(i) *Reporting requirements.* The reports submitted under this section must include the information specified in paragraph (h)(7) of this section. A final report for each chemical substance must be received by EPA by June 27, 2005, unless an extension is granted in writing pursuant to 40 CFR 790.55.

(j) *Designation of specific chemical substances for testing.* The chemical substances identified by chemical name, CAS No., and class in Table 2 of this paragraph must be tested in accordance with the testing requirements in paragraph (h) of this section and the requirements described in 40 CFR part 792.

TABLE 2—CHEMICAL SUBSTANCES DESIGNATED FOR TESTING

CAS No.	Chemical name	Class
75–05–8	Acetonitrile	1
75–15–0	Carbon disulfide	1
75–35–4	Vinylidene chloride	1
77–73–6	Dicyclopentadiene	1
78–59–1	Isophorone	1
78–87–5	Propylene dichloride	1
79–20–9	Methyl acetate	1
79–46–9	2-Nitropropane	1
91–20–3	Naphthalene	1
92–52–4	Biphenyl	1
98–29–3	*tert*-Butylcatechol	1
100–00–5	*p*-Nitrochlorobenzene	1
100–01–6	*p*-Nitroaniline	1
100–44–7	Benzyl chloride	1
106–42–3	*p*-Xylene	1
106–46–7	*p*-Dichlorobenzene	1
107–06–2	Ethylene dichloride	1
107–31–3	Methyl formate	1
108–03–2	1-Nitropropane	1
108–90–7	Chlorobenzene	1
108–93–0	Cyclohexanol	1
109–66–0	Pentane	1
109–99–9	Tetrahydrofuran	1
110–12–3	Methyl isoamyl ketone	1
111–84–2	Nonane	1
120–80–9	Catechol	1
122–39–4	Diphenylamine	1
123–42–2	Diacetone alcohol	1
127–19–5	Dimethyl acetamide	1
142–82–5	*n*-Heptane	1
150–76–5	*p*-Methoxyphenol	1
25013–15–4	Vinyl toluene	2
34590–94–8	Dipropylene glycol methyl ether.	2

(k) *Effective date* This section is effective on May 26, 2004.

[69 FR 22436, Apr. 26, 2004, as amended at 71 FR 18654, Apr. 12, 2006]

Subpart E—Product Properties Test Guidelines

§ 799.6755 TSCA partition coefficient (*n*-octanol/water), shake flask method.

(a) *Scope*—(1) *Applicability.* This section is intended to meet the testing requirements of the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) *Source.* The source material used in developing this TSCA test guideline is the Office of Prevention, Pesticides and Toxics (OPPTS) harmonized test guideline 830.7550 (August 1996, final guideline). The source is available at the address in paragraph (f) of this section.

(b) *Introductory information*—(1) *Prerequisites.* Suitable analytical method, dissociation constant, water solubility, and hydrolysis (preliminary test).

(2) *Coefficient of variation.* The coefficient of variation on the mean values reported by the participants of the Organization for Economic Coopertion and Development (OECD) Laboratory Intercomparison Testing, Part I, 1979, appeared to be dependent on the chemicals tested; it ranges from 0.17 to 1.03.

(3) *Qualifying statements.* This method applies only to pure, water soluble substances which do not dissociate or associate, and which are not surface active. In order to use the partition coefficient (P) as a screening test for bioaccumulation, it should be ascertained that the impurities in the commercial product are of minor importance. Testing of P (*n*-octanol/water) cannot be used as a screening test in the case of organometallic compounds.

(4) *Alternative methods.* High-pressure liquid chromatography (HPLC) methods described in the references in paragraphs (f)(3), (f)(4), and (f)(5) of this section may be considered as an alternative test method.

(c) *Method*—(1) *Introduction, purpose, scope, relevance, application, and limits of test.* The P of a substance between water and a lipophilic solvent (*n*-octanol) is one model variable which may be used to describe the transfer of a

substance from the aquatic environment into an organism and the potential bioaccumulation of the substance. Studies show a highly significant relationship between the P of different substances in the system water/*n*-octanol and their bioaccumulation in fish described in paragraph (f)(1) of this section.

(2) *Definitions—Partition coefficient* (P) is defined as the ratio of the equilibrium concentrations (C_i) of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. The P therefore is the quotient of two concentrations and is usually given in the form of its logarithm to base 10 (log P). In this case *n*-octanol and water:

Equation 1:

$$P_{ow} = C_{n\text{-octanol}}/C_{water}$$

(3) *Reference substances.* 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. The values presented in table 1 of this section are not necessarily representative of the results which can be obtained with this test method as they have been derived from an earlier version of the test guideline.

TABLE 1—DATA FOR REFERENCE SUBSTANCES

Tested substance [1]	P_{ow} [2]	
Di(2-ethylhexyl)phthalate (OECD)	1.3×10^5	$(4.6 \times 10^4 - 2.8 \times 10^5)$
Hexachlorobenzene (OECD)	3.6×10^5	$(1.1 \times 10^5 - 8.3 \times 10^5)$
o-Dichlorobenzene European Economic Community (EEC)	5.1×10^3	$(1.5 \times 10^3 - 2.3 \times 10^4)$
Dibutyl phthalate (EEC)	1.3×10^4	$(1.7 \times 10^3 - 2.8 \times 10^4)$
Trichloroethylene (OECD)	2.0×10^3	$(5.2 \times 10^2 - 3.7 \times 10^3)$
Urea (OECD)	6.2×10^{-2}	$(2.0 \times 10^{-2} - 2.4 \times 10^{-1})$

[1] Substances not tested: Ethyl acetate, 4-methyl-2,4-pentanediol.
[2] Total, mean, and range of mean values (in parentheses) submitted by the participants of the OECD or EEC Laboratory Intercomparison Testing.

(4) *Principle of the test method.* In order to determine a P, equilibrium between all interacting components of the system must be achieved, and the concentrations of the substances dissolved in the two phases must be determined. A study of the literature on this subject indicates that there are many different techniques which can be used to solve this problem, i.e. the thorough mixing of the two phases followed by their separation in order to determine the equilibrium concentration for the substance being examined.

(5) *Quality criteria—*(i) *Repeatability.* In order to assure the precision of the P, duplicate determinations are to be made under three different test conditions, whereby the quantity of substance specified as well as the ratio of the solvent volumes may be varied. The determined values of the P expressed as their common logarithms should fall within a range of ±0.3 log units.

(ii) *Sensitivity.* The sensitivity of the method is determined by the sensi-

tivity of the analytical procedure. This should be sufficient to permit the assessment of values of P_{ow} up to 105 when the concentration of the solute in either phase is not more than 0.01 mol/ Liter (L). The substance being tested must not be water insoluble (mass concentration $\rho \leq 10^{-6}$ gram (g)/L.

(iii) *Specificity.* The Nernst Partition Law applies only at constant temperature, pressure, and pH for dilute solutions. It strictly applies to a pure substance dispersed between two pure solvents. If several different solutes occur in one or both phases at the same time, this may affect the results. Dissociation or association of the dissolved molecules result in deviations from the Nernst Partition Law. Such deviations are indicated by the fact that the P becomes dependent upon the concentration of the solution. Because of the multiple equilibria involved, this test guideline should not be applied to

ionizable compounds without corrections being made. The use of buffer solutions in place of water should be considered for such compounds.

(iv) *Possibility of standardization.* This method can be standardized.

(d) *Description of the test procedure—* (1) *Preparations: Preliminary estimate of the P.* The size of the P can be estimated either by means of calculation or by use of published solubilities of the test substance in the pure solvents. Alternatively, it may be roughly determined by performing a simplified preliminary test. For this:

Equation 2:

$$P_{estimate} = \left(\text{saturation } C_{n\text{-octanol}} \right) / \left(\text{saturation } C_{water} \right)$$

(2) *Preparation of the solvents—*(i) *n-Octanol.* The determination of the P should be carried out with analytical grade *n*-octanol. Inorganic contaminants can be removed from commercial *n*-octanol by washing with acid and base, drying, and distilling. More sophisticated methods will be required to separate the *n*-octanol from organic contaminants with similar vapor pressure if they are present.

(ii) *Water.* Distilled water or water twice-distilled from glass or quartz apparatus should be employed. Water taken directly from an ion exchanger should not be used.

(iii) *Presaturation of the solvents.* Before a P is determined, the phases of the solvent system are mutually saturated by shaking at the temperature of the experiment. For doing this, it is practical to shake two large stock bottles of purified *n*-octanol or distilled water each with a sufficient quantity of the other solvent for 24 hours on a mechanical shaker, and then to let them stand long enough to allow the phases to separate and to achieve a saturation state.

(3) *Preparation for the test.* The entire volume of the two-phase system should nearly fill the test vessel. This will help prevent loss of material due to volatilization. The volume ratio and quantities of substance to be used are fixed by the following:

(i) The preliminary assessment of the P as discussed in paragraph (d)(1) of this section).

(ii) The minimum quantity of test substance required for the analytical procedure.

(iii) The limitation of a maximum concentration in either phase of 0.01 mol/L.

(iv) Three tests are carried out. In the first, the calculated volume ratio is added; in the second, twice the volume of *n*-octanol is added; and in the third, half the volume of *n*-octanol is added.

(4) *Test substance.* The test substance should be the purest available. For a material balance during the test a stock solution is prepared in *n*-octanol with a mass concentration between 1 and 100 milligram/milliliter (mg/mL). The actual mass concentration of this stock solution should be precisely determined before it is employed in the determination of the P. This solution should be stored under stable conditions.

(5) *Test conditions.* The test temperature should be kept constant (±1 °C) and lie in the range of 20–25 °C.

(6) *Performance of the test—*(i) *Establishment of the partition equilibrium.* Duplicate test vessels containing the required, accurately measured amounts of the two solvents together with the necessary quantity of the stock solution should be prepared for each of the test conditions. The *n*-octanol parts should be measured by volume. The test vessels should either be placed in a suitable shaker or shaken by hand. A recommended method is to rotate the centrifuge tube quickly through 180° about its transverse axis so that any trapped air rises through the two phases. Experience has shown that 50 such rotations are usually sufficient for the establishment of the partition equilibrium. To be certain, 100 rotations in 5 minutes are recommended.

(ii) *Phase separation.* In order to separate the phases, centrifugation of the mixture should be carried out. This should be done in a laboratory centrifuge maintained at room temperature, or, if a non-temperature-controlled centrifuge is used, the centrifuge tubes should be reequilibrated at the test temperature for at least 1 hour before analysis.

(7) *Analysis.* (i) For the determination of the P, it is necessary to analyze the concentrations of the test substance in both phases. This may be done by taking an aliquot of each of the two phases from each tube for each test condition and analyzing them by the chosen procedure. The total quantity of substances present in both phases should be calculated and compared with the quantity of the substance originally introduced.

(ii) The aqueous phase should be sampled by the following procedure to minimize the risk of including traces of the *n*-octanol: A glass syringe with a removable needle should be used to sample the water phase. The syringe should initially be partially filled with air. Air should be gently expelled while inserting the needle through the *n*-octanol layer. An adequate volume of aqueous phase is withdrawn into the syringe. The syringe is quickly removed from the solution and the needle detached. The contents of the syringe may then be used as the aqueous sample.

(iii) The concentration in the two-separated phases should preferably be determined by a substance-specific method. Examples of physical-chemical determinations which may be appropriate are:

(A) Photometric methods.

(B) Gas chromatography.

(C) HPLC.

(D) Back-extraction of the aqueous phase and subsequent gas chromatography.

(e) *Data and reporting*—(1) *Treatment of results.* The reliability of the determined values of P can be tested by comparison of the means of the duplicate determinations with the overall mean.

(2) *Test report.* The following should be included in the report:

(i) Name of the substance, including its purity.

(ii) Temperature of the determination.

(iii) The preliminary estimate of the P and its manner of determination.

(iv) Data on the analytical procedures used in determining concentrations.

(v) The measured concentrations in both phases for each determination. This means that a total of 12 concentrations must be reported.

(vi) The weight of the test substance, the volume of each phase employed in each test vessel, and the total calculated amount of test substance present in each phase after equilibration.

(vii) The calculated values of the P and the mean should be reported for each set of test conditions as should the mean for all determinations. If there is a suggestion of concentration dependency of the P, this should be noted in the report.

(viii) The standard deviation of individual P values about their mean should be reported.

(ix) The mean P from all determinations should also be expressed as its logarithm (base 10).

(f) *References.* For additional background information on this test guideline, the following references should be consulted. These references are available from the TSCA Nonconfidential Information Center, Rm. NE-B607, Environmental Protection Agency, 401 M St., SW., Washington, DC, 12 noon to 4 p.m., Monday through Friday, excluding legal holidays.

(1) Neely, W.B. et al. Partition Coefficients to Measure Bioconcentration Potential of Organic Chemicals in Fish. *Environmental Science and Technology* 8:1113 (1974).

(2) Leo, A. et al. Partition Coefficients and Their Uses. *Chemical Reviews* 71:525 (1971).

(3) Miyake, K. and H. Terada, Direct measurements of partition coefficients in an octanol-water system. *Journal of Chromatography* 157:386 (1978).

(4) Veith G.D. and R.T. Morris, A Rapid Method for Estimating Log P for Organic Chemicals, EPA–600/3–78–049 (1978).

(5) Mirrless, M.S. et al., Direct measurement of octanol-water partition coefficient by high pressure liquid chromatography. *Journal of Medicinal Chemistry* 19:615 (1976).

(6) EPA Draft Guidance of September 8, 1978 (F–16).

(7) Konemann H. et al. Determination of log P_{oct} values of chlorosubstituted benzenes, toluenes, and anilines by high performance liquid chromatography on ODS silica, *Journal of Chromatography* 178:559 (1979).

(8) *Organization for Economic Cooperation and Development,* Guidelines for The Testing of Chemicals, OECD 107, Partition Coefficient (*n*-octanol/water) (Shake Flask Method, Adopted 27 July 1995), OECD, Paris, France.

§ 799.6756 TSCA partition coefficient (*n*-octanol/water), generator column method.

(a) *Scope*—(1) *Applicability.* This section is intended to meet the testing requirements of the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) *Source.* The source material used in developing this TSCA test guideline is the Office of Pollution Prevention, Pesticides and Toxic Substances (OPPTS) harmonized test guideline 830.7560 (August 1996, final guideline). This source is available at the address in paragraph (e) of this section.

(b)(1) *Purpose.* (i) The measurement and estimation of the *n*-octanol/water partition coefficient (K_{ow}), has become the cornerstone of a myriad of structure-activity relationships (SAR) property. The coefficient has been used extensively for correlating structural changes in drugs with changes observed in biological, biochemical, or toxic effects. These correlations are then used to predict the effect of a new drug for which a K_{ow} could be measured.

(ii) In the study of the environmental fate of organic chemicals, the K_{ow} has become a key parameter. K_{ow} is correlated to water solubility, soil/sediment sorption coefficient, and bioconcentration and is important to SAR.

(iii) Of the three properties that can be estimated from K_{ow}, water solubility is the most important because it affects both the fate and transport of chemicals. For example, highly soluble chemicals become quickly distributed by the hydrologic cycle, have low-sorption coefficients for soils and sediments, and tend to be more easily degraded by microorganisms. In addition, chemical transformation processes such as hydrolysis, direct photolysis, and indirect photolysis (oxidation) tend to occur more readily if a compound is soluble.

(iv) Direct correlations between K_{ow} and both the soil/sediment sorption coefficient and the bioconcentration factor are to be expected. In these cases, compounds that are more soluble in *n*-octanol (more hydrophobic and lipophilic) would be expected to partition out of the water and into the organic portion of soils/sediments and into lipophilic tissue. The relationship between K_{ow} and the bioconcentration factor, are the principal means of estimating bioconcentration factors. This relationship is discussed in the reference listed in paragraph (e)(14) of this section. These factors are then used to predict the potential for a chemical to accumulate in living tissue.

(v) This section describes a method for determining the K_{ow} based on the dynamic coupled column liquid chromatographic (DCCLC) technique, a technique commonly referred to as the generator column method. The method described herein can be used in place of the standard shake-flask method specified in § 799.6755 for compounds with a $log_{10}K_{ow}$ greater than 1.0.

(2) *Definitions.* The following definitions apply to this section.

Extractor column is used to extract the solute from the aqueous solution produced by the generator column. After extraction onto a bonded chromatographic support, the solute is eluted with a solvent/water mixture and subsequently analyzed by high-performance liquid chromatography (HPLC), gas chromatography (GC), or any other analytical procedure. A detailed description of the preparation of the extractor column is given in paragraph (c)(1)(i) of this section.

Generator column is used to partition the test substance between the *n*-octanol and water phases. The column in figure 1 in paragraph (c)(1)(i)(A)(*2*) of