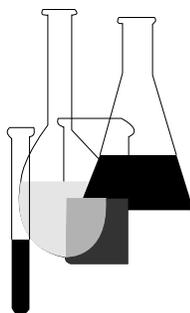




Fate, Transport and Transformation Test Guidelines

OPPTS 835.3220 Porous Pot Test



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136 *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202-512-1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202-512-0132 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from EPA's World Wide Web site (<http://www.epa.gov/epahome/research.htm>) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

OPPTS 835.3220 Porous pot test.

(a) **Scope**—(1) Applicability. This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline are the articles presented at paragraph (o) of this guideline.

(b) **Introduction.** (1) This test is intended to simulate processes in the aeration basin of the activated sludge sewage treatment process and therefore give a measure of the extent of biodegradation or removal likely to occur during sewage treatment.

(2) Information on the treatability, biodegradability, and/or removability of a test compound can be assessed by either dissolved organic carbon (DOC) measurements, specific chemical analysis, or a combination of the two. DOC measurements, relative to the controls, can be used to calculate the removal of the test compound or water-soluble degradation products by the porous pot treatment. DOC measurements do not identify the water-soluble components. Specific chemical analysis, on the other hand, is designed to determine the concentration of the parent test compound in the plant effluent and/or associated with the activated sludge solids and also, if analytical standards for the degradation products are available, the concentrations of the products. From this information, the percent removal of the test compound and the amount and identity of products resulting from primary biodegradation can be determined. A specific chemical analytical method must have a limit of detection (LOD) of ≤ 0.1 mg/L for water or ≤ 0.1 mg/kg for sludge solids.

(3) The feature that distinguishes this test from other activated sludge simulation tests is the retention of the activated sludge in a porous liner, which eliminates the need for a secondary clarifier and facilitates control of a critical parameter, the sludge retention time (SRT).

(4) Porous pots can be completely sealed and tests using ^{14}C -labeled test compounds are possible. Carbon dioxide in the exhaust gas and bicarbonate in the effluent can be used together to assess the extent of mineralization, and levels of radiolabel in the sludge and in the aqueous phase may also be determined.

(5) It is also possible to determine whether the test compound has any adverse effect on normal sewage treatment processes by simultaneously measuring the efficiency of the pots in removing DOC.

(6) This guideline may involve hazardous materials, operations and equipment. This standard does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this

standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. For specific hazard statements see paragraph (g) of this guideline.

(c) **Applicable ASTM standards.** Refer to paragraph (o)(1) of this guideline for the following standards:

(1) D1129–90 Standard Terminology Relating to Water.

(2) D1193–91 Standard Specifications for Reagent Water (Federal Test Method and Standard No. 7916).

(3) D1293–84 Standard Test Methods for pH of Water.

(4) D2579–85 Standard Test Method for Total and Organic Carbon in Water.

(5) D4375–90 Standard Terminology for Basic Statistics in Committee D–19 on Water.

(6) D4839–88 Standard Test Method for Total Organic Carbon in Water by Ultraviolet, or Persulfate Oxidation or Both, and Infrared Detection.

(7) E178–80 Standard Practice for Dealing with Outlying Observations.

(d) **Summary.** (1) The test is designed to simulate processes in the aeration basin of the activated sludge sewage treatment process and is performed using a porous pot-type laboratory-scale activated sludge apparatus, based on an original design developed by the United Kingdom Water Research Centre (WRC) (see paragraphs (o)(4) and (o)(5) of this guideline). The original design was modified (see paragraph (o)(7) of this guideline) and has been utilized in determining the effects of temperature, phosphate, and other growth media components on the growth of activated sludge and the toxicity of treated effluents (see paragraphs (o)(8) and (o)(10) of this guideline). It has also been used in the environmental safety evaluation of a new product (see paragraph (o)(9) of this guideline). The modified test system (Figure 1, under paragraph (h)(1)(i) of this guideline) facilitates control of the SRT, and the effect of this fundamental parameter on the efficiency of removal of surfactants in porous pots has been described (see paragraph (o)(2) of this guideline).

(2) The test and control pots are filled with mixed liquor from an activated sludge plant treating predominantly domestic sewage and then operated as continuous-flow systems with primary effluent or settled domestic sewage as feed.

(i) A solution or suspension of the test compound is dosed into the test pot by means of a suitable micro-metering pump. The concentration

of the test compound in the influent sewage is 10–20 mg C/L. A lower concentration of the test compound may be used if a highly sensitive analytical method is available or if radiolabeled compound is used. The total flow to the pot (sewage + test compound dosing solution) is controlled to give the required hydraulic retention time.

(ii) A similar flow of sewage and a dosing solution of a suitable reference compound such as sodium benzoate are added to the control pots. Benzoate biodegrades easily and completely in this test system, and is added at such a concentration to ensure that the total organic carbon load and the total sewage flow are the same in control and test pots. Reference compounds may also have other uses (see paragraph (f)(5) of this guideline).

(3) Air is supplied to the pots through a diffuser stone to ensure adequate oxygen transfer to the mixed liquor, and an additional flow through a 5 mm open tube is provided to ensure complete mixing of the system. The air flow should be sufficient to maintain and thoroughly mix the solids in suspension and keep the concentration of dissolved oxygen (DO) above 2 mg/L at all times. In order to do this it is necessary to maintain an air to influent flow ratio of 5 to 10/L on a volume basis.

(4) Sludge is wasted directly from the aeration chamber through the base of the pot by means of a peristaltic pump. The pump is fitted with a timer and operated intermittently to avoid problems caused by the low flow rates required. Alternatively, sludge may be wasted manually by periodically discarding the required volume.

(5) The levels of biodegradable materials remaining in the effluents are dependent on the SRT and the growth kinetics of those organisms that are involved in the metabolism of the compound under consideration. The test is therefore, in effect, a kinetic study and consequently should be conducted at a constant temperature. Further, by making measurements at two or more temperatures, the biodegradability of the test compound under summer and winter operating conditions may be established.

(6) The removal of test compound is determined by analysis of effluents and comparison of the results obtained from pots containing test compound to those from control pots treating only settled sewage and benzoate. Primary biodegradation is assessed by specific analysis of the test compound in effluents after correction for volatilization and adsorption of the parent compound onto activated sludge. Further, analysis of DOC in effluents provides a measure of ultimate biodegradation after corrections have been applied for volatilization and adsorption of parent compound and biodegradation products onto sludge.

(i) For materials that are insoluble or are sorbed or precipitated onto the activated sludge additional information will be required to distinguish between biodegradation and removal by these other processes. The addi-

tional information may be obtained by analysis of the sewage sludge or by using ^{14}C -labeled test compound.

(ii) However, in the absence of this additional information, the DOC and compound-specific analyses still provide data on the total amount of parent or residual soluble materials present in the effluents without any identification of these soluble components.

(7) The efficiency of the units may be assessed from the measurements of DOC, sludge production, and ammoniacal nitrogen in the effluent. However, the latter parameter can only be used when the SRT is sufficiently long for viable populations of nitrifying bacteria to become established in the sludge. In each case a simple Student's *t* test is applied to the data to determine if there is any significant difference between test and control pots.

(e) **Significance.** (1) Secondary wastewater treatment using activated sludge is one of the most important biological treatment processes in use today. The porous pot test employs activated sludge from a domestic activated sludge plant to assess biodegradation and treatability of organic compounds, and provides data that can be used to predict the fate of organic compounds in full-scale plants.

(2) The porous pot system provides a laboratory-scale simulation of activated sludge wastewater treatment because settled domestic sewage is used as the feed and key control parameters are maintained in the ranges typical of such treatment. These parameters include temperature, DO concentration, hydraulic retention time (HRT), and SRT. Mixed liquor volatile suspended solids (MLVSS) are monitored but not controlled. The porous pots are allowed to attain steady state at an MLVSS level commensurate with the other key control parameters.

(f) **Interpretation.** (1) Because the porous pot test system is a simulation of activated sludge wastewater treatment rather than a test to measure "ready" or "inherent" biodegradability, there are no pass or fail criteria. The levels of removal observed in the porous pot test should approximate levels of removal expected in full-scale activated sludge treatment systems.

(2) Information on the physical/chemical properties of the test compound will be useful for interpretation of results and in the selection of appropriate test compound concentrations. These properties include structure, composition, purity, molecular weight, water solubility, organic carbon content, vapor pressure, octanol/water partition coefficient, adsorption isotherm, surface tension, and Henry's law constant.

(3) Information on the toxicity of the test compound or potential toxic degradation products to activated sludge microorganisms may be useful to the interpretation of low biodegradation results and in the selection of appropriate test compound concentrations. The OECD Respiration Inhibi-

tion Test under paragraph (o)(6) of this guideline can be used to indicate such toxicity. Furthermore, chemical substances in solution or in the air that may negatively affect the growth or metabolism of sludge microorganisms, e.g., organic solvents, toxic metals, strong alkalis, and bactericides, may result in low removals and should be avoided.

(4) Use of synthetic versus natural sewage is an important consideration.

(i) It is sometimes assumed that use of synthetic sewage leads to more reproducible results; however, the microbial population that develops differs from that which is present in full-scale activated sludge plants. Generally, the most rapidly growing microorganisms will dominate the more slowly growing populations that are present in full-scale treatment plants. Natural domestic sewage varies from source to source and in nutrient content. However, it provides both the nutrients needed to support the natural microbial population and a continuous supply of fresh microorganisms to the test system.

(ii) On some occasions, particularly during periods of heavy rainfall, the strength of the primary effluent or settled domestic sewage from the treatment plant may be too low to sustain a typical biomass (MLVSS) concentration in the porous pot unit of 1.5 to 3.0 mg/L. This is not likely to happen unless the DOC concentration in the feed is <20 mg DOC/L. In this case, a blend made by supplementation of natural sewage with synthetic sewage to achieve a DOC level of at least 200 mg/L and an approximate 100:12:2 ratio of C:N:P may be desirable.

(5) Reference compounds may be useful in establishing the activity of the activated sludge and in comparing results from different laboratories. While specific reference compounds cannot be recommended for these purposes, data are available for several chemicals (see paragraphs (o)(2) and (o)(7) of this guideline).

(g) **Safety precautions.** (1) This procedure involves the use of mixed liquor and natural sewage from a domestic wastewater treatment plant. Consequently, individuals performing this test may be exposed to microbial agents that are dangerous to human health. It is recommended that porous pots be operated in a separate room and that exhaust air be vented outside the building.

(2) Personnel who work with sewage organisms may choose to keep current with pertinent immunizations such as typhoid, polio, hepatitis B, and tetanus.

(3) Effluent from the porous pots is treated with a chemical disinfectant (chlorine bleach, 5 percent) or autoclaved prior to disposal. Safety glasses and protective gloves should be worn when using sodium hypochlorite to clean pot liners.

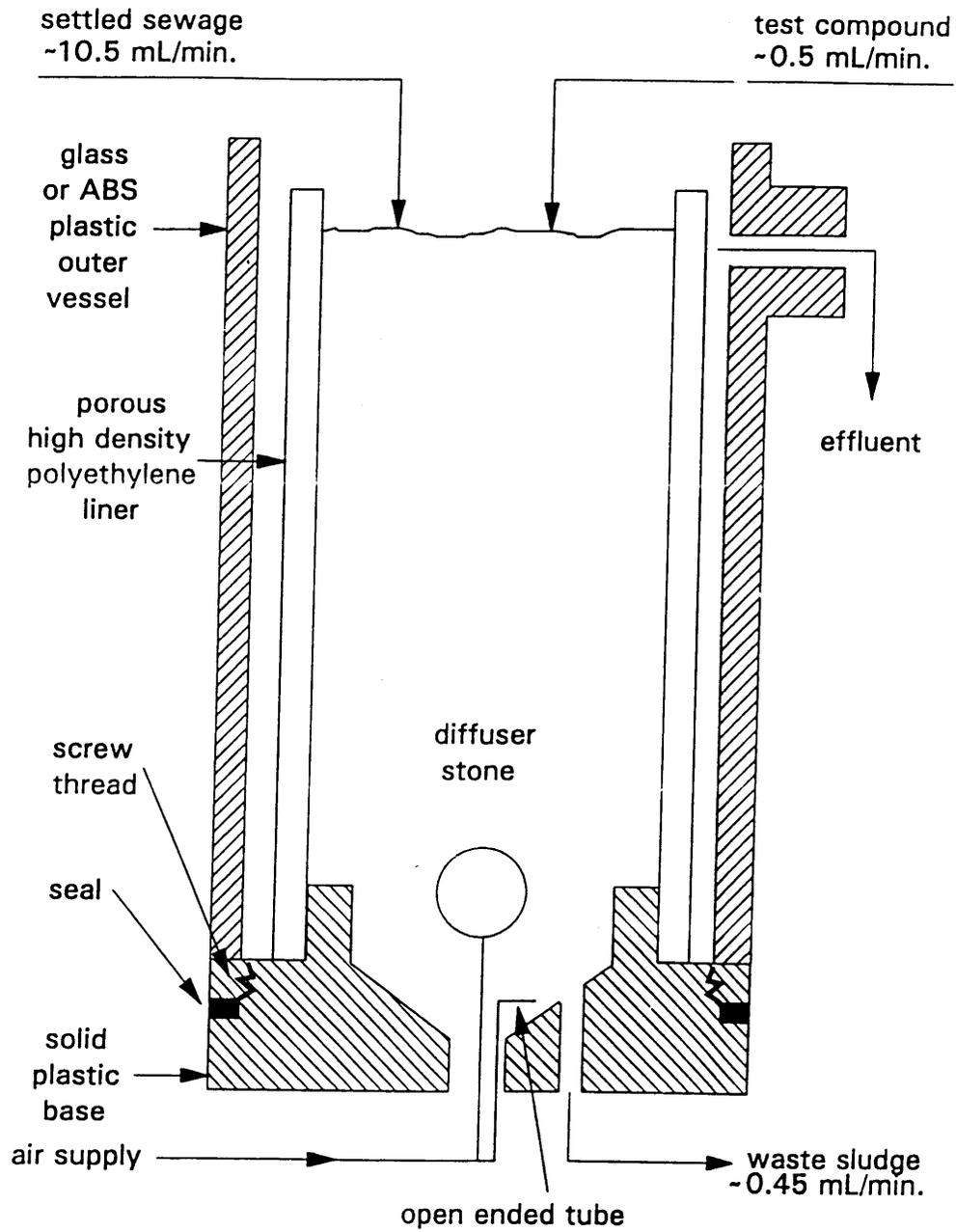
(4) Unless shown to be nontoxic, all test compounds should be treated as potentially harmful.

(h) **Apparatus.** The following apparatus are required to perform the test:

(1) Porous pot aeration vessel.

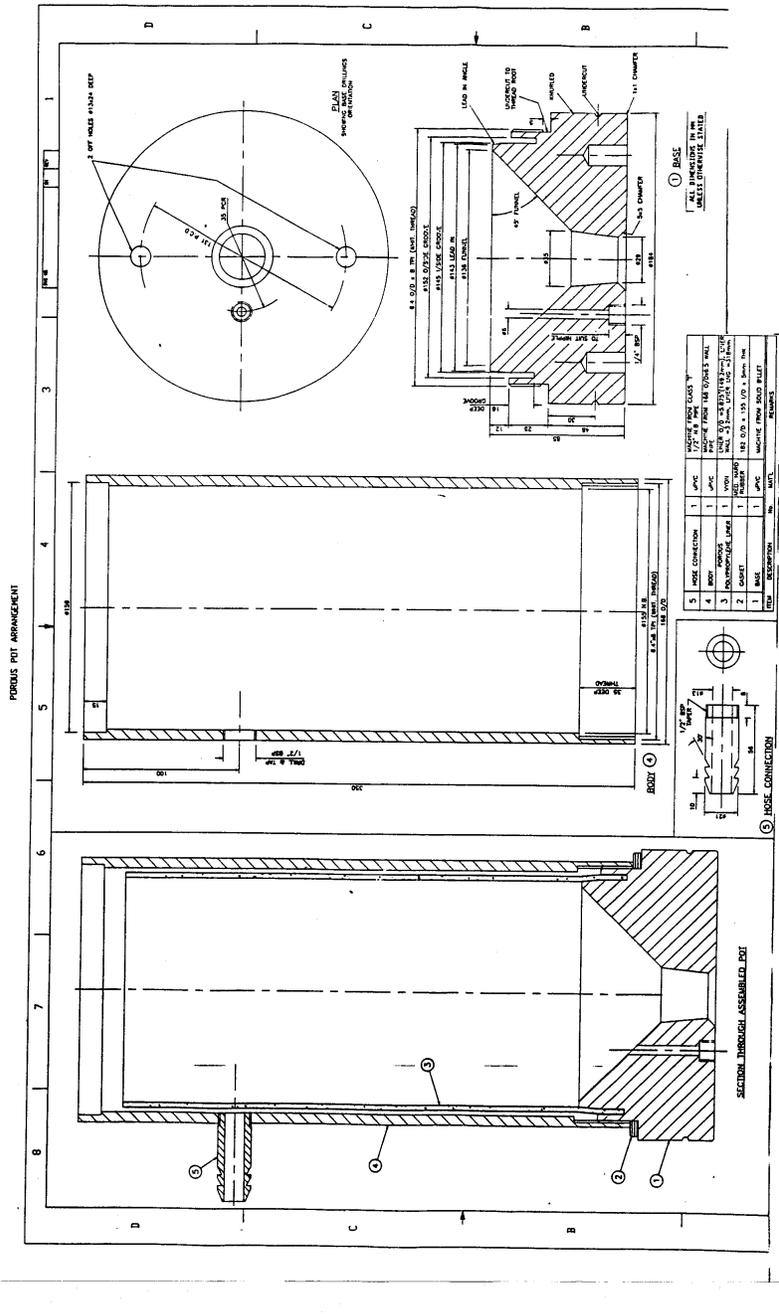
(i) URPSL design (Figure 1).

FIGURE 1. URPSL POROUS POT



(ii) Engineering drawing of URPSL design (Figure 2).

FIGURE 2. ENGINEERING DRAWING OF URPSL POROUS POT



(iii) The porous pot vessel liner is constructed from porous high density polyethylene sheets. The thickness ranges from 3.2 to 13.6 mm and pore sizes are from 65 to 90 μm . The retention of the liner is about 20 μm and all particles above this size are retained in the system. A U.S. manufacturer is Porex Technologies Corp., 500 Bohannon Rd., Fairburn, GA 30213. A U.K. supplier is Porvair Technology Ltd., Clywedog Road South, Wrexham Industrial Estate, Wrexham Cllyd, LL13 9XS, U.K. The outer vessel can be constructed of glass or an impermeable plastic such as acrylonitrile butadiene styrene (ABS) copolymer.

(2) Oil-free compressor for supplying compressed air to the aeration vessel.

(3) Suitable pumps for dosing porous pots with test substance solutions and sewage at the required rates (0–1.0 mL/min for test substance solutions, 5–20 mL/min for sewage). An additional pump is required to waste sludge from the pot, unless sludge is wasted manually. Low rates of sludge wastage are attained using a pump set at a high flow rate but operating intermittently. The actual flow is calculated as follows:

$$\text{flow} = \frac{[\text{pump throw (mL/min)} \times \text{pumping time (sec)}]}{\text{timer cycle (sec)}}$$

For example, when the pump is operating for 10 sec each minute, the timer cycle is 1 min (60 sec), and the pump throw is 3 mL/min, the wastage rate would be 0.5 mL/min.

(4) 1-L sample bottles to hold test substance dosing solutions.

(5) Silicone rubber tubing: Bore = 0.5 mm ID.

(6) Polypropylene transmission tubing.

(7) Tube connectors.

(8) Diffuser stones.

(9) 25–mL measuring cylinders.

(10) 1–mL graduated pipets.

(11) Stopwatch.

(12) 40–mL sample bottles for collection of samples for waste sludge and mixed liquor suspended solids determinations.

(13) Thermometer having range 0 to 50 $^{\circ}\text{C}$.

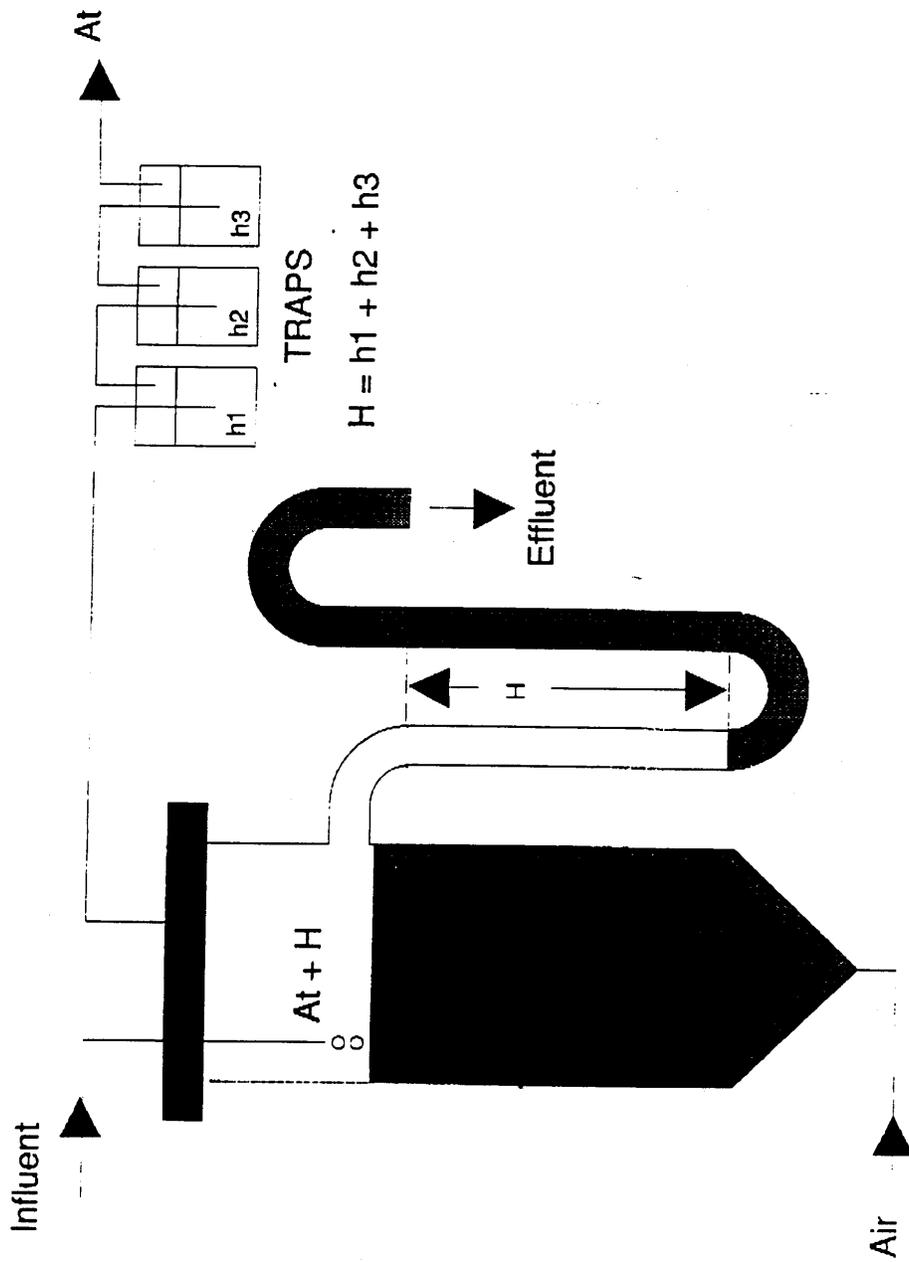
(14) 1- and 2-L measuring cylinders for each pot to collect waste sludge.

(15) Timer for sludge wastage pump allowing intermittent operation, unless sludge is wasted manually.

(16) Right-angled plastic tube to fit on one end of the air line to ensure complete mixing of activated sludge.

(17) If pots are operated in the sealed mode (see paragraph (b)(4) of this guideline), plastic tubing to fit on the pot effluent port as shown in Figure 3 to balance the back pressure caused by the CO₂ traps.

FIGURE 3. POROUS POT APPARATUS FOR SEALED MODE OF OPERATION



(i) **Reagents and materials.** The following reagents and materials are required to perform the test:

(1) Activated sludge mixed liquor collected from aeration basin or oxidation ditch of domestic wastewater treatment plant.

(2) Natural sewage feed: Primary effluent or settled domestic sewage from a domestic wastewater treatment plant. Supplementation with the synthetic sewage stock (see paragraph (i)(3) of this guideline) to achieve at least 200 mg DOC/L may be desirable in some situations, as noted in paragraph (f)(4)(ii) of this guideline.

(3) Synthetic sewage stock solution: Add 130 g glucose, 130 g nutrient broth, 130 g beef extract, 130 g dipotassium hydrogen phosphate, and 25 g ammonium sulfate to 1 L of tap water. Dissolve by heating to just below the boiling point and store in the refrigerator below 7 °C. Discard if any visual evidence of biological growth (turbidity) is observed. Synthetic sewage is created by adding 1 mL of this stock to 1 L of tap water.

(4) Compressed air (filtered for oil and water) for aeration of porous pots.

(5) Test and reference compounds of known carbon content (for DOC analyses) or composition (for specific analyses).

(6) Extraction apparatus and solvent for hydrophobic test compounds.

(7) Deionized or distilled water for preparation of test/reference compound stock solutions.

(8) Glycerol for lubricating the rollers of the peristaltic pumps.

(9) Sodium hypochlorite solution.

(10) Stock solutions of test and reference compounds. (i) For compounds that are sufficiently soluble and chemically stable, a stock solution 10× times the strength of the dosing solution may be prepared and diluted to the required strength each day.

(ii) If chemically unstable materials are being tested, it may be necessary to prepare stock/dosing solutions immediately before use.

(iii) Note: Use of a suitable stable dispersion is required when testing for insoluble compounds.

(11) Dosing solutions of test/reference compound. (i) To avoid biodegradation of the test/reference compound before it is introduced into the test system, which might occur if the test/reference compound and sewage are premixed, the test solution and the sewage are dosed into the porous pot separately.

(ii) The total flow into the pot (sewage flow + test/reference compound flow, both in milliliters per minute) is calculated as follows:

$$\text{total flow} = F = \frac{[\text{volume of porous pot (mL)}]}{[\text{required sewage retention time (h)}] \times 60 \text{ min/h}}$$

(iii) For a pot volume of 3 to 6 L, it is convenient to dose with a solution of test/reference compound at about 0.5 mL/min.

(iv) If the total flow as calculated above is F (mL/min) and the required concentration in the influent sewage is C (mg/L), the concentration of the solution (in mg/mL) to be dosed into the pot (at a rate of 0.5 mL/min) is given as follows:

Concentration of test/reference compound in dosing solution =

$$F \text{ (mL/min)} \times C \text{ (mg/L)} / 0.5 \text{ mL/min}$$

(v) The dosing solution is usually prepared daily by diluting a suitable stock solution.

(j) **Procedure.** (1) Mixed liquor should be maintained at the required working temperature (± 2 °C) throughout the test. When setting up the test pots, test/reference compound and sludge wastage rates may initially be set. The test should be started only after conditions are adjusted to the values defined in the study plan and the pots have been operating for some time under these conditions.

(2) Set up the number of pots required by the study plan. Each test should have at least one control pot (pot fed settled sewage and benzoate or other easily degradable reference compound) and it is recommended (but not required) that each test compound be tested in duplicate.

(3) Fill the aeration vessel with mixed liquor to the level of the effluent overflow. The volume required is 3.8 L for a URPSL porous pot. The initial MLVSS should be 1.5 to 3.0 g/L, and this level should be maintained throughout the test. This may necessitate supplementation of the feed with synthetic sewage as described in paragraph (f)(4)(ii) of this guideline.

(4) Start the aeration and set the air flow. The air flow should be sufficient to maintain and thoroughly mix the solids in suspension and keep the concentration of DO above 2 mg/L at all times. In order to do this it is necessary to maintain an air to influent flow ratio of 5 to 10/L on a volume basis.

(5) Place 1 L of test/reference compound dosing solution in the dosing vessel.

(6) Start the dosing pumps, lubricating the tubes with a small amount of glycerol.

(7) Start the sludge wastage pump at the rate required to give the desired SRT (the SRT (in days) is equal to the pot volume (L) divided by the sludge wastage rate (L/day)). Alternatively, sludge may be wasted manually by periodically discarding the required volume.

(8) Set the sewage dosing rate to give the required HRT and the test/reference compound dosing rate at about 0.5 ± 0.05 mL/min.

(9) Daily measurements of sewage flow rates should be made using a 25-mL measuring buret and a stopwatch. The flow rates should be adjusted to within ± 0.05 mL/min of the required flow.

(10) Dosing solution flow rates should be calculated from measuring the volume left after 24 h of dosing.

(11) The dosing rates should be recorded and corrected to the nominal value given in the study plan. The sewage flow should be adjusted if the measured flow differs by more than 0.5 mL/min from the nominal value.

(12) Sludge that gathers around the rim of the porous liner should be returned to the mixed liquor at least once per day by scraping with a large spatula. This should always be done before taking a sample of mixed liquor for MLVSS determination.

(13) The temperature, pH, and DOC of the mixed liquor should be measured at least every other day.

(14) Periodically remove a 40-mL sample of mixed liquor from the aeration vessel for MLVSS determination. Three times weekly is usually sufficient.

(15) The volume of mixed liquor wasted from the porous pot should be measured and recorded daily. Remove a representative 40-mL sample from the sludge wastage bottle at least once per week and determine the MLVSS level.

(16) The porous pot liner should be changed at the first sign of blocking of the pores, i.e., when the mixed liquor rises above the effluent overflow. To change the liner proceed as follows: Syphon the mixed liquor into a suitable container and remove any solids from the inner surface of the outer vessel. Place a fresh liner in the outer vessel. Return the mixed liquor to the aeration vessel. Scrape off and transfer any sludge adhering to the sides of the blocked liner. The blocked liner should be thoroughly cleaned before reuse by immersion in a 20 percent solution of hypochlorite

bleach for several hours. The liners must be thoroughly rinsed in clean tap and deionized water before reuse.

(17) Sewage and effluent samples should be taken twice weekly during the stabilization (“running-in”) period for organic carbon analysis and specific compound analysis if required. If necessary, ammoniacal nitrogen, nitrate, nitrite, COD and BOD₅ may also be determined.

(18) When the pots have attained steady state conditions, the effluents are analyzed periodically to determine the extent of biodegradation/removal of the test compound during treatment.

(19) If information on the effects of various operating conditions on removal is required, e.g., temperature, SRT, HRT, etc., any changes should be made gradually. Operate the pot for a period of at least three SRT under the new conditions before collecting data to determine the effect of the new condition(s).

(k) Sampling and analytical procedures—(1) Stabilization period. Over the early period of the test, influent sewage and effluent samples should be taken for organic carbon and ammoniacal nitrogen analysis to monitor the overall performance of the pots. Specific analysis for test compound or degradation products may also be performed on these samples if this is required. Normally these results are not used to assess either the biodegradability or treatability of the test compound but simply to establish that the units have reached steady state, are operating properly, and are acclimated to the test substance. However, in certain instances, such as when information is desired on treatability of test compounds that are released only intermittently to wastewater treatment systems, data gathered during the stabilization period may be useful.

(2) Calculation period. (i) When the pots have achieved steady state, the removal of the test compound is determined by specific compound analysis, measurement of DOC, or both. At least 14 results should be obtained over the calculation period at times when the process efficiency of the control pots is high. The time period over which the measurements are made is not critical but should cover at least 21 days with a maximum sampling frequency of 1 sample per day.

(ii) The treatability of the test compound may be assessed by measurement of DOC removal, removal of ammonia, sludge production, and sludge activity. Of these parameters, DOC and NH₃-nitrogen removal are the most important. Note that when pots are being operated at short SRT or reduced temperature, ammonia removal may be less than complete and will be a less reliable indicator of efficiency. However, the critical assessment of any adverse effect of the test compound on the process is always based on the absence of any significant difference between the test compound and control pots rather than the actual values of the observed parameters.

(3) **DOC analysis.** (i) DOC analysis for monitoring the porous pot test is generally employed only for test compounds whose water solubility exceeds the test concentration (e.g., a concentration equivalent to about 10 mg C/L).

(ii) Since precipitation as salts or sorption onto the sludge floc may occur even with water-soluble test compounds, DOC removal doesn't necessarily indicate biodegradation in all cases.

(iii) DOC analyses are carried out on supernatant samples removed at the end of each cycle from the test compound and control pots. Samples can either be filtered using 0.45 μm pore-size filters or centrifuged at $3,500 \times g$ for 10 min. Precaution: An aliquot of the dosing solution should be evaluated for adsorption of test compound to the filter or elution of DOC from the filter itself.

(iv) The DOC concentration of aqueous samples is determined using a suitable organic carbon analyzer (e.g., OI Corporation Model 700 TOC Analyzer equipped with an autosampler) or equivalent.

(4) **Specific compound analysis.** (i) For the assessment of primary biodegradability the porous pot method applies to water-soluble compounds provided that a suitable method of specific analysis is available.

(ii) Insoluble compounds or compounds that sorb strongly onto the activated sludge may also be examined by this procedure but it will be necessary to determine the level of the test compound associated with the activated sludge.

(iii) Nonpolar hydrophobic test compounds are usually isolated from the sludge matrix by extraction with an immiscible solvent such as methylene chloride or hexane. The extract is dried, concentrated, and analyzed by an appropriate instrumental method—GC, HPLC, GC-MS, or UV/visible spectroscopy.

(iv) Highly polar extractible or nonextractible test compounds that are associated with the mixed liquor solids require specialized testing and analytical procedures that cannot be fully documented in this guideline—use of radiolabeled materials and special apparatus. However, the porous pot operating system can be employed if appropriate mass balances can be obtained.

(v) The porous pot test is not recommended for volatile compounds (Henry's law constant $>10^{-3}$ atm-m³/mol); however, it can be used for compounds that are not completely volatilized. For compounds of moderate volatility, volatilization losses during testing may be evaluated by scrubbing aeration off-gases through a solvent train (usually three consecutive traps containing acetone, methylene chloride, or hexane) or polymeric

traps (e.g., Tenax or Sep-Pac). Specific compound analysis of each solvent trap or polymeric trap is carried out.

(1) **Treatment of results—(1) Calculation of results.** The percentage removal values obtained for the test compound during the calculation period are calculated to 0.1 percent as outlined below. Any seemingly atypical values obtained during the calculation period should be checked for rejection using Dixon's test (refer to paragraph (o)(3) of this guideline), and if rejection is statistically correct, the result should be omitted from the calculation.

(2) **Primary biodegradation/removal.** Removal is defined by the following expression:

$$\text{percent removal} = [1 - (C_E/C_0)] \times 100$$

where

C_0 = mean concentration of test compound in the influent (mg/L)

C_E = mean concentration of test compound in the effluent (mg/L).

(3) **Ultimate biodegradation/removal.** (i) The difference in DOC level (in milligrams per liter) between the control and the test effluents is assessed using a matched pairs hypothesis test. This is performed using the following formula:

$$t = (X_1 - X_2) \cdot n^{1/2}/S$$

where

t is the Student's t value

X_1 and X_2 are the means of the two sets of data

S is the standard deviation of the paired differences

n is the number of paired sets of data.

(ii) The critical value for t is obtained from statistical tables for (n - 1) degrees of freedom at the 0.05 significance level using a one-tailed test.

(iii) The percent removal is calculated from the mean difference:

$$\text{percent removal} = (C_0 - X_d)/C_0 \times 100$$

where

C_0 = concentration of test compound (expressed as milligrams of organic carbon per liter) in the test pot influent

X_d = the mean difference between the test and control effluent DOC levels over the calculation period expressed as milligrams per liter.

(iv) The 95% confidence limits for X_d are calculated as $\pm t \times S/n^{1/2}$, where S is the standard deviation (($n - 1$) degrees of freedom), t is the t value at ($n - 1$) degrees of freedom (two tailed test, $p = 0.05$), and n is the number of data pairs.

(v) 95% confidence limits for percent removal are calculated as $\pm (95\% \text{ confidence limit for } X_d \times 100)/C_0$.

(vi) Even if there is no significant difference between the test and control effluent carbon levels, it is advisable to calculate the removals since this will yield information on the variability of the results.

(vii) Efficiency may be estimated by determining organic carbon, ammoniacal nitrogen, and specific compound removal. This calculation is similar to that for primary biodegradation/removal (see paragraph (1)(2) of this guideline).

(viii) A typical data set for two control pots and three test pots is given in the following Table 1. and is used to illustrate the statistical analysis of the data. It is first necessary to establish that the two control pots are operating in parallel. As indicated in Table 1., the mean difference in effluent DOC between the two control pots is 0.21 mg/L and the standard deviation is 0.53 ($n = 17$). The Student's t value is given by:

$$t = 0.21 [(17)^{1/2}/0.53] = 1.63$$

(ix) The critical t -value at the 0.05 significance level for a two-tailed test and 16 degrees of freedom is 2.21 and since this is not exceeded by the calculated value there is no significant difference between the controls. (Note that a two-tailed test is used in this instance since there is no preconception as to which pot will have the higher effluent concentration.)

Table 1.—Sample test set

Effluent DOC (mg/L)					
Control 1	Control 2	Control Mean	Test 1	Test 2	Test 3
9.6	9.2	9.40	10.9	11.2	11.8
9.5	10.1	9.80	9.8	11.1	12.1
9.2	10.2	9.70	11.9	11.7	12.5
7.8	7.4	7.60	8.4	8.6	10.1
7.5	8.1	7.80	9.1	9.2	11.0
7.3	7.2	7.25	9.2	8.4	8.9
6.7	7.1	6.90	7.9		
11.8	13.2	12.50	13.2	12.2	14.9
12.6	12.8	12.7	11.6	11.8	14.5
18.0	17.7	17.85	13.8	14.9	16.5
11.1	11.1	11.10	9.2	11.5	14.2
8.8	9.3	9.05	7.5	8.4	10.5
8.5	8.3	8.40	7.6	9.1	9.6
10.8	10.5	10.65	9.8	11.0	11.9
12.0	11.9	11.95	9.7		
11.1	11.9	11.50	10.0	11.8	12.9
14.2	14.1	14.15	13.7	14.7	16.3

Mean of (control 2 – control 1) = 0.21

Standard deviation of (control 2 – control 1) = 0.53

(x) The test/reference compound data are treated in a similar manner but the differences between each test pot effluent DOC level and the mean control value are used. The statistical analysis for the three tests is summarized in the following Table 2.:

Table 2.—Analysis of test data

	Test 1	Test 2	Test 3
Mean difference (test-mean control)	-0.29	0.41	1.88
Standard deviation	1.69	1.25	1.10
Number of paired observations	17	15	15
Calculated student's t value	0.72	1.27	6.62
Critical student's t value	1.75	1.76	1.76
Percent removal	102.9	95.9	81.2

95% confidence intervals for percent removal: Test 1 = 94.2–111.6; Test 2 = 84.0–107.8; Test 3 = 75.1–87.3.

(xi) For Test 1, the mean is calculated for all values of the difference (test-control mean), and the calculated t-value is compared with critical t-statistics using a one-tailed test for (n-1) degrees of freedom at the 0.05 probability level. A one-tailed test is used since it is only necessary to establish if the test pot effluent has a significantly higher DOC than the control pot. The converse is of no importance and is not normally observed. Tests 2 and 3 are treated similarly.

(xii) For Test 1, the critical t-value at the 0.05 probability level for a one-tailed test and 17 paired observations (16 df) is 1.75, and since this is not exceeded by the calculated value of 0.72, the difference between Test 1 and the controls is not significantly different from zero.

(xiii) Only for Test 3 is there evidence for a non-zero difference; i.e., that the effluent DOC for the test pot is significantly greater than for the controls. In this case, however, the result is highly significant since the calculated t-value exceeds the critical value by a considerable margin. If analysis for disappearance of parent compound had been conducted simultaneously with DOC analysis and showed complete removal, then these results could be taken as evidence for formation of water-soluble degradation products in Test pot 3.

(m) **Quality assurance.** To assure the integrity of data developed using this method and to comply with current regulatory requirements, a quality assurance program meeting EPA, FDA, or OECD guidelines should be followed. This may require replicates (three or more) to be run for good laboratory practice (GLP) compliance and assessment of variability.

(n) **Documentation to be provided.** (1) A protocol giving a general overview of the study goals and procedures must be prepared before the study is initiated. If a substantive modification of this method is deemed necessary for the test compound, deviation from the method should be documented in the protocol.

(2) Final results of this study are to be documented in a final report. The final report should include the following:

(i) Names of study, investigators, and laboratory.

(ii) A brief description of the test compound including its identifying number in the log, chemical names, composition, and other appropriate information.

(iii) Summary of test method including deviations from the written method.

(iv) Summary of specific analytical methods, if employed.

(v) If applicable, tabular and graphical presentation of DOC removal data as a function of time after test initiation. Data are expressed as percent DOC removal (weekly mean for 24-h periods).

(vi) If applicable, tabular and graphical presentation of specific compound analysis data as a function of time after test initiation. Data are expressed as steady state concentrations of test compound in influents and effluents, percent DOC removal, or primary biodegradation during 24-h periods.

(vi) A listing of relevant references including all notebook pages containing raw data from this study.

(vii) The following raw data should be recorded:

(A) Date.

(B) Pot number.

(C) Sewage flow.

(D) Temperature of mixed liquor.

(E) Volume of test compound dosing solution remaining.

(F) Time of replacement of dosing solution.

(G) pH of mixed liquor.

(H) Dissolved oxygen concentration (DOC) of mixed liquor.

(I) Weight of test compound used to prepare concentrated stock solutions.

(J) Volume of activated sludge mixed liquor wasted.

(K) Date of start of calculation period.

(L) Change of porous pot liners.

(M) Faults with tubing, pumps, sewage or air supply.

(N) Signature of operator.

(O) Study number.

(P) Calibration of pH meter.

(Q) Calibration of DO meter.

(o) **References.** The following references should be consulted for additional background material on this test guideline.

(1) American Society for Testing and Materials (ASTM). Annual Book of ASTM Standards, Volumes 11.01 and 11.02 on Water and Environmental Technology, and Volume 14.02 on General Methods and Instrumentation (1993).

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(4) Department of the Environment. Standing Committee of Analysts. National Water Council. U.K. 1983. Assessment of biodegradability 1981. Methods for the examination of waters and associated materials. Continuous Simulation (Activated Sludge) Test for the Assessment of Biodegradability, Volume 1981. Her Majesty's Stationery Office, London.

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(6) Organization for Economic Cooperation and Development (OECD). Activated Sludge Respiration Inhibition Test. OECD 209, OECD, Paris (1981).

(7) Painter, H.A. and E.F. King. WRC porous pot method for assessing biodegradability. WRC Technical Report TR70 (1978).

(8) Painter, H.A. and E.F. King. The effect of phosphate and temperature on the growth of activated sludge and on the biodegradation of surfactants. *Water Research* 12: 909–915 (1978).

(9) Waters, J. et al. A new rinse conditioner active with improved environmental properties. *Tenside Surfactants Detergents* 28:460–468 (1991).

(10) Watson, H.M. Important considerations in choosing a synthetic feed for laboratory-scale wastewater treatment systems. *Environmental Toxicology and Risk Assessment: 2nd Volume*, STP 1216. J.W. Gorsuch et al., Eds., American Society for Testing and Materials, Philadelphia, PA, pp. 228-239 (1993).