remain contaminated because of PCB persistence in the environment. Although commercial PCB production has been banned by the Toxic Substances Control Act, continued use in previously existing commercial equipment can result in spills which require cleanup. EPA has become increasingly involved in the discovery, assessment, and cleanup of these sites.

The purpose of this document is to provide advisory levels for PCB cleanup, and to describe the detailed technical and scientific rationale and methods used in developing these advisory levels for PCBs in contaminated soil. This project required development of exposure and risk assesment methodology related to hazardous waste and spill sites, and analyses of health effects data. The advisory levels and the assessment methodology thus developed will help EPA set

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priorities in PCB spill and cleanup management, and address other PCB contaminant problems.

Michael Callahan, Director
Exposure Assessment Group

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PREFACE

The Exposure Assessment Group of the Office of Health and Environmental Assessment (OHEA) has prepared this development document for advisory levels for polychlorinated biphenyls (PCBs) cleanup at the request of the Office of Emergency and Remedial Response. This document summarizes the procedures concerning multimedia exposure assessments for PCB-contaminated sites, and literature information on chemical and physical properties and health effects pertinent to evaluation of exposures to PCBs. The purpose of this document is to serve as a technical and scientific basis for developing health-based advisory levels for PCBs in soil at spill or cleanup sites. The literature search supporting this document is current to May 1986.

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ABSTRACT

This document presents background information used in developing advisory levels of PCBs in soil estimated to be permissible in protecting public health. The results of exposure assessment and health effects studies are combined to arrive at the permissible levels of PCBs. Health effects studies conducted using animals for the duration of 10-30 days are used to determine the 10-day advisory levels for PCB cleanup. The long-term advisory levels are based on the carcinogenic risk evaluations.

Exposure pathways considered in estimating the 10-day and long-term average daily intakes include soil ingestion, inhalation, dermal contact, ingestion of contaminated food, and ingestion of water. Exposure to drinking water contaminants is presumed to occur independently of other pathways, because water could come from a clean public water system. The exposure pathways most pertinent to the evaluation of permissible PCB levels in soil are soil ingestion, vapor inhalation, and contaminant contact with human skin.

The currently available modeling techniques considered most appropriate within the constraints of availability of input data are used to estimate exposures. PCBs advisory levels are presented as ranges of values to reflect the difference in soil-air partition coefficients depending on soil type, different types of commercial Aroclors, and variations in the soil ingestion rate.

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U.S. Environmental Protection Agency

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1. EXECUTIVE SUMMARY

This report has been prepared in response to a memorandum dated April 9, 1985, from the Office of Emergency and Remedial Response (OERR), requesting that the Office of Health and Environmental Assessment (OHEA) develop advisory levels for polychlorinated biphenyls (PCBs) which can be used as guidelines for initiating removal action for sites contaminated with PCBs. Interested offices within EPA, including OERR, have advised OHEA that these advisory levels for PCBs cleanup should be developed based on considerations of public health protection from short-term and long-term exposures. The advisories presented in this report include permissible levels of PCBs in soil corresponding to 10-day and lifetime acceptable intakes.

Exposure routes considered in developing these advisory levels include drinking water, ingestion of PCB-contaminated soil by children and adults, and inhalation of ambient air contaminated with PCBs. Other exposure routes, such as dermal exposure, food intake, and ingestion of fish which have bioaccumulated PCBs, are considered in relation to their importance and their relevance to the present document. In view of the high bioaccumulation factor for PCBs, the consideration of bioaccumulation is important in setting PCB levels in surface water in which aquatic animals live. If one of these routes is a controlling factor in relation to the exposure route or human intake considered, the advisories need to be reevaluated.

Commercial-grade PCBs marketed as Aroclors in the United States are mixtures of many chlorinated biphenyl compounds in various proportions. Each PCB compound may exhibit its own toxicological characteristics and physical and chemical properties. This fact complicates the exposure analysis in deriving the allowable concentrations in drinking water and soil. It is conceivable

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that chemical and physical properties reported in the literature for each Aroclor designation represent an average property for the mixture. To define the variability of safe levels for contamination by different Aroclor designations, exposure analyses have been performed for several Aroclors: Aroclor 1242, 1248, 1254, and 1260. The steps used in developing the advisories include: (1) the evaluation of toxicological effect studies, (2) exposure analysis for PCB intake from drinking water, soil ingestion, air inhalation, and dermal contact, and (3) risk assessment combining the toxicology studies and exposure analysis.

Ten-day noncancer health advisories are based on the short-term acceptable intake (AI) derived from studies of animals treated with Aroclor-1254 for no more than 30 days to examine noncarcinogenic effects. This AI value, which forms the basis for establishing permissible levels of PCBs in soil, is 0.1 and 0.7 mg/day for a 10-kg child and a 70-kg adult, respectively. The permissible PCB concentrations for each carcinogenic risk level are based on the potency factor of 4 $(mg/kg \cdot day)^{-1}$ rounded off from two independent evaluations based on an Aroclor-1260 study.

It is likely that not all of the PCBs ingested or inhaled by humans are absorbed. Proper calculations of absorption rate and hence exposure should be based on realistic pharmacokinetics-type models to determine intake. Lack of experimental data with which to estimate the parameters needed in the pharmacokinetics models has prevented their applications to the analysis for PCB absorptions through human exchange boundaries. Future work should consider these models. Although most animal studies (in rats and mice) on the extent of absorption in the gastrointestinal tract show absorption in excess of 90%, there are two experiments on monkeys reporting less than 88% absorption in one case and less than 13% and 40% absorption for a specific congener in another case, based on the analysis of feces and urine. Vehicles used in administering

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PCBs were not specified. It is likely that the high adsorption characteristics of PCBs on soil could retard the absorption rate in the human intestinal tract. In the risk analysis performed in the present study, the absorption rate for humans after ingestion of PCB-contaminated soil is considered to be 30%.

Absorption from dermal exposure has been reported to be as significant as from other routes of exposure, but little information is available for the quantitative evaluation of dermal absorption rates. Five percent dermal absorption is assumed for soil contaminants in contact with human skin. The dermal absorption rate of contaminants present on soil is presumed to be less than that for contaminants spilled on skin in pure form. Inhalation studies using PCB aerosols show that the absorption of PCBs from inhalation exposure readily occurs. In the present analysis, an absorption factor of 50% is assumed for absorption of PCB vapors after inhalation into human lungs.

The circumstances under which human exposure occurs are divided into three classes depending on population distribution: (1) Exposure occurs on-site. This can be further subdivided into: (a) sites which are readily accessible to children, and, hence, the soil from which will be subject to ingestion, dermal contact, and inhalation, and (b) sites for which there is no possibility of soil ingestion, and, hence, exposure is only through inhalation; (2) sites which no population is assumed to enter within a radius of 0.1 km from the site; and (3) sites which no population is assumed to enter within a radius of 1 km from the site.

The soil ingestion rates used for Class (1)(a) evaluations are 3 and 0.6 g/day. The former is a value based on data from a study of an adult person with pica, while the latter represents a long-term average value for soil ingestion. If sites are not accessible to populations at distances of 0.1 km or 1 km from the site, as in Classes (2) and (3) above, it is assumed that

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no ingestion of contaminated soil occurs and the exposure route is that of inhalation.

The emission rate of volatilized PCBs can be considerably reduced by covering the contaminated soil by low-porosity uncontaminated soil or clay material. The reduction in the emission rate will result in a decrease in ambient air concentrations of PCBs by the action of blowing winds. When PCB-contaminated material is directly exposed to the atmosphere, the PCB levels in soil required to maintain the same level of exposure will be less than those expected when the PCB-contaminated material is covered with low-permeability material of appropriate thickness. The cover would also serve as a deterrent to soil ingestion and direct dermal contact.

The depletion of PCBs from soil caused by volatilization is accounted for in the exposure analysis by solving a partial differential equation simulating PCB vapor diffusion through the soil air-phase pores, and the distribution of PCBs between air and soil phases. Boundary conditions assume that the air-phase resistance is relatively small compared to the diffusional resistance in the soil air-phase pores. The available experimental data reasonably follow the time-emission rate relationship predicted from the models based on this assumption. Since the depletion rate varies over time, it is averaged over the exposure period. Depletion averaged over a period of time should lead to a lesser inhalation exposure than that based on the model assuming that depletion does not occur.

The worst-case emissions would occur when the contaminated soil is initially exposed to the atmosphere and the soil is contaminated up to the conditions exhibiting saturation vapor pressure. A constant emission rate can be assumed if the vapor-phase concentration maintains a constant value at the surface of

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soil contamination for time-varying emission rates. Calculations corresponding to Classes (1), (2), and (3) for exposure possibilities with surface contamination are repeated at an assumed 25-cm thickness of a soil cover initially free from PCB contamination. Among many factors affecting the emission rate (including vapor pressure, soil-air partition coefficient, Henry's Law constant, etc.), the value of the soil-air partition coefficient shows the most wide-ranging variation, because of the variation of the experimental soil-water partition coefficient available in the literature for soil textures ranging from 40 to 1,000 cm^3/g.

The method for determining the permissible PCB levels in soil, which combines the routes of soil ingestion, inhalation, and dermal exposure, has been computerized to avoid the necessity for hand calculations.

The results of these computer calculations are summarized in Tables 1 and 2, which have been prepared using different combinations of the following variables:

(1) Surface contamination representing a situation where the contaminated soil surface has been left uncovered after removal action.

(2) 25-cm (10-inch) clean cover applied, representing a situation in which clean soil material is used on top of the contaminated soil surface.

(3) Two different soil ingestion rates (3 and 0.6 g/day) for Class (1)(a), corresponding to sites accessible to children.

(4) Different AI levels (short-term AI, and AIs at different cancer risk levels).

(5) Four Aroclors (Aroclor 1242, 1248, 1254, and 1260).

(6) Two selected values of the soil-air partition coefficient, representing the high and low values.

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TABLE 1. PERMISSIBLE PCB SOIL CONTAMINATION LEVELS
(UNCOVERED SURFACE CONTAMINATION)

Location and route of human exposure	Permissible levels (µg PCB/g soil) corresponding to: Noncancer short-term[a] acceptable intake (µg/day)[b]		Cancer risk specific doses (µg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	25-100[f]	510-730	0.008-0.01	.08-0.1	0.8-2	8-17
- Soil ingestion[d], inhalation[e]	42-420	2,00-3000	0.01-0.06	0.1-0.6	1-6	35-61
- Inhalation only[e]	47-vs[g]	vs	0.01-0.2	0.1-2.0	1-20	77-470
0.1 km from contaminated site - Inhalation only[e]	vs	vs	2.0-220	90-2.2×10^4	7.7×10^3-vs	8.7×10^5-vs
1 km from contaminated site - Inhalation only[e]	vs[g]	vs	220-1.3×10^3	2.2×10^4-1.3×10^5	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without *pica* (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days' exposure per year.
[f]Ranges result in each case because 1) four PCBs (1242, 1248, 1254, 1260) are considered, each with a different vapor pressure, and 2) high and low values for soil-air partition coefficient are used in the calculations.
[g]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquids for the limit.

TABLE 2. PERMISSIBLE PCB SOIL CONTAMINATION LEVELS
(25-cm-THICK CLEAN COVER)

Location and route of human exposure	Permissible levels (µg PCB/g soil) corresponding to: Noncancer short-term[a] acceptable intake (µg/day)[b] — 100 for child	700 for adult	Cancer risk specific doses (µg/day) — 0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	110-200[f]	800-1400	0.01-0.2	0.1-2.0	1-17	22-vs
- Soil ingestion[d], - inhalation[e]	450-vs[g]	3100-vs	0.02-0.6	0.2-6.0	1.0-48	93-vs
- Inhalation only[e]	vs	vs	0.02-1.0	0.2-vs	2.0-vs	770-vs
0.1 km from contaminated site - Inhalation only[e]	vs	vs	1-vs	620-vs	vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-6, with pica (consuming 3 g soil/day).
[d]Children ages 1-6, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days' exposure per year.
[f]Ranges result in each case because 1) four PCBs (1242, 1248, 1254, 1260) are considered, each with a different vapor pressure, and 2) high and low values for soil-air partition coefficient are used in the calculations.
[g]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquids for the limit.

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(7) Exposures for 10 days after cleanup or spill of contaminants for short-term advisories.

Table 1 shows the range of values for permissible PCB concentrations in soil when the soil is contaminated up to the surface in contact with the atmosphere and is left uncovered. Table 2 represents the case where the contaminated soil left at the site, or after remediation, is covered with a 25-cm (10-inch) clean soil layer. The ranges in both tables result from the use of four Aroclors and the use of high and low values for the soil-air partition coefficient. Other factors reflected in the ranges are differences in vapor pressures and Henry's Law constants for each Aroclor. The permissible PCBs levels in soil specific to each combination of the scenarios are compiled in Appendix C, as obtained from computer simulations.

The symbol "vs" in Tables 1 and 2 indicates that no upper-bound limit for PCB concentrations in soil can be derived from the exposure evaluation, because the PCB concentration in soil is above the vapor saturation concentration. There are two reasons for such a result. First, the emission rate cannot exceed the upper-bound value which can be expected when the air-phase concentration of PCBs at the contaminated soil surface is maintained at the vapor saturation point. The concentration at the vapor saturation point corresponds to the vapor pressure concentration. Second, when the cover is applied, not only is the emission rate retarded, but also the concentration of PCBs in soil being ingested is controlled by the amount of PCBs adsorbed on soil in equilibrium with the air phase being emitted. Therefore, the concentration of PCBs in the initially clean soil material cannot exceed the concentration in equilibrium with saturated vapor.

In actuality, the "no upper limit," or the level above vapor saturation, designated by vs, should be interpreted with great care. The assumptions used

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in the exposure evaluation are critical. They include but are not limited to: (1) no soaking of clean cover by liquid PCBs for the thickness of 25 cm; (2) no disturbance of cover material by construction activities or children digging the ground; (3) no exposure to initial spills when 25 cm of clean cover (Table 2) is assumed; (4) no population enters the area within the respective radius of distances from the site; and (5) the cover material is at least equivalent to soil material.

From a practical point of view, Assumption 1 is tantamount to requiring the presence of no free liquids in the soil, which may otherwise result in the phenomenon of "wicking." Since the ranges shown in Tables 1 and 2 are dependent upon the type of Aroclors and the values of the soil-air partition coefficient, site-specific or Aroclor-specific information should be used to establish an appropriate level of PCBs for that particular condition. The methodology for performing site-specific exposure evaluations is presented. Computer outputs for the selected Aroclors under the ranges and conditions of common environmental concern are presented in Appendix C, and can easily be used to find the permissible concentrations in soil suitable to particular situations.

Table 1, for example, can be interpreted as follows:

(1) When the site is amenable to access by children with possibilities of ingesting the contaminated soil exposed to the atmosphere, and when exposure occuring to the children by inhalation and dermal contact is accounted for, the permissible PCB levels in soil should range from 25 to 100 µg/g and 42 to 420 µg/g for prevention of noncancer effects from 10-day exposure for a child with an average weight of 10 kg ingesting soil at the rates of 3 and 0.6 g/day, respectively. For cancer effects, permissible levels in soil for a lifetime exposure to PCBs resulting from ingestion of and dermal contact with contaminated soil and inhalation of contaminated air should range from 0.08 to 0.1 µg/g

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and 0.1 to 0.6 µg/g, corresponding to an upper-bound risk estimate of 10^{-6} at assumed soil ingestion rates of 3 and 0.6 g/day, respectively. The specific level will depend on the types of Aroclor present, the likely ingestion rate, and the extent of soil-air partitioning. For sites in which there is no possibility of soil ingestion, PCB levels in soil, based on the inhalation route only, should range from 47 µg/g to no limit value for a 10-day exposure for a child with an average weight of 10 kg, and correspond to no limit value for an adult with an average weight of 70 kg. The permissible levels of PCBs in soil, based on the inhalation pathway only, range from 0.1 to 2 µg/g, corresponding to a lifetime AI at a risk factor of 10^{-6}. Again, the level will be dictated by the types of Aroclor present and the specific characteristics of the site involved.

(2) If there is no possibility of a population entering the contaminated site within a radius of 0.1 km from the site, the PCB levels in soil can remain at no limit value and 90 to 2.2×10^4 µg/g, without exceeding 10-day AI and lifetime AI at 10^{-6} risk, respectively.

Similar interpretations can be made for the results applicable to sites without affected population up to 1 km from the site, and to the carcinogenic risks listed at 10^{-4}, 10^{-5} and 10^{-7}.

The short-term AI levels (100 µg/g day for a child and 700 µg/g day for an adult) used in this report to develop 10-day advisories based on noncancer effects are derived from animal studies, which collectively indicate that the experimental threshold for adverse effects of Aroclor 1254 is at or near a dose of 1.0 µg/kg body weight. Using this dose as a No Observed Adverse Effect Level (NOAEL) and a safety factor of 100, the 10-day AI levels for noncancer effects described above (100 and 700 µg/day) were computed and serve

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Advisory levels for 1-day and lifetime noncancer effects cannot be derived at this time because of the insufficiency of the available data. However, in view of the experimental duration, the 10-day advisories may well be used for the 1-day advisories.

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2. INTRODUCTION

The purpose of this document is fourfold: (1) to provide background information compiled in the process of developing permissible health advisories for polychlorinated biphenyls (PCBs) in soil and drinking water, in response to a request from the Office of Emergency and Remedial Response, (2) to outline the procedures used in developing the advisories, (3) to list pertinent input data necessary in carrying out the exposure analyses and in setting the allowable concentration limits, and (4) to present an outline summary of the results obtained from computer simulations of the techniques used.

The information and methods presented are intended for use in setting safe advisory levels to protect public health from short-term, longer-term, and lifetime exposures to PCBs released from hazardous waste facilities or from spills at previously contaminated sites. Particular interests pertain to levels of PCBs allowable in contaminated soil, and the potential of PCB migration to groundwater from PCB-contaminated or hazardous waste facilities. These advisories are not concerned with setting PCB limitations in sediments contaminating surface water, which can be a source of bioaccumulation of PCBs in aquatic animals.

The analyses presented in this report provide the basis for deriving PCB levels allowable in soil and drinking water, which are likely to be primary sources of exposure pathways. PCB problems that may exist in rivers and estuaries because of contaminated sediments are not dealt with in these analyses. The analyses mainly address health concerns at hazardous waste sites or at sites with contaminated soil. The primary health impacts considered include adverse impacts associated with ingestion of contaminated soil, inhalation of ambient air, and dermal contact with the soil. Other exposure routes, such as

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drinking water, food intake, and ingestion of bioaccumulated fish are considered to the extent that the pathways are relevant. PCBs that have migrated from contaminated sites to various exposure media are evaluated for short-term and lifetime impacts to arrive at corresponding advisory level values.

The total dose of PCBs is obtained by summing each dose from all major exposure pathways, and is compared with acceptable intakes (AI) judged from the health effects information available in the literature. A longer-term AI considered most appropriate in deriving 10-day noncarcinogenic advisories is used in the exposure evaluation. Advisories for protecting against carcinogenic risks are similarly derived at various risk levels based on the potency factor.

This report is not intended to address the achievability of the safe levels developed. Although the report contains a brief statement, taken from the available literature on analytical capability, control technology, and environmental distribution of PCBs, the data base seems insufficient to make a generalization concerning the level of PCB cleanup achievable in practice.

The Exposure Assessment Group distributed three earlier drafts of this document under the title of "Development of Health Advisories for Polychlorinated Biphenyls (PCBs)" for internal review and comment on May 9, 1985 and July 25, 1985, and under the title of "Development of Health Advisories for Polychlorinated Biphenyls (PCBs) Cleanup" on December 16, 1985. As a result of the comments from the Office of Drinking Water, on the December 16, 1985 draft, the 1-day advisories have been replaced by 10-day advisories because no data could be found indicating that 3,4,5,3',4',5'-hexachlorobiphenyl, used for development of the 1-day advisories, is a component in commerical Aroclors. This final draft reflects changes made to incorporate comments from the Office of Toxic Substances, the Office of Emergency and Remedial Response, and OHEA's

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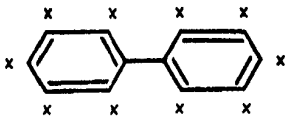
3. CHEMICAL COMPOSITIONS

A polychlorinated biphenyl (PCB) is any member in a family of organic compounds with two or more chlorine substitutions on biphenyl rings, and can be typified by the following chemical structure:



The symbol, x, in the structural formula represents possible positions of chlorine that can be substituted for hydrogen, which is one of the basic elements of aromatic hydrocarbons. Based on the possible distribution of substituted chlorine atoms on two benzene rings of biphenyl, it is calculated that there could theoretically be 209 types (congeners) of PCBs.

Patents disclose that PCBs are prepared by the chlorination of biphenyl in the presence of a catalyst. The process yields a complex mixture of chlorinated biphenyl compounds. It is unlikely, however, that all combinations of chlorinated biphenyls would be formed in the chlorination process. Although the crude mixture is purified to remove reaction impurities, the resulting product is still a mixture of chlorinated biphenyls in various proportions. Their compositions depend upon the chlorination conditions.

Commercial-grade PCBs, consisting of mixtures of different composition, are sold under the trade name Aroclors. Impurities such as chlorinated dibenzofurans and chlorinated naphthalenes are known to exist in commercial PCBs. The sole producer of Aroclors in the United States for the period 1957 to 1972 was the Monsanto Chemical Company. Their products are characterized by four-digit numbers. The first two numbers represent the type of molecule (12 = biphenyl-

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based; 54 = terphenyl-based; 25,44 = blends of PCBs and chlorinated terphenyls); and the last two digits refer to the percentage of chlorine by weight. PCB products are also manufactured in other countries, including Germany, France, Japan, and the U.S.S.R.

Table 3 illustrates approximate compositions of individual biphenyls for some Aroclors (U.S. EPA, 1976b). Although one might expect some 140 to 150 separate congeners in an Aroclor, the actual analysis of Aroclor 1248, for example, identified less than 50 peaks in the high-resolution gas chromatograph using a typical Aroclor 1248 sample (U.S. EPA, 1976b). No compounds which can be formed by addition of chlorine rather than substitution were found in a detailed study of PCBs (U.S. EPA, 1976b). It is suspected that the conditions prevailing during industrial manufacturing of PCBs do not favor the formation of addition compounds, or that these latter compounds might have been destroyed in the step used to purify the Aroclor. In constrast to the analysis shown in Table 3, another publication reports an analysis of Aroclor 1221 to contain 12.7% biphenyl, 47.1% monochlorophenyls, and 40.2% dichlorophenyls (Hutzinger et al., 1974).

Major PCB components in foreign products bearing the names of Kanechlor and Phenoclor for Japanese and French products, respectively, have been identified. The number of the major components separated from Kanechlor 400 is five, and that from Phenoclor DP6 is seven.

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TABLE 3. APPROXIMATE COMPOSITION OF AROCLORS

Chlorobiphenyl	Percent by weight for Aroclor designation					
	1221	1242	1248	1254	1260	1016
$C_{12}H_{10}$	11	<0.1		<0.1		<0.1
$C_{12}H_9Cl$	51	1		<0.1		1
$C_{12}H_8Cl_2$	32	16	2	0.5		20
$C_{12}H_7Cl_3$	4	49	18	1		57
$C_{12}H_6Cl_4$	2	25	40	21		21
$C_{12}H_5Cl_5$	0.5	8	36	48	12	1
$C_{12}H_4Cl_6$	ND	1	4	23	38	<0.1
$C_{12}H_3Cl_7$	ND	<0.1		6	41	ND
$C_{12}H_2Cl_8$	ND	ND		ND	8	ND
$C_{12}H_1Cl_9$	ND	ND		ND	1	ND
$C_{12}Cl_{10}$	ND	ND		ND		ND

ND = non-detectable.

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4. PRODUCTION

Commercial production of PCBs from the starting material benzene was begun in the 1920s by Swann Research, Inc., of Annington, Alabama, which referred to these products under the trade name Aroclor. PCBs were manufactured at that location by Swann Research, Inc., and its successor, Monsanto Chemical Company, until the plant was closed in 1971. Monsanto continued production at another plant at Sauget, Illinois, until 1977. The only other known manufacturer of PCBs is Geneva Industries of Houston, Texas, which manufactured PCBs from 1972 through 1974.

The domestic sale of Aroclor products peaked to 33,000 metric tons in 1970, and has declined since then due to restrictions on the use of PCBs (Hutzinger et al., 1974). PCBs were available commercially as mixtures (Aroclors) of 20 to 75 chlorinated biphenyls, and were marked according to the weight of chlorine contained in the mixtures. These commercial mixtures included Aroclors 1242, 1254, 1248, 1260, 1262, 1268, 1221, 1232, and 1016, in descending order according to domestic sales. In the year of peak production, 57% of the Aroclors produced were in the form of Aroclor 1242 (U.S. EPA, 1980a). The production in Japan and the annual consumption in Finland are estimated at 26 million pounds and 0.5 million pounds per year, respectively. PCB-1016 (41% chlorine) is a more recent product, and its sales prevailed for the period 1972-1976.

PCBs may be formed as side-products in other manufacturing processes involving the use of chlorinated benzene or biphenyl in the reaction step. For example, some of the trichlorobenzene used as a solvent in the manufacture of the dry pigment phthalocyanine blue is converted to PCBs during the reaction. PCBs formed can contaminate the pigment product at concentrations from a few

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parts per million to as much as 0.1%. Similarly, dichlorobiphenyl is formed in the manufacture of diarylide yellow pigments as a product of side reaction with the reactant dichlorobenzidine. The process of chlorinating water which contains biphenyl in a compound used as a dye carrier in dyeing polyester fibers can form PCBs as a side-reaction product which can contaminate the water. No natural sources of PCBs have been identified.

PCBs have been imported into the United States for use in various applications. Decachlorobiphenyl was imported from Italy for use as a wax filler in the investment casting industry until 1976. PCBs imported from France are used in mining machinery cooling systems. The percentage of imported PCBs over the total domestic sales for the period 1971 through 1975 in the United States is in the range of 1.6% to 2.7% (U.S. EPA, 1976b).

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5. USES

Products containing PCBs have been used in agriculture and industry for decades. Their use is are mainly attributable to high chemical stability and physical properties desirable in certain applications. These properties include nonflammability, high dielectric constant, plasticizing capability, and ease of volatilization under heated conditions. Since 1930, PCBs have been extensively used as dielectric fluids in electrical transformers and capacitors, and have also been used for a variety of other purposes, including use in heat transfer and hydraulic systems, in the investment casting industry, and as plasticizers and solvents in sealants and adhesives. PCBs are also used as flame retardants in the manufacture of hard plastic products in which heat resistance is desired, as a dye carrier in carbonless copy paper, and as a plasticizer in paints.

Several published sources provide a comprehensive breakdown of uses for different types of Aroclors (Hutzinger et al., 1974; Nisbet and Sarofim, 1972; Versar, Inc., 1977). The most widely used Aroclors were 1242, 1248, 1254, and 1260. Aroclor 1016 was used after 1970 but in much smaller quantities than the four types mentioned above. A Monsanto marketing bulletin on PCBs, published in the 1960s, also described their possible use as gaskets and packing materials; as vehicles in graphic arts; as impregnation agents; as moisture-proof coatings; as wax substitutes; as de-dusting agents; in insecticides; in abrasives, lubricants, and cutting oils; in inks; in mastics; and in tank coatings (Monsanto Chemical Co., undated). A number of other uses have been patented.

In the United States, there are 17 companies that have used PCBs in the manufacture of askarel capacitors, and 13 companies that have used PCBs in the manufacture of askarel transformers. According to one study, in 1976 approxi-

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mately 25 investment casting foundries (out of a total of 135 in the United States) used PCB-filled waxes in the manufacture of metal castings (U.S. EPA, 1976a).

In 1971, because of environmental concerns, the manufacturer voluntarily restricted the sale of PCB products for use only in "closed" systems, which include electrical transformers and capacitors with insulating fluids that contain PCBs. These two applications account for all of the current use of PCBs in the United States. The company was on a schedule to phase out production of all PCBs by 1979. The cessation of the production will reduce the amount of PCBs being released into the environment, but millions of pounds of PCBs are still being used in electrical insulation applications. The environmental contamination by existing PCBs, and their environmentally safe treatment or disposal, continue to be of concern.

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6. DISPOSAL

A material balance performed on the amount of PCBs produced, sold, and purchased provides an estimate of the amount of PCBs lost or disposed of in the manufacturing process. The total estimated amount reported to have been disposed of or lost for the year 1974 is about 3.8 million pounds (U.S. EPA, 1976b), of which about 1.8 million pounds are estimated to have been land-disposed, and the rest to have been incinerated.

The 1.8 million pounds of PCBs in the land-disposed wastes generated from the manufacturing process amounts to only a small fraction of the total PCBs sent to land disposal facilities. The total land-disposed amount of PCBs for the year 1976 is reported to have been about 12 million pounds (U.S. EPA, 1976b). The most important source of PCB waste has been capacitors that have failed or become obsolete, or that are contained in obsolete equipment. Other PCB wastes include solid wastes from PCB manufacturing facilities and from operations using PCBs in non-electrical applications.

The data base for the WET Model, prepared by SCS Engineers (Undated), shows that PCB fluids containing 50% PCB-1254 sent to hazardous waste treatment, storage, and disposal facilities (TSDF) amount to about 4,500 tons per year. This waste competes for the capacity of TSDF regulated under Subtitle C of the Resource Conservation and Recovery Act. Water effluents from PCB production and first-tier use facilities are relatively small compared with the amounts being disposed of in landfills. Severe local impacts are evident by the discharge into rivers of these effluents. PCBs are now found in the sediments, water column, and biota in the rivers. A few examples of current PCB problems include the Hudson River and the New Bedford, Massachusetts, harbor. As a result of a strong tendency of PCBs to adsorb on sediments, and of sediment

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migration, PCB problems are identified farther downstream from the discharge points.

Twenty spills involving PCB products have been identified (U.S. EPA, 1976c). These spills occurred in transformer installations from trucks and railroad cars while they were en route to their destinations, and from leaking drums.

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7. CHEMICAL AND PHYSICAL PROPERTIES

The chemical and physical properties of PCBs can be divided into two groups: (1) those relevant to the commercial and industrial use of PCBs, and (2) those that are needed in exposure evaluation and hence in developing media-specific safe level advisories for PCBs. The latter properties will be briefly summarized herein.

The widespread distribution of PCBs in the environment suggests that the major route by which PCBs are transported from treatment, storage, and disposal facilities is through the atmosphere in the form of volatilized vapor and adsorption on particulate matter. Vapor pressure is one of the important properties affecting volatilization. The available vapor pressure data for commercial Aroclors, as reported in the literature, have been compiled and are presented in this chapter. Vapor pressure, as distinguished from partial pressure or true pressure, refers to the maximum vapor-phase pressure achievable under equilibrium conditions at the soil-air interface.

Experimental data (U.S. EPA, 1980a) suggest that PCBs are strongly adsorbed on earth materials, including soil. PCBs adsorbed on soil, or present in the soil mixture, will be subject to ingestion if the contaminated sites are accessible to children or to adults with habitual pica. The bioaccumulation factor (in the food chain and in aquatic biota) is also an important physical parameter when there is a likelihood of PCB transport in water.

As pointed out previously, there are a number of congeners for each of the Aroclors. Thus, the properties listed herein for Aroclors represent averages over the various species that constitute the mixtures. The observation that environmental samples have contained more chlorobiphenyls with high chlorine levels than is characteristic of freshly manufactured Aroclors is attributable

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in large part to the possible metabolism and volatilization of lower chlorine species, coupled with enhanced sorption of species with more chlorine.

The more common types of Aroclors are shown in Table 4. Thirteen Aroclors were listed in a manufacturer's booklet (Monsanto Chemical Co., undated). These compounds range from oily liquids to white crystals and hard transparent resins, and generally have similar chemical and biological characteristics.

The properties and parameters commonly needed in estimating the environmental fate and transport of a given chemical are vapor pressure, solubility in water, soil-water partition coefficient, and bioaccumulation factor. These properties of PCBs, and other relevant properties, are shown in Table 4 (Burkhard et al., 1985; MacKay and Leinonen, 1975; Hutzinger et al., 1974; Monsanto Chemical Co., undated; Hwang, 1982; U.S. EPA, 1979a). Information on additional physical and chemical properties such as viscosity, softening points, and other factors, can be found in references authored by Hutzinger et al. (1974), Monsanto Chemical Co. (undated) and U.S. EPA (1980a).

The vapor pressure of PCBs and their solubility in water are low, and tend to decrease as the number of chlorine substitutions on the phenyl rings increase. Aroclors are soluble in most aliphatic and aromatic solvents, and are highly resistant to the action of strong alkali or acids, or high temperatures. Aroclors subjected to bomb tests are reported to have shown no evidence of oxidation (Hutzinger et al., 1974). PCBs have been shown to adsorb relatively rapidly and strongly to various materials, including soil, wood, plastic, and glass (Hutzinger et al., 1974).

Partition coefficients indicating a measure of partitioning under equilibrium conditions between PCBs at the interfaces of air-soil, air-water, water-soil media are important parameters in exposure analysis. Experimental data are scarce. Data for the distribution between air and water in the form of

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TABLE 4. CHEMICAL AND PHYSICAL PROPERTIES OF PCBs

PCB	Molecular weight	K_{ow}	Specific gravity	Solubility[a] in water (mg/L)	Vapor press.(mmHg) at 25°C	Henry's law constant (atm. m^3/g mol)
PCB-1016 (Arochlor 1016)	257.9	24,000		0.42	$4x10^{-4}$	
PCB-1221	200.7	12,000	1.182	15.0	$6.7x10^{-4}$	
PCB-1232	232.2	35,000	1.266	1.45	$4.06x10^{-3}$	
PCB-1242	266.5	380,000	1.380	0.24	$4.06x10^{-4}$	$5.73x10^{-4}$[b]
PCB-1248	299.5	1,300,000	1.446	$5.4x10^{-2}$	$4.94x10^{-4}$	$3.51x10^{-3}$[b]
PCB-1254	328.4	1,070,000	1.538	$1.2x10^{-2}$ ~0.03	$7.71x10^{-5}$	$8.37x10^{-3}$[c]
PCB-1260	377.5	14,000,000	1.620	$2.7x10^{-3}$	$4.05x10^{-5}$	$7.13x10^{-3}$[c]
PCB-1262			1.646			
PCB-1268			1.810			
PCB-1270			1.947			
PCB-2565			1.727			
PCB-4465			1.712			
PCB-5442			1.434			
PCB-5460			1.740			
2,2',5,6'-tetra-chloro-biphenyl				$4.6x10^{-2}$		
2,2',3,4,6,-penta-chloro-biphenyl				$2.2x10^{-2}$		

[a]Hutzinger et al., 1974; Monsanto Chemical Co., undated.
[b]MacKay and Leinonen, 1975.
[c]Hwang, 1982, and U.S. EPA, 1980c.

Bioaccumulation factor: 31,200 L/kg.

Soil-water partition coefficient (U.S. EPA, 1980a): 22 - 1938 L/kg.

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Henry's Law constant and water and soil, exist for some selected Aroclors. Experimental data measuring the distribution of PCBs between air and soil are nonexistent. Estimates of air-soil partition coefficient can be calculated based on Henry's law constant and soil-water partition coefficient using one of several empirical relationships. The Henry's Law constant and soil-water partition coefficient, in turn, are dependent on water solubility and percent organic carbon in soil, respectively.

The Henry's Law constants shown in Table 4 are based on information in MacKay and Leinonen (1975) for PCB-1242, PCB-1248, and PCB-1260; and in a U.S. EPA research report (1980c) for PCB-1254. Burkhard et al. (1985) recently published a list of calculated Henry's Law constants for PCB-1242, PCB-1248, PCB-1254, and PCB-1260. The value for PCB-1254 is an experimental value obtained in the EPA laboratory in Cincinnati, Ohio, while others represent calculated values. A comparison of Henry's Law constants for PCB-1254 shows that the values in MacKay and Leinonen (1975) and Burkhard et al. (1985) differ by a factor of 10, while those in MacKay and Leinonen (1975) and the experimental EPA value (1980c) differ by a factor of 3. Since MacKay and Leinonen's value is closer to the experimental value, Henry's Law constants for PCB-1242, PCB-1248, and PCB-1260 are taken from MacKay and Leinonen (1975).

In the absence of experimental data, the soil-water partition coefficient K_d (cm^3 water/g soil), and the air-soil partition coefficient, K_{as} (g soil/ cm^3 air) can be estimated from water solubility and percent organic carbon (%OC) in soil, using correlations presented by various researchers. For example, the values for the octanol-water partition coefficient, K_{ow}, can be used to estimate the values for the soil sorption coefficient based on soil organic carbon content, K_{OC} (cm^3 water/g organic carbon), and the bioconcentration factor (BCF) by the following formula:

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$$\log K_{OC} = 0.544 \log K_{ow} + 1.377 \quad \text{(Kenaga and Goring, 1980)} \quad (1)$$

$$\log K_{OC} = 1.00 \log K_{ow} - 0.21 \quad \text{(Karickhoff, 1979)} \quad (2)$$

$$\log BCF = 0.76 \log K_{ow} - 0.23 \quad \text{(Veith et al., 1980)} \quad (3)$$

The K_d and K_{as} values then can be estimated by

$$K_d = K_{OC} \left(\frac{\%OC}{100}\right) \quad (4)$$

$$K_{as} = \frac{H}{K_d} \quad (5)$$

where H represents Henry's Law constant. Since the common unit for H is given in atm m^3/g mol, a conversion factor of 41 (=1/2.44 x 10^{-2}) should be multiplied in the right-hand side of Eq. (5) when the units for K_{as}, H, and K_d are g soil/cm^3 air, atm m^3/g mol, and cm^3 water/g soil, respectively. The multiplication of K_{as} by the concentration of PCBs in soil will provide the concentration of PCBs in the air phase above contaminated soil of interest under equilibrium conditions. It should be recalled that the air-soil partition coefficient, K_{as}, has the unit of g soil/cm^3 air. This is equivalent to the ratio of the air-phase to soil-phase concentration, or (mg/cm^3 air)/(mg/g soil). The estimation of K_{as} requires the knowledge of Henry's Law constant and the soil-water partition coefficient as given by Eq. (5).

A listing of solubilities of each chlorinated biphenyl is shown in Table 5 (U.S. EPA, 1976c).

There has been much speculation as to the possible role of photochemical reaction in the environmental decay of PCBs. The results of a study using mercury vapor (UV) sources (U.S. EPA, 1976c) have been difficult to extrapolate

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to environmental conditions because the radiation wavelength is not within the spectrum of solar radiation at the surface of the earth. More recent experiments have been reported using a light source more closely approximating the spectral distribution of solar radiation, but the values for the photochemical reaction constants are not available.

The Monsanto Chemical Company has reported vapor pressure data only for high-temperature conditions for Aroclors 1242, 1248, 1254, and 1260 (Monsanto Chemical Co., undated). The temperature used in presenting the data ranges from 150°C to 300°C. These vapor pressure values may be extrapolated to the temperatures which are of common environmental concern, but their accuracies would be doubtful.

An EPA report presents kinetic data obtained from biodegradation experiments using water-soluble Aroclor 1242 (U.S. EPA, 1980a). The rate constants are presented for biphenyl compounds with up to the three chlorine substitutions present in Aroclor 1242. The data clearly show that many of the chlorinated biphenyls with four chlorine substitutions do not biodegrade after 48 hours of degradation run. Inferring from the compositions of Aroclors 1242 and 1254 as given in Table 3, it is conceivable that Aroclor 1242 may biodegrade to some extent because it contains a substantial amount of chlorinated biphenyls with two and three chlorine substitutions. It appears that biodegradation of Aroclor 1254 would be insignificant or may not occur because most biphenyl components have four or more substituted chlorine atoms.

PCBs have several properties which make them toxic in the environment. In addition, they can significantly bioaccumulate and concentrate in the fatty tissues of all organisms. For example, the PCB concentration in resident fish is often many times higher than that in the surrounding water. PCBs are chemically stable compounds that are able to persist in the environment for long

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TABLE 5. SOLUBILITY OF CHLOROBIPHENYLS IN WATER

Compound	Solubility mg/L (ppm)
Monochlorobiphenyls	
2-	5.9
3-	3.5
4-	1.19
Dichlorobiphenyls	
2,4-	1.40
2,2'-	1.50
2,4'-	1.88
4,4'-	0.08
Trichlorobiphenyls	
2,4,4'-	0.085
2',3,4-	0.078
Tetrachlorobiphenyls	
2,2',5,5'-	0.046
2,2',3,3'-	0.034
2,2',3,5'-	0.170
2,2',4,4'-	0.068
2,3',4,4'-	0.058
2,3',4',5-	0.041
3,3',4,4'-	0.175
Pentachlorobiphenyls	
2,2',3,4,5'-	0.022
2,2',4,5,5'-	0.031
Hexachlorobiphenyl	
2,2',4,4',5,5'-	0.0088
Octachlorobiphenyl	
2,2',3,3',4,4',5,5'-	0.0070
Decachlorobiphenyl	0.015
4,4'-Dichlorobiphenyl	
+Tween 80 0.1%	5.9
+Tween 80 1%	>10.0
+Humic acid extract	0.07

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periods. Impurities in commercial PCBs could amplify the PCB problem because of their similarity in chemical structure and toxicity (Monsanto Chemical Co., undated).

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8. ENVIRONMENTAL DISTRIBUTION

The release of PCBs into the environment through disposal on or in land, and through effluent discharges into waterways, together with their high attenuation characteristics and long half-life, has resulted in detectable levels in ambient air, soil, rivers, sediments, and in tissues of fish, wildlife, cattle, poultry, and a large portion of the human population. Measurable amounts of PCBs have been found in Antarctic ice, showing that atmospheric transport over long distances does occur (U.S. EPA, 1976b). Monitoring shows that the soils in the rural and urban areas where there is no record of PCB disposal or contamination, contain detectable amounts of PCBs (U.S. EPA, 1976c). One study estimates that 70% of the PCB load to Lake Michigan is through atmospheric transport (University of Wisconsin, 1980).

The results of soil sampling show that PCBs are more prevalent in urban soil than in agricultural soil. Data indicate that PCBs are rarely detected in agricultural soil, while urban soils showed PCB contaminations up to about 12 µg/g soil, with averages ranging from 0.01 to 0.21 µg/g (U.S. EPA, 1976c; Carey, undated). Sixty-three percent of the soil samples showed detectable PCB levels. The most prevalent PCBs in soil were Aroclor 1254, and, to a lesser extent, Aroclor 1260.

The PCB concentrations in air samples over Lake Michigan taken during 1977 (University of Wisconsin, 1980) were lower than in those taken in the urban portion of Milwaukee. The main components were identified as Aroclors 1242 and 1254, while the particulate-phase PCBs contained Aroclor 1260 in some instances. The average concentration of PCBs in the air over Lake Michigan was 0.87 ng/m^3 (0.44 to 1.33 ng/m^3). The concentrations of PCBs in the particulate samples were similar to those in the air samples. Air samples taken in later years

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from Lake Michigan showed an average concentration of 1 ng/m^3. These concentrations are lower than those reported in the ambient air in the continental United States.

The ambient air concentrations of PCBs for urban Chicago averaged 7.7 ng/m^3. The average composition in the air sampled was 86% Aroclor 1242, 13% 1254, and 1% 1260. The particulate-phase PCBs had a slightly different composition for the same Aroclors. The ambient air in Milwaukee showed an average PCB concentration of 2.25 ng/m^3.

MONS 027010

9. ENVIRONMENTAL FATE AND TRANSPORT

PCBs have been found in samples of air, water, soil, sediments, fish, birds, and mammals (including humans) all over the world (U.S. EPA, 1980a). Once released into the environment, PCBs persist and collect in animal tissues. Environmental problems caused by PCBs were largely unreported until 1966, when PCB contamination of fish, eagles, and humans was detected. The best-documented incident concerning the effects of ingested PCBs on humans is the case that occurred in Yusho, Japan, in 1968 (U.S. EPA, 1980a). Several other cases have also been reported (U.S. EPA, 1980a, U.S. EPA, 1976c).

Sediments containing PCBs have been reported in rivers, estuaries, and harbors (U.S. EPA, 1981b), in concentrations ranging from 20 to 50,000 µg/g. Leaching of PCBs could occur, although it will be constrained by the low solubility limits. Once PCBs dissolved in water enter the soil medium, it is possible that further migration will be severely retarded in view of the high soil-water partition coefficients. The retardation factors calculated from these coefficients can be used to simulate the arrival time and concentration decrease in groundwater. This will be further explained later.

Despite their low vapor pressures, PCBs can volatilize into the atmosphere. The migration of PCBs through air is considered to be one of the basic mechanisms by which the ubiquitous presence of PCBs occurs in nature (U.S. EPA, 1980a). The New York State Department of Environmental Conservation (NYSDEC) analyzed samples of PCB-contaminated air at several localities. These analyses are shown in Table 6 (NYSDEC, 1979; U.S. EPA, 1981b). Concentrations of PCBs in the ambient air as high as 300 µg/m^3 were reported at the disposal site. The average values ranged from 130 to 0.3 µg/m^3. Concentrations of suspended particulates in the air in the vicinity of dump sites were also monitored by

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TABLE 6. PCB MONITORING IN AMBIENT AIR BY NYSDEC

Site	Max. PCB conc. ($\mu g/m^3$)	Average PCB conc. ($\mu g/m^3$)
Caputo dump	300	130
Fort Miller dump	35	24
Remnant area	10	9
Moreau site	15	5.6
Buoy 212 site		0.7 (one sample)
Old Moreau Site (Summer 1979)		0.3

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NYSDEC, using high-volume air samplers (NYSDEC, 1979; U.S. EPA, 1981b). The geometric annual mean concentration of particulate matter was 36 to 63 $\mu g/m^3$, while the 24-hour averages were 71 to 144 $\mu g/m^3$. The amount of PCBs adsorbed to the collected suspended particulates was not reported.

Based on mass-transfer models, MacKay and Leinonen (1975) calculated the half-lives of PCBs present in solution in a water column of 1 m depth. Half-lives provide some indication of how fast a compound can volatilize from solution. The half-lives for Aroclor-1242, Aroclor-1248, Aroclor-1254, and Aroclor-1260 are reported to be 12.1 hr, 9.5 hr, 10.3 hr, and 10.2 hr, respectively. Half-lives will be longer when depths are greater than 1 m. The calculated half-lives are for evaporation from a calm, liquid surface. Turbulence provides exchange of contaminants between the surface layer and the bulk of the water column. This exchange results in increased emission rates, thus providing shorter half-lives.

In addition to the importance of attenuation mechanisms when PCBs interact with soil, biodegradation is also suggested as a potentially important mechanism. Biodegradation studies using pure and mixed microbial cultures, and the resulting metabolic changes in PCB compounds, have been summarized by Hutzinger et al. (1974) and Hwang (1982). Photochemical degradation of PCBs in the atmosphere is also of interest, since a number of pesticide compounds have been shown to break down through the photochemical route. However, very little information is available in the literature to determine the extent of PCB degradation in the atmosphere.

The safe disposal and treatment of PCBs discarded after their use in electrical applications is a matter of great concern with regard to human health. Incineration techniques are frequently applied to PCB material at elevated temperatures and high residence times. Several experiments involving

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pyrolysis of commercial PCBs have been reported (Buser and Rappe, 1979). The PCBs used in the pyrolysis experiments included tetrachlorobiphenyl, pentachlorobiphenyl, hexachlorobiphenyl, heptachlorobiphenyl, and octachlorobiphenyl. The analysis of the pyrolysis residues showed the presence of chlorinated furan compounds. However, the researchers concluded that the formation of furan compounds is the result of uncontrolled burning of PCBs, and that the emission of these compounds can be reduced by controlling the burning process.

The high-temperature combustion of PCBs, such as in the case of transformer fires, results in the formation of polychlorinated dibenzofurans (PCDFs) and other toxic compounds. In an experiment studying conditions favoring the formation of polychlorinated dibenzodioxins (PCDDs), researchers found that the optimum conditions for the formation of PCDFs are a temperature of near 675°C at a residence time of 0.8 seconds or longer, with 8% excess oxygen (Midwest Research Institute [MRI], 1984). No conditions for the formation of PCDDs are represented. The report states that detection of PCDDs was occasional and at low levels.

MONS 027014

10. TOXICOLOGY

The advisory levels for PCB cleanup presented in this document (i.e., the permissible PCB soil contamination levels) are health-based values. These advisories are derived through calculations which first estimate human risk-specific (cancer end point) or acceptable intake (AI) (noncancer end point) levels, and then determine the exposure rates which will effect these intake levels. Risk-specific doses are derived for the cancer end point, and a 10-day AI level is derived for an approximate 10- to 30-day exposure considering only noncancer effects. A detailed assessment of the available cancer and noncancer health effects data for PCBs is presented in Appendix D. Only a brief overview and major issues will be presented here.

The determination of risk-specific intake levels is accomplished through a mathematical process which makes use of a cancer potency factor and a reflected risk level or levels to estimate the intake level that would correspond to such risk levels. Cancer potency factors for PCBs have been determined through an exhaustive analysis of animal studies. Values have been calculated by ORD (EPA, 1980b) to be 4.34 $(mg/kg \cdot day)^{-1}$ and by OTS (EPA, 1985b) to be 3.57 $(mg/kg \cdot day)^{-1}$. An average of these values, or 4.0 $(mg/kg \cdot day)^{-1}$ is used in the calculations presented in this document. A discussion of the data and methods used to estimate cancer potency factors for PCBs is included in Appendix D. The determinations made by ORD and OTS are both based on the same animal study (Kimbrough et al., 1975), with only slightly different assumptions being incorporated.

A noncancer AI level was derived for PCBs during the preparation of this report. It must be emphasized that this AI ignores the cancer end point and is based on toxicity other than cancer. The 10-day AI level of 100 ug/day

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for a child and 700 µg/day for an adult, derived for use in this document, is based on feeding studies with rabbits and rats in which a NOAEL for decreased reproductive rate, and effects on thyroid and liver, were evaluated. These studies are described briefly below.

Villeneuve et al. (1971) found increased incidences of fetal death, resorptions, and abortions at 12.5 mg/kg/day of Aroclor 1254 in rabbits when exposed on days 1 through 28 of pregnancy. A dose of 1.0 mg/kg/day appeared to be without effect. Collins and Capen (1980a, b, c) in a series of studies on thyroid effects in rats, determined that 50 µg PCB per g of diet (~ 2.5 to 5.0 mg/kg/day) for 4 weeks was associated with clearly defined adverse effects, but that doses of 5 µg PCB per g of diet (~ 0.25 to 0.5 mg/kg/day) were not. Carter (1983) demonstrated liver hepatomegaly in rats at doses of 20 µg Aroclor 1254 per g of diet (~ 2 mg/kg/day) for 14 days; such an effect, in the absence of other signs of toxicity (e.g., fatty infiltration of the liver), might not be considered adverse. Grant and Phillips (1974) observed increased liver weights in rats at doses as low as 5 mg/kg/day Aroclor 1254 given in corn oil for 7 consecutive days. Collectively, these studies indicate that the experimental threshold for adverse effects of Aroclor 1254 in studies of 30 days' duration or less is at or near a dose of 1 mg/kg/day. Thus, it seems reasonable to use this latter dose, a No Adverse Effect dose, as a basis for health advisories for Aroclor 1254 for short exposure durations.

MDNS 027016

11. EXISTING STANDARDS AND GUIDELINES

The 1968 incident in which toxic symptoms were experienced by Japanese people exposed to contaminated cooking oil gave rise to a great deal of concern in the United States with regard to hazardous chemicals. The U.S. Food and Drug Administration (FDA) started sampling foods for possible contamination by PCBs in 1969, and detected levels of PCBs in fish from the Great Lakes, in milk caused by use of materials containing PCBs, and in chickens as a result of the existence of PCBs in the feed. The temporary tolerance levels for residues of PCBs proposed by FDA became effective June 29, 1979 (U.S. FDA, 1984).

In the early 1970s, EPA proposed the establishment of criteria for PCBs being discharged in industrial effluents, but has not so far issued effluent limitations concerning PCBs.

The Occupational Safety and Health Administration (OSHA) adopted standards for PCB exposure by industrial workers. Subsequently, the National Institute for Occupational Safety and Health (NIOSH), after their extensive assessment, recommended lowering the allowable concentration of PCBs in the workplace. However, OSHA has not acted on this recommendation. The New York State Department of Health issued an interim guideline for the allowable ambient air concentration of PCBs to which individuals may be exposed during the duration of a PCB reclamation project planned for the Hudson River (NYSDEC, 1979).

The EPA promulgated regulations relating to manufacture, processing, distribution in commerce, use, diposal, storage, and markings of PCBs and PCB items. The regulations originally became effective May 31, 1979, and were later amended. A complete presentation of the effective regulations can be found in the latest edition of 40 CFR Part 761 (U.S. EPA, 1984b). The PCBs referred to in these regulations include any chemical substances or their

MONS 027017

mixtures containing concentrations of chlorinated biphenyls of 50 ppm or greater. The regulations pertain to prohibitions on manufacturing, processing, distribution in commerce, and use, and specifically apply to PCB incinerators, chemical waste landfills disposing of PCBs, transformers, pigments, electrical and heat transfer equipment, natural gas pipeline compressors, microscopy mounting medium, capacitors, PCB containers, and hydraulic systems (U.S. EPA, 1984b).

The PCB standards and guidelines for numerical limitations of PCBs in food, drinking water, and ambient air existing at the present time are shown in Table 7. Because of the complicated nature of the EPA's regulations promulgated under TSCA, these regulations are not presented in tabular form.

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TABLE 7. EXISTING PCB STANDARDS AND GUIDELINES

Exposure pathways	Maximum allowable PCBs
Food (FDA standard)[a]	
Milk fat and dairy products	1.5 µg/g (ppm)
Poultry	3.0 µg/g (ppm)
Eggs	0.3 µg/g (ppm)
Fish and shellfish	2.0 µg/g (ppm)
Finished animal feed	1.0 µg/g (ppm)
Drinking water (New York State)	1.0 µg/L (ppb)
Ambient air	
Populated areas (New York State guideline)[b]	1.0 $\mu g/m^3$
Workplace (OSHA standard)	500 $\mu g/m^3$
Work site (NIOSH guideline)[c]	1.0 $\mu g/m^3$

[a]U.S. FDA, 1984.
[b]New York State Department of Health, 1981.
[c]NIOSH, 1977.

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12. EXPOSURE ASSESSMENT METHODOLOGY

The presence of PCBs in environmental media poses a potential health risk to humans from the following sources of intake:

- ingesting contaminated soil
- inhaling contaminated air
- ingesting contaminated food
- drinking contaminated water
- dermal absorption of PCBs in contact with skin

Other exposure pathways affecting ecological communities, such as phytotoxicity to plants, may also need to be considered. If multiple-route exposures are possible, the concentrations allowable for a single-route exposure should be adjusted to meet the acceptable levels of acute and chronic health effect exposures from all sources of intake. The amounts of each medium subject to human intake used in this analysis are as follows: daily intake of drinking water, 2 L/day; daily inhalation of air, 20 m^3/day (U.S. EPA, 1984b).

Acute effects result from short-term or long-term intakes. Carcinogenic effects are normally treated as resulting from lifetime intakes. Safe levels of PCBs in soil corresponding to 1-day and 10-day acceptable intakes should be based on consideration for preventing acute health effects from short-term and longer-term exposures. Levels of PCBs in soil corresponding to acceptable intakes for long-term effects can be derived from the acceptable daily intake (ADI) based on long-term health studies for acute effects, or from the carcinogenic potency slope based on lifetime exposure for carcinogenic effects.

The long-term risk level for ingestion of contaminated soil over a 70-year lifetime exposure can be obtained by

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$$\text{Risk} = \sum_{t=0}^{69\ \text{yrs}} \frac{(GI)\ (SM)\ Co\ e^{-kt}\ (POT)\ (IR)}{(BW)\ (LT)} \qquad (6)$$

where Risk = lifetime risk; GI = gastrointestinal tract absorption of PCBs; SM = exposure frequency over a lifetime; Co = initial concentration of PCBs in soil; k = biodegradation constant (1/day); POT = potency slope factor $(mg/kg/day)^{-1}$ for PCBs; IR = daily ingestion rate of soil; BW = body weight (70 kg for adults and 10 kg for children); and LT = exposure time over a lifetime (70 years). If the contaminant undergoes biological or chemical degradation in soil, and follows first-order kinetics in its disappearance under isothermal conditions, the contaminant concentration will change as a function of time according to $C_o e^{-kt}$. The summation in Eq. (6) is necessary in order to add all the risks associated with the daily dosage over a lifetime. The initial concentration of PCBs in the soil is calculated at an assumed lifetime risk according to Eq. (6). A computer is convenient to use to sum all the daily risks. The soil ingestion scenario will be applicable to sites which are readily accessible, especially by children. Since the population is living on or near the site, the exposure to PCBs due to inhalation of contaminated air cannot be neglected. In order to account for the inhalation exposure in determining the allowable PCB levels in soil due to the combined routes of ingestion, inhalation and dermal absorption, the ambient air concentrations at the exposure points are needed. The concentration of PCBs in the vapor phase is the result of the volatilization of the PCBs from the contaminated soil and their dilution by winds. The dilution factor for ambient air concentrations can be defined as

$$D = C_a/C_{as} \qquad (7)$$

MONS 027021

where C_a ($\mu g/m^3$) = the ambient air concentration at an exposure location, and C_{as} ($\mu g/m^3$) = the concentration of PCBs in air at the soil surface where the emission occurs. The value of C_{as} continuously increases as the PCB concentration in soil increases, until the concentration of PCBs in the air phase corresponds to that of PCB vapor pressure. Beyond this point, a further increase in PCB concentration in soil will have a minimal effect on the volatilization rate.

Once exposure pathways are identified, exposure evaluation requires information on the levels of concentration to which a given target population may be exposed. Each pathway may require route-specific evaluation. A general method for estimating exposures for contaminated sites will be described first. The method can be simplified by eliminating those pathways that are unimportant or unrelated in the evaluation of PCB advisories. The relevant assumptions for the simplification are described below.

12.1 Estimation of Exposures for Contaminated Sites

The combined human intake of contaminants from all exposure pathways should not exceed the acceptable intake (AI, in mg/day) needed for preventing adverse effects from short-term and lifetime exposures. The intake from an individual route when soil is contaminated can be expressed quantitatively as follows:

1) Intake by soil ingestion (mg/day):

$$I_1 = (C_s)(IR \times 10^{-3})(GI)(SM)(F) \qquad (8)$$

The term $(C_s)(IR)$ in Eq. (8) represents the daily amount of a contaminant ingested resulting from soil ingestion, in μg/day, because C_s repre-

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sents the contaminant concentration in soil (μg/g) or ppm (both units are equivalent), and IR is the soil ingestion rate (g/day) and GI is defined in Eq. (6). The factor 10^{-3} is needed to convert the unit from μg/day to mg/day. The fraction of the ingested contaminants that will enter human organs and systems to cause toxicity is given as GI. An individual may not always be present on the contaminated site over his lifetime. The frequency factor of exposure over a lifetime, SM, represents the fraction of a lifetime that an individual will be exposed to the contaminants under consideration. The factor F is necessary because soil ingestion only occurs during childhood (1 to 5 years of age), and the weight of the human body changes from childhood to adulthood.

ii) Intake by air inhalation of volatilized contaminants (mg/day):

$$I_2 = (K_{as})(C_s)(D)(IH \times 10^3)(ABA)(SM) \qquad (9)$$

In Eq. (9), K_{as}, D, and IH represent the soil-air partition coefficient, given by Eq. (6), g soil/cm^3; the extent of dilution as given by Eq. (7); and the average daily inhalation rate of ambient air (m^3/day), respectively. The term $(K_{as})(C_s)(D)(10^3)$ represents the ambient air concentration of a pollutant at the exposure location in mg/m^3 when K_{as} and C_s are given in g soil/cm^3 and mg/kg, respectively.

The determination of the ambient air concentration at an exposure location, $(K_{as})(C_s)(D)(10^3)$, or the dilution factor in the term, requires the estimation of transient emission rate, and the use of dispersion modeling. The emission rate will not only be transient, but it will also be retarded by soil or equivalent cover material. This phase of the problem requires solution of a

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partial differential equation, as described in the Section 16.

If the concentration of PCBs in soil is at or above saturation conditions, under which the air phase concentration of PCBs is equal to the vapor pressure concentration for a particular Aroclor or a mixture of Aroclors, the further increase in C_s will not increase the ambient air concentration, assuming that other factors, such as temperature, remain constant. Therefore, the daily intake by inhalation remains constant above the concentration of PCBs in soil providing saturated air concentration. This concentration of PCBs in soil, or the saturated concentration in soil for air inhalation, will be denoted by C_{sm}.

iii) Intake by dermal absorption (mg/day):

$$I_3 = (C_s)(CR \times 10^{-3})(ABS)(SM) \qquad (10)$$

There are many occasions when children playing in the yard or adults working in the garden will come in direct contact with contaminated soil. Dermal contact does not necessarily constitute adverse exposure. The contaminant needs to be systemic to be absorbed into the human body and to exert toxicity. In Eq. (10), the term $(C_s)(CR)$ represents the contaminant contact rate with skin in µg/day since C_s is in µg/g (=ppm) and CR is the dermal contact rate of soil in g/day. The factor 10^{-3} is used to convert the contact rate from µg/day to mg/day, and SM will be 1 when the short- or longer-term (10-day) exposure is estimated, and will be between 0 and 1 when the lifetime exposure is estimated.

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iv) Intake by drinking water (mg/day):

$$I_4 = (C_W)(IW)(SM) \tag{11}$$

In Eq. (11) it is assumed that the contaminant in drinking water is completely absorbed into the human body at the average daily water consumption rate of IW or the absorption fraction is 1. In order to relate the contaminant concentration in groundwater, C_W, to the contaminant concentration in soil, C_S, a fate and transport model can be used to estimate the concentration in the leachate entering groundwater, or

$$C_W = C_L/f_g, \text{ mg/L} \tag{12}$$

where f_g represents a functional relationship describing contaminant transport in groundwater. This function should be selected to suit the most appropriate conditions for the system. The leachate concentration, C_L, referring to the contaminant concentration in liquids just before entering groundwater, should not be confused with the contaminant concentration in groundwater, which results from mixing of the leachate with groundwater. Also, care should be exercised in using groundwater transport models, because some models will treat the leachate concentration as a boundary condition, while others require the contaminant concentration in groundwater as a boundary condition, which should be obtained by groundwater monitoring. When the units of C_W and C_L are all in mg/L, then the function f_g becomes dimensionless. Most leachate from hazardous waste land disposal sites may enter groundwater over a finite surface area, favoring area source models for simulating pollutant transport in groundwater.

There is no reliable method of predicting the leachate concentration from the contaminant concentration in soil, or vice versa. For the exposure evaluation, an equilibrium relationship vetween soil and leachate will provide a first approximation. Monitoring data can also be used relating the concentrations between leachate and soil. An equilibrium condition can be written as

$$C_s = (K_{LS})(C_L), \text{ mg/kg} \tag{13}$$

where K_{LS} is a partition coefficient in (mg/kg)/(mg/L). Eqs. (11), (12), and (13) are combined to get

$$I_4 = \frac{C_s}{(r_g)(K_{LS})}(IW)(SM) \tag{14}$$

When the equilibrium condition is not appropriate, it can be modified to include transport processes between the soil and leachate.

v) Intake by fish ingestion (mg/day):

At the average daily fish consumption rate of IF (kg/day), and under the assumption of complete absorption of the contaminant associated with the consumption of fish, the exposure can be estimated as

$$I_5 = (C_F)(IF)(SM) \tag{15}$$

where C_F is the contaminant concentration in fish. The use of the bioconcentration factor BCF, (mg/kg fish)/(mg/L water), to relate pollutant concentrations in fish and water, gives

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$$I_5 = (BCF)(C_w)(IF)(SM) \quad (16)$$

where it is assumed that contaminants are present in water in dissolved form and that bottom sediments or benthal deposits on which pollutants may be adsorbed are not directly swallowed by fish. Under the condition of equilibrium between the pollutant-containing soil and leachate which is generated from the soil, substitution of Eqs. (12) and (13) into Eq. (14) results in

$$I_5 = \frac{C_s}{(f_g)(K_{LS})}(BCF)(IF)(SM) \quad (17)$$

The transport functions, f_g, in Eqs. (14) and (17) may assume distinct mathematical descriptions, because one pertains to transport in groundwater and the other to that in surface water.

vi) Intake by inhalation of contaminants adsorbed on particulates (mg/day) may be expressed by

$$I_6 = (C_p)(IH)(C_s \times 10^{-9})(ABP)(SM) \quad (18)$$

Contaminant-containing soil can be airborne by blowing winds. In addition, toxic substances volatilized from contaminated soil can be adsorbed on particulate matter present in the ambient air. Exposure to contaminants occurs because of inhalation of air containing these particulates. The exposure location could be distant from the source of emission, or in the vicinity of the emission source. Exposure concentrations will change accordingly. Another form of exposure relates to inhalation of air con-

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taining particulate matter on which volatile constituents are adsorbed. The intake rate can be estimated based on the concentration of contaminants in wind-dispersed soil or on particulate matter, C_s µg/g (=ppm), and the concentration of the particulates in the ambient air, C_p µg/m^3, as shown in Eq. (18). The absorption fraction, ABP, 's used because contaminants present in or on soil (or particulate matter) may be bound on the solid material, reducing the contaminant's absorption rate. Finally, the factor 10^{-9} is a conversion factor to make the units consistent.

vii) Intake by ingestion of vegetables (mg/day):

The intake rate due to ingesting IV kg/day of vegetables, plants, or agricultural products containing C_v mg/L of contaminants will be

$$I_7 = (C_v)(IV)(SM) \tag{19}$$

If it is assumed that equilibrium is established between the contaminant concentrations in plant and soil, then the exposure can be modified as:

$$I_7 = (K_{sv})(C_s)(IV)(SM) \tag{20}$$

where K_{sv} is a partition coefficient defined as contaminant concentration in plant/total contaminant concentration in soil (mg/kg plant)/(mg/kg soil).

viii) Intake by ingestion of food meat:

The contaminant intake at consumption rate of IM (kg/day) of meat containing C_m (mg/kg) of pollutant is

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$$I_8 = (C_m)(IM)(SM) \tag{21}$$

Here again, an equilibrium relationship is assumed between the contaminant concentrations in the animal body and plants. Therefore, the intake rate due to meat consumption is

$$\begin{aligned} I_8 &= (K_{vm})(C_v)(IM)(SM) \\ &= (K_{vm})(K_{sv})(C_s)(IM)(SM) \end{aligned} \tag{22}$$

where K_{vm} and K_{sv} are the partition coefficients used to describe pollutant distribution between meat and vegetables, and the partition between vegetables and soil, respectively.

12.2 Determination of Permissible Pollutant Levels in Soil

The total intake from all possible exposure pathways is set equal to the acceptable intake (AI) for short-term and chronic health effects; or

$$AI = I_1 + I_2 + I_3 + \cdots \tag{23}$$

Eq. (23) can be solved for permissible contaminant levels in soil corresponding to each acceptable intake. It is possible that some exposure pathways occur independently of others. For example, a residence which is located on a contaminated site may use drinking water from a clean public water treatment system, and may thus be free of contaminants found on the site. It is also possible that domestic animals are not raised for food consumption on the contaminated site under consideration. Under such circumstances, all exposure pathways need not be considered. If exposure pathways of significant concern are related to soil ingestion, inhalation of contaminated air, or

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dermal contact with soil, as is the case for development of PCB advisories, Eqs. (8), (9), and (10) can be added to solve for C_s,

$$C_s = \frac{(AI)(1000)}{[(IR)(GI)(F) + (K_{as})(D)(IH)(ABA \times 10^6) + (CR)(ABS)]SM} \quad (24)$$

The emission rate is limited by the air phase concentration in equilibrium with the contaminant concentration in soil. Once the contaminant soil concentration reaches the level at which the vapor phase concentration in equilibrium with the soil is at the vapor pressure concentration, a further increase in contaminant concentration in soil ($C_s > C_{sm}$) does not increase the emission rate. At or above this concentration, the ambient air concentration remains constant regardless of the concentration of the contaminant in soil. Under such conditions, C_s in Eq. (9) is no longer a variable, and therefore Eq. (24) does not apply. This situation can be remedied by considering the intakes by the individual route of exposure at a constant value of C_s [$C_s = C_{sm}$ in Eq. (9)] for inhalation exposure, and solving for C_s. The form of the equation will be slightly different from that for Eq. (24).

$$C_s = \frac{(AI)(1000) - (K_{as})(C_{sm})(D)(IH \times 10^3)(ABA)(SM)}{[(IR)(GI)(F) + (CR)(ABS)]SM} \quad (25)$$

12.3 Incorporation of Time-Varying Parameters

The body weight of a human constantly changes until maturity. The calculation of AIs from the safe dose level (SL) given in mg/kg·day requires

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an assumption of body weight. For rigorous treatment, the estimation of lifetime exposure should take into account changes in body weight. In this case, it is convenient to work with SL instead of AI for exposure calculations. For carcinogens with a potency value at POT $(mg/kg \cdot day)^{-1}$, the equivalent SL at an assumed risk level, R (such as 10^{-6}, etc.), can be obtained by

$$(SL)eq. = \frac{R}{POT}, \ mg/kg \cdot day \tag{26}$$

The risk level shown represents an upper-bound estimate. An upper-bound estimate of risk of 10^{-6}, for example, means that upon lifetime exposure to a contaminant, a person experiences an increased maximum risk of developing cancer in a probability of 1 in one million.

Snyder (1975) presented data on the change of body weight as a function of age. A regression analysis on Snyder's data for average male weight provides the following relationship.

$$BW = 3.14 + 3.52 \ (age), \ kg \text{ for age } 0 - 18 \text{ yr} \tag{27}$$

$$BW = 70, \ kg \text{ for age greater than } 18 \text{ yr} \tag{28}$$

To obtain the daily exposure averaged over an individual's lifetime, intake rates given by Eqs. (8) - (10), (10), (17), (18), (20), and (24) should be divided by the body weight, and the daily intake per unit body weight should be averaged by summing the total intake per unit body weight over the period during which exposure occurs and dividing the result by LT. For purposes of illustration, Eqs. (8) and (9) are repeated below:

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i) The average daily exposure by soil ingestion per unit body weight in mg/kg·day can be determined as

$$\frac{I_1}{BW} = \sum_{1\ day}^{25550\ days} \frac{(C_o e^{-kt})(IR \times 10^3)(GI)(SM)}{(BW)(LT)} \qquad (29)$$

Again, in Eq. (29) [also in in Eq. (30)], the contaminant present in soil is assumed to disappear by biodegradation and other reactions, according to first-order kinetics. Other processes affecting the concentration in soil are considered in the exposure analyses for individual pathways.

ii) The average daily exposure by inhalation of volatilized contaminants in mg/kg·day is calculated from

$$\frac{I_2}{BW} = \sum_{1\ day}^{25550\ days} \frac{(K_{as})(C_o e^{-kt})(D)(IH \times 10^3)(ABA)(SM)}{(BW)(LT)} \qquad (30)$$

Similar expressions can be written for other exposure pathways. For conservative contaminants, the term $C_o e^{-kt}$ in Eqs. (29) and (30) can be replaced by C_s. The total dose from all exposures should not exceed SL, or $(SL)_{eq}$.

$$SL = \frac{I_1}{BW} + \frac{I_2}{BW} + \frac{I_3}{BW} + \ldots \qquad \text{for noncarcinogenic effects} \quad (30)$$

$$(SL)_{eq.} = \frac{I_1}{BW} + \frac{I_2}{BW} + \frac{I_3}{BW} + \qquad \text{for carcinogenic effects} \quad (32)$$

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As before, Eq. (31) or (32) can be solved for the permissible concentrations in soil, C_S. From Eqs. (8) and (29), one can solve for the factor F for use in Eq. (8). The use of LT = 25550 days, and the assumption that soil ingestion occurs during ages 1 through 5 (t = 365 to 1825 days), yield F = 0.323. The factor F does not depend on the soil ingestion rate. Eqs. (8) and (29) use Eqs. (28) and (27), respectively, for BW.

12.4 PCB Advisory Evaluations

Under normal conditions, significant soil ingestion is limited to children (Lepow, 1975). Although very limited information is available on the ranges of age subject to soil ingestion, one investigation presented a case study of an adult with a history of habitual eating of garden soil, which may have been associated with a pica illness (Wedeen et al., 1978). The fraction of soil contaminant absorbed by humans is dependent upon the type of compound and its soil contaminant adsorption characteristics, and is generally smaller than that which can be expected when contaminants are present in food or drinking watder.

PCBs can be removed from surface water, leaving it suitable for drinking. Well water that comes from ground water could be a direct source of drinking water. The location of the drinking water exposure does not necessarily have to be at the site of the contamination. It is assumed that the population which may be subject to PCB contamination in drinking water is remote from the PCB-contaminated sites, and the allowable water concentration is separately calculated on the basis of not eating contaminated soil and not inhaling contaminated air in the immediate vicinity of the site. The water concentration for a single-route exposure can be calculated as

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$$C_w = \frac{AI}{2\ L/day} \quad (33)$$

where C_w = concentration of PCBs in water in mg/L, and AI = the acceptable intake for prevention of acute and carcinogenic adverse health effects, in mg/day. If fish caught in PCB-contaminated surface water are eaten, and if the same water is the source of drinking water, the allowable concentration of PCBs (C_w mg/day) should be determined as

$$C_w = \frac{AI}{2\ L/day + F \cdot BCF} \quad (34)$$

where F is the daily fish consumption, BCF is the bioconcentration factor (31,200 L/kg) (U.S. EPA, 1980b; U.S. EPA, undated). The national average of fish consumption is 0.0065 kg/day (U.S. EPA, 1984b). However, it is more appropriate to use regional values where such data are available.

The variabilities of input values needed in Eq. (24) (appropriate for PCB exposure pathways) are wide-ranging for some values, and narrow for others. The inhalation rate of air used for calculation is 20 m^3/day for both adults and children (U.S. EPA, 1985d). Soil ingestion rates used for evaluating short-term exposures are 3 and 0.6 g/day, representing conditions with and without pica, respectively (further explained in Section 15). One lifetime exposure evaluation is based on an average daily rate of 0.6 g/day multiplied by factors to correct for the changing weight of the body as a person grows from a child to an adult. This exposure is assumed to occur from age 1 to 5 years. However, the soil ingestion rate of 3 g/day is also used in long-term exposure evaluation. The absorption factors for PCBs through the gastrointestinal tract for ingested soil, through the pulmonary organs for inhaled air,

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and through the skin for contacted soil are assumed to be 0.3, 0.5, and 0.05, respectively (U.S. EPA, 1984a; U.S. EPA, 1985e). The off-site factor is assumed to be 1 for longer-term (10-day) exposure evaluations, and 0.5 for lifetime exposure evaluations, using the carcinogenic potency factor. A similar approach can be used for short-term (1-day) and lifetime noncancer exposure evaluations. However, these evaluations are not performed because of a lack of data regarding health effects.

If all intake routes, including drinking water, soil ingestion, air inhalation, dermal contact, and intake of PCBs by means of fish or other food are of relevant importance, the allowable concentration levels can also be combined in similar fashion. Since the scope of the present study pertains to site cleanup, the applicable formulas for combining concentrations are not presented, but they should be considered as the situation warrants.

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13. WATER QUALITY LIMITS

The concentration levels of PCBs in drinking water are based on single-route exposures that are estimated to result in acute and chronic toxic effects. This does not imply that bioaccumulation in aquatic organisms does not occur. The assumption pertains to absence of fish contaminated with PCBs in the diet. If other routes are of concern, the allowable concentrations in water should be redefined. The following levels of PCBs in drinking water, corresponding to 10-day AIs, can be calculated for children and adults:

- 10-day health advisory:

$$\text{Safe concentration} = \frac{1\ \text{mg/kg·day} \cdot 10\ \text{kg}}{100 \cdot 1\ \text{L/day}} = 0.1\ \text{mg/L}\ (= 100\ \text{ppb})\ \text{(child)}$$

$$\text{Safe concentration} = \frac{1\ \text{mg/kg·day} \cdot 70\ \text{kg}}{100 \cdot 2\ \text{L/day}} = 0.35\ \text{mg/L}\ (= 350\ \text{ppb})\ \text{(adult)}$$

Similarly, the concentration levels at the various upper-bound cancer risks assumed are calculated, and the results can be tabulated as follows:

Upper-bound cancer risk	Advisory level (ng/L)
10^{-4}	875
10^{-5}	87.5
10^{-6}	8.75
10^{-7}	0.9

Example chronic toxicity advisory level (at 10^{-6} maximum risk)

$$\frac{10^{-6}\ \text{risk} \cdot 70\ \text{kg}}{4(\text{mg/kg·day})^{-1} \cdot 2\ \text{L/day}} = 8.75 \times 10^{-6}\ \text{mg/L}\ (= 8.75\ \text{ng/L})$$

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As indicated previously, an Aroclor constitutes a mixture of many congeners. Since each congener compound exhibits different solubility characteristics, the applicability of these limits to individual congeners is ion question. In the absence of short-term data for non-carcinogenic effects, the 10-day health advisory may be used as the 1-day health advisory for commercial Aroclors.

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14. LEACHATE CONTAMINATION OF GROUNDWATER

Contaminated leachate will impact groundwater quality. To date, groundwater monitoring data showing major contamination of groundwater by PCBs has been rarely reported. If the contaminated site is located above an unsaturated zone, soil through which leachate has to migrate to reach groundwater will adsorb PCBs and will greatly retard PCB migration, as evidenced by the high soil-water partition coefficients. Experimental work (U.S. EPA, 1980a) has shown that the adsorption characteristics vary depending upon the type of soil used. The experimental values are comparable to the partition coefficients estimated from the values of K_{ow} (water-octanol partition coefficient) given in Table 4. PCBs entering groundwater at hazardous waste sites could also be retarded as they are carried along the flow lines.

The area-source groundwater model (Hwang, 1985) has been used to evaluate the extent of retardation and dilution of contaminants in groundwater. A typical precipitation rate has been used to estimate a leachate generation rate which is a source term in the groundwater rate and transport model. Two different values of the retardation factor covering the extreme variations of the soil-water partition coefficients were considered: R_d = 127, corresponding to K_d = 22 cm^3/g; and R_d = 5715, corresponding to K_d = 1000 cm^3/g, where K_d represents the soil-water partition coefficient, and R_d is the retardation factor ($R_d = 1 + \frac{P_b}{e} K_d$, P_b = bulk density, e = porosity). The results of modeling show that when the concentration of PCBs in leachate is maintained at 0.12 mg/L, the vertically averaged PCB concentration in groundwater at 1000 cm away from the center of a disposal site after two years of release is 0.5×10^{-4} mg/L and 1.9×10^{-7} mg/L for the low and high values of the retardation factor, respectively. Other parameter values used in this simulation

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were: leachate flow rate = 23.4 cm^3/s, groundwater seepage velocity = 5 x 10^{-4} cm/s, porosity of groundwater medium = 0.35, depth of the aquifer = 300 cm, size of disposal site = 0.5 acre, and the bulk density of the medium = 2 g/cm^3. The simulation was repeated for a distance of 1 km away from the site. The concentration values at that distance were very small.

The groundwater transport analysis back-calculated allowable leachate concentrations entering groundwater below a hazardous waste facility, given the maximum allowable concentrations at a compliance point. These calculations do not account for "facilitated transport" via dissolved organics, cosolvents, etc. As indicated previously, the maximum allowable concentrations were based on the allowable daily intakes designed to prevent acute and chronic health effects. For the purposes of simulation, the maximum allowable drinking water concentrations at such distances as 1000 cm and 1 km from the contaminated site can be estimated. For acute toxicity, the drinking water concentration of 0.1 mg/L is assumed; for chronic carcinogenic toxicity, the concentration of PCB in groundwater assumed was 8.7 ng/L, corresponding to a 10^{-6} lifetime risk.

The down-gradient groundwater concentration is a complex function of leachate concentration, dispersion and retardation in groundwater, initial dilution in groundwater, biodegradation (if any), and groundwater velocity. The functional relationship can be found elsewhere (Hwang, 1985), and takes the form

$$C_L = f_g\, C_W \qquad (35)$$

where C_L represents the leachate concentration corresponding to the drinking water concentration C_W at a point of interest, and f_g is a functional relationship which incorporates fate and transport of PCB in the groundwater medium.

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At the lower end of the retardation coefficient (R_d = 127), the calculated leachate concentrations under steady-state conditions are 570 mg/L and 4.3 x 10^{-3} mg/L, for acute and chronic levels, respectively, for the concentrations maintained at the distance of 1000 cm from the site.

The solubility values in Table 4 show that the 10-day acute PCB levels in leachate are above the solubility limits. The solubilities for most Aroclors are above the leachate concentration necessary to prevent the chronic effect of PCBs in groundwater. In other words, the chances of exceeding the level of PCBs in groundwater which would cause acute toxicity would be small, while leachate can enter groundwater to exceed chronic concentration levels. It should be noted that the assumptions used in the calculations are that the points of exposure are 1000 cm and that the concentrations attainable under steady-state conditions are used. For different compliance points, the allowable leachate concentrations will change. In addition, steady-state conditions assume that the transport of PCBs in groundwater occurred for a long time, so that concentrations at the exposure point are no longer changing. However, the maximum allowable leachate concentrations for other conditions of transport, and for different locations of exposure, can also be evaluated. For individual congeners, the concentration levels should be reevaluated because of the difference in chemical and physical characteristics between an individual congener and an Aroclor consisting of an array of congener mixtures.

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15. SOIL INGESTION PATHWAY

A literature search shows that there is very limited information on the rate of likely soil ingestion by children and adults which can be used in exposure assessment. The situation for which the information is derived differs from study to study. Lepow (1975) studied the mouthing behavior of ten 2- to 6-year-old children in connection with investigations into the principal cause of the excessive lead accumulation in the children. The total soil ingestion rate for a 2-year-old child based on the average amount of street dirt, house dust, and soil ingested by the child by putting his hands and fingers in his mouth, can be summed as 0.6 g of soil per day.

Wedeen et al. (1978) observed the lead concentration in blood of a black woman with a 12-year history of habitual eating of garden soil. Using the levels of blood lead concentration and the concentrations of lead in the soil analyzed, they estimated the amount of lead the subject had consumed each year from her garden soil. From this estimate, the soil ingestion rate is estimated to have been in the range of between 1.96 and 3.9 g/day, with an average value at about 3 g/day. The lead concentration in the dried garden soil is reported to be between 690 ug/g and 700 ug/g of soil.

Investigators at the Centers for Disease Control present the lifetime ingestion rate of contaminated soil according to age group (Kimbrough et al., 1984). The paper states that the data presented are "based on work done studying lead uptake from contaminated soils." However, the writers of this report were unable to locate the original experimental work or its source to cite in this evaluation. The ingestion rate is assumed to change at different ages, and is given as 0 for the age group 0 to 9 months, as 1 g/day for the age group 9 to 18 months, as 10 g/day for the age group 1.5 to 3.5 years, as 1 g/day for

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the age group 3.5 to 5 years, and as 0.1 g/day for a 5-year-old child.

The second column of Table 8 shows the lifetime carcinogenic risk posed by ingesting soil contaminated with PCBs at various concentrations. This table is prepared using Eq. (6) at the soil ingestion rate of 3 g/day for children aged 1 through 6 and an average weight of 10 kg. The values for other parameters used are SM = 0.5, GI = 0.3, and k = 0. The risk values in the second column compare with those in the third column, which are prepared using the soil ingestion rate applicable to different age groups, as suggested by the Centers for Disease Control.

TABLE 8. MAXIMUM LIFETIME RISK FOR INGESTING SOIL CONTAMINATION AT DIFFERENT PCB LEVELS

PCB level in soil (μg/g)	Lifetime risk (IR = 3)	Lifetime risk Age-dependent IR[a]
0.1	1.54×10^{-6}	1.92×10^{-6}
1	1.54×10^{-5}	1.92×10^{-5}
5	7.7×10^{-5}	9.6×10^{-5}
10	1.54×10^{-4}	1.92×10^{-4}
20	3.08×10^{-4}	3.8×10^{-4}
50	7.7×10^{-4}	9.6×10^{-4}

[a]Taken from Kimbrough et al., 1984.

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A computer program was convenient to use in carrying out the summation of daily intakes over a lifetime period. The lifetime risk represents an upper-bound estimate of the unit risk that can occur as a result of ingesting PCB-contaminated soil over a lifetime, and indicates the risk posed by a single exposure pathway; that is, soil ingestion is the sole route for PCB intakes, and other pathways, including air, water, fish are assumed to be insignificant sources of human intake of PCBs. Since the population that will be subject to soil ingestion resides in the area and must breathe the air affected by PCB emissions, the magnitude of PCB intakes by the ingestion and inhalation routes needs to be compared to determine the significant pathway. The comparision is presented in Section 18.

Similarly, in order to determine the daily health advisory levels for a single exposure pathway, the daily PCB intakes equivalent to ingesting 3 g of soil in a day at various PCB concentrations are calculated. The results are shown in Table 9.

TABLE 9. MAXIMUM DAILY PCB INTAKE BY INGESTION OF SOIL AT VARIOUS PCB CONCENTRATIONS

PCB level in soil (µg/g)	Daily PCB intake at 30% absorption (mg/day)
0.1	0.00009
1	0.0009
5	0.0045
10	0.009
20	0.018
50	0.045

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The exposure pathways for soil ingestion, air inhalation, and other routes must be evaluated. If one pathway is found to be dominant over the other, the insignificant pathway based on short-term and long-term intake rates can be ignored. If they are comparable, the concentration levels need to be adjusted to reflect the combined intake rates by using Eq. (24) or combinations of Eqs. (8) through (22).

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16. INHALATION PATHWAY

16.1. INTAKE BY AIR EXPOSURE ROUTE

Exposure to PCBs occurs at or near contaminated sites through inhalation of ambient air contaminated with PCB vapors or particulate matter on which PCBs are adsorbed. The PCB vapors emitted from contaminated soil will be diluted by the action of winds before a person inhales the ambient air. When PCBs are adsorbed on soil, the vapor pressure of the PCBs above the soil surface will be always less than the vapor pressure exerted by PCBs when they are present in pure form. In other words, the adsorption phenomena depress the vapor pressure that can exist under saturated conditions. This true vapor pressure is referred to as "partial pressure." When adsorption reaches its saturation capacity on soil, the partial pressure will be equal to the pure PCB vapor pressure.

Estimates of PCB concentrations in the ambient air impacting the population at hazardous waste sites are discussed in this section, as well as comparison with the intake rates of PCB through soil ingestion. In calculating ambient air PCB concentrations, the first task was to estimate the emission rates of PCBs from the bulk of soil contaminated at various concentrations of PCBs. The emission rate calculations can be rigorously performed by the methods summarized by Hwang (1982) for steady state conditions, and by methods presented in the Appendix for transient conditions.

Based on the inhalation rate of 20 m^3/day, and absorption rates of 50% and 30% for inhaled and ingested PCBs (U.S. EPA, 1984b), respectively, the concentrations of PCBs in inhaled air and particulates equivalent to the dosage causing acute and chronic toxic effects can be estimated. The purpose of this exercise is to evaluate the concentrations of PCBs in the air, which are compa-

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rable to the ingestion dosage. Tables 10 and 11 show this comparison for intake rates corresponding to acute and chronic effects respectively.

Table 10, for example, shows that a daily intake of 3 g of soil containing 5 μg/g of PCBs is equivalent to a daily inhalation of air containing 0.45 $\mu g/m^3$. The concentration of PCBs on particulate matter must be as high as 7,500 μg/g for the inhalation of particulates at an assumed concentration of 60 $\mu g/m^3$ to be comparable to the ingestion of soil and inhalation of air described above. This concentration is used because the concentration should not exceed the primary ambient air quality of 75 $\mu g/m^3$ for particulate matter. Since the concentrations of PCBs on soil under consideration are in the range which is less than this concentration, it can be assumed that the PCB intake by inhalation of particulate matter at contaminated sites is relatively unimportant. Similar arguments can be made for the results shown in Table 11 for long-term intakes. The equivalent air concentrations C_e shown in Tables 10 and 11 are calculated by the following formula:

$$C_e\ (\mu g/m^3) = \frac{\text{daily intake (mg/day)} \times 10^3\ \mu g/mg}{20\ m^3/\text{day}\ 0.5\ \text{(absorption factor)}} \qquad (36)$$

16.2. EMISSION EVALUATION SCENARIOS

Emission rates are estimated for four different scenarios: Case 1--as the PCBs volatilize from the initial contaminated soil column, they are depleted from the column of soil by diffusional transfer of PCBs across the soil-air interface, resulting in unsteady-state emission rates, and the layer depleted of PCBs acts as cover material retarding the volatilization rate; Case 2--the same scenario as in Case 1 except that the contaminated soil is initially covered with 25 cm of cover material; Case 3--PCBs are volatilized from the

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TABLE 10. COMPARISON OF PCB INTAKES BY INGESTION AND INHALATION ROUTES FOR ACUTE EFFECTS

PCB levels in soil (ppm)	Daily acute intake (mg/day)	Equiv. air conc. for acute ingestion (ug/m^3)	Conc. of PCBs on particulates (ug/g)
0.1	0.00009	0.009	150
1	0.0009	0.09	1,500
5	0.0045	0.45	7,500
10	0.009	0.9	15,000
20	0.0018	1.8	30,000
50	0.045	4.5	75,000

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TABLE 11. COMPARISON OF PCB INTAKES BY INGESTION AND INHALATION ROUTES FOR CARCINOGENIC EFFECTS

PCB levels in soil (μg/g)	Lifetime risk at IR = 3	Average daily intake (mg/day)	Equiv. air conc. for the risk ($\mu g/m^3$)	Conc. of PCBs on particulates (μg/g)
0.1	1.54×10^{-6}	3.86×10^{-6}	0.00039	6.4
1	1.54×10^{-5}	3.86×10^{-5}	0.0039	64.3
5	7.7×10^{-5}	1.93×10^{-4}	0.019	322
10	1.54×10^{-4}	3.86×10^{-4}	0.039	643.7
20	3.08×10^{-4}	7.72×10^{-4}	0.077	1287
50	7.7×10^{-4}	1.93×10^{-3}	0.19	3217

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surface of contaminated soil, and the PCB concentration at the surface is kept at a constant value; Case 4--the contaminated soil is covered with 25 cm of clean cover soil to retard the volatilization rate, and the concentration of PCBs at the surface is kept at a constant value.

As pointed out previously, there exists a PCB saturation point above which the air-phase PCB concentration in equilibrium with (or partitioned with) the contaminated soil remains constant, and hence the emission rate of PCBs will also remain essentially steady over time. Below this point, a concentration profile of PCBs across the contaminated soil column starting from the surface to the depth of contamination will be created as volatilization progresses. This will result in unsteady-state emission rates which will vary over the period that exposure occurs. The period considered includes 10 days for 10-day advisory, and estimated lifetime (70 years) for long-term advisory.

The concentration of PCBs in soil corresponding to the saturation point can be estimated from the knowledge of vapor pressure and air-soil partitioning. For example, since the vapor pressure of Aroclor 1254 (7.71×10^{-5} mmHg) as reported in a publication, corresponds to the saturation concentration of 1,362.7 $\mu g/m^3$, the PCB concentration in soil at the saturation point is

$$C_s = \frac{1{,}362.7\ \mu g/m^3}{[K_{as}(g\ soil/cm^3\ air) \times 10^6\ cm^3/m^3]}$$

$$= \frac{1{,}362.7\ (2.44 \times 10^{-2})(1{,}000)}{(8.37 \times 10^{-3} \times 10^6)} \approx 4\ \mu g/g$$

for an assumed value for K_d of 1,000 cm^3/g. The saturation concentration is dependent upon the value of the air-soil partition coefficient. PCB saturation

concentrations in air, and the corresponding concentrations in soil for the Aroclors considered as part of this evaluation, are tabulated in Table 12, based on the soil-water partition coefficient of 1,000 cm^3/g for highly adsorbable earth material (U.S. EPA, 1980a), which is used in calculating the air-soil partition coefficient. A similar table can be prepared at the lower end value of the soil-water partition coefficient, which is approximately 40 cm^3/g for sandy material (U.S. EPA, 1980a).

Case 3 and perhaps Case 4 may be unrealistic, because as volatilization continues, the PCB concentration in soil decreases and the surface layer, depleted of PCBs, should act as an uncontaminated layer decreasing the emission rate. But this estimate should provide upper-bound values for emission rates. Cases 3 and 4 would be applicable in real-case situations when the concentration of PCBs in soil is high enough so that the air-phase concentration in equilibrium with soil remains constant until the concentration of PCBs in soil decreases to the saturation point (C_{sm}, as defined on p. 12-5). Below the saturation point, the air-phase concentration will no longer remain constant, but will decrease in direct proportion to the soil-phase concentration. In the emission rate calculation, the partial pressure of PCBs as a result of partitioning between the air and soil phases is used. Since the vapor pressures and Henry's Law constants are different for most PCBs, some typical PCBs are used for the purpose of illustrative calculations. Table 13 summarizes the results of calculations for emission rates for soil containing 1 μg of Aroclor-1254 and Aroclor-1242 per g of soil. The values shown for Cases 1 and 2 are the averages for one day emitted after the initial contamination at the concentration. This is for illustration only because Aroclor-1248 and Aroclor-1260 are also used for emission rate calcualations. The models used for the emission rate estimation and necessary calculations are shown in Appendix A. The emis-

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TABLE 12. CONCENTRATION OF PCBS IN SOIL AT SATURATION VAPOR PRESSURE BASED ON K_d = 1000 cm^3/g

	PCB concentration in soil with saturated vapor (µg/g)	Saturated vapor concentration (µg/m^3)
Aroclor-1254	4	1362.7
Aroclor-1242	250	5823.3
Aroclor-1260	28.2	822.8
Aroclor-1248	55.3	7962.8

TABLE 13. PCB EMISSION RATES FROM 1 µg/g PCB SOIL AT DIFFERENT CONTROL LEVELS

	Emission rates (g/cm^2·s)	
Scenario	Aroclor-1254	Aroclor-1242
Case 1	1.03×10^{-11}	2.7×10^{-12}
Case 2	3.67×10^{-13}	2.8×10^{-14}
Case 3	1.13×10^{-10}	8.57×10^{-12}
Case 4	1.67×10^{-13}[a]	1.14×10^{-14}[a]

[a]The models for estimating emissions from landfills underpredict the emission rate in comparison to Case 2. The estimates for Cases 2 and 4 are based on the mathematical model (described in Appendix A) and the empirical model (Farmer et al., 1980), respectively.

sion rates at various concentration levels of PCB in soil are made for evaluating the impact of volatilization on the exposed population at various locations. Table 13 shows that the emission rate of Aroclor-1254 is different from that of Aroclor-1242 at the same soil contamination level. The table also shows that the use of cover material is very effective in reducing the emission rate. The average emission rates over a period of 10 days, or a lifetime, for Cases 1 and 2 can be similarly estimated by the rigorous mathematical formulae provided in Appendix A. As PCBs volatilize, the partial pressure of PCBs at the soil-air interface decreases, and the soil layer, depleted of PCBs, provides the barrier for mass transfer, causing the emission rates given for Cases 3 and 4 to approach values comparable to those for Cases 1 and 2, respectively. The Thibodeaux and Hwang model (1982), originally developed for land treatment facilities, provides emission rates similar to those shown for Case 1 and 2 in Table 13.

PCBs volatilized into the atmosphere from a contamination site will impact the population in the surrounding region. The concentrations of PCBs at the point of impact need to be determined in order to evaluate the significance of the air emissions compared with the soil ingestion and dermal pathways. Acute and chronic impacts are based on the daily concentrations and the concentrations averaged over an annual period. Emission rates corresponding to all four cases of maintenance levels are used to estimate the concentrations of PCBs in the ambient air at the site and at distances of 0.1 km and 1 km.

16.3. AIR DISPERSION MODELING

Dispersion modeling is used to estimate the ambient air concentrations which may be possible for daily and annual exposures. Dispersion modeling for estimating the annually averaged concentrations makes use of six stability

classes, six wind speed classes, and 16 sectors, assigning the receptor point to one of the 16 sectors. The wind rose data consisting of 6 x 6 x 16 = 576 elements are compiled by the National Climatic Center in Asheville, North Carolina. The dispersion model for the annual concentration sums the concentration contributions over the entire range of stability classes and wind speeds for each exposure location downwind of the contamination site, and can take the following form (Bruce, 1969):

$$C(X,k) = 2.03 \times 10^6 Q \sum_{i=1}^{6} \frac{1}{(\alpha_z)_{i,X}} \sum_{j=1}^{6} \frac{f_{ijk}}{U_j} \qquad (37)$$

where C(X,k), the annual concentration located in a sector k at a distance of X downwind of the site ($\mu g/m^3$); $(\alpha)_{i,X}$ = standard deviation of the plume in the z-direction (vertical direction) at distance X for stability class i, Q = emission rate, g/s, U_j = mean wind speed for wind speed class j, m/s and f_{ijk} = frequency of wind in stability class i, wind speed class j, and direction in sector k. Both X and the standard deviation have the the units of meters.

The values for the standard deviation can be found in an air pollution textbook (Wark and Warner, 1981), or can be determined by a curve-fitting equation of the form

$$(\alpha_z)_{i,X} = aX^b + d \qquad (38)$$

where a, b, and d are empirical constants varying according to stability i and distance X (Wark and Warner, 1981; Martin, 1976). The values for these constants are given in Table 14 (Martin, 1976).

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TABLE 14. VALUES OF CONSTANTS FOR STANDARD DEVIATION EXPRESSION AS A FUNCTION OF DOWNWIND DISTANCE AND STABILITY CONDITION

Stability	X < 1 km			X > 1 km		
	a	b	d	a	b	d
A	440.8	1.941	9.27	459.7	2.094	-9.6
B	106.6	1.149	3.3	108.2	1.098	2.0
C	61.0	0.911	0	61.0	0.911	0
D	33.2	0.725	-1.7	44.5	0.516	-13.0
E	22.8	0.678	-1.3	55.4	0.305	-34.0
F	14.35	0.740	-0.35	62.6	0.180	-48.6

The estimation of the on-site ambient air concentration does not require the use of air dispersion modeling presented above. The ambient air concentration is controlled by the extent of dilution before dispersion occurs downwind of the source. The dilution can be estimated from the knowledge on the rate of PCB emissions and volumetric rate of wind being mixed with PCB vapors. The method for estimating the on-site ambient air concentrations is described in detail in Appendix A.

16.4. AIR EXPOSURE EVALUATION

Table 15 summarizes the results of dilution estimation and dispersion modeling to obtain the concentration levels of PCBs in ambient air at various locations considered for emissions of PCB-1254. This table is a summary of one set of calculations for the PCB concentration of 1 ug/g in soil for each scenario. The wind speed of 10 mph is used for both one-day and annual concentration averages. The Climatic Atlas of the United States provides information

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on annual average wind speed. A default value of 10 mph represents a typical annual wind speed in the United States. A site-specific evaluation will require detailed wind rose information based on local measurements.

TABLE 15. AMBIENT PCB CONCENTRATIONS AT DIFFERENT LOCATIONS
(PCB IN SOIL = 1 µg/g, PCB-1254)

	Concentrations ($\mu g/m^3$)				
		0.1 km from site		1 km from site	
	On-site	Daily	Annual	Daily	Annual
Case 1	0.61	0.026	0.0065	0.0016	0.0004
Case 2	1.4×10^{-3}	5.9×10^{-5}	1.47×10^{-5}	3.5×10^{-6}	8.9×10^{-7}
Case 3	11	0.48	0.12	0.03	7.3×10^{-3}
Case 4	0.017	7.1×10^{-4}	1.8×10^{-4}	4.3×10^{-5}	1.1×10^{-5}

The standard deviation curve for D stability is employed in estimating the ambient air concentrations at the distances of 0.1 and 1 km from the site as shown in Table 15, since D stability is by far the most frequently occurring stability class. Although D is the most common stability, an exposure-weighted average stability should be used for site-specific evaluations. The frequency with which winds blow toward a sector of interest is assumed to be 1 for evaluating the worst-case daily concentrations, while it should be based on the most common of the standard 16 wind directions for estimation of the average annual concentration levels. The concentrations in ambient air on-site and at distances of 0.1 km and 1 km from the site are given in the table. Calculations are performed for the ambient air concentrations of PCB-1242, PCB-1248, and

PCB-1260, but these are not tabulated here. The values in the table should not be construed as representing fixed ambient air concentrations at the location and under the mode of exposure. The values are presented by way of illustration to compare the contributions to ambient air occurring given the different assumed conditions.

The concentration of PCBs in equilibrium with soil containing 1 μg/g PCB-1254, which corresponds to the partial pressure of PCBs partitioned above the soil, is 340 $\mu g/m^3$. This represents the maximum vapor concentration when PCB is emitted from the soil surface. Based on this concentration and the estimated ambient air concentrations given in Table 15, one can calculate the dilution factors of the emissions for use in Eq. (9) or (24). For example, the air dilution factor for the on-site exposure corresponding to the Case 1 emission rate would be

$$D = 0.611/340 = 0.0018$$

The values shown in Table 15 can also be used to determine the daily intakes and lifetime risk levels corresponding to breathing each ambient air level. For example, the daily intake from the exposure to the ambient air concentrations of PCB-1254 at 0.1 km from the site for the Case 1 emission rate can be based on a daily inhalation rate of 20 m^3/day of air and 50% absorption factor for inhalation.

$$\text{Daily intake (mg/day)} = C(\mu g/m^3) \cdot 20\ m^3/\text{day} \cdot (1/1000\ \text{mg}/\mu g)$$

$$= 0.026\ (20)(1/1000)(0.5) = 0.00026\ \text{mg/day}$$

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The same daily intake can be obtained by using Eq. (9):

$$\text{Daily intake} = K_{as} \cdot C_s \cdot D \cdot IH \cdot 10^{+3} (ABA)(SM)$$

$$= (8.37 \times 10^{-3}/1000)(1/2.44 \times 10^{-2})(1)(0.026/340)(20)(10^3)(0.5)(1)$$

$$= 0.00026 \text{ mg/day}$$

Similarly, the lifetime risk associated with breathing the ambient air can be calculated as follows:

$$\text{Risk} = C(\mu g/m^3) \cdot 20\ m^3/day \cdot 1/70\ kg \cdot 1/1000\ mg/\mu g\ (4)\ (mg/kg/day)^{-1}\ (0.5)(0.5)$$

where C is the ambient air concentrations shown in Table 15 and the value 4 $(mg/kg \cdot day)^{-1}$ represents the potency factor for PCBs, and an additional factor of 0.5 is the off-site factor under the assumption that a resident stays in the area 50% of the time.

A series of calculations can be performed as shown above, or the procedure shown above can be reversed to back-calculate the PCB contaminations in soil which will provide the allowable ambient air concentrations at the locations considered and at the acute and chronic effect levels.

17. DERMAL CONTACT PATHWAY

Deposition of contaminated soil, dirt, or dust on human skin can provide another pathway for human intake of PCBs. PCBs can be absorbed through skin when PCB-contaminated particulates come into contact with skin. Exposure evaluation requires an estimation of the amount of the particulates on skin, and the extent or rate of absorption. The absorption rate is dependent upon the type of chemicals. Some chemicals are readily absorbed, while others are not.

There are many factors affecting the amount of soil which can be accumulated on skin. Factors include the exposed human skin area, contact time, type of soil, soil conditions, and type of activities. For example, the amount deposited on children playing in a contaminated area may be different from that on adults working in a garden.

OHEA (U.S. EPA, 1984b) has made an estimation of the amount of soil deposition on skin based on the studies by Lepow (1975) and Roels et al. (1980). Both investigators, using adhesive tape, measured the amount of soil and dirt accumulated by children on exposed areas such as hands, palm, and fingers. The measured amount of soil ranges from 0.5 to 1.5 mg/cm^2, with an average value of 1 mg/cm^2. It should be noted that this is an average value over the surface of the exposure area, and that some parts of the body may have more accumulation of soil than others.

The area of human skin that will come in contact with soil or dirt depends upon the protective measures used during the time that such contact occurs, as well as the age group involved. The exposed surface area of an adult is estimated to range from about 900 to 2,900 cm^2. The exposed surface area of a child may be smaller in proportion to their total surface area.

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The ranges of values associated with the factors mentioned above makes it difficult to arrive at an average value for the amount of soil and dirt accumulated on soil. An assumption of an average soil deposition at 1 mg/cm^2, and exposed surface area of about 1,000 cm^2 on a daily basis, provides an average daily deposition rate of 1 g per day. The variability is such that this value may be different by a factor of as much as two.

Investigators at the Centers for Disease Control present an estimated daily deposition of soil on skin according to age group (Kimbrough et al., 1984). Their tabular presentation shows that the daily amount of soil deposited on skin is 0 for the age group 0 to 9 months; 1 g for the age group 9 to 18 months; 10 g for the age group 1.5 to 3.5 years; 1 g for the age group 3.5 to 15 years; and 100 mg at age 15 years.

18. COMPARISON OF EXPOSURES BY SOIL INGESTION, INHALATION, AND DERMAL CONTACT

Table 16 shows comparisons of PCB intake by various exposure routes. Calculations apply for PCB-1254 because the emission rate is dependent upon the soil-air partition coefficient, which is different for each PCB. The ambient air concentration is the on-site value based on the emission rate averaged over 1 day after soil is contaminated up to the surface without cover.

TABLE 16. COMPARISON OF INTAKES BY VARIOUS EXPOSURE ROUTES[a]

Exposure route	Contact rate	Absorption fraction	Daily intake (mg/day)	Lifetime intake (mg/70 yrs)
Soil ingestion	3 g/day (with pica)	0.3	9×10^{-4}	1[d]
	0.6 g/day	0.3	1.8×10^{-4}	0.2
Inhalation	20 m^3/day[b]	0.5	6.1×10^{-3}	78[e]
Dermal absorption	1 g/day	0.05	5×10^{-5}	0.64[f]
Dust inhalation	20 m^3/day[c]	0.5	6×10^{-7}	7.7×10^{-3}[e]

[a]Used for illustration: PCB-1254 at concentration of 1 µg/g in soil.
[b]On-site ambient air concentration based on 1-day average emission rate after surface contamination (no cover).
[c]Concentration of suspended particular matter: 60 µg/m^3.
[d]183 days/year for 6 years.
[e]Off-site factor = 0.5.
[f]An average exposure of 1.5 mg/cm^2 and the exposure surface area of 1000 cm^2 assumed; off-site factor = 0.5.

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Evaluation under the scenario of the use of cover, or longer-term emission averages, may change the daily intake by inhalation considerably. For example, a calculation shows that the use of 25-cm clean soil cover will reduce the daily intake by inhalation to 1.4×10^{-5} mg/day instead of 6.1×10^{-3} mg/day. On the other hand, exposures by soil ingestion, dermal absorption, and dust inhalation will be likely to decrease because clean soil is used. The ingested soil or the soil on the surface of the cover that may be accumulated on skin is initially clean. Hence, the daily intakes by pathways other than inhalation also become small. However, the concentration of PCBs in the initially clean cover material could increase as the PCBs in the air phase being emitted are adsorbed on the cover material as the liquid PCBs rise toward the surface due to capillary potential. The table suggests that soil ingestion and inhalation are two competing exposure routes for PCB intake. The dermal contact can also become a contributing route for some conditions of exposure duration. When a high concentration of particulate matter in the ambient air is prevailing, the comparison shown in Table 16 can no longer apply. Consequently, the contribution to PCB intakes by inhalation of particulate matter will increase. There are a range of other possibilities which may result in a comparison different from that shown in Table 16.

19. RESULTS

19.1. DERIVATION OF PERMISSIBLE SOIL CONTAMINATION

Determination of the permissible PCB levels in soil for intake through the combined exposure routes makes use of 1) Eq. (24) when the soil concentration is below the saturation point; and 2) the summation of Eqs. (8), (9), and (10), equated to the acceptable intake otherwise. For each Aroclor under consideration, a separate exposure evaluation can be made for the following classes of exposure location and route: 1) Exposure occurs on-site. This can be further subdivided into:. (a) sites which are readily accessible to children, and hence for which soil ingestion is a possibility, and (b) sites for which there is no possibility of soil ingestion, and hence exposure is only through inhalation; 2) sites which no population is assumed to enter within the radius of 0.1 km from the site; and 3) sites which no population is assumed to enter within the radius of 1 km from the site.

Two classes of soil ingestion rates are evaluated when exposure occurs on-site (Class 1 above). For the first class, estimates of exposure are calculated for a person with pica who consumes 3 g per day between the ages of 1-5 years. For the second class, estimates of exposure are calculated for soil ingestion of 0.6 g per day between the ages of 1-5 years. For both classes, frequency of exposure is assumed to be every other day for lifetime exposures. For a 10-day exposure, soil consumption is assumed to occur consecutively for 10 days. No soil ingestion is assumed for sites which are not accessible to population within 0.1 km or 1 km from the contaminated site. The route of exposure in these cases is by inhalation only. For lifetime inhalation exposure estimates, it is assumed that the population is exposed 50% of the time, i.e., 12 hours/day, or 6 months/year.

The emission rate of volatilized PCBs can be considerably reduced by covering the contaminated soil by low-porosity uncontaminated soil or clay material. The reduction in the emission rate will result in a decrease in ambient air concentrations of PCBs by the action of blowing winds. When PCB material is directly exposed to the atmosphere, the PCB levels in soil required to maintain the same level of exposure will be less than those expected when the PCB material is covered with low-permeability material of appropriate thickness. The cover would also serve as a deterrent to soil ingestion and direct dermal contact.

The worst-case emissions would occur when the contaminated soil is initially exposed to the atmosphere and the soil is contaminated up to the conditions exhibiting saturation vapor pressure. Models are used to estimate emission rates which can be constant or time-varying depending upon the degree of soil contamination. The constant emission rate can be assumed if the vapor phase concentration maintains its constant value at the surface of contamination. There will be a profile along the layer of soil contamination for time-varying emission rates. The models for constant and time-varying emission rates are applied with or without cover material. Calculations corresponding to Cases 1, 2, and 3 for exposure possibilities are repeated at an assumed 25-cm (10-inch) thickness of a soil cover initially free of PCB contamination.

The ambient air concentrations given in Table 15, and the resulting air dilution factors calculated, are based on an annual average wind speed of 10 mph. When wind speed is lower than this, it is possible that the daily dilution factors could be higher than the values in the calculations (less dilution). The combined soil concentration values based on Eq. (24) will be lower when the dilution factor is higher. More accurate considerations of meteorological conditions will require site-specific evaluation.

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Among many factors affecting the emission rate (including vapor pressure, soil-air partition coefficient, and Henry's law constant), the variability associated with the soil-air partition coefficient is more pronounced than any other chemical and physical properties. This is caused by the wide variation in experimental values for the soil-water partition coefficient reported in the literature (U.S. EPA, 1980a), ranging from 22 to 2,000 cm^3 water/g soil. For clay and sandy materials, the range includes about 40 to 1,000. The values of K_d higher than 1,000 are obtained from experiments using coal chars.

For each Aroclor considered, the evaluation is performed for each set of conditions. The combination of these conditions can be summarized as follows:

(1) No cover is used. This is designated as the case of "Uncovered Surface Contamination." Contaminated soil surface is left uncovered after removal action in this case.

(2) Soil cover of 25-cm thickness initially free of PCB contamination is used. This is designated as the case of "25-cm-Thick Clean Soil Cover". Contaminated soil surface is covered with 25-cm-thick soil cover. No soaking by liquid PCBs is assumed, which will reduce the effective cover thickness.

(3) On-site exposure evaluation is conducted at two different soil ingestion rates: 0.6 and 3 g/day. The higher rate is reported for an individual with the habit of eating soil (pica). For life exposures, soil is consumed every day for ages 1-5 years. For 10-day exposures, soil is consumed every day for ages 1-5 years.

(4) Inhalation rate is 20 m^3/day for both an adult and a child. Population is exposed for 50% of the time for lifetime exposure durations. Ten-day evaluations are based on a daily inhalation rate of 20 m^3/day for 10 consecutive days.

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(5) Noncancer and cancer effects are evaluated at the acceptable intakes corresponding to 10-day exposure on the first day of cleanup and after 10 days of elapsed time upon cleanup, and lifetime permissible exposures.

(6) Two extreme values of the soil-air partition coefficients are used in the evaluation. The high values of K_d (soil-water) correspond to low values of K_{as} (soil-air).

(7) Area contaminated is 45 m x 45 m or approximately 0.5 acres.

The combinations of these evaluation conditions are presented in tabular form in Table 17. The soil ingestion rates of both 3 and 0.6 g/day are used in the evaluation pertaining to the longer-term (≥10-day) intakes. The soil ingestion rate of 0.6 g/day between ages 1 and 5 is used for lifetime exposure evaluation, and this value is averaged with respect to soil ingestion and changing body weight over a lifetime. For each Aroclor, there are 120 different situations demanding different permissible levels of PCBs in soil depending upon the location, route and duration of exposure, elapsed time after site cleanup, and the type of health effects to be protected. Table 18 shows the corresponding values for K_d (soil-water partition coefficient) and K_{as} (soil-air partition coefficient) for Aroclors considered. The high values of K_d correspond to the low values of K_{as}, and vice versa.

19.2. SUMMARY OF RESULTS

The method for determining the permissible PCB levels in soil is programmed in a personal computer to avoid hand calculations. Tables 19 through 22 summarize the results of computerized calculations for the case of "Uncovered Surface Contamination," and Tables 23 through 26 are for the case of "25-cm (10-inch)-Thick Clean Soil Cover." The ranges are presented because the low and high values for the soil-air partition coefficient are used in the evaluation.

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TABLE 17. EVALUATION CONDITIONS FOR EACH AROCLOR

Location and route of exposure / Intake rates		Values for partition coefficient (K_d cm^3/g) and cover depth (L cm)									
		On-site						0.1 km from site		1 km from site	
		Soil ingestion (3 g/d) inhalation dermal		Soil ingestion (0.6 g/d) inhalation dermal		Inhalation only		Inhalation		Inhalation	
Ten-day intake, child (100 µg/d)	K_d[a]	1,000	40	1,000	40	1,000	40	1,000	40	1,000	40
	L	0[b]	25[c]	0	25	0	25	0	25	0	25
Ten-day intake, adult (700 µg/d)	K_d	1,000	40	1000	40	1,000	40	1,000	40	1,000	40
	L	0	25	0	25	0	25	0	25	0	25
10^{-7} risk (0.00175 µg/d)	K_d	1,000	40	1,000	40	1,000	40	1,000	40	1,000	40
	L	0	25	0	25	0	25	0	25	0	25
10^{-6} risk (0.0175 µg/d)	K_d	1,000	40	1,000	40	1,000	40	1,000	40	1,000	40
	L	0	25	0	25	0	25	0	25	0	25
10^{-5} risk (0.175 µg/d)	K_d	1,000	40	1,000	40	1,000	40	1,000	40	1,000	40
	L	0	25	0	25	0	25	0	25	0	25
10^{-4} risk (1.75 µg/d)	K_d	1,000	40	1,000	40	1,000	40	1,000	40	1,000	40
	L	0	25	0	25	0	25	0	25	0	25

[a] K_d = soil-water partition coefficient in units of cm^3 water/g soil (= conc. in soil/conc. in water), high values close to clays, low values close to sand.
[b] Means no cover designated as "surface contamination."
[c] Means 25 cm (10") clean soil cover applied immediately after remedial action.

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TABLE 18. LOW AND HIGH VALUES OF AIR-SOIL PARTITION COEFFICIENT USED IN THE EVALUATION

PCB type	K_d (cm^3 water/g soil)[a]		K_{as} (g soil/cm^3 air)[b]	
	High	Low	Low	High
1242	1,000	40	2.35×10^{-5}	5.87×10^{-4}
1248	1,000	40	1.44×10^{-4}	3.6×10^{-3}
1254	1,000	40	3.43×10^{-4}	8.58×10^{-3}
1260	1,000	40	2.92×10^{-4}	7.31×10^{-3}

[a] K_d is the soil-water partition coefficient and has the unit of cm^3 water/g soil which is equivalent to concentration in soil/concentration in water.
[b] K_{as} is the soil-air partition coefficient and has the unit of g soil/cm^3 air, which is equivalent to concentration in air/concentration in soil. This is calculated by (H/K_d) $(1/2.44 \times 10^{-2})$.

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TABLE 19. PERMISSIBLE PCB-1242 SOIL CONTAMINATION LEVELS (UNCOVERED SURFACE CONTAMINATION)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b] — 100 for child	700 for adult	Cancer risk specific doses (µg/day) — 0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	55-60[f]	510-690	0.008-0.01	0.08-0.1	0.8-1.0	8-13
- Soil ingestion[d], inhalation[e]	92-247	2100-2800	0.03-0.06	0.3-0.6	3.0-6.0	35-61
- Inhalation only[e]	116-vs[g]	vs	0.04-0.2	0.4-2.0	4.0-20	110-200
0.1 km from contaminated site - Inhalation only[e]	vs	vs	4.0-20	110-200	1.1×10^{4}	vs
1 km from contaminated site - Inhalation only[e]	vs[g]	vs	310-430	3.1×10^{4}	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]Ranges result in each case because 1) four PCBs (1242, 1248, 1254, 1260) are considered, each with a different vapor pressure, and 2) high and low values for soil-air partition coefficient are used in the calculations.
[g]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquids for the limit.

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TABLE 20. PERMISSIBLE PCB-1248 SOIL CONTAMINATION LEVELS (UNCOVERED SURFACE CONTAMINATION)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b] — 100 for child	700 for adult	Cancer risk specific doses (µg/day) — 0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	32-80[f]	612-710	0.01	0.1	1.0	8-10
- Soil ingestion[d], inhalation[e]	42-330	2500-2900	0.02-0.04	0.2-0.5	2.0-5.0	37-49
- Inhalation only[e]	47-vs[g]	vs	0.02-0.08	0.2-0.8	2.0-8.0	87-110
0.1 km from contaminated site - Inhalation only[e]	vs	vs	2.0-8.0	90-110	8.7×10^3	8.7×10^5-vs
1 km from contaminated site - Inhalation only[e]	vs	vs	250-270	2.4×10^4-2.5×10^4	vs	vs

[a]Short-term x 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]Ranges result in each case because 1) four PCBs (1242, 1248, 1254, 1260) are considered, each with a different vapor pressure, and 2) high and low values for soil-air partition coefficient are used in the calculations.
[g]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquids for the limit.

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TABLE 21. PERMISSIBLE PCB-1254 SOIL CONTAMINATION LEVELS
(UNCOVERED SURFACE CONTAMINATION)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b] — 100 for child	700 for adult	Cancer risk specific doses (µg/day) — 0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[g]	90-100[f]	720-730	0.009-0.01	0.09-0.1	1.0-2.0	12
- Soil ingestion[d], inhalation[g]	370-420	2980-3000	0.01-0.04	0.1-0.4	3.0-4.0	36-59
- Inhalation only[e]	vs[h]	vs	0.01-0.05	0.1-0.5	5.0-7.0	460-470
0.1 km from contaminated site - Inhalation only[e]	vs	vs	5.0-7.0	460-470	4.7×10^4	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	1.3×10^3	1.3×10^5	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]Ranges result in each case because 1) four PCBs (1242, 1248, 1254, 1260) are considered, each with a different vapor pressure, and 2) high and low values for soil-air partition coefficient are used in the calculations.
[g]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquids for the limit.

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TABLE 22. PERMISSIBLE PCB-1260 SOIL CONTAMINATION LEVELS
(UNCOVERED SURFACE CONTAMINATION)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b] — 100 for child	700 for adult	Cancer risk specific doses (µg/day) — 0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	25-87[f]	640-710	0.01	0.1	1.0	12-17
- Soil ingestion[d], inhalation[e]	61-360	2670-2900	0.01-0.04	0.1-0.4	1.0-4.0	40-48
- Inhalation only[e]	vs[g]	vs	0.01-0.06	0.1-0.6	1.0-6.0	77-91
0.1 km from contaminated site - Inhalation only[e]	vs	vs	6-220	90-2.2×10^{4}	7.7×10^{3}-vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	220-240	2.2×10^{4}	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]Ranges result in each case because 1) four PCBs (1242, 1248, 1254, 1260) are considered, each with a different vapor pressure, and 2) high and low values for soil-air partition coefficient are used in the calculations.
[g]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquids for the limit.

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TABLE 23. PERMISSIBLE PCB-1242 SOIL CONTAMINATION LEVELS
(25-cm-THICK CLEAN SOIL COVER)

Location and route of human exposure	Permissible levels (µg/g) corresponding to					
	Noncancer short-term[a] acceptable intake µg/day[b]		Cancer risk specific doses (µg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	170-200[f]	1200-1400	0.03-0.2	0.3-2.0	3-17	170-vs
- Soil ingestion[d], inhalation[e]	450-820	3100-5700	0.1-0.6	1.0-6.0	12-48	260-vs
- Inhalation only[e]	vs[g]	vs	0.9-1.0	9-vs	86-vs	vs
0.1 km from contaminated site - Inhalation only[e]	vs	vs	85-vs	vs	vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-6, with pica (consuming 3 g soil/day).
[d]Children ages 1-6, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]Ranges result in each case because 1) four PCBs (1242, 1248, 1254, 1260) are considered, each with a different vapor pressure, and 2) high and low values for soil-air partition coefficient are used in the calculations.
[g]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquids for the limit.

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TABLE 24. PERMISSIBLE PCB-1248 SOIL CONTAMINATION LEVELS
(25-cm-THICK CLEAN SOIL COVER)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b] — 100 for child	700 for adult	Cancer risk specific doses (µg/day) — 0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	160-190[f]	1100-1300	0.01-.09	0.1-1.0	1-10	26-460
- Soil ingestion[d], inhalation[e]	650-vs[g]	4500-vs	0.02-0.1	0.2-1	2.0-10	93-2,500
- Inhalation only[e]	vs	vs	0.02-0.1	0.2-1	2.0-14	1.9×10^4
0.1 km from contaminated site - Inhalation only[e]	vs	vs	2-14	1.9×10^4	vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term v 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]Ranges result in each case because 1) four PCBs (1242, 1248, 1254, 1260) are considered, each with a different vapor pressure, and 2) high and low values for soil-air partition coefficient are used in the calculations.
[g]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquids for the limit.

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TABLE 25. PERMISSIBLE PCB-1254 SOIL CONTAMINATION LEVELS
(25-cm-THICK CLEAN SOIL COVER)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b] — 100 for child	700 for adult	Cancer risk specific doses (µg/day) — 0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	140-180[f]	970-1300	0.01-0.09[f]	0.1-0.2	2.0-7.0	vs
- Soil ingestion[d], inhalation[e]	520-vs[g]	4000-vs	0.02-0.06	0.2-0.6	8.0-9.0	vs
- Inhalation only[e]	vs	vs	0.02-0.08	0.2-0.8	10-14	vs
0.1 km from contaminated site - inhalation only[e]	vs	vs	10-14	vs	vs	vs
1 km from contaminated site - inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term x 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]Ranges result in each case because 1) four PCBs (1242, 1248, 1254, 1260) are considered, each with a different vapor pressure, and 2) high and low values for soil-air partition coefficient are used in the calculations.
[g]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquids for the limit.

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TABLE 26. PERMISSIBLE PCB-1260 SOIL CONTAMINATION LEVELS
(25-cm-THICK CLEAN SOIL COVER)

Location and route of human exposure	Permissible levels (μg/g) corresponding to: Noncancer short-term[a] acceptable intake μg/day[b] — 100 for child	700 for adult	Cancer risk specific doses (μg/day) — 0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	110-184[f]	800-1300	0.01-0.02	0.1-1.0	1.0-2.0	22-360
- Soil ingestion[d], inhalation[e]	550-800	4000-5000	0.02-0.07	0.2-0.7	1.0-7.0	120-550
- Inhalation only[e]	vs[g]	vs	0.02-0.08	0.2-0.8	1.0-8	620-770
0.1 km from contaminated site - Inhalation only[e]	vs	vs	1-8	620-770	vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]Ranges result in each case because 1) four PCBs (1242, 1248, 1254, 1260) are considered, each with a different vapor pressure, and 2) high and low values for soil-air partition coefficient are used in the calculations.
[g]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquids for the limit.

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The symbol "vs" indicates that no upper-bound limit for PCB concentrations in soil can be derived from the exposure evaluation. This results mainly for two reasons. First, the emission rate cannot exceed the upper-bound value which can be expected when the air phase concentration of PCBs at the contaminated soil surface is maintained at the saturation point. The concentration at the saturation point corresponds to the vapor pressure concentration. Second, when the cover is applied, the emission rate is not only retarded, but also the concentration of PCBs in soil being ingested is controlled by the amount of PCBs adsorbed on soil in equilibrium with the air phase being emitted. Hence, the concentration of PCBs in the initially clean soil material cannot exceed the saturation point concentration. The PCB concentrations in soil corresponding to vapor saturation concentrations are 250, 55, 4, and 28 µg/g when K_d = 1000; and 10, 2.2, 0.2, and 1.1 when K_d = 40; for PCB-1242, PCB-1248, PCB-1254, and PCB-1260, respectively.

In actuality, the "no upper limit," or the level above vapor saturation, designated by vs, should be interpreted with great care. The assumptions used in the exposure evaluation are critical. They include, but are not limited to: 1) no soaking of clean cover by liquid PCBs for the thickness of 25 cm; 2) no disturbance of cover material by construction activities or children digging the ground; 3) no exposure to initial spills when the values applicable for 25-cm clean cover (Tables 23 through 26) are considered; 4) no population enters the area within the respective radius of distances from the site; and 5) the cover material is at least equivalent to soil material. The existence of free flowing PCBs liquids when placing clean soil cover material will have an effect of wetting the cover, resulting in the reduction of effective cover thickness. Hence, Assumption 1 will be tantamount to requiring the presence of no free liquids in the contaminated soil.

Since the ranges shown in these tables are dependent upon the values of the soil-air coefficient, the site-specific or contaminant-specific information will help find an appropriate level of PCBs for that particular condition. This can be done either by using the procedure outlined in the main body of the report, or can be conveniently done by looking up the values listed in the Appendix for each Aroclor at low and high values of soil-air partition coefficient.

The results in Tables 23 through 26 for each Aroclor assume that the 25-cm clean cover material is placed on top of contaminated soil. In this case, the intake rate by exposure to soil ingestion is calculated based on the estimated concentration profile existing in the cover material. This profile exists because of the establishment of the vapor-solid adsorption equilibrium between the vapors being emitted and the soil. The concentration profile, which changes as a function of time, is estimated by mathematical models. the concentration used for soil ingestion is the average concentration along the thickness of the initially clean cover material.

If the prevailing contaminants at a site are PCB-1242, for example, Table 19 can be interpreted as follows:

(1) When the site is amenable to access by children with possibilities of ingesting the contaminated soil exposed to the atmosphere, the permissible PCB concentrations levels in soil should range from 55 to 60 μg/g, and 92 to 247 μg/g for prevention of noncancer effects from 10-day exposures at soil ingestion rates of 3 g/day and 0.6 g/day, respectively.

When the site is accessible to children and the population has the potential of on-site exposures to the contaminated soil and air over a lifetime, the permissible PCB levels in soil should range from 0.008 to 0.01, 0.08 to 0.1, 0.8 to 1.0, and 8 to 13 μg/g, corresponding to the best estimate of an upper-

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bound oncogenic risk at 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4}, respectively. The specific level will be dependent upon the likely soil ingestion rate and the extent of soil-air partitioning. Because of the PCB concentration profile being established in the soil column as volatilization occurs, the PCB concentration averaged over the depth will gradually decrease over time. Hence, if the population is allowed to enter the site at some time after site cleanup, the permissible levels for preventing 10-day noncancer health effects can change. Again, the specific level will be dictated by site-specific characteristics such as the soil-air partition coefficient.

(2) If there is no possibility of population entering the contaminated site within a radius of 0.1 km from the site, the PCB levels in the soil can remain at the no theoretical upper-bound limit value (vs µg/g) without exceeding the 10-day AI upon inhalation exposure for 10 days; and at 110-200 µg/g without exceeding the average daily dose corresponding to a 10^{-6} risk for lifetime exposure. Similar interpretations can be made for the results applicable to the carcinogenic risk listed at 10^{-4}, 10^{-5}, and 10^{-7}, and to sites without affected population up to 1 km from the site.

20. LIMITATIONS OF APPLICATION

It is assumed that the 25-cm (10-inch) clean cover material used remains undisturbed in the process of human activities on the site. At times this assumption may be found arbitrary, because an opportunity could exist that would expose the contaminated soil surface in contact with the atmosphere by inadvertent disturbances of soil surfaces, construction activities, utility installation, precipitation, or children playing on the site, to name a few. In this case, additional thickness of cover material should be used, or the site should be made inaccessible to children or should be kept from any activities that would lead to disturbance of the soil surfaces. Spills on top of the clean cover will result in a situation equivalent to the surface contamination case, requiring a more stringent concentration limit in soil. In this case, the results given for the 25-cm-thick clean cover material do not apply.

The tabulated results are intended to be applicable under certain specific conditions. Under conditions similar to those used in preparing the tables, the values can be used without additional evaluations. A particular situation may warrant a site-specific evaluation which may require the use of conditions different from what has been assumed in preparing the tables. If the analysis is available to show the specific type of Aroclor contaminating the soil, the individual table should be used. If the value for the soil-air partition coefficient can be better defined, the range of the permissible PCB concentration should be further narrowed.

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DERIVATION OF MODELS FOR ESTIMATING VOLATILE EMISSIONS FROM CONTAMINATED SOIL COLUMNS UNDER TRANSIENT CONDITIONS

Because of the limited aqueous solubility and high soil affinity of PCBs, it has been assumed that these compounds move vertically in soils, primarily by diffusion in the vapor phase. If transport of PCBs is by vapor phase diffusion through interstitial spaces between soil particles, a mass balance over an infinitesimal vertical element of soil can be written as follows:

$$A\left(-D_{ei}\frac{\partial C}{\partial z}\right)_{z,t} - A\left(-D_{ei}\frac{\partial C}{\partial z}\right)_{z+\Delta z,t} = A\Delta z\frac{\partial C}{\partial t} + A\Delta z\overline{P}_s\frac{\partial C_s}{\partial t} \qquad \text{(A-1)}$$

where:

- A = cross-sectional area of interest, cm^2
- C = concentration of PCBs in the vapor phase in soil pores, g/cm^3
- C_s = concentration of PCBs in soil, g/g
- D_i = molecular diffusivity, cm^2/s
- D_{ei} = effective diffusivity, cm^2/s ($=Di \cdot E^{1/3}$)
- E = pore porosity
- $\overline{P}_s$ = bulk density of soil = true density of soil, Ps, multiplied by (1-E), g/cm^3
- t = time, seconds
- z = depth measured from the soil-air interface, cm

In Eq. (A-1), the effective diffusivity, D_{ei}, is used in place of $E^{1/3} \cdot D_i$ to account for the tortuosity effect in porous media. The use of effective diffusivity is consistent with the findings which describe emission rates of volatile chemicals from landfills and soils (Hwang, 1982; Thibodeaux, 1979; Farmer et al., 1980). The effective porosity for dry soil is used for simplicity. The effect of moisture can be incorporated in the porosity term as shown by Farmer et al. (1980).

Since changes in soil and vapor phase PCB concentrations occur slowly, it can be assumed that vapor phase concentrations and soil concentrations of PCBs

are in local equilibrium. If PCB concentrations in soil and in interstitial vapors approach equilibrium, they are related by the following equation:

$$C_s = \frac{K_d}{H} \cdot C \qquad \text{(A-2)}$$

where

K_d = soil/water partition coefficient

H = Henry's constant

Rearranging Eq. (A-1) and substituting Eq. (A-2) into the resulting relationship yields

$$D_{ei} \frac{\partial^2 C}{\partial z^2} = \left(1 + \frac{\tilde{P}_s \cdot K_d}{E \cdot H}\right) \frac{\partial C}{\partial t} \qquad \text{(A-3)}$$

or

$$\alpha \frac{\partial^2 C}{\partial z^2} = \frac{\partial C}{\partial t} \qquad \text{(A-3)}$$

where

$$\alpha = \frac{D_{ei} \cdot E}{(E + P_s \cdot [1-E] \cdot K_d/H)} \qquad \text{(A-4)}$$

α can also be defined as

$$\alpha = \frac{D_{ei}}{1 + K \cdot S} \qquad \text{(A-5)}$$

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where

$$K = \frac{K_d}{H} \cdot \rho_s$$

Eq. (A-3) can be solved to estimate PCB soil concentration, vapor phase concentration, and emission rate into air above soil for the various cases described in this report if initial and boundary conditions are specified for each of these cases.

Case 1. Surface is exposed to the atmosphere. The boundary and initial conditions are

1. I.C. $C = (H/K_d)C_{SO}$, at $t = 0$, $z > 0$
2. B.C. $C = (H/K_d)C_{SO}$, at $z = \infty$, $t > 0$
3. B.C. $C = 0$, at $z = 0$, $t > 0$

where C_{SO} is the initial concentration of PCBs in soil. The solution to Eq. (A-3) for the above initial and boundary conditions is

$$C = (H/K_d)C_{SO} \cdot \text{erf}\left(\frac{z}{2\sqrt{\alpha t}}\right) \qquad \text{(A-6)}$$

where

$$\text{erf}(n) = \text{error function} = \frac{2}{\sqrt{\pi}} \int_0^n \exp(-n^2)\, dn$$

The flux rate at the soil-air interface (N_A) can be estimated as a function of time from equation (A-6) by using the concentration gradient

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as follows:

$$N_A = -E \cdot D_{ei} \left.\frac{\partial C}{\partial z}\right|_{z=0} = \frac{E\, D_{ei}}{\sqrt{\pi \alpha t}} \cdot \frac{H}{K_d} \cdot C_{SO} \qquad (A\text{-}7)$$

The boundary conditions used here are superior to those used by Jury et al. (1983), assuming that the vapor-phase boundary layer is rate-controlling. Experiments by DuPont (1985) on emission rates from contaminated soil show that when the emission rates for volatile organics are plotted against the reciprocal of $\sqrt{t}$, a straight line is obtained. This observation is consistent with the relationships given by Eq. (A-7), and Thibodeaux and Hwang (1982). The model derived by Jury et al. (1983), based on the boundary conditions of the controlling boundary layer in the air phase, does not provide a straight-line relationship between emission rate and $1/\sqrt{t}$. For this reason, the relationship derived in this report is used for exposure evaluation.

The average flux rate, N_A, over an exposure interval, T, can be calculated using Eq. (A-7).

$$\overline{N_A} = \frac{\int_0^T N_A \, dt}{T} = \frac{2 \cdot E \cdot D_{ei}}{\sqrt{\pi \alpha T}} \frac{H}{K_d} C_{SO} \qquad (A\text{-}8)$$

or

$$\overline{N_A}(T) = 2\, N_A(T) \qquad (A\text{-}9)$$

To estimate the total average emission rate, Q, the flux rate defined in Eq. (A-9) must be multiplied by the area of soil contaminated.

$$Q = A \cdot \overline{N}_A \qquad \text{(A-10)}$$

Furthermore, while Eq. (17) in Section 16 can be used at any distance X from the site to estimate air concentrations of PCBs, it cannot be used on-site. Although at present there is no generally accepted methodology for estimating on-site concentrations from an area source, on-site PCB air concentration was estimated based on a "box model" approach, by using the equation

$$C = \frac{Q}{LS \cdot V \cdot H} \qquad \text{(A-11)}$$

where

H = mixing height = 2 m

V = average wind speed within mixing zone

= 0.5 wind speed at the mixing height = 0.5 x 4.5 meter/sec = 2.25 m/s

LS = width dimension of contaminated area perpendicular to the wind direction = $\sqrt{A}$ = 45 m

A need exists for development of a more rigorous approach to estimating on-site ambient air concentrations. Time constraints did not allow development and validation of a rigorous model.

Estimation of ingestion of contaminated soil required the calculation of an appropriate soil concentration. This concentration was calculated by determining the average concentration of PCB in soil to a depth of or 25.4 cm (10 inches) for a period of 6 years beginning at time 0. Because the error function has no closed-form solution it was approximated by

$$C_s = \frac{4\,C_{SO}}{\pi} \sum_{n=0}^{\infty} \frac{e^{-\frac{\alpha(2n+1)^2\pi^2 t}{L^2}}}{2n+1} \sin\left(\frac{(2n+1)\pi}{2}\frac{z}{L}\right) \qquad \text{(A-12)}$$

where L is a depth which was selected such that

$$C_s\,(L,t) = C_{SO}$$

for all exposure durations. In calculations reported in this report, L was set equal to 250 cm. Integrating C_s of the exposure duration t_D (5 years) and depth ℓ (25 cm) yields an equation for average PCB soil concentration, $\bar{C}_s$

$$\bar{C}_s = \frac{1}{t_D \cdot \ell} \int_0^{t_D} \int_0^{\ell} C_s\, dz = \frac{32L^3\,C_{SO}}{\pi^4 \alpha \ell t_D} \sum_{n=0}^{\infty} \left\{1-\cos\left(\frac{2n+1}{2}\pi\frac{\ell}{L}\right)\right\} \cdot \left\{\frac{1-e^{-\frac{\alpha(2n+1)^2\pi^2 t_D}{4L^2}}}{(2n+1)^4}\right\} \qquad \text{(A-13)}$$

Case 2. The contaminated surface is covered with PCB-free soil material. Let ℓ = thickness of cover, cm, and L = the depth of contamination measured from the top of cover material, cm. The initial and boundary conditions become:

1.	I.C.	$C = 0$,	$0 \leq z \leq \ell$, at $t = 0$
2.	I.C.	$C = C_0$,	$\ell < z \leq L$, at $t = 0$
3.	B.C.	$C = 0$,	$z = 0$, at $t > 0$
4.	B.C.	$\frac{\partial C}{\partial z} = 0$,	$z = L$, at $t > 0$

where C_0 is the initial concentration of PCBs in the vapor phase, which can be obtained by $C_0 = (H/K_d)C_{SO}$. Eq. (A-3) with these initial and boundary conditions can be solved using the Fourier Series technique. The solution is

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$$C = \frac{4C_0}{\pi} \sum_{n=0}^{\infty} e^{\frac{-\alpha(2n+1)^2 \pi^2 t}{4L^2}} \cdot \sin\left\{\frac{2n+1}{2}\pi \frac{z}{L}\right\} \cos\left\{\frac{2n+1}{2}\pi \frac{x}{L}\right\} \qquad \text{(A-14)}$$

The flux rate at the soil-air interface (N_A) can be estimated as a function of time from equation (A-14)

$$N_A = E \cdot D_{ei} \left.\frac{\partial C}{\partial z}\right|_{z=0} = \frac{2\, C_0\, E \cdot D_{ei}}{L} \sum_{n=0}^{\infty} e^{-\frac{\alpha (2n+1)^2 \pi^2 t}{4L^2}} \cos\left\{\frac{(2n+1)\pi x}{L}\right\} \qquad \text{(A-15)}$$

The average emission rate over a time period, T, can be obtained by integration of Eq. (A-15). The result is

$$\bar{N}_A = \frac{B(H/K_d) \cdot C_{SO} \cdot E \cdot D_{ei} \cdot L}{\alpha \pi^2 T} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \left[e^{-\frac{\alpha (2n+1)^2 \pi^2) t_1}{4 \cdot L^2}} - e^{-\frac{\alpha (2n+1)^2 \pi^2) t_2}{4 \cdot L^2}}\right] \cdot \cos\left\{\frac{(2n+1)}{2} \frac{\pi x}{L}\right\} \qquad \text{(A-16)}$$

or

$$\bar{N}_A = \frac{B(H/K_d) \cdot C_{SO} \cdot E \cdot (1+K \cdot S) \cdot D_{ei}\, L}{\pi^2 T} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \left[e^{-\frac{D_i E^{1/3} (2n+1)^2 \pi^2) t_1}{(1+K \cdot S) L^2 \cdot 4}} - e^{-\frac{D_i E^{1/3} (2n+1)^2 \pi^2) t_2}{(1+K \cdot S) \cdot L^2 \cdot 4}}\right] \cos\left\{\frac{(2n+1)}{2} \frac{\pi x}{L}\right\} \qquad \text{(A-17)}$$

The summation of terms given in Eq. (A-16) can be conveniently carried out by means of computer simulation. The time interval t_2-t_1 should be set equal to the exposure interval. In calculating exposures, the maximum average exposure was estimated. This was achieved by calculating N_A as a function of time and determining the time at which the maximum value of N_A occurred; t_1 was then set equal to this time.

It should be noted that when the value of the expression $\frac{\alpha(2n+1)^2\pi^2}{4L^2}$ in the exponential term of Eq. (A-16) is small or considerably less than 1, the average of the exponential term over a time, t, will be close to 1. In this situation, averaging of the exponential term of Eq. (A-15) by the integration formulae given by Eq. (A-16) or Eq. (A-17) may easily result in an erroneous answer because one has to evaluate very precise numbers of many decimal points for the values of the exponential term. It is more practical to numerically average Eq. (A-15) than to obtain the average value by using the integration formula given by Eq. (A-16) or Eq. (A-17):

$$\overline{N_A} = \frac{2(H/K_d)C_{SO}\cdot E\cdot D_{ei}}{L}\,\frac{1}{T}\sum_{n=0}^{\infty}\left[\int_{t_1}^{t_2} e^{-\frac{\alpha(2n+1)^2\pi^2 t}{4L^2}}\right]\,dt\cdot\cos\left\{\frac{(2n+1)\pi z}{L}\right\} \qquad \text{(A-18)}$$

The steps of the summation and the integration with respect to n and t, respectively, need to be carried out by means of a computer.

As in Case 1, Eqs. (A-10) and (A-11) are used to estimate emissions rate and on-site air concentration of PCBs. However, Eq. (A-17) or (A-18) is substituted into Eq. (A-10) as an estimate of flux rate.

Also as in Case 1, the average soil concentration used to estimate ingestion of soil must be calculated. This can be accomplished by noting that C_{SO}

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$= C_0 \cdot K_d/H$, substituting this relationship into Eq. (A-14) and integrating the resulting equation over the depth interval ℓ and over the time interval t_1 to $t_1 + t_D$. The result is

$$\bar{C}_s = \frac{1}{t_D \ell} \int_{t_1}^{t_1+t_D} \int_0^{\ell} C_s \, dz = \frac{32 L^3 C_{SO}}{\pi^4 \alpha \ell t_D} \sum_{n=0}^{\infty} \left\{1 - \cos\left(\frac{(2n+1)\pi \ell}{2L}\right)\right\} \left\{e^{\frac{-\alpha (2n+1)^2 \pi^2 t_1}{4 \cdot L^2}}\right.$$

$$\left. - e^{\frac{\sim (2n+1)^2 \pi^2 (t_1+t_D)}{4 \cdot L^2}}\right\} \frac{\cos\left(\frac{(2n+1)\pi \ell}{2L}\right)}{(2n+1)^4} \qquad \text{(A-19)}$$

where t_D = 5 years and ℓ = 25 cm.

When the initial PCB soil concentration used in estimating exposures exceeds the concentration at which the vapor pressure of PCB is achieved, a different model must be used in both Case 1 and Case 2. The vapor phase PCB concentration that can be achieved in the interstitial voids in soil is limited to the concentration corresponding to the vapor pressure. While this limits the emission rate, it should be noted that as the soil zones nearest the air-soil interface become depleted of PCB, the emission rate decreases. If PCB is present in soil concentrations that produce the vapor pressure in the vapor phase, the average emission rate may be increased because soil near the surface is depleted less rapidly.

In modeling this phenomenon it has been assumed that at any given time, the concentration profile of PCB in soil as a function of depth is a steady-state profile. As in the previous models, the concentration of PCB in the interstitial soil void space is assumed to be in equilibrium with PCB soil

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concentrations. Given these assumptions and the initial conditions that

$$C_0 = \frac{H \cdot C_{ss}}{K_d}$$

$$C_s = C_{so}$$

where

C_{ss} = PCB soil concentration at which the vapor pressure is achieved.

A mass balance can be written to determine the rate of depletion from soil. If the soil concentration profile is as defined in Figure A-1, this mass balance is

$$\frac{dz}{dt} = \frac{E \cdot D_{ef} \cdot \frac{\partial C}{\partial z}}{\frac{1}{2}\left[C_{ss}[P(1-E)+E \cdot K_d/H] + P_s(1-E)(C_{so}-C_{ss}) \right.} \qquad \text{(A-20)}$$

Because we assume that any any time the soil and vapor PCB approach their steady-state concentrations,

$$\frac{\partial C}{\partial z} = \frac{C_0}{z} \qquad \text{(A-21)}$$

substituting equation (A-21) into equation (A-20) and integrating the resulting equation over the time interval 0 to t and the corresponding depth interval 0 to z yields the result

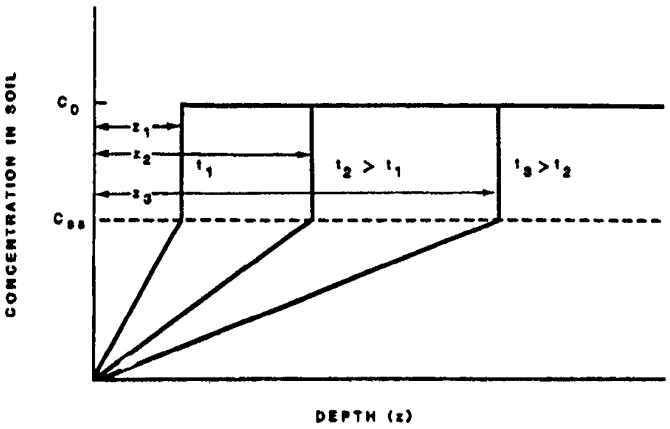

Figure A-1. Model of chemical vapor movement through soil when partial pressure is equal to vapor pressure.

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$$z = 2\sqrt{\frac{E \cdot D_{ei} \cdot C_{ss} \cdot t}{E \cdot C_{ss} + 2 \cdot P \cdot (1-E) \cdot C_{SO} \cdot K_d/H - (1-E) \cdot P \cdot C_{ss} \cdot K_d/H}} \qquad \text{(A-22)}$$

As in the previous case, the flux rate can be calculated as

$$N_A = E \cdot D_{ei} \frac{\partial C}{\partial x} = E \cdot D_{ei} \frac{C_O}{z} = \frac{E \cdot D_{ei} \cdot C_{ss} \cdot H}{z} K_d \qquad \text{(A-23)}$$

or

$$N_A = \frac{\sqrt{E \cdot D_{ei} \cdot C_{ss} + \{2 \cdot (1-E) \cdot P \cdot C_{SO} + E \cdot C_{ss} \cdot H/K_d - (1-E) \cdot P \cdot C_{ss}\}}}{2\sqrt{t \cdot K_d/H}} \qquad \text{(A-24)}$$

If the average flux is determined for the time interval T, it is easy to show that

$$\overline{N}_A(T) = 2N_A(T) \qquad \text{(A-25)}$$

As indicated previously, Eqs. (A-10) and (A-11) can be used to estimate emission rate and on-site air concentrations. However, Eq. (A-24) is substituted into Eq. (A-10) as an estimate of flux rate in this case.

The average soil concentration to a depth of 25 cm over the exposure duration of up to 5 years of exposure must be determined to estimate ingested dose of PCBs. The equation used to estimate this average depends on whether the depth z in Eq. (A-22) is less or greater than L_1 (25 cm) at the end of the ingestion exposure periods. The time T_5 at which $z = L_1$ is easily

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calculated using the following equation:

$$T_5 = 0.25 \cdot L_1^2 \cdot (E \cdot C_{ss} - 2(1-E)P \cdot C_{SO} \cdot K_d/H - P \cdot (1-E) \cdot C_{ss} \cdot K_d/H)/(E \cdot D_{e1} \cdot C_{ss}) \qquad \text{(A-26)}$$

If the ingestion exposure period, T, is less than T_5, the depth, z, will always be less than L_1, and the average soil PCB concentration, C_s, can be calculated as follows:

$$\bar{C}_S = 2 \cdot (C_{ss} - 2 \cdot C_{SO}) \{E \cdot D_{e1} \cdot C_{ss} \cdot T/[E \cdot C_{ss} + 2(1-E) \cdot P \cdot C_{SO} \cdot K_d/H - (1-E) \cdot P \cdot C_{ss} \cdot K_d/H]\}^{1/2} /3L_1 + C_{SO} \qquad \text{(A-27)}$$

If the ingestion exposure period is greater than T_5, the depth, z, will be greater than L_1 at the end of the exposure period, and the average soil PCB concentration can be calculated as follows:

$$\bar{C}_S = 2 \cdot (C_{ss} - 2 \cdot C_{SO}) \{E \cdot D_{e1} \cdot C_{ss}/[E \cdot C_{ss} + 2(1-E) \cdot P \cdot C_{SO} \cdot K_d/H - (1-E) \cdot C_{ss} \cdot P \cdot K_d/H]\}^{0.5} \cdot T_5^{1.5} /(3 \cdot L_1 \cdot T) + C_{SO} \cdot T_5/T + 2 \cdot (C_{ss} - L_1) \{[E \cdot C_{ss} + 2(1-E) \cdot P \cdot C_{SO} - (1-E) \cdot P \cdot C_{ss} \cdot K_d/H] / [E \cdot D_{e1} \cdot C_{ss}]\}^{.5} \{T^{0.5} - T_5^{0.5}\}/T \qquad \text{(A-28)}$$

When clean cover is placed over contaminated soil, a similar model can be developed as in the case where soil is contaminated to the surface. For such situations, assuming that local equilibrium between vapor and solid phases and steady-state concentration distributions at any time are attained, the following mass balance which yields relationships illustrated in Figures A-2 and A-3, which define the concentrations of PCB in soils as a function of depth

$$L_4 = \{2 \cdot L_1 \cdot (1-E) \cdot P (C_{SO} - C_{ss}) + \{4 \cdot [(1-E) \cdot L_1 \cdot (C_{SO} - C_{ss}) \cdot P]^2$$

$$+ 4 L_1^2 \cdot (E \cdot H/K_d + (1-E) \cdot P) \cdot C_{ss} \cdot [2(1-E) \cdot P \cdot (C_{SO} - C_{ss}) + E \cdot H/K_d + (1-E) \cdot P) \cdot C_{ss}]\}^{0.5}$$

$$/ \{2 \cdot [2 \cdot (1-E) \cdot P \cdot (C_{SO} - C_{ss}) + [E \cdot H/K_d + (1-E) \cdot P] \cdot C_{ss}]\} \qquad \text{(A-29)}$$

The time at which PCB reaches the air-soil interface, T_b, can be estimated by rearranging Eq. (A-22) and substituting $L_4 + L_1$ for z, as follows:

$$T_b = (L_4 + L_1)^2 \cdot \{E \cdot C_{ss} + 2 \cdot (1-E) \cdot P \cdot C_{SO} \cdot K_d/H - (1-E) \cdot P \cdot C_{ss} \cdot K_d/H\}$$

$$\cdot \{4 \cdot E \cdot D_{ei} \cdot C_{ss}\}^{-1} \qquad \text{(A-30)}$$

Integrating Eq. (A-24) over the exposure time interval T_b to $T+T_b$ and dividing the result by T yields the following expression for the average flux over the exposure period:

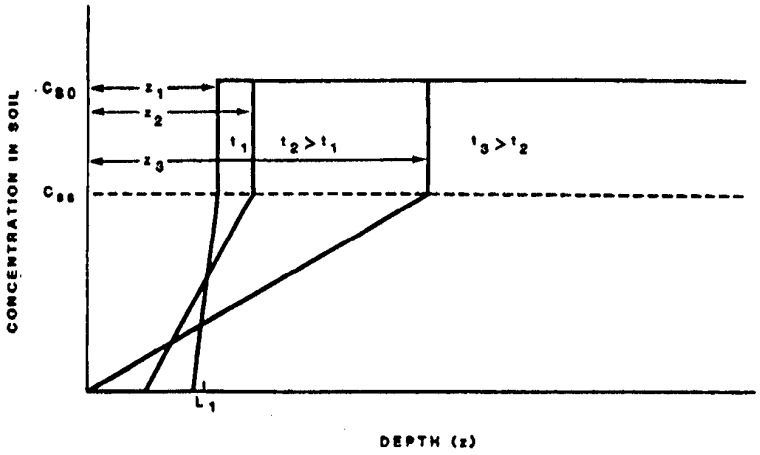

Figure A-2. Model of chemical vapor movement through soil when partial pressure is equal to vapor pressure.

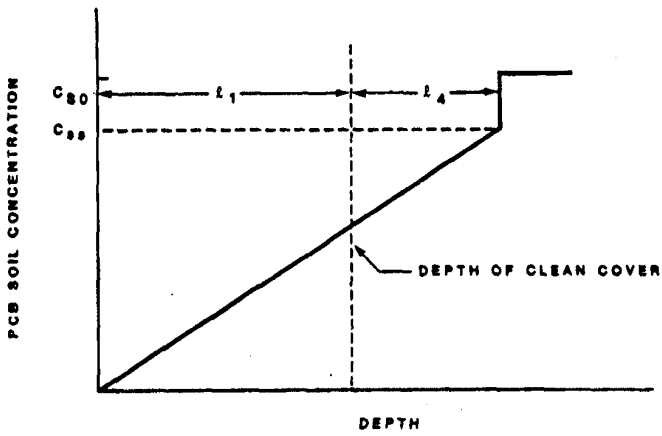

Figure A-3. Mass balance for vapor movement through soil when partial pressure is equal to vapor pressure.

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$$\bar{N}_A = \frac{1}{T}\int_{T_b}^{T_b+T} N_A\, dt =$$

$$\left\{ \frac{\sqrt{E \cdot D_{ei} \cdot C_{ss}}\,\{2 \cdot (1-E) \cdot P \cdot C_{SO} + E \cdot C_{ss} \cdot H/K_d - (1-E) \cdot P \cdot C_{ss}}{\sqrt{K_d/H}} \right\}$$

$$\left\{ \frac{(T_b+T)^{1/2} - T_b^{1/2}}{T} \right\} \qquad \text{(A-31)}$$

As before, Eqs. (A-10) and (A-11) can be used to estimate emission rates and on-site air concentrations.

Finally, the average soil concentration to a depth of 25 cm over the exposure of duration up to 5 years must be determined in order to estimate ingested dose of PCBs. The equation used to estimate this average is:

$$\bar{C}_s = C_{ss} \cdot L_1 \cdot T_5^{1/2}\{(T_5+T)^{1/2} - T_5^{1/2}\} / \{T \cdot (L_1+L_4)\} \qquad \text{(A-32)}$$

Calculation of the Depth-Averaged Concentration for Uncovered Surface

We want to find the average concentration of PCBs in soil over the exposure period. As time progresses, the concentration in soil decreases because of volatilization. First, we want to find the time when the emission rate at any time equals the average emission rate. We equate Eqs. (A-7) and (A-8). Then

$$\frac{E \cdot D_{ei}}{\sqrt{\pi \alpha t}} \cdot \frac{H}{K_d} \cdot C_{SO} = \frac{2E \cdot D_{ei}}{\sqrt{\pi \alpha T}} \cdot \frac{H}{K_d} \cdot C_{SO} \qquad \text{(A-33)}$$

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The emission rate equals the average emission rate at t = T/4, where T is the exposure time (1 day, 10 days, or 70 years), and t is any time. From Eq. (A-6), the vertically-averaged concentration over a depth of 2 cm is:

$$\bar{C} = \frac{H}{K_d} \cdot C_{SO} \frac{1}{z} \int_0^z \operatorname{erf}\left(\frac{z}{2\sqrt{\alpha t}}\right) dz \qquad \text{(A-34)}$$

or

$$\bar{C}_s = C_{SO} \frac{1}{z} \int_0^z \operatorname{erf}\left(\frac{z}{2\sqrt{\alpha t}}\right) dz \qquad \text{(A-35)}$$

The integral should be numerically evaluated at t = T/4, T being the exposure period for developing health advisories, and at an appropriate depth within which the concentration average is desired.

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APPENDIX B

EXAMPLE EMISSION RATE CALCULATIONS FOR FOUR STUDIED SCENARIOS

Example calculations for estimating volatile emissions of PCBs based on the models presented in Appendix A for unsteady-state conditions, and under steady-state conditions are presented below.

Case 1. Unsteady-state emission: no cover.

The contaminated soil is exposed to the atmosphere, and no clean soil cover is applied on top of the contaminated surface. PCB-1254 is used as an example. The average emission rate, which is obtained by averaging the instantaneous emission rate over a time period, t (sec), can be obtained by:

$$\overline{N_A} = \frac{H}{K_d}\left(\frac{g\ soil}{cm^3\ air}\right) E \frac{2\, D_{ei}\left(\frac{cm^2}{s}\right) C_{SO}\left(\frac{g}{g}\right)}{\sqrt{\pi \alpha \left[\frac{cm^2}{s}\right] t(s)}}$$

where

$$\alpha = \frac{E\, D_{ei}}{E + P_s(1-E)\frac{K_d}{H}}$$

$$= \frac{D_{ei}}{1 + S \cdot K}$$

with $S = \frac{1-E}{E}$, and $K = \frac{K_d}{H} P_s$

$$D_{ei} = 0.05\ (0.35)^{1/3} = 0.0352\ cm^2/s$$

$$\frac{H}{K_d} \text{ in } \frac{g\ soil}{cm^3\ air} = \frac{8.37 \times 10^{-3}}{1000} \times 41 = 0.000343\ \frac{mg/cm^3\ air}{mg/g\ soil}$$

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Hence:

$$\alpha = \frac{0.0123}{0.35 + 2.65(0.65)\frac{1}{0.000343}} = 2.45 \times 10^{-6} \text{ cm}^2/\text{s}$$

The average emission rate when the initial concentration in soil, $C_{S)} = 10^{-6}$ g/g (= 1 ppm) can be obtained by averaging over 10 days or 70 years:

- <u>10 day (= 86,400 sec) average</u>:

$$\bar{N}_A = 0.00034(0.35)\frac{2(0.0352)(10^{-6})}{\sqrt{3.14(2.45\text{x}10^{-6})(864{,}000)}} = 3.25 \times 10^{-12} \text{ g/cm}^2\cdot\text{s}$$

- <u>70 years (= 2.2075 x 10^9 sec) average</u>:

$$\bar{N}_A = 0.00034(0.35)\frac{2(0.0352)(10^{-6})}{\sqrt{3.14(2.45\text{x}10^{-7})(2.2075\text{x}10^{9})}} = 6.4 \times 10^{-14} \text{ g/cm}^2 \cdot \text{s}$$

Case 2. Unsteady-state emission--soil cover applied.

Clean soil is applied on top of contaminated soil surface. Emissions will occur through the cover material, and will remain unsteady below the vapor saturation point. The average emission rate under the assumption of the initial

contamination depth, L = 200 cm, can be obtained by Eq. (A-13):

$$\bar{N}_A = \frac{2(H/K_d)C_{SO}\cdot E\cdot Dei}{L} \cdot \frac{1}{t}\sum_{n=0}^{\infty}\left[\int_0^t e^{-\frac{\alpha(2n+1)^2\pi^2 t}{4L^2}}\, dt\cdot\cos\left\{\frac{(2n+1)\pi z}{L}\right\}\right]$$

For an average emission over a period of 10 days (864,000 sec)

$$\bar{N}_A = \frac{2(0.000342)(10^{-6})(0.35)(0.0352)}{(200)(864,000)}$$

$$\sum_{n=0}^{\infty}\int_0^{864,000} e^{-\frac{2.45\times10^{-6}(2n+1)^2}{4(200)^2}\pi^2 t}\, dt \cdot \cos\left\{\frac{(2n+1)\pi(25.4)}{2\ 200}\right\}$$

$$= 7.6 \times 10^{-14}\ g/cm^2\cdot s$$

Similarly, the average emission rate over a period of 70 years

$$= 5 \times 10^{-14}\ g/cm^2\cdot s$$

NOTE: The emission rate equation is programmed in a computer, and the summation is carried out using the program.

Case 3. Steady-state emission--no cover.

The same scenario assumes that the contaminated surface is exposed to the atmosphere, and its surface is maintained at the concentration of interest over the period of emission. This will apply to the case where a large reservoir of PCBs is available for emission until the concentration at the surface reaches

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the saturation value. This will be the upper-bound emission rate for contaminated soil with no clean cover applied.

PCB-1254

$$\frac{H}{K_d} \cdot C_s = \frac{0.34 \text{ g/cm}^3 \text{ air/g/cm}^3 \text{ water}}{1000 \text{ mg/g soil/mg/cm}^3 \text{ water}} C_s$$

$$= 0.00034 \frac{\text{mg/cm}^3 \text{ air}}{\text{mg/g soil}} \quad 0.001 \text{ mg/g soil}$$

$$= 3.4 \times 10^{-7} \text{ mg/cm}^3 \text{ air} = 3.4 \times 10^{-7} \times 10^{-3} \times 10^{-6} \text{ g/m}^3$$

$$= 340 \text{ µg/m}^3$$

Partitioned vapor concentration: 340 µg/m^3

Vapor pressure at 25°C = 7.71 x 10 $^{-5}$ mmHg

$$C^* = \frac{PMW}{RT} = \frac{14.7 \ (7.71 \times 10^{-5}/760) \cdot 328.4}{10.73 \ (460 + 77)} = 8.5 \times 10^{-8} \text{ \#/ft}^3$$

where F = 1.8 (25) + 32 = 77°F,
C* = saturation concentration of PCB-1254 in #/ft^3 or µg/m^3,
P = vapor pressure,
MW = molecular weight,
R = gas constant.

$$\frac{8.5 \times 10^{-8} \ (454) \times 10^6 \text{ ug}}{(0.3048)^3 \text{ m}^3} = 1362.7 \text{ µg/m}^3$$

When PCB-1254 is greater than 4 ppm in soil, the partial pressure in the air phase is equal to vapor pressure. An average temperature of 25°C is used for evaluating the saturation concentration in the vapor phase. Since the vapor

pressure is dependent upon temperature, a specific evaluation will require a temperature of interest. The temperature in the subsurface soil may not fluctuate considerably over the average value.

PCB-1242

Concentration in air above soil with 1 µg/g PCB

$$340 \times \frac{5.73\times10^{-4}}{8.37\times10^{3}} = 23.3 \text{ µg/m}^3$$

Saturated vapor concentration

$$1362.7 \frac{4.06\times10^{-4}}{7.71\times10^{-5}} \frac{266.5}{328.4} = 5823.3 \text{ µg/m}^3$$

Note: When the PCB concentration in soil = 250 µg/g = $(\frac{5823.3}{23.3})$, the vapor phase is saturated.

In order to calculate the emission rate, the values for gas-phase mass transfer coefficients are estimated using the relationship given by Hwang (1982).

$$\text{PCB-1254; } kg = 5.8\times10^{-5} \left(\frac{18}{328.4}\right)^{0.5} = 1.36\times10^{-5} \text{ g mol/cm}^2 \cdot \text{s}$$

$$\text{PCB-1242; } kg = 5.8\times10^{-5} \left(\frac{18}{266.5}\right)^{0.5} = 1.51\times10^{-5} \text{ g mol/cm}^2 \cdot \text{s}$$

Mole fractions of PCBs in the gas phase (y) when the concentrations are retained at 340 µg/m^3 and 23.3 µg/m^3 for PCB-1254 and PCB-1242, respectively, are:

PCB-1254 $y = 340\ \mu g/m^3 \cdot 0.0742\ ppb/(\mu g/m^3) \times 10^{-9} = 2.52 \times 10^{-8}$

PCB-1242 $y = 23.3\ \mu g/m^3 \cdot 0.0916\ ppb/(\mu g.m^3) \times 10^{-9} = 2.13 \times 10^{-9}$

The emission rate, Q, can be obtained from the formula $Q = MW \cdot kg \cdot y$, where MW = molecular weight (Hwang, 1982). Thus, the emission rate for each Aroclor is:

PCB-1254 $Q = 328.4\ (1.36 \times 10^{-5})(2.52 \times 10^{-8}) = 1.13 \times 10^{-10}\ g/s\ cm^2$

PCB-1242 $Q = 266.5\ (1.51 \times 10^{-5})\ (2.13 \times 10^{-9}) = 8.57 \times 10^{-12}\ g/s\ cm^2$

Case 4. Steady-state emission--cover applied.

It is assumed that the contaminated site is covered with PCB-free soil cover, and that the concentration at the top of the contaminated soil is maintained at 1 µg/g over the period of emission. The emission rate can be obtained from the formula (Hwang, 1982).

$$Q = D_1\ P_T^{4/3}\ \frac{C_1^*}{h}\ ,\ g/cm^2 \cdot s$$

where D_1 = diffusivity, cm^2/s,
P_T = total porosity,
C_1^* = true vapor pressure in equilibrium with soil, g/cm^3,
h = cover thickness, cm.

Using the diffusivity value of 0.05 cm^2/s, one can get for PCB-1254, an emission rate of

$$Q = (0.05)(0.35)^{4/3}\ \frac{340\ \mu g/m^3\ 10^{-6}\ g/\mu g \times 10^{-6}\ cm^3/m^3}{25.4\ cm}$$

$$= 1.67 \times 10^{-13}\ g/cm^2 \cdot s$$

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Similarly, for PCB-1242:

$$Q = (0.05)\ (0.35)^{4/3}\ \frac{23.3\times10^{-6}\ \times\ 10^{-6}}{25.4} = 1.14\times10^{-14}\ g/cm^2 \cdot s$$

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APPENDIX C

SUMMARY OF COMPUTER RUNS FOR EACH AROCLOR AND AT EACH VALUE OF SOIL-AIR PARTITION COEFFICIENTS

TABLE C-1. PERMISSIBLE PCB-1242 SOIL CONTAMINATION LEVELS[a]
(UNCOVERED SURFACE CONTAMINATION, K_d = 1000)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b] — 100 for child	700 for adult	Cancer risk specific doses (µg/day) — 0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[g]	55	510	0.008	0.08	0.8	8
- Soil ingestion[d], inhalation[g]	92	2100	0.06	0.6	6	61
- Inhalation only[g]	116	vs	0.2	2	20	204
0.1 km from contaminated site - Inhalation only[g]	vs[f]	vs	20	204	1.1×10^4	vs
1 km from contaminated site - Inhalation only[g]	vs	vs	428	3.1×10^4	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= $H.41/K_d = 5.73 \times 10^{-4}$ (41)/1000 = 2.35×10^{-5}).

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TABLE C-2. PERMISSIBLE PCB-1242 SOIL CONTAMINATION LEVELS[a]
(UNCOVERED SURFACE CONTAMINATION, K_d = 40)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b]		Cancer risk specific doses (µg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	60	690	.01	0.1	1.0	13
- Soil ingestion[d], inhalation[e]	247	2800	.03	0.3	3.0	35
- Inhalation only[e]	vs[f]	vs	0.04	.4	4	110
0.1 km from contaminated site - Inhalation only[e]	7	vs	4	110	1.1×10^4	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	310	3.1×10^4	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= $H.41/K_d = 5.73 \times 10^{-4}$ (41)/1000 = 2.35×10^{-5}).

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TABLE C-3. PERMISSIBLE PCB-1248 SOIL CONTAMINATION LEVELS[a]
(UNCOVERED SURFACE CONTAMINATION, K_d = 1000)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b]		Cancer risk specific doses (µg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	32	612	0.01	0.1	1	10
- Soil ingestion[d], inhalation[e]	42	2500	0.04	0.5	5	49
- Inhalation only[e]	47	vs	0.08	0.8	4	110
0.1 km from contaminated site - Inhalation only[e]	vs[f]	vs	8.0	110	8,700	8.7×10^5
1 km from contaminated site - Inhalation only[e]	vs	vs	270	2.5×10^4	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= H.41/K_d = 5.73×10^{-4} (41)/1000 = 2.35×10^{-5}).

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TABLE C-4. PERMISSIBLE PCB-1248 SOIL CONTAMINATION LEVELS[g]
(UNCOVERED SURFACE CONTAMINATION, K_d = 40)

Locatio and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b] — 100 for child	700 for adult	Cancer risk specific doses (µg/day) — 0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	80	710	0.01	0.1	1	8
- Soil ingestion[d], inhalation[e]	330	2900	0.02	0.2	2.0	37
- Inhalation only[e]	vs[f]	vs	0.02	0.2	2.0	87
0.1 km from contaminated site - Inhalation only[e]	vs	vs	2.0	90	8,700	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	250	2.5×10^4	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= H.41/K_d = 5.73×10^{-4} (41)/1000 = 2.35×10^{-5}).

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TAULE C-5. PERMISSIBLE PCB-1254 SOIL CONTAMINATION LEVELS[g]
(UNCOVERED SURFACE CONTAMINATION, K_d = 1000)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b]		Cancer risk specific doses (µg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	90	720	0.01	0.1	1	12
- Soil ingestion[d], inhalation[e]	370	2980	0.04	0.4	4	59
- Inhalation only[e]	vs[f]	vs	0.05	0.5	7	460
0.1 km from contaminated site - Inhalation only[e]	vs	vs	7	460	4.7×10^4	4.7×10^6
1 km from contaminated site - Inhalation only[e]	vs	vs	1.2×10^3	1.3×10^5	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= $H.41/K_d = 6.73 \times 10^{-4}$ (41)/1000 = 2.35×10^{-5}).

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TABLE C-6. PERMISSIBLE PCB-1254 SOIL CONTAMINATION LEVELS[g]
(UNCOVERED SURFACE CONTAMINATION, K_d = 40)

Location and route of human exposure	Permissible levels (μg/g) corresponding to: Noncancer short-term[a] acceptable intake μg/day[b]		Cancer risk specific doses (μg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	100	730	0.009	0.09	2.0	12
- Soil ingestion[d], inhalation[e]	420	3000	0.01	0.1	3	36
- Inhalation only[e]	vs[f]	vs	0.01	0.1	5	470
0.1 km from contaminated site - Inhalation only[e]	vs	vs	5	470	4.7×10^4	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	1300	1.3×10^5	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= H.41/K_d = 5.73×10^{-4} (41)/1400 = 2.35×10^{-5}).

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TABLE C-7. PERMISSIBLE PCB-1260 SOIL CONTAMINATION LEVELS[g]
(UNCOVERED SURFACE CONTAMINATION, K_d = 1000)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b]		Cancer risk specific doses (µg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	25	640	0.01	0.1	1	12
- Soil ingestion[d], inhalation[e]	61	2670	0.04	0.4	4	48
- Inhalation only[e]	vs[f]	vs	0.06	0.6	6	91
0.1 km from contaminated site - Inhalation only[e]	vs	vs	6	91	7.7×10^3	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	240	2.2×10^4	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-6, with pica (consuming 3 g soil/day).
[d]Children ages 1-6, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= H.41/K_d = 5.73×10^{-4} (41)/1000 = 2.35×10^{-5}).

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TABLE C-8. PERMISSIBLE PCB-1260 SOIL CONTAMINATION LEVELS[g]
(UNCOVERED SURFACE CONTAMINATION, K_d = 40)

Location and route of human exposure[f]	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b]		Cancer risk specific doses (µg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	87	710	0.01	0.1	1.0	17
- Soil ingestion[d], inhalation[e]	360	2900	0.01	0.1	1.0	40
- Inhalation only[e]	vs[f]	vs	0.01	0.1	1.0	77
0.1 km from contaminated site - Inhalation only[e]	vs	vs	1.0	76	7600	7.6×10^5
1 km from contaminated site - Inhalation only[e]	vs	vs	220	2.2×10^4	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 5 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposure: assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= H.41)/K_d = 5.73×10^{-4} (41)/1000 = 2.35×10^{-5}).

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TABLE C-9. PERMISSIBLE PCB-1242 SOIL CONTAMINATION LEVELS[g]
(25-cm-THICK CLEAN SOIL COVER, K_d = 1000)

Location and route of human exposure	Permissible levels (μg/g) corresponding to					
	Noncancer acceptable short-term[a] intake μg/day[b]		Cancer risk specific doses (μg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	200	1400	0.2	2	17	170
- Soil ingestion[d], inhalation[e]	820	5700	0.6	6	48	260
- Inhalation only[e]	vs[f]	vs	0.9	9	86	vs
0.1 km from contaminated site - Inhalation only[e]	vs	vs	85	vs	vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= $H.41/K_d$ = 5.73×10^{-4} (41)/1000 = 2.35×10^{-5}).

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TABLE C-10. PERMISSIBLE PCB-1242 SOIL CONTAMINATION LEVELS[a]
(25-cm-THICK CLEAN SOIL COVER, K_d = 40)

Location and route of human exposure	Permissible levels (μg/g) corresponding to					
	Noncancer short-term[a] acceptable intake μg/day[b]		Cancer risk specific doses (μg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[g]	170	1200	0.03	0.3	3.0	vs
- Soil ingestion[d], inhalation[g]	450	3100	0.1	1.0	12	vs
- Inhalation only[e]	vs[f]	vs	1.0	vs	vs	vs
0.1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.36×10^{-5} g soil/cm^3 air (= H.4i/K_d = 5.73×10^{-4} (41)/1000 = 2.35×10^{-5}).

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TABLE C-11. PERMISSIBLE PCB-1248 SOIL CONTAMINATION LEVELS[a]
(25-cm-THICK CLEAN SOIL COVER, K_d = 1000)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b] — 100 for child	700 for adult	Cancer risk specific dose (µg/day) — 0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	190	1300	0.09	1	10	26
- Soil ingestion[d], inhalation[e]	650	4500	0.1	1	10	93
- Inhalation only[e]	vs[f]	vs	0.1	1	14	19,000
0.1 km from contaminated site - Inhalation only[e]	vs	vs	14	19,000	vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= $M.W./K_d = 5.73 \times 10^{-4}$ (41)/1000 = 2.35×10^{-5}).

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TABLE C-12. PERMISSIBLE PCB-1248 SOIL CONTAMINATION LEVELS[a]
(25-cm-THICK CLEAN SOIL COVER, K_d = 40)

Location and route of human exposure	Permissible levels (μg/g) corresponding to					
	Noncancer short-term[a] acceptable intake μg/day[b]		Cancer risk specific doses (μg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	160	1100	0.01	0.1	1.0	460
- Soil ingestion[d], inhalation[e]	vs[f]	vs	0.02	0.2	2.0	2500
- Inhalation only[e]	vs	vs	0.02	0.2	2.0	1.9×10^4
0.1 km from contaminated site - Inhalation only[e]	vs	vs	2.0	1.9×10^4	vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-6, with pica (consuming 3 g soil/day).
[d]Children ages 1-6, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= $H.41/K_d$ = 5.73×10^{-4} (41)/1000 = 2.35×10^{-5}).

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TABLE C-13. PERMISSIBLE PCB-1254 SOIL CONTAMINATION LEVELS[a]
(25-cm-THICK CLEAN SOIL COVER, K_d = 1000)

Location and route of human exposure	Permissible levels (μg/g) corresponding to: Noncancer short-term[a] acceptable intake μg/day[b]		Cancer risk specific doses (μg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	180	1300	0.02	0.2	2	vs
- Soil ingestion[d], inhalation[e]	620	4000	0.06	0.6	8	vs
- Inhalation only[e]	vs[f]	vs	0.08	0.8	14	vs
0.1 km from contaminated site - Inhalation only[e]	vs	vs	14	vs	vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= $H.41/K_d = 5.73 \times 10^{-9}$ (41)/1000 = 2.35×10^{-5}).

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TABLE C-14. PERMISSIBLE PCB-1254 SOIL CONTAMINATION LEVELS[a]
(25-cm-THICK CLEAN SOIL COVER, K_d = 40)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b]		Cancer risk specific doses (µg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[g]	140	970	0.01	0.1	1	vs
- Soil ingestion[d], inhalation[g]	vs	vs	0.02	0.2	9	vs
- Inhalation only[e]	vs	vs	0.02	0.2	10	vs
0.1 km from contaminated site - Inhalation only[e]	vs	vs	10	vs	vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.36×10^{-5} g soil/cm^3 air (= H.41/K_d = 5.73×10^{-4} (41))/1000 = 2.35×10^{-5}).

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TABLE C-15. PERMISSIBLE PCB-1260 SOIL CONTAMINATION LEVELS[a]
(25-cm-THICK CLEAN SOIL COVER, K_d = 1000)

Location and route of human exposure	Permissible levels (µg/g) corresponding to: Noncancer short-term[a] acceptable intake µg/day[b]		Cancer risk specific doses (µg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	184	1300	0.02	0.2	2.0	22
- Soil ingestion[d], inhalation[e]	550	4000	0.07	0.7	7	120
- Inhalation only[e]	vs[f]	vs	0.08	0.8	8.0	770
0.1 km from contaminated site - Inhalation only[e]	vs	vs	8.0	770	vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-6, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35×10^{-5} g soil/cm^3 air (= H.41/K_d = 5.73×10^{-4} (41)/1000 = 2.35×10^{-5}).

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TABLE C-16. PERMISSIBLE PCB-1260 SOIL CONTAMINATION LEVELS[g]
(25-cm-THICK CLEAN SOIL COVER, K_d = 40)

Location and route of human exposure	Permissible levels (μg/g) corresponding to					
	Noncancer short-term[a] acceptable intake μg/day[b]		Cancer risk specific doses (μg/day)			
	100 for child	700 for adult	0.00175 (10^{-7} risk)	0.0175 (10^{-6} risk)	0.175 (10^{-5} risk)	1.75 (10^{-4} risk)
On the contaminated site						
- Soil ingestion[c], inhalation[e]	110	800	0.01	0.1	1.0	360
- Soil ingestion[d], inhalation[e]	800	5000	0.02	0.2	1.0	550
- Inhalation only[e]	vs[f]	vs	0.02	0.2	1.0	620
0.1 km from contaminated site - Inhalation only[e]	vs	vs	1.0	620	vs	vs
1 km from contaminated site - Inhalation only[e]	vs	vs	vs	vs	vs	vs

[a]Short-term = 10-day intake.
[b]Based on average weights of 10 and 70 kg for a child and an adult, respectively.
[c]Children ages 1-5, with pica (consuming 3 g soil/day).
[d]Children ages 1-5, without pica (consuming 0.6 g soil/day).
[e]Inhalation rates are assumed to be 20 m^3/day for the short-term and longer-term noncancer exposures; all other (more chronic) exposures assumed to be 10 m^3/day as a result of 182 days exposure per year.
[f]vs denotes no theoretical upper-bound limit. Practical reasons require no free-flowing PCB liquid for the limit.
[g]Soil-air partition coefficient = 2.35 x 10^{-5} g soil/cm^3 air (= H.41/K_d = 5.73 x 10^{-4} (41)/1000 = 2.35 x 10^{-5}).

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APPENDIX D

HEALTH ADVISORIES FOR PCBs IN SOIL

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APPENDIX D

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EXISTING GUIDELINES, RECOMMENDATIONS, AND STANDARDS

The manufacture, sale, and distribution of PCBs have been restricted under Section 6(e) of the Toxic Substances Control Act (TSCA) (P.L. 94-469). PCBs were restricted to sealed systems as of 1977, and manufacture and distribution were banned in 1979. Rules for the disposal of PCBs were proposed in 1978 (43 FR 7150).

The U.S. EPA (1980) has set ambient water quality criteria for PCBs for the protection of humans from increased risk of cancer over a lifetime of 10^{-5}, 10^{-6} and 10^{-7} at 0.79, 0.079, and 0.0079 ng/L. As a result of the large bioconcentration factor in fish, these criteria apply regardless of whether exposure occurs through consumption of 2 L of water and 6.5 g of fish/day or through consumption of fish alone. The Food and Drug Administration (FDA) has set temporary tolerances for PCBs in food and food related products, as shown in Table D-1.

Occupational exposure limits for PCBs have been recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 1980), and criteria have been set for PCBs in workplace air by the National Institute for Occupational Safety and Health (NIOSH, 1977). The time-weighted average (TWA) and short-term exposure limit (STEL) for Aroclor 1254, are, respectively, 0.5 and 1.0 mg/m^3, and for Aroclor 1242, 1 and 2 mg/m^3 (ACGIH, 1980). The NIOSH (1977) criterion is 1.0 $\mu g/m^3$ for a 10 hours/day, 40 hours/week exposure.

The National Academy of Sciences developed a 24-hour suggested no adverse response level (SNARL) for PCBs of 350 µg/L based on the induction of mixed-function oxidase enzymes in the liver of rats administered Aroclor at doses of 1 to 2 mg/kg (NAS, 1980). For this analysis, an uncertainty factor of 100 was used, since only enzyme induction was reported in this dose range.

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TABLE D-1. FDA REGULATIONS FOR PCBs

Commodity	Temporary tolerances (ppm)
Milk (fat basis)	1.5
Manufactured dairy products (fat basis)	1.5
Poultry (fat basis)	3.0
Eggs	0.3
Finished animal feeds	0.2
Animal feed components of animal origin	2.0
Edible portion of fish and shellfish	5.0
Infant and junior foods	0.2
Paper food packaging material	10.0

SOURCE: 21 CFR 109.30.

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NONCARCINOGENIC EFFECTS

Tests of the acute lethality of PCB products in laboratory animals, with the exception of the guinea pig, suggest that, in general, PCB products have similar toxicity regardless of route of administration, species, or age of animal. The single dose oral LD_{50} value in rats, rabbits, mice, and mink ranged from 0.5-20 g/kg bw (Grant and Phillips, 1974; Bruckner et al., 1973; Kimbrough et al., 1978; Fishbein, 1974; Garthoff et al., 1981, Aulerich and Ringer, 1977). Route of administration also had little effect (less than one order of magnitude) on lethality, with the lethal dose for dermal administration in rabbits ranging from 0.7-3 g/kg bw (Nelson et al., 1972), while the lethal dose in mice administered PCBs by intraperitoneal injection ranged from 0.8-1.2 g/kg bw (Lewin et al., 1972).

There are only two indications of major differences in the acute toxicity of PCBs. First, there is limited evidence that the guinea pig may be more sensitive to the lethality of PCBs than other species. Miller (1944) observed a 100% mortality in a small group of guinea pigs receiving two oral doses of PCB (43% chlorine) at levels of 67 mg/animal at an interval of 7 days apart; and McConnell and Kinney (1978) reported an LD_{50-30} of 0.5 mg/kg for the PCB isomer 3,4,5-sym-hexachlorobiphenyl (HCB) in guinea pigs. This indication of possible large interspecies differences in sensitivity is of concern in species-to-species extrapolation when there is insufficient data to indicate which experimental animal most accurately reflects the sensitivity of humans. The second problem concerns the possible large difference in toxicity of specific isomers of PCBs. There are indications, on the basis of limited data available from Biocca et al. (1981), that four different hexa-PCBs differ in LD_{50} value from 19 mg/kg to >64 mg/kg after oral administration to mice. It

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would not be unreasonable to assume that even larger differences will be encountered as more isomers are tested. Variation in toxicity of the different isomers is not of great concern in defining acceptable environmental levels, since individual isomers were not commercially made and released to the environment. The analytical methods used to measure environmental PCBs determine the levels, in mg, of specific Aroclors. Since, as described above, the Aroclors do not differ greatly in acute toxicity, using data from the most toxic Aroclor should be protective without overly penalizing the other Aroclors.

Some data are available on the nonlethal acute toxicity of PCBs administered by the oral route for periods of 30 days or less. The effects described in these studies were alterations of the liver, thyroid, and reproductive system. Rosin and Martin (1983) reported that a dose of 500 mg/kg of Aroclor 1254 for 14 days to CD-1 mice decreased pentobarbital sleeping time, indicating a substantial induction of microsomal enzymes. At lower doses, Sanders et al. (1974) reported that exposure of ICR mice to diets containing 250 to 62.5 ppm of Aroclor 1254 for 14 days resulted, respectively, in hepatomegaly and elevated serum corticosterone (the latter presumably as a result of altered liver steroid metabolism). These exposures would result in doses of 32.8 and 8.1 mg/kg bw/day, assuming that a mouse consumes 13% of its body weight per day.

Similarly, Narbonne (1979) reported decreases in phenobarbital sleeping time in Sprague-Dawley rats maintained fro 8 days on a diet containing 100 ppm of Phenoclor DP6 (dose of 5 mg/kg bw/day, assuming that a rat consumes 5% of its body weight/day). PCB-induced increases in liver enzymes were suggested as the reason for the increase in testicular acid phosphatase observed by Dikshith et al. (1975) in Sprague-Dawley rats fed Aroclor 1254 at a dose of 50 mg/kg bw/day for 7 days. Increases in liver-to-body weight ratio appear to be one of

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the sensitive indicators of PCB exposure. Carter and Mercer (1983) reported that 64 mg/kg bw/day of Aroclor 1254 caused increased liver weight, while Grant and Phillips (1974) observed increased liver weight at doses as low as 12.5 and 5 mg/kg bw/day in male and female Wistar rats, respectively, receiving Aroclor 1254 in the diet for 14 days. Carter (1983) observed hepatomegaly in rats ingesting diets containing as little as 20 ppm Aroclor 1254 (1 mg/kg bw/day) for 14 days. Doses as low as 1 ppm in diet of 3,4,5,3',4',5'-hexachlorobiphenyl (345 HCB) for 28 days caused liver microabscesses and an increased liver weight in 18-20 g 5-week-old C57B1/6J mice (Biocca et al., 1981). Although adverse this study, 0.3 ppm in diet could be considered a lowest observed adverse effects were observed at concentrations as low as 0.3 ppm in diet in this study, there is no documentation indicating that commercial Aroclors contain 345 HcB. Hence, this study for 345 HcB cannot be used for establishing the short-term Health Advisory (HA), corresponding to an acceptable intake (AI) level.

Besides changes in the liver, other effects reported for exposure to low levels of PCBs were increased thyroid activity in Sherman rats maintained on diets containing 250 ppm of Aroclor 1254 (12.5 mg/kg bw) for 14 days; in Sprague-Dawley rats, administration of Aroclor 1254 by gavage for 21 days at a dose of 0.05 g/kg bw/day resulted in weight loss and decreased body temperature (Komives, 1979; Komives and Alayoku, 1980). Enlarged thyroid has also been found in Osborne-Mendel rats maintained on diets containing 5 ppm of Aroclor 1254 (0.25 mg/kg bw/day) for 4 weeks (Collins and Capen, 1980b). This exposure level also resulted in increased liver enzymes in Holtzman rats (Garthoff et al., 1977).

The toxicity of PCBs resulting from exposures of between 30 and 90 days has been more extensively studied. Alterations in liver histopathology

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occurred at doses as low as 5 ppm in diet for 5 weeks in Holtzman rats (Kasza et al., 1978b). In the mouse (MNRI), a dose of Clophen A-60, as low as 0.025 mg/mouse (0.8 mg/kg bw/day, assuming a mouse weight of 0.03 kg) for 62 days, increased the estrous cycle, probably as a result of PCB-induced changes in liver steroid metabolism (Orberg and Kihlstrom, 1973). At higher dietary concentrations of 167 ppm (22 mg/kg bw) for 6 weeks, Aroclor 1016 and 1242 decreased the immunologic capabilities of BALB/CJ mice (Loose et al., 1978).

Species other than rats and mice have been tested to a lesser extent for this duration. Rabbits exposed to diets containing 3 ppm of Aroclor 1254 (0.15 mg/kg bw/day, assuming that a rabbit consumes 4.9% of its body weight per day) for 8 weeks developed hepatomegaly and immunosuppression. In the guinea pig, Vos and van Genderen (1973) reported that diets containing 250 ppm of Clophen A-60 (7 mg/kg bw/day, assuming that a guinea pig consumes 2.8% of its body weight per day) for 4-7 weeks was lethal, while diets containing 50 ppm of Clophen A-60 or Aroclor 1260 (1.4 mg/kg bw) for 4 to 7 weeks produced immunosuppression. Allen et al. (1974) and Allen (1975) observed comedones and facial edema in rhesus monkeys ingesting diets containing 25 of ppm Aroclor (1.1 mg/kg bw, assuming that a monkey consumes 4.2% of its body weight per day) for 2 months.

Studies of subchronic and chronic exposure (i.e., $\geq$90 days) to PCBs have failed to use sufficiently low doses to define a no observed adverse effect level (NOAEL) in rats. In Sprague-Dawley rats, Allen et al. (1976) and Allen and Abrahamson (1979) reported that a 52-week exposure to diets containing 100 ppm of Aroclor 1248, 1254, or 1262 (5 mg/kg bw/day) followed by a 13-week observation period resulted in hepatomegaly and liver necrosis. At a lower exposure of 75 ppm in the diet (3.75 mg/kg bw/day) for 36 weeks, Sprague-Dawley rats developed focal necrosis (Jonsson et al., 1981). Elevated liver porphy-

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rine levels were detected by both Kimbrough et al. (1972) and Zinkl (1977) after exposure of Sherman rats for 8 months to 20 ppm of Aroclor 1254 or 1260 (1 mg/kg bw/day) and of CD rats for 20 weeks to 10 ppm of Aroclor 1254 (0.4 mg/kg bw/day). In a study employing near-lifetime exposure (2 years), Morgan et al. (1981) reported an increase in mortality (33% as compared with 8% in controls) in Fischer 344 rats at the lowest dose tested (25 ppm; 1.25 mg/kg bw/day). The subchronic studies demonstrated increasing liver pathology over the dose ranges studied, 0.5-5 mg/kg bw/day; while in the only chronic study, the lowest dose tested (1.25 mg/kg bw/day) resulted in early deaths.

In mice, dietary exposure levels to Kanechlor-300, -400 or -500 or Arochlor 1254 of between 100 and 500 ppm (13-65 mg/kg bw/day) for periods from 23 weeks to 11 months produced hepatomegaly (Ito et al., 1973; Bell, 1983; Kimbrough and Linder, 1974). Koller (1977) used groups of BALB/CJ mice which were maintained for 9 months on diets containing 0, 3.75, 37.5, or 375 ppm of the Aroclors 121, 1242, or 1254 (0.46, 4.57, or 47.75 mg/kg bw/day). The Aroclor with the lowest chlorine content (1221) produced no liver lesions, while exposure to Aroclor 1242 resulted in increased liver weight in the high-dose group. In mice exposed to Aroclor 1254, increased mortality was observed in the high-dose group, mild hepatopathology was observed in the median dose group, and no liver lesions were detected in the low-dose group. The no observed effect level (NOEL) in this study in mice of 0.45 mg/kg bw/day is nearly identical to the lowest observed effect level (LOEL) of 0.5 mg/kg bw/ day associated with porphyria in rats (Kimbrough et al., 1972; Zinkl, 1977).

The only other species tested in chronic bioassays was the monkey, and it proved to be highly sensitive to the toxic effects of PCBs. The most common observations in monkeys exposed to Aroclor 1248 in the diet for a period of from 8 to 39 months were skin lesions, palpebral edema, and erythema (Barsotti

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and Allen, 1975; Allen and Barsotti, 1976; Allen et al., 1980; Becker et al., 1979). These effects were observed at the lowest doses tested, ranging from 2.5 to 3 ppm in the diet (0.095-0.126 mg/kg bw/day). In addition, Becker et al. (1979) reported that monkeys fed diets containing 3 ppm of PCBs had gastric lesions, body weight loss, and reduced hemoglobin and leukocytes. In the monkey, doses as low as 0.1 mg/kg bw/day produced frank toxic effects; no studies have been conducted from which a NOAEL can be derived or to indicate how close 0.1 mg/kg bw/day is to the NOAEL for monkeys.

Although PCBs have not been demonstrated to be animal teratogens following oral exposure, these compounds have been demonstrated to adversely affect reproduction. When adminstered to pregnant Wistar rats at a dose of 100 mg/kg bw/day on days 6 to 15 of gestation, Villeneuve et al. (1971) observed no adverse effects; however, using the same treatment schedule, Spencer (1982) reported that Sprague-Dawley rats were infertile after receiving 15 mg/kg bw/day, that animals receiving 5 mg/kg bw/day had reduced litter weights, and that 2.5 mg/kg bw/day was the NOEL. Rabbits had resorptions, abortions, and fetuses at similar dose levels of 12.5 mg/kg bw/day administered on days 0 to 28 of gestation; however, slightly smaller doses of 10 mg/kg bw/day were reported to be the NOEL. The Hartly guinea pig, which has been shown to have greater sensitivity to the toxicity of PCBs than most other species, had macerated fetuses after receiving 2.2 mg/day (6/5 mg/kg bw/day) of Clophen A-50 on days 10 to 60 of gestation (Brunstroem et al., 1982).

Effective doses of PCBs were lower than exposure occurred before and during gestation. In a two-generation study, Sherman rats maintained on diets containing 20 ppm Aroclor 1254 (1 mg/kg bw/day) had reduced litter size, and at 100 ppm (5 mg/kg bw/day) the pups that were born died during nursing (Linder et al., 1974). In this study, 5 ppm (0.25 mg/kg bw/day) was the NOEL. Complete

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loss of fertility was observed in male and female Wistar rats caged together for 9 weeks while ingesting 6.4 mg/kg bw/day of Aroclor 1254 emulsified in their drinking water (Baker et al., 1977). Males regained normal fertility after removal from treatment for 2 weeks. When Aroclor 1254 was administered to lactating Holtzman rats at 32 mg/kg bw/day on days 3, 5 and 7 of lactation, the future mating behavior of nursing male pups was adversely affected (Sager, 1983). A lower dose of 8 mg/kg bw/day was a NOEL.

Of the species tested, the mink and the monkey are the most sensitive to the reproductive toxicity of PCBs. Bleavins et al. (1980) maintained mink on diets containing 5 ppm Aroclor 1242 or 20 ppm Aroclor 1016 (doses of 0.75 and 3 mg/kg bw/day, assuming that a mink consumes 15% of its body weight per day) for 18 months and observed complete reproductive failure in the Aroclor 1242 group and 25% mortality and infertility in the Aroclor 1016 group. A more recent study by Aulerich et al. (1985) tested yet lower doses fed to mink via diet. Aroclor 1254 at 2.5 ppm; 3,4,5,3',4',5'-hexachlorobiphenyl (345 HCB) at 0.1 or 0.5 ppm; 2,4,5,2',4',5'-hexachlorobiphenyl (245 HCB) at 2.5 or 5.0 ppm; or 2,3,6,2',3',6'-hexachlorobiphenyl (236 HCB) at 2.5 or 5.0 ppm of diet were fed to groups of 10 standard dark mink (proven breeders). A group of 20 animals served as controls. All of the mink fed 0.5 ppm 345 HCB died within 60 days, while those fed 0.1 ppm showed 50% mortality after 3 months. One stillborn kit was whelped in the Aroclor 1254 group. 245 HCB and 236 HCB did not affect reproductive performance at either dose.

In a limited study (8 animals/group), Allen et al. (1980) maintained rhesus monkeys on diets containing 2.5 or 5 ppm (0.1 or 0.2 mg/kg bw/day) of Aroclor 1248 for 18 months. In the low-dose group, increased abortions were observed, while in the high-dose group, the mothers showed overt signs of toxicity and no live births occurred. After removal from exposure for 1 year,

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fertility had still not returned to normal, and some pups died during nursing. It is apparent that frank effects in reproduction were observed in mink at lower doses than in monkeys and still lower than the NOEL in rats, rabbits, and guinea pigs following repeated exposure to PCBs.

QUANTIFICATION OF SHORT-TERM HEALTH ADVISORY LEVELS

PCBs belong to a class of chemically stable, multi-use industrial chemicals that have been widely distributed in the ecosystem. Technical preparations consist of complex mixtures of discrete PCB isomers. Because of their physicochemical properties, PCBs have been used as heat exchangers, dielectric, hydraulic and lubricating fluids, plasticizers, pesticide extenders, adhesives, printing inks and surface coatings.

The physical and chemical properties and the chemical formulations of PCBs vary considerably, depending on the amount and position of chlorine substitution. Such properties as stability, volatility, and water solubility are particularly important in regard to frequency of occurrence in the environment. The higher chlorinated biphenyls are less volatile than the lower chlorinated biphenyls (Mieure et al., 1976). PCBs are extremely insoluble in water. The solubility of commercial mixtures (for example, the Aroclors) ranges from 25 to 200 ppb (25 to 200 µg/L), depending on the chlorine content (Nisbet and Sarofim, 1972; Haque et al., 1974). The solubility of discrete PCB isomers has been examined, and ranges from 1 to 600 ppb (1 to 600 µg/L) depending on the degree of chlorine substitution in the biphenyl ring (Haque and Schmeddig, 1975).

PCBs elicit a variety of adverse health effects. This is true of even partially well-defined compositions such as Aroclor 1254. For example, toxi-

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cology studies on Aroclor 1254 of less less than 30 days' duration report liver toxicity (Grant and Phillips, 1974; Carter, 1983), thyroid toxicity (Collins and Capen, 1980a, b, c) and reproductive toxicity (Villeneuve et al., 1971), as well as other types of toxicity (U.S. EPA, 1985b). At first, it would seem that this variety in elicited adverse effects would make it difficult to distinguish the critical toxic effects of PCBs. However, it appears that the experimental thresholds for these effects may be similar, at least for studies of 30 days' duration or less.

Villeneuve et al. (1971) found increased incidences of fetal death, resorptions, and abortions at 12.5 mg/kg/day of Aroclor 1254 in rabbits when exposed on days 1 through 28 of pregnancy. A dose of 1.0 mg/kg/day appeared to be without effect. Collins and Capen (1980a, b, c), in a series of studies on thyroid effecs in rats, determined that 50 ppm of diet (~ 2.5 to 5.0 mg/kg/day) for 4 weeks was associated with clearly defined adverse effects, but that doses of 5 ppm of diet (~ 0.25 to 0.5 mg/kg/day) were not. Carter (1983) demonstrated liver hepatomegaly in rats at doses of 20 ppm Aroclor 1254 of diet (~ 2 mg/kg/day) for 14 days; such an effect in the absence of toxicity (e.g., fatty infiltration of the liver) might not be considered adverse. Grant and Phillips (1974) observed increased liver weights at doses as low as 5 mg/kg/day Aroclor 1254 given in corn oil for 7 consecutive days. Collectively, these studies indicate that the experimental threshold for adverse effects of Aroclor 1254 in studies of 30 days' duration or less is at or near a dose of 1 mg/kg/day. Thus, it seems reasonable to use this latter dose as a basis for health risk assessments for Aroclor 1254 for short durations.

Utilizing a dose of 1 mg/kg/day weight as a No Adverse Effect dose, a 10-day exposure level for PCBs may be calculated as follows:

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$$\text{10-day exposure level} = \frac{1 \text{ mg/kg/day} \times 10 \text{ kg}}{100} = 0.1 \text{ mg/day for a 10-kg child.}$$

where

10 kg = assumed body weight of a child, and

100 = uncertainty (safety) factor chosen in accordance with the National Academy of Sciences guidelines, in which a NOAEL from an animal study is employed.

For a 70-kg adult the 10-day exposure level would be 0.7 mg/day.

CARCINOGENIC EFFECTS

A number of short-term assays predictive of carcinogenic potential have been performed using the Aroclors and individual isomers of PCBs. Negative results have been obtained in the reverse mutation assay using S. typhimurium (Schoeny et al., 1979; Schoeny, 1982; Heddle and Bruce, 1977), and in the dominant lethal assay in rats (Green et al., 1975). PCB products also did not produce chromosomal changes in D. melanogaster (Nilsson and Ramel, 1974) or in the sperm and bone marrow cells of rats (Green et al., 1975; Garthoff et al., 1977; Dikshith et al., 1975). Wyndham et al. (1976), however, observed increases in reversion frequency in S. typhimurium exposed to 4-chlorobiphenyl and to a lesser extent with Aroclor 1221, while the more highly chlorinated 2,2',5,5'-tetrachlorobiphenyl and Aroclor 1254 were negative. The positive response was observed in one strain, TA1538, in the presence of a metabolic activation system derived from rabbit liver. In addition, a weak positive response was observed by Peakall et al. (1972) in an assay of chromosomal aberration in the embryos of ring doves fed Aroclor 1254. The variable data observed with PCBs is consistent with the poor response and lack of correlation

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with animal carcinogenicity data reported for many highly chlorinated compounds in short-term assay.

Early bioassays of PCBs were inadequate as a result of small group size or periods of exposure extending for less than 1 year. These studies failed to demonstrate that PCBs were carcinogenic when fed to rats or mice (Kimura and Baba, 1973; Ito et al., 1973; Ito et al., 1974; Kimbrough and Linder, 1974). The study by Kimbrough and Linder (1974) in which 50 BALB/CJ male mice were fed diets containing 300 ppm of Aroclor 1254 for 6 or 11 months was suggestive that Aroclor 1254 was a liver carcinogen. Of the 22 animals surviving PCB treatment for 11 months, all had areas of adenofibrosis in the liver, and seven had histologically identified hepatomas. In animals surviving 11 months which are maintained on PCB-contaminated diets for 6 months, and in control animals, there were, respectively, only 0/24 and 0/58 livers with areas of adenofibrosis and 1/24 and 0/58 animals with hepatomas. There were no histologically identified hepatocellular carcinomas in any group.

In a later study in rats using a larger group size and a longer exposure period, Kimbrough et al. (1975) reported an increased incidence in hepatocellular carcinomas in animals exposed to PCBs. In this study, 200 female Sherman rats were exposed to diets containing a nominal 100 ppm (range 70-107 ppm) of Aroclor 1260 for 630 days. The incidence of hepatocellular carcinomas in the treated group compared with control animals was 26/184 and 1/173, respectively; while the incidence of hepatic neoplastic nodules was 144/184 and 0/173, respectively.

In the only other chronic bioassay performed (NCI, 1978), 24 male and 24 female Fischer 344 rats/group were maintained on diets containing 0, 25, 50, or 100 ppm of Aroclor 1254. Although dose-related increases in nodular hyperplasia were observed, there was no significant increase in neoplastic lesions.

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With respect to the tumor incidence reported by Kimbrough et al. (1975), the number of rats/group in the NCI study may have been too small to detect a carcinogenic response.

Very limited information is available on the carcinogenicity of PCBs in humans. In a survey of 1,200 patients in Yusho, Japan, 5.5 years after exposure to PCBs, 41% of the 22 deaths were attributed to neoplasia (Kuratsune, 1976; Urabe, 1974). The relevance of these findings is unclear since the tumors were at various common sites, and comparable incidences for an unexposed population were not presented. In two letters to the editor, Bahn et al. (1976, 1977) described suspected increases in malignant melanomas in a small group of workers exposed to Aroclor 1254. In the 31 "heavily exposed" workers, there were two cases of melanoma, while in the 41 "less heavily" exposed workers, there was one melanoma. The International Agency for Research on Cancer (IARC) estimates that only 0.04 cases would be expected in this number of individuals (IARC, 1978). The only epidemiologic study (Davidorf and Knupp, 1979) examined the association between ocular melanomas and populations residing in areas of known high environmental levels of PCBs. The authors concluded that a causal relationship between PCBs and ocular melanomas was not demonstrated. The IARC (1978) considered the association between exposure to Aroclor 1254 and malignant melanomas described in the two letters to the editor of the New England Journal of Medicine (Bahn et al., 1976, 1977) to be suggestive evidence that PCBs are human carcinogens.

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QUANTIFICATION OF CARCINOGENIC RISK

WEIGHT OF EVIDENCE FOR HUMAN CARCINOGENICITY

The likelihood that a chemical such as PCB is a human carcinogen is expressed through a characterization or stratification of the "weight of evidence" (human, animal, short-term test) and a final indiction of the "overall weight of evidence" for human carcinogenicity.

The IARC has characterized the evidence for the carcinogenicity of PCBs in humans as "inadequate," the evidence for carcinogenicity in animals as "sufficient," and the supportive evidence from short-term tests as "inadequate," (IARC, 1982). The overall weight-of-evidence designation for PCBs under the IARC scheme is 2B (probably carcinogenic in humans; evidence inadequate in humans and sufficient in animals). The EPA has recently proposed a similar scheme, which is an adaptation of the IARC scheme (U.S. EPA, 1984a). There are some differences in the two schemes; however, for PCBs the requirements for inadequate evidence of carcinogenicity in humans and for sufficient evidence in animals are essentially identical. Similarly, the overall weight-of-evidence under the new EPA scheme would be designated as B2, which has the same requirements as the 2B designation of the IARC scheme.

To comply with the EPA proposed guidelines for carcinogen risk assessment (U.S. EPA, 1984a), any final risk estimate developed or used in risk characterization should be coupled with the EPA classification of the qualitative weight-of-evidence. Thus, those risk levels used in this paper are understood to carry the "B2" designation; for example, 1×10^{-5} (B2), 1×10^{-6} (B2), etc.

POTENCY SLOPE FACTORS

The use of a potency slope factor, necessary in calculating risk or back-calculating permissible PCB soil concentrations from selected risk levels, does

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not definitely indicate that the chemical is a human carcinogen. The likelihood that the agent is a human carcinogen is a function of the weight-of-evidence described above. The proposed EPA guidelines for carcinogen risk assessment (U.S. EPA, 1984a) suggest that agents falling into Groups A and B are suitable for quantitative risk assessments.

The U.S. EPA Carcinogen Assessment Group (CAG) has used the data from female rats in the study by Kimbrough et al. (1975) to quantify the carcinogenic risk from exposure to PCBs (U.S. EPA, 1980). In this analysis, the TWA concentration of PCB (Aroclor 1260) in the diet was determined to be 88.4 ppm, associated with a daily dose of 4.42 mg/kg bw/day, by assuming that an adult rat consumes food equal to 5% of its body weight per day. In addition, for this analysis, the incidence of hepatocellular carcinomas (26/184 in treated animals and 1/173 in controls) and neoplastic nodules (144/184 in treated animals and 0/173 in controls) were combined to produce tumor incidences of 170/184 and 1/173 in the treated and control groups, respectively. Using these data and the linearized multistage model, a cancer potency value (q_1^*) for human exposure to Aroclor 1260 of 4.34 $(mg/kg/day)^{-1}$ was calculated using the data in Table D-2. The U.S. EPA Office of Toxic Substances (OTS) has also used the data from the same study, but by altering two of the variables (see Table D-2), calculated a q_1^* of 3.57 $(mg/kg/day)^{-1}$ (U.S. EPA, 1985a). The average of these two values is 4.0 $(mg/kg/day)^{-1}$, and this value has been adopted by this health advisories appendix for use in developing advisory levels for PCBs clean-up.

Using this q_1^*, a risk-specific dose (RSD) of Aroclor 1260 that would result in an increased lifetime risk level of 10^{-5} for a 70-kg man can be calculated as follows:

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TABLE D-2. DATA USED AS THE BASIS FOR THE q_1^*

Species	rat
Strain	Sherman
Sex	female
Body weight (assumed)	0.4 kg (0.35 kg)[a]
Length of exposure	645 days (730 days)[a]
Length of experiment	730 days
Tumor site	liver
Tumor type	combined hepatocellular carcinomas and neoplastic nodules
PCB product tested	Aroclor 1260

Dose (mg/kg/day)	Incidence (No. responding/No. tested)
0	1/173
4.42	170/184

[a]The data in parentheses indicate the alternative values used by OTS in computing a cancer potency factor.

SOURCE: Kimbrough et al., 1975.

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$$RSD = 1 \times 10^{-5} \times 70 \text{ kg bw} \div q_1^* \text{ (mg/kg/day)}^{-1}$$

$$= 0.175 \text{ µg/day.}$$

Thus, using the q_1^* for humans of 4.0 $(mg/kg/day)^{-1}$, the RSDs corresponding to lifetime risks of 10^{-4}, 10^{-5}, and 10^{-6} are 1.75, 0.175, and 0.0175 µg/day, respectively. The adoption of these potency and concentration levels for all PCBs requires a further assumption that all PCB compounds are carcinogenic and that the potency of Aroclor 1260 is representative of any mixture of any other PCB compound.

SPECIAL CONSIDERATIONS

HIGH-RISK SUBPOPULATION

Two separate groups of high-risk subpopulations for exposure to PCBs may be identified. The first group includes those persons with the potential for frequent or high exposure, namely, occupationally-exposed workers and breast-fed infants, as PCBs are excreted in the breast milk of lactating humans (Miller, 1977; Rogan et al., 1980; Wickizer et al., 1981; Mes and Davies, 1979; Kuwabara et al., 1979; Hofvander et al., 1981). The second group includes those persons with an inability to oxidize PCBs via glucuronidation to facilitate detoxification and elimination of these toxicants, such as embryos, fetuses, and neonates (2 to 3 months old) (Calabrese and Sorenson, 1977; Gillette, 1967; Nyhan, 1961), especially breast-fed infants who receive a steroid via human breast milk that inhibits glucuronyl transferase activity (Calabrese and Sorenson, 1977; Gartner and Arias, 1966), children simultaneously exposed to the antibiotic novobiocin (Lokietz et al., 1963; Calabrese and Sorenson, 1977), persons with Gilbert's syndrome or Crigler and Najjar

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syndrome (Lester and Schmid, 1964; Calabrese and Sorenson, 1977), or persons with hepatic infections such as infectious hepatitis (Calabrese and Sorenson, 1977).

COCARCINOGENESIS, INITIATION, AND PROMOTION

DiGiovanni et al. (1978) demonstrated that Aroclor 1254 had weak tumor initiating activity in the mouse two-stage tumorigenesis models. Promoting activity was not indicated for this Aroclor in a study by Berry et al. (1978). Using other experimental systems, Ito et al. (1973) observed an increased incidence of liver tumors in rats co-administered PCBs and benzene hexachloride as compared with benzene hexachloride treatment alone. Co-administration of other potent live carcinogens, 3'-methyl-4-dimethylaminoazobenzene, N-2-fluorenylacetamide and diethylnitrosamine, with PCBs, however, has resulted in the inhibition of the tumorigenic response (Ito et al., 1973). Similarly, antineoplastic effects were reported by Nishizumi (1980) for pups of dams administered Kanechlor-500 and diethylnitrosamine. These studies make it apparent that exposure to PCBs can affect the carcinogenicity of other xenobiotics.

SUMMARY

For the purposes of setting advisory levels for PCBs contaminating soils, acceptable intake (AI) levels have been developed which are based on both the toxicity and the carcinogenicity of PCBs. The 10-day health advisories (HA) for toxicity other than cancer have been developed. The 1-day and lifetime HAs could not be evaluated. Advisories for the cancer end point are expressed in terms of 10^{-4} to 10^{-7} lifetime individual excess risk levels. See Table D-3 for a summary of these AIs and risk-specific doses.

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TABLE D-3. SUMMARY OF RISK SPECIFIC DOSES (RSDs) FOR CANCER RISKS OF PCBs, OR OF ACCEPTABLE INTAKES FOR PROTECTION AGAINST THE NONCARCINOGENIC EFFECTS OF PCBs

Description	Value (μg/day)
10^{-4} RSD	1.75
10^{-5} RSD	0.175
10^{-6} RSD	0.0175
Short-term AI (1-day)	Not estimated
Longer-term AI (10-day)	100 for a child
	700 for an adult
Lifetime AI	Not estimated

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The toxicity and carcinogenicity of PCBs have recently been critically evaluated in a number of EPA documents (U.S. EPA, 1980, 1983, 1984b, 1985b). The approach taken here in developing a 10-day exposure advisory for noncancer toxicity has been to select from the available literature the animal study that addresses the critical toxic effect and yields the most appropriate no observed effect, no observed adverse effect, lowest observed effect, or lowest observed adverse effect level. This dose was then divided by an appropriate uncertainty factor to obtain the 10-day HA.

The calculation of a 10-day HA for noncarcinogenic toxicity should make use of animal data derived after an exposure period ranging from 10 to 30 days. The literature contains several animal studies which address this length of exposure. The studies chosen as a basis for the 10-day HA (Villeneuve et al., 1971; Grant and Phillips, 1974; Collins and Capen, 1980a, b, c; Carter, 1983) yield a No Observed Adverse Effect Level (NOAEL). These studies involved the feeding of Aroclor 1254 to rabbits and rats. Collectively, in these studies, a NOAEL can be ascribed to a dose of 1 mg/kg/day). Dividing this NOAEL by an uncertainty factor of 100 yields a 10-day HA of 100 µg/kg/day for a child, and 700 ug/day for a 70-kg adult.

The 1-day HA could not be estimated based on animal data derived from studies with an exposure duration of 1 to 5 days. The lifetime HA noncancer toxicity, likewise, could not be estimated.

The cancer risk specific doses (intake levels) have been calculated by solving for exposure in the equation

$$\text{Risk} = \text{Potency} \times \text{Exposure}$$

and multiplying by 70 kg. A cancer potency factor of 4.0 $(mg/kg/day)^{-1}$ was

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adopted for use in these calculations. This value is the mean of the values determined by OTS (3.57 $(mg/kg/day)^{-1}$) and by ORD (4.34 $(mg/kg/day)^{-1}$) for PCBs.

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