Activated Sludge Primary Biodegradation of Polychlorinated Biphenyls

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Since the identification of polychlorinated biphenyl (PCB) residues in the environment in Sweden (Jensen, 1966) an effort has been made to determine the level and distribution of these materials as well as their effects upon living organisms. The widespread distribution of low but detectable levels of some PCBs in the environment is now well documented and a substantial amount of data have been accumulated regarding their acute, subacute, and chronic toxicity (Peakall and Linder, 1970; Edwards, 1970).

While much is known about the effects of PCBs on living organisms, there is relatively little information available regarding the effects that living organisms have upon PCBs, i.e. their biodegradability.

The biodegradability or susceptibility of an organic compound to biological degradation, especially by bacteria, is a prime determinant of its environmental residence time. The finding of PCB residues in the environment suggests to many that PCBs as a class of compounds are resistant to microbial degradation. However, in order to fairly evaluate the persistence of PCBs it must be understood that PCBs are not a single entity, but complex mixtures made up of many entities which may undergo biological degradation at different rates.

Chlorinated biphenyls are commercially produced by the direct chlorination of biphenyl. The resultant mixtures can theoretically have as many as 210 different components containing 0-10 chlorine atoms per biphenyl molecule. Of the possible isomers, 103 are considered most probable (Widmark, 1968). The complexity of the PCBs, therefore, complicates the determination of both environmental levels and their impact on the biota.

Environmental monitoring programs have demonstrated that, with the exception of direct high level controllable contamination near points of manufacture or use,

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the PCBs generally found in the environment are the more highly chlorinated biphenyls, i.e., those containing 5 or more chlorine atoms per biphenyl molecule (Jensen and Widmark, 1967; Holmes, et al, 1967; Koeman, et al, 1969). This is true even though the more highly chlorinated biphenyls constitute only about 35% of all the PCBs manufactured over the years (Monsanto Company, 1971). This is strong evidence that the less chlorinated materials degrade more rapidly than the more highly chlorinated ones. It also suggests that even under conditions of unrestricted use and without special precautions to prevent entry into the environment that the less chlorinated materials degraded rapidly enough to prevent accumulation.

It is obvious, therefore, that to obtain a more complete understanding of the environmental behavior of PCBs that information concerning their susceptibility to microbial degradation is needed.

It has been shown that biphenyl can be degraded by gram-negative bacteria through 2,3-dihydro -2,3-dihydroxybiphenyl, α-hydroxy-β-phenylmuconic semi-aldehyde and phenyl pyruvate (Lunt and Evans, 1970), and it has been reported that Pseudomonas putida oxidizes biphenyl through 2,3-dihydro - 2,3-dihydroxybiphenyl and benzoic acid (Catelani, et al, 1971). More recently Gibson and co-workers isolated a bacterium, tentatively identified as a Beijerinckia species from a polluted stream, capable of utilizing biphenyl as a sole source of carbon and energy for growth (Gibson, et al, 1973) and organisms capable of oxidizing biphenyl and p-chloro biphenyl have been shown to be widely distributed in the natural environment (Ohmori, et al, 1973).

Degradation studies have also been carried out on some selected PCB isomers with two species of Achromobacter isolated from sewage (Ahmed and Focht, 1973a,b) and with Arocior 1242 (Kaiser and Wong, 1974). To date, no work has been reported on the biodegradation of commercial PCB mixtures by activated sludge.

In the study reported here, the susceptibility of commercial polychlorinated biphenyl mixtures to primary degradation by activated sludge microorganisms was investigated.

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METHODS AND MATERIALS

Polychlorinated Biphenyl Mixtures

The following polychlorinated biphenyl mixtures manufactured by Monsanto Company were studied: Aroclor[1] 1254, Aroclor 1242, Aroclor 1016, MCS 1043 (not a commercial mixture), and Aroclor 1221. In Table I, the typical per cent composition of the mixtures studied is given in terms of the number of chlorine atoms per biphenyl.

TABLE I
TYPICAL % COMPOSITION OF POLYCHLORINATED BIPHENYL PRODUCTS*

# Cl Bi-phenyl	Aroclor 1221 (21% Cl)	MCS 1043 (30% Cl)	Aroclor 1016 (41% Cl)	Aroclor 1242 (42% Cl)	Aroclor 1254 (54% Cl)
0	11	0.1	<0.1	<0.1	<0.1
1	51	22	1	1	<0.1
2	32	72	20	16	<0.5
3	4	6	57	49	1
4	2	ND	21	25	21
5	<0.5	ND	1	8	48
6	ND	ND	<0.1	1	23
7	ND	ND	ND	<0.1	6
8	ND	ND	ND	ND	ND

*Per cent (w/w) by GC/Mass using area correction factors.

ND - None Detected, <0.01%

Biodegradation Method

The primary degradation rate of the PCB mixtures was determined using the Soap and Detergent Association semi-continuous activated sludge (SCAS) procedure and modified feed (S.D.A., 1965, 1969). The sludge unit used was a cylindrical glass chamber (85 mm O.D.) of 1500 ml working volume with provisions for aeration, magnetic stirring, sampling and draining. The activated sludge culture was obtained from a local municipal sewage treatment plant and acclimated on synthetic sewage (300 mg of glucose + 200 mg nutrient broth + 130 mg KH_2PO_4 per liter) for several weeks prior to the start of actual feeding of the polychlorinated biphenyl mixtures. The mixed liquor (sludge + aqueous phase)

[1]Registered trademark of Monsanto Company

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was initially adjusted in each sludge unit to a suspended solids concentration of about 2500 mg/liter and during the course of the tests readjusted to this value on a weekly basis.

Each cycle was initiated by the addition of synthetic sewage and the PCB being studied.

Because of their low water solubility, the PCB mixtures being tested were fed via syringe injection of 200 µl of an ethanol solution. In this manner, homogeneous dispersions of the PCBs on the bacterial sludge were obtained.

After about 30 minutes of aeration, a 20 ml aliquot of the mixed liquor was withdrawn and analyzed for the PCB mixture in question. Aeration was continued until the end of the cycle when a second 20 ml sample of mixed liquor was withdrawn for analysis. At this point, the aeration was stopped and the sludge allowed to settle. After noting the sludge volume, two-thirds (1000 ml) of the supernatant was withdrawn and replaced with tap water. Monitoring of the sludge volume and supernatant pH provided some indication of satisfactory operation of the unit. The units were generally operated on two 48-hour and one 72-hour cycle per week.

Analytical Methods

The PCBs in the mixed liquor samples were isolated from the sample matrix by solvent extraction using either nanograde or spectrograde hexane. After concentrating in a Kuderna-Danish evaporative concentrator, the extracts were analyzed for PCBs by either electron-capture gas chromatography (EC/GC) or ultraviolet (UV) spectrophotometry. The UV analyses were made with a Cary Model 14 recording spectrophotometer and matched 2.0 cm quartz cells. The gas chromatographic analyses were carried out with a Hewlett-Packard 5750 chromatograph equipped with a Ni^{63} electron-capture detector. A 2 m X 4 mm glass column packed with 4% XE-60 on 80/100 mesh Chromosorb W, H.P., wes employed. The temperatures of the injection port, column and detector were 220°C, 170°-220°C, and 300°C, respectively. Calibration curves were prepared using standard solutions of the appropriate PCB mixture.

In Table II, the wavelength of the absorption maxima and absorptivities employed in the UV analyses are given. Aroclor 1254 did not have sufficient UV absorption for analytical purposes.

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TABLE II
ULTRAVIOLET ABSORPTION AND RECOVERY DATA FOR BIPHENYL AND POLYCHLORINATED BIPHENYL MIXTURES

Material	λ Max.,nm	Absorptivity Liters/g-cm	Per Cent Recovery
Biphenyl	246	110.9	93 ± 3
Aroclor 1221	245	66.4	96 ± 1
MCS 1043	244	48.9	95 ± 1
Aroclor 1016	246	36.1	92 ± 1
Aroclor 1242	245	37.0	84 ± 1
Aroclor 1254*	-	-	76 ± 2

*Analyzed by EC/GC

In order to demonstrate the efficiency of the extraction procedure, samples of activated sludge were spiked in duplicate at two levels, 2.5 and 5 ppm, with each of the materials and then carried through the entire analytical procedure. The recovery data for each PCB fluid studied are given in Table II. No isomer distribution changes were observed upon comparison of the electron-capture chromatograms of the PCB reference materials to those of the spiked sludge extracts.

In order to show that the PCBswere not irreversibly adsorbed on and/or stored within the bacterial cells of the activated sludge end therefore not recovered via extraction, a sample of acclimated Aroclor 1016 mixed liquor was homogenized with a Polytron Sonic Homogenizer to lyse the bacterial cells and then extracted. A 100 ml sample of homogenized mixed liquor gave a PCB level of 0.51 mg compared to 0.58 mg for an identical mixed liquor sample treated in the normal manner.

RESULTS AND DISCUSSION

In Figure 1, the per cent degradation rates and 95% confidence limits obtained in this study are given for biphenyl and the PCB fluids. It is apparent from the plot of degradation rate vs weight per cent chlorine that the level of chlorination of the mixture is the most significant factor in the relative degradability of the PCBs.

The degradation rates reported here were obtained after the sludge units had been acclimated for about 5 months to the appropriate PCB. Initially the PCB mixtures were fed at a rate of 1 mg per 24-hour cycle, but because of the relatively slow rate of degradation observed in spot checks, the cycle time was increased to 48 hours.

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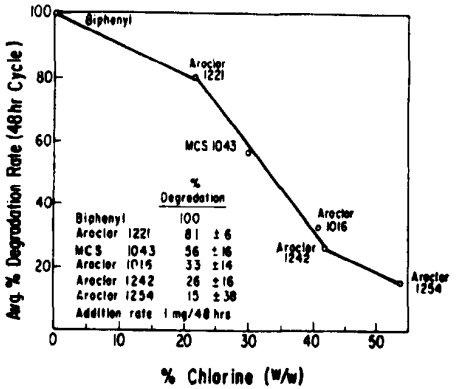

Figure 1. SCAS primary biodegradation rates of commercial PCBs as a function of the weight per cent chlorine.

Since Aroclor 1221 was found to be quite degradable, the effects of both time cycle and feed level were studied briefly. In Table III disappearance rate data obtained for 24 and 48-hour time cycles at 1 and 5 mg feed levels are given. It is apparent that for Aroclor 1221, most of the degradation occurs during the first 24 hours. This can be explained by the fact that the lower chlorinated biphenyls degrade more rapidly than the higher chlorinated biphenyls.

To verify that the disappearance of the PCBs was due predominantly to degradation and not to volatilization, off-gases from the Aroclor 1221, MCS 1043, and Aroclor 1016 units were passed through a train of three hexane scrubbers during several complete cycles. At the 0.1 cubic foot per hour aeration rate, the disappearance rates due to volatility were 4.2, 6.1, and 3.6% for Aroclor 1221, MCS 1043, and Aroclor 1016, respectively. These losses are well within the 95% confidence limits of the overall disappearance rates.

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TABLE III
EFFECT OF TIME CYCLE AND FEED LEVEL ON THE AROCLOR 1221 DISAPPEARANCE RATE

Feed Level	Disappearance Rate, % 24-Hr Cycle	48-Hr Cycle
1 mg	73 ± 21	81 ± 6
5 mg	89 ± 17	87 ± 5

In order to observe changes in the distribution of the chlorinated biphenyls in the PCBs mixtures after exposure to the activated sludge, selected samples of Aroclor 1221, MCS 1043, Aroclor 1016 and Aroclor 1242 were analyzed by EC/GC. The chromatogram for Aroclor 1221 is shown in Figure 2. The top trace is a chromatogram of the Aroclor 1221 standard, representative of the feed material. The center trace is that of a concentrated sludge extract taken at the end of a degradation cycle. At the bottom is a trace of an Aroclor 1242 standard run under equivalent conditions. The numbers above each peak indicate the dominant PCB represented by the peak as determined by GC/Mass spectrometry. Comparison of the extract chromatograms to that of the Aroclor 1242 standard shows that the minor components in Aroclor 1221, which do not degrade as rapidly, are the major components in Aroclor 1242.

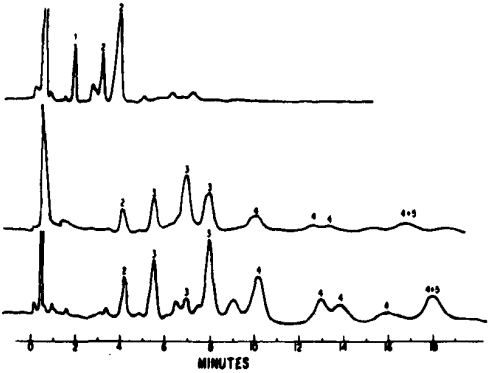

Figure 2. Typical electron capture chromatograms for Aroclor 1221, after exposure to activated sludge for 24 hours, and Aroclor 1242.

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It is important to note that the electron-capture detector does not have the same response for all components. With PCBs, the sensitivity of the detector generally increases as the degree of chlorination increases. From the Aroclor 1221 chromatograms, it is apparent that the dominant monochlorobiphenyl and dichlorobiphenyl components are readily degraded. Once the major components of Aroclor 1221 are degraded, the minor more slowly degrading components are easily observed after concentration of the sludge extracts.

Similar, less dramatic, alterations were noted for MCS 1043 and Aroclor 1242. No differences were observed in the extract and Aroclor 1254 standard chromatograms.

CONCLUSIONS

The results of this study demonstrate that commercial PCB mixtures which contain predominantly mono- and dichlorobiphenyls readily undergo primary biodegradation under the experimental conditions employed.

The data also illustrates that as the levels of tri-, tetra-, and pentachlorobiphenyls increase, the degradation rates decrease accordingly. This resistance of the more highly chlorinated biphenyls, particularly those containing 5 or more chlorine atoms per molecule, explains in part their detection as residues in weathered biological and environmental samples.

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ACKNOWLEDGEMENT

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TABLE I

PRIMARY AND ULTIMATE BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS

Test Material	Structural Composition	Ultimate Biodegradability (% Theoretical Co-Evolution)		Primary Biodegradability						
				Sub-Continuous Activated Sludge (ASCS)						
		Thomson-Dutrie-Sturm procedure	Pulsqueo Shake Flask procedure	Addition Rate (ppm added per cycle)	Primary Degradation Rate ±95% C.L.	Comments	Initial Conc(ppm)	Primary Degradation	[illegible]	
				24-Hour Cycle		[illegible] indicate moderate, strong, and very-thin inhibition of sludge growth rate [illegible]				
MCS 1412 (p-chlorodiphenylmethane)		86 (35-Day)		3 13	> 97 > 99	Volatility loss ≤ 4%. + For more detail see AC-73-SS-18.			82.3 ± 8.9	AC-72-M-3
MCS 1413 (p-chlorodiphenylmethane)		80 (35-Day)		3 13	> 97 > 99	+ + For more detail see AC-73-SS-18.			88.1 ± 5.4	AC-72-M-3
MCS 1310 (3,4-Dichlorodiphenylmethane)				3 13	> 98 > 99	Volatility loss ≤ 1%. +For more detail see AC-73-SS-18.			91.6 ± 3.7	AC-72-M-3
MCS 1584 (2,4-Dichlorodiphenylmethane)		83 (35-Day)		3 13	> 97 > 99	Volatility loss ≤ 1%. + For more detail see AC-73-SS-18.			76.2 ± 5.5	AC-72-M-3

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TABLE I

PRIMARY AND ULTIMATE BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS

Test material	Structural formulation	Biodegradability (Shake-flask method) — Static Exposure — Screening test — Sturm procedure	Biodegradability (Shake-flask method) — Dynamic Exposure — Shake flask procedure	Sewage addition Rate (ml/liter per cycle)	Biodegradability (Shake-flask method) — Dynamic Exposure — Semi-batch flow test (% BOD)	Remarks	Solubility (mg/liter)	Toxicity Threshold Concentration (ppm)	Reference
				24-Hour Cycle		A ... and w/- ... dielectric materials ... strongly, the varia... rate ... although ... rate ... reasonably ...			
HCS 1560 (Mixture of 2,4- and 3,4-Dichlorodiphenyl methane)				3	> 97	Volatility loss < 1%.		79.6 ± 1.6	AC-72-M-9
				13	> 99	For more detail see AC-73-55-18.			
HCS 1559 (Mixture of 2,4'- and 4,4'-Dichlorodiphenyl methane)				3	3 ± 6	Volatility loss < 1%. For more detail see AC-73-55-18.		89.7 ± 2.3	AC-72-M-3
HCS 1558 (Trichlorodiphenyl methane)		26 (35-Day)		3	> 99	No detectable volatility loss (40.1%)		31.3 ± 6.7	AC-73-M-5
				13	63 ± 10	+ (13th-23rd week)			
					27 ± 2	+ (24th-31st week) Slowly degrading trichlorophenyl-acetic acid metabolite formed.			
HCS 1414 (Mixture of 2,3',4-, 3,3',4'-, and 4,3',4'-trichlorodiphenyl methane)				3	10 ± 19	Volatility loss < 1%. For more detail see AC-73-55-18.		84.2 ± 5.1	AC-72-M-3

TABLE I

PRIMARY AND ULTIMATE BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS

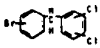
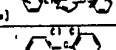
Test Material	Structural Formula	Ultimate Biodegradability (% of Theoretical CO_2 Evolution) Thornton-Buttle-Sturm procedure	Ultimate Biodegradability (% of Theoretical CO_2 Evolution) Mantoto Shake Flask procedure	Primary Biodegradability: Synchronous Activated Sludge (SCAS) Weak Rate (ppm added per cycle)	Primary Biodegradability: Synchronous Activated Sludge (SCAS) Primary 4 Degradation Rate ±95% C.L.	Primary Biodegradability: Comments	Primary Biodegradability: Rate of Initial Conclusion	Primary Biodegradability: Rate for 50% Primary Degradation	[illegible]	[illegible]
				24-Hour Cycle		+, ++ and +/- indicate moderate, strong, and variable inhibition of sludge growth rate respectively.				
PCS 1355 (Trichlorodiphenyl methane mixture)				3	6 ± 11	Volatility loss ≤ 1%. For more detail see AC-73-SS-18.			77.5 ± 2.2	AC-72-M-3
PCS 1356 (Bromodichloro-diphenyl methane mixture)				3	1 ± 10	Volatility loss ≤ 1%. For more detail see AC-73-SS-18.			94.1 ± 4.3	AC-72-M-1
PCS 1358 (Bis(trichloro-benzoyl) benzene)		1 (35-Day)		3	7 ± 10	Volatility loss ≤ 1%.			74.4 ± 4.5	AC-73-M-13
2,2'-Dichloro-diphenyl Ether				3	10 ± 4	+			87.3 ± 4.2	AC-71-M-17
				48-Hour Cycle						
PCS 1315 (Trichlorodiphenyl Ether)				3	24 ± 9	Volatility loss ~7%. ++ For more detail see AC-72-SS-13.			95.1 ± 6.3	AC-72-M-24

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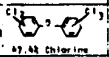
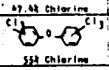
TABLE I

PRIMARY AND ULTIMATE BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS

Test Material	Structure/ Composition	Ultimate Biodegradability (% of Theoretical Ion Evolution)		Primary Biodegradation Rate						
				Semi-Continuous Activated Sludge (SCAS)			Lower Diet Organisms			
		Thomson -Duthie -Sturm procedure	Monsanto Shake Flask procedure	Reaction Rate (ppm added per cycle)	Primary Degradation Rate (ppm/cycle)	Comments	Initial Conc (ppm)	Cycle Conc (ppm)	Primary Degradation Rate (ppm/cycle)	
				24-Hour Cycle		[illegible], and +/- indicate moderate, strong, and variable inhibition of sludge growth rate respectively.				
Pentachlorodiphenyl ether mixture	 47.4% Chlorine			1	1 ± 12	Volatility loss not significant.			72.8 ± 2.6	AC-74-[illegible]-24
Hexachlorodiphenyl ether mixture	 55% Chlorine			1	-4 ± 15	Volatility loss not significant.			78.9 ± 5.3	AC-74-[illegible]-24
MCS 1311 (m-Trichloromethyldiphenyl ether)				3 13	68 ± 15 35 ± 12	Volatility loss 8-10%. ++ ++ For more detail see AC-72-55-13.			83.9 ± 3.7	AC-72-[illegible]-24
MCS 1335 (Mixture of 2-chlorophenyl-n-butylether and 2,4-dichlorophenyl-n-butyl ether)				3	45 ± 6	Volatility loss [illegible]32%. ++ For more detail see AC-72-55-13.			103.4 ± 11.2	AC-72-[illegible]-24
MCS 1343 (n-Butylcentachlorophenyl) ether				1	-1 ± 17	Volatility loss ≤ 1%. +			70.2 ± 2.8	AC-74-[illegible]-5

MONS 004019

TABLE I

PRIMARY AND ULTIMATE BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS

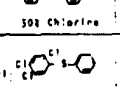
Test Material	Structure/ Composition	Ultimate Biodegradability (% of Theoretical CO2 Evolution)		Primary Biodegradability						
				Semi-Continuous Activated Sludge (SCAS)			Biodegradability			
		Incubation -Quartz -Sturm procedure	Incubation Sludge Flask procedure	Addition Rate (mg/l added per cycle)	Primary Degradation Rate ±95% C.L.	Comments	Initial Conditions	Time Duration of SCAS Primary Interaction	Ultimate Biodegradability Extraction	
				24-Hour Cycle		+, ++, and +/- indicate moderate, strong, and variable inhibition of sludge growth rate respectively				
MCS 1646 (Mixture of 2,3,4,5-Tetrachloroanisole and senior chloro anisole)	 87% 13%	82(28-Day)		1 3 13	> 95 > 99 > 99				78.8 ± 3.2	AC-74-M-6
MCS 1647 [2,3,4,6-Tetrachlorophenyl-1-(2-Chloro-2-propenyl) ether]				1	9 ± 5	Volatility loss ≤ 1%.			69.4 ± 3.7	AC-74-M-7
MCS 1648 (Pentachlorodiphenyl sulfide mixture	50% Chlorine			1	5 ± 11	Volatility loss ≤ 1%. ++			100.6 ± 13.1	AC-74-M-8
MCS 1649 (2,4,5-Trichlorodiphenyl sulfide)				1	4 ± 6	Volatility loss ≤ 1%.			83.2 ± 1.1	AC-74-M-9

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TABLE I

PRIMARY AND ULTIMATE BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS

Test material	Structural Description	Ultimate Biodegradation (% of Theoretical CO2 Evolution) Thirteen-Bottle Manometric Respirometer procedure	Ultimate Biodegradation (% of Theoretical CO2 Evolution) Sealed Flask procedure	Primary Biodegradability: Activated Sludge (ASG) Addition Rate (ppm added per cycle)	Primary Biodegradability: Activated Sludge (ASG) Percent Degradation Rate ±5% C.I.	Primary Biodegradability: Activated Sludge (ASG) Comments	Primary Biodegradability: River Die-away Initial Conc (ppm)	Primary Biodegradability: River Die-away Percent Degradation	Primary Biodegradability: Other (Specify)
				24-Hour Cycle		[illegible]			
PCS 1650 (Dichlorodiphenyl sulfide mixture)				1	19 ± 5	Volatility loss ≤ 13%.			101.6 ± [illegible] AC-74-M-40
PCS 1663 (Tetrachlorothiophene)				1 3	Not detectable	Very high volatility losses.			71.4 ± 7.7 AC-71-M-11
PCS 1664 (Hexachlorotetrahydrothiopyrone)				1 3	Not detectable	Very high volatility losses.			68.8 ± 7.4 AC-71-M-12
Diphenyl Sulfone				3	1 ± 6	Volatility loss ≤ 13%. For more detail see AC-72-SS-16.			98.7 ± 3.4 AC-72-M-6
PCS 1302 (Tolyltolyl sulfone)				3 48-Hour Cycle 3	23 ± 10 42 ± 12	Volatility loss ≤ 13%. For more detail see AC-72-SS-16.			98.8 ± 4.4 AC-71-M-29 AC-72-M-4

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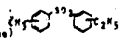
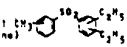
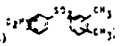
TABLE I

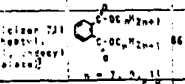
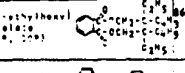
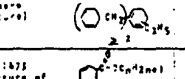
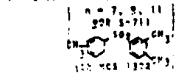
PRIMARY AND ULTIMATE BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS

Test Sample	Structure/ Composition	Ultimate Biodegradability (% of Theoretical Cyc Evolution)		Primary Biodegradability					
		Modified Sturm procedure	Modified Sluka Flask procedure	Sludge (Mixed)					
				Sludge Ratio (ppm added per cycle)	Primary Biodegradation Rate ±95% C.L.	Comments	Initial Condition	Fluid for 30% Primary Biodegradation	Total Biodegradation Rate
				24-Hour Cycle		+, ++, and +/- indicate measurable, strong, and variable inhibition of sludge growth rate respectively.			
MCS 1313 (Tolylethyl phenyl sulfone)				3	36 ± 6	Volatility loss ≤ 1%. For more detail see AC-72-SS-16.			105.6 ± 14.3 AC-72-M-4
MCS 1312 (Tolyldiethyl phenyl sulfone)				3	13 ± 4	Volatility loss ≤ 1%. For more detail see AC-72-SS-16.			97.6 ± 3.8 AC-72-M-5
MCS 1314 (Tolyl isopropyl phenyl sulfone)				3	27 ± 8	Volatility loss ≤ 1%. For more detail see AC-72-SS-16.			111.2 ± 10.0 AC-72-M-6
MCS 1738 (Ethylphenyl xylyl sulfone)				3	17 ± 6	Volatility loss ≤ 1%.			96.8 ± 1.4 AC-75-M-16

MONS 004022

TABLE I

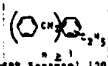
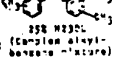
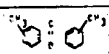
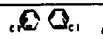
PRIMARY AND ULTIMATE BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS

Test Material	Structure/ Specification	Ultimate Biodegradability (% of Theoretical) by Evolution: Carbon-Dioxide Sturm procedure	Shake Flask procedure	Primary Biodegradability: Semi-Continuous Activated Sludge (SCAS): Initial Rate (ppm aqueous per cycle)	Degradation Rate 95% C.I.	Comments	Initial Concentration	Test Period for Primary Degradation	Viscosity, centistokes
				24-Hour Cycle		n, m, and o/p ratios reported; strong, and variable inhibition of sludge growth rate respectively.			
Santicizer 711 [Di(heptyl, nonyl, undecyl) phthalate]	 n = 7, 9, 11	86 (27-Day)		3 7 13	52 ± 14 48 ± 9 54 ± 7	For more detail see AC-73-55-20 and AC-75-55-8.	1	> 15	100.7 ± 3.1 AC-73-55-5 AC-73-55-8
Di-2-ethylhexyl phthalate (DEHP, DOP)		86 (27-Day)		3 3	70 ± 11 78 ± 3	For more detail see AC-73-55-20 and AC-75-55-8.	1	10	75.1 ± 5.9 AC-73-55-6
Santosol 100 [Para and poly-benzylethyl - benzene mixture]				8	61 ± 8	Volatility loss ~52. Monobenzyl ethyl benzene degraded much more rapidly than polybenzyl components.			96.9 ± 2.6 AC-73-55-19 AC-73-55-20 AC-75-55-8
HCB 1477 [Mixture of 55% 1-771 and 13% HCB 1308]	 n = 7, 9, 11 55% 1-771 13% HCB 1308			3	38 ± 7	Volatility loss 6.18. For more detail see AC-73-55-16.			97.8 ± 5.1 AC-73-55-16

MONS 004023

TABLE 1

PRIMARY AND ULTIMATE BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS

Test Material	Structure/ Composition	Ultimate Biodegradability (% of Theoretical CO_2 Evolution) Sturm Flask procedure	Monsanto Shake Flask procedure	Primary Biodegradability: Semi-Continuous Activated Sludge (SCAS) Addition Rate (ppm acid per cycle)	Primary Biodegradability: Semi-Continuous Activated Sludge (SCAS) Primary Degradation Rate ±95% C.L.	Primary Biodegradability: Semi-Continuous Activated Sludge (SCAS) Comments	Primary Biodegradability: Kow (Log P) Shake Flask (pH)	Primary Biodegradability: Kow (Log P) Partition Coef. Measurement	Primary Biodegradability: Kow (Log P) Calculated Value	Primary Biodegradability: Kow (Log P) Structure Nr.
	 n ≥ 1 50% Santosol 130			24-Hour Cycle		+, ++, and +/- indicate moderate, strong, and varied inhibition of sludge growth rate respectively.				
MCS 1476 (Mixture of 50% Santosol 130, 25% MCS 1302, and 25% MCS 1301)	 25% MCS 1301 (Complex alkylbenzene mixture)			3	21 ± 12	Volatility loss ≤ 1%.			91.0 ± 4.1	AC-73-M-11
MCS 1362 (Diphenyl benzophenone)				3 13	85 ± 9 93 ± 6 52 ± 21	Volatility loss ≤ 1%. + For more detail see AC-73-55-16.			88.0 ± 4.4	AC-73-M-25
Aroclor 1221 (Polychlorinated biphenyl)	 21% Cl 11% Biphenyl 51% Monochloro- 32% Dichloro- 3% Trichloro- 2% Tetrachloro-	45(28-Day)		24-Hour Cycle 1 3 48-Hour Cycle 1 8	 73 ± 21 52 ± 17 51 ± 6 87 ± 5	Volatility loss ≤ 6%. Lot AK-2 For more detail see AC-71-55-6 and AC-72-55-17.			95.9 ± 1.6	AC-71-M-17

MONS 004024

TABLE I

(19)

PRIMARY AND ULTIMATE BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS

Test Material	Structure/ Composition	Ultimate Biodegradability (Final Microbial Population) 30-Day Exposure: Percent Stable Chlorine Groups	Ultimate Biodegradability (Final Microbial Population) 30-Day Exposure: Percent Stable Phenyl Groups	Primary Biodegradability (Semi-Continuous Activated Sludge Test) Addition Rate (ppm added per cycle)	Primary Biodegradability (Semi-Continuous Activated Sludge Test) Primary Biodegradation Rate (ppm C.L. per hour)	Comments	Concentration	Half-Life for Primary Biodegradation	Reference
				24-Hour Cycle		Data and information indicate primary, and ultimate biodegradation of fluids greater than current levels.			
PCB 1063 (Polychlorinated biphenyl)	 39% Cl 0.1% Biphenyl 23% Monochloro 72% Dichloro 6% Trichloro	43(35-Day) Lot QA47		1	74 ± 7	+ Lot QA47			94.7 ± 1.9 AC-71-M-17
				48-Hour Cycle					
				1	56 ± 16	Sample OR15A91 Volatility loss ~69.			
Arochlor 1232 (Poly Brominated-biphenyl)	 21% Cl, 18% Br			48-Hour Cycle					
				1	54 ± 19	Sample OR16B82 For more detail see AC-71-SS-6.			93.8 ± 1.7 AC-71-M-17
Arochlor 1016 (Polychlorinated biphenyl)	 42% Cl 1% Monochloro- 23% Dichloro- 27% Trichloro- 21% Tetra- 1% Penta-	4(28-Day) 12(35-Day)		48-Hour Cycle					
				1	33 ± 14	Volatility loss ~ 88. Sample OR15B91 For more detail see AC-71-SS-2, AC-71-SS-6 and AC-72-SS-19.			91.6 ± 1.2 AC-71-M-17

MONS 004025

TABLE I

PRIMARY AND ULTIMATE BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS

Test Material	Structure/ Composition	Ultimate Biodegradability (% of Theoretical CO_2 Evolution) Thompson -Double -Sturm procedure	Ultimate Biodegradability (% of Theoretical CO_2 Evolution) Monsanto Shake Flask procedure	Primary Biodegradability: Semi-Continuous Activated Sludge (SCAS) Addition Rate (ppm added per cycle)	Primary Biodegradability: Semi-Continuous Activated Sludge (SCAS) Primary & Degradation Rate ±55° C.C.	Primary Biodegradability: Comments	Primary Biodegradability: Initial Conc(ppm)	Primary Biodegradability: [illegible]	Primary Biodegradability: [illegible]	
						+, ++, and +/- indicate moderate, strong, and marginable inhibition of sludge growth rate respectively.				
Aroclor 1242 (Polychlorinated Biphenyl)	 42% Cl 1% Monochloro- 16% Dichloro- 49% Trichloro- 25% Tetrachloro- 8% Pentachloro- 1% Hexachloro-	11(35-Day)		48-Hour Cycle 1	26 ± 15	Volatility loss ~4%. Lot AK-255 For more detail see AC-71-SS-6 and AC-72-SS-19.			83.4 ± 1.3	AC-71-M-1
Aroclor 1254 (Polychlorinated Biphenyl)	 54% Cl 1% Trichloro- 21% Tetrachloro- 45% Pentachloro- 23% Hexachloro- 6% Heptachloro-			48-Hour Cycle 1	15 ± 38	Volatility loss ≤ 1%. Lot AK-38 For more detail see AC-71-SS-6.			75.5 ± 2.4	AC-71-M-1

MONS 004026

TABLE 1

PRIMARY AND ULTIMATE BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS

Test Material	Structure/ Composition	Ultimate Biodegradability (% of Theoretical CO_2 Evolution) Thompson -Duthie -Sturm procedure	Ultimate Biodegradability (% of Theoretical CO_2 Evolution) Manzanto Shake Flask procedure	Primary Biodegradability: Semi-Continuous Activated Sludge (SCAS) Addition Rate (ppm added per cycle)	Primary Biodegradability: Semi-Continuous Activated Sludge (SCAS) Primary % Degradation Rate ±95% C.L.	Primary Biodegradability: Semi-Continuous Activated Sludge (SCAS) Comments	Primary Biodegradability: Sludge (SCAS) Initial Condition	Primary Biodegradability: Sludge (SCAS) Recip. or 50% Primary Degradation	Primary Biodegradability: Sludge (SCAS) % Recovery (Hexane Extractable)	Primary Biodegradability: Sludge (SCAS) % Recovery (Hexane Extractable)
				24-Hour Cycle		+/-, +, and -/- indicate moderate, strong, and very-slow inhibition of sludge growth rate respectively.				
Dow-Corning 705 (Methylchenyl polysiloxane)				3	16 ± 13	No detectable volatility loss. For more detail see AC-73-55-23.			75.6 ± 8.2	AC-73-M-
HCT 1288 (Bromobichloro benzene)				1	14 ± 8	Volatility loss ~4%. +/- For more detail see AC-72-55-15.			77.3 ± 2.6	AC-[illegible]-M-





MONS 004027