C.23. AEROBIC AND ANAEROBIC TRANSFORMATION IN SOIL

1. METHOD

This Test Method is a replicate of the OECD TG 307 (2002)

1.1 INTRODUCTION

This Test Method is based on existing guidelines (1)(2)(3)(4)(5)(6)(7)(8)(9). The method described in this Test Method is designed for evaluating aerobic and anaerobic transformation of chemicals in soil. The experiments are performed to determine (i) the rate of transformation of the test substance, and (ii) the nature and rates of formation and decline of transformation products to which plants and soil organisms may be exposed. Such studies are required for chemicals which are directly applied to soil or which are likely to reach the soil environment. The results of such laboratory studies can also be used to develop sampling and analysis protocols for related field studies.

Aerobic and anaerobic studies with one soil type are generally sufficient for the evaluation of transformation pathways (8)(10)(11). Rates of transformation should be determined in at least three additional soils (8)(10).

An OECD Workshop on soil and sediment selection, held at Belgirate, Italy in 1995 (10) agreed, in particular, on the number and types of soils for use in this test. The types of soils tested should be representative of the environmental conditions where use or release will occur. For example, chemicals that may be released in subtropical to tropical climates should be tested with Ferrasols or Nitosols (FAO system). The Workshop also made recommendations relating to collection, handling and storage of soil samples, based on the ISO Guidance (15). The use of paddy (rice) soils is also considered in this method.

1.2 DEFINITIONS

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

Transformation products: all substances resulting from biotic or abiotic transformation reactions of the test substance including CO_2 and products that are in bound residues.

Bound residues: "Bound residues" represent compounds in soil, plant or animal, which persist in the matrix in the form of the parent substance or its metabolite(s)/transformation products after extraction. The extraction method must not substantially change the compounds themselves or the structure of the matrix. The nature of the bond can be clarified in part by matrix-altering extraction methods and sophisticated analytical techniques. To date, for example, covalent ionic and sorptive bonds, as well as entrapments, have been identified in this way. In general, the formation of bound residues reduces the bioaccessibility and the bioavailability significantly (12) [modified from IUPAC 1984 (13)].

Aerobic transformation: reactions occurring in the presence of molecular oxygen (14).

Anaerobic transformation: reactions occurring under exclusion of molecular oxygen (14).

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

(a) undisturbed, as it has developed with time, in characteristic layers of a variety of soil types;
(b) disturbed, as it is usually found in arable fields or as occurs when samples are taken by digging and used in this test method (14).

Mineralisation: is the complete degradation of an organic compound to CO_2 and H_2O under aerobic conditions, and CH_4, CO_2 and H_2O under anaerobic conditions. In the context of this test method, when ^{14}C-labelled compound is used, mineralisation means extensive degradation during which a labelled carbon atom is oxidised with release of the appropriate amount of $^{14}CO_2$ (14).

Half-life: $t_{0.5,}$ is the time taken for 50% transformation of a test substance when the transformation can be described by first-order kinetics; it is independent of the concentration.

DT_{50} (Disappearance Time 50): is the time within which the concentration of the test substance is reduced by 50%; it is different from the half-life $t_{0.5}$ when transformation does not follow first order kinetics.

DT_{75} (Disappearance Time 75): is the time within which the concentration of the test substance is reduced by 75%.

DT_{90} (Disappearance Time 90): is the time within which the concentration of the test substance is reduced by 90%.

1.3 REFERENCE SUBSTANCES

Reference substances should be used for the characterisation and/or identification of transformation products by spectroscopic and chromatographic methods.

1.4 APPLICABILITY OF THE TEST

The method is applicable to all chemical substances (non-labelled or radiolabelled) for which an analytical method with sufficient accuracy and sensitivity is available. It is applicable to slightly volatile, non-volatile, water-soluble or water-insoluble compounds. The test should not be applied to chemicals which are highly volatile from soil (e.g. fumigants, organic solvents) and thus cannot be kept in soil under the experimental conditions of this test.

1.5 INFORMATION ON THE TEST SUBSTANCE

Non-labelled or labelled test substance can be used to measure the rate of transformation. Labelled material is required for studying the pathway of transformation and for establishing a mass balance. ^{14}C-labelling is recommended but the use of other isotopes, such as ^{13}C, ^{15}N, ^{3}H, ^{32}P, may also be useful. As far as possible, the label should be positioned in the most stable part(s) of the molecule[1]. The purity of the test substance should be at least 95 %.

Before carrying out a test on aerobic and anaerobic transformation in soil, the following information on the test substance should be available:

(a) solubility in water (Method A.6)
(b) solubility in organic solvents;
(c) vapour pressure (Method A.4) and Henry's law constant;
(d) n-octanol/water partition coefficient (Method A.8);
(e) chemical stability in dark (hydrolysis) (Method C.7);
(f) pK_a if a molecule is liable to protonation or deprotonation [OECD Guideline 112] (16).

Other useful information may include data on toxicity of the test substance to soil micro-organisms [Testing Methods C.21 and C.22] (16).

Analytical methods (including extraction and clean-up methods) for quantification and identification of the test substance and its transformation products should be available.

[1] For example, if the test substance contains one ring, labelling on this ring is required; if the test substance contains two or more rings, separate studies may be needed to evaluate the fate of each labelled ring and to obtain suitable information on formation of transformation products.

1.6 PRINCIPLE OF THE TEST METHOD

Soil samples are treated with the test substance and incubated in the dark in biometer-type flasks or in flow-through systems under controlled laboratory conditions (at constant temperature and soil moisture). After appropriate time intervals, soil samples are extracted and analysed for the parent substance and for transformation products. Volatile products are also collected for analysis using appropriate absorption devices. Using ^{14}C-labelled material, the various mineralisation rates of the test substance can be measured by trapping evolved $^{14}CO_2$ and a mass balance, including the formation of soil bound residues, can be established.

1.7 QUALITY CRITERIA

1.7.1 **Recovery**

Extraction and analysis of, at least, duplicate soil samples immediately after the addition of the test substance gives a first indication of the repeatability of the analytical method and of the uniformity of the application procedure for the test substance. Recoveries for later stages of the experiments are given by the respective mass balances. Recoveries should range from 90% to 110% for labelled chemicals (8) and from 70% to 110% for non-labelled chemicals (3).

1.7.2 **Repeatability and sensitivity of analytical method**

Repeatability of the analytical method (excluding the initial extraction efficiency) to quantify test substance and transformation products can be checked by duplicate analysis of the same extract of the soil, incubated long enough for formation of transformation products.

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

1.7.3 **Accuracy of transformation data**

Regression analysis of the concentrations of the test substance as a function of time gives the appropriate information on the reliability of the transformation curve and allows the calculation of the confidence limits for half-lives (in the case of pseudo first order kinetics) or DT_{50} values and, if appropriate, DT_{75} and DT_{90} values.

1.8 DESCRIPTION OF THE TEST METHOD

1.8.1 **Equipment and chemical reagents**

Incubation systems consist of static closed systems or suitable flow-through systems (7)(17). Examples of suitable flow-through soil incubation apparatus and biometer-type flask are shown in Figures 1 and 2, respectively. Both types of incubation systems have advantages and limitations (7)(17).

Standard laboratory equipment is required and especially the following:

— Analytical instruments such as GLC, HPLC, TLC-equipment, including the appropriate detection systems for analysing radiolabelled or non-labelled substances or inverse isotopes dilution method;
— Instruments for identification purposes (e.g. MS, GC-MS, HPLC-MS, NMR, etc.);
— Liquid scintillation counter;
— Oxidiser for combustion of radioactive material;
— Centrifuge;
— 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);
— Water bath;
— Mechanical mixing device (e.g. kneading machine, rotating mixer).

Chemical reagents used include, for example:

— NaOH, analytical grade, 2 mol · dm^{-3}, or other appropriate base (e.g. KOH, ethanolamine);
— H_2SO_4, analytical grade, 0.05 mol · dm^{-3};
— Ethylene glycol, analytical grade;
— Solid absorption materials such as soda lime and polyurethane plugs;
— Organic solvents, analytical grade, such as acetone, methanol, etc.;
— Scintillation liquid.

1.8.2 **Test substance application**

For addition to and distribution in soil, the test substance can be dissolved in water (deionised or distilled) or, when necessary, in minimum amounts of acetone or other organic solvents (6) in which the test substance is sufficiently soluble and stable. However, the amount of solvent selected should not have a significant influence on soil microbial activity (see sections 1.5 and 1.9.2-1.9.3 The use of solvents which inhibit microbial activity, such as chloroform, dichloromethane and other halogenated solvents, should be avoided.

The test substance can also be added as a solid, e.g. mixed in quartz sand (6) or in a small sub-sample of the test soil which has been air-dried and sterilised. If the test substance is added using a solvent the solvent should be allowed to evaporate before the spiked sub-sample is added to the original non-sterile soil sample.

For general chemicals, whose major route of entry into soil is through sewage sludge/farming application, the test substance should be first added to sludge which is then introduced into the soil sample. (see sections 1.9.2 and 1.9.3)

The use of formulated products is not routinely recommended. However, e.g. for poorly soluble test substances, the use of formulated material may be an appropriate alternative.

1.8.3 **Soils**

1.8.3.1 *Soil selection*

To determine the transformation pathway, a representative soil can be used; a sandy loam or silty loam or loam or loamy sand [according to FAO and USDA classification (18)] with a pH of 5.5-8.0, an organic carbon content of 0.5-2.5% and a microbial biomass of at least 1% of total organic carbon is recommended (10).

For transformation rate studies at least three additional soils should be used representing a range of relevant soils. The soils should vary in their organic carbon content, pH, clay content and microbial biomass (10).

All soils should be characterised, at least, for texture (% sand, % silt, % clay) [according to FAO and USDA classification (18)], pH, cation exchange capacity, organic carbon, bulk density, water retention characteristic[2] and microbial biomass (for aerobic studies only). Additional information on soil properties may be useful in interpreting the results. For determination of the soil characteristics the methods recommended in references (19)(20)(21)(22)(23) can be used. Microbial biomass should be determined by using the substrate-induced respiration (SIR) method (25)(26) or alternative methods (20).

[2] Water retention characteristic of a soil can be measured as field capacity, as water holding capacity or as water suction tension (pF). For explanations see Annex 1. It should be reported in the test report whether water retention characteristics and bulk density of soils were determined in undisturbed field samples or in disturbed (processed) samples.

1.8.3.2 *Collection, handling, and storage of soils*

Detailed information on the history of the field site from where the test soil is collected should be available. Details include exact location, vegetation cover, treatments with chemicals, treatments with organic and inorganic fertilisers, additions of biological materials or other contamination. If soils have been treated with the test substance or its structural analogues within the previous four years, these should not be used for transformation studies (10)(15).

The soil should be freshly collected from the field (from the A horizon or top 20 cm layer) with a soil water content which facilitates sieving. For soils other than those from paddy fields, sampling should be avoided during or immediately following long periods (> 30 days) of drought, freezing or flooding (14). Samples should be transported in a manner which minimises changes in soil water content and should be kept in the dark with free access of air**,** as much as possible. A loosely-tied polyethylene bag is generally adequate for this purpose.

The soil should be processed as soon as possible after sampling. Vegetation, larger soil fauna and stones should be removed prior to passing the soil through a 2 mm sieve which removes small stones, fauna and plant debris. Extensive drying and crushing of the soil before sieving should be avoided (15).

When sampling in the field is difficult in winter (soil frozen or covered by layers of snow), it may be taken from a batch of soil stored in the greenhouse under plant cover (e.g. grass or grass-clover mixtures). Studies with soils freshly collected from the field are strongly preferred, but if the collected and processed soil has to be stored prior to the start of the study storage conditions must be adequate and for a limited time only (4 ± 2°C for a maximum of three months) to maintain microbial activity[3]. Detailed instructions on collection, handling and storage of soils to be used for biotransformation experiments can be found in (8)(10)(15)(26)(27).

Before the processed soil is used for this test, it should be pre-incubated to allow germination and removal of seeds, and to re-establish equilibrium of microbial metabolism following the change from sampling or storage conditions to incubation conditions. A pre-incubation period between 2 and 28 days approximating the temperature and moisture conditions of the actual test is generally adequate (15). Storage and pre-incubation time together should not exceed three months.

1.9 PERFORMANCE OF THE TEST

1.9.1 **Test conditions**

1.9.1.1 *Test temperature*

During the whole test period, the soils should be incubated in the dark at a constant temperature representative of the climatic conditions where use or release will occur. A temperature of 20 ± 2 °C is recommended for all test substances which may reach the soil in temperate climates. The temperature should be monitored.

For chemicals applied or released in colder climates (e.g. in northern countries, during autumn/winter periods), additional soil samples should be incubated but at a lower temperature (e.g. 10 ± 2 °C).

[3] Recent research results indicate that soils from temperate zones can also be stored at –20°C for more than three months (28)(29) without significant losses of microbial activity.

1.9.1.2 *Moisture content*

For transformation tests under aerobic conditions, the soil moisture content[4] should be adjusted to and maintained at a pF between 2.0 and 2.5 (3). The soil moisture content is expressed as mass of water per mass of dry soil and should be regularly controlled (e.g. in 2 week intervals) by weighing of the incubation flasks and water losses compensated by adding water (preferably sterile-filtered tap water). Care should be given to prevent or minimise losses of test substance and/or transformation products by volatilisation and/or photodegradation (if any) during moisture addition.

For transformation tests under anaerobic and paddy conditions, the soil is water-saturated by flooding.

1.9.1.3 *Aerobic incubation conditions*

In the flow-through systems, aerobic conditions will be maintained by intermittent flushing or by continuously ventilating with humidified air. In the biometer flasks, exchange of air is maintained by diffusion.

1.9.1.4 *Sterile aerobic conditions*

To obtain information on the relevance of abiotic transformation of a test substance, soil samples may be sterilised (for sterilisation methods see references 16 and 29), treated with sterile test substance (e.g. addition of solution through a sterile filter) and aerated with humidified sterile air as described in section 1.9.1.3. For paddy soils, soil and water should be sterilised and the incubation should be carried out as described in section 1.9.1.6.

1.9.1.5 *Anaerobic incubation conditions*

To establish and maintain anaerobic conditions, the soil treated with the test substance and incubated under aerobic conditions for 30 days or one half-life or DT_{50} (whichever is shorter) is then water-logged (1-3 cm water layer) and the incubation system flushed with an inert gas (e.g. nitrogen or argon)[5]. The test system must allow for measurements such as pH, oxygen concentration and redox potential and include trapping devices for volatile products. The biometer-type system must be closed to avoid entrance of air by diffusion.

1.9.1.6 *Paddy incubation conditions*

To study transformation in paddy rice soils, the soil is flooded with a water layer of about 1-5 cm and the test substance applied to the water phase (9). A soil depth of at least 5 cm is recommended. The system is ventilated with air as under aerobic conditions. pH, oxygen concentration and redox potential of the aqueous layer should be monitored and reported. A pre-incubation period of at least two weeks is necessary before commencing transformation studies (see section 1.8.3.2).

[4] The soil should neither be too wet nor too dry to maintain adequate aeration and nutrition of soil microflora. Moisture contents recommended for optimal microbial growth range from 40-60% water holding capacity (WHC) and from 0.1-0.33 bar (6). The latter range is equivalent to a pF-range of 2.0 – 2.5. Typical moisture contents of various soil types are given in Annex 2.

[5] Aerobic conditions are dominant in surface soils and even in sub-surface soils as shown in an EU sponsored research project [K. Takagi et al. (1992). Microbial diversity and activity in subsoils: Methods, field site, seasonal variation in subsoil temperatures and oxygen contents. Proc. Internat. Symp. Environm. Aspects Pesticides Microbiol., 270-277, 17-21 August 1992, Sigtuna, Sweden]. Anaerobic conditions may only occur occasionally during flooding of soils after heavy rainfalls or when paddy conditions are established in rice fields.

1.9.1.7 *Test duration*

The rate and pathway studies should normally not exceed 120 days[6] (3)(6)(8), because thereafter a decrease of the soil microbial activity with time would be expected in an artificial laboratory system isolated from natural replenishment. Where necessary to characterise the decline of the test substance and the formation and decline of major transformation products, studies can be continued for longer periods (e.g. 6 or 12 months) (8). Longer incubation periods should be justified in the test report and accompanied by biomass measurements during and at the end of these periods.

1.9.2 **Performance of the test**

About 50 to 200 g of soil (dry weight basis) are placed into each incubation flask (see Figures 1 and 2 in Annex 3) and the soil treated with the test substance by one of the methods described in section 1.8.2. When organic solvents are used for the application of the test substance, they should be removed from soil by evaporation. Then the soil is thoroughly mixed with a spatula and/or by shaking of the flask. If the study is conducted under paddy field conditions, soil and water should be thoroughly mixed after application of the test substance. Small aliquots (e.g. 1 g) of the treated soils should be analysed for the test substance to check for uniform distribution. For alternative method, see below.

The treatment rate should correspond to the highest application rate of a crop protection product recommended in the use instructions and uniform incorporation to an appropriate depth in the field (e.g. top 10 cm layer[7] of soil). For example, for chemicals foliarly or soil applied without incorporation, the appropriate depth for computing how much chemical should be added to each flask is 2.5 cm. For soil incorporated chemicals, the appropriate depth is the incorporation depth specified in the use instructions**.** For general chemicals, the application rate should be estimated based on the most relevant route of entry; for example, when the major route of entry in soil is through sewage sludge, the chemical should be dosed into the sludge at a concentration that reflects the expected sludge concentration and the amount of sludge added to the soil should reflect normal sludge loading to agricultural soils. If this concentration is not high enough to identify major transformation products, incubation of separate soil samples containing higher rates may be helpful, but excessive rates influencing soil microbial functions should be avoided (see sections 1.5 and 1.8.2).

Alternatively, a larger batch (i.e. 1 to 2 kg) of soil can be treated with the test substance, carefully mixed in an appropriate mixing machine and then transferred in small portions of 50 to 200 g into the incubation flasks (for example with the use of sample splitters). Small aliquots (e.g. 1 g) of the treated soil batch should be analysed for the test substance to check for uniform distribution. Such a procedure is preferred since it allows for more uniform distribution of the test substance into the soil.

Also untreated soil samples are incubated under the same conditions (aerobic) as the samples treated with the test substance. These samples are used for biomass measurements during and at the end of the studies.

6 Aerobic studies might be terminated much before 120 days provided that ultimate transformation pathway and ultimate mineralisation are clearly reached at that time. Termination of the test is possible after 120 days, or when at least 90% of the test substance is transformed, but only if at least 5% CO_2 is formed.

7 Calculation of the initial concentration on an area basis using the following equation:

$$C_{soil}[mg/kg_{soil}] = \frac{A[kg/ha]\cdot 10^6[mg/kg]}{l[m]\cdot 10^4[m^2/ha]\cdot d[kg_{soil}/m^3]}$$

C_{soil} = Initial concentration in soil [$mg \cdot kg^{-1}$]

A = Application rate [$kg \cdot ha^{-1}$]; 1 = thickness of field soil layer [m]; d = dry bulk density of soil [$kg \cdot m^{-3}$].

As a rule of thumb, an application rate of 1 $kg \cdot ha^{-1}$ results in a soil concentration of approximately 1 $mg \cdot kg^{-1}$ in a 10 cm layer (assuming a bulk density of 1 $g \cdot cm^{-3}$).

When the test substance is applied to the soil dissolved in organic solvent(s), soil samples treated with the same amount of solvent(s) are incubated under the same conditions (aerobic) as the samples treated with the test substance. These samples are used for biomass measurements initially, during and at the end of the studies to check for effects of the solvent(s) on microbial biomass.

The flasks containing the treated soil are either attached to the flow-through system described in Figure 1 or closed with the absorption column shown in Figure 2 (see Annex 3).

1.9.3 **Sampling and measurement**

Duplicate incubation flasks are removed at appropriate time intervals and the soil samples extracted with appropriate solvents of different polarity and analysed for the test substance and/or transformation products. A well-designed study includes sufficient flasks so that two flasks are sacrificed at each sampling event. Also, absorption solutions or solid absorption materials are removed at various time intervals (7-day intervals during the first month and after one month in 17 -day intervals) during and at the end of incubation of each soil sample and analysed for volatile products. Besides a soil sample taken directly after application (0-day sample) at least 5 additional sampling points should be included. Time intervals should be chosen in such a way that pattern of decline of the test substance and patterns of formation and decline of transformation products can be established (e.g. 0, 1, 3, 7 days; 2, 3 weeks; 1, 2, 3 months, etc.).

When using ^{14}C-labelled test substance, non-extractable radioactivity will be quantified by combustion and a mass balance will be calculated for each sampling interval.

In the case of anaerobic and paddy incubation, the soil and water phases are analysed together for test substance and transformation products or separated by filtration or centrifugation before extraction and analysis.

1.9.4 **Optional tests**

Aerobic, non-sterile studies at additional temperatures and soil moistures may be useful for the estimation of the influence of temperature and soil moisture on the rates of transformation of a test substance and/or its transformation products in soil.

A further characterisation of non-extractable radioactivity can be attempted using, for example, supercritical fluid extraction.

2 **DATA**

2.1 TREATMENT OF RESULTS

The amounts of test substance, transformation products, volatile substances (in % only), and non-extractable should be given as % of applied initial concentration and**,** where appropriate, as mg·kg^{-1} soil (based on soil dry weight) for each sampling interval. A mass balance should be given in percentage of the applied initial concentration for each sampling interval. A graphical presentation of the test substance concentrations against time will allow an estimation of its transformation half-life or DT_{50}. Major transformation products should be identified and their concentrations should also be plotted against time to show their rates of formation and decline. A major transformation product is any product representing ≥ 10% of applied dose at any time during the study.

The volatile products trapped give some indication of the volatility potential of a test substance and its transformation products from soil.

More accurate determinations of half-lives or DT_{50} values and, if appropriate, DT_{75} and DT_{90} values should be obtained by applying appropriate kinetic model calculations. The half-life and DT_{50} values should be reported together with the description of the model used, the order of kinetics and the determination coefficient (r^2). First order kinetics is favoured unless $r^2 < 0.7$. If appropriate, the calculations should also be applied to the major transformation products. Examples of appropriate models are described in references 31 to 35.

In the case of rate studies carried out at various temperatures, the transformation rates should be described as a function of temperature within the experimental temperature range using the Arrhenius relationship of the form:

$$k = A \cdot e^{-B/T} \quad \text{or} \quad \ln k = \ln A - \frac{B}{T},$$

where ln A and B are regression constants from the intercept and slope, respectively, of a best fit line generated from linearly regressing ln k against 1/T, k is the rate constant at temperature T and T is the temperature in Kelvin. Care should be given to the limited temperature range in which the Arrehenius relationship will be valid in case transformation is governed by microbial action.

2.2 EVALUATION AND INTERPRETATION OF RESULTS

Although the studies are carried out in an artificial laboratory system, the results will allow estimation of the rate of transformation of the test substance and also of rate of formation and decline of transformation products under field conditions (36)(37).

A study of the transformation pathway of a test substance provides information on the way in which the applied substance is structurally changed in the soil by chemical and microbial reactions.

3 REPORTING

TEST REPORT

The test report must include:

Test substance:

- common name, chemical name, CAS number, structural formula (indicating position of label(s) when radiolabelled material is used) and relevant physical-chemical properties (see section 1.5);
- purity (impurities) of test substance;
- radiochemical purity of labelled chemical and specific activity (where appropriate);

Reference substances:

- chemical name and structure of reference substances used for the characterisation and/or identification of transformation product;

Test soils:

- details of collection site;
- date and procedure of soil sampling;
- properties of soils, such as pH, organic carbon content, texture (% sand, % silt, % clay), cation exchange capacity, bulk density, water retention characteristic, and microbial biomass;
- length of soil storage and storage conditions (if stored);

Test conditions:

— dates of the performance of the studies;
— amount of test substance applied;
— solvents used and method of application for the test substance;
— weight of soil treated initially and sampled at each interval for analysis;
— description of the incubation system used;
— air flow rates (for flow-through systems only);
— temperature of experimental set-up;
— soil moisture content during incubation;
— microbial biomass initially, during and at the end of the aerobic studies;
— pH, oxygen concentration and redox potential initially, during and at the end of the anaerobic and paddy studies;
— method(s) of extraction;
— methods for quantification and identification of the test substance and major transformation products in soil and absorption materials;
— number of replicates and number of controls.

Results:

— result of microbial activity determination;
— repeatability and sensitivity of the analytical methods used;
— rates of recovery (% values for a valid study are given in section 1.7.1);
— tables of results expressed as % of applied initial dose and, where appropriate, as $mg \cdot kg^{-1}$ soil (on a dry weight basis);
— mass balance during and at the end of the studies;
— characterisation of non-extractable (bound) radioactivity or residues in soil;
— quantification of released CO_2 and other volatile compounds;
— plots of soil concentrations versus time for the test substance and, where appropriate, for major transformation products;
— half-life or DT_{50} , DT_{75} and DT_{90} for the test substance and, where appropriate, for major transformation products including confidence limits;
— estimation of abiotic degradation rate under sterile conditions;
— an assessment of transformation kinetics for the test substance and, where appropriate, for major transformation products;
— proposed pathways of transformation, where appropriate;
— discussion and interpretation of results;
— raw data (i.e. sample chromatograms, sample calculations of transformation rates and means used to identify transformation products).

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ANNEX 1

WATER TENSION, FIELD CAPACITY (FC) AND WATER HOLDING CAPACITY (WHC)(1)

Height of Water Column [cm]	pF[(a)]	bar[(b)]	Remarks
10^7	7	10^4	Dry Soil
$1.6 \cdot 10^4$	4.2	16	Wilting point
10^4	4	10	
10^3	3	1	
$6 \cdot 10^2$	2.8	0.6	
$3.3 \cdot 10^2$	2.5	0.33[(c)]	
10^2	2	0.1	Range of
60	1.8	0.06	Field capacity[(d)]
33	1.5	0.033	
10	1	0.01	WHC (approximation)
1	0	0.001	Water saturated soil

(a) pF = log of cm water column.
(b) 1 bar = 10^5 Pa.
(c) Corresponds to an approximate water content of 10% in sand, 35% in loam and 45% in clay.
(d) Field capacity is not constant but varies with soil type between pF 1.5 and 2.5.

Water tension is measured in cm water column or in bar . Due to the large range of suction tension it is expressed simply as pF value which is equivalent to the logarithm of cm water column.

Field capacity is defined as the amount of water which can be stored against gravity by a natural soil 2 days after a longer raining period or after sufficient irrigation. It is determined in undisturbed soil in situ in the field. The measurement is thus not applicable to disturbed laboratory soil samples. FC values determined in disturbed soils may show great systematic variances.

Water holding capacity (WHC) is determined in the laboratory with undisturbed and disturbed soil by saturating a soil column with water by capillary transport. It is particularly useful for disturbed soils and can be up to 30 % greater than field capacity (1). It is also experimentally easier to determine than reliable FC-values.

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ANNEX 2

SOIL MOISTURE CONTENTS (g water per 100 g dry soil) OF VARIOUS SOIL TYPES FROM VARIOUS COUNTRIES

Soil Type	Country	Soil Moisture Content at		
		WHC[1]	pF = 1.8	pF = 2.5
Sand	Germany	28.7	8.8	3.9
Loamy sand	Germany	50.4	17.9	12.1
Loamy sand	Switzerland	44.0	35.3	9.2
Silt loam	Switzerland	72.8	56.6	28.4
Clay loam	Brazil	69.7	38.4	27.3
Clay loam	Japan	74.4	57.8	31.4
Sandy loam	Japan	82.4	59.2	36.0
Silt loam	USA	47.2	33.2	18.8
Sandy loam	USA	40.4	25.2	13.3

[1] Water Holding Capacity

ANNEX 3

Figure 1

Example of a flow-through apparatus to study transformation of chemicals in soil (1)(2)

1: needle valve

2: gas washing bottle containing water

3: ultramembrane (sterile conditions only), pore size 0.2 µm

4: soil metabolism flask (water-logged only for anaerobic and paddy conditions;)

5: ethylene glycol trap for organic volatile compounds

6: sulphuric acid trap for alkaline volatile compounds

7, 8: sodium hydroxide trap for CO_2 & other acidic volatiles

9: flow meter.

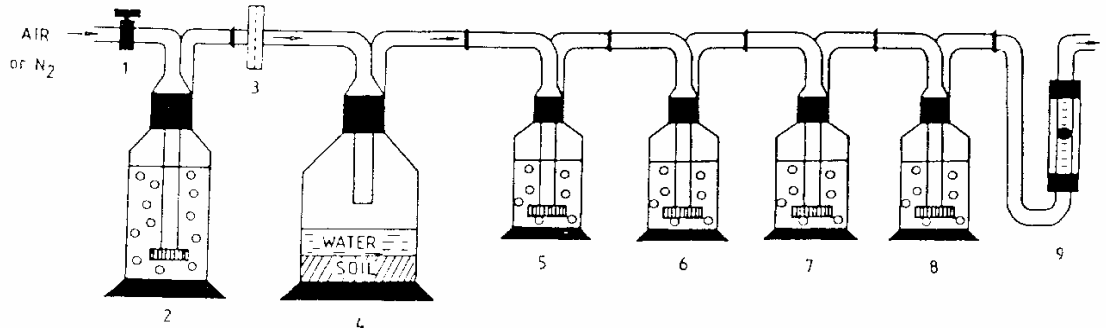
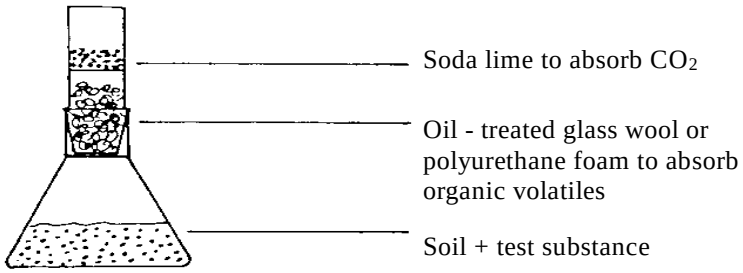


Figure 2

Example of a biometer-type flask for studying the transformation of chemicals in soil (3)



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