

OECD GUIDELINES FOR THE TESTING OF CHEMICALS

Leaching in Soil Columns

INTRODUCTION

1. Man-made chemicals may reach soil directly via deliberate application (e.g. agrochemicals) or via indirect routes (e.g. via waste water → sewage sludge → soil or air → wet/dry deposition). For risk assessment of these chemicals, it is important to estimate their potential for transformation in soil and for movement (leaching) into deeper soil layers and eventually into groundwater.
2. Several methods are available to measure the leaching potential of chemicals in soil under controlled laboratory conditions, i.e. soil thin-layer chromatography, soil thick-layer chromatography, soil column chromatography, and adsorption - desorption measurements (1)(2). For non-ionised chemicals, the n-octanol-water partition coefficient (P_{ow}) allows an early estimation of their adsorption and leaching potential (3)(4)(5).
3. The method described in this Test Guideline is based on soil column chromatography in disturbed soil (see Annex 1 for definition). Two types of experiments are performed to determine (i) the leaching potential of the test substance, and (ii) the leaching potential of transformation products (study with aged residues) in soils under controlled laboratory conditions¹. The method is based on existing guidelines (6)(7)(8)(9)(10)(11).
4. An OECD Workshop on soil/sediment selection, held at Belgirate, Italy in 1995 (12) agreed on the number and type of soils for use in this test. It also made recommendations with regard to collection, handling and storage of soil samples for leaching experiments.

PRINCIPLE OF THE TEST

5. Columns made of suitably inert material (e.g. glass, stainless steel, aluminium, teflon, PVC, etc.) are packed with soil and afterwards saturated and equilibrated with an “artificial rain” solution (for definition see Annex 1) and allowed to drain. Then the surface of each soil column is treated with the test substance and/or with aged residues of the test substance. Artificial rain is then applied to the soil columns and the leachate is collected. After the leaching process the soil is removed from the columns and is sectioned into an appropriate number of segments depending on the information required from the study. Each soil segment and the leachate are then analysed for the test substance and, if appropriate, for transformation products or other chemicals of interest.

¹ Column leaching studies with crop protection products may provide mobility information on a test substance and its transformation products and may supplement batch sorption studies.

APPLICABILITY OF THE TEST

6. The method is applicable to test substances (unlabelled or radio-labelled: e.g. ^{14}C) for which an analytical method with sufficient accuracy and sensitivity is available. The test should not be applied to chemicals which are volatile from soil and water and thus do not remain in soil and/or leachate under the experimental conditions of this test.

INFORMATION ON THE TEST SUBSTANCE

7. Unlabelled or radio-labelled test substances can be used to measure the leaching behaviour in soil columns. Radio-labelled material is required for studying the leaching of transformation products (aged residues of the test substance) and for mass balance determinations. ^{14}C -labelling is recommended but other isotopes, such as ^{13}C , ^{15}N , ^3H , ^{32}P , may also be useful. As far as possible, the label should be positioned in the most stable part(s) of the molecule. The purity of the test substance should be at least 95%.

8. Most chemicals should be applied as single substances. However, for active ingredients in plant protection products, formulated products may be used to study the leaching of the parent test substance but their testing is particularly required when the formulation is likely to affect the release rate (e.g. granular or controlled release formulations). Regarding formulation specific requirements for test design, it may be useful to consult with the regulatory authority prior to conducting the test. For aged residue leaching studies, the pure parent test substance should be used.

9. Before carrying out leaching tests in soil columns, the following information on the test substance should preferably be available:

- (a) solubility in water [OECD Guideline 105] (13);
- (b) solubility in organic solvents;
- (c) vapour pressure [OECD Guideline 104] (13) and Henry's Law constant;
- (d) n-octanol/water partition coefficient [OECD Guidelines 107 and 117] (13);
- (e) adsorption coefficient (K_d , K_f or K_{OC}) [OECD Guideline 106 and/or 121] (13);
- (f) hydrolysis [OECD Guideline 111] (13);
- (g) dissociation constant (pK_a) [OECD Guideline 112] (13);
- (h) aerobic and anaerobic transformation in soil [OECD Guideline 307] (13)

Note: The temperature at which these measurements were made should be reported in the respective test reports.

10. The amount of test substance applied to the soil columns should be sufficient to allow for detection of at least 0.5 % of the applied dose in any single segment. For active substances in plant protection products, the amount of test substance applied may correspond to the maximum recommended use rate (single application).

11. An appropriate analytical method of known accuracy, precision and sensitivity for the quantification of the test substance and, if relevant, of its transformation products in soil and leachate must be available. The analytical detection limit for the test substance and its significant transformation products (normally at least all transformation products ≥ 10 % of applied dose observed in transformation pathway studies, but preferably any relevant transformation products of concern) should also be known (see paragraph 17).

REFERENCE SUBSTANCES

12. Reference substances with known leaching behaviour such as atrazine or monuron which can be considered moderate leachers in the field should be used for evaluating the relative mobility of the test substance in soil (1)(8)(11). A nonsorbing and non degradable polar reference substance (e.g. tritium, bromide, fluorescein, eosin) to trace the movement of water in the column may also be useful to confirm the hydrodynamic properties of the soil column.

13. Analytical standard substances may also be useful for the characterisation and/or identification of transformation products found in the soil segments and in the leachates by chromatographic, spectroscopic or other relevant methods.

DEFINITIONS AND UNITS

14. See Annex 1.

QUALITY CRITERIA**Recovery**

15. The sum of the percentages of the test substance found in the soil segments and the column leachate after leaching gives the recovery for a leaching experiment. Recoveries should range from 90 % to 110 % for radio-labelled substances (11) and from 70 % to 110 % for non-labelled substances (8).

Repeatability and sensitivity of analytical method

16. Repeatability of the analytical method to quantify test substance and transformation products can be checked by duplicate analysis of the same extract of a soil segment or of a leachate (see paragraph 11).

17. The limit of detection (LOD) of the analytical method for the test substance and for the transformation products should be at least $0.01 \text{ mg} \cdot \text{kg}^{-1}$ in each soil segment or leachate (as test substance) or 0.5 % of applied dose in any single segment whichever is lower. The limit of quantification (LOQ) should also be specified.

DESCRIPTION OF THE TEST METHOD**Test system**

18. Leaching columns (sectionable or non-sectionable) made of suitably inert material (e.g. glass, stainless steel, aluminium, teflon, PVC) with an inner diameter of at least 4 cm and a minimum height of 35 cm are used for the test. Column materials should be tested for potential interactions with the test substance and/or its transformation products. Examples of suitable sectionable and non-sectionable columns are shown in Annex 2.

19. Spoon, plunger and vibration apparatus are used for filling and packing the soil columns.

20. For application of artificial rain to the soil columns, piston or peristaltic pumps, showering heads, Mariotte bottles or simple dropping funnels can be used.

Laboratory equipment and chemicals

21. Standard laboratory equipment is required, in particular the following:

- analytical instruments such as GLC, HPLC and TLC equipment, including the appropriate detection systems for analysing labelled or unlabelled substances or inverse isotope dilution method;
- instruments for identification purposes (e.g. MS, GC-MS, HPLC-MS, NMR, etc.);
- liquid scintillation counter for radio-labelled test substance;
- oxidiser for combustion of labelled material;
- extraction apparatus (for example, centrifuge tubes for cold extraction and Soxhlet apparatus for continuous extraction under reflux);
- instrumentation for concentrating solutions and extracts (e.g. rotating evaporator);

22. Chemicals used include: organic solvents, analytical grade, such as acetone, methanol, etc.; scintillation liquid; 0.01 M CaCl₂ solution in distilled or deionised water (= artificial rain).

Test substance

23. To apply the test substance to the soil column it should be dissolved in water (deionised or distilled). If the test substance is poorly soluble in water, it can be applied either as formulated product (if necessary after suspending or emulsifying in water) or in any organic solvent. In case an organic solvent is used, it should be kept to a minimum and should be evaporated from the surface of the soil column prior to start of leaching procedure. Solid formulations, such as granules, should be applied in the solid form without water; to allow a better distribution over the surface of the soil column, the formulated product may be mixed with a small amount of quartz sand (e.g. 1 g) before application.

24. The amount of test substance applied to the soil columns should be sufficient to allow for detection of at least 0.5 % of the applied dose in any single segment. For active substances in plant protection products, this may be based on the maximum recommended use rate (single application rate) and, for both parent and aged leaching, should be related to the surface area of the soil column used².

² The amount to be applied to cylindrical soil columns can be calculated by the following formula:

$$M [\mu\text{g}] = \frac{A [\text{kg} / \text{ha}] \bullet 10^9 [\mu\text{g} / \text{kg}] \bullet d^2 [\text{cm}^2] \bullet \pi}{10^8 [\text{cm}^2 / \text{ha}] \bullet 4}$$

where:

M = amount applied per column [μg]

A = rate of application [$\text{kg} \cdot \text{ha}^{-1}$]

d = diameter of soil column [cm]

$\pi = 3.14$

Reference substance

25. A reference substance should be used in the leaching experiments (see paragraph 12). It should be applied to the soil column surface in a similar way as the test substance and at an appropriate rate that enables adequate detection either as an internal standard together with the test substance on the same soil column or alone on a separate soil column. It is preferred that both substances be run on the same column, except when both substances are similarly labelled.

Soils**Soil selection**

26. For leaching studies with the parent test substance 3 to 4 soils with varying pH, organic carbon content and texture should be used (12). Guidance for selection of soils for leaching experiments is given in Table 1 below. For ionisable test substances the selected soils should cover a wide range of pH, in order to evaluate the mobility of the substance in its ionised and unionised forms; at least 3 soils should have a pH at which the test substance is in its mobile form.

Table 1: Guidance for selection of soils for leaching studies

Soil No.	pH value	Organic carbon %	Clay content %	Texture*
1	> 7.5	3.5 - 5.0	20 - 40	clay loam
2	5.5 - 7.0	1.5 - 3.0	15 - 25	silt loam
3	4.0 - 5.5	3.0 - 4.0	15 - 30	loam
4	<4.0 - 6.0 §	<0.5 - 1.5 §‡	<10 - 15 §	loamy sand
5	< 4.5	> 10 [#]	< 10	loamy sand/sand

* According to FAO and USDA systems (14).

§ The respective variables should preferably show values within the range given. If, however, difficulties in finding appropriate soil material occur, values below the indicated minimum are accepted.

‡ Soils with less than 0.3 % organic carbon may disturb correlation between organic content and adsorption. Thus, it is recommended to use soils with a minimum organic carbon content of 0.3 %.

Soils with very high carbon content (e.g. >10%) may not be acceptable legally for example for pesticide registration purposes.

27. Other soil types may sometimes be necessary to represent cooler, temperate and tropical regions within the OECD countries. Therefore, if other soil types are preferred, they should be characterised by the same parameters and should have similar variations in properties as those described in the guidance for selection of soils for leaching studies (see Table 1 above), even if they do not match the criteria exactly.

28. For leaching studies with “aged residues“, one soil should be used (12). It should have a sand content > 70% and an organic carbon content between 0.5 - 1.5 % (e.g. soil No. 4 in Table 1). Use of more soil types may be necessary if data on the transformation products are important.

29. All soils should be characterised at least for texture [% sand, % silt, % clay according to FAO and USDA classification systems (14)], pH, cation exchange capacity, organic carbon content, bulk density (for disturbed soil) and water holding capacity. Measurement of microbial biomass is only required for the soil which is used in the ageing/incubation period carried out before the aged leaching experiment. Information on additional soil properties (e.g. soil classification, clay mineralogy, specific

surface area) may be helpful for interpreting the results of this study. For determination of soil characteristics the methods recommended in references (15)(16)(17)(18)(19) can be used.

Collection and storage of soils

30. The soils should be taken from the top layer (A-horizon) to a maximum depth of 20 cm. Remains of vegetation, macro-fauna and stones should be removed. The soils (except those used for ageing the test substance) are air-dried at room temperature (preferably between 20-25 °C). Disaggregation should be performed with minimal force, so that the original texture of the soil will be changed as little as possible. The soils are sieved through a ≤ 2 mm sieve. Careful homogenisation is recommended, as this enhances the reproducibility of the results. Before use the soils can be stored at ambient temperature and kept air dried (12). No limit on storage time is recommended but soils stored for more than 3 years should be re-analysed prior to use with respect to their organic carbon content and pH.

31. Detailed information on the history of the field sites from where the test soils are collected should be available. Details include exact location [exactly defined by UTM (Universal Transversal Mercator-Projection/European Horizontal Datum) or geographical co-ordinates], vegetation cover, treatments with crop protection chemicals, treatments with organic and inorganic fertilisers, additions of biological materials or accidental contamination (12). If soil have been treated with the test substance or its structural analogues within the previous four years, these soils should not be used for leaching studies.

Test conditions

32. During the test period, the soil leaching columns should be kept in the dark at ambient temperature as long as this temperature is maintained within a range of $\pm 2^\circ\text{C}$. Recommended temperatures are between 18 and 25°C.

33. Artificial rain (0.01 M CaCl_2) should be applied continuously to the surface of the soil columns at a rate of 200 mm over a period of 48 hours³; this rate is equivalent to an application of 251 ml for a column with an inner diameter of 4 cm. If needed for the purpose of the test, other rates of artificial rainfall and longer duration may additionally be used.

Performance of the test

Leaching with parent test substance

34. At least duplicate leaching columns are packed with untreated, air-dried and sieved soil (< 2 mm) up to a height of approximately 30 cm. To obtain uniform packing, the soil is added to the columns in small portions with a spoon and pressed with a plunger under simultaneous gentle column vibration until the top of the soil column does not sink in further. Uniform packing is required for obtaining reproducible results from leaching columns. For details on column packing techniques, see references (20)(21) and (22). To control the reproducibility of the packing procedure, the total weight of the soil packed in the columns is determined⁴; the weights of the duplicate columns should be similar.

³ This simulates an extremely high rainfall. The average yearly rainfall, for example, in Central Europe is of the order of 800-1000mm.

⁴ Examples of bulk densities for disturbed soils are as follows:

for a sand soil $1.66 \text{ g} \cdot \text{ml}^{-1}$	for a loamy sand soil $1.58 \text{ g} \cdot \text{ml}^{-1}$
for a loam soil $1.17 \text{ g} \cdot \text{ml}^{-1}$	for a silt soil $1.11 \text{ g} \cdot \text{ml}^{-1}$

35. After packing, the soil columns are pre-wetted with artificial rain (0.01 M CaCl₂) from bottom to top in order to displace the air in the soil pores by water. Thereafter the soil columns are allowed to equilibrate and the excess water is drained off by gravity. Methods for column saturation are reviewed in reference 23.

36. Then the test substance and/or the reference substance are applied to the soil columns (see also paragraphs 23-25). To obtain a homogeneous distribution the solutions, suspensions or emulsions of the test and/or reference substance should be applied evenly over the surface of the soil columns. If incorporation into soil is recommended for the application of a test substance, it should be mixed in a small amount (e.g. 20 g) of soil and added to the surface of the soil column.

37. The surfaces of the soil columns are then covered by a glass sinter disk, glass pearls, glass fibre filters or a round filter paper to distribute the artificial rain evenly over the entire surface and to avoid disturbance of the soil surface by the rain drops. The larger the column diameter the more care is needed for the application of the artificial rain to the soil columns to ensure an even distribution of the artificial rain over the soil surface. Then the artificial rainfall is added to the soil columns drop-wise with the aid of a piston or a peristaltic pump or a dropping funnel. Preferably, the leachates should be collected in fractions and their respective volumes are recorded⁵.

38. After leaching and allowing the columns to drain, the soil columns are sectioned in an appropriate number of segments depending on the information required from the study, the segments are extracted with appropriate solvents or solvent mixtures and analysed for the test substance and, when appropriate, for transformation products, for total radioactivity and for the reference chemical. The leachates or leachate fractions are analysed directly or after extraction for the same products. When radio-labelled test substance is used, all fractions containing ≥ 10 % of the applied radioactivity should be identified.

Leaching with aged residues

39. Fresh soil (not previously air-dried) is treated at a rate corresponding to the surface area of the soil columns (see paragraph 24) with the radio-labelled test substance and incubated under aerobic conditions according to OECD Test Guideline 307 (13). The incubation (ageing) period should be long enough to produce significant amounts of transformation products; an ageing period of one half-life of the test substance is recommended⁶, but should not exceed 120 days. Prior to leaching, the aged soil is analysed for the test substance and its transformation products.

40. The leaching columns are packed up to a height of 28 cm with the same soil (but air-dried) as used in the ageing experiment as described in paragraph 34 and the total weight of the packed soil columns is also determined. The soil columns are then pre-wetted as described in paragraph 35.

41. Then the test substance and its transformation products are applied to the surface of the soil columns in the form of aged soil residues (see paragraph 39) as a 2 cm soil segment. The total height of the soil columns (untreated soil + aged soil) should preferably not exceed 30 cm (see paragraph 34).

⁵ Typical leachate volumes range from 230-260 ml corresponding to approx. 92-104 % of total artificial rain applied (251 ml) when using soil columns of 4 cm diameter and 30 cm length.

⁶ More than one major transformation product may be formed in soil which also may appear at different time points during a transformation study. In such cases, it may be necessary to conduct leaching studies with aged residues of different age.

42. The leaching is carried out as described in paragraph 37.

43. After leaching, soil segments and leachates are analysed as indicated in paragraph 38 for the test substance, its transformation products and not-extracted radioactivity. To determine how much of the aged residue is retained in the top 2-cm layer after leaching, this segment should be analysed separately.

DATA AND REPORTING

Treatment of results

44. The amounts of test substance, transformation products, non-extractables and, if included, of the reference substance should be given in % of applied initial dose for each soil segment and leachate fraction. A graphical presentation should be given for each column plotting the percentages found as a function of the soil depths.

45. When a reference substance is included in these column leaching studies, the leaching of a chemical can be evaluated on a relative scale using relative mobility factors (RMF; for definition see Annex 3) (1)(11) which allows the comparison of leaching data of various chemicals obtained with different soil types. Examples of RMF-values for a variety of crop protection chemicals are given in Annex 3.

46. Estimates of K_{oc} (organic carbon normalised adsorption coefficient) and K_{om} (organic matter normalised distribution coefficient) can also be obtained from column leaching results by using average leaching distance or established correlations between RMF and both K_{om} and K_{oc} respectively (4) or by applying simple chromatographic theory (24). However, the latter method should be used with caution especially when considering that the leaching process does not solely involve saturated flow conditions, but rather unsaturated systems.

Interpretation of results

47. The column leaching studies described in this Guideline allow determining the leaching or mobility potential in soil of the test substance (in the parent leaching study) and/or its transformation products (in the aged residue leaching study). These tests do not quantitatively predict leaching behaviour under field conditions, but they can be used to compare the 'leachability' of one chemical with others whose leaching behaviour may be known (24). Likewise, they do not quantitatively measure the percentage of applied chemical that might reach ground water (11). However, the results of column leaching studies may assist in deciding whether additional semi-field or field testing has to be carried out for substances showing a high mobility potential in laboratory tests.

Test report

48. The report must include:

Test substance and reference substance (when used):

- common name, chemical name (IUPAC and CAS nomenclature), CAS number, chemical structure (indicating position of label when radio-labelled material is used) and relevant physical-chemical properties;
- purity (impurity) of test substance;

- radiochemical purity of labelled chemical and specific activity (where appropriate).

Test soils:

- details of collection site;
- properties of soils, such as pH, organic carbon and clay content, texture and bulk density (for disturbed soil);
- soil microbial activity (only for soil used for ageing of test substance);
- length of soil storage and storage conditions.

Test conditions:

- dates of the performance of the studies;
- length and diameter of leaching columns;
- total soil weight of soil columns;
- amount of test substance and, if appropriate, reference substance applied;
- amount, frequency and duration of application of artificial rain;
- temperature of experimental set-up;
- number of replications (at least two);
- methods for analysis of test substance, transformation products and, where appropriate, of reference substance in the various soil segments and leachates;
- methods for the characterisation and identification of transformation products in the soil segments and leachates.

Test results:

- tables of results expressed as concentrations and as % of applied dose for soil segments and leachates;
- mass balance, if appropriate;
- leachate volumes;
- leaching distances and, where appropriate, relative mobility factors;
- graphical plot of % found in the soil segments versus depth of soil segment;
- discussion and interpretation of results.

LITERATURE

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ANNEX 1DEFINITIONS AND UNITS

Aged soil residue: Test substance and transformation products present in soil after application and following a period long enough to allow transport, adsorption, metabolism, and dissipation processes to alter the distribution and chemical nature of some of the applied chemical (1).

Artificial rain: 0.01 M CaCl₂ solution in distilled or deionised water.

Average Leaching Distance: Bottom of soil section where cumulative recovered substance = 50% of total recovered test substance [normal leaching experiment], or; (bottom of soil section where cumulative recovered substance = 50% of total recovered test substance) – ((thickness of aged residue layer)/2) [aged residue leaching study]

Leachate: Aqueous phase percolated through a soil profile or a soil column (1).

Leaching: Process by which a chemical moves downward through the soil profile or a soil column (1).

Leaching distance: Deepest soil segment in which ≥ 0.5 % of the applied test substance or aged residue was found after the leaching process (equivalent to penetration depth).

Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection (LOD) is the concentration of a substance below which the identity of the substance cannot be distinguished from analytical artefacts. The limit of quantification (LOQ) is the concentration of a substance below which the concentration cannot be determined with an acceptable accuracy.

RMF Relative Mobility Factor: (leaching distance of test substance (cm)) / (leaching distance of reference substance (cm))

Test substance: Any substance, whether the parent substance or relevant transformation products.

Transformation product: All substances resulting from biotic or abiotic transformation reactions of the test substance including CO₂ and products that are bound in residues.

Soil: A mixture of mineral and organic chemical constituents, the latter containing compounds of high carbon and nitrogen content and of high molecular weights, populated by small (mostly micro-) organisms. Soil may be handled in two states:

- undisturbed, as it has developed with time, in characteristic layers of a variety of soil types;
- disturbed, as it is usually found in arable fields or as occurs when samples are taken by digging and used in this guideline (2).

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ANNEX 2

Figure 1: Example of non- sectionable leaching columns made of glass
With a length of 35 cm and an inner diameter of 5 cm (1)



← Dropping funnels for application of artificial rain

← Glass sinter disc to avoid disturbance of the soil surface and to evenly distribute the artificial rain

← Glass column filled with test soil (when testing photolabile products columns should be wrapped in aluminium foil)

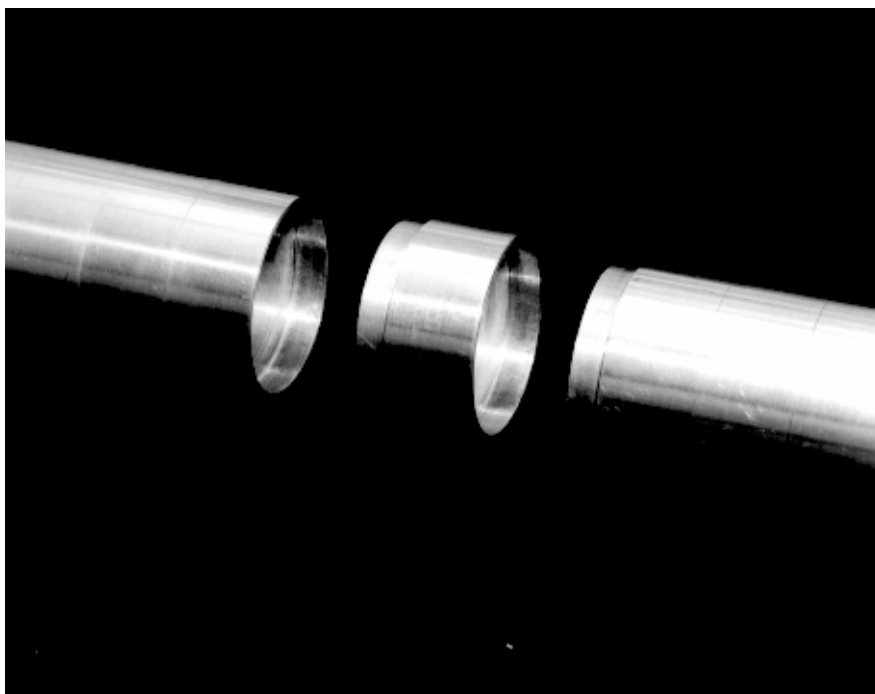
← Layer of quartz sand

← Glass wool plug to keep the soil in the column

← Round-bottom flask for collection of leachate; wrapped in aluminium foil to exclude photolysis

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Figure 2: Example of a sectionable metal column with 4 cm inner diameter (1)



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ANNEX 3**EXAMPLES OF RELATIVE MOBILITY FACTORS* (RMF) FOR A VARIETY OF CROP PROTECTION SUBSTANCES (1)(2) AND CORRESPONDING MOBILITY CLASSES⁺**

RMF-Range	Compound (RMF)	Mobility Class
≤ 0.15	Parathion (< 0.15), Flurodifen (0.15)	I immobile
0.15 - 0.8	Profenophos (0.18), Propiconazole (0.23), Diazinon (0.28), Diuron (0.38), Terbutylazine (0.52), Methidathion (0.56), Prometryn (0.59), Propazine (0.64), Alachlor (0.66), Metolachlor (0.68)	II slightly mobile
0.8 - 1.3	Monuron** (1.00), Atrazine (1.03), Simazine (1.04), Fluometuron (1.18)	III moderately mobile
1.3 - 2.5	Prometon (1.67), Cyanazine (1.85), Bromacil (1.91), Karbutilate (1.98)	IV fairly mobile
2.5 - 5.0	Carbofuran (3.00), Dioxacarb (4.33)	V mobile
> 5.0	Monocrotophos (> 5.0), Dicrotophos (> 5.0)	VI very mobile

* The Relative Mobility Factor is derived as follows (3):

$$\text{RMF} = \frac{\text{leaching distance of test substance (cm)}}{\text{leaching distance of reference substance (cm)}}$$

** Reference substance

+ Other systems to classify a chemical's mobility in soil are based on R_f values from soil thin-layer chromatography (4) and on K_{oc} values (5)(6).

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