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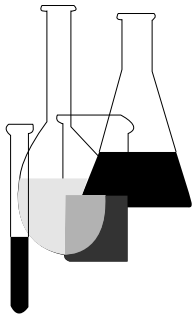
Fate, Transport and Transformation Test Guidelines

OPPTS 835.1110 Activated Sludge Sorption Isotherm



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.*).

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OPPTS 835.1110 Activated sludge sorption isotherm.

(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 referenced under paragraphs (f)(1) through (f)(10) of this guideline.

(b) **Introduction.** The sorption of chemical compounds to activated sludge biomass in biological wastewater treatment systems is an important process that affects the distribution of the compounds between solid, aqueous, and vapor phases. If a chemical compound is sorbed to sludge biomass, it may be removed from the system along with other solids by clarification. If a compound is not sorbed, it will remain in the aqueous phase where it is subject to removal via biodegradation, chemical interactions, and/or volatilization. A nonsorbing, nonbiodegradable, noninteracting, nonvolatile compound will pass through a biological treatment system unaffected. Information on sorption potential is needed to assess the possibility for the removal of chemical compounds in biological wastewater treatment systems. This test guideline describes a procedure for the determination of the sorption potential of activated sludge solids for removal of specific chemical compounds.

(c) **Discussion.** (1) This guideline describes a procedure for measuring the extent to which a chemical compound distributes itself between activated sludge as the sorbent and water as the solvent. The equation describing that relationship is called a sorption isotherm. The chemical compound solubilized at a constant concentration in the solvent is contacted with a measured quantity of the sorbent for a time period sufficient to attain sorption equilibrium. Data are obtained for various sorbent dosages. The amount of compound remaining in solution is determined analytically and the amount of compound sorbed is calculated for each sorbent dosage. A range of sorbent dosages is selected to achieve optimal spread of the data and to reflect biomass concentrations in an actual wastewater treatment system.

(2) There are several common models for describing a sorption isotherm. Historically, each model has been based on either an empirical or a theoretical equation. The three most common models are the Freundlich, the Langmuir, and the Brunauer, Emmett, and Teller (BET). The Freundlich model is most widely used to describe sorption in dilute wastewater systems and is employed in this test guideline.

(i) The basic form of the Freundlich equation is as follows:

$$X/M = K \cdot C_e^{1/n}$$

where

C_0 = initial concentration of the chemical compound in solution expressed in grams per liter

C_e = final concentration of the chemical compound in solution expressed in grams per liter

X = amount of test compound sorbed =

$$[(C_0 \times \text{solution volume (L)}) - (C_e \times \text{solution volume (L)})]$$

M = mass of sorbent expressed in grams

K = Freundlich sorption coefficient

1/n = exponent where n is a constant

(ii) K and 1/n are the sorption constants and are unique for each isotherm. In order to determine K and 1/n graphically, the Freundlich equation is plotted on a log-log scale to yield a linear relationship. X/M is plotted along the Y-axis and C_e along the X-axis. The data will fit the logarithmic form of the Freundlich equation, as follows:

$$\log X/M = \log K + 1/n \log C_e$$

(iii) K is the X/M intercept when $C_e = 1$, and 1/n is the slope of the line when plotted. The logarithmic plot of the equation is termed the isotherm plot.

(3) In order to assess the potential for relative sorptive removal of the chemical compound, the values K and 1/n are compared with the values for other chemicals whose behavior in activated sludge treatment systems is documented.

(4) A sorption isotherm is dependent on the conditions under which it is determined. Sorption can be greatly affected by changes in temperature, pH, and test chemical concentration. Therefore, each of these parameters must be held constant during a sorption isotherm test.

(d) **Sorption isotherm test**—(1) **General.** (i) The papers by Aharonson and Kafkafi (1975), Goring and Hamaker (1972), Harvey (1974), Lieberman (1986), Murray (1975), Saltzman (1972), Weber (1971), and Wu (1975), under paragraphs (f)(1) through (f)(8) of this guideline, served as the basis for this test guideline. For additional information on conducting sorption isotherm experiments, the reader is referred to OPPTS 835.1220, Sediment and Soil Adsorption/Desorption Isotherm. The soil and colloid chemistry literature and the analytical chemistry lit-

erature substantiate the experimental conditions and procedures specified in this guideline as accepted, standard procedures. These procedures have been modified slightly to allow the use of activated sludge solids.

(ii) Any procedure designed to determine the sorption potential of activated sludge for the test compound should use lyophilized, desiccated sludge solids as the sorbent. Lyophilization freeze-dries the microorganisms in sludge and allows the sorption potential to be measured without interference by biodegradation of the test compound. After lyophilization many of the microorganisms are still viable, but desiccation (drying in an oven) should inactivate the remaining microorganisms. The flocculation and settling properties of lyophilized, desiccated sludge resemble those of active sludge biomass upon rehydration. Photomicrographs of this material reveal the presence of structurally intact microorganisms (see the report referenced in paragraph (f)(9) of this guideline). Furthermore, sludge subjected to this treatment has been shown to have a sorption capacity similar to that of viable sludge solids (see paragraph (f)(10) of this guideline).

(iii) Lyophilization is an expensive process with a significant capital investment. For the purpose of this test guideline, it is assumed that the lyophilization process will be contracted to a qualified vendor, for which cost should be less significant (less than $500 for several grams of sludge).

(2) **Activated sludge collection and preparation.** (i) The activated sludge to be used as a sorbent should be collected from the most concentrated source available within an activated sludge treatment process; i.e., the return activated sludge (RAS) line. If the solids concentration of the sludge to be collected and the amount of sorbent required for the isotherm test are known, the volume of sludge that needs to be collected can be determined. About 2 to 3× the volume calculated should be collected as a safety measure. The shelf life of lyophilized sludge has not been determined systematically, but based on empirical observation it should be approximately 6 mo. As a result, excess sludge may be collected, prepared, and held for future testing as outlined under paragraphs (d)(2)(ii) through (d)(2)(v) of this guideline.

(ii) The sludge solids to be lyophilized need to be separated from the wastewater present in the sludge. In the first step collected sludge is allowed to settle for 15 to 30 min before the supernatant is decanted. Care must be taken to remove only the supernatant and leave the solids.

(iii) Further mechanical separation (e.g. centrifugation) of the solids from the water is required in order to achieve a sludge suitable for lyophilization. To accomplish this, settled solids are placed in a centrifuge vessel and centrifuged for 5 min. After centrifugation the supernatant is decanted and the sludge solids remain in the centrifuge vessel.

(iv) Washing of the solids is recommended to remove any color or matrix materials. Washing can be accomplished by filling the centrifuge

vessel with laboratory-grade water and suspending the sludge solids in the water, followed by centrifugation and decantation of the supernatant. Sludge solids should be washed and centrifuged 3 times. They can be stored at 4 °C until lyophilization can be undertaken by a vendor, but sludge should not be held for longer than 24 hr prior to starting lyophilization because changes in the sludge are likely to occur after this even when the sludge is held at 4 °C.

(v) After the lyophilization process is complete, the solids will be in the form of a dry cake. This cake should be broken into a dry powder by gently passing it through a mesh screen. The powder should be desiccated at 103 °C for 3 h or more prior to use as a sorbent.

(3) **Procedure**—(i) **Test conditions.** (A) The isotherm test should be run at constant temperature and pH. Temperature control can be accomplished using a bath or constant temperature room. pH should be controlled using buffers. Preliminary tests should be run to assure that there are no interactions between test compound and buffer (such as complexation with the buffer) that could affect sorption of the test compound. This test is accomplished by dosing the test compound into a mixture of buffer and clean water at the isotherm test's target concentration. The dosed mixture should be mixed for a time period equal to the anticipated duration of the isotherm test. Analytical tests and visual observation of the test compound in the buffered mixture should be performed to verify that no chemical interactions or buffer-catalyzed degradation of the test compound has occurred.

(B) A sorption isotherm plot can be generated with two sorbent dosages. However, the accuracy of the isotherm plot is increased if more data points are generated. Generally, six to eight points are needed to produce an accurate plot.

(C) Normal quality assurance/quality control practices (QA/QC) should be employed when conducting the isotherm test in order to assure that valid data are generated. A test solution control and a sorbent control should be included as part of the isotherm test. The test solution control consists of a sample of the test compound dosed at the target concentration into buffered water with no sorbent present. This control is used to verify the initial concentration of test compound for each reaction vessel and to indicate if any degradation or interaction of the test compound with buffer occurs during the test period. The sorbent control consists of a sample of the sorbent in buffered water without test compound. Analysis of this sample will indicate if any color or matrix interferences were caused by the sorbent.

(ii) **Equilibration.** (A) Weigh sludge solids into six to eight individual isotherm reaction vessels to yield final concentrations of sludge solids that will achieve varying degrees of sorption of the test compound. Test

vessel volume is not specified in this test guideline because it is not critical as long as the vessel is large enough to allow for good mixing. The vessels should be of a constant and known volume.

(B) Place a stirring bar or other mixing device in each reaction vessel. Contents of test vessels must not be mixed by bubbling gas through them because this could remove test compound by stripping.

(C) If necessary, bring the buffered test substance/solids mixtures in the reaction vessels up to the correct volume with measured quantities of laboratory-grade water and buffer.

(D) Stir mixtures in the reaction vessels for 0.5 h. Stirring should be vigorous enough to keep the sorbent in suspension without being so vigorous as to break it up.

(E) Measure the pH and temperature of the contents of each reaction vessel while stirring to verify the target conditions for the test.

(F) Dose each reaction vessel and the test solution control while stirring by pipeting in a calculated volume of test compound. This will bring the solution in the vessel up to a final specified volume and test compound concentration. The dosing process should be completed quickly and with precision, in order to achieve a uniform starting time for all reaction vessels.

(G) Stir the test solutions until sludge and test compound have reached equilibrium. How long this takes may have to be determined by trial and error.

(iii) **Centrifugation.** (A) At the end of the stirring period, transfer a representative sample of the mixture from each reaction vessel into a clean centrifuge tube. The volume of mixture that needs to be collected is determined by the analytical method employed to detect and quantify the test compound. Contents of the reaction vessels should be stirred continuously while samples are being collected.

(B) Centrifuge the contents of each tube at 2,000 × g for 5 min in order to provide adequate separation of solid and liquid phases for subsequent analysis.

(4) **Analysis for test compound.** (i) After centrifugation, remove a volume of the supernatant sufficient for analysis from each tube, pass it through a 0.45 μm pore-size filter previously shown not to sorb test compound, and place the filtrate in a clean sample bottle.

(ii) Analyze the aqueous phase for the presence and amount of test compound using an appropriate analytical method. QA/QC procedures should be employed for verification of analytical precision.

(iii) If the analysis for test compound must be delayed for any reason after the samples have been filtered, store samples at 0 to 4 °C. Samples should not be stored for more than 24 h before analysis.

(5) **Analysis of test data.** (i) After the isotherm test has been performed and analysis for test compound is complete, tabulate the data generated using the following format, representative of a Freundlich model:

Data Collection Format

M	C_e	X	X/M
Activated sludge	Test chemical	Test chemical sorbed [(C_0 × sol'n volume in L) – (C_e × sol'n volume in L)]	Grams test chemical sorbed per gram sludge
mass in grams	g/L	mass in grams	
.....	.....	.....	.....
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(ii) Plot X/M vs. C_e on log-log paper, and calculate the line of best fit to yield the factors K and 1/n for the isotherm.

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

(1) Test conditions: Temperature and pH at which the test was conducted.

(2) Detailed description of the analytical techniques used in the recovery and quantitative analysis for test compound.

(3) Amount of test compound dosed (C_0 × solution volume), and the amount recovered in each reaction vessel (C_e × solution volume).

(4) Volume of each reaction vessel and total volume of the sorbent/sorbate mixture.

(5) QA/QC data such as duplicate analyses, background interferences, spikes, matrix spikes, etc.

(6) Graphical plots of log X/M as a function of log C_e, and the values of K and 1/n determined from the plots.

(7) Sludge solids information: Sampling location, observations, calculations for volume sampled, lyophilization vendor, sample custody, description of the lyophilized sludge, desiccation time.

(8) Any unusual observations made during the experiments.

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

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(2) Goring, C.A. and J.W. Hamaker, Eds. *Organic chemicals in the soil environment*, vol. I and II. Marcel Dekker, NY (1972).

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(4) Lieberman, R.J. Technical status report for adsorption of azo dyes onto activated sludge solids. Report prepared under EPA contract no. 68–03–3183. USEPA Risk Reduction Engineering Laboratory, Cincinnati, OH (1986).

(5) 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).

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

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

(9) USEPA. Prediction of the Fate of Organic Chemicals in Activated Sludge Wastewater Treatment Processes. EPA/600/2–85–102 (1985).

(10) Hopkins, B.T. *et al.* Activated sludge adsorption methodology: effect of biomass inactivation and incubation conditions. Presented at the Society of Environmental Chemistry and Toxicology (SETAC) 13th Annual Meeting, November 8–12, Cincinnati, OH. Abstract no. WA4A18 (1992).