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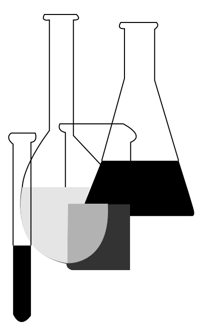
Fate, Transport and Transformation Test Guidelines

OPPTS 835.1210
Soil Thin Layer Chromatography



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 835.1210 Soil thin layer chromatography.

(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 is 40 CFR 796.2700 Soil Thin Layer Chromatography.

(b) **Introduction—**(1) **Background and purpose.** (i) Leaching of chemicals through soil is an important process which affects a chemical's distribution in the environment. If a chemical is tightly adsorbed to soil particles, it will not leach through the soil profile but will remain on the soil surface. If a chemical is weakly adsorbed, it will leach through the soil profile and may reach ground waters and then surface waters. Knowledge of the leaching potential is essential under certain circumstances for the assessment of the fate of chemicals in the environment.

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

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

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

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

Name	Diameter range (mm)
Very coarse sand	2.0 to 1.0
Coarse sand	1.0 to 0.5
Medium sand	0.5 to 0.25
Fine sand	0.25 to 0.125
Very fine sand	0.125 to 0.062

Name	Diameter range (mm)
Silt	0.062 to 0.002
Clay	<0.002

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

(iv) *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.

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

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

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

(viii) *Soil order* is the broadest category of soil classification and is based on general similarities of physical/chemical properties. The formation by similar genetic processes causes these similarities. The soil orders found in the United States are: Alfisol, aridisol, entisol, histosol, inceptisol, mollisol, oxisol, spodosol, ultisol, and vertisol.

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

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

(xi) *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).

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

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

(ii) Papers by Helling (1968, 1971) under paragraphs (e) (5), (6), and (7) of this guideline and Helling and Turner (1968) under paragraph (e)(3) of this guideline were the basis of this test guideline. The soil and colloid chemistry literature and the analytical chemistry literature substantiate the experimental conditions specified in the guideline.

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

(4) **Applicability and specificity.** (i) Soil TLC can be used to determine the soil mobility of sparingly to infinitely water soluble chemicals. In general, a chemical having a water solubility of less than 0.5 ppm need not be tested since the literature indicates that these chemicals are, in general, immobile (see Goring and Hamaker (1972) under paragraph (e)(1)) of this guideline. However, this does not preclude future soil adsorption/transformation testing of these chemicals if more refined data are needed for the assessment process.

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

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

(c) **Test procedures—**(1) **Test conditions.** (i) Equipment required—distilled-deionized water adjusted to pH 7 by boiling to remove CO_2, clean glass plates (TLC plates), glass rods or a variable thickness plate spreader, masking tape, closed chromatographic chambers, and analytical instrumentation necessary and appropriate for the detection and quantitative analysis of the test chemical.

(ii) The test procedure may be run at 23±5 °C.

(iii) It is recommended that three replicate plates for each soil be used.

(2) **Test procedures.** (i) To reduce aggregate size before or during sieving, crush and grind the air-dried soil very, very gently.

(ii) Sieve air-dried soils with a 250 µm sieve.

(iii) Add water to the sieved soil until a smooth, moderately fluid slurry is attained (approximately 0.75 mL H_2O added for each gram of soil).

(iv) Spread the slurry evenly and quickly across the clean glass plate using a variable thickness plate spreader, a glass rod, or other available method. If a glass rod is used, control the layer thickness by affixing multiple layers of masking tape along the plate edges. Soil layer thickness should be 0.50–0.75 mm.

(v) Air dry the plates at 25 °C for a minimum of 24 h after uniform slurry application is achieved.

(vi) Scribe a horizontal line 11.5 cm above the base through the soil layer down to the glass to stop solvent movement.

(vii) Spot the test chemical, in solution, 1.5 cm above the base. For radiolabeled materials, 0.5–5 µg containing 0.01–0.03 µCi of ^{14}C-labeled compound may be used.

(viii) If the compound is volatile, it is extremely important that a clean plate be placed over the soil TLC plate to impede volatilization.

(ix) Immerse the plate with the base down at some angle from the vertical in a closed chromatographic chamber containing water at a height of 0.5 cm.

(x) Allow the solvent front to migrate to the 11.5 cm line before removing the plates from the chamber.

(xi) Determine the R_f values. Zonal extraction, plate scanning, or any other method or combination of methods suitable for detection of the parent test chemical may be used.

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

(d) **Data and reporting.** Report the following information. (1) Temperature at which the test was conducted.

(2) Amount of the test chemical applied and amount recovered from the plates.

(3) Detailed description of the analytical technique used in the R_f determination, the chemical extraction, and the quantitative recovery and analysis of the parent chemical.

(4) The mean frontal R_f value with the standard deviation for each soil tested.

(5) A photograph or diagram of the TLC plate which shows the entire leaching pattern (from 1.5 to 11.5 cm).

(6) Soil information: Soil order, series, texture, sampling location, horizon, general clay fraction mineralogy.

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

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

(1) Goring, C.A.I. and Hamaker, J.W. *Organic Chemicals in the Soil Environment.* Vol. I & II. Marcel Dekker, NY (1972).

(2) Helling, C.S. Pesticide mobility investigations using soil thin layer chromatography. *American Society for Agronomy Abstracts* (1968).

(3) Helling, C.S. and Turner, B.C. Pesticide mobility: Determination by soil thin layer chromatography, *Science* 162:562 (1968).

(4) Helling, C.S., Movement of *S*-triazine herbicides in soils, *Residue Review* 32:175–210 (1970).

(5) Helling, C.S. Pesticide mobility in soils I. Parameters of soil thin layer chromatography. *Soil Science Society of America Proceedings* 35:732–737 (1971).

(6) Helling, C.S. Pesticide mobility in soils II. Applications of soil thin layer chromatography. *Soil Science Society of America Proceedings* 35:737–743 (1971).

(7) Helling, C.S. Pesticide mobility in soils III. Influence of soil properties, *Soil Science of America Proceedings*, 35:743–748 (1971).