(ii) Summary data for each group shall include:

(A) The number of animals at the start of the test.

(B) Body weights of the dams during gestation and lactation.

(C) Litter size and mean weight at birth.

(D) The number of animals showing each observation score at each observation time.

(E) The percentage of animals showing each abnormal sign at each observation time.

(F) The mean and standard deviation for each continuous end point at each observation time. These will include body weight, motor activity counts, acoustic startle responses, performance in active avoidance tests, and brain weights (both absolute and relative).

(G) The number of animals in which any lesion was found.

(H) The number of animals affected by each different type of lesion, the average grade of each type of lesion, and the frequency of each different type and/or location of lesions.

(3) *Evaluation of data.* An evaluation of the test results shall be made. The evaluation shall include the relationship between the doses of the test substance and the presence or absence, incidence, and severity of any neurotoxic effect. The evaluation shall include appropriate statistical analyses. The choice of analyses shall consider tests appropriate to the experimental design and needed adjustments for multiple comparisons.

(e) *References.* For additional background information on this test guideline, the following references should be consulted:

(1) Adams, J., Buelke-Sam, J., Kimmel, C.A., Nelson, C.J., Reiter, L.W., Sobotka, T.J., Tilson, H.A., and Nelson, B.K. ''Collaborative behavioral teratology study: Protocol design and testing procedure.'' *Neurobehavioral Toxicology and Teratology.* 7: 579–586. (1985).

(2) Brush, F.R. ''The effects of intertrial interval on avoidance learning in the rat.'' *Journal of Comparative Physiology and Psychology.* 55: 888–892. (1962).

(3) Brush, F.R. ''Retention of aversively motivated behavior.'' In: ''Adverse Conditioning and Learning.''

Brush, F.R., ed., New York: Academic Press. (1971).

(4) Brush, F.R. and Knaff, P.R. ''A device for detecting and controlling automatic programming of avoidance-conditioning in a shuttle-box.'' *American Journal of Psychology.* 72: 275–278 (1959).

(5) Dixon, W.J. and Massey, E.J. ''Introduction to Statistical Analysis.'' 2nd ed. New York: McGraw-Hill. (1957).

(6) Glowinski, J. and Iversen, L.L. ''Regional studies of catecholamines in the rat brain-I.'' *Journal of Neurochemistry.* 13: 655–669. (1966).

(7) Ison, J.R. ''Reflex modification as an objective test for sensory processing following toxicant exposure.'' *Neurobehavioral Toxicology and Teratology.* 6: 437–445. (1984).

(8) Jensen, D.R. ''Some simultaneous multivariate procedures using Hotelling's T2 Statistics.'' *Biometrics.* 28: 39–53. (1972).

(9) McAllister, W.R. and McAllister, D.E. ''Behavioral measurement of conditioned fear.'' In: ''Adverse Conditioning and Learning.'' Brush, F.R., ed., New York: Academic Press (1971).

(10) Neter, J. and Wasserman, W. ''Applied Linear Statistical Models.'' Homewood: Richard D. Irwin, Inc. (1974).

(11) Sokal, R.P. and Rohlf, E.J. ''Biometry.'' San Francisco: W.H. Freeman and Co. (1969).

(12) Spencer, P.S., Bischoff, M.C., and Schaumburg, H.H., ''Neuropathological methods for the detection of neurotoxic disease.'' In: ''Experimental and Clinical Neurotoxicology.'' Spencer, P.S. and Schaumburg, H.H., eds., Baltimore, MD: Williams & Wilkins, pp. 743–757. (1980).

[53 FR 5957, Feb. 26, 1988]

PART 796—CHEMICAL FATE TESTING GUIDELINES

Subpart A [Reserved]

Subpart B—Physical and Chemical Properties

79

Subpart C—Transport Processes

Subpart D—Transformation Processes

AUTHORITY: 15 U.S.C. 2603.

Subpart A [Reserved]

Subpart B—Physical and Chemical Properties

§ 796.1050 Absorption in aqueous solution: Ultraviolet/visible spectra.

(a) *Introductory information*—(1) *Guidance information*. (i) Molecular formula.

(ii) Structural formula.

(2) *Standard documents*. The spectrophotometric method is based on national standards and consensus methods which are applied to measure the absorption spectra.

(b) *Method*—(1)(i) *Introduction, purpose, scope, relevance, application and limits of test*. (A) The primary environmental purpose in determining the ultraviolet-visible (UV-VIS) absorption spectrum of a chemical compound is to have some indication of the wavelengths at which the compounds may be susceptible to photochemical degradation. Since photochemical degradation is likely to occur in both the atmosphere and the aquatic environment, spectra appropriate to these media will be informative concerning the need for further persistence testing.

(B) Degradation will depend upon the total energy absorbed in specific wavelength regions. Such energy absorption is characterized by both molar absorption coefficient (molar extinction coefficient) and band width. However, the absence of measurable absorption does not preclude the possibility of photodegradation.

(ii) *Definitions and units*. The *UV-VIS absorption spectrum* of a solution is a function of the concentration, c_1, expressed in mol/L, of all absorbing species present; the path length, d, of the spectrophotometer cell, expressed in cm; and the molar absorption (extinction) coefficient, ε_i, of each species. The absorbance (optical density) A of the solution is then given by:

$$A = d\sum_{i}\varepsilon_{ici}$$

For a resolvable absorbance peak, the band width λ is the wavelength range, expressed in nm = 10^{-9} m, of the peak at half the absorbance maximum.

(iii) *Reference substances*. (A) The reference substances need not be employed in all cases when investigating a new substance. They are provided primarily so that calibration of the method may be performed from time to time and to offer the chance to compare the results when another method is applied.

(B) Reference compounds appropriate for the calibration of the system are:

(*1*) Potassium dichromate (in 0.005 mol/L, H_2SO_4 solution) from J.A.A. Ketelaar, paragraph (d)(2) of this section:

log ε	3.56	3.63	3.16	3.50
λ in nm	235	257	313	350

(*2*) Fluoranthene (in methanol) from *C.R.C. Atlas of Spectral Data*, paragraph (d)(3) of this section:

log ε	4.75	4.18	4.73	3.91	3.92
λ in nm	237	236	288	339	357

(*3*) 4-nitrophenol (in methanol) from *C.R.C. Atlas of Spectral Data*, paragraph (d)(3) of this section:

log ε	3.88	4.04
λ in nm	288	311

See also paragraph (d)(1) of this section.

(iv) *Principle of the test method*. This method utilizes a double-beam spectrophotometer which records only the absorption differences between the blank and test solutions to give the spectrum of the chemical being tested.

(v) *Quality criteria—Reproducibility and sensitivity*. (A) Reproducibility and sensitivity, need not be measured directly. Instead, the accuracy of the system in measuring the spectra of reference compounds will be defined so as to assure appropriate reproducibility and sensitivity. It is preferable to use a recording double-beam spectrophotometer to obtain the UV-VIS spectrum of the test compound. Such an instrument should have a photometric accuracy of ±0.02 units over the absorbance

range of 0 to 2 units. It should be capable of recording absorbances at wavelengths of 200 to 750 nanometers nm with a wavelength accuracy of ±0.5 nm. The cells employed with the instrument must necessarily be transparent over this wavelength range and must have a path length determined to within 1 percent. To ensure that the instrument is performing satisfactorily, spectra for test solutions of $K_2Cr_2O_7$ (for absorbance accuracy) and holmium glass (for wavelength accuracy) should be run periodically.

(B) In the event that a recording double-beam instrument is not available, it will be necessary to determine the absorbance of the test solution in a single-beam instrument at 5-nm intervals over the entire wavelength range and at 1-nm intervals where there are indicated absorbance maxima. Wavelength and absorbance tests should be done as with the double-beam instrument.

(2) *Description of the test procedure*—(i) *Preparation*—(A) *Preparation of test solutions.* (*1*) Solutions should be prepared by accurately weighing an appropriate amount of the purest form of the test substance available. This should be made up in a concentration which will result in at least one absorbance maximum in the range 0.5 to 1.5 units.

(*2*) The absorption of a compound is due to its particular chemical form. It is often the case that different forms are present, depending on whether the medium is acidic, basic, or neutral. Consequently, spectra under all three conditions are required where solubility and concentration allow. Where it is not possible to obtain sufficient concentrations in any of the aqueous media, a suitable organic solvent should be used (methanol preferred).

(*3*) The acid medium should have a pH of less than 2, and the basic medium should be at least pH 10. The solvent for the neutral solution, and for preparing the acidic and basic ones, should be distilled water, transparent to ultraviolet radiation down to 200 nm. If methanol must be used, acidic and basic solutions can be prepared by adding 10 percent by volume of HCl or NaOH in aqueous solution ([HCl], [NaOH] = 1 mol/L).

(*4*) In theory, all chemical species other than that being tested are present in both beams and would therefore not appear in the recorded spectrum of a double-beam instrument. In practice, because the solvent is usually present in great excess, there is a threshold value of wavelength below which it is not possible to record the spectrum of the test chemical. Such a wavelength will be a property of the solvent or of the test medium. In general, distilled water is useful from 200 nm (dissolved ions will often increase this), methanol from 210 nm, hexane from 210 nm, acetonitrile from 215 nm and dichloromethane from 235 nm.

(B) *Blank solutions.* A blank must be prepared which contains the solvent and all chemical species other than the test chemical. The absorption spectrum of this solution should be recorded in a manner identical to that of the test solution and preferably on the same chart. This ''baseline'' spectrum should never record an absorbance reading varying more than ±0.05 from the nominal zero value.

(C) *Cells.* Cell pathlengths are usually between 0.1 cm and 10 cm. Cell lengths should be selected to permit recording of at least one maximum in the absorbance range of 0.5 to 1.5 units. Which set of cells should be used will be governed by the concentration and the absorbance of the test solution as indicated by the Beer-Lambert Law. The cells should be transparent over the range of the spectrum being recorded, and the path-lengths should be known to an accuracy of at least 1 per cent. Cells should be thoroughly cleaned in an appropriate manner (chromic acid is useful for quartz cells) and rinsed several times with the test or blank solutions.

(ii) *Performance of the test.* Both cells to be employed should be rinsed with the blank solution and then filled with same. The instrument should be set to scan at a rate appropriate for the required wavelength resolution and the spectrum of the blank recorded. The sample cell should then be rinsed and filled with the test solution and the scanning repeated, preferably on the same spectrum chart, to display the baseline. The test should be carried out at 25 °C.

(c) *Data and reporting*—(1) *Treatment of results.* (i) The molar absorption coefficient ε should be calculated for all

absorbance maxima of the test substance. The formula for this calculation is:

$$\varepsilon = \frac{A}{c_{,i} \times d},$$

where the quantities are as defined above (see Definitions and units).

(ii) For each peak which is capable of being resolved, either as recorded or by extrapolated symmetrical peaks, the bandwidth should be recorded.

(2) *Test report.* (i) The report should contain a copy of each of the three spectra (3 pH conditions). If neither water nor methanol solutions are feasible, there will be only one spectrum. Spectra should include a readable wave-length scale. Each spectrum should be clearly marked with the test conditions.

(ii) For each maximum in each spectrum, the ε value and bandwidth (when applicable) should be calculated and reported, along with the wavelength of the maximum. This should be presented in tabular form.

(iii) The various test conditions should be included, such as scan speed, the name and model of the spectrophotom-eter, the slit width (where available), cell type and path length, the concentrations of the test substance, and the nature and acidity of the solvent medium. A recent test spectrum on appropriate reference materials for photometric and wavelength accuracy should also be submitted (see Reproducibility and sensitivity).

(d) *Literature references.* For additional background information on this test guideline, the following references should be consulted:

(1) Milazzo, G., Caroli, S., Palumbo-Doretti, M., Violante, N., *Analytical Chemistry,* 49: 711 (1977).

(2) Katelaar, J.A.A., *Photoelectric Spectrometry Group Bulletin,* 8, (Cambridge, 1955).

(3) Chemical Rubber Company, *Atlas of Spectral Data,* (Cliffland, Ohio).

[50 FR 39472, Sept. 27, 1985]

§ 796.1950 Vapor pressure.

(a) *Introduction*—(1) *Background and purpose.* (i) 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.

(ii) 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.

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

(2) *Definitions and units.* (i) "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.

(ii) "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.

(iii) The "torr" is a unit of pressure which equals 133.3 pascals or 1 mm Hg at 0 °C.

(iv) "Vapor pressure" is the pressure at which a liquid or solid is in equilibrium with its vapor at a given temperature.

(v) "Volatilization" is the loss of a substance to the air from a surface or from solution by evaporation.

(3) *Principle of the test methods.* (i) 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.

(ii) 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) under paragraph (d)(2) of this section. 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

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

where R is the gas constant (8.31 Pa m^2 mol^{-1} K^{-1}) when the pressure is in pascals (Pa) and the volume is in cubic meters. As noted by Spencer and Cliath (1970) under paragraph (d)(3) of this section, direct vapor pressure measurements by gas saturation techniques are more directly related to the volatilization of chemicals than are other techniques.

(iii) In an effort to improve upon the procedure described by Spencer and Cliath (1969) under paragraph (d)(2) of this section, 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) under paragraph (d)(1) of this section. 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.

(4) *Applicability and specificity.* (i) 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 section, 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.

(ii) 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.

(iii) 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.

(b) *Test procedures*—(1) *Test conditions.* (i) The apparatus in the isoteniscope method is described in paragraph (b)(2)(i) of this section.

(ii) The apparatus used in the gas saturation method is described in paragraph (b)(2)(ii) of this section.

(2) *Performance of the tests*—(i) *Isoteniscope Procedure.* The isoteniscope procedure described as ANSI/ASTM Method D 2879–86 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). ASTM D 2879–86 is available for inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.* This incorporation by reference was approved by the Director of the Office of the Federal Register. This material is incorporated as it exists on the date of approval and a notice of any change in this material will be published in the FEDERAL REGISTER. Copies of the incorporated material may be obtained from the Director, Environmental Assistance Division (7408), Office of Pollution Prevention and Toxics, Environmental Protection Agency, Room E–543B, 1200 Pennsylvania Ave. NW., Washington, DC 20460–0001, or from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103. 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 ±0.5 °C and at any other suitable temperatures (±0.5°). 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.

(ii) *Gas saturation procedure.* (A) The test procedures require the use of a constant-temperature box as depicted in the following Figure 1.
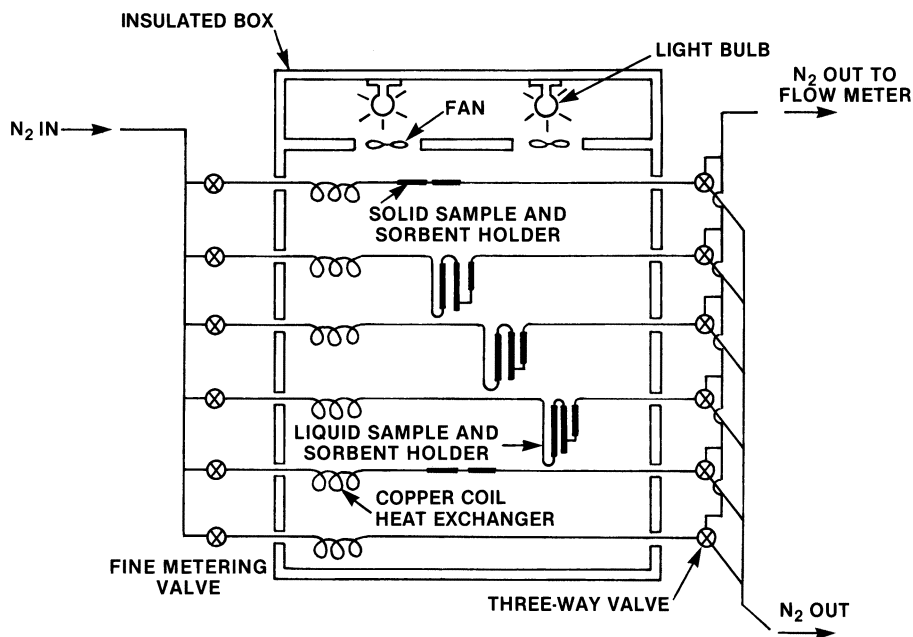
FIGURE 1—SCHEMATIC DIAGRAM OF VAPOR SATURATION APPARATUS

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 ±0.5° 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 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) × 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

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

In this equation, R is the gas constant (8.31 Pa $m^3 mol^{-1}$ 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 (°C).

(B) Solid samples are loaded into 5 mm i.d. glass tubing between glass wool plugs. The following Figure 2 depicts a drawing of a sample holder and absorber system.
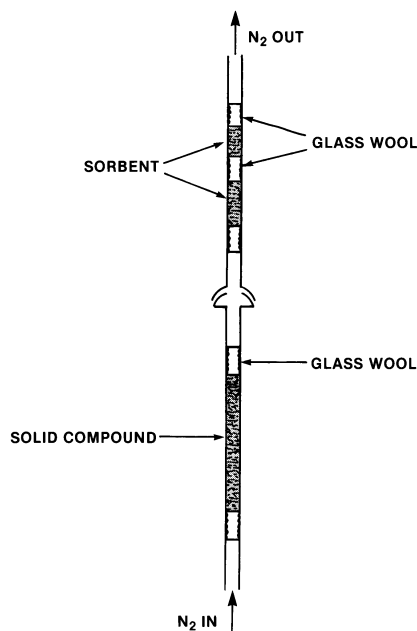
85

FIGURE 2—SOLID COMPOUND SAMPLING
SYSTEM

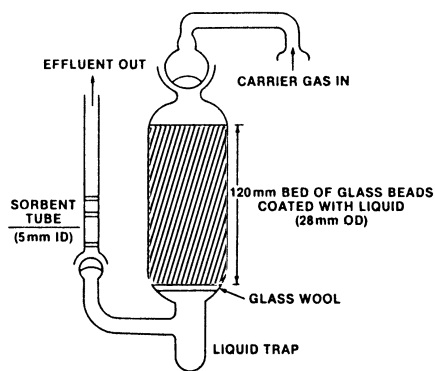(C) Liquid samples are contained in a holder as shown in the following Figure 3.

FIGURE 3—LIQUID COMPOUND SAMPLING
SYSTEM

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.

(D) 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.

(E) 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 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.

(F) 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.

(G) 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.

(c) *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.

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) U.S. Environmental Protection Agency. *Evaluation of Gas Saturation Methods to Measure Vapor Pressures: Final Report,* EPA Contract No. 68–01–5117 with SRI International, Menlo Park, California (1982).

(2) Spencer, W.F. and Cliath, M.M. ''Vapor Density of Dieldrin,'' *Journal of Agricultural and Food Chemistry*, 3:664–670 (1969).

(3) Spencer, W.F. and Cliath, M.M. ''Vapor Density and Apparent Vapor Pressure of Lindane,'' *Journal of Agricultural and Food Chemistry*, 18:529–530 (1970).

[50 FR 39252, Sept. 27, 1985, as amended at 53 FR 12525, Apr. 15, 1988; 53 FR 21641, June 9, 1988; 60 FR 34466, July 3, 1995; 69 FR 18803, Apr. 9, 2004; 77 FR 46293, Aug. 3, 2012]

Subpart C—Transport Processes

§ 796.2750 Sediment and soil adsorption isotherm.

(a) *Introduction*—(1) *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 section describes procedures that will enable sponsors to determine the adsorption isotherm of a chemical on sediments and soils.

(2) *Definitions and units.* (i) 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.

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

(iii) ''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.

(iv) ''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:

Name	Diameter range
Very coarse sand	2.0 to 1.0 mm

Name	Diameter range
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

(v) 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.

(vi) 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.

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

(viii) "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.

(ix) "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.

(x) "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.

(xi) 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.

(xii) "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.

(xiii) "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).

(xiv) "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.

(3) *Principle of the test method.* (i) 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:

Equation 1

$$x/m = C_s = KC_e^{1/n}$$

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.

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

Equation 2

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

(ii) 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.

(iii) 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.

(iv) The papers by Aharonson and Kafkafi (1975) under paragraph (d)(1) of this section, Harvey (1974) under paragraph (d)(3) of this section, Murray (1975) under paragraph (d)(4) of this section, Saltzman (1972) under paragraph (d)(5) of this section, Weber (1971) under paragraph (d)(6) of this section, and Wu (1975) under paragraph (d)(7) of this section served as the basis for this section. 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.

(4) *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 solu-

bility 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, see Goring and Hamaker (1972) under paragraph (d)(2) of this section. However, this does not preclude future soil adsorption/transformation testing of these chemicals if more refined data are needed for the assessment process.

(b) *Test procedures*—(1) *Test conditions*—(i) *Special laboratory equipment.* (A) Equilibrating solutions that contain, besides the test chemical, 0.01M calcium nitrate dissolved in sterilized, distilled-deionized H_2O adjusted to neutral pH 7 by boiling to remove CO_2.

(B) Containers shall be 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 micron (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 micron from aqueous solution.

(ii) *Temperature.* The test procedure shall be performed at 23 ±5 °C.

(iii) *Replications.* Three replications of the experimental treatments shall be used.

(iv) *Soil pretreatment.* The following soil pretreatment steps shall 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.

(v) *Sediment pretreatment.* The following sediment pretreatment steps shall be performed under the following conditions:

(A) Decrease the H_2O 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.

(vi) *Solid/solution ratio.* The solid/solution ratio shall 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. If an insufficient amount of chemical remains in the water phase for quantification, the solid/solution ratio should be adjusted so that measurable amounts of the test chemical remain in solution.

(vii) *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. The equilibration time shall 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 equal to or greater than 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.

(viii) *Centrifugation time.* Calculate the centrifugation time, t_c, necessary to remove particles from solution greater than approximately 0.5 μm (5×10^{-5} μm) equivalent diameter (which represents all particles except the fine clay fraction) using the following equation:

Equation 3

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

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.

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

(x) *Solvents for extraction.* It is important that the solvent used to extract the chemical from the sediment or soil is reagent grade or better. Solvents shall contain no impurities which could interfere with the determination of the test compound.

(2) *Test procedure*—(i) *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 does not exceed 10 ppm, is at least one order of magnitude greater than the lowest concentration reported, and 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.

(ii) *Centrifugation.* When the equilibration time has expired, centrifuge the containers for t_c minutes.

(iii) *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.

(iv) *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.

(c) *Reporting.* Report the following information:

(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 (i) the equilibration time determination and (ii) the isotherm determination.

(6) The quantities K, n, and l/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/H_2O); and cation exchange capacity.

(10) The procedures used to determine the physical/chemical properties listed under paragraphs (c) (7) through (9) of this section.

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Aharonson, N., Kafkafi, U. ''Adsorption, mobility and persistence of thiabendazole and methyl 2-benzimidasole carbamate in soils,'' *Journal of Agricultural and Food Chemistry*, 23:720–724 (1975).

(2) Goring, C.A.I., Hamaker, J.W., (eds). *Organic Chemicals in the Soil Environment.* Vol. I & II (New York: Marcel Dekker, Inc., 1972).

(3) Harvey, R.G. et al. ''Soil adsorption and volatility of dinitroaniline herbicides,'' *Weed Science*, 22:120–124 (1974).

(4) Murray, D.S. et al. ''Comparative adsorption, desorption, and mobility of dipropetryn and prometryn in soil,'' *Journal of Agricultural and Food Chemistry*, 23:578–581 (1973).

(5) Saltzman, S.L. et al. ''Adsorption, desorption of parathion as affected by soil organic matter,'' *Journal of Agricultural and Food Chemistry*, 20:1224–1226 (1972).

(6) Weber, J.B. ''Model soil system, herbicide leaching, and sorption,'' *Weed Science*, 19:145–160 (1971).

(7) Wu, C.H., et al. ''Napropamide adsorption, desorption, and movement in soils,'' *Weed Science*, 23:454–457 (1975).

Subpart D—Transformation Processes

§ 796.3100 Aerobic aquatic biodegradation.

(a) *Introduction*—(1) *Purpose.* (i) 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.

(ii) 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.

(2) *Definitions.* (i) "Adaptation" is the process by which a substance induces the synthesis of any degradative enzymes necessary to catalyze the transformation of that substance.

(ii) "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.

(iii) "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.

(3) *Principle of the test method.* This Guideline method is based on the method described by William Gledhill (1975) under paragraph (d)(1) of this section. The method consists of a 2-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 $Ba(OH)_2$ 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.

(4) *Prerequisites.* The total organic carbon (TOC) content of the test sub-

stance shall 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.

(5) *Guideline information.* (i) 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."

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

(6) *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 CO_2 and show a removal of 70 percent DOC within 28 days. Otherwise the test is regarded as invalid and shall be repeated using an inoculum from a different source.

(7) *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.

(8) *Sensitivity.* The sensitivity of the method is determined by the ability to measure the endogenous CO_2 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 ^{14}C-labeled test substances, test substance concentrations can be much lower.

(9) *Possibility of standardization.* This possibility exists. The major difficulty is to standardize the inoculum in such a way that interlaboratory reproducibility is ensured.

(10) *Possibility of automation.* None at present, although parts of the analyses may be automated.

(b) *Test procedures*—(1) *Preparations*—(i) *Apparatus.* The shake flask apparatus under the following Figure 1 contains 10 mL of 0.2N $Ba(OH)_2$ in an open container suspended over 1 liter of culture medium in a 2-liter Erlenmeyer flask.
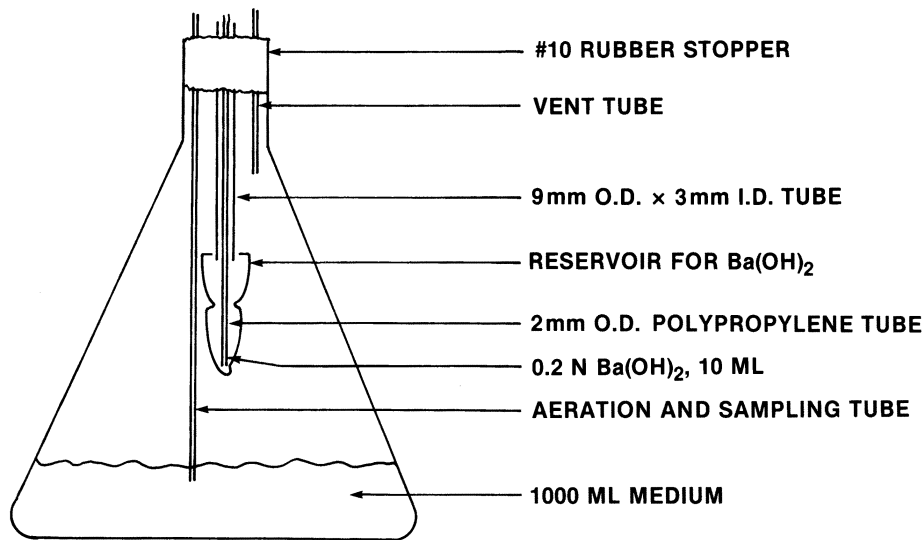
#10 RUBBER STOPPER

VENT TUBE

9mm O.D. × 3mm I.D. TUBE

RESERVOIR FOR $Ba(OH)_2$

2mm O.D. POLYPROPYLENE TUBE

0.2 N $Ba(OH)_2$, 10 ML

AERATION AND SAMPLING TUBE

1000 ML MEDIUM

FIGURE 1—SHAKE-FLASK SYSTEM FOR CARBON DIOXIDE EVOLUTION

The $Ba(OH)_2$ 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. × 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 $Ba(OH)_2$, while the constriction permits transferal of the flask to and from the shaker without $Ba(OH)_2$ 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 $Ba(OH)_2$ 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.

(ii) *Reagents and stock solutions.* (A) Stock solutions, I, II, and III under the following Table 1.
(B) Yeast extract.
(C) Vitamin-free casamino acids.
(D) 70 percent O_2 in nitrogen or CO_2-free air.
(E) 0.2N $Ba(OH)_2$.
(F) 0.1 N HCl.
(G) 20 percent H_2SO_4.
(H) Phenolphthalein.
(I) Dilution water—distilled, deionized water (DIW).
(iii) *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) under paragraph (d)(2) of this section. The soil surface is cleared of litter and a soil sample is obtained 10 to 20 cm below the surface. The sample is screened through a sieve with 2 to 5 mm openings and stored in a polyethylene bag at 2 to 4 °C for not more

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than 30 days prior to use. The soil is never allowed to air-dry, and shall not be frozen during storage.

TABLE 1—MEDIUM EMPLOYED FOR ASSAY OF CO_2 EVOLUTION

Solution [1]	Compound	Stock Solution Conc. (g/L)
I	NH_4Cl	35
	KNO_3	15
	$K_2HPO_4·3H_2O$	750
	$NaH_2PO_4·H_2O$	25
II [2]	KCl	10
	$MgSO_4$	20
	$FeSO_4·7H_2O$	1
III	$CaCl_2$	5
	$ZnCl_2$	0.05
	$MnCl_2·4H_2O$	0.5
	$CuCl_2$	0.05
	$CoCl_2$	0.001
	H_3BO_3	0.001
	MoO_3	0.0004

[1] = Each liter of test medium contains 1 mL of each solution.
[2] = Final pH is adjusted to 3.0 with 0.10 N HCl.

(iv) *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 in Table 1 in paragraph (b)(1)(iii) of this section, 1.0 gm of soil inoculum (prepared according to paragraph (b)(1)(iii) of this section), 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.

(2) *Procedures.* (i) Inoculum (100 mL of acclimation medium) is added to 900 mL DIW containing 1 mL each of solutions I, II, and III in Table 1 under paragraph (b)(1)(iii) of this section 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 Ba $(OH)_2$ are added to the suspended reservoir in each flask and duplicate 10 mL samples of $Ba(OH)_2$ 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 (±10 percent) 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 to 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, $Ba(OH)_2$ 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 $Ba(OH)_2$ sample to be analyzed. Flasks are resealed and placed on the shaker. On the day prior to terminating the test, 3 mL of 20 percent H_2SO_4 are added to the medium to release carbonate bound CO_2.

(ii) For each set of experiments, each test substance shall be tested in triplicate.

(iii) 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.

(iv) For each test set, triplicate controls receiving inoculated medium and no test compound, plus all test and reference flasks, are analyzed for CO_2 evolution and DOC removal. Results from analysis of the control flasks (DOC, CO_2 evolution, etc.) are subtracted from corresponding experimental flasks containing test compound in order to arrive at the net effect due to the test compound.

(v) 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 ($HgCl_2$) to inhibit microbial activity.

(vi) Flasks shall 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/1 $HgCl_2$, are needed to correct for interferences due to nonbiological 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 to 3 hours of sampling to minimize interferences which may occur in storage. All glassware should be free of organic carbon contaminants.

(3) *Analytical measurements.* The quantity of CO_2 evolved is measured by titration of the entire $Ba(OH)_2$ sample (10 mL $Ba(OH)_2$ + 10 mL rinse water) with 0.1 N HCl to the phenolphthalein end point. $Ba(OH)_2$ 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.

(c) *Data and reporting*—(1) *Treatment of results.* (i) Test compound (10 mg carbon) is theoretically converted to 0.833 mmol CO_2.$^{-1}$ Absorbed CO_2 precipitates as $BaCO_3$ from $Ba(OH)_2$, 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:

Percent CO_2 evolution=[(TF−CF)/16.67] 100 (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.

(ii) The cumulative percent CO_2 evolution at any sample time is calculated as the summation of the percent CO_2 evolved at all sample points of the test.

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

Percent 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.

(iv) 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 to 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 shall be rerun beginning with the acclimation phase.

(v) CO_2 evolution in the reference flasks is also indicative of the activity of the microbial test system. The suggested reference compounds should all yield final CO_2 evolution values of at

least 60 percent of theoretical CO_2. If, for any test set, the percent theoretical CO_2 evolution value for the reference flasks is outside this range, the test results are considered invalid and the test is rerun.

(vi) Inhibition by the test compound is indicated by lower CO_2 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.

(vii) The use of ^{14}C-labeled chemicals is not required. If appropriately labeled test substance is readily available and if the investigator chooses to use this procedure with labeled test substance, this is an acceptable alternative. If this option is chosen, the investigator may use lower test substance concentrations if those concentrations are more representative of environmental levels.

(2) *Test report.* (i) For each test and reference compound, the following data shall be reported.

(ii) 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).

(iii) 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.

(iv) Average cumulative percent theoretical CO_2 evolution over the test duration.

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

(vi) Average percent DOC removal at each sampling time.

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

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Gledhill, W.E. ''Screening Test for Assessment of Ultimate Biodegradability: Linear Alkyl Benzene Sulfonate,'' *Applied Microbiology*, 30:922–929 (1975).

(2) Pramer, D., Bartha, R. ''Preparation and Processing of Soil Samples for Biodegradation Testing,'' *Environmental Letters*, 2:217–224 (1972).

[50 FR 39252, Sept. 27, 1985, as amended at 52 FR 19058, May 20, 1987]

§ 796.3500 Hydrolysis as a function of pH at 25 °C.

(a) *Introduction*—(1) *Background and purpose.* (i) 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.

(ii) 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.

(2) *Definitions and units.* (i) ''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).

(ii) ''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.

(iii) 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.

(iv) 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.

(v) ''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:

$$RX + HOH \rightarrow ROH + HX.$$
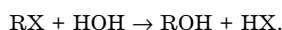
(A) Another result of hydrolysis can be the incorporation of both H and OH in a single product. An example of this is the hydrolysis of epoxides, which can be represented by
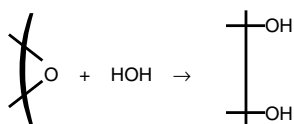
(B) The hydrolysis reaction can be catalyzed by acidic or basic species, including OH^- and $H_3O^=$ ($H^=$). The promotion of the reaction by H_3O^- or OH^- is called specific acid or specific base catalysis, respectively, as contrasted with general acid or base catalysis encountered with other cationic or anionic species. Usually, the rate law for chemical RX can be written as:

Equation 1

$$-d[RX]/_{d=} = k_h[RX] = k_A[H^=]\,[RX]$$
$$+ k_B[OH^-]\,[RX] + k'_N\,[H_2O]\,[RX],$$

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 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:

Equation 2

$$k_h = k_A\,[H^=] + k_B\,[OH^-] + k_N,$$

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:

Equation 3

$$t_{1/2} = 0.693/k_h.$$

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

Equation 4

$$\log_{10}C = -(k_h\,t/_{2.303}) + \log_{10}C_o,$$

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

(C) At a given pH, Equation 2 under paragraph (a)(2)(v)(B) of this section 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

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

Equation 5

$$k_A = 10^3 \left[k_h\,(3) - k_h\,(7) + 10^{-4}\,k_h\,(11) \right]$$
$$k_B = 10^3 \left[k_h\,(11) - k_h\,(7) + 10^{-4}\,k_h\,(3) \right]$$
$$k_N = k_h\,(7) - 10^{-4} \left[k_h\,(3) + k_h\,(11) \right]$$

The calculated rate constants from equation 5 under this paragraph can be employed in equation 2 under paragraph (a)(2)(v)(B) of this section to calculate the hydrolysis rate of a chemical at any pH of environmental concern.

(D) The equations under paragraph (a)(2) of this section apply whether the test chemical has one or more hydrolyzable groups. In the latter case, the rate may be written as:

Equation 6

$$-d[RX]/_{dt} = [RX] = k_2\,[RX] + \ldots + k_n$$
$$[RX] = (k_1 + k_2 + \ldots \ldots k_n)\,[RX] = k_h$$
$$[RX].$$

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 section.

(3) *Principle of the test method.* Procedures described in this section 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. The latter constants can then be employed in determining the hydrolysis rates of chemicals at any pH of environmental concern at 25 °C.

(4) *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 section.

(b) *Test procedures*—(1) *Test conditions*—(i) *Special laboratory equipment.* (A) A thermostatic bath that can be maintained at a temperature of 25 ±1 °C.

(B) A pH meter that can resolve differences of 0.05 pH units or less.

(C) Stoppered volumetric flasks (no grease) or glass ampoules that can be sealed.

(ii) *Purity of water.* Reagent-grade water (e.g., water meeting ASTM Type IIA standards or an equivalent grade) shall be used to minimize biodegradation. ASTM Type IIA water is described in ASTM D 1193–77 (Reapproved 1983), ''Standard Specification for Reagent Water.'' ASTM D 1193–77 (Reapproved 1983) is available for inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.* This incorporation by reference was approved by the Director of the Office of the Federal Register. This material is incorporated as it exists on the date of approval and a notice of any change in this material will be published in the FEDERAL REGISTER. Copies of the incorporated material may be obtained from the Director, Environmental Assistance Division (7408), Office of Pollution Prevention and Toxics, Environmental Protection Agency, Room E–543B, 1200 Pennsylvania Ave. NW., Washington, DC 20460–0001, or from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103.

(iii) *Sterilization.* All glassware shall be sterilized. Aseptic conditions shall be used 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.

(iv) *Precautions for volatility.* If the chemical is volatile the reaction vessels shall be almost completely filled and sealed.

(v) *Temperature controls.* All hydrolysis reactions shall be carried out at 25

°C (±1 °C) and with the temperature controlled to ±0.1 °C.

(vi) *pH conditions.* It is recommended that all hydrolysis experiments be performed at pH 3.00, 7.00, and 11.00 ±0.05 using the appropriate buffers described in paragraph (b)(2)(i)(A) of this section.

(vii) *Concentration of solutions of chemical substances.* The concentration of the test chemical shall be less than one-half the chemical's solubility in water but not greater than 10^{-3} M.

(viii) *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 900 ±0.05 using the appropriate buffers described in paragraphs (b)(2)(i) (A) and (B) of this section. 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 paragraph (b)(2)(i)(B) of this section.

(ix) *Buffer catalysis.* For certain chemicals, buffers may catalyze the hydrolysis reaction. If this is suspected, hydrolysis rate determination shall be carried out with the appropriate buffers and the same experiments 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, the test chemical concentrations also shall 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 for two different solutions differing in buffer or test chemical concentration by a factor of five.

(x) *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, the hydrolysis experiment shall 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 under § 796.1050.

(xi) *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.

(2) *Preparation*—(i) *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 0.100M 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 paragraph (b)(1)(viii) of this section, prepare buffers using reagent-grade water and reagent-grade chemicals as follows:

(*1*) pH 5.00: use 250 mL of 0.100M 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 ($Na_2 B_4O_7$); 23 mL of 0.100M hydrochloric aid; 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 concentration to be employed in carrying out hydrolysis measurements. If the initial concentration of the test chemical is less than 10^{-3} M, the buffer concentration shall be lowered by a corresponding amount; e.g., if the initial test chemical concentration is 10^{-4} M, the concentration of the above buffers shall be reduced by a factor of 10. In addition, for those reactions in which an acid or base is not a reaction product, the minimum buffer concentration necessary for maintaining the pH within + 0.05 units shall be employed.

(*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.* (*1*) If the test chemical 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, the aliquot shall 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. The final concentration shall not be greater than one-half the chemical's solubility in water and not greater than 10^{-3} M.

(*2*) 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 and then added to an aliquot of the acetonitrile solution. Do not employ heat to dissolve the chemical in acetonitrile. The final concentration of the test chemical shall not be greater than one-half the chemical's solubility in water and not greater than 10^{-3} M. In addition, the final concentration of the acetonitrile shall be one volume percent or less.

(3) *Performance of the test.* Carry out all hydrolysis experiments by employing one of the procedures described in this paragraph. Prepare the test solutions as described in paragraph (b)(2)(i) of this section at pH 3.00, 7.00, and 11.00 ±0.05, and determine the initial test chemical concentration (C_o) in triplicate. Analyze each reaction mixture in triplicate at regular intervals, employing one of the following procedures:

(i) *Procedure 1.* Analyze each test solution at regular intervals to provide a minimum of six measurements with the extent of hydrolysis between 20 to 70 percent. Rates should be rapid enough so that 60 to 70 percent of the chemical is hydrolyzed in 672 hours.

(ii) *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.

(iii) *Procedure 3.* (A) If chemical hydrolysis is less than 20 percent after 672 hours, determine the concentration (C) after this time period.

(B) 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.

(iv) *Analytical methodology.* Select an analytical method that is most applicable to the analysis of the specific chemical being tested under paragraph (b)(1)(xi) of this section.

(c) *Data and reporting*—(1) *Treatment of results.* (i) If Procedure 1 or 2 were employed in making concentration measurements, use a linear regression analysis with Equation 4 under paragraph (a)(2)(v)(B) of this section 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 under paragraph (a)(2)(v)(B) of

this section to calculate the hydrolysis half-life using k_h.

(ii) If Procedure 3 was employed in making rate measurements, use the mean initial concentration (C_o) and the mean concentration of chemical (C) in Equation 4 under paragraph (a)(2)(v)(B) of this section to calculate k_h for each pH used in the experiments. Calculate the hydrolysis half-life using k_h in Equation 3 under paragraph (a)(2)(v)(B) of this section.

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

(iv) 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.

(2) *Specific analytical and recovery procedures.* (i) Provide a detailed description or reference for the analytical procedure used, including the calibration data and precision.

(ii) 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.

(3) *Test data report.* (i) 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.

(ii) 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.

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

PART 797—ENVIRONMENTAL EFFECTS TESTING GUIDELINES

Subpart A [Reserved]

Subpart B—Aquatic Guidelines

AUTHORITY: 15 U.S.C. 2603.

SOURCE: 50 FR 39321, Sept. 27, 1985, unless otherwise noted.

Subpart A [Reserved]

Subpart B—Aquatic Guidelines

§797.1050   Algal acute toxicity test.

(a) *Purpose.* The guideline in this section is intended for use in developing data on the acute toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*). This guideline prescribes test procedures and conditions using freshwater and marine algae to develop data on the phytotoxicity of chemicals. The United States Environmental Protection Agency (U.S. EPA) will use data from these tests in assessing the hazard of a chemical to the environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. The following definitions also apply to this guideline:

(1) *Algicidal* means having the property of killing algae.

(2) *Algistatic* means having the property of inhibiting algal growth.

(3) *ECx* means the experimentally derived chemical concentration that is calculated to effect X percent of the test criterion.

(4) *Growth* means a relative measure of the viability of an algal population based on the number and/or weight of