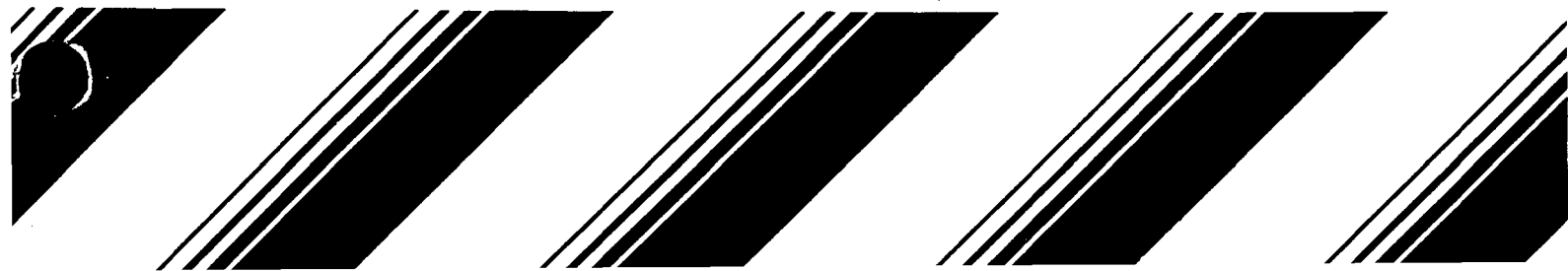

Toxic Substances



Chemical Fate Test Guidelines



CHEMICAL FATE TESTING GUIDELINES AND
SUPPORT DOCUMENTS

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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16. Abstract (Limit: 200 words) These documents constitute a set of 21 chemical fate test guidelines (and, in some cases, support documents) that may be cited as methodologies to be used in chemical specific test rules promulgated under Section 4(a) of the Toxic Substances Control Act (TSCA). These guidelines cover testing for physical and chemical properties, transport processes and transformation processes. The guidelines will be published in loose leaf form and updates will be made available as changes are dictated by experience and/or advances in the state-of-the-art.			
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PREAMBLE

The following guidelines describe methods for performing testing of chemical substances under the Toxic Substances Control Act (TSCA). These methods include the state-of-the-art for evaluating certain properties, processes and effects of chemical substances. They are intended to provide guidance to test sponsors in developing test protocols for compliance with test rules issued under Section 4 of the TSCA. They may also provide guidance for testing which is unrelated to regulatory requirements. Support documentation is included for some of these guidelines. It is expected that additional guidelines and support documentation will be incorporated later as the state-of-the-art evolves or the need for them warrants.

Since these guidelines are divided into three sections which cover the diverse areas of health effects, environmental effects and chemical fate testing, there are some differences in the ways they are presented. These differences are explained in an introduction prepared for each section.

INTRODUCTION TO CHEMICAL FATE TESTING GUIDELINES

In these guidelines, methods have been categorized under the general headings (1) physical and chemical properties, (2) transport processes and, (3) transformation processes. These categories are arbitrary and are only a convenient classification. As complex microcosms are developed and validated for use in fate studies, it may be appropriate to add a fourth category for them since they often include the simultaneous evaluation of various transport and transformation processes. The categorization and numbering of the chemical fate guidelines allow for future supplementations with methods for additional parameters (e.g. gas phase photolysis) or other approaches to parameters already addressed.

The environmental impact of a chemical substance depends on the environment into which it is released, the concentration of the chemical, the duration and nature of any exposure, and its toxicity to organisms at risk or its effects on abiotic structures or processes. Potential adverse effects on populations or inanimate receptors at risk are highly dependent upon the environmental fate of the chemical substance, where fate is defined as the disposition of the substance resulting from transport and transformation processes.

An assessment of the fate of a chemical released to the environment will depend, in part, upon laboratory data used to evaluate properties and processes which influence transport and transformations. Test procedures identified in these guidelines are the first in a series that will be needed to develop reliable

and adequate data on the physical, chemical and environmental persistence characteristics of chemical substances or mixtures. Many of the fate guidelines contain complete descriptions of appropriate laboratory procedures. For some of the guidelines, it is believed that readily citable and widely available methods described by the Organization for Economic Cooperation and Development (OECD), the American Society for Testing and Materials (ASTM) and others provide adequate examples, and in those guidelines the reader is directed to appropriate sources.

Efforts are underway to develop and validate additional methods of varying levels of complexity. This includes the development of estimation techniques for many of the chemical and physical characteristics to guide in the selection of test methods or to preclude the necessity for certain laboratory testing. Also under development are complex testing methods designed to provide kinetic (rate) data and information on likely transformation products in simulations of selected environments. Between these two extremes, in method complexity, there is a need for appropriate methods for additional properties and processes and for improvements in existing guidelines. For example, it is expected that methods for determining gas phase absorption spectra, volatilization rates from soil and water, and gas phase photolysis will be available soon. When their development and validation have been completed, they will be added to these guidelines. It is also anticipated that individual methods described in these guidelines will be revised, discarded or replaced, when appropriate.

CHEMICAL FATE TESTING GUIDELINES

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PHYSICAL AND CHEMICAL PROPERTIES

CG-1050
August, 1982

ABSORPTION IN AQUEOUS SOLUTION:
ULTRAVIOLET/VISIBLE SPECTRA

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

ABSORPTION IN AQUEOUS SOLUTION:
ULTRAVIOLET/VISIBLE SPECTRA

I. PURPOSE

This Test Guideline references methodology to develop ultraviolet and visible absorption spectra of a chemical in aqueous solution. The data may be used to evaluate the potential for sunlight photochemical transformation in aqueous media.

II. TEST PROCEDURES

Examples of methods for determining the absorption spectra of chemicals in solution are given in OECD Guideline No. 101, (OECD), "UV-VIS Absorption Spectra" and in the U.S. EPA Discussion of premanufacture testing (USEPA 1979). The U.S. sales agent for the OECD guidelines is OECD Publications and Information Center, Suite 1207, 2750 Pennsylvania Ave. NW, Washington, DC 20006.

III. REFERENCE

OECD. 1981. Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals.

USEPA. 1979. U.S. Environmental Protection Agency. Office of Toxic Substances. Toxic Substances Control: Discussion of premanufacture testing policy and technical issues; request for comment. (44 FR 16267-8).

CG-1100
August, 1982

BOILING TEMPERATURE

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

BOILING TEMPERATUREI. PURPOSE

This Test Guideline references methodology to develop data on the equilibrium boiling temperature of chemical substances and mixtures at environmentally relevant pressures. The data may be used to characterize the physical state of the material, to evaluate the manner and extent that the chemical will be transported in the environment, and as a guide in the selection and design of other tests.

II. TEST PROCEDURES

Examples of methods for determining the boiling temperature or boiling point range of chemical substances or mixtures are listed in Table 1, "Test Procedures for Equilibrium Boiling Temperature" and in OECD Guideline No. 103, (OECD), "Boiling Point/Boiling Range." The codes to standardized bodies listed in Table 1 are:

ANSI	American National Standards Institute
ASTM	American Society for Testing and Materials
BSI	British Standards Institution
CIPAC	Collaborative International Pesticides Analytical Council
DIN	Das Ist Norm (earlier Deutsche Industrienormen)
IP	Institute of Petroleum
ISO	International Organization for Standardization
USP	United States Pharmacopeia XVIII

These Test Guidelines are available for purchase as follows:

- (1) ANSI, BSI, DIN, and ISO standards are available from:
Sales Department, American National Standards Institute,
1430 Broadway, New York, NY. 10018.
- (2) ASTM standards are available from: American Society for
Testing and Materials, 1916 Race St., Philadelphia, PA.
- (3) CIPAC standards are available from: National Agricultural
Chemicals Association, 1155 Fifteenth Street, NW,
Washington, DC 20005.

- (4) IP standards are available from: Hayden and Son Ltd., Spectrum House, Alderton Cres., London NW4 3XX UK.
- (5) OECD methods are available from: OECD Publications and Information Center, Suite 1207, 2750 Pennsylvania Ave. NW, Washington, DC 20006.
- (6) USP standards are available from: U.S. Pharmacopeia, Bethesda, MD 20014.

Table 1. Test Procedures for Equilibrium Boiling Temperature

Instrumental Technique	Standard Method or Other Description	Applicability		
		Pure Liquid	Pure Solids	Liquid Mixtures & Impure Liquids

Boiling temperature of liquid (Ebulliometric)

Cottrell distillation	ASTM D 1088 ^a	X		X
Ramsay/Young	Ref. A ^b pp. 56, 57	X		
Immersed thermometer	ASTM D 1120 ^c	X		X
Osborn/Douslin	Ref. B ^b pp. 222-225	X		X
	Ref. A ^b pp. 66-69	X		X
Herington/Martin/ Ambrose	Ref. A ^b pp. 66-69	X		X
Swietoslawski	Ref. B ^b pp. 225-227	X		X
	Ref. D ^b	X		X

Condensation temperature of vapor (Ebulliometric)

Distillation flask	ASTM D 86/E 133 ^d	X		X
or Claisson flask	ASTM D 1078/E 133	X		X
	ASTM D 1160	X		X
	Ref. A ^b pp. 57-59	X		X
	Ref. B ^b pp. 218-221	X		X
	Ref. C ^b pp. 984,1000- 1003	X		X

Hoover/John/Mellon	Ref. A ^b pp. 54-56	X	X
Hickman/Weyarts	Ref. A ^b pp. 59, 71-74	X	X
Fenske	Ref. A ^b p. 59	X	X
Willingham et al./ Stull	Ref. A ^b p. 60	X	X
Swietoslowski	Ref. B ^b p. 228-230	X	X
Fractionating column	ASTM D 285	X	X
	ASTM D 2892	X	X
	USP I or II	X	X
	ISO R 918 ^e	X	X

Pressure equilibration (Static methods)

Isoteniscope	ASTM D 2879	X	X
	Ref. A ^b pp. 49-50	X	X
Submerged bulb or capillary			
Sowoloboff/ Rosenblum	Ref. A ^b pp. 79-81	X	
Smith/Menzies	Ref. A ^b pp. 79-81	X	X
Garcia	Ref. A ^b pp. 79-83	X	
	Ref. D ^b	X	X

^a Equivalent standard: ANSI 1088

^b Reference A, Thomson, Douslin (1971); Reference B, Anderson (1971);
Reference C, Raw et al. 1970. Reference D, OECD 1979a,b

^c Equivalent standard: ANSI 1120

^d Equivalent standards: ANSI D86/E133, IP 123, BS 4349, DIN
51751, CIPAC MT70

^e Equivalent standards: BS 4591. DIN 53171

III. REFERENCES

ANSI. (Latest Edition). American National Standards Institute. Book of Standards.

ASTM. (Latest Edition). American Society for Testing and Materials. Annual Book of ASTM standards.

BSI. (Latest Edition). British Standards Institute. Book of Standards.

CIPAC. 1970. Collaborative International Pesticides Analytical Council. CIPAC Handbook, Volume 1. Analysis of Technical and Formulated Pesticides.

DIN. (Latest Edition). Das Ist Norm. Book of Standards.

IP. (Latest Edition). Institute of Petroleum. Book of Standards.

ISO. (Latest Edition). International Organization for Standardization.

OECD. 1981. Organization for Economic Cooperation and Development. OECD Guidelines for Testing Chemicals.

USP. 1970. Pharmacopeia of the United States of America. Eighteenth Revision.

CG-1150

August, 1982

DENSITY/RELATIVE DENSITY

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

DENSITY/RELATIVE DENSITYI. INTRODUCTION

This Test Guideline references methodology to develop data on density and relative density (specific gravity) of chemical substances and mixtures. The data may be used to evaluate the manner and extent that chemicals will be transported in the environment and the places they will be deposited.

II. TEST PROCEDURES

Examples of methods for determining density and relative density of gaseous, liquid, or solid chemical substances are listed in Table 1, "Standard Density - Measurement Techniques Referenced in this Test Guideline" and in OECD Guideline No. 109, (OECD 1981), "Density of Liquids and Solids." The codes to standardizing bodies listed in Table 1 are:

ANSI - American National Standards Institute.

ASTM - American Society for Testing and Materials.

BSI - British Standards Institution.

IP - Institute of Petroleum.

CIPAC - Collaborative International Pesticides Analytical
Council.

DIN - Das Ist Norm (earlier Deutsche Industrienormen).

API - American Petroleum Institute.

ISO - International Organization for Standardization.

These Test Guidelines are available for purchase as follows:

- (1) ANSI, BSI, ISO, and DIN standards are available from:
Sales Department, American National Standards Institute,
1430 Broadway, New York, NY 10018.
- (2) ASTM standards are available from: American Society
for Testing and Materials, 1916 Race Street,
Philadelphia PA 19103.
- (3) API methods are available from: American Petroleum
Institute 2101 L Street NW., Washington, DC 20037.
- (4) IP methods are available from: Hayden and Son Ltd.,
Spectrum House, Alderton, Cres., London NW4 3XX U.K.
- (5) CIPAC methods are available from: National Agricultural
Chemicals Association, 1155 Fifteenth Street, NW.,
Washington, DC 20005.
- (6) OECD methods are available from: OECD Publications and
Information Center, Suite 1207, 2750 Pennsylvania Ave. NW,
Washington, DC 20006.

Table 1
Standard Density-Measurement Techniques Referenced in this Standard

Technique	Class of Substance			Standardizing Body and Identification Number					
	Gas	Liq	Solid	ANSI	ASTM	BSI	CIPAC	DIN	ISO
ideal gas calculation	X								
gas density balance	X			Z77.12	D 1070 (26) ^a				
hydrometer		X		Z11.84 Z11.147 D 891	D 1298 (23, 40) ^b D 1657 (23) D 891 (29)	4714	MT3	51757 12791 -2, 3	R387 R649
hydrostatic displacement		X	chunks	K65.8 C 830 D 891	D 792 (35) C 830 (17) D 891 (29) C 693 (17)			53479	R1183
sink-float comparator			chunks	C 729	C 729 (17)				
pycnometer-narrow-mouth	Sprengel-Ostwald	X							
	Lipkin bicapillary	X		Z11.62 Z11.120 D 3505	D 941 (23) ^c D 1481 (23, 40) D 3505 (29)	4699	MT3	51757 12798	
	Bingham	X		D 891 D 1217 Z11.119	D 891 (29) D 1217 (23) D 1480 (23)			12807	
pycnometer-wide-mouth	volumetric flask	X	powder crystals chunks	D 153 D 1076	D 153 (28) D 1076 (37)				
	thermometer stoppered	X	powder crystals chunks	C 135 K65.8	C 135 (17) D 792 (35)				
	capillary stopper	X	powder crystals chunks	D 1076 D 153 D 1817 K65.8 D 891	D 1076 (37) D 153 (28) D 1817 (37) D 792 (35) D 891 (29)	5093	MT3	12797 12809	
	Johnson and Adams	X	powder crystals chunks						
	gas comparison pycnometer		powder	C 604	C 604 (17)				

^a In parentheses by ASTM Test Standard number is the volume number in which the standard appeared in the 1978 Annual Book of ASTM Standards.

^b Adopted by American Petroleum Institute as API Standard No. 2547 and by the Institute of Petroleum as IP Standard No. 160.

^c Adopted by the General Services Administration as Method 402, Federal Test Method Standard 791b.

III. REFERENCES

ANSI. (Latest Edition). American National Standards Institute. Book of Standards.

ASTM. (Latest Edition). American Society for Testing and Materials. Annual Book of ASTM Standards.

API. (Latest Edition). American Petroleum Institute. Book of Test Standards.

BSI. (Latest Edition). British Standards Institution. Book of Standards.

CIPAC. 1970. Collaborative International Pesticides Council. CIPAC Handbook, Volume 1. Analysis of Technical and Formulated Pesticides.

DIN. (Latest Edition). Das Ist Norm. Book of Standards.

IP. (Latest Edition). Institute of Petroleum. Book of Standards.

ISO. (Latest Edition). International Organization for Standards. Book of Standards.

OECD. 1981. Organization of Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals.

CG-1200
August, 1982

DISSOCIATION CONSTANTS IN WATER

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

DISSOCIATION CONSTANTS IN WATERI. PURPOSE

This Test Guideline references methodology to develop data on acid dissociation constants of chemical substances that are acids or bases. The data may be used to evaluate the transport of a substance in the environment, the kinds of reactions the substance will undergo, the effects of pH on those reactions, the probable sites and modes of action of the substance in humans and the environment, and as a guide in the design of other tests for physical and chemical properties and for effects on human health and the environment.

II. TEST PROCEDURES

Examples of methods for determining dissociation constants are cited by Serjeant and Dempsey (1979), Perrin (1965, 1969), Kortum Vogel and Andrussow (1961), and in OECD Guideline No. 112, (OECD), "Dissociation Constants in Water." The U.S. sales agent for this OECD Guideline is OECD Publications and Information Center, Suite 1207, 1750 Pennsylvania Ave. NW, Washington, DC 20006.

III. REFERENCES

- Kortum G, Vogel W, Andrussow K. 1961. Dissociation Constants for Organic Acids in Aqueous Solution. London: Butterworths.
- Kortum G, Vogel W, Andrussow K. 1960. Dissociation constants of organic acids in aqueous solution. Pure Appl Chem No. 2, 3. 186-536.
- OECD. 1981. Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals.
- Perrin DD. 1965. Dissociation Constants of Organic Bases in Aqueous Solution. London: Butterworths.
- Perrin DD. 1969. Dissociation Constants of Inorganic Acids in Bases in Aqueous Solution. London: Butterworths. Also, Pure Appl Chem 20, No. 2.
- Serjeant EP, Dempsey B. 1979. Ionization Constants of Organic Acids in Aqueous Solution. IUPAC Data Series No. 23. Oxford: Pergamon Press.

CG-1250
August, 1982

HENRY'S LAW CONSTANT

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

HENRY'S LAW CONSTANTI. PURPOSE

This Test Guideline references methodology to develop data on Henry's law constant of chemical substances. This data may be used to evaluate the potential for volatilization from water and is essential in determining rates of transfer from water.

II. TEST PROCEDURES

An example of a method for determining Henry's law constant is described by MacKay, Shiu, and Sutherland (1979).

III. REFERENCE

Mackay D, Shiu WY, and Sutherland RP. 1979. Determination of air-water Henry's law constant for hydrophobic pollutants. Environ Sci Technol 13:333-337.

CG-1300
August, 1982

MELTING TEMPERATURE

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

MELTING TEMPERATUREI. INTRODUCTION

This Test Guideline references methodology to develop data on the melting temperature or melting temperature range of chemical substances and mixtures. The data may be used to assess the potential for movement of materials in the environment, to determine the physical state of the substance under environmental conditions and to evaluate possible health and environmental effects.

II. TEST PROCEDURES

Examples of methods for determining the melting temperature or melting temperature range of chemical substances or mixtures are listed in Table 1, "Standard Temperature Technique Referenced in this Test Guideline" and in OECD Guideline No. 102, (OECD 1981), "Melting Point/Melting Range." The codes to standardized bodies listed in Table 1 are:

ANSI - American National Standards Institute.

ASTM - American Society for Testing and Materials.

TAPPI - Technical Association for the Pulp and Paper
Industry.

FTS - Federal Test Standards.

BSI - British Standards Institution.

IP - Institute of Petroleum.

CIPAC - Collaborative International Pesticides Analytical
Council.

These Test Guidelines are available for purchase as follows:

- (1) ANSI and BSI standards are available from: Sales Department, American National Standards Institute, 1430 Broadway, New York, NY 10018.
- (2) ASTM standards are available from: Testing and Materials 1916 Race St., Philadelphia PA 19103.
- (3) CIPAC standards are available from: National Agricultural Chemicals Association, 1155 Fifteenth Street, NW., Washington, DC 20005.
- (4) FTS standards are identical to the corresponding ASTM standards and should be obtained from the same source as ASTM standards.
- (5) IP standards are available from: Hayden and Son Ltd., Spectrum House, Alderton Cres., London NW4 3XX U.K.
- (6) OECD methods are available from: OECD Publications and Information Center, Suite 1205, 2750 Pennsylvania Ave. NW, Washington, DC 20006.
- (7) TAPPI standards are available from: TAPPI Press, Technical Association of the Pulp and Paper Industry. One Dunwoody Park, Atlanta GA 30338.

**TABLE 1-STANDARD MELTING TEMPERATURE TECHNIQUES
REFERENCED IN THIS STANDARD**

TECHNIQUE	STANDARDIZING BODY ¹					
	ANSI/ASTM ^{2,3}	TAPPI	FST ⁴	BSI	IP	CIPAC
Thiele Tube	E 324-79 [30] D 1519-68 ⁵ (1974) [37]					MT2
Cooling Curve	D 87-77 [23] [20]	T630-os-71	791B#1402.5	4695	55	MT1
Fisher-Johns	D 789-78a [36] D 1457-78 [36] D 2116-79 [36] D 2133-78 [36]					
Microscope Hot Stage	D 2117-64 (1978) [35]					
Kofler Hot Bench	D 3451-76 [27]					
Drop Melting Point	D 127-63 (1977) [23] [20]	T634-os-70	791B#1401.4	5090	133	
Pour Point	D 97-66 (1978) [23] [40]		791B#201.9	4452	15	
Congealling Point	D 938-71 (1976) [23]			5088	76	

¹Names of standardizing bodies are given in text.

²Joint standard unless otherwise specified.

³In brackets by ASTM Test Standard number is the volume number in which the standard appeared in the 1979 or 1980 Annual Book of ASTM Standards. Numbers in parentheses indicate the year of last reapproval.

⁴Identical to ANSI/ASTM standard.

⁵ASTM only.

III. REFERENCES

ANSI. (Latest Edition). American National Standards Institute. Book of Standards.

ASTM. (Latest Edition). American Society for Testing and Materials. Annual Book of ASTM Standard.

BSI. (Latest Edition). British Standards Institution. Book of British Standards.

CIPAC. 1970. Collaborative International Pesticides Analytical Council. CIPAC Handbook, Volume 1. Analysis of Technical and Formulated Pesticides.

FTS. (Latest Edition). Federal Test Standards. Book of Standards.

IP. (Latest Edition). Institute of Petroleum. Book of Standards.

OECD. 1981. Organization for Economic Cooperation and Development. OECD Guideline for Testing of Chemicals.

TAPPI. 1971. Technical Association of the Pulp and Paper Industry. Book of Standards.

CG-1350
August, 1982

PARTICLE SIZE DISTRIBUTION/FIBER LENGTH AND DIAMETER
DISTRIBUTIONS

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

PARTICLE SIZE DISTRIBUTION/FIBER LENGTH AND DIAMETER DISTRIBUTIONSI. PURPOSE

This Test Guideline references methodology to (1) develop data on the effective hydrodynamic radius or effective Stokes' radius (R_s) and/or, (2) provide histograms of the length and diameter distributions of fibers. The data may be used to evaluate the transportation and sedimentation of insoluble particles in water and air. In the special case of materials which can form fibers, an additional set of measurements is also recommended to help identify potential health hazards arising from inhalation or ingestion.

II. TEST PROCEDURES

Appropriate methods are described in OECD Guideline No. 110, (OECD), "Particle Size Distribution/Fiber Length and Diameter Distributions". The U.S. sales agent for the OECD guidelines is OECD Publications and Information Center, Suite, 1207, 1750 Pennsylvania Ave. NW. Washington DC 20006.

III. REFERENCES

OECD. 1981. Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals.

CG-1400
August, 1982

PARTITION COEFFICIENT (n-OCTANOL/WATER)

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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PARTITION COEFFICIENT (n-OCTANOL/WATER)I. INTRODUCTIONA. Background and Purpose

Bioconcentration, the accumulation of a substance in living tissues or other organic matter as a result of net chemical uptake from the medium (e.g., water), is a factor in determining the movement of a chemical in the environment and the potential effects of the chemical on biota. Hydrophobic chemicals that are present in the aqueous environment at subtoxic concentrations may accumulate to toxic levels once inside organisms, presumably through diffusion into nonpolar cell components, where they accumulate because of their greater solubility. Further movement of the substance in living tissues may occur as a result of ingestion of lower trophic level organisms, i.e., food chain effects.

The tendency of an organic chemical to bioconcentrate in living cells can be inferred from the value of the octanol/water partition coefficient, K_{OW} (Neely et al. 1974). Chemicals with K_{OW} less than 10 will not significantly partition into, or tend to accumulate in, living cells. Chemicals with K_{OW} greater than 10^6 will tend to accumulate. Chemicals that exist in the environment at subtoxic levels may bioconcentrate to toxic levels once inside organisms.

This test guideline describes a detailed and commonly used procedure for determining the octanol/water partition coefficient of organic chemicals.

B. Definitions and Units

The octanol/water partition coefficient (K_{OW}) is defined as the equilibrium ratio of the molar concentrations of a chemical in n-octanol and water, in dilute solution. K_{OW} is a constant for a given chemical at a given temperature. Since K_{OW} is the ratio of two molar concentrations, it is a dimensionless quantity. Sometimes K_{OW} is reported as $\log_{10} K_{OW}$.

The mathematical statement of K_{OW} is:

$$K_{OW} = \frac{C_{\text{octanol}}}{C_{\text{water}}} , \quad (1)$$

where C is the molar concentration of the solute in n-octanol and water at equilibrium at a given temperature.

According to Nernst (1891) the distribution law applies only to individual molecular species in solution. If a molecule dissociates or associates in octanol and water, then equation (1) must be modified. In general, if α represents the fraction of the total solute that is dissociated or associated, assuming that either association or dissociation occurs in each solvent, then

$$K_{OW} = \frac{(1 - \alpha_{\text{oct.}}) C_{\text{oct.}}}{(1 - \alpha_{\text{water}}) C_{\text{water}}} , \quad (2)$$

since $(1-\alpha)$ gives the fraction of unchanged molecules in each phase. For the special case where no association takes place in octanol, equation (2) reduces to

$$K_{ow} = \frac{C_{oct.}}{(1 - \alpha_{water}) C_{water}} , \quad (3)$$

where α_{water} represents the fraction of the total solute that has dissociated in water.

C. Principle of the Test Method

The conventional method for determining the octanol/water partition coefficient is carried out by distributing a chemical between n-octanol and water in a vessel at constant temperature and measuring the concentration in the two liquid phases after equilibration. Numerous researchers use the conventional method for determining K_{ow} and have published papers using this method (e.g., Fujita et al. 1964; Hansch and Anderson 1967; Leo et al. 1971; Chiou et al. 1977).

D. Applicability and Specificity

The test guideline is designed to determine the octanol/water partition coefficient of solid or liquid organic chemicals in the range 10 to 10^6 . For chemicals whose values lie outside this range, K_{ow} should be characterized as less than 10 or greater than 10^6 with no further quantification.

II. TEST PROCEDURES

A. Test Conditions

1. Special Laboratory Equipment

- (1) A thermostatic bath, chamber, or room with a shaker and temperature control as specified in Temperature Control below;
- (2) an ultracentrifuge with temperature control as specified in Temperature Control below;
- (3) stainless steel or glass centrifuge tubes with sealable caps. Special glass centrifuge tubes can be used up to approximately 12,000 G and stainless steel tubes can be used at high G values;
- (4) a mechanical shaker; and
- (5) a pH meter capable of resolving differences of 0.1 pH unit or less.

2. Temperature Control

It is recommended that the temperature of the water bath, or chamber, or room, and the ultracentrifuge be controlled to $(25 \pm 1)^{\circ}\text{C}$.

3. Solvents

It is extremely important that n-octanol, purified as described in Section II.B.1.a, and distilled or reagent grade water, i.e., ASTM Type II water or an equivalent grade, be used. ASTM Type II water is described in ASTM D-1193-77, "Standard Specification for Reagent Water."

4. Concentration of Solute

It is extremely important that all experiments be carried out at solute concentration $C < 0.01M$ (Molar) in octanol and water and well below the solubility in either phase.

5. Equilibration Time

In general, 1 hour of gentle agitation is sufficient to reach equilibrium. For surfactants, at least 16 hours is required to reach equilibrium.

6. Octanol/Water Volume Ratio

It is recommended that the ratio of the volumes of the two liquids be adjusted as appropriate for the relative solubility of the chemical in octanol and water. By adjusting the volumes, concentration errors (resulting from analytical errors) are minimized and errors resulting from dividing large numbers by small numbers are kept to a minimum.

7. Chemical Analysis of the Octanol and Water Phases

In determining the K_{OW} value for any given solute, it is important that both the octanol and water phases be analyzed for the chemical. An analytical method should be selected that is most applicable to the analysis of the specific chemical. Chromatographic methods are preferable because of their compound specificity in analyzing the parent chemical without interference from impurities. Whenever practicable, the chosen analytical method should have a precision with ± 5 percent.

8. Emulsification and Ultracentrifugation

It is important that gentle shaking be used to minimize the formation of emulsions. Ultracentrifugation is necessary to separate troublesome emulsions and to separate the octanol and water phases. Therefore, it is very important that ultracentrifugation be carried out at 25°C for 20 minutes in a temperature controlled ultracentrifuge. The acceleration (G) value required to break the emulsion and to achieve complete separation of the octanol and water phases can be determined by trial-and-error experimentation.

9. Equilibration Vessel

If feasible, equilibration should be carried out in a centrifuge tube (stainless steel or glass) with a sealable cap. It is important that the centrifuge tubes be almost completely full. In this way, partitioning with air will be minimized,

especially for volatile chemicals, and the mixture will be completely mixed.

Very hydrophobic chemicals, with K_{OW} in the order of 10^4 to 10^6 , require relatively large volumes of the aqueous phase. Hence, for these chemicals, it is recommended that equilibration be carried out in a large ground-glass stoppered flask.

10. Speciation Effects

The octanol/water partition coefficient, K_{OW} , has been defined in Section I.B. The mathematical statement of K_{OW} is given by equation (1).

If the chemical does not associate or dissociate in octanol and water, then use equation (1) and determine K_{OW} at molar concentrations $C < 0.01M$ and $C_1 = 0.01C$.

If the chemical associates in octanol or water or in both liquids, then use equation (1) and determine K_{OW} at molar concentrations $C < 0.01M$, $C_1 = 0.1C$, $C_2 = 0.01C$, $C_3 = 0.001C$ When K_{OW} is constant at two molar concentrations differing by a factor of 10, then the effect of association has been minimized or eliminated.

If a molecule dissociates or associates in octanol and water, then it is extremely important that equation (1) be modified to take into account such speciation changes as ionization, aggregation, and hydration. For the special case, where no association takes place in octanol and only dissociation takes

place in water, equation (3) can be used. For chemicals that reversibly ionize or pronate (e.g., carboxylic acids, phenols, or anilines), use equation (3) with water buffered at pH 5.0, 7.0, and 9.0. It is recommended that buffers described in Section II.B.1.b be used.

11. Prerinsing of all Transfer Vessels

It is important that all transfer vessels be prerinsed with a portion of the equilibrium phase prior to transfer for analysis. This is especially important for very hydrophobic chemicals.

B. Preparations

1. Reagents and Solutions

a. Octanol and Water

Very pure n-octanol can be obtained as follows: wash pure n-octanol (minimum 98 percent pure) sequentially with 0.1N H₂SO₄, with 0.1N NaOH, then with distilled water until neutral. Dry the n-octanol with magnesium sulfate and distill twice in a good distillation column under reduced pressure [b.p. about 80°C at 0.27 kPa (2 torr)]. It is important that the octanol produced be at least 99.9 percent pure. Alternatively, a grade equivalent to Fisher Scientific Co. No. A-402 "Certified Octanol-1" can be used. It is important that distilled or reagent grade (ASTM Type II) water be used.

b. Buffer Solutions

Prepare buffer solutions using reagent grade chemicals in distilled or reagent grade water as follows:

pH 5.0 - To 250 mL of 0.1M potassium hydrogen phthalate add 113 mL of 0.1M sodium hydroxide; adjust final volume to 500 mL with reagent grade water.

pH 7.0 - To 250 mL of 0.1M potassium dihydrogen phosphate add 145 mL of 0.1M sodium hydroxide; adjust final volume to 500 mL with reagent grade water.

pH 9.0 - To 250 mL of 0.07M borax add 69 mL of 0.1M HCl; adjust final volume to 500 mL with reagent grade water.

Check the pH of each buffer solution at 25°C with a pH meter and adjust to pH 5.0, 7.0, or 9.0, if necessary.

c. Presaturation of the Solvents

Before a partitioning experiment is carried out, prepare octanol saturated with water and water saturated with octanol. Add purified n-octanol to a large stock bottle and sufficient distilled water to saturate it. Shake the flask for 24 hours on a mechanical shaker. Then allow sufficient time for the mixture to stand so that the two phases separate. Repeat this procedure using another large stock bottle containing distilled water and

sufficient octanol to saturate it. The desired quantities of the presaturated solvents can be taken from these stock bottles for each partition experiment.

d. Preparation of Test Solution

Prepare a 10^{-2} to 10^{-3} M solution of the test material in octanol.

C. Performance of the Test

- (1) Add a small volume of the octanol test solution (1 to 5 mL) to a centrifuge tube with a sealable cap as described in Section II.A.9.
- (2) Add the required volume of water to the centrifuge tube as described in Section II.A.6. The volume of water required is variable, depending upon the amount of chemical required for the analysis. Generally, 20-40 mL of water should be sufficient. Make sure that the centrifuge tube is almost completely full. In this way, partitioning with air will be minimized. This is important, especially when determining K_{ow} for volatile chemicals.
- (3) Equilibrate the samples at 25°C in a constant temperature bath, chamber, or room by gently shaking the centrifuge tube for 1 hour. Avoid vigorous shaking that may cause troublesome emulsions to form. For surfactants, a minimum of 16 hours of shaking is required as described in Section II.A.5.

- (4) Centrifuge the samples at 25°C for 20 minutes to break any emulsion and to separate the octanol and water phases. Evidence for breaking the emulsion and separation of the water and octanol phases can be obtained using a turbidimeter. The acceleration (G) value required to break the emulsion and to achieve complete separation of the octanol and water phases can be determined by trial-and-error experimentation.
- (5) Sample the octanol and water phases as follows:
- o Withdraw by pipet a known volume of the octanol phase (approximately 1/2 or less of the total octanol phase) and transfer to an analysis cell or diluting solvent. Before transferring the aliquot of the octanol phase, wipe the outside of the pipet with a paper tissue.
 - o Remove by pipet the remainder of the octanol phase including the interfacial layer and discard.
 - o Insert another clean pipet close to the bottom of the centrifuge tube and carefully withdraw a known volume of the aqueous phase. Wipe the bottom exterior part of the pipet with a tissue and discharge the aqueous sample directly into an analysis cell or extraction solvent. Do not allow the extraction solvent to contact the pipet stem.
- (6) Select an analytical method that is most applicable to the analysis of the specific chemical as described in Section II.A.7. Determine the concentration in the

octanol and water phases. Express the concentration of the chemical in octanol and water in moles/liter (M).

- (7) Determine the partition coefficient in triplicate (steps 1 through 7) at two concentrations of the test material $C < 0.01M$ and $C_1 = 0.1C$ as described in Section II.A.10. If K_{ow} is not constant at C and C_1 , then association effects should be considered. Therefore, follow steps 1 through 7 at lower concentrations until K_{ow} is constant at two concentrations differing by a factor of 10 as described in Section II.A.10.
- (8) Very hydrophobic chemicals (with K_{ow} on the order of 10^4 to 10^6) require relatively large volumes of the aqueous phase as described in Section II.A.6 and section II.A.9. Hence, for very hydrophobic materials, equilibrate the octanol and water phases in a large ground-glass stoppered flask as described above in step (3). For the final phase separation, transfer the two phase mixture to centrifuge tubes that have been prerinsed with some of the aqueous phase; centrifuge as described in step (4); withdraw aliquots from each centrifuge tube as described in step (5); and recombine for analysis. [Note: Prerinse all transfer tubes with the water phase.] Complete steps (6) and (7) to determine K_{ow} .
- (9) For materials that reversibly ionize or protonate, determine K_{ow} at pH 5.0, 7.0, and 9.0 as described in Section II.A.10. Follow steps (1) through (7) using the

buffered aqueous solutions described in Section II.B.1.b. Using the acid dissociation constant and the concentration of the chemical in the aqueous phase $[C_{\text{water}}]$, the term α can be calculated. The concentration of undissociated chemical can be determined from α and C_{water} .

III. DATA AND REPORTING

A. Test Report

For each individual determination, report the octanol/water partition coefficient at each concentration of the test substance, including the molar concentration of chemical in each phase [C_{oct} and C_{water}]. In addition, report the mean value of K_{ow} , and the standard deviation.

Summarize all the data on the data sheets listed in Appendix 1.

B. Specific Analytical and Recovery Procedures

- (1) Provide a detailed description or reference for the analytical procedure used, including the calibration data and precision; and
- (2) if extraction methods were used to separate the solute from the octanol and aqueous phases, provide a description of the extraction data.

C. Other Test Conditions

Report the experimental (G) value required to break the emulsion and to achieve separation of the octanol and water phases.

IV. REFERENCES

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Chiou CT, Freed VH, Schmedding DW, Kohnert RL. 1977. Partition coefficient and bioaccumulation of selected organic chemicals. Environ Sci Tech 11:475.

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Leo A, Hansch C, Elkins D. 1971. Partition coefficients and their uses. Chem Rev 71:525.

Neely WB, Branson DR, Blau GE. 1974. Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ Sci Tech 8:113.

Nernst W. 1891. Z Phys Chem 8:110.

V. APPENDIX 1: DATA FORMAT SHEETSInstructions

- (1) If multiple pH values are required, complete multiple copies of the Test Results Data page - one set of test results should be reported for each pH tested. If only unbuffered pure water is used, number 3 should be checked.
- (2) If K_{OW} is not constant at C and C_1 , and the chemical associates in water or octanol or both, additional concentrations must be used ($C_2, C_3 \dots$) until K_{OW} is constant at two molar concentrations differing by a factor of ten. These additional concentrations should be reported on duplicates of the Test Results page substituting C_2 and C_3 for C and C_1 .
- (3) This test guideline is designed to determine the octanol/water partition coefficient of the test chemical in the range 10 to 10^6 . For chemicals outside this range, the octanol/water partition coefficient should be reported as $K_{OW} < 10$ or $K_{OW} > 10^6$ with no further quantification.

TEST RESULTS SUMMARY

1. Chemical lot number 2. pH of water 3. Unbuffered pure water ☐

Conc.	Det. No.	C_{octanol} (mol dm ⁻³)			C_{water} (mol dm ⁻³)		
C	1	4. <input type="text"/>			5. <input type="text"/>		
	2	Exp <input type="text"/>			Exp <input type="text"/>		
	3	Exp <input type="text"/>			Exp <input type="text"/>		
C ₁	1	<input type="text"/>			<input type="text"/>		
	2	Exp <input type="text"/>			Exp <input type="text"/>		
	3	Exp <input type="text"/>			Exp <input type="text"/>		

Conc.	Det. No.	Partition Coefficient	K_{ow} Mean	K_{ow} Standard Deviation
C	1	6. <input type="text"/> Exp <input type="text"/>	7. <input type="text"/> Exp <input type="text"/>	8. <input type="text"/> Exp <input type="text"/>
	2	<input type="text"/> Exp <input type="text"/>		
	3	<input type="text"/> Exp <input type="text"/>		
C ₁	1	<input type="text"/> Exp <input type="text"/>	<input type="text"/> Exp <input type="text"/>	<input type="text"/> Exp <input type="text"/>
	2	<input type="text"/> Exp <input type="text"/>		
	3	<input type="text"/> Exp <input type="text"/>		

9. Partition coefficient mean (C and C₁) Exp 10. Partition coefficient standard deviation (C and C₁) Exp 11. Acceleration value (G)

CS-1400
August, 1982

PARTITION COEFFICIENT (n-OCTANOL/WATER)

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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PARTITION COEFFICIENT (n-OCTANOL/WATER)I. NEED FOR THE TEST

Bioconcentration, the accumulation of a substance in living tissues or other organic matter as a result of net chemical uptake from the medium (e.g., water), is a factor in determining the movement of a chemical in the environment and the potential effects of the chemical on biota. Hydrophobic chemicals that are present in the aqueous environment at subtoxic concentrations may accumulate to toxic levels once inside organisms, presumably through diffusion into nonpolar cell components, where they accumulate because of their greater solubility. Further movement of the substance in living tissues may occur as a result of ingestion of low trophic level organisms, i.e., food chain effects.

The octanol/water partition coefficient K_{ow} has been shown to be a good predictor of the tendency of chemicals to bioconcentrate in fish (Neely et al. 1974). Since 1974, K_{ow} has been used as a measure of bioconcentration potential in fatty tissues in aquatic and other living organisms. The numerical value of the octanol/water partition coefficient is one factor to be considered in determining whether to conduct fish bioconcentration studies. Other factors must also be taken into account. For example, transformation rates (e.g., rates of biodegradation, hydrolysis, photolysis, and oxidation) must also be considered. If a chemical transforms readily by one of these processes, the potential for bioconcentration will be reduced significantly and fish bioconcentration studies may not be needed.

The octanol/water partition coefficient has been introduced by Hansch to correlate biological activity and chemical structure (Hansch 1969; Hansch and Fujita 1964). Numerous papers have been published by Hansch and his coworkers on this subject in the ensuing years. A monograph has been published on the Hansch approach (Gould 1972).

A recent publication has indicated that the sorption of several hydrophobic pollutants on natural sediments can be related to the octanol/water partition coefficient. Karickhoff et al. (1979) showed that a reasonable estimate (within a factor of two) of the sorption behavior of hydrophobic pollutants can be made from knowledge of the particle size distribution and associated organic content of the sediment and the octanol/water partition coefficient.

Another recent publication has described a novel method for estimating the distribution of a chemical in the environment (Mackay 1979). K_{OW} is used in this partitioning analysis. This partitioning analysis will be used as a guide to ecological and health effects testing.

II. SCIENTIFIC ASPECTS

A. Rationale for the Use of the Octanol/Water Partition Coefficient to Estimate Bioconcentration Potential

Intuitively, the absorption and fat storage of xenobiotic chemicals in living organisms seem to be related to lipophilicity or preferential solubility in fats as compared to water. By definition, the octanol/water partition coefficient K_{OW} expresses the equilibrium concentration ratio of an organic chemical partitioned between octanol and water in dilute solution. If one assumes that octanol simulates fats in its solubilizing effect on organic chemicals, then K_{OW} should be a potential measure of the ease of storage of organic chemicals in fats. For example, a large value of K_{OW} indicates that an organic chemical is not very soluble in water but soluble in octanol. Hence, this would suggest the potential for a large storage of the organic chemical in fats. Davies et al. (1975) reported human pesticide poisoning by a fat-soluble organophosphate, dichlofenthion. The octanol/water partition coefficient K_{OW} was found to be very high (1.37×10^5), which correlated with the high fat storage of this chemical.

Neely et al. (1974) found a pronounced correlation between K_{OW} and the bioconcentration in trout muscle. Specifically, these researchers obtained a linear correlation between the log of bioconcentration and the log of calculated K_{OW} for a series of organic chemicals. Since that time, K_{OW} has been used by researchers as an index of bioconcentration potential in living

organisms. The Office of Pesticide Programs (OPP) [EPA 1975, 1978] has proposed, and the Organization for Economic Cooperation and Development (OECD 1981) is using K_{ow} as a measure of bioconcentration potential in aquatic organisms.

B. Rationale for the Selection of the Test Method

1. The Conventional Method of Determining the Octanol/ Water Partition Coefficient K_{ow}

The conventional method for determining a distribution coefficient is carried out by distributing a chemical between two immiscible liquids in a vessel and measuring the concentration of the chemical in the two liquid phases after equilibration (Glasstone 1946; Leo et al. 1971). This method can be applied to the determination of the octanol/water partition coefficient K_{ow} . Numerous researchers use the conventional method of determining K_{ow} and have published papers using this method (e.g., Fujita et al. 1964; Hansch and Anderson 1967; Leo et al. 1971; Chiou et al. 1977). EPA (EPA 1975, 1978) has proposed, and OECD (1981) is using the conventional method of determining K_{ow} . Most chemical companies that determine octanol/water partitioning use the conventional method of determining K_{ow} . Hence, the test guideline uses the conventional method of determining the octanol/water partition coefficient K_{ow} . It should be noted that there is no validated standard test method for determining K_{ow} (e.g., an ASTM method). The method in this guideline was

developed from a thorough review of the research literature on the experimental determination of K_{ow} and by talking to researchers who have considerable experience in carrying out these experiments.

2. Other Experimental Methods of Determining K_{ow}

a. Reverse-Phase High-Pressure Liquid Chromatography as a Method of Estimating K_{ow}

A rapid method based on reverse-phase high-pressure liquid chromatography has been developed by Veith (Veith and Morris 1978; Veith et al. 1979) to estimate the octanol/water partition coefficient of organic chemicals. Using the solvent mixture water/methanol (15/85 v/v) as the elutant, the log of the retention time [$\log(t_R)$] of organic chemicals on a permanently bonded (C-18) reverse-phase high-pressure liquid chromatographic system has been found to be linearly related to $\log K_{ow}$. This relationship has been expressed by the equation

$$\log K_{ow} = A \log(t_R) - B, \quad (1)$$

where A and B are constants determined from the experimental data for some organic chemicals. Using a mixture of the chemicals benzene, bromobenzene, biphenyl, p,p'-DDE [2,2-bis(p-chlorophenyl)-1,1-dichloroethylene] and 2,4,5,2',5'-pentachlorobiphenyl, A and B were found to be 5.106 and 1.258, respectively, with a coefficient

of determination of 0.975. It must be emphasized that this correlation is limited with respect to being representative of the organic chemicals encountered. This calibration mixture was selected largely on the basis of the $\log K_{ow}$ values reported in the literature, and the correlation is linear over five orders of magnitude of K_{ow} . To determine the accuracy of this method of estimating $\log K_{ow}$ by comparison with data reported in the literature, Veith and coworkers measured the retention time of 18 chemicals, and the standards and $\log K_{ow}$ values were calculated from the regression equation (1). The results indicated that $\log K_{ow}$ can be estimated to within (22.8 ± 20.0) percent when compared with the values reported in the literature from measurements using other methods. The percent error was calculated assuming the literature value is the correct $\log K_{ow}$; these researchers had some reservations about this assumption. It should be noted that some of the greatest relative errors were observed with polar chemicals that dissociate in water (e.g., m-chlorobenzoic acid, 2,4,5-trichlorophenol, and diphenylamine). This method has a definite advantage, since the estimation of K_{ow} can be made rapidly and relatively easily in comparison to the determination of K_{ow} by the conventional method. Furthermore, K_{ow} can be estimated for individual chemicals in complex mixtures (e.g., solid wastes) without knowing the specific chemical structure of each chemical.

Other researchers have developed high-pressure liquid chromatographic methods to determine K_{OW} (Mirrless et al. 1976; Yamana et al. 1977; Carlson et al. 1975; Hulshoff and Perrin 1976; McCall 1975). However, these methods are based on a very limited number of experiments and considerably more work is needed to develop them.

b. Thin-Layer Chromatography as a Method of
Estimating K_{OW}

It has been reported that thin-layer chromatography can be used to estimate K_{OW} (Mirrless et al. 1976; Hulshoff and Perrin 1976). However, high-pressure liquid chromatography (HPLC) is far superior to thin-layer chromatography (TLC) because of its accuracy (i.e., definition of the peak, reproducibility, ease of detection in many cases, and above all the range of applicability (HPLC is applicable over 5 orders of magnitude of K_{OW} while TLC is only applicable over 1.5 orders of magnitude of K_{OW}) (Mirrless et al. 1976).

c. Estimation of K_{OW} from Water Solubility Data

The octanol/water partition coefficient is defined as the ratio of the equilibrium molar concentration of the chemical in octanol and water. Thus, low molecular mass (i.e., molecular weight) organic chemicals with a low water solubility should have

a high value of K_{OW} (e.g., hydrophobic organic chemicals). Therefore, there should be a correlation between K_{OW} and water solubility. Chiou et al. (1977) studied the relationship between K_{OW} and the water solubility, S , and found that, for 34 organic chemicals, an excellent linear correlation was observed between $\log K_{OW}$ and $\log S$ that extended to more than eight orders of magnitude in water solubility (10^{-3} to 10^4 ppm), and six orders of magnitude in K_{OW} (10 to 10^7). Chiou et al. (1977) found the following regression equation

$$\log K_{OW} = 5.00 - 0.670 \log S, \quad (2)$$

where K_{OW} is the octanol/water partition coefficient S is the water solubility in mol/L, and the coefficient of determination (r^2) was 0.970 for these 34 chemicals. Thus, K_{OW} can be estimated from the experimental value of the water solubility of an organic chemical. This method would have a definite advantage in that K_{OW} could be estimated directly from water solubility data without having to experimentally measure K_{OW} . Thus, the octanol/water test guideline could eventually be eliminated, thereby reducing the cost of testing. However, considerably more experimental work is necessary to extend the correlation to a large number of organic chemicals with different structures before it can be used as a test guideline.

C. Rationale for the Selection of Test Conditions

A detailed study of the theory of the distribution law, the partition coefficient, and the published literature on the conventional determination of K_{OW} indicates that it is extremely important that numerous factors (or test conditions) be standardized. In order to establish these factors clearly, the theory of the distribution law and its relation to these factors are discussed in detail in the following sections.

1. Theory of the Distribution Law and the Octanol/Water Partition Coefficient

The distribution coefficient or partition coefficient can be derived using thermodynamic theory (Glasstone 1946). Consider a mixture of two immiscible liquids that is shaken with a solute (organic chemical). The solute distributes itself between the two liquids in such a way that at equilibrium, in dilute solution, the ratio of the concentrations of the solute in the two layers is a constant at a given temperature. The tendency of a chemical to distribute itself between two immiscible liquids with a constant concentration ratio, in dilute solution, is a direct consequence of the thermodynamic requirements for equilibrium. To illustrate this, consider a pair of immiscible liquids A and B in contact with each other containing the same solute in solution. The chemical potential of a solute in solvent A is given by

$$\mu_A = \mu_A^\circ + RT \ln a_A, \quad (3)$$

where μ_A is the chemical potential of the solute in solvent A, μ_A° is the standard chemical potential of the solute in the same solvent (i.e., the value of μ_A at $a_A = 1$), while a_A , the activity of the solute in the solvent A is the effective concentration taking into account intermolecular interactions of the solute in the solvent. R is the gas constant and is equal to 8.314 joules/°K/mol, while T is the absolute temperature in °K. Similarly, for solvent B

$$\mu_B = \mu_B^\circ + RT \ln a_B, \quad (4)$$

where all the quantities have the same significance as in equation (3). At equilibrium between the layers $\Delta\mu = 0$; hence

$$\Delta\mu = \mu_B - \mu_A = 0,$$

and

$$\mu_B = \mu_A. \quad (5)$$

Using equations (3) and (4) in (5) yields

$$\begin{aligned} \mu_B^\circ + RT \ln a_B &= \mu_A^\circ + RT \ln a_A \\ \ln \frac{a_B}{a_A} &= \frac{\mu_A^\circ - \mu_B^\circ}{RT} \end{aligned} \quad (6)$$

However, at a given temperature, μ_B° and μ_A° are constants for a given solute in a particular solvent; hence

$$\ln \frac{a_B}{a_A} = K,$$

and

$$\frac{a_B}{a_B} = K. \quad (7)$$

Equation (7) is the mathematical statement of the distribution law that states that a substance will distribute itself between two solvents until at equilibrium the ratio of the activities of a chemical in the two layers is a constant at a fixed temperature, irrespective of the absolute values of a_A or a_B . The activity a_A can be written as

$$a_A = \mu_A C_A, \quad (8)$$

where μ_A is the activity coefficient and takes into account the interaction between molecules A in solution, and C_A is the molar concentration. In dilute solution as

$$C_A \rightarrow 0,$$

$$\gamma_A \rightarrow 1; \quad (9)$$

hence,

$$\lim_{C_A \rightarrow 0} (a_A) = C_A.$$

The same argument follows for the solute in solvents B and
 $C_B \xrightarrow{\text{limit } 0} (a_B) = C_B$. Using these results in equation (7), the
 distribution coefficient K, in dilute solution, becomes

$$K = \lim_{C \rightarrow 0} \left(\frac{C_B}{C_A} \right) \quad (11)$$

For the specific case for the octanol/water partition
 coefficient, B is the solvent n-octanol, A is the solvent water,
 and $K^\circ = K_{ow}$. Thus, equation (11) becomes

$$K_{ow} = \frac{C_{\text{octanol}}}{C_{\text{water}}} \quad (12)$$

According to Nernst (1891), the distribution law applies only
 to individual molecular species in solution. If a molecule
 dissociates or associates in octanol and water, then equation (12)
 must be modified. In general, if α represents the fraction of the
 total solute that is dissociated or associated, assuming that
 either association or dissociation occurs in each solvent, then

$$K_{ow} = \frac{(1 - \alpha_{\text{oct.}}) C_{\text{oct.}}}{(1 - \alpha_{\text{water}}) C_{\text{water}}}, \quad (13)$$

since $(1 - \alpha)$ gives the fraction of unchanged molecules in each
 phase. For the special case where no association takes place in
 octanol, equation (13) reduces to

$$K_{ow} = \frac{C_{\text{oct.}}}{(1 - \alpha_{\text{water}}) C_{\text{water}}} \quad (14)$$

2. Factors that Affect the Value of K_{OW}

a. Effect of Temperature.

From the theory of the distribution law as outlined in Section II.C.1, the distribution coefficient K is a function of the temperature (equation (6)), and is a constant at a fixed temperature (equation (7)). Since K_{OW} is a distribution coefficient, it should also vary with temperature and is a constant at a fixed temperature. Hence, in carrying out octanol/water partition coefficient experiments by the conventional method, the temperature should be controlled. However, variations due to temperature are small compared to those inherent in the errors in the other measurements, e.g., the errors in measuring the concentration of solute in octanol and water. Therefore, for reasonably accurate determinations of K_{OW} , it is sufficient to control the temperature to $\pm 1^\circ\text{C}$. Since most physical properties of chemicals are reported at 25°C , this guideline requires that K_{OW} be determined at this temperature.

b. Purity of the Solvents

Trace amounts of impurities present in n-octanol tend to produce emulsions and must be removed (Fujita et al. 1964; Hansch and Anderson 1967; Chiou et al. 1977). Emulsions give poor phase separation and result in a wide scatter in the value of K_{OW} . In addition, impurities in octanol may affect the analysis for the solute. Hence it is extremely important that the octanol be

99.9 percent pure. Distilled or reagent grade water (ASTM Type II) should be used.

c. Concentration of Solute

From the theory of the distribution law, as outlined in Section II.C.1, equations (12), (13), and (14) only apply in dilute solution. Hence, it is extremely important that all experiments be carried out at molar concentration $C < 0.01M$ in octanol and water.

d. Equilibration Time

For many chemicals, 5 minutes of gentle agitation of the two-phase system established equilibrium and produced consistent results (Leo et al. 1971). Studies by Craig and Craig (1950) indicated that when the phases were of about equal volume, equilibrium was rapidly attained. When high ratios of water to octanol (100:1) were used, longer shaking was necessary to establish equilibrium. High ratios of water to octanol are used to determine K_{ow} for very hydrophobic organic chemicals (Sections II.C.1.e and h as described below). Therefore, for most chemicals, gentle agitation for 1 hour should be adequate to reach equilibrium. For surfactants, at least 16 hours of agitation is necessary to reach equilibrium. This is an empirical observation obtained by researchers who have carried out experiments with surfactants. It is undoubtedly due to the nature of surfactant chemicals.

e. Octanol/Water Volume Ratio

Depending upon the solubility of the solute in octanol and water, the ratio of the volume of octanol to water should be adjusted. For hydrophobic solutes, which are very insoluble in water, considerably more water than octanol should be used. Adjustment of solvent volumes can decrease the effect of analytical errors and consequently decrease the error in determining K_{ow} (Leo et al. 1971). The following example will illustrate this point. Consider a chemical with a molecular mass (i.e., molecular weight) MW (mg/mmol), $K_{ow} = 200$. Twenty mg of chemical are dissolved in 100 mL of octanol, and 100 mL of water are added to this system. After equilibration, the mass of chemical in each phase can be calculated as follows. The chemical will partition with x mg in the water phase and (20-x) mg in the octanol phase.

$$C_{oct.} = \frac{(20-x)mg}{\frac{MW (mg/mmol)}{100 \text{ mL}}} = \frac{(20-x)}{100 \text{ MW}} \frac{\text{mmol}}{\text{mL}}$$

$$= \frac{(20-x)}{100 \text{ MW}} \text{ Molar}$$

$$C_{water} = \frac{\frac{x \text{ mg}}{MW(mg/mmol)}}{100 \text{ mL}} = \frac{x}{100 \text{ MW}} \frac{\text{mmol}}{\text{mL}}$$

$$= \frac{x}{100 \text{ MW}} \text{ Molar}$$

Since

$$K_{ow} = \frac{C_{oct.}}{C_{water}},$$

$$200 = \frac{\frac{(20-x)}{100 \text{ MW}}}{\frac{x}{100 \text{ MW}}} = \frac{(20-x)}{x}.$$

$$\text{Then } x \frac{20}{201} = 0.0996 = 0.10 \text{ mg} = \text{mass of chemical in the water phase;}$$

and

$$(20-x) = 19.9 \approx 20 \text{ mg} = \text{mass chemical in the octanol phase.}$$

Consider an analytical error of ± 0.05 mg in the aqueous phase (i.e., $0.10 - 0.05 = 0.05$ and $0.10 + 0.05 = 0.15$).

$$K_{ow} = \frac{\frac{20}{\text{MW}}}{\frac{100}{\frac{0.05}{\text{MW}}}} = \frac{20}{0.05} = 400$$

$$K_{ow} = \frac{\frac{20}{\text{MW}}}{\frac{100}{\frac{0.15}{\text{MW}}}} = \frac{20}{0.15} = 133.$$

Therefore, an analytical error of = 0.05 mg in the aqueous phase means that K_{ow} can range from 400 to 133, and a very large error in K_{ow} occurs.

Consider the same example as above, but now the solvents are adjusted to 200 mL of water and 5 mL of octanol. After equilibration, the mass of chemical in each phase is now:

$$C_{\text{oct.}} = \frac{\frac{(20-x)}{5}}{\text{MW}} = \frac{(20-x)}{5 \text{ MW}} \text{ Molar}$$

$$C_{\text{H}_2\text{O}} = \frac{\frac{x}{200}}{\text{MW}} = \frac{x}{200 \text{ MW}} \text{ Molar}$$

$$K_{ow} = \frac{C_{\text{oct.}}}{C_{\text{water}}}$$

$$200 = \frac{\frac{(20-x)}{5 \text{ MW}}}{\frac{x}{200 \text{ MW}}} = \frac{(20-x)(40)}{x}$$

Then

$$x = \frac{20}{6} = 3.33 \text{ mg} = \text{mass of chemical in the water phase}$$

and

$$(20-x) = 16.7 \text{ mg} = \text{mass of chemical in the octanol phase.}$$

Now consider the same analytical error of ± 0.05 mg in the aqueous phase (i.e., $3.33 - 0.05 = 3.28$ and $3.33 + 0.05 = 3.38$)

$$K_{ow} = \frac{\frac{\frac{16.7}{MW}}{5}}{\frac{3.28}{\frac{MW}{200}}} = \frac{16.7}{3.38}(40) = 203$$

$$K_{ow} = \frac{\frac{\frac{16.7}{MW}}{5}}{\frac{3.28}{\frac{MW}{200}}} = \frac{16.7}{3.28}(40) = 197$$

Now the analytical error of ± 0.05 mg in the aqueous phase means that K_{ow} can range from 197 to 203 and the error in K_{ow} has been reduced dramatically.

f. Chemical Analysis of the Octanol and Water Phases

Consider a partitioning experiment in which a chemical is dissolved in octanol at a low concentration (less than 0.01 molar). The conventional partitioning experiment is carried out, and only one phase is analyzed for the molar concentration of the solute. Using a mass balance, the molar concentration of the solute in the other phase is obtained by difference. However, if there is a loss of chemical by adsorption to the surface of the glass walls, a serious error will occur at this low concentration. This is especially true for very hydrophobic chemicals (Chiou et al. 1977) and for ionic solutes (Leo et al. 1971). Therefore, it is important that both the octanol and water phases be analyzed.

An analytical method should be selected that is the most applicable to the analysis of the specific chemical. However, large errors can occur as a result of traces of more-water-soluble contaminants that are not analytically distinguishable from the parent chemical. This error is very significant when the analytical method is ultraviolet absorption spectroscopy or radiometry, since these methods can be nonspecific for many solutes. Therefore, chromatographic methods are preferable because of their compound specificity in analyzing the parent chemical without interference from impurities (Karickhoff and Brown 1979). Wherever practicable, the chosen analytical method should have a precision with ± 5 percent.

g. Emulsification and Ultracentrifugation

Many chemicals can cause troublesome emulsions to form between octanol and water and emulsification can result in large errors in K_{ow} (Leo et al. 1971; Chiou et al. 1977). This is especially true for hydrophobic chemicals. Therefore, it is important that gentle shaking be used to minimize the formation of emulsions. In addition, incomplete separation of the two phases is one of the most serious sources of error. To break any emulsion formed and to separate completely the octanol and water phases, it is extremely important that the two-phase system be ultracentrifuged at 25°C for 20 minutes. The acceleration G value required to break an emulsion and to separate completely the octanol and water phases can be determined by trial-and-error experimentation. Since the visual clarity of the two phases is

not a dependable criterion of the absence of an emulsion and complete separation of the two-phase system, it is recommended that a turbidimeter be used to make sure that the emulsion is broken and the octanol and water phases have been completely separated.

h. Equilibration Vessel

To simplify the experimental procedure, it is recommended that equilibration be carried out in a centrifuge tube (special glass tubes can be used up to approximately 12,000 G and stainless steel centrifuge tubes can be used at higher G values) with a sealable cap. This will avoid a transfer step and volatile chemicals can be handled easily. It is important that the centrifuge tube be almost completely filled with the two-phase mixture to minimize partitioning with air. This is especially important when determining K_{ow} for volatile chemicals (Hansch and Anderson 1967).

Very hydrophobic chemicals, with K_{ow} on the order of 10^4 to 10^6 , require relatively large volumes of the aqueous phase (Section II.C.2.e). Hence, for these chemicals, it is recommended that equilibration be carried out in a large ground-glass stoppered flask.

i. Speciation Effects

The details of speciation have been discussed in the theory of the distribution law and the octanol/water partition coefficient, Section II.C.1.

If the chemical does not associate or dissociate in octanol and water, then the test guideline requires that equation (12) be used and K_{OW} be determined at concentrations $C < 0.01M$ and $C_1 = 0.1C$. Under these experimental conditions, if K_{OW} is constant, then association or dissociation has been minimized or eliminated.

If the chemical associates in octanol or water or in both liquids, then the test guideline requires that equation (13) be used and K_{OW} be determined at concentrations $C < 0.01M$, $C_1 = 0.1C$, $C_2 = 0.01C$, $C_3 = 0.001C, \dots$. When K_{OW} is constant at two concentrations differing by a factor of 10, then the effect of association has been minimized or eliminated.

For chemicals that reversibly ionize or protonate (e.g., carboxylic acids, phenols or anilines), the test guideline requires that equation (14) be used with water buffered at pH 5.0, 7.0, and 9.0, the pHs of environmental concern.

j. Presaturation of the Solvents

Presaturation of octanol with water and water with octanol is required for this test guideline. The preparation of these saturated solutions is very simple to carry out. This requirement

is extremely important when determining K_{OW} for very hydrophobic chemicals, since the ratio of water to octanol will be very large. In this case, if the experiment is carried out without presaturation of the water with octanol, then all the octanol will dissolve in the aqueous phase and K_{OW} cannot be determined.

D. Reference Compounds

It would be very desirable to have reference compounds that cover a K_{OW} range of 10 to 10^6 . These reference compounds would provide the experimenter with comparative reference values to determine how well the test has been conducted. Unfortunately, these reference compounds are not currently available. When appropriate reference compounds have been identified they will be recommended for use in this test guideline. In the interim, it is recommended that the book by Hansch and Leo (1979) be used for the selection of potential reference compounds.

E. Test Data Required

The tendency of an organic chemical to partition out of water into other environmental compartments containing hydrophobic constituents (e.g., aquatic organisms) can be inferred from the values of the octanol/water partition coefficient K_{OW} . Chiou et al. (1977) developed regression equations relating $\log K_{OW}$ with water solubility S (in mol/L) and bioconcentration in rainbow trout (BF). Assuming $\log K_{OW}$ is between 1 and 6, S and BF can be

calculated; these results are summarized in Table 1 (note that S has been converted to mol/L). Furthermore, assuming that the average molecular mass (i.e., molecular weight) of an organic chemical is 300 gm/mol, the water solubility can be converted to ppm; these results are also summarized in Table 1. It is apparent that for $\log K_{OW} = 6$ (i.e., $K_{OW} = 10^6$), the water solubility will be extremely low (9.7×10^{-3} ppm or 9.7 ppb) and the predicted BF is 1.48×10^4 . Hence, the data indicate that the chemical will partition out of the water phase and into the fat of the fish (i.e., the hydrophobic phase). For $\log K_{OW} = 1$ (i.e., $K_{OW} = 10$), the water solubility will be very high (2.80×10^5 mg/L or 280 gm/L) and the predicted BF is 2.4. Hence, these data indicate that the chemical will remain in the water phase and will not partition significantly into the fat of the fish (i.e., the hydrophobic phase). Therefore, the test guideline is designed to determine the value of K_{OW} in the range 10 to 10^6 . Low molecular mass organic chemicals with a K_{OW} value less than 10 will not partition significantly into or tend to accumulate in, any hydrophobic environmental compartments. Low molecular mass organic chemicals with K_{OW} in excess of 10^6 will tend to accumulate into all hydrophobic environmental compartments. For low molecular mass organic chemicals outside the range 10 to 10^6 , the test guideline requires that K_{OW} be characterized as 10 or 10^6 with no further quantification.

Specific analytical and recovery procedures should be reported to determine whether acceptable data have been generated.

Table 1. Summary of Calculated Values of Water Solubility and Bioconcentration in Rainbow Trout as a Function of Log K_{ow} ^a

log K_{ow}	S(mol/L) ^b	S(mg/L or ppm) ^c	BF ^d
6	3.24×10^{-8}	9.7×10^{-3}	1.48×10^4
5	1×10^{-6}	0.30	2.57×10^3
4	3.09×10^{-5}	9.3	4.47×10^2
3	9.77×10^{-4}	2.93×10^2	7.76×10^1
2	3.02×10^{-2}	9.06×10^3	1.35×10^1
1	9.3×10^{-1}	2.80×10^5	2.4

^aRegression equations taken from Chiou et al. (1977).

^bWater solubility

^cWater solubility in ppm assuming a molecular mass of an organic chemical is 300 gm/mol.

^dBioconcentration in rainbow trout

F. Statistical Analysis of the Data

Numerous researchers have published data on the determination of the octanol/water partition coefficient by the conventional method (e.g., Chiou et al. 1977; Davies et al. 1975; Fujita et al. 1964; Hansch and Anderson 1967; Leo et al. 1971). However, none of these researchers has analyzed the data statistically and the precision of K_{ow} as determined by the conventional method has not been clearly established. The precision is, in part, a function of the nature of the specific chemical. As the hydrophobicity of the chemical increases, K_{ow} increases and the precision of K_{ow} decreases. Furthermore, the precision is also a function of the analytical procedure used. In general, the lower the concentration to be measured, the poorer is the precision of the analytical procedure. Therefore, no reliable precision can be stated at this time for determining K_{ow} . Obviously, the precision can be improved by making numerous replicate determinations. However, in order to minimize cost, it has been decided to determine K_{ow} with three replicates. Therefore, it is important that the submitter of the test results analyze the data statistically. When a large number of chemicals have been determined by the proposed method, the data will be analyzed statistically and the level of precision can be defined for various ranges of K_{ow} .

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pH OF WATER SOLUTION OR SUSPENSION

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
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pH OF WATER SOLUTION OR SUSPENSIONI. PURPOSE

This Test Guideline references methodology to develop data on the pH of aqueous solutions and suspensions of chemical substances and mixtures. The data may be used to calculate the degree of acidity or basicity of an aqueous solutions or suspensions that will be formed by a chemical substance and to evaluate the resulting effects the substance will have on human health and the environment. The pH may be used in the design of other tests.

II. TEST PROCEDURES

The electrometric method is recommended for determining the pH of an aqueous solution or suspension. The recommended apparatus should consist of a pH meter and electrodes, meeting performance standards and specifications as described, for example, in ANSI/ASTM E 70-7700 and in ASTM D 1293-78.

It is recommended that the water used in preparing solutions or suspensions meet the standards for Type I or Type II reagent water specified by ANSI/ASTM D 1193-77 and be free of CO₂. For chemical substances that are readily soluble in water, the Test Guideline recommends that a solution of 1% by mass be used. For chemical substances that are soluble in water to an extent of less than 1% by mass at 25°C, then an aqueous suspension of the chemical substance should be tested.

The recommended test methods are available for purchase from the American Society for Testing and Materials, 1916 Race St., Philadelphia, PA 19103.

III. REFERENCE

ASTM. (Latest Edition). American Society for Testing and Materials. Annual Book of ASTM Standards.

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WATER SOLUBILITY

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
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WATER SOLUBILITYI. INTRODUCTIONA. Background and Purpose

The water solubility of a compound can be defined as the equilibrium concentration of the compound in a saturated aqueous solution at a given temperature and pressure. The water solubility of a chemical is an important factor in determining the environmental movement and distribution of any substance. Chemicals that are relatively water soluble are more likely to be widely distributed by the hydrologic cycle than those which are relatively insoluble.

Water provides the medium in which many organisms live, and water is a major component of the internal environment of all living organisms (except for dormant stages of certain life forms). Even organisms which are adapted to life in a gaseous environment require water for normal functioning. Water is thus the medium through which most other chemicals are transported to and into living cells. As a result, the extent to which chemicals dissolve in water will be a major determinant for movement through the environment and entry into living systems.

The water solubility of a chemical has an effect on its adsorption on and desorption from soils and sediments and on volatilization from aqueous media. The more soluble a chemical substance is, the more likely it is to desorb from soils and sediments and the less likely it is to volatilize from water. The extent of chemical transformations via hydrolysis,

photolysis, oxidation, reduction, and biodegradation in water depends on the chemical being soluble in water (i.e., homogeneous kinetics). Finally, the design of most chemical tests and many ecological and health tests requires precise knowledge of the water solubility of the chemical to be tested.

Procedures in this test guideline have been described to enable sponsors to determine the water solubility for solid and liquid organic compounds.

B. Definitions and Units

(1) "Colloidal dispersion" is a mixture resembling a true solution but containing one or more substances that are finely divided but large enough to prevent passage through a semipermeable membrane. It consists of particles which are larger than molecules, which settle out very slowly with time, which scatter a beam of light, and which are too small for resolution with an ordinary light microscope.

(2) A "concentration vs. time study" results in a graph which plots the measured concentration of a given compound in a solution as a function of elapsed time. Usually, it provides a more reliable determination of equilibrium water solubility of hydrophobic compounds than can be obtained by single measurements of separate samples.

(3) "Concentration" of a solution is the amount of solute in a given amount of solvent and can be expressed as a weight/weight or weight/volume relationship. The conversion from a weight relationship to one of volume incorporates density as a factor. For dilute aqueous solutions, the density of the solvent is approximately equal to the density of the solutions; thus, concentrations in mg/dm^3 are approximately equal to $10^{-3} \text{ g}/10^3 \text{ g}$ or parts per million (ppm); ones in $\mu\text{g/dm}^3$ are approximately equal to $10^{-6} \text{ g}/10^3 \text{ g}$ or parts per billion (ppb). In addition, concentration can be expressed in terms of molarity, normality, molality, and mole fraction. For example, to convert from weight/volume to molarity one incorporates molecular mass as a factor.

(4) "Density" is the mass of a unit volume of a material. It is a function of temperature, hence the temperature at which it is measured should be specified. For a solid, it is the density of the impermeable portion rather than the bulk density. For solids and liquids, suitable units of measurement are g/cm^3 . The density of a solution is the mass of a unit volume of the solution and suitable units of measurement are g/cm^3 .

(5) An "oversaturated (supersaturated) solution" is a solution that contains a greater concentration of a solute than is possible at equilibrium under fixed conditions of temperature and pressure.

(6) A "saturated solution" is a solution in which the dissolved solute is in equilibrium with an excess of undissolved solute; or a solution in equilibrium such that at a fixed temperature and pressure, the concentration of the solute in the solution is at its maximum value and will not change even in the presence of an excess of solute.

(7) A "solution" is a homogeneous mixture of two or more substances constituting a single phase.

C. Principle of the Test Method

The test method is based on the conventional method of preparing saturated aqueous solutions. The method involves the coating of the compound to the walls of a vessel, adding water (i.e., very pure water, buffer solution, or artificial seawater), and determining the concentration of the compound in the water as a function of time at a fixed temperature. When the concentration reaches a plateau, equilibrium has been achieved, and the water is saturated with the compound. Specific procedures have been incorporated in this test guideline to measure the water solubility of very hydrophobic compounds and to alleviate the problems of colloids and emulsions usually formed.

D. Applicability and Specificity

Procedures have been described in this test guideline to determine the saturated water solubility for liquid or solid compounds. The water solubility can be determined in very pure

water, buffer solution for compounds that reversibly ionize or protonate, or in artificial seawater as a function of temperature (i.e., in the range of temperatures of environmental concern). Water solubility is usually not useful for gases because their solubility in water is measured when the gas above the water is at a partial pressure of one atmosphere which is several orders of magnitude greater than those existing under environmental conditions. A more important parameter for gases is Henry's law constant which is the ratio of the vapor pressure of the compound to solution concentration at low partial pressures.

This test guideline is designed to determine the saturated water solubility of a solid or liquid test chemical in the range infinity to 10 parts per billion (ppb). For chemicals whose solubility is below 10 ppb, the water solubility should be characterized as "less than 10 ppb" with no further quantification.

II. DESCRIPTION OF THE TEST PROCEDURE

A. Test Conditions

1. Special Laboratory Equipment

- (1) A thermostatic bath with temperature control ($\pm 1^{\circ}\text{C}$) in the approximate range of 5-30°C;

- (2) an ultracentrifuge with temperature control ($\pm 1^{\circ}\text{C}$) in the approximate range of $5\text{--}30^{\circ}\text{C}$ and capable of obtaining acceleration (G) values to 39,000 or higher;
- (3) a pH meter capable of resolving differences of 0.1 pH units or less; and
- (4) centrifuge tubes with sealable caps: special glass tubes can be used up to approximately 12,000 G; tubes to be used at G values $> 12,000$ should be made of stainless steel.

2. Purity of Water

Reagent grade water, e.g., water meeting ASTM Type IIA standards or an equivalent grade, is highly recommended to minimize biodegradation and to minimize the effects of dissolved salts on water solubility. ASTM Type IIA water is described in ASTM D1193-77, "Standard Specification for Reagent Water".

3. Purity of Solvents

It is important that all solvents used for coating test compounds on the walls of vessels and in separation and analytical technique be reagent grade and contain no impurities which will interfere with the determination of the test compound.

4. Seawater

It is recommended that artificial seawater be used to determine the saturated water solubility in seawater. The preparation of artificial seawater is described in Section II.B.2.

5. Agitation and Equilibration Time

It is important that contact time of test compounds with water be sufficient to obtain a saturated solution. The length of time necessary will depend upon such variables as the size of the vessel, the extent and degree of agitation, the properties of the compound and particle size. To increase the rate of solution of hydrophobic compounds, mild agitation is recommended. For hydrophobic compounds a minimum time of one day is required.

6. Effects of Colloids and Emulsions: Centrifugation

It is important that gentle shaking be used to minimize the formation of colloids. The presence of colloids and emulsions will lead to solubility values that are higher than those in a true saturated solution. This is a common problem with hydrophobic solids and liquids but can usually be overcome by centrifugation. It is recommended that centrifugation be conducted in tightly sealed tubes that are almost filled to capacity to avoid partitioning with air.

It is extremely important that centrifugation be carried out at two or three different G values (minimum of 12,000 G) for at least 30 minutes at 25°C until concentration changes are small. For hydrophobic compounds (solubility \leq 10 ppm), it is extremely important that the acceleration G values differ by 10,000 G and include a determination of 39,000 or higher.

7. Effect of pH on Solubility

It is recommended that all experiments be carried out at pH's 5.0, 7.0, and 9.0 for any chemical which reversibly ionizes or protonates (e.g., carboxylic acids, phenols, amines). Buffers described in Section II.B.2. can be used.

8. Analysis of Saturated Solutions

Any suitable analytical method may be used; where practicable, precision should be within ± 5 percent. Preferred analytical methods are those that are specific for the compound to be tested, to the exclusion of other compounds. Chromatographic methods which incorporate separation, and therefore, specification, are recommended.

9. Adsorption to Glass or Other Surfaces

Hydrophobic compounds have a tendency to adsorb to glass or other surfaces, e.g., stainless steel. Thus, when transferring the solution to any glass vessel or container, it is essential to pre-rinse the surfaces of the vessel or container with the

solution. Failure to do so will lead to solubility values that are lower than those of true equilibrium water solubility because the compound will adsorb to the unrinsed surface. However, when hydrophobic compounds are extracted with organic solvent, the extraction vessels should not be pre-rinsed since this would lead to solubility values that are greater than those of true equilibrium water solubility.

B. Preparation of Reagents and Solutions

1. Buffer Solutions

Prepare buffer solutions using reagent grade water as follows:

pH 5.00-- To 250 mL of 0.1M potassium hydrogen phthalate add 113 mL of 0.1M sodium hydroxide; adjust the final volume to 500 mL with reagent grade water.

pH 7.00-- To 250 mL of 0.1M potassium dihydrogen phosphate add 145 mL of 0.1M sodium hydroxide; adjust the final volume to 500 mL with reagent grade water.

pH 9.00-- To 250 mL of 0.075M borax add 69 mL of 0.1M HCl; adjust the final volume to 500 mL with reagent grade water.

Check the pH of each buffer solution with a pH meter at 25°C and adjust to pH 5.0, 7.0, or 9.0, if necessary. If the pH of the solution has changed by ± 0.2 pH units or more after the addition of the test compound, then a more concentrated buffer is required for that pH determination. The sponsor should then choose a more suitable buffer.

2. Artificial Seawater

Add the reagent-grade chemicals listed in Table 1 in the specified amounts and order to 890 mL of reagent-grade water. It is important that each chemical be dissolved before another one is added.

C. Performance of the Test

Determine the saturated water solubility of the test compound at 25°C in reagent grade water or buffer solution, if appropriate. Under certain circumstances, it may be necessary to determine the water solubility of a test compound at 25°C in artificial seawater. The water solubility can also be determined at other temperatures of environmental concern by adjusting the temperature of the water bath to the appropriate temperature.

Table 1--Constituents of Artificial Seawater¹

Chemical	Amount
NaF	3 mg
SrCl ₂ ·6H ₂ O	20 mg
H ₃ BO ₃	30 mg
KBr	1.00 mg
KCl	700 mg
CaCl ₂ ·2H ₂ O	1.47 g
Na ₂ SO ₄	4.00 g
MgCl ₂ ·6H ₂ O	10.78 g
NaCl	23.50 g
Na ₂ SiO ₃ ·9H ₂ O	20 mg
NaHCO ₃	200 mg

¹If the resulting solution is diluted to 1 cubic decimeter (1 liter), the salinity should be 34 ± 0.5 g/kg and the pH 8.0 ± 0.2 . The desired test salinity is attained by dilution at time of use.

1. Procedure for the Determination of
Solids and Liquids in Water at 25°C

Dissolve a sufficient amount of the solid compound in a suitable volatile organic solvent and coat on the walls of a vessel. Viscous liquids may be coated on vessels in a similar fashion; non-viscous liquids do not require solvents. Remove the solvent under reduced pressure or with a pure nitrogen gas stream. When all the solvent is removed, add reagent grade water or, for compounds which reversibly ionize or protonate, the appropriate buffer solution and slowly stir or agitate the mixture under temperature control. Mixing may be accomplished by use of a teflon coated stirring bar and should be continued for a minimum of 24 hours before aliquots are withdrawn. Prior to taking aliquots, the mixture should be left to stand at constant temperature for at least one hour to permit separation of any small particles. To determine the concentration of the compound in the aqueous phase, aliquots should be centrifuged at two or three different G values (minimum of 12,000 G) for at least 30 minutes at 25°C until concentration changes are small. The concentration value so obtained is plotted against the time of mixing. At a later time, aliquots are again taken and analyzed in the same fashion to produce another data point on a concentration vs. time plot. When the concentration reaches a plateau, equilibrium is assumed. For hydrophobic compounds (solubility ≤ 10 ppm) it is extremely important that the acceleration (G) values differ by 10,000 G and include a determination at 39,000 G or higher.

For more soluble compounds (solubility \geq 500 ppm) coating the walls of the vessel is not necessary and filtration may be substituted for centrifugation. Use filters which are adequate to remove suspended particles. If the concentration of the solute exceeds 10 g/dm^3 , then determine the density of the solution. This can be done by weighing known volumes of the solution at the same temperature as the constant temperature bath. Sufficient solution should be used so that each determination is made on a fresh aliquot. Carry out solubility and density experiments in triplicate.

2. Modification of Procedures for Potential Problems

a. Interference of Soluble Impurities

Interference by soluble impurities in the test sample can be avoided by the use of an analytical technique that is specific to the compound being tested. If this is not practical, interferences can sometimes be minimized by repeatedly preparing saturated solutions from the same sample chemical until the concentration of the impurity has been depleted.

b. Decomposition of the Test Compound

If the test compound decomposes in one or more of the aqueous solvents required during the period of the test at a rate such that an accurate value for water solubility cannot be obtained, then it will be necessary to carry out detailed

transformation studies e.g., hydrolysis. If decomposition is due to aqueous photolysis, then it will be necessary to carry out water solubility studies in the dark, under red or yellow lights, or by any other suitable method to eliminate this transformation process.

III. DATA AND REPORTING

A. Test Report

For each set of conditions, (e.g., temperature, pure water, buffer solution, artificial seawater) required for the study, provide the water solubility value for each of three determinations, the mean value, and the standard deviation.

For compounds that decompose at a rate such that a precise value for the water solubility cannot be obtained, provide a statement to that effect.

For compounds with water solubility below 10 ppb, report the value as "less than 10 ppb".

For compounds with water solubility greater than 10 g/dm³, report the density of the solution at each required temperature.

Summarize all the data in the data sheets listed in Appendix 1.

B. Specific Analytical and Recovery Procedures

- (1) Provide a detailed description or references for the analytical procedure used, including the calibration data and precision; and
- (2) if extraction methods were used to separate the solute from the aqueous solution provide a description of the extraction method as well as the recovery data.

IV. REFERENCES

ASTM. 1978. Annual Book of ASTM Standards, American Society for Testing and Materials, Philadelphia, Pa., Part 31, Method D 1193-77.

V. APPENDIX 1: DATA FORMAT SHEETS

Instructions

If multiple temperatures are required, complete multiple copies of the Test Results pages, one set of test results reported for each temperature at which the test was conducted.

If multiple pH values are required, complete multiple copies of the Test Results pages, one set of test results reported for each pH tested.

TEST RESULTS

1. Coating solvent (if used) _____
2. Solubility determined in

--	--	--

Reagent grade Water 101
 Buffer solution 102
 Artificial seawater 103

3. If #2 is buffer solution, pH of solution

--	--	--

4. Acceleration (G) values for centrifugation

5. If filtration was substituted for centrifugation, describe the type filters used.

6. Concentration units
 7. Time units

 Abbrev. _____
 Abbrev. _____

8. Temperature

--	--

 °C ±

--	--

 °C

Sampling
 Time

9.

Plateau

Run 1

10.

Run 2

11.

Run 3

12.

13. Mean Saturated Equilibrium Concentration
 14. Standard Deviation

±

--	--	--	--	--

TEST RESULTS CONTINUED

Report the density of the solution for concentrations equal to or greater than 10 gdm^{-3} , and the temperature at which it was measured.

15. Temperature \pm $^{\circ}\text{C}$

Density (gcm^{-3})				
Run 1	Run 2	Run 3	Mean	Std. Dev.
16. <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	17. <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	18. <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	19. <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	20. \pm <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>

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WATER SOLUBILITY

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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WATER SOLUBILITYI. NEED FOR THE TEST

The water solubility of a compound can be defined as the equilibrium concentration of the compound in a saturated aqueous solution at a given temperature. The water solubility is a fundamental physical property of a compound. It is an essential characteristic for determining that compound's movement and distribution in the environment and, therefore, its potential effects on living organisms. Highly soluble compounds are more likely to be distributed by the hydrologic cycle than less soluble compounds. The degree of water solubility of a compound can affect its adsorption and desorption on soils and sediments and ease of volatility from aquatic systems. Substances which are more soluble in water are more likely to desorb from soils and sediments and less likely to volatilize from water. Transformations such as hydrolysis, photolysis, oxidation, reduction, and biodegradation in water proceed more rapidly when the compound is dissolved in water (i.e., homogeneous kinetics). The potential importance of these transformations is, therefore, a function of the ability of a compound to dissolve in water. The design of most chemical tests and many ecological and health tests requires accurate knowledge of the water solubility of the compound to be tested.

Virtually all modeling systems devised to determine the distribution of a compound in the environment require water solubility data. Although attempts have been made to correlate water solubility with other physical parameters such as

structure, enthalpy of fusion, melting point and molar volume, in order to predict or calculate the water solubility of a compound, it is generally agreed that an actual physical measurement of water solubility is a far more accurate and desirable approach (Mader and Grady 1970). In addition, values of water solubility have been shown to correlate with the octanol/water partition coefficient (K_{ow}) for a number of compounds (Chiou et al. 1977, Yalkowsky and Valvani 1979, 1980, 1981). These correlations enable a quite accurate estimate of a compound's K_{ow} to be made from its water solubility.

Water provides the medium in which many organisms live, and water is a major component of the internal portion of all living organisms (except for dormant stages of certain life forms). Even organisms which are adapted to life in a gaseous environment require water for normal functioning. Water is thus the medium through which most chemicals are transported to and into living cells. As a result, the extent to which chemicals dissolve in water will be a major determinant for movement through the environment and entry into living systems.

Water solubility is an essential parameter for assessing the environmental partitioning of all solid or liquid chemicals. Water solubility is usually not useful for gases because their solubility in water is measured when the gas above the water is at a partial pressure of one atmosphere which is several orders

of magnitude greater than those existing under environmental conditions. Thus, water solubility of gases does not generally apply to environmental assessment because the actual partial pressure of a gas in the environment is extremely low.

II. SCIENTIFIC ASPECTS

A. Rationale for the Selection of the Test Method

Analytical methods for the determination of water solubility consist of obtaining an equilibrium saturated aqueous solution of the compound and analyzing the solution by some suitable physical or chemical method. Equilibrium may be obtained by intimately mixing the solute and solvent. Separation of the phases can then be accomplished by filtration or decantation, usually followed by centrifugation.

The solubility of a compound in water at equilibrium is a function of temperature, pressure, and purity of solute and water (Mader and Grady 1970). A major practical difficulty is determining that equilibrium has actually been attained. There are two general methods to determine the attainment of equilibrium. The concentration vs. time method (Mader and Grady 1970) involves periodic sampling over meaningful periods of time; when the concentration has reached a plateau, equilibrium has been attained. The undersaturation/oversaturation method (Mader and Grady 1970) refers to approaching equilibrium from both possible non-equilibrium conditions. One of two identical

solutions is heated to a temperature well above the required test temperature so that the solubility is increased. Then both solutions are placed in a thermostatic bath to equilibrate at the required test temperature. The heated solution becomes supersaturated (oversaturated) when its temperature decreases and reaches equilibrium as the concentration of the dissolved solute decreases to a plateau at the required test temperature. The other solution is undersaturated and reaches equilibrium as the concentration of dissolved solute increases to a plateau at the required test temperature. If identical concentrations in aliquots of these two samples are obtained after a period of time, equilibrium has been reached. Research sponsored by EPA at the Battelle Institute demonstrated that the under/oversaturation method will give poor analytical results if the compound tested undergoes a phase transition in the temperature range utilized (EPA 1981). Thus, a low melting solid may recrystallize at a very slow rate after being heated to elevated temperatures and make separation of excess solute from the aqueous phase extremely difficult. Other potential problems include accelerated decomposition of the test material at elevated temperatures and dramatically different changes in solubility rates of impurities vs. test compound with change in temperature.

The method described in the test guideline involves coating the compound on the walls of a vessel, adding very pure water, and determining the change in concentration of the compound in the water over a period of time at a fixed temperature (Haque and Schmedding 1975, Karickhoff and Brown 1979). Typically, the

concentration of a compound dissolved in the aqueous phase will increase with time. As the solution progresses toward saturation, the rate of change of concentration will decrease and ultimately, the concentration will reach a plateau. The formation of that plateau after a suitable period of time has elapsed is generally considered an accurate indication that equilibrium has been established. The coating of solids on the walls of the vessel is considered to be more practical than suspending the solid as small particles in water since the coating technique minimizes the formation of microcrystals that are difficult to remove.

The use of a single procedure to cover the entire range of solubilities from infinity down to 10 ppb has the obvious advantage of not having to change procedures near the cut-off of two ranges. Sufficient flexibility has been incorporated into the test guideline to accomodate a wide range of solubilities.

The use of a "suitable solvent" for coating the vessel walls allows the sponsor to choose a solvent that is more appropriate for the test compound in recognition of the possible variation in the physical properties of different compounds. Typically, solvents such as acetone, methanol, ether, dichloromethane, and hexane can be employed.

It has been shown that constant but erroneous solubility values can be obtained when very small droplets or particles remain dispersed in water (Biqgar and Biqqs 1974). To separate

these colloidal particles, centrifugation (or ultracentrifugation) is necessary (Biggar and Riggs 1974). If constant concentration values are obtained after centrifugation at several different acceleration (G) values, one can assume that removal of colloidal particles has been achieved. The centrifuge tubes should be tightly sealed because the test compound can escape from the solution through volatilization, especially for those compounds with an appreciable vapor pressure. In addition, the tubes should be filled almost to capacity to minimize partitioning to air.

The less soluble the test compound, the greater the error introduced by the presence of suspended particles. For example, the suspension of $100 \mu\text{g}/\text{dm}^3$ of undissolved material is small if the true solubility is $100 \text{ mg}/\text{dm}^3$ (error of 0.1 percent) but is extremely large if the true solubility is $100 \mu\text{g}/\text{dm}^3$ (error of 100 percent). Thus, more extensive ultracentrifuge procedures will be required for more hydrophobic compounds. It has been demonstrated that reliable solubility determinations can be made for very hydrophobic chemicals by centrifuging the suspension at two or three different G values for one-half hour at constant temperature until concentration changes are small (Biggar and Riggs 1974). For several pesticides, centrifugation at 39,000 G removed the colloidal particles corresponding to $0.1 \mu\text{m}$ particle size. For DDT, it had been demonstrated that one hour of centrifugation at 84,000 G removed all the colloidal particles (Bowman, Acree, and Corbett 1960). For chemicals that are soluble in the range 500 to 10 ppm, aliquots of the suspension

must be centrifuged at two or three different G values for at least 30 minutes at constant temperature until concentration changes are small. For hydrophobic chemicals (solubility ≤ 10 ppm), it is extremely important that acceleration (G) values differ by 10,000 G and include a determination at 39,000 G or higher. When determining the solubility of relatively soluble compounds (>500 ppm), large solid particles can be conveniently separated by filtration, and macropore filters may be employed as part of the separation technique.

It is recommended that the analytical technique be selected by the sponsor and/or testing laboratory in recognition of the many different techniques available and the advantage of being able to match one to the properties of the compound (e.g., the degree of solubility, the spectroscopic properties of the compound, and its chromatographic behavior). Analytical techniques that allow the quantitative determination of the test compound to the exclusion of impurities are recommended to the extent practicable. Therefore, chromatographic techniques are recommended.

B. Other Methods of Determining Water Solubility

1. Interferometry

This method has been used to determine the solubility of slightly soluble solids and liquids in water in the ppm range (Adams 1915, Mader and Grady 1970). Limited research has been

done on this method, and the technique has only been applied to a few compounds. It does not have general applicability.

2. Nephelometry

The nephelometric (turbidity) method calls for preparation of stable suspensions of the organic chemical at several different concentrations exceeding the nominal solubility. The turbidities of the resulting suspensions are then measured and plotted against total concentration. A Beer's Law relationship is assumed; thus, a straight line drawn through the points yields an intercept equal to the solubility, i.e., the concentration at which the turbidity vanishes (Davis and Parke 1942).

In tests of this method a complete suspension of excess solute could not be accomplished by mechanical means such as sonication. In order to obtain a complete suspension, the procedure requires that the compound be dissolved in a solvent that is miscible with water. Initial amounts of dissolved solute that are added tend to disperse in the water and form a true solution. However, as the solubility limit is approached, turbid zones are produced that persist briefly depending on the stirring efficiency, and a permanent turbidity is formed at the solubility limit.

In principle, this approach can be used to give a rough initial estimate of the solubility. The resulting solubility is subject to several conditions and limitations, including the

inaccuracy and lack of reproducibility of the individual turbidity measurements, the instability of the original particle size distribution (a function of time and dilution), and the effect of the dispersing agent (solvent) on the solubility. These conditions and limitations are not independent of each other. For example, the use of solvent pairs to form a liquid having solvent properties intermediate between those of the parent solvents is a well-recognized technique (e.g., as applied with gradient elution HPLC). Thus, not only is it to be expected that the use of a dispersing agent might influence the true solubility, but the solubility and hence the particle size distribution might be expected to change as a function of dilution of the stock solution. Also, in cases where the solubility is strongly influenced by the presence of the dispersing solvent, evaporative loss of the dispersing solvent can lead to continued precipitation of the solute and thus influence the particle size distribution and apparent turbidity.

The stability of the particle size distribution can also be affected by the nature of the solute. With a solute that has a relatively high mobility, it can be expected that the large particles will grow at the expense of the smaller particles thus altering the apparent turbidity. If the solute is volatile, an appreciable fraction of the solute may be lost from the suspension, and recrystallization may occur on the surface of the container or on other surfaces external to the bulk liquid.

Therefore, the nephelometric method has been rejected as a general method for determining the water solubility of organic compounds.

3. Coupled Column Liquid Chromatography

This is a recently published method used to determine the aqueous solubility of hydrophobic polycyclic aromatic hydrocarbons (May, Wasik, and Freeman 1978a,b). The method consists of pumping water through a column containing glass beads coated with the compound being studied. The beads are prepared by adding them to a 0.1 percent methylene chloride solution of the test compound and stripping the solvent with a rotary evaporator. The coated beads are packed into stainless steel tubes. Saturated solutions are generated by pumping water through these thermostated columns at flow rates ranging between 0.1 and 5 mL/min. These saturated solutions are extracted by flowing a measured volume of solution through a stainless steel extactor column where the material is adsorbed to a special column packing. Another solvent system is then used to elute the test material from the extactor column onto an analytical column where its concentration is measured by a standard liquid chromatography detector system. It is necessary to calibrate the detector signal by using solutions of known concentration. Precision in the ppb range is excellent (± 3 percent). The method appears promising but needs more experimental work on a variety of hydrophobic chemicals before it can be determined whether or not to recommend the procedure.

C. Rationale for the Selection of the
Test Conditions

1. Temperature

The equilibrium water solubility of a substance is a constant at a fixed temperature and pressure. For most solid and liquid compounds, an increase in temperature results in an increase in water solubility. The rate of change in water solubility with temperature is not linear for a given chemical and varies dramatically from one substance to another (Mader and Grady 1970). For many compounds the following relationship describes concentration (water solubility) as a function of temperature:

$$\ln \frac{1}{X_a} = \frac{\Delta H}{R} \left(\frac{1}{T} - \frac{1}{T_f} \right),$$

where X_a is the mole fraction of compound A, ΔH is the enthalpy of fusion, T_f is the thermodynamic temperature of fusion and R is the universal gas constant (Bigger and Riggs 1974). The equation can be put into the form $\log_{10} C = -\frac{A}{T} + B$, where C = molar concentration, T is the thermodynamic temperature and A and B are constants. By determining C as a function of T (e.g., at 5, 15, 25°C), the data can be used to determine A and B (i.e., plot

$\log_{10}C$ vs. $1/T$; the slope of the line is A and the intercept is B). Thus, one can use this equation to estimate C at any environmental temperature of concern. As a working estimate, scientists often anticipate a doubling of solubility with an increase of 10°C . The temperature chosen for this test guideline is 25°C since this is the temperature traditionally used for reporting physical properties. Solubility determinations may be requested at additional temperatures of environmental concern for specific compounds or classes of compounds.

Since water solubility values will change with temperature (Mader and Grady 1970), the temperature should be controlled to $\pm 1^{\circ}\text{C}$. This is a condition easily achieved by standard temperature control devices for water baths and is a practical range for temperature controlled centrifuges.

2. Purity of Water

Dissolved salts can affect the water solubility of a compound, necessitating the use of very pure water (Mader and Grady 1970). In addition, the water should be relatively free of bacteria which may consume or alter the organic test material during extended periods of testing. ASTM Type II reagent grade water (ASTM 1978), or an equivalent grade containing less than 1 mg/dm^3 total organic carbon, has been recommended for use in this test guideline to minimize biodegradation and the effects of impurities.

3. Purity of Solvents

Organic solvents that come into contact with the test material and aqueous solution must be as pure as practicable. Trace organic impurities that are soluble in water can alter the water solubility of the test material as well as interfere with the analysis of the concentration of the test material.

4. Seawater

Organic compounds are often released to seawater. The presence of dissolved salts will usually alter the true water solubility of a compound. Typically, the water solubility of an organic compound will be less in seawater than in pure water at a fixed temperature (Long and McDevit 1952). Data on solubility in seawater is needed to design ecological testing of marine species and for modeling. Salinity varies in different marine environments. In order to determine solubility under uniform conditions, a formulation for artificial seawater is specified in the test guideline (Kester et al. 1967, EPA 1975a).

5. Agitation and Equilibration Time

To increase the rate of solution of hydrophobic compounds, mild agitation is recommended. The use of strong agitation, while increasing the rate of solution, also increases the formation of emulsions and colloids by producing very small particles which remain suspended in solution. For hydrophobic

compounds, experience has shown that a minimum of one day with mild agitation is necessary to reach equilibrium, and several weeks are often required (Haque and Schmedding 1975).

6. Effects of Colloids and Emulsions: Centrifugation

The use of a centrifuge allows the rapid separation of small particles suspended in water. The size of the particles separated depends on the acceleration factor (G value) and the length of time the sample is centrifuged. Centrifugation at two or three different G values will partition suspended particles to two or three different distributions. When the water solubility value obtained is relatively constant at two different G values, this indicates that any suspended particles present are relatively insignificant (Biggar and Riggs 1974). It is important that centrifugation be conducted in tightly sealed tubes that are almost filled to capacity to avoid partitioning with air and loss of chemical via volatilization.

7. Effect of pH on Solubility

It is known that the water solubility of some organic compounds will alter with change in the pH of the solution (Cheung and Biggar 1974). This change will not be dramatic unless the compound contains readily ionizable or protonated groups. For carboxylic acids, amines and compounds which reversibly ionize or protonate in water, it is necessary to

measure the water solubility at pH values of 5.0, 7.0, and 9.0, since these pH values are representative of those found in natural aquatic ecosystems, groundwater, and rainwater.

The specified buffer solutions contain an adequate concentration of buffer to neutralize approximately 100-500 ppm of dissolved test compound (depending on its molecular weight) without a significant change in pH and, therefore, will be satisfactory for most test compounds. A change in pH of 0.2 units signifies that a more concentrated buffer solution is needed. In such cases, the choice of buffer solution should be made by the sponsor in order for it to be compatible with their analytical scheme.

8. Analysis of Saturated Solutions

The diversity of compounds to be tested precludes the specification of a limited number of analytical techniques; the choice is best left to the sponsor. A great many analytical procedures are potentially useful. Nonetheless, the use of a compound-specific analytical procedure has been recommended for use in this test guideline. Since chromatographic techniques entail separation as well as quantification for many organic compounds, these methods have been recommended. Where procedures are available, chromatographic techniques are cost effective. However, determining the proper chromatographic procedure can be time consuming. Depending upon the physical properties exhibited by the compound and its equilibrium concentration in water, other

standard techniques can be more efficient with respect to ease of analysis, precision, and cost. Whenever practicable, an analytical procedure should be used having a precision within ± 5 percent. Scientists are generally aware of the wide variety of standard techniques available today and of the large number of methods being developed, so that a discussion of even a small percentage of analytical possibilities would serve little purpose. This test guideline does require, however, that the specific analytical technique utilized be adequately described. Some procedures involve the use of extracting solvents and, when so used, it is important that these extraction procedures be adequately described and recovery information be submitted.

9. Adsorption to Glass or Other Surfaces

Hydrophobic compounds will have a tendency to adsorb to glass or other surfaces (e.g., stainless steel). Thus, when transferring the test solution to any vessel or container, it is essential to pre-rinse the surfaces with the solution. Failure to do so will lead to solubility values that are lower than those of true equilibrium water solubility. However, when hydrophobic compounds are extracted with organic solvent, their containers should not be pre-rinsed since this would lead to solubility values that are greater than those of true equilibrium water solubility.

D. Test Data Required

Present analytical techniques allow the determination of the concentration of organic compounds in water as low as the parts per billion range and, in time, this capability may be extended. However, using current techniques the reliability and precision below 10 ppb will be ± 50 percent at best. Because of these inherent inaccuracies, the greatly increased costs associated with quantification below 10 ppb would be difficult to justify. Furthermore, organic substances that are so hydrophobic that their water solubility is less than 10 ppb may disperse in water forming micelles rather than being truly soluble. Thus, for substances whose water solubility is below 10 ppb, the test guideline requires that the water solubility be characterized as "less than 10 ppb" with no further quantification.

When the solubility of a compound is equal to or greater than 10 g/dm^3 , it is important that the density of the saturated solution be determined experimentally and reported. For solutions of such concentration, density is needed to convert from a concentration of g/dm^3 to other units (e.g., ppm, mole fraction, molality).

E. Statistical Analysis of the Data

Numerous researchers have published measurements of water solubility using a variety of methods (Gunther et al. 1968). For many methods, good precision (i.e., repeatable values) can be obtained but the comparison of one method to another may give

larger differences than would be expected from the precision of each one. Therefore, though precise, the water solubility data may not be accurate (i.e., correct). For example, the water solubility of DDT has been investigated by many researchers. It was finally demonstrated that values obtained depended upon the size distribution of suspended particles in the "saturated aqueous solution". Suspended particle size distribution was a function of time of centrifuging and acceleration (G) values (Biggar and Riggs 1974). Therefore, significantly different values (each with good precision) could be obtained for the different sets of conditions. This problem is more significant for very hydrophobic compounds than for those which are more water soluble. It is one of the reasons why the method does not require quantification below 10 ppb.

The precision of the water solubility data generated by the proposed general method has not been clearly established. As the solubility decreases, the precision is expected to become poorer. The precision is also a function of the nature of the specific compound and the analytical procedure used. Therefore, no reliable precision can be stated at this time for determining the water solubility. Obviously, the precision can be improved by making numerous replicate determinations. However, in order to minimize costs, it has been decided to determine water

solubility with three replicates. Therefore, it is extremely important that the submitter of the test results analyze the data statistically. After the water solubility of a large number of compounds of various types has been determined by this method, the level of precision can be defined for various ranges.

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VAPOR PRESSURE

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
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VAPOR PRESSUREI. INTRODUCTIONA. Background and Purpose

Volatilization, the evaporative loss of a chemical, depends upon the vapor pressure of chemical and on environmental conditions which influence diffusion from a surface.

Volatilization is an important source of material for airborne transport and may lead to the distribution of a chemical over wide areas and into bodies of water far from the site of release. Vapor pressure values provide indications of the tendency of pure substances to vaporize in an unperturbed situation, and thus provide a method for ranking the relative volatilities of chemicals. Vapor pressure data combined with water solubility data permit the calculation of Henry's law constant, a parameter essential to the calculation of volatility from water.

Chemicals with relatively low vapor pressures, high adsorptivity onto solids, or high solubility in water are less likely to vaporize and become airborne than chemicals with high vapor pressures or with low water solubility or low adsorptivity to solids and sediments. In addition, chemicals that are likely to be gases at ambient temperatures and which have low water solubility and low adsorptive tendencies are less likely to transport and persist in soils and water. Such chemicals are

less likely to biodegrade or hydrolyze and are prime candidates for atmospheric oxidation and photolysis (e.g., smog formation or stratospheric alterations). On the other hand, nonvolatile chemicals are less frequently involved in atmosphere transport, so that concerns regarding them should focus on soils and water.

Vapor pressure data are an important consideration in the design of other chemical fate and effects test; for example in preventing or accounting for the loss of volatile chemicals during the course of the test.

B. Definitions and Units

- (1) "Desorption efficiency" of a particular compound applied to a sorbent and subsequently extracted with a solvent is the weight of the compound which can be recovered from the sorbent divided by the weight of the compound originally sorbed.
- (2) "Pascal" (Pa) is the standard international unit of vapor pressure and is defined as newtons per square meter (N/m^2). A Newton is the force necessary to give acceleration of one meter per second squared to one kilogram of mass.
- (3) The "torr" is a unit of pressure which equals 133.3 pascals or 1 mm Hg at 0°C.

- (4) "Vapor pressure" is the pressure at which a liquid or solid is in equilibrium with its vapor at a given temperature.
- (5) "Volatilization" is the loss of a substance to the air from a surface or from solution by evaporation.

C. Principle of the Test Methods

The isoteniscope procedure uses a standardized technique [ASTM 1978] that was developed to measure the vapor pressure of certain liquid hydrocarbons. The sample is purified within the equipment by removing dissolved and entrained gases until the measured vapor pressure is constant, a process called "degassing." Impurities more volatile than the sample will tend to increase the observed vapor pressure and thus must be minimized or removed. Results are subject to only slight error for samples containing nonvolatile impurities.

Gas saturation (or transpiration) procedures use a current of inert gas passed through or over the test material slowly enough to ensure saturation and subsequent analysis of either the loss of material or the amount (and sometimes kind) of vapor generated. Gas saturation procedures have been described by Spencer and Cliath (1969). Results are easy to obtain and can be quite precise. The same procedures also can be used to study volatilization from laboratory scale environmental simulations. Vapor pressure is computed on the assumption that the total pressure of a mixture of gases is equal to the sum of the

pressures of the separate or component gases and that the ideal gas law is obeyed. The partial pressure of the vapor under study can be calculated from the total gas volume and the weight of the material vaporized. If v is the volume which contains w grams of the vaporized material having a molecular weight M , and if p is the pressure of the vapor in equilibrium at temperature T (K), then the vapor pressure, p , of the sample is calculated by

$$p = (w/M)(RT/v) \quad ,$$

where R is the gas constant ($8.31 \text{ Pa m}^2\text{mol}^{-1} \text{ K}^{-1}$) when the pressure is in pascals (Pa) and the volume is in cubic meters. As noted by Spencer and Cliath (1970), direct vapor pressure measurements by gas saturation techniques are more directly related to the volatilization of chemicals than are other techniques.

In an effort to improve upon the procedure described by Spencer and Cliath (1969) and to determine the applicability of the gas saturation method to a wide variety of chemical types and structures, EPA has sponsored research and development work at SRI International (EPA 1982). The procedures described in this Test Guideline are those developed under that contract and have been evaluated with a wide variety of chemicals of differing structure and vapor pressures.

D. Applicability and Specificity

A procedure for measuring the vapor pressure of materials released to the environment ideally would cover a wide range of vapor pressure values, at ambient temperatures. No single procedure can cover this range, so two different procedures are described in this Test Guideline, each suited for a different part of the range. The isoteniscope procedure is for pure liquids with vapor pressures from 0.1 to 100 kPa. For vapor pressures of 10^{-5} to 10^3 Pa, a gas saturation procedure is to be used.

With respect to the isoteniscope method, if compounds that boil close to or form azeotropes with the test material are present, it is necessary to remove the interfering compounds and use pure test material. Impurities more volatile than the sample will tend to increase the observed vapor pressure above its true value but the purification steps will tend to remove these impurities. Soluble, nonvolatile impurities will decrease the apparent vapor pressure. However, because the isoteniscope procedure is a static, fixed-volume method in which an insignificant fraction of the liquid sample is vaporized, it is subject to only slight error for samples containing nonvolatile impurities. That is, the nonvolatile impurities will not be concentrated due to vaporization of the sample.

The gas saturation method is applicable to solid or liquid chemicals. Since the vapor pressure measurements are made at ambient temperatures, the need to extrapolate data from high

temperatures is not necessary and high temperature extrapolation, which can often cause serious errors, is avoided. The method is most reliable for vapor pressures below 10^3 Pa. Above this limit, the vapor pressures are generally overestimated, probably due to aerosol formation. Finally, the gas saturation method is applicable to the determination of the vapor pressure of impure materials.

II. TEST PROCEDURES

A. Test Conditions

- (1) The apparatus in the isoteniscope method is described in Section II.B.1.
- (2) The apparatus used in the gas saturation method is described in Section II.B.2.

B. Performance of the Tests

1. Isoteniscope Procedure

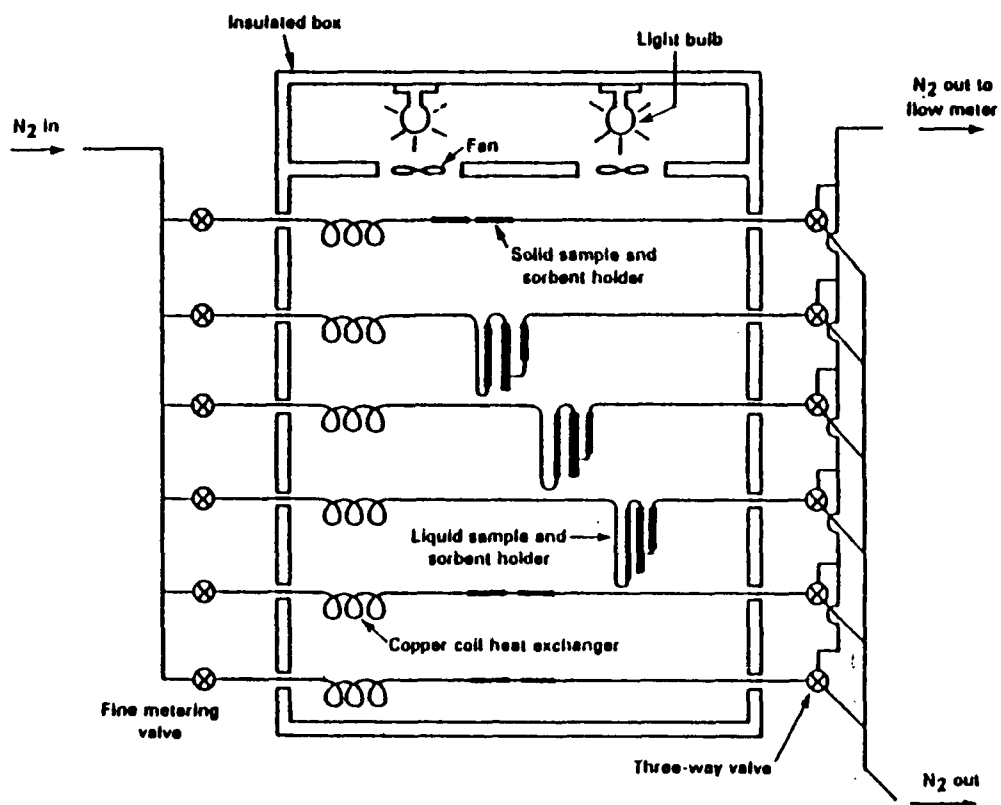
The isoteniscope procedure described as ANSI/ASTM Method D 2879-75 is applicable for the measurement of vapor pressures of liquids with vapor pressures of 0.1 to 100 kilopascals (kPa) (0.75 to 750 torr). The isoteniscope method involves placing liquid sample in a thermostated bulb (the isoteniscope) connected to a manometer and a vacuum pump. Dissolved and entrained gases are removed from the sample in the isoteniscope by degassing the

sample at reduced pressure. The vapor pressure of the sample at selected temperatures is determined by balancing the pressure due to the vapor of the sample against a known pressure of an inert gas. The vapor pressure of the test compound is determined in triplicate at $25 \pm 0.5^{\circ}\text{C}$ and at any other suitable temperatures ($\pm 0.5^{\circ}$). It is important that additional vapor pressure measurements be made at other temperatures, as necessary, to assure that there is no need for further degassing, as described in the ASTM method.

2. Gas Saturation Procedure

- (1) The test procedures require the use of constant-temperature box as depicted in Figure 1. The insulated box, containing sample holders, may be of any suitable size and shape. The sketch in Figure 1 shows a box containing three solid sample holders and three liquid sample holders, which allows for the triplicate analysis of either a solid or liquid sample. The temperature within the box is controlled to $\pm 0.5^{\circ}$ or better. Nitrogen gas, split into six streams and controlled by fine needle valves (approximately 0.79 mm orifice), flows into the box via 3.8 mm (0.125 in.) i.d. copper tubing. After temperature equilibration, the gas flows through the sample and the sorbent trap and exits from the box. The flow rate of the effluent

FIGURE 1 - SCHEMATIC DIAGRAM OF VAPOR SATURATION APPARATUS



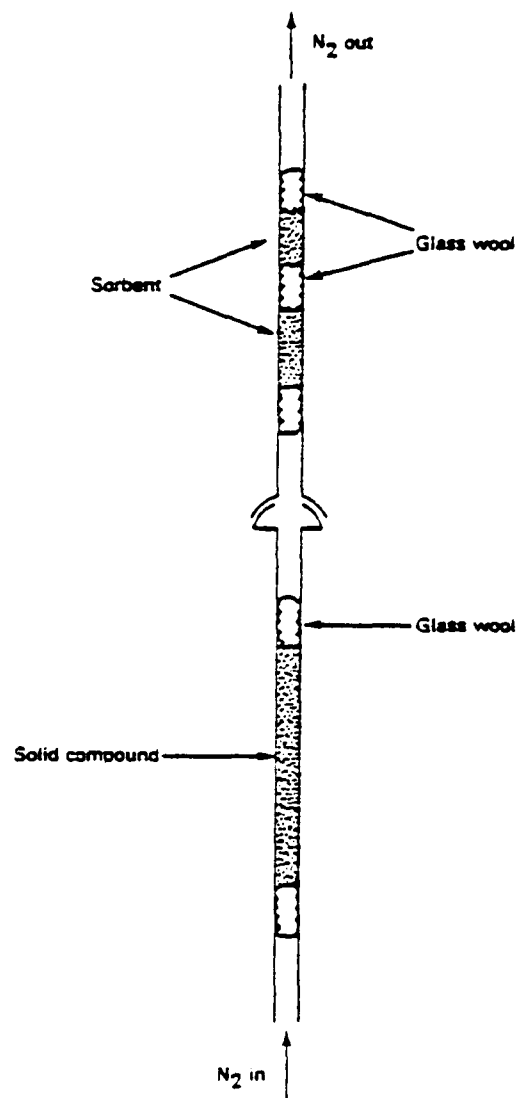
carrier gas is measured at room temperature with a bubble flow meter or other suitable device. The flow rate is checked frequently during the experiment to assure that there is an accurate value for the total volume of carrier gas. The flow rate is used to calculate the total volume (at room temperature) of gas that has passed through the sample and sorbent [(vol/time) x time = volume]. The vapor pressure of the test substance can be calculated from the total gas volume and the mass of sample vaporized. If v is the volume of gas that transported mass w of the vaporized test material having a molecular weight M , and if p is the equilibrium vapor pressure of the sample at temperature T , then p is calculated by the equation

$$p = (w/M)(RT/v) \quad .$$

In this equation, R is the gas constant ($8.31 \text{ Pa m}^3\text{mol}^{-1} \text{K}^{-1}$). The pressure is expressed in pascals (Pa), the volume in cubic meters (m^3), mass in grams and T in kelvins (K). $T = 273.15 + t$, if t is measured in degrees Celsius ($^{\circ}\text{C}$).

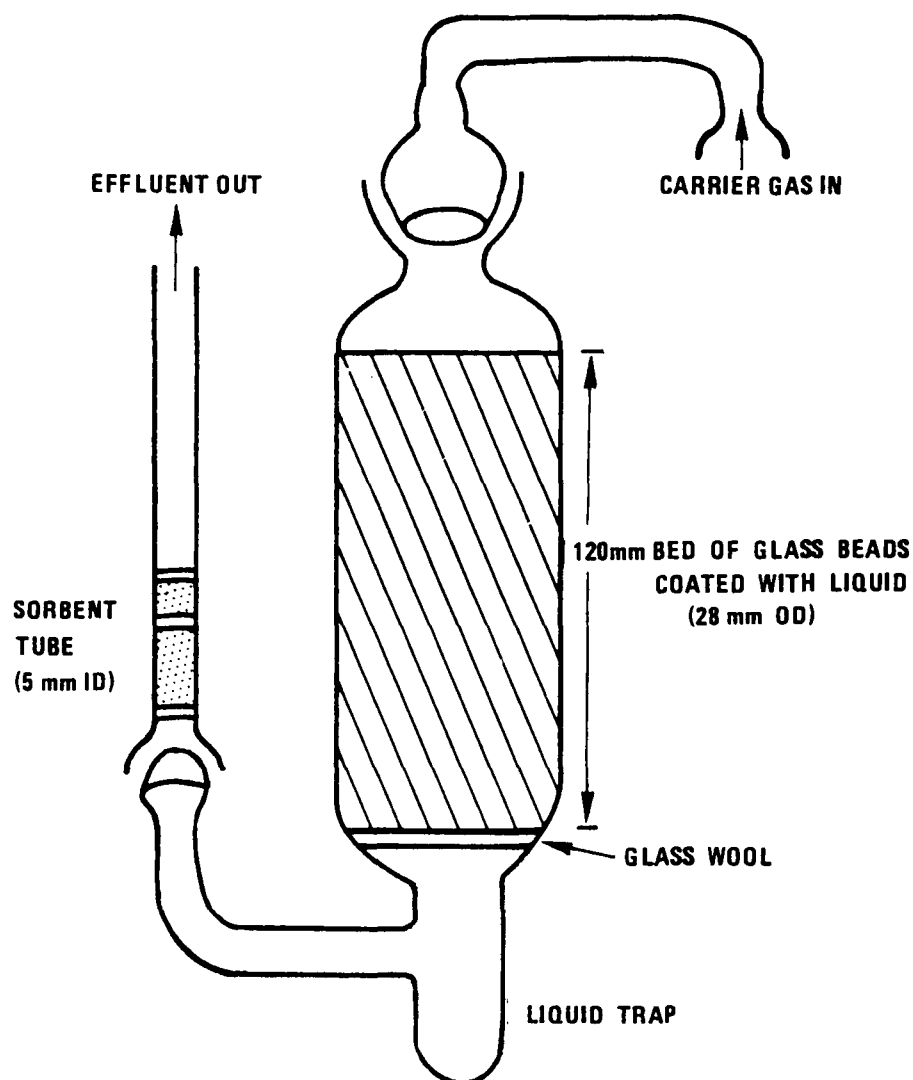
- (2) Solid samples are loaded into 5mm i.d. glass tubing between glass wool plugs. Figure 2 depicts a drawing of a sample holder and absorber system.

FIGURE 2 - SOLID COMPOUND SAMPLING SYSTEM



- (3) Liquid samples are contained in a holder as shown in Figure 3. The most reproducible method for measuring the vapor pressure of liquids is to coat the liquid on glass beads and to pack the holder in the designated place with these beads.
- (4) At very low vapor pressures and sorbent loadings, adsorption of the chemical on the glass wool separating the sample and the sorbent and on the glass surfaces may be a serious problem. Therefore, very low loadings should be avoided whenever possible. Incoming nitrogen gas (containing no interfering impurities) passes through a coarse frit and bubbles through a 38 cm column of liquid sample. The stream passes through a glass wool column to trap aerosols and then through a sorbent tube, as described above. The pressure drop across the glass wool column and the sorbent tube are negligible.
- (5) With both solid and liquid samples, at the end of the sampling time, the front and backup sorbent sections are analyzed separately. The compound on each section is desorbed by adding the sorbent from that section to 1.0 ml of desorption solvent in a small vial and allowing the mixture to stand at a suitable temperature until no more test compound desorbs. It is extremely important that the desorption solvent contain no impurities which would interfere with the analytical method of choice. The resulting solutions are analyzed

FIGURE 3 – LIQUID COMPOUND SAMPLING SYSTEM



quantitatively by a suitable analytical method to determine the weight of sample desorbed from each section. The choice of the analytical method, sorbent, and desorption solvent is dictated by the nature of the test material. Commonly used sorbents include charcoal, Tenax GC, and XAD-2. Describe in detail the sorbent, desorption solvent, and analytical methods employed.

- (6) Measure the desorption efficiency for every combination of sample, sorbent, and solvent used. The desorption efficiency is determined by injecting a known mass of sample onto a sorbent and later desorbing it and analyzing for the mass recovered. For each combination of sample, sorbent, and solvent used, carry out the determination in triplicate at each of three concentrations. Desorption efficiency may vary with the concentration of the actual sample and it is important to measure the efficiency at or near the concentration of sample under gas saturation test procedure conditions.
- (7) To assure that the gas is indeed saturated with test compound vapor, sample each compound at three differing gas flow rates. Appropriate flow rates will depend on the test compound and test temperature. If the calculated vapor pressure shows no dependence on flow rate, then the gas is assumed to be saturated.

III. DATA AND REPORTING

- (1) Report the triplicate calculated vapor pressures for the test material at each temperature, the average calculated vapor pressure at each temperature, and the standard deviation.
- (2) Provide a description of analytical methods used to analyze for the test material and all analytical results.
- (3) For the isoteniscope procedure, include the plot of p vs the reciprocal of the temperature in K, developed during the degasing step and showing linearity in the region of 298.15 K (25°C) and any other required test temperatures.
- (4) For the gas saturation procedure, include the data on the calculation of vapor pressure at three or more gas flow rates at each test temperature, showing no dependence on flow rate. Include a description of sorbents and solvents employed and the desorption efficiency calculations.
- (5) Provide a description of any difficulties experienced or any other pertinent information.

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VAPOR PRESSURE

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VAPOR PRESSUREI. NEED FOR THE TEST

The vapor pressure of a chemical is an important parameter in determining the environmental fate of the chemical.

The atmosphere is a major route for the widespread distribution of chemicals. There are several ways by which chemicals may become airborne and subsequently be transported by wind currents. Airborne solids and foamy emulsions are commonly observed, but these are not believed to be major factors in atmospheric transport because they involve particulate matter which may be of sufficient size to settle out in a relatively short time (Seiber et al. 1975). Aerosols, from spray applications, manufacturing and formulation sites, and aerated waste treatment systems may constitute more important sources of chemical for air transport since very small droplets (5 micrometers or less in diameter) may be formed and carried considerable distances (Edwards 1973). However, it appears that volatilization from land and water surfaces is the most important source of material for airborne transport (Hartley 1969, Lichtenstein 1971, MacKay and Wolkoff 1973, Seiber et al. 1975).

Volatilization is the evaporative loss of a chemical compound. Volatilization rates are dependent on the vapor pressure of the chemical and the environmental factors which influence diffusion from the evaporative surface. Harper et al. (1976, p. 236) noted that "volatilization is probably the single largest means by which pesticides are lost and transported over wide areas and into bodies of water far from the application

location." The airborne vapors of a hazardous chemical may present a threat to plant and animal life exposed to those vapors, not only in the area of chemical release but also at sites remote from the volatilization site. This occurs when vapors are removed from the air, primarily by precipitation with rain or snow.

Volatilization rates are related to vapor pressure, which varies with temperature. However, volatilization from soil or water is also influenced by other environmental conditions and the effective vapor pressure may be considerably lower than the potential vapor pressure. Nevertheless, vapor pressure is the one common factor governing the tendency of a compound to volatilize.

According to kinetic theory there is a continuous flight of molecules from the surface of a liquid or solid into the free space above it. At the same time vapor molecules return to the surface at a rate depending on the concentration of the vapor. If there is no removal of vapor from the surface (for example, by air currents), equilibrium will be established where the rate of vaporization is exactly equal to the rate of condensation. The pressure exerted by the equilibrium vapor is known as the vapor pressure (Daniel and Alberty 1955) and is dependent upon temperature.

Knowledge of the vapor pressure of a compound allows the ranking of a chemical as relatively nonvolatile, highly volatile, or of some intermediate volatility. When vapor pressure data are

combined with solubility data to calculate Henry's Law constants, as described by MacKay and Leinonen (1975) and Dilling (1977), rates of the evaporation of dissolved chemicals from water can be estimated.

Evaporation from an exposed surface will depend upon other factors such as wind speed (which reduces the vapor density above the surface) and adsorption (which may act to hold the substance on the surface). Volatilization from aqueous systems also depends on the solubility of the compound and its movement to the water surface. In soils the rate of volatilization of a chemical will depend upon such factors as adsorption on soil, solubility in soil water, and on the amount of soil water and its rate of evaporation. Volatilization from soils can become a diffusion controlled process as mass transfer to the soil surface is reduced by low water evaporation due to high humidity or to the lack of soil water in a dry soil (Bailey et al. 1974).

Chemicals that have relatively low vapor pressures and that sorb readily to solids or dissolve readily in water are not likely to vaporize significantly at ambient temperatures. For that reason, airborne transport is not a major transport mechanism for these chemicals and assessment of them should be focused on their chemical fate and environmental effects in soils, sediments, and water. However, chemicals with high vapor pressures or with relatively low water solubility and low adsorptivity to solids are less likely to reside only in soils, sediments or water, since volatilization can be a potentially significant

factor in their environmental transport. Chemicals that are gases at ambient temperatures and that have low water solubility and low adsorptive tendencies will be transported to a significant degree in the atmosphere and are prime candidates for photolysis and for involvement in adverse atmospheric effects such as smog formation or stratospheric alterations. Furthermore, effects testing of those chemicals should also focus on inhalation and surface contact as potential routes for direct exposure.

An understanding of how a chemical is likely to partition among the various environmental media (air, water, soil, and sediment) is needed in judging whether or not a chemical will be subject to various transformation possibilities, such as oxidation by hydroxyl radicals or ozone in the atmosphere. Vapor pressure data can influence decisions on whether or not it is appropriate to conduct photolysis, adsorption/desorption, partition coefficient, and certain biodegradation tests. Vapor pressure data are an important consideration in the design of other fate and effects tests, for example in preventing or accounting for the loss of volatile materials during the course of the test. Clearly, a knowledge of vapor pressure combined with information on water solubility and adsorptive tendencies is necessary in predicting environmental transport and in providing guidance as to which persistence and effects tests need to be considered and how those tests should be designed.

II. SCIENTIFIC ASPECTS

A. Test Methods

A procedure for measuring the vapor pressure of materials released to the natural environment ideally would cover a range of vapor pressure values, at ambient temperatures, of about 10^{-5} Pa to 10^5 Pa (approximately 10^{-7} to 760 torr). Because no single procedure can cover this range, two different procedures are described, each suited for a different part of the range. The isoteniscope procedure (ASTM 1978) is for pure liquids with vapor pressures from 0.1 to 100 kPa. For vapor pressures of 10^{-5} to 10^3 Pa, a gas saturation procedure may be used. The Knudsen effusion procedure (Thomson and Douslin 1971) may be used for low vapor pressure values.

It is important that each of the tests be performed under conditions of normal laboratory room temperatures in order to allow for careful control of the temperatures in thermostated baths or chambers containing the test apparatus.

The isoteniscope procedure uses a standardized technique that was developed to measure the vapor pressure of certain liquid hydrocarbons. It is applicable to pure liquids with vapor pressures of 0.1 kPa (0.75 torr) or more at ambient temperatures. The sample is purified within the equipment by removing dissolved and adsorbed gases until the measured vapor pressure is constant. This process is called "degassing." The procedures do not remove higher boiling impurities, decomposition products, or

compounds that boil close to or form azeotropes with the material under test. If compounds that boil close to or form azeotropes with the test material are present, it is necessary to remove the interfering compounds and use pure test material. Impurities more volatile than the sample will tend to increase the observed vapor pressure above its true value but the purification steps will tend to remove these impurities. Soluble, nonvolatile impurities will decrease the apparent vapor pressure. However, because the isoteniscope procedure is a static, fixed-volume method in which an insignificant fraction of the liquid sample is vaporized, it is subject to only slight error for samples containing nonvolatile impurities. That is, the nonvolatile impurities will not be concentrated due to vaporization of the sample.

Gas saturation (or transpiration) procedures use a current of inert gas passed through or over the test material slowly enough to ensure saturation and subsequent analysis of either the loss of material or the amount (and sometimes kind) of vapor generated (Bellar and Lichtenberg 1974, Thomson and Douslin 1971).

The gas saturation procedures have been described by Spencer and Cliath (1969). Results are easy to obtain and can be quite precise. The same procedures can also be used to study volatilization from laboratory scale environmental simulations. Vapor pressure is computed on the assumptions that the total pressure of a mixture of gases is equal to the sum of the

pressures of the separate or component gases and that the ideal gas law is obeyed. The partial pressure of the vapor under study can be calculated from the total gas volume and the weight of the material vaporized. If v is the volume which contains w grams of the vaporized material having a molecular weight M , and if p is the pressure of the vapor in equilibrium at temperature T (K), then the vapor pressure, p , of the sample is calculated by

$$p = (w/M)(RT/v) \quad ,$$

where R is the gas constant ($8.31 \text{ Pa M}^3\text{mol}^{-1} \text{ K}^{-1}$) when the pressure is in pascals (Pa) and the volume is in cubic meters. As noted by Spencer and Cliath (1970), direct vapor pressure measurements by gas saturation techniques are more directly related to the volatilization of chemicals than are other techniques.

In addition to the above methods, other procedures have been described for the measurement of vapor pressure (Daniels et al. 1956, Glasstone 1946, Thomson and Douslin 1971). These include boiling point procedures, effusion techniques, and many highly specialized techniques that are restricted to the determination of very precise vapor pressure values or to the measurement of vapor pressures of specific kinds of materials. These highly specialized methods do not have general applicability to either a wide variety of chemicals or a relatively broad range of vapor pressure values at ambient temperatures.

Boiling point procedures, such as that using Ramsey and Young apparatus, have very poor accuracy below 10^3 Pa (Thomson and Douslin 1971) and provide inaccurate estimates of the vapor pressures at ambient temperatures if there is a change of state or a transition temperature between the boiling and ambient temperatures.

Effusion techniques, particularly those employing the Knudsen effusion apparatus, are used to measure vapor pressure from about 10^{-5} to 1 Pa and have provided some good data (Hamaker and Kerlinger 1969). Those procedures require working with systems under vacuum and it is necessary to saturate the capsule space with vapor during the measurement periods. The lack of equilibrium saturation has been postulated as a reason for inaccurate published vapor pressure data (Spencer and Cliath 1970). However, it must be recognized that there are laboratories which have employed Knudsen effusion techniques successfully and which have considerable experience with the method, especially for determining very low vapor pressure values, such as 10^{-5} to 10^{-3} Pa. For such laboratories, the Knudsen effusion methods are a satisfactory alternative to the gas saturation method in the determination of low vapor pressure values. However, it seems reasonable to require that the laboratory using effusion methods supply documentation to substantiate successful utilization of the effusion procedures with other compounds.

B. Test Procedures

1. Temperature of the Test

The test procedures generally require a thermostated bath or test chamber temperature of $25 \pm 0.5^{\circ}\text{C}$. Laboratories should be able to carry out vapor pressure measurements without the need for elaborate temperature control devices. Control of the bath or chamber to $\pm 0.5^{\circ}\text{C}$ will permit substantial confidence in the data without requiring unnecessarily costly apparatus.

The International Union of Pure and Applied Chemistry has for many years (IUPAC 1972) recommended the reporting of physical-chemical properties measurements at the temperature of 25°C . A temperature of 25°C is slightly above most laboratory room temperatures and this allows for convenient adjustment and maintenance of constant temperature baths and enclosures.

Because of the nature of the isoteniscope procedure, it is necessary in that test to conduct some measurements at temperatures above and/or below 25°C in order to determine whether the sample needs further degassing. Also, for some chemicals, it may be necessary to require vapor pressure data at temperatures other than 25°C . Examples of when this requirement may be applicable include situations where there is evidence that the vapor pressure may change significantly with relatively small changes in ambient temperature or when the boiling temperature for a chemical is at an ambient temperature below 25°C .

2. Sorbent for Gas Saturation Procedure

The choice of sorbent and desorption solvent is dictated by the nature of the compound being evaluated. Charcoal sorbent is inexpensive and may be desorbed with carbon disulfide, a convenient solvent for use with a flame ionization detector. Many compounds, however, do not desorb efficiently from charcoal and more expensive sorbents, such as Tenax GC and XAD-2, are recommended. The desorption efficiency of a particular compound from a sorbent with a solvent is defined as the weight of the compound which can be recovered from the sorbent divided by the weight of the compound originally adsorbed. It is extremely important that the desorption efficiency be measured for every combination of sample, sorbent, and solvent used. Desorption efficiency may vary with concentration, so it is important to measure it at or near the concentration of the actual sample. It is sometimes necessary to interpolate between two measured efficiencies.

3. Gas Flow Rates in Gas Saturation Procedure

Accurate control of gas flow rates is essential to assure that a known volume of carrier gas is passed through the system. Very long sampling times are required for compounds with low vapor pressures, and it is difficult to control very low flow rates for very long times. It is necessary to use fine needle valves to control the flow rates and to measure the flow rates

frequently during the test period in order to make corrections for variation which can occur, e.g. due to changes in atmospheric pressure.

4. Calculations of Vapor Pressure in the Gas Saturation Procedure

The calculation of vapor pressure is straightforward. The weight of the sample desorbed from a sorbent section is divided by the desorption efficiency to give the weight of the sample collected by the sorbent trap. With the volume of carrier gas calculated from the flow rate, the ideal gas law is used to calculate the vapor pressure of the sample. To assure that the carrier gas is indeed saturated with the compound vapor, each compound is sampled at three different gas flow rates. If the vapor pressure calculated shows no dependence on flow rate, then the gas is assumed to be saturated. The method also assumes that there are no interactions between vaporized sample and the carrier gas and that the molecular weight of the vaporized sample is the same as for the sample liquid or solid. If there are any indications that these may not be valid assumptions, the vapor should be analyzed both qualitatively and quantitatively using such techniques as gas chromatography combined with mass spectrometry (Heller et al. 1975).

C. Test Data Required

The Test Guideline requires that the average calculated vapor pressure for the test material at each required test temperature be reported, including the individual values from triplicate determinations and the calculated standard deviation for each average calculated vapor pressure. It might be preferable for assessment purposes to require that each vapor pressure determination be made in sufficient replication to provide a given degree of reproducibility. However, the precision attainable will vary not only with the number of replications but also with the procedure employed and the test chemical. For a given chemical, the only way to determine how many replications of a given procedure are necessary to provide vapor pressure data with some specified precision is to repeat the procedure until the data provide that precision. This may take a few or many replications and a requirement for numerous replications is not justified unless the specified precision is needed for assessment purposes with an individual chemical. The minimum requirement EPA would impose would be a statistical analysis of vapor pressure data to provide standard deviation calculations based on triplicate determinations.

For the isoteniscope method, the Test Guideline requires that the vapor pressure data generated during the degassing operation, including a plot of $\log p$ vs $1/T$, be included to provide evidence of successful degassing. For the gas saturation method, the Test Guideline requires that the data showing that

vapor pressure does not vary with flow rate be included to provide evidence of saturation of the carrier gas with the sample vapor. Furthermore, it is extremely important that the data also include a complete description of all analytical techniques and results, a description of the sorbents and desorption solvents used and the desorption efficiency calculations.

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TRANSPORT PROCESSES

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August, 1982

SOIL THIN-LAYER CHROMATOGRAPHY

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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SOIL THIN-LAYER CHROMATOGRAPHYI. INTRODUCTIONA. Background and Purpose

Leaching of chemicals through soil is an important process which affects a chemical's distribution in the environment. If a chemical is tightly adsorbed to soil particles, it will not leach through the soil profile but will remain on the soil surface. If a chemical is weakly adsorbed, it will leach through the soil profile and may reach ground waters and then surface waters. Knowledge of the leaching potential is essential under certain circumstances for the assessment of the fate of chemicals in the environment.

Chemical leaching also affects the assessment of ecological and human health effects of chemicals. If a chemical reaches ground water, deleterious human health effects may arise due to the consumption of drinking water. If a chemical remains at the soil surface, deleterious environmental and human health effects may arise due to an increased concentration of the chemical in the zone of plant growth, possibly resulting in contamination of human food supplies.

Soil thin layer chromatography (TLC) is a qualitative screening tool suitable for obtaining an estimate of a chemical's leaching potential. This test is one of several tests which can be used in obtaining a rough estimation of a chemical's leaching potential.

B. Definitions and Units

"Cation exchange capacity" (CEC) is the sum total of exchangeable cations that a soil can adsorb. The CEC is expressed in milliequivalents of negative charge per 100 grams (meq/100g) or milliequivalents of negative charge per gram (meq/g) of soil.

"Particle size analysis" is the determination of the various amounts of the different particle sizes in a soil sample (i.e., sand, silt, clay) usually by sedimentation, sieving, micrometry or combinations of these methods. The names and size limits of these particles as widely used in the United States are:

very coarse sand	2.0 to 1.0 mm dia.
coarse sand	1.0 to 0.5 mm
medium sand	0.5 to 0.25 mm
fine sand	0.25 to 0.125 mm
very fine sand	0.125 to 0.062 mm
silt	0.062 to 0.002 mm
clay	<0.002 mm

" R_f " is the furthest distance traveled by a test material on a thin-layer chromatography plate divided by the distance traveled by a solvent front (arbitrarily set at 10.0 cm in soil TLC studies).

"Soil" is the unconsolidated mineral material on the immediate surface of the earth that serves as a natural medium for the growth of land plants; its formation and properties are determined by various factors such as parent material, climate, macro- and microorganisms, topography, and time.

"Soil aggregate" is the combination or arrangement of soil separates (sand, silt, clay) into secondary units. These units may be arranged in the profile in a distinctive characteristic pattern that can be classified on the basis of size, shape, and degree of distinctness into classes, type, and grades.

"Soil classification" is the systematic arrangement of soils into groups or categories. Broad groupings are made on the basis of general characteristics, subdivisions, on the basis of more detailed differences in specific properties. The soil classification system used today in the United States is the 7th Approximation Comprehensive System. The ranking of subdivisions under the system is: order, suborder, greatgroup, family and series.

"Soil horizon" is a layer of soil approximately parallel to the land surface. Adjacent layers differ in physical, chemical, and biological properties or characteristics such as color, structure, texture, consistency, kinds, and numbers of organisms present, and degree of acidity or alkalinity.

"Soil order" is the broadest category of soil classification and is based on general similarities of physical/chemical properties. The formation by similar genetic processes causes these similarities. The soil orders found in the United States are: Alfisol, Aridisol, Entisol, Histosol, Inceptisol, Mollisol, Oxisol, Spodosol, Ultisol, and Vertisol.

"Soil organic matter" is the organic fraction of the soil; it includes plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by the microbial population.

"Soil pH" is the negative logarithm to the base 10 of the hydrogen ion activity of a soil as determined by means of a suitable sensing electrode coupled with a suitable reference electrode at a 1:1 soil:water ratio.

"Soil series" is the basic unit of soil classification and is a subdivision of a family. A series consists of soils that were developed under comparable climatic and vegetational conditions. The soils comprising a series are essentially alike in all major profile characteristics except for the texture of the "A" horizon (i.e. the surface layer of soil).

"Soil texture" refers to the classification of soils based on the relative proportions of the various soil separates present. The soil textural classes are: clay, sandy clay, silty clay, clay loam, silty clay loam, sandy clay loam, loam, silt loam, silt, sandy loam, loamy sand, and sand.

C. Principle of the Test Method

Before 1968, methods of investigating the mobility of nonvolatile organic chemicals within soils were based on the use of field analysis, soil adsorption isotherms, and soil columns. In 1968, Helling and Turner introduced soil thin layer chromatography (soil TLC) as an alternative procedure; it is analogous to conventional TLC, with the use of soil instead of silica gels, oxides, etc., as the adsorbent phase.

The papers by Helling (1968, 1971a, 1971b, 1971c) and Helling and Turner (1968) were the basis of this test guideline. The soil and colloid chemistry literature and the analytical chemistry literature substantiates the experimental conditions specified in the guideline.

The soil TLC offers many desirable features. First, mobility results are reproducible. Mass transfer and diffusion components are distinguishable. The method has relatively modest requirements for chemicals, soils, laboratory space, and equipment. It yields data that are amenable to statistical analyses. A chemical extraction-mass balance procedure to elicit information on degradation and chemical transformations occurring at colloid interfaces can be incorporated into this test. The ease with which the R_f and mass balance are performed will depend upon the physical/chemical properties of the test chemical and the availability of suitable analytical techniques for measuring the chemical.

D. Applicability and Specificity

Soil TLC can be used to determine the soil mobility of sparingly water soluble to infinitely soluble chemicals. In general, a chemical having a water solubility of less than 0.5 ppm need not be tested since the literature indicates that these chemicals are, in general, immobile (Goring and Hamaker, 1972). However this does not preclude future soil adsorption/transformation testing of these chemicals if more refined data are needed for the assessment process.

Soil TLC may be used to test the mobility of volatile chemicals by placing a clean plate over the spotted soil TLC plate and then placing both plates in a closed chromatographic chamber.

Soil TLC was originally designed for use with soils. The literature shows no published use of this method with sediments as the absorbent phase, probably due to the fact that sediment surface properties change significantly during air drying. It is extremely important that the TLC plate with the adsorbent be air dried before leaching studies can be undertaken.

II. TEST PROCEDURES

A. Test Conditions

- (1) Equipment required: Distilled-deionized water adjusted to pH 7 by boiling to remove CO₂; clean glass plates (TLC); glass rods or a variable thickness plate spreader; masking tape; closed chromatographic chambers; analytical instrumentation necessary and appropriate for the detection and quantitative analysis of the test chemical;
- (2) the test procedure may be run at $23 \pm 5^{\circ}\text{C}$; and
- (3) it is recommended that three replicate plates for each soil be used.

B. Test Procedures

- (1) To reduce aggregate size before or during sieving, crush and grind the air dried soil very, very gently;
- (2) sieve air dried soils with a 250 micrometer sieve;
- (3) add water to the sieved soil until a smooth, moderately fluid slurry is attained (approximately 3/4 ml H₂O added for each gram of soil);

- (4) spread the slurry evenly and quickly across the clean glass plate using a variable thickness plate spreader, a glass rod, or other available method. If a glass rod is used, control the layer thickness by affixing multiple layers of masking tape along the plate edges. Soil layer thickness should be 0.50 - 0.75 mm;
- (5) air dry the plates at 25°C for a minimum of 24 hours after uniform slurry application is achieved;
- (6) scribe a horizontal line 11.5 cm above the base through the soil layer down to the glass so as to stop solvent movement;
- (7) spot the test chemical, in solution, 1.5 cm above the base. For radiolabeled materials, 0.5 - 5 g containing 0.01 - 0.03 Ci of ^{14}C labeled compound may be used;
- (8) if the compound is volatile, it is extremely important that a clean plate be placed over the soil TLC plate to impede volatilization;
- (9) immerse the plate with the base down at some angle from the vertical in a closed chromatographic chamber containing H_2O at a height of 0.5 cm;
- (10) allow the solvent front to migrate to the 11.5 cm line before removing the plates from the chamber;
- (11) determine the R_f values. Zonal extraction, plate scanning, or any other method or combination of methods suitable for detection of the parent test chemical may be used; and

- (12) determine the amount of the parent test chemical on the entire soil TLC plate after test chemical migration. Any method or combination of methods suitable for the extraction and quantitative detection of the parent test chemical may be used.

III. DATA AND REPORTING

Report the following information as shown in Figures 1 and 2.

- (1) Temperature at which the test was conducted;
- (2) amount of the test chemical applied and amount recovered from the plates;
- (3) detailed description of the analytical technique used in the R_f determination, the chemical extraction, and the quantitative recovery and analysis of the parent chemical;
- (4) the mean frontal R_f value with the standard deviation for each soil tested;
- (5) a photograph or diagram of the TLC plate which shows the entire leaching pattern (from 1.5 to 11.5 cm);
- (6) soil information: soil order, series, texture, sampling location, horizon, general clay fraction mineralogy; and

- (7) soil physical/chemical properties: percent sand,
percent silt and percent clay (particle size analysis);
percent organic matter; pH (soil to water ratio, 1:1);
and cation exchange capacity.

FIGURE 1 - SOIL TLC DATA FORMAT

SOIL:



PLATE 1



PLATE 2



PLATE 3

QUANTITY	PLATE 1	PLATE 2	PLATE 3
R_f			
Amount Applied			
Amount Recovered			
% Recovered			
Mean R_f			
Standard Deviation			

Figure 2. Soil Physical, Chemical, and Classification
Data Format

	Soil 1	Soil 2
SOIL ORDER:	Alfisol	
SOIL SERIES:	Crider	
SOIL TEXTURE:	Silt Loam	
LOCATION:	Gallatin County, IL	
HORIZON:	A	
% SAND:	1.2	
% SILT:	86.6	
% CLAY:	12.2	
% ORGANIC MATTER:	1.74	
pH (1:1 H ₂ O):	7.20	
CEC (MEQ/100GMS):	13.5	
CLAY FRACTION	75% Montmorillonite	
MINERALOGY:	5-20% Mica	
	5% Kaolinite	
	(36-120 cm depth)	

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SOIL THIN LAYER CHROMATOGRAPHYI. NEED FOR THE TEST

Leaching of chemicals through soil is an important process which affects a chemical's distribution in the environment. If a chemical is tightly adsorbed to soil particles, it will not leach through the soil profile but will remain on the soil surface. If a chemical is weakly adsorbed, it will leach through the soil profile and may reach ground waters and then surface waters. Knowledge of the leaching potential is essential under certain circumstances for the assessment of the fate of chemicals in the environment.

Chemical leaching also affects the assessment of ecological and human health effects of chemicals. If a chemical reaches ground water, deleterious human health effects may arise due to the consumption of drinking water. If a chemical remains at the soil surface, deleterious environmental and human health effects may arise due to an increased concentration of the chemical in the zone of plant growth, possibly resulting in contamination of human food supplies.

Soil thin layer chromatography (TLC) is a qualitative screening tool suitable for obtaining an estimate of a chemical's leaching potential. This test is one of several tests which can be used in obtaining a rough estimation of a chemical's leaching potential.

II. SCIENTIFIC ASPECTS OF SOIL LEACHING

A. Introduction

Since chemical leaching in soils is affected by a large number of interacting processes (Hamaker 1975) this section of the support document will discuss these processes as they relate to this phenomenon of soil leaching.

B. Basic Processes Affecting Soil Leaching

The general equation (Guenzi 1974) for chemical movement through porous media under steady state soil-water flow conditions is:

$$\frac{B\delta S}{\theta\delta t} + \frac{\delta C'}{\delta t} = D' \frac{\delta^2 C'}{\delta X^2} - V \frac{\delta C'}{\delta X} \quad (1)$$

where

- B = soil bulk density (g/cm³)
- θ = volumetric water content (cm³/cm³)
- S = amount of chemical adsorbed at the soil/water interface (g/g soil).
- t = time (sec.)
- C' = solution concentration of chemical
- D' = dispersion coefficient (cm²/sec)
- V = average pore-water velocity (cm/sec)
- X = space coordinate measured normal to the section

Most mass transport equations represent simplifications of "real world" conditions. Equation 1 and similar mathematical expressions try to describe the chromatographic distribution of the chemical in the soil profile; however, they are gross simplifications of a phenomenon affected by a number of complex interacting processes including but not limited to precipitation, evaporation, evapotranspiration and hydrodynamic dispersion.

In general, chemical leaching is dependent upon three major processes: the mass transport of water (the direction and rate of water flow), diffusion, and the adsorption characteristics of the chemical in soil (Guenzi 1974). Diffusion is the transport of matter resulting from random molecular motion caused by molecular thermal energy. This random motion will lead to the uniform distribution of molecules in a closed system since there is net movement from regions of higher to lower concentrations. In this document, adsorption refers to the equilibrium distribution of a molecule between a solid phase and a solution phase. As the degree of adsorption increases, the concentration of the chemical in the soil water and the soil air decreases. This equilibrium process is governed by two opposing rate processes. The adsorption rate is the rate to which molecules from the liquid phase transfer into the adsorbed state in the solid phase. The desorption rate is the opposite process, i.e., the rate at which molecules transfer from the adsorbed state in the solid phase into the liquid phase. In general, the mass transport, diffusion, and adsorption processes produce the observed leaching pattern of a chemical in soil.

C. Chemical Properties Affecting Leaching

The main process of the three processes discussed above which determines a chemical's leaching potential (as described mathematically in equation 1) is adsorption. Adsorption is governed by the properties of both the adsorbent and the adsorbate. The important properties of the adsorbate affecting adsorption by soil colloids (Bailey and White 1970) are: (1) chemical structure and conformation (2) acidity or basicity of the molecule (pK_a or pK_b), (3) water solubility, (4) permanent charge, (5) polarity, (6) molecular size, and (7) polarizability. There are many ways in which each of these adsorbate properties interact and are manifested in the overall adsorption reaction (Bailey and White 1970).

D. Soil Properties Affecting Leaching

Soil is the unconsolidated organic and mineral material on the immediate surface of the earth which serves as a natural medium for the growth of plants. The combined actions of climate, microorganisms and macroorganisms over long periods of time on different parent geologic and biotic materials form soils that differ widely in their physical, chemical, and morphological characteristics. The wide variations in the amounts and types of clay and organic matter, soil pH, primary and secondary minerals, structure, texture, and exchange capacity create soils of

substantial heterogeneity within the United States. There are currently 10 Soil Orders, at least 43 Suborders, over 200 Great Groups and over 7,000 soil series recognized in the United States (Buckman and Brady 1969).

The soil properties affecting the adsorption and desorption of organics include organic matter content, type and amount of clay, exchange capacity, and surface acidity (Adams 1973; Bailey and White 1970; and Helling 1970). Soil organic matter is a primary soil parameter responsible for the adsorption of many pesticides. Helling (1970) lists many examples where the organic matter primarily influenced the adsorption of pesticides. Although organic matter and clay are the soil components most often implicated in pesticide adsorption, the individual effects of either organic matter or clay are not easily ascertained. Since the organic matter in most soil is intimately bound to the clay as a clay-metal-organic complex (Stevenson 1973), two major types of adsorbing surfaces are normally available to the chemical, namely, clay-organic and clay alone. Clay and organic matter function more as a unit than as separate entities and the relative contribution of organic and inorganic surfaces to adsorption will depend on the extent to which the clay is coated with organic substances. Comparative studies between known clay minerals and organic soils suggest that most, but not all, pesticides have a greater affinity for organic surfaces than for mineral surfaces (Stevenson 1973). Since typical soil studies compare soils in which both clay and organic matter increase and do not utilize multiple regression analyses to isolate the

governing parameter (Helling 1970), only generalizations concerning the relative importance of clay and organic matter in the adsorption process can be made.

The activity of protons in the bulk suspension (i.e., as measured by pH) and the activity of protons at or in close proximity to the colloidal surface (i.e., the acidity in the interfacial region) may differ significantly. The term "surface acidity" as applied to soil systems is the acidity at or in close proximity to the colloidal surface and reflects the ability of the system to act as a Lewis acid. Surface acidity is a composite term which reflects both the total number of acidic sites and their relative degree of acidity. Surface acidity is probably the most important property of the soil or colloidal system in determining the extent and nature of adsorption of basic organic chemicals as well as determining if acid-catalyzed chemical transformation occurs (Bailey and White 1970). There is overwhelming evidence, mainly from infrared studies as well as other studies, pointing to the fact that there is protonation of basic chemicals by clays having hydrogen and aluminum as the predominant exchangeable cation and by clays saturated with alkali, alkaline earth, and transition metal cations. A summary of recent investigations indicates that the protonation of chemicals in the interfacial region of clays is a function of the basicity of the molecule, the nature of the exchangeable cation on the clay, water content of the clay system, and the origin of negative charge in the aluminosilicate clay (Bailey and White 1970).

In summary, the chemical properties discussed in (C) and the soil properties discussed in (D) both govern the extent of adsorption in soils.

E. Types of Adsorptive Forces

The specific type of interaction of organic molecules with soil will depend on the specific chemical properties of the organic molecule and the type of soil. These specific interactions or adsorptive forces are usually classified as: van der Waals forces, charge transfer, ion exchange, and hydrophobic bonding (Adams 1975, Goring and Hamaker 1972).

The van der Waals forces arise from the fluctuations in a molecule's electron distribution as the electrons circulate in their orbitals. These fluctuations produce instantaneous dipoles which cause that molecule's attraction to other atoms and molecules. Charge transfer involves the formation of a donor-acceptor complex between an electron donor molecule and an electron acceptor molecule with partial overlap of their respective molecular orbitals and a partial exchange of electron density. Ion exchange refers to the exchange between counterions balancing the surface charge on the soil colloid and the ions in the soil solution. The driving force for this interaction is the requirement for electroneutrality: the surface electric charge must be balanced by an equal quantity of oppositely charged counterions. In general, ion exchange is reversible, diffusion controlled, stoichiometric and, in most cases, exhibits some

selectivity or preferential adsorption for one ion over another competing ion. Hydrophobic solvation, the process commonly referred to as hydrophobic bonding, refers to the preference of an organic molecule for a hydrocarbon solvent or hydrophobic region of a colloid over a hydrophilic solvent. This preference is due to the fact that hydrocarbon regions of a molecule have greater solubility in liquid hydrocarbons (or most organic solvents) than in water. In general, one or more of these specific interactions or adsorptive forces may occur at the same time depending on the presence and magnitude of the chemical and soil properties discussed above.

F. Surface Transformations

A special type of interaction between organic molecules and soils deals with the transformation of organic chemicals into new compounds containing different chemical structures through the catalytic activity of the soil colloid surfaces. Although several theories exist to account for the mechanism of these transformations, no scheme predicting the occurrence of such surface reactions presently exists. Therefore, it is extremely important that parent compound mass balances be performed and reported in order to ascertain the extent of such transformations during soil leaching experiments. Also, the leaching pattern (a diagram or photograph of the TLC plate showing the position of the chemical) can give a qualitative indication of the extent of such transformations and should be reported. The scientific literature shows that a number of chemicals and chemical classes

undergo colloid surface induced chemical transformations. Poly-(dimethylsiloxane) fluids in intimate contact with many soils undergo siloxane bond redistribution and hydrolysis, resulting in the formation of low molecular weight cyclic and linear oligomers (Buch and Ingebrightson 1979). S-triazines (White 1976) and organophosphorus pesticides (Yaron 1978, and Mingelgrin et al. 1977) undergo clay colloid induced hydrolysis. Benzene and phenol polymerize into high molecular weight species by adsorption and reaction at the surface of smectite saturated with transition metal cations (Mortland and Halloran 1976). Gallic acid, pyrogallol, protocatechuic acid, caffeic acid, orcinol, ferulic acid, p-coumaric acid, syringic acid, vanillic acid and p-hydroxybenzoic acid undergo oxidative polymerization in the presence of various clay minerals (Wang and Li 1977, and Wang et al. 1978). In general, testing methods that do not take into account surface transformations should not be used in determining the leaching potential of chemicals.

In summary, the interfacial region is important in determining the adsorption mechanism, the energy by which the adsorbate is held, and in determining if the adsorbed chemical is transformed. This information is important in determining the persistence and ultimate toxicity of the molecule since the transformation product(s) (1) may be more or less toxic than the original compound, (2) may be more or less tightly bound than the original compound, and (3) may have a water solubility either greater than or less than the original compound, thereby affecting its leaching and movement into the groundwater.

III. SCIENTIFIC ASPECTS OF THE TEST

A. Development of Soil Thin Layer Chromatography (TLC)

Before 1968, methods of investigating the mobility of nonvolatile organic chemicals within soils were based on the use of field analysis, soil adsorption isotherms, and soil columns. In 1968, Helling and Turner introduced soil thin layer chromatography (soil TLC) as an alternate procedure. It is analogous to conventional TLC, with the use of soil instead of silica gels, oxides, etc. as the adsorbent phase.

In their initial report, Helling and Turner used Lakeland sandy loam, Chillum silt loam, and Hagerstown silty clay loam. Medium sand (250 m dia.) was removed from Chillum and Hagerstown soils and coarse sand (500 m) from Lakeland soil by dry-sieving. Aqueous slurries were prepared and 0.50 mm (silt loam, silty clay loam) or 0.75 mm (sandy loam) thick layers were spread on TLC plates using conventional TLC apparatus. After drying, six or seven radiolabelled pesticides were applied near the base of a 20 x 20 cm plate and developed ten cm with water by ascending chromatography. Pesticide movement was visualized by autoradiography. Movement was expressed by the conventional R_f designation, although this referred to the front of pesticide movement rather than its maximum concentration. The soil TLC data are most appropriately compared with other mobility data

which indicate the depth to which an organic chemical may be leached. The ranking of pesticides in order of mobility is in good agreement with general trends previously reported.

Absolute movement on soil TLC plates cannot be transposed directly to field or soil column experiments. Since soil structure in the TLC system is considerably more homogeneous than in most other systems, band spreading will be somewhat less than in field or column regimes. Flow rates are also higher than those occurring naturally. For example, infiltration into Hagerstown silty clay loam was equivalent to rainfall of about 1.2 cm/hr (Helling 1970). High flow rates are usually associated with increased mobility, as later correlations (Helling 1968) bore out. In spite of these problems, monitoring data utilizing certain reference chemicals has provided the necessary information to relate soil TLC data to column and field data. In general, Helling and Turner (1968) indicated that soil TLC offered a rapid, simple, and inexpensive procedure for establishing a general mobility classification of pesticides and organic chemicals.

Simple chromatographic theory can be used to correlate adsorption coefficients with soil TLC R_f values. If chromatographic movement through a soil column is treated according to the distillation theoretical plate theory (Block et al. 1958, Martin and Synge 1941), a formula for R_f is obtained in

terms of the relative cross-sectional areas of the liquid and solid phases and partitioning of a chemical between solid and liquid phases (Hamaker 1975):

$$R_f = A_L / (A_L + A_S) = 1 / [1 + \alpha (A_L + A_S)] \quad (2)$$

where A_S and A_L are cross-sectional areas of solid and liquid phases and α is the ratio of volume concentration in the solid phase to that in the liquid phase. For saturated conditions which will be assumed for a soil plate, $A_L + A_S = A$ (cross-sectional area), this can be written:

$$R_f = 1 / [1 + \alpha (A / [A_L - 1])] \quad (3)$$

When reexpressed in terms of the pore fraction of the soil θ , density of soil solids (d_s), and a soil adsorption coefficient K , this equation becomes:

$$R_f = (1 + K(d_s)(1/\theta^{2/3} - 1))^{-1} \quad (4)$$

This ratio, A/A_L , is set equal to $1/\theta^{2/3}$ by analogy to the treatment of soil diffusion by Millington and Quirk (1961) where it serves to correct for the tortuosity of flow through the porous medium. In this case, it serves to relate the pore volume to the cross sectional area of the liquid phase in a saturated

soil. In general, equation 4 has shown that an inverse relationship exists between the soil adsorption coefficient K and R_f (Hamaker 1975).

Riley (1976) presented a general relationship between the soil/solution distribution coefficient K and the depth of pesticide leaching. Relating the data of Riley (1976) with the R_f values of Helling (1968, 1971a, 1971b, 1971c) and the average K values of Goring and Hamaker (1972) for selected pesticides, the general relationship shown in Table 1 was developed between the soil/solution partition coefficient, R_f , and soil mobility.

B. Rationale for the Selection of Soil TLC

A number of laboratory tests - the soil thin layer chromatography, soil adsorption isotherm, and soil columns - have been developed to obtain an estimate of a chemical's leaching potential (Hamaker 1975). Soil TLC is the least expensive of the available tests which measures leaching potential, and is widely used; furthermore, it offers many desirable features. First, mobility results are reproducible. Mass transfer and diffusion components are distinguishable. The method has relatively modest requirements for chemicals, soils, laboratory space, and equipment. It yields data that are amenable to statistical analyses. A chemical extraction-mass balance procedure to elicit information on degradation and chemical transformations occurring at colloid interfaces can be incorporated into this test. The ease with which the R_f and mass balance are performed will depend

Table 1. The General Relationship Between the Soil/Solution
Partition Coefficient K , R_f and Soil Mobility

K	R_f	Mobility class	Distance surface applied chemical may leach
0.1.....	0.95	Very Mobile.....	
1.....	0.60	Much of chemical leaches through top 20 cm soil into subsoil.
10.....	0.25	Mobile.....	Much of chemical leached into soil but peak concentration in top 20 cm soil.
10^2	0.10	Low mobility.....	Only small amount of leaching and peak concentration normally in top 5 cm soil.
$10^{2.5}$	0.00	Immobile.....	No significant leaching.
10^3	0.00	
10^4	0.00	

upon the physical/chemical properties of the test chemical and the availability of suitable analytical techniques for measuring the chemical.

C. Rationale for Selection of Experimental
Conditions and Procedures

The papers by Helling (1968, 1971a, 1971b, 1971c) and Helling and Turner (1968) were the basis of this test guideline. The soil and colloid chemistry literature and the analytical chemistry literature substantiates the experimental conditions specified in this Test Guideline as accepted, standard procedures. A few of these conditions will be discussed in greater detail below.

Soil TLC can be used to determine the soil mobility of sparingly water soluble to infinitely soluble chemicals. In general a chemical having a water solubility of less than 0.5 ppm need not be tested since the literature indicates that these chemicals are, in general, immobile (Goring and Hamaker 1972). However, this does not preclude advanced soil adsorption/transformation testing of these chemicals if more refined data are needed for the assessment process.

Soil TLC may be used to test the mobility of volatile chemicals by placing a clean plate over the spotted soil TLC plate and then placing both plates in a closed chromatographic chamber.

Soil TLC was originally designed for use with soils. The literature shows no published use of this method with sediments as the adsorbent phase, probably due to the fact that sediment surface properties change significantly during air drying. It is extremely important that the TLC plate with the adsorbent be air dried before leaching studies can be undertaken.

Distilled-deionized H₂O is required in order to minimize competition effects for soil exchange sites by cationic and anionic species normally present in tap and distilled H₂O.

It is extremely important that the test chemical be of the purest grade available. Impurities may produce migration patterns on the TLC plate independent of the parent chemical and may be misinterpreted as transformation products. Transformation product identification is an expensive analytical procedure that may be unnecessarily required as a result of the presence of impurities.

The sieving of soils will remove the coarse (500-2,000 μ m) and medium (250-500 μ m) sand fractions. Published testing results showed that removal of a portion of sand had no affect on the mobility of a test compound but aided in achieving a more cohesive uniform soil layer and more reproducible results (Helling 1971a).

Gentle crushing and grinding should be used to reduce soil aggregate size. Fine particles (silt and clay) in excess of the amount originally present may be created if excessive pressure is exerted on the aggregates.

It is important that application of the soil slurry to clean glass plates be done quickly to prevent particle size segregation. A specific method of soil slurry application was not identified since a number of methods which produce the acceptable layer thickness are in use today.

Replication of the basic experimental unit was necessary in order to estimate the standard deviation of the treatment mean. Three replicates are considered to be the minimum number of replicates for a statistically acceptable estimation. The soils literature indicates that, in general, the standard deviation should be less than 0.01 R_f units for soil TLC.

Since the available literature indicated that pesticide mobility on soil TLC plates did not significantly change when temperature varied from 2° to 25°C (Helling 1971a), only a room temperature range was suggested.

The Soil Order, Series, and general clay fraction mineralogy data may be found in Soil Survey Reports published after approximately 1970. Pre-1970 reports may not contain mineralogy data. Soil Survey Reports have been issued for most U.S. counties and may be obtained from County Extension Offices; the State Office of the U.S. Department of Agriculture Soil

Conservation Service; or the USDA-Soil Conservation Service, Publications and Information Division, P.O. Box 2890, Washington, DC 20013. If mineralogy data are not printed in a report, the State Office of the U.S. Department of Agriculture Soil Conservation Service may be contacted for assistance in obtaining general clay mineral data of a particular soil.

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SEDIMENT AND SOIL ADSORPTION ISOTHERM

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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SEDIMENT AND SOIL ADSORPTION ISOTHERMI. INTRODUCTIONA. Background and Purpose

The adsorption of chemicals to sediments and soils is an important process that affects a chemical's distribution in the environment. If a chemical is adsorbed to soil particles, it will remain on the soil surface and will not reach ground water. If a chemical is not adsorbed, it will leach through the soil profile and may reach ground waters and then surface waters. Similarly, if a chemical adsorbed to sediment, it will accumulate in the bed and suspended load of aquatic systems. If a chemical is not adsorbed to sediment, it will accumulate in the water column of aquatic systems. Information on the adsorption potential is needed under certain circumstances to assess the transport of chemicals in the environment. This Test Guideline describes procedures that will enable sponsors to determine the adsorption isotherm of a chemical on sediments and soils.

B. Definitions and Units

The "cation exchange capacity" (CEC) is the sum total of exchangeable cations that a sediment or soil can adsorb. The CEC is expressed in milliequivalents of negative charge per 100 grams (meq/100g) or milliequivalents of negative charge per gram (meq/g) of soil or sediment.

"Clay mineral analysis" is the estimation or determination of the kinds of clay-size minerals and the amount present in a sediment or soil.

"Organic matter" is the organic fraction of the sediment or soil; it includes plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by the microbial population.

"Particle size analysis" is the determination of the various amounts of the different particle sizes in a sample (i.e., sand, silt, clay), usually by sedimentation, sieving, micrometry, or combinations of these methods. The names and diameter range commonly used in the United States are:

<u>Name</u>	<u>diameter range</u>
very coarse sand	2.0 to 1.0 mm dia.
coarse sand	1.0 to 0.5 mm
medium sand	0.5 to 0.25 mm
fine sand	0.25 to 0.125 mm
very fine sand	0.125 to 0.062 mm
silt	0.062 to 0.002 mm
clay	<0.002 mm

The "pH" of a sediment or soil is the negative logarithm to the base ten of the hydrogen ion activity of the sediment or soil suspension. It is usually measured by a suitable sensing electrode coupled with a suitable reference electrode at a 1/1 solid/solution ratio by weight.

The adsorption ratio, " K_d ," is the amount of test chemical adsorbed by a sediment or soil (i.e., the solid phase) divided by the amount of test chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio.

"Sediment" is the unconsolidated inorganic and organic material that (a) is suspended in and being transported by surface water, or (b) has settled out and has deposited into beds.

"Soil" is the unconsolidated mineral material on the immediate surface of the earth that serves as a natural medium for the growth of land plants. Its formation and properties are determined by various factors such as parent material, climate, macro- and microorganisms, topography, and time.

"Soil aggregate" is the combination or arrangement of soil separates (sand, silt, clay) into secondary units. These units may be arranged in the soil profile in a distinctive characteristic pattern that can be classified according to size, shape, and degree of distinctness into classes, types, and grades.

"Soil classification" is the systematic arrangement of soils into groups or categories. Broad groupings are based on general soil characteristics while subdivisions are based on more detailed differences in specific properties. The soil classification system used in this standard and the one used today in the United States is the 7th Approximation-Comprehensive System. The ranking of subdivisions under this system is: Order, Suborder, Great group, family, and series.

A "soil horizon" is a layer of soil approximately parallel to the land surface. Adjacent layers differ in physical, chemical, and biological properties such as color, structure, texture, consistency, kinds and numbers of organisms present, and degree of acidity or alkalinity.

"Soil Order" is the broadest category of soil classification and is based on the general similarities of soil physical/chemical properties. The formation of soil by similar general genetic processes causes these similarities. The Soil Orders found in the United States are: Alfisol, Aridisol, Entisol, Histosol, Inceptisol, Mollisol, Oxisol, Spodosol, Ultisol, and Vertisol.

"Soil series" is the basic unit of soil classification and is a subdivision of a family. A series consists of soils that were developed under comparable climatic and vegetational conditions. The soils comprising a series are essentially alike in all major profile characteristics except for the texture of the "A" horizon (i.e., the surface layer of soil).

"Soil texture" is a classification of soils that is based on the relative proportions of the various soil separates present. The soil textural classes are: clay, sandy clay, silty clay, clay loam, silty clay loam, sandy clay loam, loam, silt loam, silt, sandy loam, loamy sand, and sand.

C. Principle of the Test Method

The extent of adsorption of a chemical onto sediment or soil is measured, using this test guideline, by equilibrating aqueous solutions containing different, but environmentally realistic, concentrations of the test chemical with a known quantity of sediment or soil. After equilibrium is reached, the distribution of the chemical between the water phase and the solid phase is quantitatively measured by a suitable analytical method. Then, sorption constants are calculated by using the Freundlich equation:

$$x/m = C_s = KC_e^{1/n} \quad (1)$$

where

C_e = Equilibrium concentration of the chemical in the solution phase

C_s = Equilibrium concentration of the chemical in the solid phase

K = Freundlich adsorption coefficient

m = The mass of the solid in grams

$1/n$ = Exponent where n is a constant

x = The mass in micrograms of the chemical adsorbed by m grams of solid.

Logarithmic transformation of the Freundlich equation yields the following linear relationship:

$$\log C_s = \log K + (1/n) \log C_e \quad (2)$$

In order to estimate the environmental movement of the test chemical, the values K and $1/n$ are compared with the values of other chemicals whose behavior in soil and sediment systems is well-documented in scientific literature.

The adsorption isotherm (AI) test has many desirable features. First, adsorption results are highly reproducible. The test provides excellent quantitative data readily amenable to statistical analyses. Also, it has relatively modest requirements for chemicals, soils, laboratory space, and equipment. It allows solution phase organic chemical determinations that are relatively uncomplicated. A chemical extraction-mass balance procedure to elicit information on chemical transformations occurring at colloid interfaces can be incorporated into this test. The ease of performing the isotherm test and mass balance will depend upon the physical/chemical properties of the test chemical and the availability of suitable analytical techniques to measure the chemical.

The papers by Aharonson and Kafkafi (1975), Harvey (1974), Murray (1975), Saltzman (1972), Weber (1971), and Wu (1975) served as the basis for this Test Guideline. The soil and colloid

chemistry literature and the analytical chemistry literature substantiate the experimental conditions and procedures specified in this guideline as accepted, standard procedures.

D. Applicability and Specificity

The AI Test Guideline can be used to determine the soil and sediment adsorption potential of sparingly water soluble to infinitely soluble chemicals. In general, a chemical having a water solubility of less than 0.5 ppm need not be tested with soil as the solid phase, since the literature indicates that these chemicals are, in general, immobile in soils. (Goring and Hamaker, 1972). However, this does not preclude future soil adsorption/transformation testing of these chemicals if more refined data are needed for the assessment process.

II. TEST PROCEDURES

A. Test Conditions

1. Special laboratory equipment

- a. Equilibrating solutions that contain, besides the test chemical, 0.01M calcium nitrate dissolved in sterilized, distilled-deionized H₂O adjusted to neutral pH 7 by boiling to remove CO₂.
- b. Containers that are composed of material that (1) adsorb negligible amounts of test chemical, and (2) withstand high speed centrifugation. The volume of the container is not a major consideration; however, it is extremely important that the amount of soil or sediment and the solid/solution ratio used in the study result in minimal container headspace. It is also extremely important that the containers be sterilized before use.
- c. A 150 μ m (100 mesh) stainless steel or brass sieve.

- d. Drying oven, with circulating air, that can attain 100°C.
- e. Vortex mixer or a comparable device.
- f. Rotary shaker or a comparable device.
- g. High speed temperature-controlled centrifuge capable of sedimenting particles greater than 0.5 μ m from aqueous solution.

2. Temperature

It is recommended that the test procedure be performed at 23 \pm 5°C.

3. Replications

It is recommended that three replications of the experimental treatments be used.

4. Soil Pretreatment

It is extremely important that these soil pretreatment steps be performed under the following conditions:

- a. Decrease the water content, air or oven dry soils at or below 50°C.

- b. Reduce aggregate size before and during sieving, crush and grind dried soil very gently.
- c. Eliminate microbial growth during the test period using a chemical or physical treatment that does not alter or minimally alters the soil surface properties.
- d. Sieve soils with a 100 mesh stainless steel or brass sieve.
- e. Store all solutions and soils at temperatures between 0 and 5°C.

5. Sediment Pretreatment

It is extremely important that these sediment pretreatment steps be performed under the following conditions:

- a. Decrease the H₂O content by air or oven drying sediments at or below 50°C. Sediments should not be dried completely and should remain moist at all times prior to testing and analysis.
- b. Eliminate microbial growth during the test period by using a chemical and/or physical treatment that does not alter or minimally alters the colloid surface's properties.

- c. Store at temperatures between 0 and 5°C.

6. Solid/Solution Ratio

It is recommended that the solid/solution ratio be equal to or greater than 1/10. If possible, the ratios should be equal to or greater than 1/5. The sediment or soil dry weight after drying for a 24 hour minimum at 90°C is recommended for use as the weight of the solid for ratio and data calculations.

7. Equilibration Time

The equilibration time will depend upon the length of time needed for the parent chemical to attain an equilibrium distribution between the solid phase and the aqueous solution phase. It is recommended that the equilibration time be determined by the following procedure:

- a. Equilibrate one solution containing a known concentration of the test chemical with the sediment or soil in a solid/solution ratio not exceeding 1/10 and preferably equal to or greater than 1/5. It is important that the concentration of the test chemical in the equilibrating solution (1) does not exceed one half of its solubility and (2) should be 10 ppm or less at the end of the equilibration period.

- b. Measure the concentration of the chemical in the solution phase at frequent intervals during the equilibration period.
- c. Determine the equilibration time by plotting the measured concentration versus time of sampling; the equilibration time is the minimum period of time needed to establish a rate of change of solution concentration of 5 percent or less per 24 hours.

8. Centrifugation Time

Calculate the centrifugation time, t_c , necessary to remove particles from solution greater than approximately $0.5 \mu\text{m}$ ($5 \times 10^{-5} \text{ m}$) equivalent diameter (which represents all particles except the fine clay fraction) using the following equation:

$$t_c(\text{min}) = 1.41 \times 10^9 [\log(R_2/R_1)]/N^2 \quad (3)$$

where

t_c = centrifuge time in minutes

R_2 = distance from centrifuge spindle to deposition
surface of centrifuge

R_1 = distance from spindle to surface of the sample

N = number of revolutions of the centrifuge per minute.

9. Storage of Solutions

If the chemical analysis is delayed during the course of the experiment, store all solutions between 0 and 5°C.

10. Solvents for Extraction

It is extremely important that (1) the purity of the solvent used to extract the chemical that is adsorbed on the sediment or soil is analytical grade or better and (2) the minimum solubility of the test chemical in the solvent is 10 g/l.

B. Test Procedure

1. Equilibration

Add six solutions containing different concentrations of the test chemical to at least one gram of each solid. The initial concentration of the test chemical in these solutions will depend on the affinity the chemical has for the sediment or soil. Therefore, after equilibrium is attained, it is extremely important that the highest concentration of the test chemical in the equilibrating solution (a) does not exceed 10 ppm, (b) is at least one order of magnitude greater than the lowest concentration reported, and (c) does not exceed one half of its solubility.

- a. Immediately after the solutions are added to the solids, tightly cap the containers and vigorously agitate them for several minutes with a vortex mixture or similar device.
- b. Shake the containers throughout the equilibration period at a rate that suspends all solids in the solution phase.

2. Centrifugation

When the equilibration time has expired, centrifuge the containers for t_c minutes.

3. Chemical Extraction

- a. After centrifugation, remove the supernatant aqueous phase from the solid-solution mixture.
- b. Extract the chemical adsorbed on the sediment or soil colloid surfaces with solvent.

4. Chemical Analysis

Determine the amount of parent test chemical in the aqueous equilibrating solution and organic solvent extractions. Use any method or combination of methods suitable for the identification and quantitative detection of the parent test chemical.

III. REPORTING

Report the following information using Tables 1 and 2 or a similar format:

- (1) Temperature at which the test was conducted.
- (2) Detailed description of the analytical technique(s) used in the chemical extraction, recovery, and quantitative analysis of the parent chemical.
- (3) Amount of parent test chemical applied, the amount recovered, and the percent recovered.
- (4) Extent of adsorption by containers and the approach used to correct the data for adsorption by containers.
- (5) The individual observations, the mean values, and graphical plots of x/m as a function of C_e for each sediment or soil for (a) the equilibration time determination, and (b) the isotherm determination.
- (6) The quantities K , n , and $1/n$.
- (7) Soil information: Soil Order, series, texture, sampling location, horizon, general clay fraction mineralogy.

- (8) Sediment information: sampling location, general clay fraction mineralogy.
- (9) Sediment and soil physical-chemical properties: percent sand, silt, and clay (particle size analysis); percent organic matter; pH (1/1 solids/H₂O); and cation exchange capacity.
- (10) The procedures used to determine the physical/chemical properties listed above.

TABLE 1SEDIMENT AND SOIL ADSORPTION ISOTHERM DATA FORMAT

Sediment/Soil No. and Name :

K :

n :

1/n :

Temperature :

Solid/Solution Ratio :

Adsorption Isotherm Determination

1	2	3	4	5	6
---	---	---	---	---	---

Amount of Chemical Applied :

Standard Deviation :

Percent Recovered (Mean) :

Standard Deviation :

x/n

Replication 1 :

Replication 2 :

Replication 3 :

Mean :

Standard Deviation :

TABLE 1 (continued)

	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
C_e						
Replication 1 :						
Replication 2 :						
Replication 3 :						
Mean :						
Standard Deviation :						

Equilibration Time Determination

Sampling Time (hrs)* :	<u>1</u>	<u>2</u>	<u>4</u>	<u>8</u>	<u>12</u>	<u>24</u>	<u>36</u>	<u>48</u>	<u>60</u>	<u>72</u>
C_e :										
x/m :										
Original Concentration :										

*Suggested Sampling Times.

TABLE 2SEDIMENT PHYSICAL, CHEMICAL, AND CLASSIFICATION DATA FORMAT

<u>Sediment #1</u>	<u>Sediment #2</u>
--------------------	--------------------

Location:

Percent SAND:

Percent SILT:

Percent CLAY:

Percent ORGANIC MATTER:

pH (1/1 Sediment H₂O):

CEC (meq/100g):

CLAY MINERAL ANALYSIS:

TABLE 3SOIL PHYSICAL, CHEMICAL, AND CLASSIFICATION DATA FORMAT

	<u>Soil #1</u>	<u>Soil #2</u>	<u>Soil #3</u>
SOIL ORDER:	Alfisol		
SOIL SERIES:	Crider		
SOIL TEXTURE:	Silt Loam		
LOCATION:	Gallatin County, Ill		
HORIZON:	A		
Percent SAND:	1.2		
Percent SILT:	6.6		
Percent CLAY:	12.2		
Percent ORGANIC MATTER:	1.74		
pH (1:1 soil:H ₂ O):	7.20		
CEC (meg/100g):	13.5		
CLAY MINERAL ANALYSIS:	75 percent Montmorillonite		
	5 - 20 percent Mica		
	5 percent Kaolinite		
	(25-120 cm depth)		

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SEDIMENT AND SOIL ADSORPTION ISOTHERMI. NEED FOR THE TEST

The Sediment and Soil Adsorption Isotherm (AI) is a screening test suitable for obtaining an estimate of the sediment adsorption potential of a chemical and its soil leaching potential. The adsorption affects the distribution of a chemical in the environment. Knowledge of the adsorption potential is essential under certain circumstances for the assessment of the fate of chemicals in the environment. If a chemical is tightly adsorbed to soil particles, it will not leach through the soil profile but will remain on the soil surface. If a chemical is weakly adsorbed, it may leach through the soil profile and may reach ground waters and then surface waters. Similarly, if a chemical is tightly adsorbed to sediment, it will accumulate in the bed and suspended load of aquatic systems. If a chemical is weakly adsorbed to sediment, it may be found predominately in the water column of aquatic systems.

Since adsorption can affect the distribution of a chemical in the environment, it may have a profound effect on a chemical's effect on man, the ecosystem in question, and on species within the ecosystem. If a chemical reaches ground and/or surface waters, it may cause deleterious human health effects by contaminating the drinking water. If a chemical remains at the soil surface, it may cause deleterious environmental and human health effects by contaminating the drinking water. If a chemical remains at the soil surface, it may cause deleterious environmental and human health effects due to its presence in the zone of plant growth that may result in contaminated feed and food.

II. SCIENTIFIC ASPECTS OF SOIL LEACHING

A. Introduction

The leaching of chemicals in soils is affected by several interacting processes, including adsorption, that occur at the soil-water interface. The interfacial region is important for two reasons. First, it determines the adsorption mechanism and the energy by which the chemical is held. Second, it may catalyze the transformation of the original compound. Transformation product(s) are of particular concern, since they (1) may be toxic to a greater or lesser degree than the original compound, (2) may be absorbed either greater than or less than the original compound, and (3) may have a water solubility either greater than or less than the original compound, thereby affecting its leaching and movement into the ground water. This section of the support document will discuss these processes as they relate to leaching and the AI Test Guideline.

B. Basic Processes Affecting Soil Leaching

The leaching of chemicals through soil is a complex phenomenon consisting of several major processes (Hamaker 1975). One general equation (Guenzi 1974) for chemical movement through porous media under steady state soil-water flow conditions for water in soil is:

$$\partial S / \partial t + \partial c' / \partial t = D' \partial^2 c' / \partial x^2 - v \partial c' / \partial x \quad (1)$$

where B = soil bulk density (g/cm^3)
 θ = volumetric water content (cm^3/cm^3)
 S = mass fraction of test chemical adsorbed at the
soil/water interface ($\text{g test chemical}/\text{g soil}$)
 t = time (s)
 c' = concentration of test chemical in solution (g/cm^3)
 D' = dispersion coefficient (cm^2/s)
 V = average pore-water velocity (cm/s)
 X = space coordinate measured normal to the section

Most mass transport equations represent simplifications of "real world" conditions that attempt to describe the chromatographic distribution of the chemical in the soil profile. They are gross simplifications of a phenomenon that is affected by complex, interacting processes. In general, chemical leaching is dependent upon three major processes: the mass transport of water (the direction and rate of water flow), the rate of diffusion, and the adsorption characteristics of the chemical in soil (Guenzi 1974).

Diffusion is the transport of matter resulting from random molecular motion, which is caused by molecular thermal energy. This random motion leads to the uniform distribution of molecules in a closed fluid system, since a net movement of molecules from regions of higher to lower concentrations occurs.

Adsorption is the accumulation of molecules by the attractive forces of the surface of a solid phase. When adsorption is a

significant factor, there is a higher concentration of a chemical in an extremely thin layer at the surface of a sediment or soil than is present in the bulk aqueous solution associated with the sediment and soil. The equilibrium adsorption-desorption process is governed by two opposing rate processes. The adsorption rate is the rate at which molecules from the liquid phase transfer into the adsorbed state in the solid phase and the rate increases as the concentration of dissolved species increases. The desorption rate is the rate of the opposite process, i.e., the rate at which molecules transfer from the adsorbed state in the solid phase into the liquid phase. Equilibrium is established when the rates of these two processes are equal.

C. Chemical Properties Affecting Adsorption

Adsorption is the main process that determines a chemical's leaching potential as described mathematically in equation 1. It is governed by the properties of both the solid phase and the adsorbate. The important properties of the adsorbate that affect adsorption by soil (Bailey and White 1970) are: (1) chemical structure and conformation (2) molecular size, (3) acidity or basicity of the molecule (pK_a or pK_b), (4) water solubility, (5) permanent charge, (6) polarity, and (7) polarizability.

D. Soil Properties Affecting Adsorption

The soil properties affecting the adsorption and desorption of organics include the organic matter content, type and amount of

region of clays is a function of the basicity of the molecule, the nature of the exchangeable cation on the clay, water content of the clay system, and the origin of negative charge in the aluminosilicate clay (Bailey and White 1970).

E. Types of Adsorptive Forces

The specific type of interaction that organic molecules have with soil depends on the chemical properties of the organic molecules and the type of soil. These interactions or adsorptive forces are classified as: van der Waals forces, charge transfer, ion exchange, and hydrophobic bonding (Adams 1975, Goring and Hamaker 1972). In general, one or more of these specific interactions or adsorptive forces may occur simultaneously.

The attractive van der Waals forces or polarizability forces arise from the random fluctuations in a molecule's electron distribution. These fluctuations theoretically produce instantaneous dipoles due to the concentration of charges in one region of the molecule and cause that molecule's attraction to other atoms and molecules.

Charge transfer involves the formation of a donor-acceptor complex between an electron donor molecule and an electron acceptor molecule with partial overlap of their respective molecular orbitals and a partial exchange of electron density.

Ion exchange refers to the exchange between counterions balancing the surface charge of the soil colloid and the ions in

the soil solution. The driving force for this interaction is the requirement for electroneutrality: the surface charge must be balanced by an equal quantity of oppositely charged counterions. In general, ion exchange is reversible, diffusion controlled, and stoichiometric in most cases. It exhibits some selectivity or preferential adsorption for one ion over another competing ion.

Hydrophobic bonding refers to the greater affinity of an organic molecule for a hydrocarbon solvent or hydrophobic region of a colloid than for a hydrophilic solvent. Hydrocarbon regions of a molecule have greater solubility in liquid hydrocarbons (or most organic solvents) than in water.

F. Surface Transformations

A special type of interaction between organic molecules and soils deals with the transformation of organic chemicals into new compounds containing different chemical structures through the catalytic activity of the soil colloid surfaces. Although several theories exist to account for the mechanism of these transformations, no scheme that predicts the occurrence of these surface reactions presently exists. Therefore, it is extremely important that mass balance calculations for the test chemical are performed to ascertain the extent of these transformations during soil leaching experiments.

The scientific literature shows that a number of chemicals and chemical classes undergo colloid surface induced chemical transformations. Poly-(dimethylsiloxane) fluids in intimate

clay, exchange capacity, and surface acidity (Adams 1973, Bailey and White 1970, and Helling 1970). The combined actions of climate, micro-, and microorganisms over long periods of time on different parent geologic and biotic materials form soils that differ widely in their physical, chemical, morphological, and adsorption characteristics. The amounts and types of clay and organic matter, soil pH, primary and secondary minerals, structure, texture, and exchange capacity vary considerably for U.S. soils. There are currently 10 Soil Orders, at least 43 Suborders, over 200 Great groups and over 7,000 soil series recognized in the United States (Buckman and Brady 1969).

Soil organic matter is a primary soil property responsible for the adsorption of many chemicals. Helling (1970) lists many examples where the organic matter primarily influenced the adsorption of pesticides. Organic matter and clay are the soil components most often implicated in pesticide adsorption. However, the individual effects of either organic matter or clay are not easily ascertained. Since the organic matter in most soil is intimately bound to the clay as a clay-metal-organic complex (Stevenson 1976), two major types of adsorbing surfaces are normally available to the chemical, namely, clay-organic and clay alone. Clay and organic matter function more as a unit than as separate entities and the relative contribution of organic and inorganic surfaces to adsorption will depend on the extent to which the clay is coated with organic substances. Comparative studies between known clay minerals and organic soils suggest that most, but not all, pesticides have a greater affinity for organic

surfaces than for mineral surfaces (Stevenson 1973). Since typical studies compare soils in which both clay and organic matter increase and do not use multiple regression analyses to isolate the governing parameter (Helling 1970), only generalizations concerning the relative importance of clay and organic matter can be made.

Surface acidity is another soil property affecting the adsorption of many organic chemicals. Surface acidity is probably the most important property of the soil or colloidal system in determining the extent and nature of adsorption of basic organic chemicals, as well as determining if acid-catalyzed chemical transformation occurs (Bailey and White 1970). The activity of protons in the bulk suspension, which is expressed by pH, and the activity of protons at or in close proximity to the colloidal surface (i.e., the acidity in the interfacial region) may differ significantly. The term "surface acidity," when applied to soil systems, is the acidity at or in close proximity to the colloidal surface that reflects the ability of the system to act as a Lewis acid. Surface acidity is a composite term that reflects both the total number of acidic sites and their relative degree of acidity.

Overwhelming evidence, mainly from infrared and other studies, indicates that the clays that protonate basic chemicals either have hydrogen and aluminum as the predominant exchangeable cations, or are saturated with alkali metal, alkaline earth metal, and transition metal cations. A summary of recent investigations indicates that the protonation of chemicals in the interfacial

contact with many soils undergo siloxane bond redistribution and hydrolysis that result in the formation of low molecular weight cyclic and linear oligomers (Buch and Ingebrightson 1979). Substituted (White 1976) and organophosphorus pesticides (Yaron 1978, Mingelrin et al. 1977) undergo clay colloid induced hydrolysis. Benzene and phenol polymerize into high molecular weight chemicals by adsorption and reaction at the surface of smectite saturated with transition metal cations (Mortland and Halloran 1976). Gallic acid, pyrogallol, protocatechuic acid, caffeic acid, orcinol, ferulic acid, p-coumaric acid, syringic acid, vanillic acid, and p-hydroxybenzoic acid undergo oxidative polymerization in the presence of various clay minerals (Wang and Li 1977, Wang et al. 1978).

III. SCIENTIFIC ASPECTS OF SEDIMENT-CHEMICAL TRANSPORT AND ADSORPTION

A. Introduction

The transport and adsorption of chemicals by sediment are affected by a large number of interacting processes. Furthermore, chemical movement in aquatic systems, unlike soil systems, is dependent not only on the extent of adsorption but on the movement of sediment. This Test Guideline will develop data on the extent of a chemical's adsorption onto sediments. Sediment movement can be mathematically estimated in several ways for a specific situation provided hydrologic and meteorologic information is available. This section of the support document will discuss the transport and adsorption of chemicals by sediment as they relate to the Test Guideline.

B. Basic Sedimentation Processes Affecting Chemical Movement

Sediment is the unconsolidated inorganic and organic material that is being transported or has been transported by and deposited in beds from water. Synthesis, erosion, transportation, and deposition of sediment are natural processes that have occurred throughout geologic time. The extent of biologic activity and the extent of erosion will govern the amount of sediment that enters a watershed (Chow 1964). In general, every sediment particle that passes a particular cross section of a water body must satisfy two

conditions: (a) it must have been eroded or synthesized somewhere in the watershed above the cross section and (b) it must have been transported by the flow of water from the place of erosion or synthesis to the cross section. (Chow 1964). The wash load is the finer sediment fraction that the flow can easily carry in large quantities. The bed-material load is the coarser sediment fraction that is difficult to move by the flow and is limited in its movement by the transporting ability of the flow between source and section. The bed load is the sediment in the bed layer that cannot be suspended in the water column for fluid-dynamic reasons. The basic difference between wash load and bed-material load can best be visualized in a concrete-lined channel. If the flow is large and fast, the flow condition is not in any way affected by adding small amounts of a fine and easily transported material. This added material, the wash load, moves in suspension with the flow at the same average velocity and does not settle. If the flow velocity and discharge are now reduced and/or if the material is increased in size and rate, sediment will begin to deposit on the channel bottom and a granular sediment bed will develop. These sedimenting particles are designated as the bed-material load.

Sediment particle size is the single most important physical parameter affecting sediment transport and deposition. The exemplified differences between wash load, bed-material load, and bed load show that different sediment sizes behave differently in the same hydrologic system. Similar quantitative differences in behavior exist between different sizes of the bed-material load.

The grain size distributions in the bed and in transport are quite often different, even within the size range of the bed-material load. Also, many streams have heterogeneous beds with individual bars having very different composition and appearance. Finally, many stream and river beds exhibit another type of segregation in which all coarse particles are concentrated in lenses, or layers, at a greater or lesser depth below the bed surface.

C. Chemical Properties Affecting Adsorption

A chemical's adsorption potential is governed by the properties of both the sediment phase and the adsorbate. The important properties of the adsorbate affecting adsorption onto sediments are basically the same properties affecting adsorption onto soil colloids as discussed in Section II.C. They are: (1) chemical structure and conformation, (2) acidity or basicity of the molecule (pK_a or pK_b), (3) water solubility, (4) permanent charge, (5) polarity, (6) molecule size, and (7) polarizability.

D. Sediment Properties Affecting Adsorption

The inorganic chemical composition of sediments includes most primary and secondary minerals. In general, it is composed of mineral fragments having particle sizes ranging from clay and silt to sand, gravel, and boulders. The mineralogy of original source rocks, together with chemical weathering processes, mechanical weathering processes, and precipitation processes, determine the ultimate size, weight, shape, and, therefore, the adsorptive

capacity of inorganic sediment particles. The mineral content contributes to the sorting phenomena. Heavy minerals will deposit at higher flow velocities compared to lighter materials of equal size. Hard minerals, such as quartz, will resist abrasion to a greater degree than soft, chemically unstable minerals, such as gypsum or limestone. Some minerals and rocks disintegrate along crystal faces or cleavage planes to form platelike particles while others form equidimensional particles.

The organic sediment particles are composed of plant and animal tissue in various stages of chemical and microbial decomposition. The highly varied composition and the continuous fluctuation of the hydrologic conditions found in many aquatic systems create heterogeneous sediments with a widely range of characteristics.

A review of the literature on the adsorption of chemicals reveals that pesticide-soil studies dominate the literature. Most of the principles applicable to the adsorption and transformation of chemicals in soils (Section II, Parts C, D, E, and F) are applicable to sediments. In general, sediments have a finer texture than soils. They contain more amorphous organic matter due to the biotic activity in water and contain more clay due to the erodability of the finer soil components. Therefore, sediments generally show higher sorption tendencies than soils (Pionke and Chesters 1973, Lotse et al. 1968). However, Pionke and Chesters (1973) state that little information exists on sediment-chemical interactions to indicate differences from typical soil-chemical interactions.

IV. SCIENTIFIC ASPECTS OF THE TEST

A. Development of the Adsorption Isotherm

The fact that solids can remove salts and color from solution by adsorption has been known from the earliest times. For example, Aristotle knew that seawater lost some of its taste by filtration through sand (Forrester and Giles 1971b). Since then, the phenomenon of adsorption has been used to solve various water purification problems. In the 17th and 18th centuries, seawater purification by soil was used on the Barbary Coast to produce fresh water (Forrester and Giles 1971b). During the 19th century, a number of soil chemists published studies on the adsorption of both basic and acidic compounds onto soils. In 1881, van Bemmelen published the first solute-solid adsorption diagram based on his studies of sulphuric acid adsorption by metastannic acid (Forrester and Giles 1972). The only previously published adsorption diagrams were those for gas-solid adsorption plotted by Chappius and by Kayser in 1880. The term "adsorption" appeared for the first time in the English language in an 1882 abstract of Kayser's work in Nature (Forrester and Giles 1971a). The term "isotherm" was first used by Ostwald in 1885 to describe the plot of pressure (abscissa) versus the amount of gas adsorbed (ordinate) (Forrester and Giles 1971a). Soon afterwards, the term "adsorption isotherm" was adopted to describe solute-solid adsorption diagrams. In the first decade of this century, adsorption isotherms began appearing frequently in the published literature, often as adjuncts to other investigations. Since then, the

isotherm has been used for innumerable adsorption studies using an extremely wide variety of solutes, solvents, and solids over the entire range of experimentally obtainable pressures and temperatures.

Since adsorption is the major retention mechanism for most organic and inorganic compounds in soils (Section II), the magnitude of any mathematical or empirical estimation of a chemical's soil leaching potential (e.g., equation (1)) is, in general, proportional to the magnitude of the adsorption coefficient. Therefore, one need only estimate the adsorption coefficient to obtain an estimate of a chemical's leaching potential in soil. Similarly, a chemical's distribution in aquatic system will, in general, depend upon the magnitude of its adsorption to sediment. Therefore, an estimate of the adsorption potential is needed to estimate a chemical's distribution in the water column.

B. Rationale for the Selection of the Adsorption Isotherm Test

Soil thin layer chromatography, soil AI, and soil columns have been developed to obtain an estimate of a chemical's leaching potential (Hamaker 1975). The AI test is applicable to obtaining both sediment adsorption potential data and soil adsorption and mobility data.

This Test Guideline is developed upon the basic principles comprising most adsorption isotherm tests found in scientific literature. The extent of adsorption of a chemical onto sediments

or soils is measured by equilibrating aqueous solutions containing different but environmentally realistic concentrations of the test chemical with a known quantity of sediment or soil. After equilibrium is reached, the distribution of the chemical between the water phase and the solid phase is quantitatively measured by a suitable analytical method. Then, sorption constants are calculated by using the Freundlich equation:

$$x/m = C_s = K C_e^{1/n} \quad (1)$$

where

C_e =equilibrium concentration of the chemical in the solution phase

C_s =equilibrium concentration of the chemical in the solid phase

K =Freundlich adsorption coefficient

m =the mass of the solid in grams

$1/n$ =exponent where n is a constant

x =the mass in micrograms of the chemical adsorbed by m grams of solid.

A logarithmic transformation of the Freundlich equation yields the following linear relationship:

$$\log C_s = \log K + (1/n) \log C_e \quad (2)$$

In order to estimate the environmental movement of the chemical in surface water systems and soils, the values K and $1/n$ are compared with the values of other chemicals whose behavior in soil and sediment systems is well-documented in scientific literature.

The adsorption isotherm (AI) test was selected because it contains many desirable features. First, adsorption results are highly reproducible. The test provides excellent quantitative data readily amenable to statistical analyses. Also, it has relatively modest requirements for chemicals, soils, laboratory space, and equipment. It allows solution phase organic chemical determinations that are relatively uncomplicated. A chemical extraction-mass balance procedure to elicit information on chemical transformations occurring at colloid interfaces can be incorporated into this test. The ease of performing the isotherm test and mass balance will depend upon the physical/chemical properties of the test chemical and the availability of suitable analytical techniques to measure the chemical.

C. Rationale for the Selection of Experimental Conditions and Procedures

The papers by Aharonson and Kafkafi (1975), Harvey (1974), Murray (1975), Saltzman (1972), Weber (1971), and Wu (1975) served as the basis for this Test Guideline. The soil and colloid chemistry literature and the analytical chemistry literature substantiate the experimental conditions and procedures specified in the suggested Test Guideline as accepted, standard procedures. The rationale for the selection of these conditions and procedures will be discussed in greater detail below.

The AI Test Guideline can be used to determine the soil adsorption potential of sparingly water soluble to infinitely water soluble chemicals. In general, a chemical having a water solubility of less than 0.5 ppm need not be tested, since the literature indicates that these chemicals are, in general, immobile (Goring and Hamaker 1972). However, this does not preclude future soil adsorption/transformation testing of these chemicals if more refined data are needed for the assessment process.

The 0.01M calcium nitrate is required to insure colloid flocculation during the experiment.

Distilled-deionized H₂O and glassware are required to minimize competition effects for exchange sites by unidentified cationic and anionic species normally present in tap and distilled H₂O.

Sterile water and glassware are required to minimize the potential for microbial growth in the test containers.

The absorption isotherm standard can be used to determine the adsorption potential of volatile chemicals, since the standard requires the use of containers that are capped and the use of a solid/solution ratio that minimizes container headspace.

A room temperature range of 23±5°C was adopted since the available literature indicated that pesticide adsorption in general does not significantly change in the temperature range of 1° to 30°C.

Replication of the basic experimental unit was necessary in order to estimate the standard deviation of the treatment mean. Three replicates are considered to be the minimum number of replicates for a statistically acceptable estimation.

It is important that gentle crushing and grinding be used to reduce soil aggregate size. Fine particles (silt and clay) in excess of the amount originally present may be created if excessive pressure is exerted on the aggregates.

Soil sieving with a 100 mesh stainless steel or brass sieve is required to remove debris and coarse fragments. Removal of these components should improve the reproducibility of the adsorption test and will aid in obtaining a more uniform sample.

In order to obtain reproducible results, it is necessary to: (1) sterilize the soil or sediment to prevent microbial growth and degradation of the test chemical; (2) choose the appropriate sterilization technique to make sure that the soil or sediment surface properties are not altered; and (3) store the pretreated soil or sediment at 0 to 5°C to minimize microbial effects.

It is extremely important that the solvent chosen for extraction be: (1) analytically pure or better; and (2) the minimum solubility of the test chemical in the solvent be at least 10 g/l. The first condition is necessary to minimize the effects of solvent impurities on the analytical determination of the concentration of chemical on the soil or sediment and in aqueous

solution. The second condition is necessary to make sure that the test chemical is essentially completely extracted so that an accurate value of the concentration of chemical adsorbed on the soil or sediment and in aqueous solution is obtained.

In carrying out the adsorption experiments, it is recommended that the solid/solution ratio be approximately equal to 1/10 or greater. This ratio will give the best reproducible results. However, for certain chemicals which do not adsorb readily, it is preferable to use the ratio of approximately 1/5 or slightly greater to give the best reproducible results. However, it is not practical to use ratios much greater than 1/5, since the mixture would be too viscous and true equilibrium would not be achieved. Hence, under these conditions, the experimental results would be erroneous.

In general, a majority of chemicals should attain equilibrium between the solid and solution phases within 24 to 48 hours. For unknown reasons, however, many chemicals require longer equilibration periods. Since it is not presently possible to predict the proper equilibration period for a chemical, the Test Guideline recommends that the equilibration period be determined as a part of the test procedure for all chemicals.

In general, the test chemical should be of the purest grade readily available. Impurities may produce mass balance data indicating the presence of transformation products. Transformation product identification is an expensive analytical procedure that may be unnecessarily required due to the presence of impurities.

The Soil Order, series, and general clay fraction mineralogy data may be found in Soil Survey Reports published after approximately 1970. Pre-1970 reports may not contain mineralogy data. Soil survey reports have been issued for most U.S. counties and may be obtained from County Extension Offices; the State Office of the U.S. Department of Agriculture Soil Conservation Service; or the USDA-Soil Conservation Service, Publications and Information Division, P.O. Box 2890, Washington, DC 20013. If mineralogy data are not printed in a report, the State Office of the U.S. Department of Agriculture Soil Conservation Service may be contacted for assistance in obtaining general clay mineral data of a particular soil. The Test Guideline does not require soil mineral analysis since general clay mineralogy data may already exist for the test soil.

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TRANSFORMATION PROCESSES

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AEROBIC AQUATIC BIODEGRADATION

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
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AEROBIC AQUATIC BIODEGRADATIONI. NEED FOR THE TEST

The transformation of organic substances by living organisms is an important factor in determining their environmental fate. Organic substances may be transformed by nonbiological as well as biological mechanisms, such as photolysis, hydrolysis and oxidation. There is little doubt, however, that biodegradation is the predominant mechanism for the transformation of many organic compounds in soil and water. Evidence indicates that microorganisms are responsible for converting many complex organic substances to inorganic products (Alexander 1973, Howard et al. 1975 p. 37).

Biodegradation is often the most desirable mechanism for decomposing organic substances. This is especially true if biodegradation is rapid and if degradation products are inorganic molecules and metabolites that may be used for energy and microbial growth. Photochemical degradation and other chemical processes usually do not completely mineralize organic substances, and resulting products of unknown toxicity and/or persistence may be generated (Alexander 1967).

Laboratory evaluations for determining biodegradability are an important part of testing to indicate whether a substance is likely to persist in the presence of microorganisms in the natural environment or in biological treatment processes. If the substance does not persist, it may be necessary to determine whether the substance degrades to innocuous molecules or whether some relatively persistent and toxic intermediate is formed. It

also may be important to obtain better estimates for biodegradation rates under various environmental conditions in order to more precisely assess the persistence of a substance. The assessment of risk from environmental exposure to organic substances depends upon estimates of environmental concentrations of the parent substances and potentially toxic transformation products. Because biodegradation plays a vital role in the transformation of most organic compounds in the environment, knowledge of biodegradation rates and products is an important element in the assessment process.

Chemical substances can enter natural waters in a variety of ways. These include runoff from land, discharges of industrial wastes, home and commercial use with disposal into sewers, spills and leaks, leaching from landfills and transfer from the atmosphere through rainfall or particulate deposition. Surface waters normally contain bacterial populations which are continually replenished from sewer outfalls and land runoff and which are capable of the uptake and metabolism of many of these chemical substances. Microbes account for a rapid turnover and substantial breakdown of such substances, particularly those organic substances of relatively low molecular weight (R. T. Wright 1979).

It is therefore important to obtain an understanding of whether or not chemical substances which are released to or which may be transported to aerobic surface waters will be degraded by the microbial populations in those waters. The most cost-effective way to obtain that knowledge is through laboratory studies using aerated water containing the test substance and representative microorganisms.

II. SCIENTIFIC ASPECTS

A. Test Methods

Laboratory techniques which are used to study microbial degradation processes in aerobic aquatic environments include (1) those which test for the biodegradability potential of a substance without any attempt to carefully simulate any particular portion of the aquatic environment and (2) those which do attempt to simulate natural water bodies (e.g. a given lake or stream) in an effort to determine the rate and extent of biodegradation at a specific site.

The purpose of the procedure in this guideline is to screen for the biodegradation potential of substances in aerobic aqueous environments in general. The method is applicable to a wide variety of substances and is not intended to simulate any particular aquatic environment. Those procedures which do attempt to simulate a specific site (for example by carefully controlling most of the important variables such as pH, salinity and nutrient concentration) are more appropriately employed at a higher tier or step in a testing program.

The screening methods for biodegradability potential in aerobic, aquatic environments may be subdivided into tests for ready biodegradability and for inherent biodegradability, as in the OECD Level I and Level II methods (OECD 1979). Methods to test for ready biodegradability are designed so that positive results are unequivocal and lead to the reasonable assumption

that the substance will undergo rapid and ultimate biodegradation in the environment (biodegradation to inorganic compounds and products associated with the normal metabolic processes of microorganisms). This assumption is supported by the features of test methods used for this purpose such as exclusion of organic substrates other than the test substance and the absence of any adaptation steps. Methods to test for inherent biodegradability, on the other hand, using more favorable conditions, are designed to assess if a substance has any potential for biodegradation.

The method in this guideline is fundamentally a method to test for ready biodegradability with an option to employ some features of methods for inherent biodegradability such as an adaptation of the microorganisms to the chemical substance. Such flexibility is highly desirable for the purposes of Test Rules for specific chemical substances. A review of the available information on a specific substance may reveal patterns of disposal that make the inclusion of a method with an adaptation step advisable. If a substance is being released to the aquatic environment on a rather continuous basis and at some steady concentration, then the natural microbial population will have ample opportunity to adapt to the transformation of the substance and laboratory studies should include an adaptation step.

Laboratory methods which have been employed to screen for biodegradation potential in aerobic aquatic environments include (1) those which follow the uptake of dissolved oxygen by the

microbial population, (2) river water die-away tests and, (3) aerobic culture tests. These have been discussed in some detail by Howard et al. (1975, p. 49-117).

Dissolved oxygen methods included dilution methods such as the standard BOD test (APHA 1975) and respirometric techniques. The dilution methods employ closed bottles containing appropriate dilutions of the test substance in inoculated water which generally contains a buffered essential salts mixture. The uptake of dissolved oxygen by the microorganisms is followed by chemical analyses for dissolved oxygen in a series of replicate bottles over a period of time, or by the use of an oxygen-sensitive electrode. Respirometric methods follow the uptake of dissolved oxygen by manometric techniques, commonly in a system where the carbon dioxide evolved by the microorganisms is trapped in an alkaline solution contained in a well or side arm. The respirometric methods are more difficult to set up and interpret than the dilution methods and they require the use of relatively costly equipment which must be recalibrated frequently. For these reasons, the dilution methods are preferable for screening purposes.

The dilution methods of the OECD Guidelines (1981) are validated methods with proven reproducibility. The OECD Closed Bottle Test (OECD 1981) is a modification of the standard BOD test (APHA 1975). For those investigators who are more familiar with the procedures of the standard BOD test, that test should be an acceptable substitute for the OECD method provided that the

BOD test is continued for a nominal 28-day incubation period. In either method, the use of an oxygen-sensitive electrode is preferred to chemical analysis for dissolved oxygen because repetitive dissolved oxygen readings may be made on a single bottle and, in addition, the electrode is more accurate and less subject to interferences than the chemical titration methods (Reynolds 1969, Hwang and Forsberg 1973). The oxygen uptake methods are highly desirable in the screening level group of methods because they are the only simple methods available which can handle volatile substances.

River water die-away methods, although used by some for biodegradability screening, are, in fact, static simulation tests which employ raw water collected from a river or lake and follow the disappearance of an added amount of the test substance. Variations include the use of added nutrients and/or microorganisms to fortify the natural water. Generally, biodegradation is followed by using an analytical method which is specific for the test substance. Although a number of investigators have used die-away methods to study biodegradability, there is no standard version of the procedure and the use of specific natural waters tends to make the methods less amenable to standardization than other screening methods. The difficulties of standardization combined with the requirement for chemical-specific analytical methods preclude the inclusion of river water die-away methods in a screening-level or base-set of test methods.

Aerated culture methods generally employ flasks or bottles containing an aqueous medium plus the test substance and a suitable inoculum. The vessels may be placed on a shaker to promote aeration of the aqueous medium and contact of the microorganisms with both substrate and dissolved oxygen, or they may be aerated by an air stream bubbled into the liquid medium. Analysis for biodegradation may be by analytical methods which are specific for the test substance (e.g. colorimetric or chromatographic techniques), by the use of appropriately radiolabeled compounds, or by the use of such non-specific procedures as following the loss of dissolved organic carbon (DOC) or the evolution of respiratory carbon dioxide (CO_2).

The use of an analytical method specific for the test substance or of an appropriately labeled test substance would be too costly for requirement in an initial evaluation of biodegradation in aerobic waters. Thus, this screening-level guideline method and other screening-level or first tier methods are limited to aerated culture methods which employ non-specific analytical methods to follow the biodegradation of the test substance. This guideline method, which is based on work by Gledhill (1975), is a shake flask method which uses the test substance as the sole carbon source in a mineral nutrient medium, and follows biodegradation by both DOC analyses and CO_2 evolution. This method includes an adaptation step as a routine procedure. The guideline method has been evaluated in several laboratories and has proven reproducibility.

Another type of aerated culture method is that employing continuous culture, with the bacterial population density regulated by automatic additions of fresh medium and test substance to the reaction vessel. Advantages of such procedures are that toxic products and metabolic wastes will not accumulate and their effects on biodegradation processes will not increase with time. These systems are more like those natural aquatic environments where a continual input of chemical and removal or dilution of toxic products is likely. These methods invariably require the use of special equipment and specific analytical procedures or radiolabeled test substances, and they tend to simulate rather specific kinds of aquatic environments. For these reasons, such methods were rejected for inclusion in the screening level of test methods for aerobic aquatic biodegradation.

The method cited in the guideline is applicable to organic substances. Substances which are highly volatile cannot be studied readily in the aerated systems and are restricted to the Closed Bottle or BOD test. Substances with very low water solubility (less than a few mg/L) cannot be used for those methods that rely on DOC measurements. The information already known about a specific substance will aid in the specification of the most appropriate biodegradation method(s) to use for the substance.

B. Test Conditions

1. Incubation Temperatures

The incubation temperatures specified in the guideline method are at or slightly above most laboratory room temperatures and this allows for convenient adjustment and maintenance of constant temperature baths and enclosures. For some substances it may be necessary to require biodegradation data at temperatures other than those specified. Examples of when this requirement may be applicable include situations where there is evidence that the substance is being released in significant amounts to environmental sites where the ambient temperatures are commonly well below or above the guideline temperatures.

C. Test Procedures

1. Reference Compounds

Reference compounds are suggested to evaluate the biodegradation potential of the microbial inoculum. For that purpose it is necessary to use a reference compound that will be biodegradable under the test conditions but not so readily biodegradable that the material is completely degraded within a small fraction of the normal test period. For that reason, some traditional reference materials such as glucose and mixtures of glucose and glutamic acid are not appropriate since they would biodegrade too rapidly. Aniline appears to be a good general

choice for the aqueous aerobic methods. Sodium citrate, phthalic acid and trimellitic acid are also suitable reference substances and, like aniline, will exhibit ultimate biodegradation in this test method. However, for some purposes, the use of a specific reference compound that is analogous to the test substance may be required.

2. Inhibited Systems

Tests which rely on DOC removal to evaluate biodegradation require the use of inhibited systems which allow determinations to be made with regard to whether or not such losses were due to non-biological processes such as adsorption and volatilization. Estimates of test substance removed by sorption and volatilization can be achieved in two ways: (1) use of uninoculated flasks or (2) use of inoculated flasks that contain the test substance and a metabolic inhibitor such as HgCl_2 to prevent microbial activity (inhibited systems). The first option is more difficult because it requires the maintenance of sterile conditions in units which are aerated. The use of a chemically inhibited system provides a practical method for controlling microbial activity and estimating loss of the test substance by sorption or volatilization.

3. Replication

Three inoculated cultures containing test substance are desirable at each incubation temperature, which follows the recommendation for the use of 2 to 4 replicates in biodegradation testing (Gledhill 1975). The precision of the test data is related to the number of replicates, and three replicates are considered to be the minimum for statistically acceptable mean and standard deviation calculations.

4. Sampling Frequency and Duration

Samples should be taken according to a schedule appropriate to the rates of degradation of the test substance and the reference compound. They should be sufficiently frequent to establish plots of degradation vs time, in order to properly judge the nature of the biodegradation and whether or not it is possible to identify such aspects as an adaptation phase, a degradation phase and a plateau. A nominal test time of 28 days was selected to allow for a reasonable period for observations with more slowly degraded substances, to permit some adaptation to occur, and to be consistent with the requirements of the OECD (1981). Tests may be terminated prior to 28 days if an end-point plateau is observed and if that plateau is consistent ($\pm 10\%$) over 3 consecutive days.

5. Filtration

The use of membrane filters with 0.45 micrometer (μm) pore diameter to prepare samples for DOC analysis is recommended to standardize the process of sample preparation and to insure that all particulate matter $> 0.45 \mu\text{m}$ is excluded from the sample.

D. Test Data

The written, tabular and graphical data represent the minimum acceptable information necessary to evaluate the biodegradation of the test substance under the conditions of the guideline.

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AEROBIC AQUATIC BIODEGRADATION

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
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AEROBIC AQUATIC BIODEGRADATIONI. INTRODUCTIONA. Purpose

This Guideline is designed to develop data on the rate and extent of aerobic biodegradation that might occur when chemical substances are released to aquatic environments. A high biodegradability result in this test provides evidence that the test substance will be biodegradable in natural aerobic freshwater environments.

On the contrary, a low biodegradation result may have other causes than poor biodegradability of the test substance. Inhibition of the microbial inoculum by the test substance at the test concentration may be observed. In such cases further work is needed to assess the aerobic aquatic biodegradability and to determine the concentrations at which toxic effects are evident. An estimate of the expected environmental concentration will help to put toxic effects into perspective.

B. Definitions

1. Adaptation is the process by which a substance induces the synthesis of any degradative enzymes necessary to catalyze the transformation of that substance.

2. Ultimate Biodegradability is the breakdown of an organic compound to CO_2 , water, the oxides or mineral salts of other elements and/or to products associated with normal metabolic processes of microorganisms.

3. Ready Biodegradability is an expression used to describe those substances which, in certain biodegradation test procedures, produce positive results that are unequivocal and which lead to the reasonable assumption that the substance will undergo rapid and ultimate biodegradation in aerobic aquatic environments.

C. Principle of the Test Method

This Guideline Method is based on the method described by William Gledhill (1975). The method consists of a two-week inoculum buildup period during which soil and sewage microorganisms are provided the opportunity to adapt to the test compound. This inoculum is added to a specially equipped Erlenmeyer flask containing a defined medium with test substance. A reservoir holding barium hydroxide solution is suspended in the test flask. After inoculation, the test flasks are sparged with CO_2 -free air, sealed and incubated with shaking in the dark. Periodically, samples of the test mixture containing water soluble test substances are analyzed for dissolved organic carbon (DOC) and the $\text{Ba}(\text{OH})_2$ from the reservoirs is titrated to measure the amount of CO_2 evolved. Differences in the extent of DOC disappearance and CO_2 evolution

between control flasks, containing no test substance, and flasks containing test substance are used to estimate the degree of ultimate biodegradation.

D. Prerequisites

The total organic carbon (TOC) content of the test substance should be calculated or, if this is not possible, analyzed, to enable the percent of theoretical yield of carbon dioxide and percent of DOC loss to be calculated.

E. Guideline Information

Information on the relative proportions of the major components of the test substance will be useful in interpreting the results obtained, particularly in those cases where the result lies close to a "pass level".

Information on the toxicity of the chemical may be useful in the interpretation of low results and in the selection of appropriate test concentrations.

F. Reference Substances

Where investigating a chemical substance, reference compounds may be useful and an inventory of suitable reference compounds needs to be identified. In order to check the activity of the inoculum the use of a reference compound is desirable. Aniline, sodium citrate, dextrose, phthalic acid and trimellitic

acid will exhibit ultimate biodegradation under the conditions of this Test Guideline method. These reference substances must yield 60 percent of theoretical maximum CO₂ and show a removal of 70 percent DOC within 28 days. Otherwise the test is regarded as invalid and should be repeated using an inoculum from a different source.

G. Reproducibility

The reproducibility of the method has not yet been determined; however it is believed to be appropriate for a screening test which has solely an acceptance but no rejective function.

H. Sensitivity

The sensitivity of the method is determined by the ability to measure the endogenous CO₂ production of the inoculum in the blank flask and by the sensitivity limit of the dissolved organic carbon analysis. If the test is adapted to handle ¹⁴C-labelled test substances, test substance concentrations can be much lower.

I. Possibility of Standardization

This possibility exists. The major difficulty is to standardize the inoculum in such a way that interlaboratory reproducibility is ensured.

J. Possibility of Automation

None at present, although parts of the analyses may be automated.

II. TEST PROCEDURES

A. Preparations

1. Apparatus

The shake flask apparatus (Figure 1) contains 10 mL of 0.2N $\text{Ba}(\text{OH})_2$ in an open container suspended over 1-liter of culture medium in a 2-liter Erlenmeyer flask. The $\text{Ba}(\text{OH})_2$ container is made by placing a constriction just above the 10 mL mark of a 50 mL heavy duty centrifuge tube and attaching the centrifuge tube to a 2 mm I.D. x 9 mm O.D. glass tube by means of 3 glass support rods. The centrifuge tube opening is large enough to permit CO_2 to diffuse into the $\text{Ba}(\text{OH})_2$, while the constriction permits transferal of the flask to and from the shaker without $\text{Ba}(\text{OH})_2$ spillage into the medium. For periodic removal and addition of base from the center well, a polypropylene capillary tube, attached at one end to a 10 ml disposable syringe, is inserted through the 9 mm O.D. glass tube into the $\text{Ba}(\text{OH})_2$ reservoir. The reservoir access port is easily sealed during incubation with a serum bottle stopper. Two glass tubes are added for sparging, venting, and medium sampling. The tops of these tubes are connected with a short section of flexible tubing during incubation.

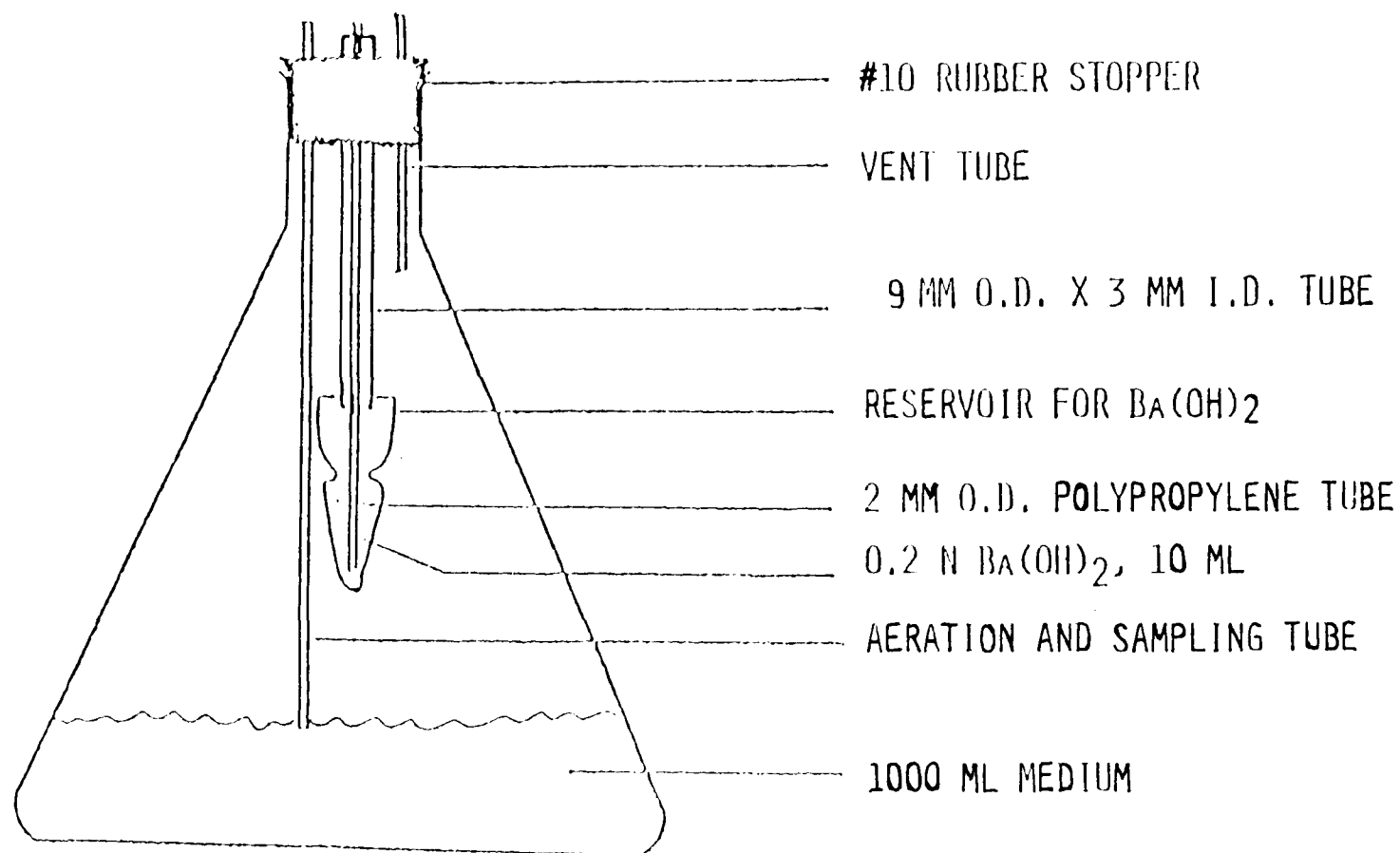


FIGURE 1. SHAKE FLASK SYSTEM FOR
CARBON DIOXIDE EVOLUTION

2. Reagents and Stock Solutions

Stock solutions, I, II, and III (Table 1)

Yeast Extract

Vitamin-free Casamino Acids

70% O₂ in nitrogen or CO₂-free air

0.2N Ba(OH)₂

0.1 N HCl

20% H₂SO₄

Phenolphthalein

Dilution water - distilled, deionized water (DIW)

3. Soil Inoculum

A fresh sample of an organically rich soil is used as the inoculum in the ultimate biodegradation test. Soil is collected, prepared, and stored according to the recommendations of Pramer and Bartha (1972). The soil surface is cleared of litter and a soil sample is obtained 10-20 cm below the surface. The sample is screened through a sieve with 2-5 mm openings and stored in a polyethylene bag at 2-4°C for not more than 30 days prior to use. The soil is never allowed to air dry, and should not be frozen during storage.

TABLE 1
MEDIUM EMPLOYED FOR ASSAY OF CO₂ EVOLUTION

<u>SOLUTION^a</u>	<u>COMPOUND</u>	STOCK SOLUTION
		<u>CONC. (g/L)</u>
I	NH ₄ Cl	35
	KNO ₃	15
	K ₂ HPO ₄ ·3H ₂ O	75
	NaH ₂ PO ₄ ·H ₂ O	25
II ^b	KCl	10
	MgSO ₄	20
	FeSO ₄ ·7H ₂ O	1
III	CaCl ₂	5
	ZnCl ₂	0.05
	MnCl ₂ ·4H ₂ O	0.5
	CuCl ₂	0.05
	CoCl ₂	0.001
	H ₃ BO ₃	0.001
	MoO ₃	0.0004

^a = Each liter of test medium contains 1 mL of each solution.

^b = Final pH is adjusted to 3.0 with 0.10 N HCl.

4. Acclimation Medium

Acclimation medium is prepared by adding, for each liter of distilled, deionized water (DIW): 1 mL each of solutions I, II, and III (Table 1), 1.0 gm of soil inoculum (prepared according to 3, above), 2.0 mL of aerated mixed liquor (obtained from an activated sludge treatment plant not more than 2 days prior to commencing the acclimation phase, and stored in the interim at 4°C) and 50 mL raw domestic influent sewage. This medium is mixed for 15 minutes and filtered through a glass wool plug in a glass funnel. The filtrate is permitted to stand for 1 hour, refiltered through glass wool, and supplemented with 25 mg/L each of Difco vitamin-free casamino acids and yeast extract. Appropriate volumes are added to 2-liter Erlenmeyer flasks. Test compounds are added incrementally during the acclimation period at concentrations equivalent to 4, 8, and 8 mg/L carbon on days 0, 7, and 11, respectively. On day 14, the medium is refiltered through glass wool prior to use in the test. For evaluating the biodegradability of a series of functionally or structurally related chemicals, media from all inoculum flasks may be combined before final filtration.

B. Procedures

Inoculum (100 mL of acclimation medium) is added to 900 mL DIW containing 1 mL each of solutions I, II, and III (Table 1) in a 2-liter Erlenmeyer flask. Test compound equivalent to 10 mg/liter carbon is added to each of the replicate flasks

containing the test medium. Ten mL of 0.2 N $\text{Ba}(\text{OH})_2$ are added to the suspended reservoir in each flask and duplicate 10 mL samples of $\text{Ba}(\text{OH})_2$ are also saved as titration blanks for analysis with test samples. Flasks are sparged with CO_2 -free air (for volatile test materials, sparging is done prior to addition of the chemical), sealed, and placed on a gyrotary shaker (approximately 125 rpm) at 20 to 25°C in the dark. For each set of experiments, each test, reference, inhibited and control system should be analyzed at time zero and at a minimum of four other times from time zero through day 28. Sampling must be made with sufficient frequency to allow for a smooth plot of biodegradation with time. Sampling times should be varied by the investigator as deemed appropriate to match the rate of degradation of the test substance. Tests may be terminated when biodegradation reaches a plateau and is consistent ($\pm 10\%$) over 3 consecutive days or on day 28, whichever occurs first. For chemicals which are water soluble at the test concentration, an adequate volume (5-10 mL) of medium is removed for DOC analysis. Each sample for DOC analysis should be filtered through a membrane filter of 0.45 micrometer pore diameter before DOC analysis. For all test and reference compounds, $\text{Ba}(\text{OH})_2$ from the center well is removed for analysis. The center well is rinsed with 10 mL CO_2 -free DIW and is refilled with fresh base. Rinse water is combined with the $\text{Ba}(\text{OH})_2$ sample to be analyzed. Flasks are resealed and placed on the shaker. On the day prior to terminating the test, 3 mL of 20% H_2SO_4 are added to the medium to release carbonate bound CO_2 .

For each set of experiments, each test substance should be tested in triplicate.

For each set of experiments, one or two reference compounds are included to assess the microbial activity of the test medium. Duplicate reference flasks are prepared by adding reference compound equivalent to 10 mg/liter carbon to each of two flasks containing the test medium. Reference compounds which are positive for ultimate biodegradability include: sodium citrate, dextrose, phthalic acid, trimellitic acid and aniline.

For each test set, triplicate controls receiving inoculated medium and no test compound, plus all test and reference flasks, are analyzed for CO₂ evolution and DOC removal. Results from analysis of the control flasks (DOC, CO₂ evolution, etc.) are subtracted from corresponding experimental flasks containing test compound in order to arrive at the net effect due to the test compound.

A test system containing a growth inhibitor should be established as a control for each substance tested for biodegradation by this method. That inhibited system must contain the same amount of water, mineral nutrients, inoculum and test substance used in the uninhibited test systems, plus 50 mg/L mercuric chloride (HgCl₂) to inhibit microbial activity.

Flasks should be incubated in the dark to minimize both photochemical reactions and algal growth. Appropriate sterile controls or controls containing a metabolic inhibitor, such as 50

mg/L HgCl_2 , are needed to correct for interferences due to non-biological degradation. With volatile organic materials, sparging with CO_2 -free air is performed only once, just prior to addition of the test chemical. Analyses for CO_2 evolution and DOC removal are conducted within 2-3 hours of sampling to minimize interferences which may occur in storage. All glassware should be free of organic carbon contaminants.

C. Analytical Measurements

The quantity of CO_2 evolved is measured by titration of the entire $\text{Ba}(\text{OH})_2$ sample (10 mL $\text{Ba}(\text{OH})_2$ + 10 mL rinse water) with 0.1 N HCl to the phenolphthalein end point. $\text{Ba}(\text{OH})_2$ blanks are also supplemented with 10 mL CO_2 -free DIW and titrated in a similar manner. Samples (5 mL) for DOC are centrifuged and/or filtered and supernatant or filtrate analyzed by a suitable total organic carbon method.

III. DATA AND REPORTING

A. Treatment of Results

Test compound (10 mg carbon) is theoretically converted to 0.833 mmol CO_2 . Absorbed CO_2 precipitates as BaCO_3 from $\text{Ba}(\text{OH})_2$, causing a reduction in alkalinity by the equivalent of 16.67 mL of 0.1 N HCl for complete conversion of the test compound carbon to CO_2 . Therefore, the percent theoretical CO_2 evolved from the test compound is calculated at any sampling time from the formula:

chosen, the investigator may use lower test substance concentrations if those concentrations are more representative of environmental levels.

B. Test Report

For each test and reference compound, the following data should be reported.

Information on the inoculum, including source, collection date, handling, storage and adaptation possibilities (i.e., that the inoculum might have been exposed to the test substance either before or after collection and prior to use in the test).

Results from each test, reference, inhibited (with HgCl_2) and control system at each sampling time, including an average result for the triplicate test substance systems and the standard deviation for that average.

Average cumulative percent theoretical CO_2 evolution over the test duration.

Dissolved organic carbon due to test compound at each sampling time (DTF-DCF).

Average percent DOC removal at each sampling time.

Twenty-eight day standard deviation for percent CO_2 evolution and DOC removal.

IV. REFERENCES

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$$\% \text{ CO}_2 \text{ evolution} = [(TF - CF)/16.67] 100 \text{ (for 10 mg/L test compound carbon)}$$

where:

TF = mL 0.1 N HCl required to titrate Ba(OH)_2 samples from the test flask

CF = mL 0.1 N HCl required to titrate Ba(OH)_2 samples from the control flask.

The cumulative % CO_2 evolution at any sample time is calculated as the summation of the % CO_2 evolved at all sample points of the test.

The percent DOC disappearance from the test compound is calculated from the following equation:

$$\% \text{ DOC Removal} = [1 - (DTF_x - DCF_x)/(DTF_o - DCF_o)] 100$$

where:

DTF = Dissolved organic carbon from test flask
 DCF = Dissolved organic carbon from control flask
 o = Day zero measurements
 x = Day of measurements during test.

The difference between the amount of 0.1 N HCl used for the Ba(OH)_2 titration blank samples and the Ba(OH)_2 samples from the control units (no test compound) is an indication of the activity of the microorganisms in the test system. In general, this

difference is approximately 1-3 mL of 0.1 N HCl at each sampling time. A finding of no difference in the titration volumes between these two samples indicates a poor inoculum. In this case, the validity of the test results is questionable and the test set should be rerun beginning with the acclimation phase.

CO₂ evolution in the reference flasks is also indicative of the activity of the microbial test system. The suggested reference compounds should all yield final CO₂ evolution values in the range 80-100% of theoretical CO₂. If, for any test set, the percent theoretical CO₂ evolution value for the reference flasks is outside this range, the test results are considered invalid and the test is rerun.

Inhibition by the test compound is indicated by lower CO₂ evolution in the test flasks than in the control flasks. If inhibition is noted, the study for this compound is rerun beginning with the acclimation phase. During the test phase for inhibitory compounds, the test chemical is added incrementally according to the schedule: Day 0 - 0.5 mg/liter as organic carbon, Day 2 - 1 mg/liter C, Day 4 - 1.5 mg/liter C, Day 7 - 2 mg/liter C, Day 10 - 5 mg/liter C. For this case, the Ba(OH)₂ is sampled on Day 10, and weekly thereafter. The total test duration remains 28 days.

The use of ¹⁴C-labelled chemicals is not required. If appropriately labelled test substance is readily available and if the investigator chooses to use this procedure with labelled test substance, this is an acceptable alternative. If this option is

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ANAEROBIC BIODEGRADATION

OFFICE OF TOXIC SUBSTANCES
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August, 1982

COMPLEX FORMATION ABILITY IN WATER

OFFICE OF TOXIC SUBSTANCES
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COMPLEX FORMATION ABILITY IN WATERI. PURPOSE

This Test Guideline references methodology to develop data on the ability of a chemical substance to form soluble metal complexes. It is applicable only to pure chemical substances and is not applicable to the determination of mercury complexes. The data may be used to evaluate the potential increase in availability to food chains or drinking water of metals that might otherwise become inaccessible.

II. TEST PROCEDURES

Appropriate methods are found in OECD Guideline No. 108, (OECD), "Complex Formation Ability in Water". The U.S. sales agent for the OECD guidelines is OECD Publications and Information Center, Suite 1207, 1750 Pennsylvania Ave. NW, Washington, DC 20006.

III. REFERENCE

OECD. 1981. Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals.

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HYDROLYSIS AS A FUNCTION OF pH AT 25°C

OFFICE OF TOXIC SUBSTANCES
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HYDROLYSIS AS A FUNCTION OF pH AT 25°CI. INTRODUCTIONA. Background and Purpose

Water is one of the most widely distributed substances in the environment. It covers a large portion of the earth's surface as oceans, rivers, and lakes. The soil also contains water as does the atmosphere in the form of water vapor. As a result of this ubiquitousness, chemicals introduced into the environment almost always come into contact with aqueous media. Certain classes of these chemicals, upon such contact, can undergo hydrolysis, which is one of the most common reactions controlling chemical stability and is, therefore, one of the main chemical degradation paths of these substances in the environment.

Since hydrolysis can be such an important degradation path for certain classes of chemicals, it is necessary, in assessing the fate of these chemicals in the environment, to know whether, at what rate, and under what conditions a substance will hydrolyze. Some of these reactions can occur so rapidly that there may be greater concern about the products of the transformation than about the parent compounds. In other cases, a substance will be resistant to hydrolysis under typical environmental conditions, while, in still other instances, the substance may have an intermediate stability that can result in the necessity for an assessment of both the original compound and

its transformation products. The importance of transformation of chemicals via hydrolysis in aqueous media in the environment can be determined quantitatively from data on hydrolysis rate constants. This hydrolysis Test Guideline represents a test to allow one to determine rates of hydrolysis at any pH of environmental concern at 25°C.

B. Definitions and Units

1. "Hydrolysis" is defined as the reaction of an organic chemical with water, such that one or more bonds are broken and the reaction products of the transformation incorporate the elements of water (H_2O).

2. "Elimination" is defined in this Test Guideline to be a reaction of an organic chemical (RX) in water in which the X group is lost. These reactions generally follow the same type of rate laws that hydrolysis reactions follow and, thus, are also covered in this Test Guideline.

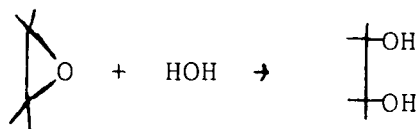
3. A "first-order reaction" is defined as a reaction in which the rate of disappearance of the chemical substance being tested is directly proportional to the concentration of the chemical substance and is not a function of the concentrations of any other substances present in the reaction mixture.

4. The "half-life" of a chemical is defined as the time required for the concentration of the chemical substance being tested to be reduced to one-half its initial value.

Hydrolysis refers to a reaction of an organic chemical with water such that one or more bonds are broken and the reaction products incorporate the elements of water (H_2O). This type of transformation often results in the net exchange of a group X, on an organic chemical RX, for the OH group from water. This can be written



Another result of hydrolysis can be the incorporation of both H and OH in a single product. An example of this is the hydrolysis of epoxides, which can be represented by



The hydrolysis reaction can be catalyzed by acidic or basic species, including OH^- and H_3O^+ (H^+). The promotion of the reaction by H_3O^+ or OH^- is called specific acid or specific base catalysis, respectively, as contrasted with general acid or base catalysis encountered with other cationic or anionic species. Usually, the rate law for chemical RX can be written as:

$$\begin{aligned} \frac{-d[\text{RX}]}{dt} = & k_h [\text{RX}] = k_A [\text{H}^+] [\text{RX}] \\ & + k_B [\text{OH}^-] [\text{RX}] + k'_N [\text{H}_2\text{O}] [\text{RX}], \end{aligned} \quad (1)$$

where k_A , k_B and k'_N are the second-order rate constants for acid and base catalyzed and neutral water processes, respectively. In dilute solutions, such as are encountered in following this Test Guideline, water is present in great excess and its concentration

is, thus, essentially constant during the course of the hydrolysis reaction. At fixed pH, the reaction, therefore, becomes pseudo first-order, and the rate constant (k_h) can be written as:

$$k_h = k_A [H^+] + k_B [OH^-] + k_N, \quad (2)$$

where k_N is the first-order neutral water rate constant. Since this is a pseudo first-order process, the half-life is independent of the concentration and can be written as:

$$t_{1/2} = 0.693/k_h. \quad (3)$$

At constant pH, Equation 1 can be integrated to yield the first order rate expression

$$\log_{10} C = - (k_h t / 2.303) + \log_{10} C_0, \quad (4)$$

where C is the concentration of the test chemical at time t and C_0 is the initial chemical concentration ($t = 0$).

At a given pH, Equation 2 contains three unknowns, k_A , k_B , and k_N . Therefore, three equations (i.e., measurements at three different pH's at a fixed temperature) are required if one wishes to solve for these quantities. Making suitable approximations for

quantities that are negligible, the expressions for k_A , k_B , and k_N using values of k_h measured at pH 3, 7, and 11 are:

$$\begin{aligned} k_A &= 10^3 [k_h (3) - k_h (7) + 10^{-4} k_h (11)] \\ k_B &= 10^3 [k_h (11) - k_h (7) + 10^{-4} k_h (3)] \\ k_N &= k_h (7) - 10^{-4} [k_h (3) + k_h (11)] \end{aligned} \quad (5)$$

The above calculated rate constants can be employed in equation 2 to calculate the hydrolysis rate of a chemical at any pH of environmental concern.

The above equations apply whether the test chemical has one hydrolyzable group or several. In the latter case, the rate may be written:

$$\begin{aligned} \frac{-d[RX]}{dt} &= k_1 [RX] + k_2 [RX] + \dots + k_n [RX] \\ &= (k_1 + k_2 + \dots + k_n) [RX] = k_h [RX]. \end{aligned} \quad (6)$$

Equation 6 applies to the hydrolysis rate of a molecule having n hydrolyzable groups, each of which follows first-order reaction kinetics. The measured k_h is now the sum of the individual reaction rates and is the only rate constant required in this Test Guideline.

C. Principle of the Test Method

Procedures described in this Test Guideline enable sponsors to obtain quantitative information on hydrolysis rates through a

determination of hydrolysis rate constants and half-lives of chemicals at pH 3.00, 7.00, and 11.00 at 25°C. The three measured rate constants are used to determine the acidic, basic, and neutral rate constants associated with a hydrolytic reaction. These latter constants can then be employed in determining the hydrolysis rates of chemicals at any pH of environmental concern at 25°C.

D. Applicability and Specificity

There are several different common classes of organic chemicals that are subject to hydrolysis transformation, including esters, amides, lactones, carbamates, organophosphates, and alkyl halides. Processes other than nucleophilic displacement by water can also take place. Among these are elimination reactions that exhibit behavior similar to hydrolysis and, therefore, are also covered in this Test Guideline.

II. TEST PROCEDURES

A. Test Conditions

1. Special Laboratory Equipment

- (1) A thermostatic bath that can be maintained at a temperature of $25 \pm 1^{\circ}\text{C}$.
- (2) A pH meter that can resolve differences of 0.05 pH units or less.
- (3) Stoppered volumetric flasks (no grease) or glass ampoules that can be sealed.

2. Purity of Water

Reagent-grade water (e.g., water meeting ASTM Type IIA standards or an equivalent grade) is highly recommended to minimize biodegradation. ASTM Type IIA water is described in ASTM D-193-77, "Standard Specification for Reagent Water."

3. Sterilization

It is extremely important to sterilize all glassware and to use aseptic conditions in the preparation of all solutions and in carrying out all hydrolysis experiments to eliminate or minimize biodegradation. Glassware can be sterilized in an autoclave or by any other suitable method.

4. Precautions for Volatility

If the chemical is volatile, it is extremely important that the reaction vessels be almost completely filled and sealed.

5. Temperature Controls

It is important that all hydrolysis reactions be carried out at 25°C and the temperature is controlled to $\pm 1^\circ\text{C}$.

6. pH Conditions

It is recommended that all hydrolysis experiments be performed at pH 3.00, 7.00, and 11.00 \pm 0.05 using the appropriate buffers described in Section II.B.1.a.

7. Concentration of Solutions of Chemical Substances

It is extremely important that the concentration of the test chemical be less than one-half the chemical's solubility in water and not greater than 10^{-3} M.

8. Effect of Acidic and Basic Groups

Complications can arise upon measuring the rate of hydrolysis of chemicals that reversibly ionize or are protonated in the pH range 3.00 to 11.00. Therefore, for these chemicals, it is recommended that these hydrolysis tests be performed at pH 5.00, 7.00, and 9.00 \pm 0.05 using the appropriate buffers described in Sections II.B.1.a and II.B.1.b. If a test chemical reversibly

ionizes or protonates in the pH range 5.00 to 9.00, then it is recommended that additional hydrolysis tests should be carried out at pH 6.00 and 8.00 ± 0.05 using the buffers described in Section II.B.1.b.

9. Buffer Catalysis

For certain chemicals, buffers may catalyze the hydrolysis reaction. If this is suspected, it is extremely important that hydrolysis rate determinations be carried out with the appropriate buffers and that the same experiments be repeated at buffer concentrations lowered by at least a factor of five. If the hydrolysis reaction produces a change of greater than 0.05 pH units in the lower concentration buffers at the end of the measurement time, then it is extremely important that the test chemical concentrations also be lowered by at least a factor of five. Alternatively, test chemical concentrations and buffer concentrations may both be lowered simultaneously by a factor of five. A sufficient criterion for minimization of buffer catalysis is an observed equality in the hydrolysis rate constant of two different solutions differing in buffer or test chemical concentration by a factor of five.

10. Photosensitive Chemicals

The solution absorption spectrum can be employed to determine whether a particular chemical is potentially subject to photolytic transformation upon exposure to light. For chemicals that absorb

light of wavelengths greater than 290 nm, it is important that the hydrolysis experiment be carried out in the dark, under amber or red safelights, in amber or red glassware, or employing other suitable methods for preventing photolysis. The absorption spectrum of the chemical in aqueous solution can be measured by OECD Test Guideline No. 101, "UV-VIS Absorption Spectra."

11. Chemical Analysis of Solutions

In determining the concentrations of the test chemicals in solution, any suitable analytical method may be employed, although methods which are specific for the compound to be tested are preferred. Chromatographic methods are recommended because of their compound specificity in analyzing the parent chemical without interferences from impurities. Whenever practicable, the chosen analytical method should have a precision within ± 5 percent.

B. Preparation

1. Reagents and Solutions

a. Buffer Solutions

Prepare buffer solutions using reagent-grade chemicals and reagent-grade water as follows:

- (1) pH 3.00 use 250 mL of 0.100M potassium hydrogen phthalate;
111 mL of 0.100M hydrochloric acid;

and adjust volume to 500 mL with reagent-grade water.

(2) pH 7.00 use 250 mL of 0.100M potassium dihydrogen phosphate; 145 mL of 0.100M sodium hydroxide; and adjust volume to 500 mL with reagent-grade water.

(3) pH 11.00 use 250 mL of 0.0500M sodium bicarbonate; 113 mL of 0.100 M sodium hydroxide; and adjust volume to 500 mL with reagent-grade water.

b. Additional Buffer Solutions

For chemicals that ionize or are protonated as discussed in Section II.A.8, prepare buffers using reagent-grade water and reagent-grade chemicals as follows:

(1) pH 5.00 use 250 mL of 0.100 M potassium hydrogen phthalate; 113 mL of 0.100M sodium hydroxide; and adjust volume to 500 mL with reagent-grade water.

- (2) pH 6.00 use 250 mL of 0.100M potassium dihydrogen phosphate;
28 mL of 0.100M sodium hydroxide; and
adjust volume to 500 mL with reagent-grade water.
- (3) pH 8.00 use 250 mL of 0.100M potassium dihydrogen phosphate;
234 mL of 0.100M sodium hydroxide; and
adjust volume to 500 mL with reagent-grade water.
- (4) pH 9.00 use 250 mL of 0.0250M borax ($\text{Na}_2\text{B}_4\text{O}_7$);
23 mL of 0.100M hydrochloric acid; and
adjust volume to 500 mL with reagent-grade water.

c. Adjustment of Buffer Concentrations

- (1) The concentrations of all the above buffer solutions are the maximum concentrations to be employed in carrying out hydrolysis measurements. If the initial concentration of the test chemical is less than 10^{-3}M , it is extremely important that the buffer concentrations be lowered by a corresponding amount; e.g., if the initial test chemical

concentration is 10^{-4}M , then reduce the concentration of the above buffers by a factor of 10. In addition, for those reactions in which an acid or base is not a reaction product, then employ the minimum buffer concentration necessary for maintaining the pH within ± 0.05 units.

- (2) Check the pH of all buffer solutions with a pH meter at 25°C and adjust the pH to the proper value, if necessary.

d. Preparation of Test Solution

If the test substance is readily soluble in water, prepare an aqueous solution of the chemical in the appropriate buffer and determine the concentration of the chemical. Alternatively, a solution of the chemical in water may be prepared and added to an appropriate buffer solution and the concentration of the chemical then determined. In the latter case, it is important that the aliquot be small enough so that the concentration of the buffer in the final solution and the pH of the solution remain essentially unchanged. Do not employ heat in dissolving the chemical. It is extremely important that the final concentration not be greater than one-half the substance's solubility in water and not greater than 10^{-3}M .

If the test chemical is too insoluble in pure water to permit reasonable handling and analytical procedures, it is recommended

that the chemical be dissolved in reagent-grade acetonitrile and buffer solution then added to an aliquot of the acetonitrile solution. Do not employ heat to dissolve the chemical in acetonitrile. It is extremely important that the final concentration of the test substance not be greater than one-half the chemical's solubility in water and not greater than $10^{-3}M$. In addition, it is extremely important that the final concentration of the acetonitrile be one volume percent or less.

C. Performance of the Test

Carry out all hydrolysis experiments by employing one of the procedures described below. Prepare the test solutions as described in Section II.B.1 at pH 3.00, 7.00, and 11.00 ± 0.05 , and determine the initial test chemical concentration (C_0) in triplicate. Analyze each reaction mixture in triplicate at regular intervals, employing one of the following procedures:

1. Procedure 1

Analyze each test solution at regular intervals to provide a minimum of six measurements with the extent of hydrolysis between 20-70 percent. Rates should be rapid enough so that 60-70 percent of the chemical is hydrolyzed in 672 hours.

2. Procedure 2

If the reaction is too slow to conveniently follow hydrolysis to high conversion in 672 hours but still rapid enough to attain

at least 20 percent conversion, take 15 to 20 time points at regular intervals after 10 percent conversion is attained.

3. Procedure 3

If chemical hydrolysis is less than 20 percent after 672 hours, determine the concentration (C) after this time period.

If the pH at the end of concentration measurements employing any of the above three procedures has changed by more than 0.05 units from the initial pH, repeat the experiment using a solution having a test chemical concentration lowered sufficiently to keep the pH variation within 0.05 pH units.

4. Analytical Methodology

Select an analytical method that is most applicable to the analysis of the specific chemical being tested (Section II.A.11).

III. DATA AND REPORTING

A. Treatment of Results

(1) If Procedures 1 or 2 were employed in making concentration measurements, use a linear regression analysis with equation 4 to calculate k_h at 25°C for each pH employed in the hydrolysis experiments. Calculate the coefficient of determination (R^2) for each rate constant. Use equation 3 to calculate the hydrolysis half-life using k_h .

(2) If Procedure 3 was employed in making rate measurements, use the mean initial concentration (C_0) and the mean concentration of chemical (C) in equation 4 to calculate k_h for each pH used in the experiments. Calculate the hydrolysis half-life using k_h in equation 3.

(3) For each set of three concentration replicates, calculate the mean value of C and the standard deviation.

(4) For test chemicals that are not ionized or protonated between pH 3 and 11, calculate k_A , k_B , and k_N using equation 5.

B. Specific Analytical and Recovery Procedures

(1) Provide a detailed description or reference for the analytical procedure used, including the calibration data and precision.

(2) If extraction methods were used to separate the solute from the aqueous solution, provide a description of the extraction method as well as the recovery data.

C. Test Data Report

(1) For procedures 1 and 2, report k_h , the hydrolysis half-life ($t_{1/2}$), and the coefficient of determination (R^2) for each pH employed in the rate measurements. In addition, report the individual values, the mean value, and the standard deviation for each set of replicate concentration measurements. Finally, report k_A , k_B , and k_N .

(2) For Procedure 3, report k_h and the half-life for each pH employed in the rate measurements. In addition, report the individual values, the mean value, and the standard deviation for each set of replicate concentration measurements. Finally, report k_A , k_B , and k_N .

(3) If, after 672 hours, the concentration (C) is the same as the initial concentration (C_0) within experimental error, then k_h cannot be calculated and the chemical can be reported as being persistent with respect to hydrolysis.

IV. REFERENCES

ASTM. 1978. Annual Book of ASTM Standards. American Society for Testing and Materials. Philadelphia, PA., Part 31, Method D1193-77.

OECD. 1981. Organization of Economic Cooperation and Development. OECD Guidelines for Testing Chemicals: No. 101 - UV-VIS Absorption Spectra.

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HYDROLYSIS AS A FUNCTION OF pH AT 25°C

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
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HYDROLYSIS AS A FUNCTION OF pH AT 25°CI. NEED FOR THE TEST

Water is one of the most widely distributed substances in the environment. It covers a large portion of the earth's surface as oceans, rivers and lakes. The soil also contains water as does the atmosphere in the form of water vapor. As a result of this ubiquitousness, chemicals introduced into the environment almost always come into contact with aqueous media. Certain classes of these chemicals, upon such contact, can undergo hydrolysis which is one of the most common reactions controlling chemical stability and is, therefore, one of the main chemical degradation paths of these substances in the environment. There are several different, common classes of chemicals which are subject to this type of degradation including esters, amides, lactones, carbamates, organophosphates, alkyl halides, epoxides, etc. Processes other than nucleophilic displacement by water can also take place. Among these are elimination reactions which exhibit rate behavior similar to hydrolysis and, thus, are also covered in this Test Guideline.

Since hydrolysis can be such an important degradation path for certain classes of chemicals, it is necessary, in assessing the fate of these chemicals in the environment, to know whether, at what rate, and under what conditions a substance will hydrolyze. Some of these reactions can occur so rapidly that there may be greater concern about the products of the

transformation than about the parent compounds. In other cases a substance will be resistant to hydrolysis under typical environmental conditions, while in still other instances, the substance may have an intermediate stability which can result in the necessity for an assessment of both the original compound and its transformation products. The importance of transformation of chemicals via hydrolysis in aqueous media in the environment can be determined quantitatively from data on hydrolysis rate constants. This hydrolysis Test Guideline represents a procedure to allow one to determine the rate constants for acid catalyzed, base catalyzed, and neutral hydrolysis at 25°C. The results can be used to calculate the rate constant for hydrolysis at any pH of environmental concern at this temperature. Future Test Guidelines will extend the temperature range of testing conditions and will cover other environmental factors which might affect hydrolysis such as general acid-base catalysis involving transition metal ions or nucleophilic species, salt effects in sea water, etc. In addition, more advanced Test Guidelines may be concerned with determining the identity and fate of the transformation products.

II. SCIENTIFIC ASPECTS

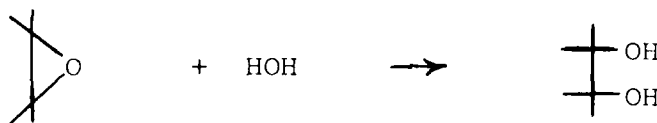
A. General Background

Hydrolysis refers to a reaction of an organic chemical with water such that one or more bonds are broken and the reaction products incorporate the elements of water (H_2O). This type of transformation often results in the net exchange of a group X, on

an organic chemical RX, for the OH group from water. This can be written as:



Another result of hydrolysis can be the incorporation of both H and OH in a single product. An example of this is the hydrolysis of epoxides which can be represented by:



The hydrolysis reaction can be catalyzed by acidic or basic species, including OH^- and H_3O^+ (H^+). The promotion of the reaction by H_3O^+ or OH^- is called specific acid or specific base catalysis, as contrasted to general acid or base catalysis encountered with other cationic or anionic species, respectively. The rate law for hydrolysis of chemical RX usually can be written

$$\frac{-d[\text{RX}]}{dt} = k_h[\text{RX}] = k_B[\text{OH}^-][\text{RX}] \quad (1)$$

$$+ k_A[\text{H}^+][\text{RX}] + k'_N[\text{H}_2\text{O}][\text{RX}],$$

where k_A , k_B , and k'_N are the second-order rate constants for acid and base catalyzed and neutral water processes, respectively. In the environment, hydrolysis of organic chemicals occurs in dilute solution. Under these conditions,

water is present in large excess, and the concentration of water is essentially constant during the hydrolysis reaction. Thus, at fixed pH, this process follows pseudo first-order kinetics and the rate of disappearance of the chemical is dependent only on its concentration. The hydrolysis rate constant can thus be written as:

$$k_h = k_B[\text{OH}^-] + k_A[\text{H}^+] + k_N, \quad (2)$$

where k_A and k_B are the second-order rate constants and k_N is a first order-rate constant. At a given pH, expression (2) contains three unknowns, k_A , k_B , and k_N . Therefore, three equations (i.e., measurements at three different pH's at a fixed temperature) are required to solve for these quantities. Making suitable approximations for quantities which are negligible, the expressions for k_A , k_B , and k_N using values of k_h measured at three environmental pH's (e.g., pH = x, x+y, and x+y+z) are (Mill et al. 1981a)

$$k_A = 10^x [k_h(x) - k_h(x+y)] + 10^{x-z} k_h(x+y+z)$$

$$k_N = k_h(x+y) - 10^{-y} k_h(x) - 10^{-z} k_h(x+y+z) \quad (3)$$

$$k_B = 10^{14-x-2y-z} k_h(x) - 10^{14-x-y-z} [k_h(x+y) - k_h(x+y+z)].$$

At a fixed temperature, the determination of rate constants at three different pH's, therefore, can be used to determine k_A , k_B ,

and k_N . For example, using the three measurements at pH 3.0, 7.0, and 11.0 required by the protocol ($x=3$, $y=4$, $z=4$), one obtains:

$$\begin{aligned} k_A &= 10^3 [k_h(3) - k_h(7) + 10^{-4} k_h(11)] \\ k_B &= 10^3 [k_h(11) - k_h(7) + 10^{-4} k_h(3)] \\ k_N &= k_h(7) - 10^{-4} [k_h(3) + k_h(11)]. \end{aligned} \quad (4)$$

Once the above three rate constants have been calculated, equation (2) can be used to calculate rate constants at any pH of environmental significance at a fixed temperature. After k_h has been determined, the half-life of a chemical is easily obtained since at fixed pH the hydrolysis reaction is pseudo-first order, and the half-life of the substrate is independent of its concentration. Thus:

$$t_{1/2} = 0.693/k_h. \quad (5)$$

The dependence of rate on pH can be conveniently expressed graphically. Figure (1) shows a typical $\log k_h$ vs. pH plot for substances which undergo acid, water, and base-promoted hydrolysis (Mill et al. 1981a). Most pH-rate profiles are found to have one or two areas of curvature corresponding to pH values where two kinds of rate processes contribute to the overall hydrolysis rate. For molecules for which acid catalyzed processes do not play an important role, the low pH region will have near zero slope, while the same will be true in the high pH region if base-catalyzed hydrolysis is unimportant. The lower curve in Figure (1) results when $k_N \ll k_A[H^+]$ and $k_B[OH^-]$.

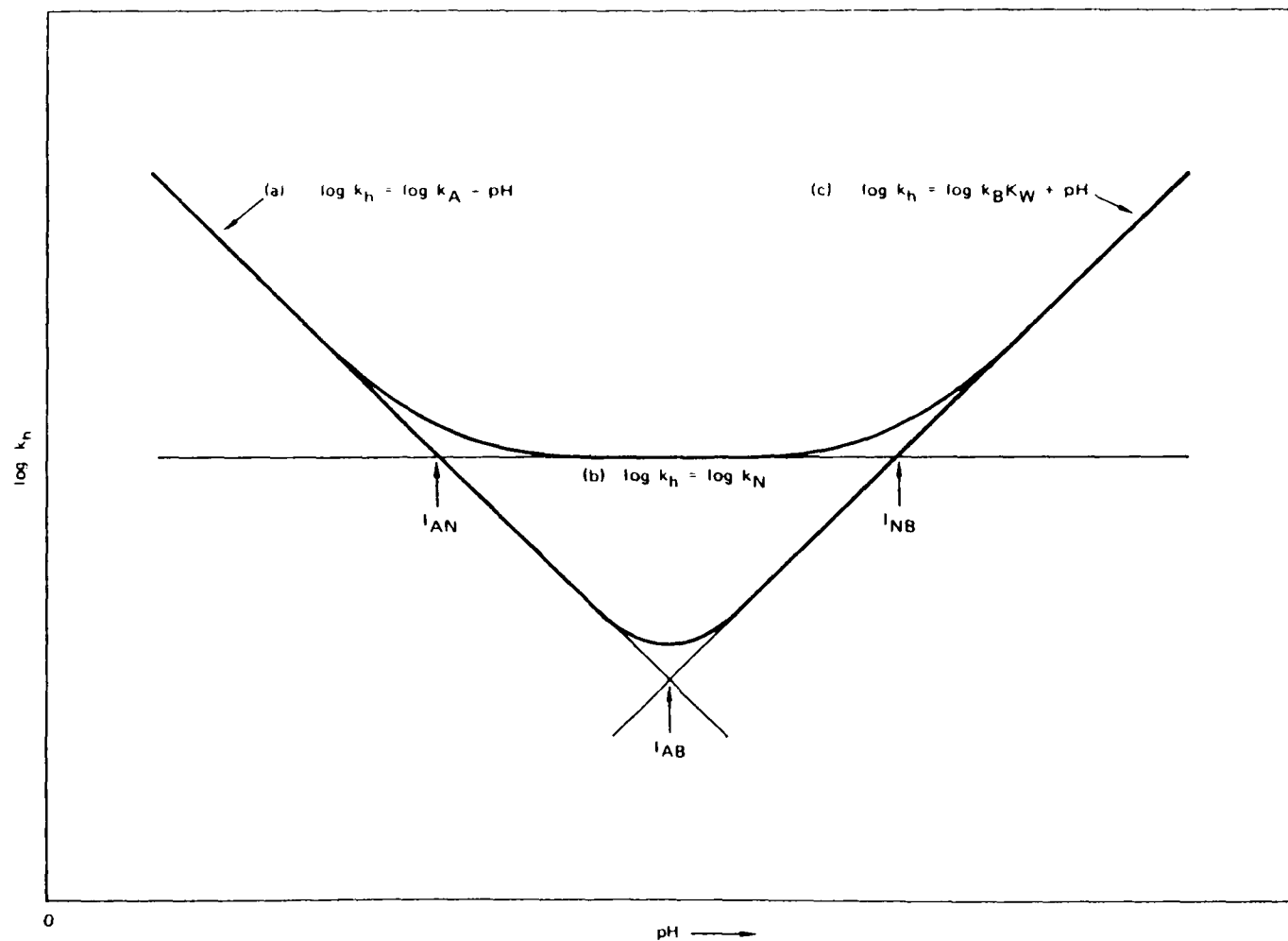


Figure .1 pH Dependence of k_h for Hydrolysis by (a) Acid-, (b) Water-, and (c) Base-Promoted Processes

The equations discussed in this section are equally applicable to molecules having one or many hydrolyzable groups. In the latter case, the rate of reaction may be written as:

$$\begin{aligned} \frac{-d[RX]}{dt} &= k_1[RX] + k_2[RX] + \dots + k_n[RX] \\ &= (k_1 + k_2 + \dots + k_n) [RX] = k_h[RX]. \end{aligned} \quad (6)$$

The above equation applies to the hydrolysis rate of a molecule having n hydrolyzable groups, each of which reacts according to first-order kinetics. The measured k_h , which is still the rate constant for the disappearance of RX , is now the sum of the individual hydrolysis rate constants. Measurements of concentration of any particular reaction product vs. time, permits the determination of the rate constants associated with this product (Frost and Pearson 1961). This determination is not required in this Test Guideline.

Details of the hydrolysis reactions of various types of compounds can be found in many kinetics texts (e.g., Laidler 1965, Frost and Pearson 1961). Discussions of hydrolysis from an environmental point of view have also been published (Mabey and Mill 1978, Tinsley 1979). Finally, examples of experiments in which hydrolysis rate constants were measured in an environmentally relevant fashion can be found in the papers by Wolfe et al. (1976, 1977), Smith et al. (1977, 1978).

B. Rationale for Selection of Test Method

An extensive amount of information has been published on the hydrolysis of a wide variety of organic chemicals. However, most of the literature relating to hydrolysis of chemicals in the environment concerns pesticides. Many of these data are incomplete for the range of pH and temperature of environmental concern. Effects of buffer salts are often unrecognized. A detailed literature search indicates that numerous factors must be considered in determining the hydrolysis rate constants of certain organic chemicals. At present, no validated procedure exists for determining the hydrolysis rate constant at environmental pH's and temperatures. The proposed method in this Test Guideline was developed from a detailed review of the literature on hydrolysis, from consultations with researchers having considerable experience in carrying out these types of measurements, and from the results of a contract with SRI International on optimization of hydrolysis protocols (Mill et al. 1981). The method was selected on the basis of the following criteria: (1) The test method should be based on the fundamentals of the kinetics of hydrolysis. (2) The test method should yield quantitative data in the pH and temperature range of environmental concern. (3) The effects of buffer salts on the rates of hydrolysis should be minimized. (4) The test should be designed to insure that only hydrolysis takes place. For example, the experiment should be designed to make sure that other processes such as biodegradation, loss by volatilization, or photolysis are eliminated.

The test method requires that rate constants be measured in buffered, distilled water solutions rather than in natural waters and, thus, makes the assumption that hydrolysis rates are the same in the two media. This assumption has been confirmed by the published data to date [e.g., Smith et al. (1977, 1978), Wolfe et al. (1976, 1977), Zepp et al. (1975)], in which comparable rates of hydrolysis were found for various chemicals in sterile natural waters and in buffered distilled water at the same temperature and pH.

Assuming a sufficient number of time points, n , are taken, the percent standard deviation in the measurement of the hydrolysis rate constant, k_h , is proportional to $1/(n)^{1/2}$ and $1/t$, where t represents the number of half-lives over which measurements were taken (Mill et al. 1981a). The number of half-lives, or in other words the extent of conversion, is thus a more important factor than the number of time points in reducing the error in k_h . In order to obtain a reasonably good determination of k_h , and, also to clearly demonstrate that the hydrolysis reaction is following first-order kinetics, the reaction should be followed for at least one half-life (50% conversion). For a reaction which can attain at least 60% conversion in four weeks, Procedure 1 which requires a minimum of six data points is adequate. If the reaction is too slow to follow to high conversion, but at least 20% hydrolysis is attained in four weeks, the number of data points must be increased in order to decrease the uncertainty of the rate constant determination. Thus, for example, a reaction which is only 30% complete at the

end of concentration sampling has a percent standard deviation twice that of a reaction which is followed until it is 60% complete. To offset this increase in error, the number of data points must be increased $(2)^2$ or fourfold. The Procedure 2 requirement of 15 to 20 data points is an approximately threefold increase in the number of measurements and represents a compromise between the need to reduce uncertainty in a slow hydrolysis and the need to avoid an undue number of experimental determinations. If less than 20% of the chemical is hydrolyzed at the end of four weeks, a significant reduction in the uncertainty in k_h becomes more difficult to attain than is deemed necessary for the procedure described in this Test Guideline. In this case, a determination of only one data point is required.

C. Rationale for the Selection of Experimental Conditions

1. Purity of Water

Dissolved impurities can catalyze or affect the rate of hydrolysis. In addition, the water should be free of bacteria which may consume or alter the organic test material during the prolonged periods of testing which may occur in the course of a rate determination. Thus, very pure water [e.g., water comparable to that meeting ASTM Type II specification (ASTM 1979), or an equivalent grade], is required in this Test Guideline.

2. Sterilization

It is extremely important to sterilize all glassware, to use aseptic conditions in the preparation of all solutions, and in carrying out all hydrolysis experiments to eliminate or minimize biodegradation. Glassware can be sterilized in an autoclave or by any other suitable method.

3. Precautions for Volatility

Loss of a test chemical through volatilization will result in the determination of excessively large rate constants. Therefore, for volatile chemicals, it is extremely important that the reaction flasks be filled almost completely and sealed in order to avoid this type of loss.

4. Temperature

Since hydrolysis rates are a function of temperature, it is extremely important that the temperature of a hydrolysis reaction be kept constant during the course of measurement. The relationship between temperature and rate constant can be found in any chemical kinetics text (e.g., Laidler 1965, Benson 1960).

In aquatic systems, temperatures commonly encountered range from close to freezing in some lakes during winter to 30°C in some ponds during summer. Since this test method is only a screening test, the rate constant for hydrolysis is, ordinarily, required only at 25°C. This temperature was chosen since it is

in the temperature range of environmental concern, is the temperature at which most physical and chemical properties are reported, and, being at the higher end of the environmental temperature range, allows one to measure rates which are close to an upper limit for environmental hydrolysis of a particular substance and, thus, are more practical to determine within 672 hours.

In general, hydrolysis rates can be expected to vary by factors of 2 to 5 with each 10°C change in temperature (Mill et al. 1981a). Therefore, variations of temperature of $\pm 1^{\circ}\text{C}$ can typically lead to changes of hydrolysis rate on the order of ± 7 to $\pm 18\%$. This is an acceptable fluctuation for the proposed method and, thus, temperature control is set at $\pm 1^{\circ}\text{C}$ in the Test Guideline. This is a condition easily achieved by standard control devices for water baths.

5. pH

It is recommended that hydrolysis experiments be carried out at pH's of 3.00, 7.00, and 11.00. The first and last pH's are not ordinarily found under environmental conditions, and the rate constants measured at these pH's cannot therefore be directly used to yield environmentally relevant rates. The three measured rate constants are instead used in expression (4) to calculate k_A , k_B , and k_N . These latter constants are then used in expression (2) to determine the hydrolysis rate at 25°C of a test chemical at pH's commonly found in the environment.

Although the above procedure is less direct than performing rate measurements at environmental pH's such as 5, 7, and 9 as required previously (EPA 1979), an examination of equation (1) indicates that the rates of hydrolysis at pH 3 and 11 are as much as two orders of magnitude greater than at pH 5 and 9. This reduces the possibility of the intrusion of unwanted processes such as biodegradation, failure of control equipment, intermittent power failure, etc., which could tend to be a large factor in the errors associated with the slower rate processes at pH 5 and 9. The potential increase in accuracy of the pH 3 and 11 measurements is great enough so that the indirect measurement of environmentally relevant hydrolysis rates described in this Test Guideline can actually lead to more reliable results than a direct measurement (Mill et al. 1981). In addition, the potential decrease in measurement time can lead to a large reduction in the cost and effort of obtaining a rate constant. For these reasons, measurements at pH 3 and 11 are specified in the Test Guideline rather than pH 5 and 9.

The pH of a test solution cannot vary by more than 0.05 units. Examination of equation (1) shows that such a variation can lead to changes in the rate of as much as 12 percent. This is considered a maximum acceptable variation for this hydrolysis test method.

6. Initial Concentration of Chemicals

Typical concentrations of trace organics in the environment, except perhaps near release sites, do not ordinarily exceed 10^{-6}M (Mill 1981a). Changes in concentration at these low levels are difficult to monitor, however, so that the required 10^{-3}M concentration represents a compromise between the desirability of easily following the reaction rate and the desire to reproduce environmental conditions. Simple first-order processes at higher concentrations are expected to remain the same at lower concentrations so that the rate constants found at 10^{-3}M will be valid at environmental concentrations. In addition, since hydrolysis is almost always first-order (Laidler 1965, Frost and Pearson 1961), the actual half-life of the chemical is a function only of the rate constant and not the initial concentration.

If the test chemical is not sufficiently soluble to permit reasonable handling and analytical procedures, then test solutions can be prepared from a chemical dissolved in reagent grade acetonitrile. The solution can then be diluted with buffer to an appropriate concentration. It is extremely important that the final acetonitrile concentration be no more than one volume percent in order to avoid acetonitrile solvent effects (Smith et al. 1977, 1978; Mabey and Mill 1978).

7. Ionized or Protonated Groups

Certain chemicals reversibly ionize or are protonated in the pH range 3 to 11. As a result, complications can arise when measuring hydrolysis rates. The pH rate profiles of these substances will be more complicated than typical profiles and will often have a maximum or minimum. For chemicals which exhibit ionization or are protonated in the pH range 3 to 11, it is not possible to use k_A , k_B and k_N to obtain rate constants at environmental pH's. In this case, it is therefore necessary to measure k_h at pH 5, 7, and 9 directly. If the test chemical ionizes or is protonated between pH 5 and 9, the resulting complexity of the pH rate profile necessitates 2 additional measurements at pH 6 and 8.

8. Buffers

Nucleophilic salts can increase hydrolysis rates through a general acid or base catalyzed process (Jencks 1969), the magnitude of which is dependent on the nature and concentration of the salt. Thus, the results from experiments which utilize high concentrations of buffer are suspect unless carefully examined for buffer catalysis effects. The buffers listed for pH 3, 7, and 11 in this method were tested and used at low concentrations in order to minimize this type of catalysis (Mabey and Mill 1978). The hydrolysis of some chemicals, however, may still be affected by nucleophilic buffers at the concentrations specified in this Test Guideline. If there is any reason to

believe that this is occurring, it is recommended that hydrolysis experiments be carried out at buffer concentrations reduced by a factor of five. If the pH changes by more than 0.05 pH units at the end of these latter experiments, then the buffer capacity has been exceeded. Therefore, it is extremely important that the test chemical concentrations then also be reduced by a factor of five. Alternatively, one may reduce the buffer and test chemical concentration by the above factors simultaneously.

9. Light Sensitive Compounds

Chemicals which absorb light at wavelengths greater than 290 nm may be subject to photolysis, especially over the relatively long periods of time in which the hydrolysis experiments may be run. Therefore, it is recommended that precautions be taken, to insure that these substances are protected from light, including sunlight. Appropriate measures could include use of an amber or red safelight, use of amber or red colored glassware, or any other suitable technique which will eliminate the possibility of photolytic transformation.

10. Chemical Analysis of Solutions

The analytical techniques employed in the determination of test chemical concentrations are left to the sponsor to select. This is in recognition of the many different techniques available and the practical advantage of being able to make particular use of one of the properties of the compounds; e.g., the NMR or UV

spectrum of the substance, or the chromatographic behavior. Analytical techniques that permit the determination of the test compound to the exclusion of impurities or reaction products are recommended to the extent practicable. Therefore, chromatographic techniques are particularly desirable. Whenever practicable, an analytical procedure having a precision of $\pm 5\%$ should be used. The Test Guideline requires that the specific technique utilized be adequately described.

D. Test Data Required

The rate constant data required will be used to help assess the environmental fate and persistence of the test materials. It is essential data which is needed to make a risk assessment. If hydrolysis is a relatively important transformation process and the initial risk assessment indicates that the material poses a threat to the health of humans and/or to the environment, then advanced tests may be necessary to obtain more extensive data.

E. Statistical Analysis of the Data

In the case of Procedure 1, assuming 7 points have been measured over the course of one half-life and the error in individual concentration measurements is approximately 5%, one can anticipate an uncertainty in the rate constant of about twice that of the individual measurements (Mill et al. 1981a). This is an ideal case, however, in which temperature is assumed to have remained strictly constant and that no adventitious processes

have occurred. In cases in which errors due to environmental fluctuations (e.g., temperature) are larger than the uncertainties due to individual measurements, a case of cumulative errors arises which can lead to greater uncertainties than the aforementioned factor of two (Mandel 1964, Daniels and Johnston 1921). As was pointed out in section B, greater uncertainties are also expected when employing Procedure 2 in which the total measurement time covers less than one half-life. Finally, Procedure 3 (in which only one point is measured over less than 20% conversion) yields the greatest degree of uncertainty in the rate constant.

The exact magnitudes of all of the aforementioned errors are a function of the details of the hydrolysis kinetics of the particular chemical being tested. These uncertainties have not been clearly established and, thus, the Test Guideline makes no specific requirements for degree of precision in rate constants other than that of a ± 5 percent uncertainty in individual concentration measurements, if at all possible. The final precision can be improved by making numerous replicate determinations. The minimum requirement EPA would impose would be a statistical analysis of the data to provide standard deviations based on triplicate determinations. When a large number of chemicals have been determined by the proposed methods and the practical effect of factors such as fluctuations of temperature over a one degree range, measurement of very slow

reactions over a long time period, measurement of rates of relatively insoluble, highly volatile chemicals, etc. have been better defined, the level of precision in the rate constants can be given for chemicals having varying ranges of hydrolysis rates and differing physical properties.

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PHOTOLYSIS IN AQUEOUS SOLUTION IN SUNLIGHT

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PHOTOLYSIS IN AQUEOUS SOLUTION IN SUNLIGHTI. INTRODUCTIONA. Background and Purpose

Numerous chemicals have entered natural aquatic systems from a variety of sources. For example, chemical wastes have been discharged directly into natural water bodies. Chemicals have leached into natural water bodies from landfills. Pesticides have been applied directly into water bodies. Pesticides have been applied to soils and vegetation and have subsequently leached into water bodies. Pollutants present in aqueous media can undergo photochemical transformation in the environment (i.e., in sunlight by direct photolysis or by sensitized photolysis). As a result, there has been considerable interest in photolysis in solution, especially the photolysis of pesticides. However, most of these studies have been qualitative in nature and involved the identification of photolysis products. Quantitative data in the form of rate constants* and half-lives are needed to determine the importance of photochemical transformation of pollutants in aqueous media. This Test

* The EPA is developing test methods to determine the transport and transformation of chemicals in the environment. These methods will yield rate constants and equilibrium constants which can be extrapolated to a variety of environmental scenarios. Thus, this data can be used in models along with production, volume, distribution, etc. to predict an expected environmental concentration (EEC).

Guideline describes a method for determining direct photolysis rate constants and half-lives of chemicals in water in the presence of sunlight.

B. Definitions and Units

Direct photolysis is defined as the direct absorption of light by a chemical followed by a reaction which transforms the parent chemical substance into one or more products.

Numerous papers have been published on the photolysis of chemicals in solution. However, only recently has work been published on the determination of rate constants and half-lives for direct photolysis of chemicals in water under environmental conditions, i.e., in sunlight.

Zepp and Cline (1977) published a paper on the rates of direct photolysis in aquatic environments. The rates of all photochemical processes in a water body are affected by solar spectral irradiance at the water surface, radiative transfer from air to water, and the transmission of sunlight in the water body. It has been shown that in dilute solution (i.e., with the absorbance of a chemical less than 0.02 in the reaction cell at all wavelengths greater than 290 nm) at shallow depths, the kinetic expression for direct photolysis of a chemical at a molar concentration C is

$$- \frac{dC}{dt} = \phi k_a C = k_p C, \quad (1)$$

where
$$k_p = \phi k_a , \quad (2)$$

ϕ is the reaction quantum yield of the chemical in dilute solution, and $k_a = \sum k_{a\lambda}$, the sum of $k_{a\lambda}$ values of all wavelengths of sunlight that are absorbed by the chemical. The term k_p represents the photolysis rate constant in sunlight in the units of reciprocal time. Integrating equation 1 yields

$$\log_{10} \frac{C_0}{C} = \frac{k_p t}{2.30} , \quad (3)$$

where C is the molar concentration of chemical at time t during photolysis and C_0 is the initial molar concentration. By measuring the concentration of chemical as a function of the time t during photolysis in sunlight, k_p can be determined using equation 3. Since equation 1 is a first-order rate equation, the half-life for direct photolysis in sunlight is given by

$$t = \frac{0.693}{k_p} . \quad (4)$$

Zepp and Cline (1977) derived equations that describe the direct photolysis rates of pollutants in aquatic environments. These equations translate readily obtained laboratory data, such as the quantum yield ϕ as measured by Zepp (1978) and extinction coefficients of the chemical in aqueous solution, into rate constants and half-lives for photolysis in sunlight. Rate constants and half-lives can be calculated (by computer) as a function of season, latitude, time-of-day, depth in water bodies,

and the ozone layer. Several published papers concerning the photolysis of chemicals in sunlight verified this method (Zepp et al. 1977, Wolfe et al. 1976, Zepp et al. 1976, Zepp et al. 1975, Smith et al. 1977, 1978).

The absorbance of a chemical substance is the logarithm (base 10) of the ratio of the intensity of light entering a cell containing a solution of chemical to that leaving the cell.

A photolysis "day" is defined as the period of time from sunrise to sunset when sunlight photolysis of a chemical may take place. A fraction of a day is defined as a fraction of the daylight period. Based on this definition, the term k_p , used in equation 3, is then the sunlight photolysis rate constant expressed in the units day^{-1} . The half-life of the chemical is defined as the time in days corresponding to the disappearance of one-half of the initial concentration of the chemical in sunlight. The half-life can be calculated using equation 4. The rate constant and half-life determined by this method are relevant to the day mid-way between the beginning and termination of the photolysis experiment and, therefore, represent an "average" rate constant and half-life for the chemical during a certain period of time. Obviously, the longer the experiment, the greater the difference in the average value of the rate constant and half-life during the experimental time period. Therefore, all photolysis data reported should carry appropriate information on the duration of the experiment.

C. Principle of the Test Method

This Test Guideline is based on the principles developed by Zepp and Cline (1977) and the U.S. EPA Premanufacture Test Guideline (1979). A simple aqueous photolysis screening test has been developed to determine rate constants and half-lives in the presence of sunlight using equations (3) and (4). Sunlight was chosen as the irradiation source because of its relevancy as well as its low cost in comparison to artificial light sources. A small error (approximately 3%) is introduced in this methodology assuming an absorbance of 0.1 instead of 0.02 and first order kinetics are still applicable. Therefore, this screening test method is applicable to homogeneous chemical solutions having an absorbance of less than 0.10 in the reaction cell at all wavelengths greater than 290 nm and at shallow depths. The experiments have been designed to make sure that only photolysis occurs. For example, the experiments have been designed to eliminate biodegradation and volatilization. The experiment must be carried out during a warm time of the year (i.e., May, June, July, or August in the northern hemisphere, weather permitting) and the measured rate constant and half-life is characteristic of that time of year and the latitude of the site where the experiment was carried out.

This preliminary screening test has a limitation in that it fails to measure sunlight intensities incident on the sample during photolysis. Sunlight actinometers are being developed to evaluate sunlight intensities. The screening test will then be

modified to use simultaneous photolysis of a chemical and actinometer to evaluate sunlight intensities on the sample. The modified procedure will quantify sunlight photolysis of a chemical at a specific time of year and latitude and will give a useful measure of seasonal variation of photolysis.

D. Applicability and Specificity

This Test Guideline is applicable to all chemicals which have uv-visible absorption maxima at 290nm or greater. Some chemicals absorb light significantly below 290nm and consequently will not undergo direct photolysis in sunlight (e.g., alkanes, alkenes, alkynes). Some chemicals have absorption maxima significantly below 290nm but have measureable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290nm. Photolysis experiments should also be carried out for these chemicals. This screening test method is applicable to the photolysis of chemicals in dilute solution in which the absorbance is less than 0.10 in the reaction cell at all wavelengths greater than 290nm and at shallow depths. Furthermore, these experiments are limited to the direct photolysis of chemicals in air-saturated pure water. The water must be air-saturated to simulate environmental conditions.

II. TEST PROCEDURES

A. Test Conditions

1. Special Laboratory Equipment

- (1) A variable wavelength uv-visible spectrophotometer capable of measuring accurate absorbances at 0.10 (absorbance) units or less. Refer to OECD Guideline No. 101, "UV-VIS Absorption Spectra", for details on the use of the spectrophotometer;
- (2) a pH meter capable of resolving differences of 0.1 pH unit or less; and
- (3) the absorption spectrum of the chemical substance in aqueous solution (as determined by OECD Guideline No. 101) can be used to determine the type of reaction vessel to be employed for these photolysis experiments. It is strongly recommended that quartz vessels be used for the photolysis of chemical substances which absorb at wavelengths below 340nm. Chemical substances that absorb at wavelengths greater than 340nm may be tested in borosilicate glass vessels.

Thin walled borosilicate or quartz tubes are recommended. Disposable culture tubes (13 x 100 mm) with teflon lined screw caps or quartz tubes with ground glass stoppers (no grease) may be used as reaction vessels. Tubes of 11 mm i.d. are recommended. For some chemical substances it may difficult to

analyze the concentration of the chemical substance in reaction vessels of such small volume. For these chemical substances larger reaction vessels are recommended providing that the cell walls are thin and the pathlength of radiation through the vessel is less than 0.5 meter. Reaction vessels should be filled as completely as possible and sealed to minimize volatilization.

2. Purity of Water

Reagent grade water, e.g., water meeting ASTM Type II A Standards, or an equivalent grade, is highly recommended to minimize biodegradation. ASTM Type II A water is described in ASTM D 1193-77, "Standard Specification for Reagent Water". It is important to saturate water with bacteria-free air just prior to the preparation of the test and control solutions to simulate environmental conditions. The air can be filtered through a 0.2 μm (pore size) filter to remove bacteria.

3. Sterilization

It is extremely important to sterilize all glassware and to use aseptic conditions in the preparation of all solutions and in carrying out all photolysis experiments to eliminate or minimize biodegradation. Glassware can be sterilized in an autoclave or by any other suitable method.

4. pH Effects

It is recommended that all photolysis experiments be carried out at pHs 5.0, 7.0, and 9.0 for any chemical which reversibly ionizes or protonates (e.g., carboxylic acids, phenols, and amines). Buffers described in Section II.B.1.b. may be used.

5. Chemical Analysis of Solutions

In determining the concentration of the chemical in solution, an analytical method should be selected which is most applicable to the analysis of the specific chemical substance. Chromatographic methods are generally recommended because of their chemical specificity in analyzing the parent chemical substance without interference from impurities. Whenever practicable the chosen analytical method should have a precision of ± 5 percent.

6. Volatile Chemical Substances

Special care should be taken when testing a volatile chemical so that the chemical substance is not lost due to volatilization during the course of the photolysis experiment. Thus, it is important to effectively seal the reaction vessels. Tubes with ground-glass stoppers (no grease) or with plastic screw tops with teflon inserts are recommended. In addition, the reaction vessels should be as completely filled as is possible to prevent volatilization to any air space.

7. Control Solution

It is extremely important to take certain precautions to prevent loss of chemical from the reaction vessels by processes other than photolysis. For example, biodegradation and volatilization can be eliminated or minimized by use of sterile conditions and minimal air space in sealed vessels. Hydrolysis is a process which cannot be minimized by such techniques. Thus, control vessels containing test substance which are not exposed to sunlight are required. By suitable analysis of the concentration of test substance in the control vessels, corrections, if any, can be made to the measured photolysis rates. If hydrolysis is found to be significant, hydrolysis studies should be carried out first. (Test Guideline CG-5000).

8. Absorption Spectrum as a Criterion for Performing the Photolysis in Aqueous Solution in Sunlight Test

The Photolysis in Aqueous Solution in Sunlight Test is applicable to all chemicals which have uv-visible absorption maxima at 290nm, or greater. Some chemicals have absorption maxima significantly below 290nm but have measureable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290nm. Photolysis experiments must also be carried out for these chemicals. The absorption spectrum of the chemical in aqueous solution can be measured by OECD Test Guideline No. 101, "UV-VIS Absorption Spectra".

B. Preparations

1. Reagents and Solutions

a. Preparation of Test Chemical Solution

Prepare homogeneous solutions with the chemical at less than one-half its solubility in water and at a concentration such that the absorbance is less than 0.10 in the photolysis reaction vessel at wavelengths greater than 290nm. For very hydrophobic chemicals, it is difficult and time consuming to prepare aqueous solutions. To facilitate the preparation of aqueous solutions containing very hydrophobic chemicals and to allow for easier analytical measurement procedures, the following procedure may be used to aid in the dissolution of the chemical. Dissolve the pure chemical in reagent grade acetonitrile. Add pure water as described under Test Conditions, Section II.A.2., or buffer solution as described under Preparations, Section II.B.1.b., for chemical substances which reversibly ionize or protonate, to an aliquot of the acetonitrile solution. Do not exceed one volume-percent of acetonitrile in the final solution. Place the reaction solution in the photolysis reaction vessels.

b. Preparation of Buffer Solutions

Prepare buffer solutions using reagent grade chemicals and pure water as described under Test Conditions, Section II.A.2., as follows:

pH 5.0--0.1 molar sodium acetate ($\text{NaC}_2\text{H}_3\text{O}_2$) adjusted to pH 5.0 with 0.1 molar acetic acid ($\text{CH}_3\text{CO}_2\text{H}$).

pH 7.0--0.01 molar potassium dihydrogen phosphate (KH_2PO_4) adjusted to pH 7.0 with 0.1 molar sodium hydroxide (NaOH)

pH 9.0--0.025 molar sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7$) adjusted to pH 9.0 with 0.1 molar hydrochloric acid (HCl)

The pH of all buffer solutions must be checked with a pH meter at 25°C and adjusted to the proper value if necessary.

C. Performance of the Test

For all experiments, prepare an aqueous solution of the chemical substance and a sufficient number of samples in quartz or borosilicate glass vessels to perform all the required tests. Fill the vessels as completely as possible and seal them. Prepare three control samples in the absence of

ultraviolet light and totally exclude light by wrapping the vessels with aluminum foil or by other suitable methods. These samples are analyzed for the chemical substance immediately after completion of the experiment to measure the loss of chemical in the absence of light. Place the samples, including the controls, outdoors in an area free of shade and reflections of sunlight from windows and buildings. Place the samples on a black, non-reflective background and inclined at approximately 30° from the horizontal with the upper end pointing due north. Conduct the photolysis experiments during a warm time of year (i.e., May, June, July, and August in the northern hemisphere--weather permitting) and start the experiments initially before sunrise. Record the date and time the experiment was begun, the date and time completed, the time of sunrise and sunset on all days when photolysis experiments were performed, the times exposure was stopped and restarted for intermittent exposure, the weather conditions during this period, and the latitude of the site. For chemical substances that reversibly ionize or protonate, carry out photolysis experiments at pH 5.0, 7.0, and 9.0 as described under Test Conditions, Section II.A.4.

1. Procedures

Use one of the following procedures, depending on how fast the chemical substance photolyzes.

a. Procedure 1

If the chemical substance degrades 50-80% within 28 days, measure the concentration of the chemical substance, in triplicate, at time $t = 0$ and periodically (at least three data points at approximately equal time intervals) at 12 o'clock noon until at least 50% of the substance has been consumed. Determine the concentration of test chemical from three, freshly opened, reaction vessels for each time point. Determine the concentration in each of the three control solutions as soon as the photolysis experiments are completed.

b. Procedure 2

If the chemical substance degrades in the range of 20-50% in 28 days, determine the concentration of the chemical substance, in triplicate, at time $t = 0$. Determine the concentration of the three separate reaction vessels and the three control vessels after 28 days of photolysis.

c. Procedure 3

For chemical substances that degrade in sunlight 50-80% within two days, place the samples outside before sunrise and analyze triplicate samples of the concentration of the chemical substance at $t = 0$, and in three, freshly opened reaction vessels after sunset the first day, and again, in three, freshly opened, reaction vessels after sunset the second day. Determine the

concentration in each of the three control solutions as soon as the photolysis experiments are completed. Carry out the above experiment on clear sunny days a total of three times.

d. Analytical Methodology

Select an analytical method which is most applicable to the analysis of the specific chemical being tested [Section II.A.5.1].

III. DATA AND REPORTING

A. Treatment of Results

If loss of test substance in the control vessels has occurred, use this data to make corrections to the measured photolysis rate. Note the site of photolysis and its latitude and the weather conditions. For Procedures 1 and 2 note the dates and times of actual exposure including times of sunrise and sunset and, in case the cells are moved to prevent freezing or for other reasons, make sure that these times are recorded and that the cells are kept in a dark place when exposure is not in progress.

- (1) For chemical substances which degrade 50-80% within 28 days, use a concentration C , which corresponds to no more than 50% of the initial concentration of chemical substance remaining, and the corresponding time t , in days, along with the initial molar concentration C_0 , in

equation 3 to calculate k_p in days^{-1} . From the analysis of the three samples at time t , calculate a mean value of C and a value of k_p . Calculate the half-life, t , using the value of k_p in equation 4;

- (2) for chemical substances which degrade 20-50% in 28 days, use the mean concentration C remaining at t 28 days along with C_0 to calculate k_p . Use the same procedure as described above to calculate the value of k_p and t . If less than 20% of the chemical substance degrades in 28 days, report C and C_0 and the mean concentration of C and C_0 . In this case the apparent half-life is greater than 3 months; and
- (3) for chemical substances which degrade 50% or more in the first day, as described in Procedure 3, calculate a full day k_p value using the concentration C of chemical substance remaining after sunset the first day along with C_0 using equation 3. For chemical substances which degrade less than 50% by the end of the first day but 50% or more by the end of the second day, calculate k_p using the mean concentration of chemical substances remaining after sunset the second day. Repeat these calculations for the three separate full-day photolysis experiments. Calculate a mean value of k_p from the results of the three separate experiments. Calculate the half-life, t , using the mean value of k_p in Equation 4. If loss of test substance in the control

vessels has occurred, use this data to make corrections to the measured photolysis rate. Note the dates of photolysis, the latitude, and the site.

3. Specific Analytical and Recovery Procedures

- (1) Provide a detailed description or reference for the analytical procedure used, including the calibration data and precision; and
- (2) if extraction methods were used to separate the solute from the aqueous solution, provide a description of the extraction method as well as the recovery data.

C. Other Test Conditions

- (1) Report the size, shape, approximate cell wall thickness, and type of glass used for the reaction vessels;
- (2) report the initial pH of all test solutions;
- (3) for all Procedures, report the dates of photolysis, the time of sunrise and sunset on each photolysis day, the site of photolysis and its latitude, and the weather conditions. For Procedures 1 and 2 submit the dates and times of actual exposure, and the duration of exposure, and, for intermittent exposure, the fraction of each day photolyzed; and

- (4) if acetonitrile was used to solubilize the test substance, report the percent, by volume, of acetonitrile which was used.

D. Test Data Report

- (1) For each photolysis experiment, report the initial concentration (C_0), and the mean value for test and control solutions.
- (2) After the completion of the photolysis experiments, report the concentration of chemical in each test and control vessel, the time(s) for experiment termination and the mean concentration value
- (3) For Procedures 1 and 2, report the value of k_p and the half-life, t , calculated using the value of k_p .
- (4) For Procedure 3, from the analysis of triplicate samples, report a value of k_p for each series of experiments. Report these data for the three separate full-day photolysis experiments. Report the mean value of k_p from the three separate full-day experiments. Report the half-life, t , calculated from the mean value of k_p .

Summarize all the data in the data sheets listed in Appendix 1.

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Zepp RG, Wolfe NL, Gordon JA, and Baughman GL. 1975. Dynamics of 2,4-D esters in surface water. Hydrolysis, photolysis, and vaporization. Environ Sci Tech 9:1145.

V. APPENDIX 1: DATA FORMAT SHEETSInstructions

1. On the first Test Results page, time in columns 5, 6, 8, and 9 are to be based on a 24-hour clock. Times of sunrise and sunset are to be recorded for all photolysis days. Stop and start times for intermittent exposure are to be recorded only when exposure is not continuous from sunrise to sunset for any day of the experiment (columns 8 and 9). More than one line may be required to record this information if several intermittent exposure periods occur on the same day. Exposure duration (column 10) is the total number of hours and fractions of hours of sunlight exposure of the samples from sunrise to sunset for every day of photolysis. If photolysis is carried out uninterrupted for a complete day, the number of hours in column 10 equals the number of hours in column 7. The fraction of day exposed (column 11) is obtained by dividing the value in column 10 by the value in column 7 for each photolysis day.
2. To report data for procedures 1 or 2, complete the first and second Test Results pages. To report data for procedure 3, complete the first, third, and fourth Test Results pages. For procedure 3, multiple copies of the first Test Results page should be used, one copy for each determination.

3. If multiple pH values are required, complete one set of the appropriate Test Results pages for each pH tested.
4. If multiple test substance initial concentrations are used, complete one set of the appropriate Test Results pages, for each concentration tested.

TEST RESULTS

1. Photolysis site _____ 2. Latitude Degrees N S

3. Determination number (if procedure 3 was used) ☐

EXPOSURE PERIODS

[illegible]

12. Weather conditions:

TEST RESULTS
CONTINUED

PROCEDURE 3

29. pH 30. % Acetonitrile used in solvent system

Determination 1

Elapsed Time	C O N C E N T R A T I O N (moldm ⁻³)											
	Run 1		Run 2		Run 3		Mean		Std. Dev.			
	31.	Exp	32.	Exp	33.	Exp	34.	Exp	35.	Exp		
Initial	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>		
Day 1	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>		
Day 2	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>		

Concentration of controls at the end of experiment:

36. 37. 38. 39. 40.

41. Kp Exp from date on Day 42. Half-life ($t_{1/2}$) days

Determination 2

	C O N C E N T R A T I O N (moldm ⁻³)											
	Run 1		Run 2		Run 3		Mean		Std. Dev.			
	43.	Exp	44.	Exp	45.	Exp	46.	Exp	47.	Exp		
Initial	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>		
Day 1	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>		
Day 2	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>		

Concentration of controls at the end of experiments:

48. 49. 50. 51. 52.

53. Kp Exp from date on Day 54. Half-life ($t_{1/2}$) days

CG-6000

TEST RESULTS
CONTINUED

PROCEDURE 3

Determination 3

	C O N C E N T R A T I O N (moldm ⁻³)									
Eapsed Time	Run 1		Run 2		Run 3		Mean		Std. Dev.	
	55.	Exp	56.	Exp	57.	Exp	58.	Exp	59.	Exp
Initial	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Day 1	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Day 2	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

Concentration of controls at the end of experiments:

60.
 61.
 62.
 63.
 64.

65. Kp Exp from date on Day 66. Half-life (t_{1/2}) days

Procedure 3 summary:

67. Kp Mean Exp days⁻¹

68. Half-life days

CG-6000

CS-6000
August, 1982

PHOTOLYSIS IN AQUEOUS SOLUTION IN SUNLIGHT

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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PHOTOLYSIS IN AQUEOUS SOLUTION IN SUNLIGHTI. NEED FOR THE TEST

The majority of the earth's surface is covered by water in the form of oceans, seas, rivers, lakes, streams, ponds, etc. As a result, chemicals are likely to enter aqueous media and can then undergo transformation via direct aqueous photolysis.

Direct aqueous photolysis represents the transformation of a chemical substance by direct absorption of radiant energy (sunlight) into new chemicals different from their precursors. Chemical substances which are present in aqueous media photolyze at different rates depending upon the solar irradiance and the chemical substance's molar extinction coefficient at each wavelength of solar radiation and its photolysis quantum yield at the wavelengths of concern. Chemical substances which photolyze rapidly under environmental conditions have relatively short lifetimes in the environment. Consequently, the Agency's assessment may focus on the degradation products to a greater extent than on the parent compound. On the other hand, if the chemical substance is resistant to photolysis as well as to all the other possible transformation processes, the assessment should focus on the parent chemical.

A cost-effective aqueous photolysis test is needed to assess quantitatively the transformation of chemical substances in sunlight. The importance of direct photolysis in sunlight as a transformation process of chemical substances in aqueous media in the environment can be determined quantitatively from data on photolysis rate constants and half-lives.

The photolysis in aqueous solution test represents a screening test to allow one to determine how rapidly photolysis will take place in aqueous media under certain environmental conditions. If the photolysis test data indicate that photolysis is a relatively important transformation process and the initial assessment indicates that there is a threat to the health of humans and/or to the environment, then detailed tests may be required to obtain more precise aqueous photolysis data over a wide range of environmental conditions. These more detailed tests will also be concerned with determining the identity and fate of the transformation products.

II. SCIENTIFIC ASPECTS

A. Rationale for the Selection of the Test Method

1. Historical Discussion

The scientific literature contains a number of publications dealing with the photolysis in solution of various chemical substances. Unfortunately, for one or more reasons, most of the data contained in the literature is of little or no use to EPA in determining aqueous photolysis rate constants and half-lives. Reasons for this include: (1) Many of the publications deal primarily with the products which form from direct photolysis reactions and the detailed mechanisms involved rather than photolysis rates. (2) The publications give no quantitative data on the rates of photolysis under environmental conditions, i.e.,

many researchers used sources of light which do not simulate sunlight or performed experiments in solvents other than air-saturated water. (3) Some studies are not based upon the fundamental laws of photochemistry. (4) Many publications report the effects of certain sensitizers which are environmentally unimportant, or the effect of other chemicals present in solution, on the chemical substance being studied. (5) Many of the studies suffer from poor experimental design, overlooking the controls necessary to make sure that photolysis and not biodegradation, volatility, or other competing processes are taking place to remove or transform the chemical substance. The following paragraphs cite a few representative examples which illustrate the points mentioned above, as well as the publication which forms the basis for this Test Guideline.

A paper by Grunwell and Erickson (1973) deals with the photochemistry of parathion. This report is of little relevance in the evaluation of photolysis rates of chemical substances for several reasons. The main emphasis of this work is in the identification of major products formed by the photolysis of parathion rather than in the measurement of rate constants and half-lives. Photolysis was performed at three wavelengths (254, 300, and 350 nm), of which only the last two are environmentally relevant. The chemical was dissolved in solutions of 20 percent water and either 80 percent ethanol or tetrahydrofuran (by weight), neither of which is environmentally relevant.

A paper by Langford et al. (1973) claims environmental relevance by working under environmental conditions. Radiation of 350 nm as well as sunlight was used. Pure water and river water were used as solvents. The authors presented no quantitative data on the rate of photolysis of nitrilotriacetic acid, the chemical they studied. Detailed quantitative measurement of the photolysis rate constant and half-life, along with adequate controls, would be necessary to make this research useful for the purposes of this Test Guideline.

Benson et al. (1971) photolyzed chlordane with both mercury arc radiation and sunlight. Acetone was used as both a solvent and photosensitizer. Sunlight photolysis was done only on the pure compound applied as a thin layer under quartz glass. The main emphasis of this work was to look at the chemical structure of the reaction products. Considering that rate constants were not measured, acetone was used as both a solvent and sensitizer, and a mercury arc lamp was used as radiation source, this publication has minimal applicability as a test method to screen for photolysis rates.

Su and Zabik (1972) studied the photochemistry of arylamidine derivatives in distilled natural water. A high pressure mercury arc, filtered to remove radiation below 286 nm was used. Products of the reaction were determined but no kinetic studies were performed and no rate constants or half-lives were reported.

Mancini (1978) presented a theoretical framework for the first order photodecomposition of picloram in aqueous solution and tested the framework using some experimental data on the photolysis of picloram in sunlight. In reality, the Mancini framework is only an empirical approach describing the rate of photolysis of picloram in sunlight.

Zepp and Cline (1977) published a paper on direct photolysis in aqueous environments with equations for the determinations of direct photolysis rates in sunlight. This paper avoids the problems illustrated above and serves as a basis for the proposed photolysis in aqueous solution in sunlight test. These equations translate readily obtained laboratory data into rate constants and half-lives for sunlight photolysis. Photolysis half-lives can be calculated as a function of season, latitude, time of day, depth in water bodies, and thickness of the atmospheric ozone layer. Several published papers concerning the photolysis of chemicals in sunlight have verified this method. (Wolfe et al. 1976, Smith et al. 1977, 1978, Zepp et al. 1975, 1976).

2. Selection of the Test Method

The method in this Test Guideline was developed from a thorough review of the research literature on the experimental determination of aqueous photolysis rate constants and by talking to researchers who have considerable experience in carrying out these experiments. In the development of this Test Guideline on aqueous photolysis, the principles outlined by Zepp and Cline (1977) have been taken into account.

The proposed test method for the measurement of direct solar photolysis of a chemical substance in aqueous solution is based upon four fundamental criteria. These criteria are: (1) The test method should be based upon the fundamentals of photochemistry. (2) The test method should yield quantitative data on direct photolysis rates of chemical substances in aqueous media. (3) Sunlight should be used as the irradiation source because of its obvious relevance as well as its low cost in comparison to artificial light sources (Zepp 1980). (4) The test method should be designed to account for degradation or chemical losses by mechanisms other than photolysis. For example, the experiments should be designed to account for or minimize hydrolysis, biodegradation, and volatilization as factors in the estimation of test substance losses. The proposed method has a limitation since it does not measure sunlight intensity on the sample during photolysis. A careful study has been made on the use of insolation methods for measuring solar irradiance [e.g., radiometry, photometry, and actinometry (Mill et al. 1981)]. The most suitable method for measuring sunlight intensity was found to be actinometry. This method has the advantage that it conforms to the geometry of the reaction cell and measures the actinic flux directly, under known sensitivity conditions. When reference compounds (i.e., sunlight actinometers) are developed, the proposed test will be modified to use simultaneous photolysis of a chemical substance and an actinometer to evaluate sunlight intensities on the sample. The modified procedure will quantify

sunlight photolysis of a chemical substance at a specific time of year and latitude and will give a useful measure of seasonal variation of photolysis.

This photolysis in aqueous solution Test Guideline allows one to determine how rapidly a chemical will photolyze in sunlight. Future Test Guidelines need to be developed to obtain detailed data on the direct photolysis of a chemical under a wide variety of environmental conditions. These detailed tests will provide improved translation of laboratory data into rate constants and half-lives for photolysis in sunlight as a function of season, latitude, time of day, depth in water bodies, effect of other dissolved materials (e.g., clays or humic acids) or suspended solids (e.g., sediments) in water, and the thickness of the ozone layer.

3. Theoretical Aspects of the Selected Test Method

The theory of the method of Zepp and Cline (1977) is briefly discussed to show that the proposed test method is based upon the fundamental criteria given in Section II.A.2. These discussions lay the foundation for the proposed method, show how the method can be used to obtain direct sunlight photolysis rate constants and half-lives, and indicate what test conditions must be standardized in order to obtain meaningful aqueous photolysis rate data.

For the direct photolysis of a chemical substance, the rate of decrease of the concentration of the chemical with time is given by

$$-\frac{dC}{dt} = \phi I_a \quad , \quad (1)$$

where C is the molar concentration of the chemical substance, t is time, ϕ is the photolysis quantum yield of the chemical substance, and I_a is the absorbed radiation intensity. For the direct sunlight photolysis of a chemical substance in dilute aqueous solution (an absorbance of less than 0.10 units)^a in pure water at shallow depth (less than 0.5 meter), the kinetic expression for direct photolysis is

$$-\frac{dC}{dt} = \phi k_a C = k_p C \quad , \quad (2)$$

where k_a equals $\sum k_{a\lambda}$, the sum of the $k_{a\lambda}$ values for all wavelengths of sunlight that are absorbed by the chemical substance, and k_p represents the photolysis rate constant in sunlight (summed over all wavelengths of sunlight) in units of reciprocal time. The expression in equation (2) is a first order rate equation. Integration of equation (2) yields

^a Zepp and Cline showed that the kinetics are first order under the conditions of absorbance less than 0.02. Only a small error (approximately 3%) is introduced in the proposed test method assuming an absorbance of 0.1 and first order kinetics is still applicable.

$$\log_{10} \frac{C_0}{C} = \frac{k_p t}{2.30} \quad (3)$$

where C is the molar concentration of chemical at time t during photolysis and C₀ is the initial molar concentration. It then follows that the half-life for a first order equation is

$$t = \frac{0.693}{k_p} \quad (4)$$

Thus, by measuring the initial molar concentration of a chemical substance and measuring C as a function of the time, t, it is possible to calculate both the sunlight photolysis rate, k_p, and the half-life, t, for that chemical substance.

B. Rationale for the Selection of the Test Conditions

1. Special Laboratory Equipment

- (1) A variable wavelength uv-visible absorption spectrophotometer. This instrument, which must be capable of measuring accurate absorbances to 0.10 or less, is necessary for use in the aqueous photolysis screening test to measure an accurate uv-visible absorption spectrum of each chemical substance. There are two reasons why accurate absorption spectra are necessary. These are: one; the theory upon which this Test Guideline is based, as discussed in detail in Section II.A.3., is only applicable at low

absorbances. Therefore, a complete uv-visible absorption spectrum of the aqueous solution of each chemical substance, at the concentration at which it is being tested, is required to make sure that these conditions are met so that the photolysis experiments are valid and two; the determination of which chemical substances must be tested in the aqueous photolysis screening test is based upon information obtained from the chemical substance's uv-visible absorption spectrum in aqueous solution. Test chemical substances which have absorption maxima at wavelengths of 290 nm or greater, as determined from the chemical substance's uv-visible absorption spectrum, are applicable. The justification for the use of a chemical substance's absorption spectrum as a means of determining whether a chemical substance should be tested for aqueous photolysis is discussed in Section II.B.5.

- (2) In this test method, special reaction vessels are necessary to contain the reaction solutions during photolysis. Reaction vessels of 11 mm inside diameter are recommended as they are inexpensive and easily obtained in the form of culture tubes. For some chemical substances it may be difficult to analyze the concentration of the chemical substance in the small volume present in 11 mm i.d. reaction vessels. For such chemical substances the use of larger reaction vessels is permissible as long as the pathlength is

less than 0.5 meter (see Section II.A.3.). Reaction vessels of either quartz or thin walled borosilicate glass may be used. The absorption spectrum of the chemical in aqueous solution as determined by OECD Guideline No. 101 (OECD 1981), can be used to determine the type of reaction vessel to be employed for these photolysis experiments.

All reaction vessels must be capable of being sealed (without the use of grease) and must be filled as completely as possible to prevent volatilization or other losses of the test substance or water (see Section II.B.9.). Grease must be avoided because it might absorb or react with the substance being tested. Caps lined with teflon inserts must be used to avoid adsorption of hydrophobic chemicals.

2. Purity of Water

Pure water is used because dissolved impurities could sensitize or otherwise affect the rate of photolysis. In addition, the water needs to be sterile because bacteria may consume or alter the chemical substance during the prolonged periods of testing which may occur in the course of a rate determination. Thus, pure water [e.g., ASTM Type II A (ASTM 1979)], is recommended in this Test Guideline. Furthermore, it is important that the water be saturated with air prior to

preparation of the test and control solutions to simulate environmental conditions. It is important that this air be filtered through a 0.2 μm (pore size) filter to remove bacteria.

3. Sterilization

Sterilization is necessary to kill all bacteria and therefore limit or reduce biodegradation which could interfere with the photolysis rate determination. The presence of bacteria in either the test solutions or controls may cause biodegradation of the test substance. This may make concentration determinations difficult and less accurate, calculations difficult, and in general increase sources of error in the experiment. Thus, it is extremely important to use aseptic conditions in carrying out all photolysis experiments to minimize biodegradation.

4. Concentration of Solution

Solutions of chemical substances used in this Test Guideline must be prepared at low concentrations in order to both approximate environmental conditions and to allow first-order kinetics assumptions to apply (see Section II.A.3.).

If the chemical substance is too difficult to dissolve in pure form to permit reasonable handling and analytical procedures, then test solutions can be prepared more easily from a chemical dissolved in reagent grade acetonitrile. The final acetonitrile concentration in the test solution should be no more

than one volume percent in order to avoid acetonitrile solvent effects (Smith et al. 1977, 1978). Acetonitrile was chosen as a solvent as it is soluble in water, is non-polar and thus effective in dissolving many substances which are insoluble in water, it does not absorb radiation over the wavelength range of 290 to 800nm, and it causes minimal solvent effects or shifts in absorbance wavelength for test substances.

5. Absorption Spectrum

The absorption spectrum of the chemical substance is used as a criterion for determining the necessity of performing this aqueous photolysis test. Solar radiation reaching the earth's surface has a sharp cutoff at a wavelength of approximately 290 nm (Leighton 1961, Zepp and Cline 1977). Photolysis does not occur unless there is absorption of radiant energy. Thus, if an aqueous solution of a chemical substance does not absorb light at a wavelength of 290 nm or greater, it will not undergo direct photolysis under natural conditions. The uv-visible absorption spectrum of a chemical substance in aqueous solution will give a good indication of whether it would be useful to carry out this aqueous photolysis test.

If the absorption spectrum of a chemical substance, as determined in OECD Test Guideline No. 101 (OECD, 1981), exhibits an absorption maximum at a wavelength of 290 nm or greater, the chemical substance may undergo direct photolysis in sunlight. Thus, this Test Guideline is applicable to all chemicals which

have an absorption maximum at a wavelength of 290 nm or greater. If the chemical substance absorbs radiation only at wavelengths appreciably below 290 nm, then it cannot undergo direct photolysis in sunlight and therefore need not be tested. A few examples of classes of chemicals that do not need to be tested in this Test Guideline are alkanes, alkenes, and alkynes because they only absorb uv radiation substantially below 290 nm. It is possible that some chemicals will absorb radiation mainly at wavelengths below 290 nm but may have an absorption tail that extends above 290 nm. Photolysis experiments should be carried out for these chemicals.

6. pH Effects

The molecular structure of a chemical substance which ionizes or protonates is a function of the pH. As a result, the absorption spectrum and consequently the rate of photolysis may change with pH. In general, the pH range of environmental concern is from 5 to 9; hence, for chemical substances that reversibly ionize or protonate (e.g., carboxylic acids, phenols, and amines), the aqueous photolysis test should be carried out at pHs of 5.0, 7.0, and 9.0. Since buffers could influence the rate of photolysis, the recommended buffers for use in this Test Guideline were carefully chosen to be transparent to radiation between 290 and 800 nm and are kept at very low concentrations to avoid buffer effects which may cause transformation of the substance by, for example, catalysis.

7. Outdoor Experimental Conditions

It is important that the photolysis reaction vessels containing the chemical substance be placed in an area free from shade and reflections and on a black, non-reflecting, background to insure that they receive direct and sky radiation from the sun. The reaction vessels should be tilted at 30° from horizontal with the upper end pointing due north so that they present a large surface area and minimum pathlength to the sun and create minimal internal reflections.

It is recommended that the photolysis experiments be carried out during the warm time of the year (i.e., May, June, July, and August in the northern hemisphere--temperature permitting). This period of time was chosen because the solar intensity is a maximum and consequently the rate of photolysis will be a maximum. Thus, the kinetics of photolysis will be easier to follow, especially for chemicals that photolyze slowly. Furthermore, in many parts of the United States, the temperature falls below 0° C during the winter months. Therefore, if the photolysis experiments are carried out in the winter, the dilute aqueous reaction solution would freeze, the tubes would break, and the samples would be destroyed. It may be possible to avoid this problem by placing the reaction tubes in a housing transparent to the appropriate wavelengths of radiation and thermostated to control the temperature at $25 \pm 5^\circ \text{C}$. Thus, with this device it may be possible to carry out photolysis experiments at any time of the year.

8. Chemical Analysis of Solutions

The analytical techniques employed in the determination of the concentration of the test substances are left to selection by the sponsor. This is in recognition of the many different techniques available and the practical advantage of being able to make particular use of one of the properties of the substances; e.g., the NMR or UV spectrum of the substance, or its chromatographic behavior. Analytical techniques that permit the determination of the test compound to the exclusion of impurities or photolysis reaction products are recommended to the extent practicable. Therefore, chromatographic techniques are particularly desirable. Whenever practicable, an analytical procedure should be used which has a precision of $\pm 5\%$. The specific technique which is utilized should be adequately described.

9. Precautions for Losses Due to Processes Other Than Photolysis

Undetected loss of a test substance through volatilization, hydrolysis, or other processes during the course of the photolysis experiment will result in the determination of excessively large rate constants for aqueous photolysis. Therefore, for volatile chemical substances, it is important that the reaction vessels and control vessels be filled as completely as possible and sealed in order to avoid evaporative losses. To correct for possible losses, control solutions of test substance,

in darkened vessels, are placed side by side with the photolysis vessels and the contents of the control vessels are analyzed at the end of the experiment. In this way the loss rate for processes other than photolysis may be determined and subtracted from the overall rate of disappearance of the chemical substance to give the corrected direct photolysis rate.

C. Test Data Required

This Test Guideline is designed to obtain direct photolysis rate constants and half-lives (of less than one day to 3 months) for chemical substances in aqueous solution. These data will be used to assist in the determination of the environmental fate of the chemical substance. It is important for each photolysis experiment to keep a complete record of the time the vessels are exposed to solar radiation including the times of sunrise and sunset. For each experiment, the initial concentrations (C_0) of three test solutions and the mean value of the initial concentrations are required. During the course of the experiment, the concentration of chemical substance in each test vessel must be reported at each time that it is measured. After completion of each photolysis experiment the concentrations of chemical substance in each test vessel and control and the mean values of the concentrations of both test and control solutions must be reported. These data are needed to calculate appropriate photolysis rate data and to make sure that the test substance is not lost by other processes (e.g., biodegradation or volatility),

or, if lost, is accounted for and that only the rate of photolysis, k_p , is determined. Using the value of k_p , the half-life ($t_{1/2}$) can be calculated and reported.

The rate constant and half-life determined in this Test Guideline are relevant to the day midway between the beginning and the end of the experiment and thus represent an "average" value for the test chemical substance during a certain period of time. Therefore, all photolysis experiments should include the duration of exposure. Since the rate of photolysis can vary with a number of conditions, the latitude, dates of exposure, weather conditions, and pH for all test and standard solutions, it is important that these data be reported.

D. Statistical Analysis of Data

Several groups of researchers have published experimental data on the determination of direct aqueous photolysis rate constants and half-lives of chemical substances using solar radiation (Smith et al. 1978, Wolfe et al. 1976, Zepp et al. 1975, 1976, 1977). However, the precision in measuring the sunlight rate constant has not been clearly established.

In general, when measuring direct photolysis rate constants or half-lives by this Test Guideline, there are many factors which will influence the values obtained. For the purposes of this Test Guideline it is impossible to accurately evaluate the effects of these factors on the rate constant data obtained. Solar intensity may vary due to ozone layer thickness,

meteorological conditions, time of day and year, latitude, etc. Therefore, no reliable precision can be stated at this time for the determining the sunlight photolysis rate constant. For the purpose of this Test Guideline and to minimize costs, the test procedure is limited to the determination of triplicate samples and a statistical analysis of the data.

As stated above, the variability in the rate data is a function of the variability in the solar intensity. As sunlight actinometers are developed to quantify solar intensities, the test method will be modified to use simultaneous photolysis of a chemical substance and an actinometer to evaluate sunlight intensity on the sample.

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