

33. The test should be performed in the dark at a constant temperature in the range of 10 to 30 °C. A temperature of  $(20 \pm 2)^\circ\text{C}$  is appropriate. Where appropriate, an additional lower temperature (e.g. 10 °C) may be considered on a case-by-case basis, depending on the information required from the test. Incubation temperature should be monitored and reported.

#### Treatment and application of test substance

34. One test concentration of chemical is used<sup>5</sup>. For crop protection chemicals applied directly to water bodies, the maximum dosage on the label should be taken as the maximum application rate calculated on the basis of the surface area of the water in the test vessel. In all other cases, the concentration to be used should be based on predictions from environmental emissions. Care must be taken to ensure that an adequate concentration of test substance is applied in order to characterise the route of transformation and the formation and decline of transformation products. It may be necessary to apply higher doses (e.g. 10 times) in situations where test substance concentrations are close to limits of detection at the start of the study and/or where major transformation products could not readily be detected when present at 10% of the test substance application rate. However, if higher test concentrations are used they should not have a significant adverse effect on the microbial activity of the water-sediment system. In order to achieve a constant concentration of test substance in vessels of differing dimensions an adjustment to the quantity of the material applied may be considered appropriate, based on the depth of the water column in the vessel in relation to the depth of water in the field (which is assumed to be 100 cm, but other depths can be used). See Annex 5 for an example calculation.

35. Ideally the test substance should be applied as an aqueous solution into the water phase of the test system. If unavoidable, the use of low amounts of water miscible solvents (such as acetone, ethanol) is permitted for application and distribution of the test substance, but this should not exceed 1% v/v and should not have adverse effects on microbial activity of the test system. Care should be exercised in generating the aqueous solution of the test substance - use of generator columns and pre-mixing may be appropriate to ensure complete homogeneity. Following addition of the aqueous solution to the test system, gentle mixing of the water phase is recommended, disturbing the sediment as little as possible.

36. The use of formulated products is not routinely recommended as the formulation ingredients may affect the distribution of the test substance and/or transformation products between water and sediment phases. However, for poorly water-soluble test substances, the use of formulated material may be an appropriate alternative.

37. The number of incubation vessels depends on the number of sampling times (see paragraph 38). A sufficient number of test systems should be included so that two systems may be sacrificed at each sampling time. Where control units of each aquatic sediment system are employed, they should not be treated with the test substance. The control units can be used to determine the microbial biomass of the sediment and the total organic carbon of the water and sediment at the termination of the study. Two of the control units (i.e. one control unit of each aquatic sediment) can be used to monitor the required parameters in the sediment and water during the acclimation period (see Table in paragraph 26). Two additional control units have to be included if the test substance is applied by means of a solvent to measure adverse effects on the microbial activity of the test system.

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<sup>5</sup> Test with a second concentration can be useful for chemicals that reach surface waters by different entry routes resulting in significantly different concentrations, as long as the lower concentration can be analysed with sufficient accuracy.

### **Test duration and sampling**

38. The duration of the experiment should normally not exceed 100 days (6), and should continue until the degradation pathway and water/sediment distribution pattern are established or when 90 % of the test substance has been removed by transformation and/or volatilisation. The number of sampling times should be at least six (including zero time), with an optional preliminary study (see paragraph 40) being used to establish an appropriate sampling regime and the duration of the test, unless sufficient data are available on the test substance from previous studies. For hydrophobic test substances, additional sampling points during the initial period of the study may be necessary in order to determine the rate of distribution between water and sediment phases.

39. At appropriate sampling times, whole incubation vessels (in replicate) are removed for analysis. Sediment and overlying water are analysed separately<sup>6</sup>. The surface water should be carefully removed with minimum disturbance of the sediment. The extraction and characterisation of the test substance and transformation products should follow appropriate analytical procedures. Care should be taken to remove material that may have adsorbed to the incubation vessel or to interconnecting tubing used to trap volatiles.

### **Optional preliminary test**

40. If duration and sampling regime cannot be estimated from other relevant studies on the test substance, an optional preliminary test may be considered appropriate, which should be performed using the same test conditions proposed for the definitive study. Relevant experimental conditions and results from the preliminary test, if performed, should be briefly reported.

### **Measurements and analysis**

41. Concentration of the test substance and the transformation products at every sampling time in water and sediment should be measured and reported (as a concentration and as percentage of applied). In general, transformation products detected at  $\geq 10\%$  of the applied radioactivity in the total water-sediment system at any sampling time should be identified unless reasonably justified otherwise. Transformation products for which concentrations are continuously increasing during the study should also be considered for identification, even if their concentrations do not exceed the limits given above, as this may indicate persistence. The latter should be considered on a case by case basis, with justifications being provided in the report.

42. Results from gases/volatiles trapping systems (CO<sub>2</sub> and others, i.e. volatile organic compounds) should be reported at each sampling time. Mineralisation rates should be reported. Non-extractable (bound) residues in sediment are to be reported at each sampling point.

## **DATA AND REPORTING**

### **Treatment of results and calculations**

43. Total mass balance or recovery (see paragraph 13) of added radioactivity is to be calculated at every sampling time. Results should be reported as a percentage of added radioactivity. Distribution of radioactivity between water and sediment should be reported as concentrations and percentages, at every sampling time.

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<sup>6</sup> In cases where rapid re-oxidation of anaerobic transformation products may readily occur, anaerobic conditions should be maintained during sampling and analysis.

44. Half-life,  $DT_{50}$  and, if appropriate,  $DT_{75}$  and  $DT_{90}$  of the test substance should be calculated along with their confidence limits (see paragraph 16). Information on the rate of dissipation of the test substance in the water and sediment can be obtained through the use of appropriate evaluation tools. These can range from application of pseudo-first order kinetics, empirical curve-fitting techniques which apply graphical or numerical solutions and more complex assessments using, for example, single- or multi-compartment models. Further details can be obtained from the relevant published literature (32)(33)(34).

45. All approaches have their strengths and weaknesses and vary considerably in complexity. An assumption of first-order kinetics may be an oversimplification of the degradation and distribution processes, but when possible gives a term (the rate constant or half-life) which is easily understood and of value in simulation modelling and calculations of predicted environmental concentrations. Empirical approaches or linear transformations can result in better fits of curves to data and therefore allow better estimation of half-lives,  $DT_{50}$  and, if appropriate,  $DT_{75}$  and  $DT_{90}$  values. The use of the derived constants, however, is limited. Compartment models can generate a number of useful constants of value in risk assessment that describe the rate of degradation in different compartments and the distribution of the chemical. They should also be used for estimation of rate constants for the formation and degradation of major transformation products. In all cases, the method chosen must be justified and the experimenter should demonstrate graphically and/or statistically the goodness of fit.

#### Test report

46. The report must include the following information:

##### Test substance:

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

##### Reference substances:

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

##### Test sediments and waters:

- location and description of aquatic sediment sampling site(s) including, if possible, contamination history;
- all information relating to the collection, storage (if any) and acclimation of water-sediment systems;
- characteristics of the water-sediment samples as listed in Table in paragraph 26.

##### Test conditions:

- test system used (flow-through, biometer, way of ventilation, method of stirring, water volume, mass of sediment, thickness of both water and sediment layer, dimension of test vessels, etc.)
- application of test substance to test system: test concentration used, number of replicates and controls mode of application of test substance (e.g. use of solvent if any), etc.

- incubation temperature;
- sampling times;
- extraction methods and efficiencies as well as analytical methods and detection limits;
- methods for characterisation/identification of transformation products;
- deviations from the test protocol or test conditions during the study.

#### Results:

- raw data figures of representative analyses (all raw data have to be stored in the GLP-archive);
- repeatability and sensitivity of the analytical methods used;
- rates of recovery (% values for a valid study are given in paragraph 13);
- tables of results expressed as % of the applied dose and in  $\text{mg}\cdot\text{kg}^{-1}$  in water, sediment and total system (% only) for the test substance and, if appropriate, for transformation products and non-extractable radioactivity;
- mass balance during and at the end of the studies;
- a graphical representation of the transformation in the water and sediment fractions and in total system (including mineralisation);
- mineralisation rates;
- half-life,  $\text{DT}_{50}$  and, if appropriate,  $\text{DT}_{75}$  and  $\text{DT}_{90}$  values for the test substance and, where appropriate, for transformation products including confidence limits in water, sediment and in total system;
- an assessment of the transformation kinetics of the test substance and, where appropriate, the transformation products;
- a proposed pathway of transformation, where appropriate;
- discussion of results.

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ANNEX 1GUIDANCE ON THE AEROBIC AND THE ANAEROBIC TEST SYSTEMSAerobic test system

The aerobic test system described in this test guideline consists of an aerobic water layer (typical oxygen concentrations range from 7 to 10 mg·L<sup>-1</sup>) and a sediment layer, aerobic at the surface and anaerobic below the surface (typical average redox potentials ( $E_h$ ) in the anaerobic zone of the sediment range from -80 to -190 mV). Moistened air is passed over the surface of the water in each incubation unit to maintain sufficient oxygen in the head space.

Anaerobic test system

For the anaerobic test system, the test procedure is essentially the same as that outlined for the aerobic system with the exception that moistened nitrogen is passed above the surface of the water in each incubation unit to maintain a head space of nitrogen. The sediment and water are regarded as anaerobic once the redox potential ( $E_h$ ) is lower than -100 mV.

In the anaerobic test, assessment of mineralisation includes measurement of evolved carbon dioxide and methane.

ANNEX 2DEFINITIONS AND UNITS

Standard International (SI) units should be used in any case.

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

Transformation products: all substances resulting from biotic and abiotic transformation reactions of the test substance including CO<sub>2</sub> and bound residues.

Bound residues: "Bound residues" represent compounds in soil, plant or animal that persist in the matrix in the form of the parent substance or its metabolite(s) after extractions. 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 (1) [modified from IUPAC 1984 (2)].

Aerobic transformation (oxidising): reactions occurring in the presence of molecular oxygen (3).

Anaerobic transformation (reducing): reactions occurring under exclusion of molecular oxygen (3).

Natural waters are surface waters obtained from ponds, rivers, streams, etc.

Sediment is a mixture of mineral and organic chemical constituents, the latter containing compounds of high carbon and nitrogen content and of high molecular masses. It is deposited by natural water and forms an interface with that water.

Mineralisation is the complete degradation of an organic compound to CO<sub>2</sub>, H<sub>2</sub>O under aerobic conditions, and CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>O under anaerobic conditions. In the context of this guideline, when radiolabelled compound is used, mineralisation means extensive degradation of a molecule during which a labelled carbon atom is oxidised or reduced quantitatively with release of the appropriate amount of <sup>14</sup>CO<sub>2</sub> or <sup>14</sup>CH<sub>4</sub>, respectively.

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ANNEX 2 (continued)

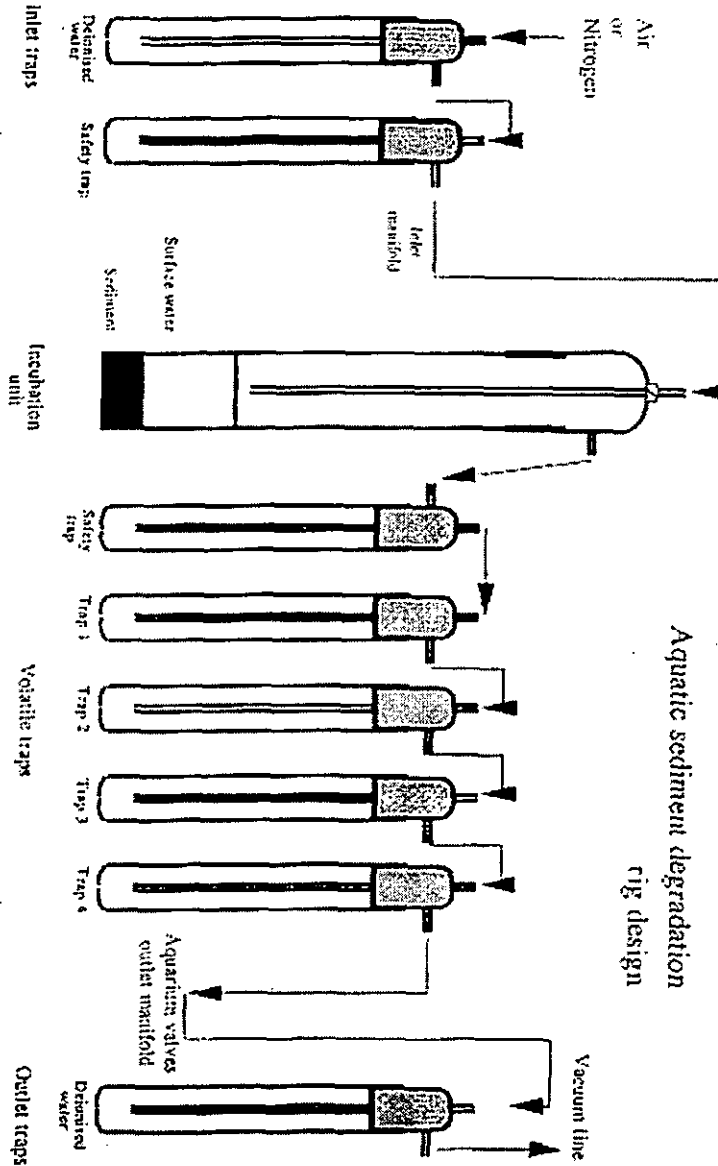
Half-life,  $t_{0.5}$ , is the time taken for 50% transformation of a test substance when the transformation can be described by first-order kinetics; it is independent of the initial concentration.

DT<sub>50</sub> (Disappearance Time 50) is the time within which the initial concentration of the test substance is reduced by 50%.

DT<sub>75</sub> (Disappearance Time 75) is the time within which the initial concentration of the test substance is reduced by 75%.

DT<sub>90</sub> (Disappearance Time 90) is the time within which the initial concentration of the test substance is reduced by 90%.

**ANNEX 3**  
**EXAMPLE OF A GAS FLOW-THROUGH APPARATUS**



Safety trap, empty

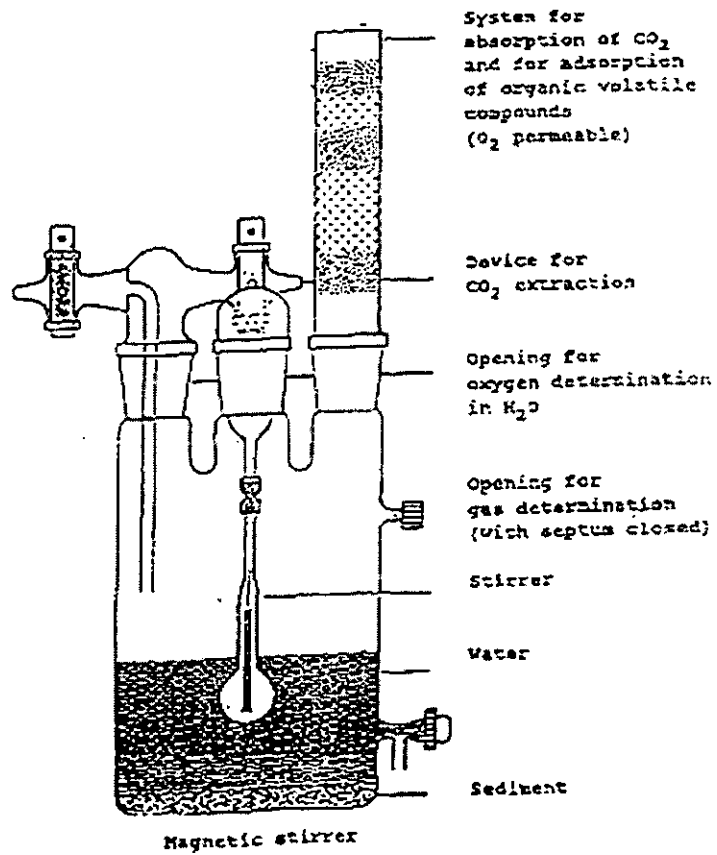
Trap 1:  
ethyleneglycol to  
trap organic  
volatiles

Trap 2:  
sulphuric acid 0.1  
M to trap alkaline  
volatiles

Aquatic sediment degradation  
rig design

ANNEX 4

EXAMPLE OF A BIOMETER APPARATUS



ANNEX 5EXAMPLE CALCULATION FOR APPLICATION DOSE TO TEST VESSELS

Cylinder internal diameter:	= 8 cm
Water column depth not including sediment:	= 12 cm
Surface area: $3.142 \times 4^2$	= $50.3 \text{ cm}^2$
Application rate: 500 g test substance/ha corresponds to $5 \text{ } \mu\text{g}/\text{cm}^2$	
Total $\mu\text{g}$ : $5 \times 50.3$	= 251.5 $\mu\text{g}$
Adjust quantity in relation to a depth of 100 cm: $12 \times 251.5 \div 100$	= 30.18 $\mu\text{g}$
Volume of water column: $50.3 \times 12$	= 603 mL
Concentration in water: $30.18 \div 603$	= 0.050 $\mu\text{g}/\text{mL}$ or 50 $\mu\text{g}/\text{L}$

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