OECD GUIDELINES FOR THE TESTING OF CHEMICALS

PROPOSAL FOR A NEW GUIDELINE

Phototransformation of Chemicals on Soil Surfaces

INTRODUCTION

1. Man-made chemicals may reach soil directly via deliberate application (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 distribution in and between the various environmental compartments (soil, water, air).

2. Chemicals can be transformed in soil by microbial, chemical and/or photochemical processes. The method described in this Test Guideline deals with the photochemical transformation of chemical substances on soil surfaces. It is based on existing guidelines (1) (2) (3) (4) (5).

PRINCIPLE OF THE TEST

3. Thin-layers of soil are treated with the test substance and irradiated by simulated sunlight (Xenon lamp) under controlled laboratory conditions. Simultaneously, not-irradiated dark controls are kept under identical conditions to distinguish between photochemical and other reactions. After appropriate time intervals, the soil thin-layers are extracted and analysed for the parent test substance and/or for major phototransformation products. Volatile products are collected by appropriate absorption devices and are also analysed for at time intervals. Using ^{14}C-labelled material, phototransformation products can be identified and a mass balance, including the formation of soil bound (non-extractable) residues, can be established.

APPLICABILITY OF THE TEST

4. The method is applicable to all test substances (unlabelled and radiolabelled) for which an analytical method with sufficient accuracy and sensitivity is available. It is applicable to slightly volatile or 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.

INFORMATION ON THE TEST SUBSTANCE

5. Unlabelled or labelled test substances can be used to measure the rate of phototransformation. Labelled material is required for studying the pathway of phototransformation and for establishing a mass balance. ^{14}C-labelling is recommended but 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 %.

6. Before carrying out a phototransformation test on soil surfaces, the following information on the test substance should be available:

(a) UV-VIS Absorption Spectra [OECD Guideline 105] (6);
(a) solubility in water [OECD Guideline 105] (6);
(b) solubility in organic solvents;
(c) vapour pressure [OECD Guideline 104] (6) and Henry's Law constant;
(d) n-octanol/water partition coefficient [OECD Guidelines 107 and/or 117] (6);
(e) adsorption coefficient (k_d, k_f or K_{OC}) [OECD Guideline 106 and/or Guideline 121] (6);
(f) chemical stability in the dark (hydrolysis) [OECD Guideline 111] (6);
(g) aerobic and anaerobic transformation in soil [OECD Guideline 307] (6);
(h) phototransformation in water [OECD Guideline, in preparation](7).

The temperature at which these measurements were made should be reported.

7. An appropriate analytical method of known accuracy, precision and sensitivity for the quantification and identification of the test substance and its phototransformation products in soil should be available. The analytical detection limit for the test substance and its phototransformation products ($\geq$10 % of the applied dose) should also be known (see paragraph 12).

REFERENCE SUBSTANCES

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

DEFINITIONS AND UNITS

9. See Annex 1.

QUALITY CRITERIA

Recovery

10. 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 (5) and from 70 % to 110 % for non-labelled chemicals (3).

[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 phototransformation products.

Repeatability and sensitivity of analytical method

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

12. The limit of detection (LOD) of the analytical method for the test substance and for the phototransformation products should be at least 0.01 mg · kg^{-1} soil (as test substance) or 1 % of the applied dose which ever is lower. The limit of quantification should also be specified.

Accuracy of transformation data

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

DESCRIPTION OF THE TEST METHOD

Light source

14. Simulation of sunlight with solar simulators under well-controlled laboratory conditions is considered the optimal light source. A characteristic of spectral power distribution for sunlight is given elsewhere (8). The cut-off of radiation of the light source used has to be 295 nm. Xenon lamps equipped with appropriate filters, such as the Hanau Suntest apparatus (see Annex 2) are recommended as irradiation source (5)(8) because they simulate very closely natural sunlight in the 295-800 nm region (see Annex 2). Whereas mercury-metal halide arc lamps or equivalents can be used for other tests requiring lower level of energy than xenon lamps, such lamps are not suitable to evaluate the photransformation of chemicals because they have less power than xenon lamps. For the assessment of phototransformation of chemicals, generally higher level of irradiation power is required which can only be provided by xenon lamps for the time being.

15. Since light in the wavelength range of 295 to 400 nm is most relevant for phototransformation of chemicals in the environment, the spectral energy distribution of the Xenon light source (i.e. the incident light available at the soil surface level) in this range is recorded using a portable spectroradiometer[2], consisting of a holographic grating monochromator and a Teflon cosine diffuser in combination with glass fibre optics. Typical light intensities (irradiance) between 300 and 400 nm at different exposure positions are given in Annex 3.

Conversion of laboratory irradiation time into days of natural summer sunlight

16. In order to relate the rate of phototransformation caused by the Xenon light to the rate likely to occur in sunlight, it is necessary to compare the irradiance of the Xenon lamp with sunlight (9). For this purpose, the integral of light intensity between 295 to 400 nm is measured at the various positions of the soil thin-layers in the test system described in paragraph 16 - 18 before and after the test. These values are

[2] For example, Model LI-1800, LICOR Ltd., Lincoln, NE 68504, USA can be used. This information is given for the convenience of readers. Equivalent instruments may be used if they can be shown to lead to the same result.

compared to energy values of natural summer sunlight (midday) at latitude 30 to 50° N[3] which can be obtained from reference (10) or which can also be measured with the spectroradiometer (see Annex 3).

17. Furthermore, it is assumed that the average daily sun radiation intensity is approx. 75 % of the maximum intensity over a 12-hour period (10), whereas irradiation intensity of the Xenon lamp is constant over time. The equivalent days d of natural summer sunlight can be calculated by the following equation:

$$d = \frac{h \cdot r}{0.75 \cdot 12}$$

where d = days of summer sunlight
h = hours of irradiation by the Xenon lamp
r = ratio of intensity (irradiance) of the Xenon radiation to that of summer sunlight
0.75 = correction for diurnal variation of natural sunlight
12 = conversion factor of hours to days.

Test system

18. The treated soil thin-layers (for preparation see paragraphs 27 – 28) are incubated and irradiated under temperature-controlled conditions in a flow-through system to trap any volatile products formed during the experiments. An example of a useful flow-through system is shown in Annex 4 (9). Other test systems are described in references (11) (12) (13).

19. This test system consists of a stainless steel cooling tank containing the soil plates. This tank is sealed with a quartz lid, using teflon tape between and bound around the lid/tank interface, and placed beneath the Xenon lamp. Cooling water at a pre-set temperature is pumped through the base of the tank to control the temperature of the irradiated soil-coated plates. The temperature is monitored in the soil during the experiments using an electronic thermologger[4].

20. In order to trap any volatile products formed during irradiation, air is sucked through the tank at a certain flow rate (e.g. 10 – 12 ml/min) using a peristaltic pump. The incoming air is bubbled through traps containing 2 M potassium hydroxide solution and then through traps containing water to ensure that moist, carbon dioxide free air enters the system. The effluent air is bubbled through a series of traps containing, for example, 1 M sulphuric acid solution (to trap volatile basic compounds), methoxyethanol (to trap volatile organosoluble compounds) and ethanolamine (to trap $^{14}CO_2$ or other acidic volatile compounds).

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 isotop dilution method;

[3] Intensities of sunlight depend on the geographical latitude in spring, autumn and winter, but are practically not influenced in summer.
[4] For example, Grant Instruments Cambridge Ltd., Barrington, Cambridge, UK. This information is given for the convenience of readers. Equivalent instruments may be used if they can be shown to lead to the same result.

- instruments for identification purposes (e.g. MS, GC-MS, HPLC-MS, NMR, etc.);
- liquid scintillation counter for radiolabelled 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)
- water bath

22. Chemicals used include: organic solvents, analytical grade such as acetone, methanol, etc.; scintillation liquid; various absorption solutions (see paragraph 20).

Soil

Soil selection

23. For phototransformation studies on soil surfaces, the use of one soil is sufficient. As these experiments require glass or metal plates to be covered with thin-layers of soil, only those soils can be used which can form thin and solid layers on a glass or metal surface. Generally, a silty loam or clay loam soil rather than a sandy soil should be selected (5).

24. The selected soil should be characterised for texture [according to FAO and USDA classification systems (14)], pH, organic carbon content and water holding capacity. For determination of soil characteristics the methods recommended in references (15) (16) (17) (18) (19) can be used.

Collection and storage of soil

25. The soil should be taken from the top layer (A-horizon) to a maximum depth of 20 cm. Remains of vegetation, macrofauna and stones should be removed. The soils are air-dried at room temperature (preferably between 20 - 25°C). Desaggregation 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 $\leq$ 2 mm sieve. Before use the soils can be stored at ambient temperature and kept air-dried as microbial activity is of little relevance when studying physico-chemical processes on the soil surface. 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.

26. Detailed information on the history of the field sites from where the test soil is 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 contaminations (20).

Preparation of soil thin-layer plates

27. Soil thin-layers are prepared by applying an aqueous slurry of soil to a glass or metal plate[5] in such a way that a soil layer of about 2 mm thickness is formed. Thereafter, the soil layers are allowed to air-dry to reach a soil moisture of about 75 % of field capacity (about 24 hours).

[5] An appropriate plate size is 5.5 cm × 3.5 cm. Parts of the soil thin-layers are scraped off at the edges of the plate, so that a soil area of 5 cm × 2.5 cm is left which corresponds to approx. 12.5 cm^2.

28. An additional set of soil thin-layers is allowed to completely dry-out before test substance application. Theses samples are used to measure phototransformation on irradiated dry soil surfaces.

Test substance application

29. To apply the test substance to the soil thin-layers it should be dissolved in water (deionised or distilled) or in a suitable organic solvent. Each soil thin-layer is then evenly treated with the test substance by distributing small droplets of the test substance solution over the whole soil surface by means of an appropriate syringe. In case an organic solvent is used, it should be evaporated from the soil thin-layers prior to the start of the irradiation process.

30. The amount of test substance applied to the soil thin-layers should correspond, for general chemicals, to the estimated amount normally reaching the soil, or for crop protection chemicals, to the maximum recommended use rate, and should be related to the surface area of the soil thin-layers used[6].

Test conditions

Test temperature

31. During the test, the treated soil thin-layers are kept in the test system described in paragraphs 18 - 20 at a constant temperature of 20 ± 2°C. The temperature is monitored during the test by a thermocouple which is inserted in the slurry of one soil thin-layer before the slurry dries out.

Moisture content

32. Soil thin-layers are kept either moist (75 % of the field capacity[7]) or air-dried during the irradiation period. Soil moisture is adjusted in the moist samples each time when samples are removed for analysis.

Dark controls

33. Additional moist soil thin-layers are kept under identical conditions but not irradiated to distinguish between photochemical and other reactions (5).

Test duration

[6] The amount to be applied to the soil thin-layers can be calculated by the following formula:

$$M[\mu g] = \frac{A[kg/ha] \cdot 10^9[\mu g/kg] \cdot F[cm^2]}{10^8[cm^2/ha]}$$

where: M = amount applied on soil thin-layer [µg]
A = rate of application [kg · ha^{-1}]
F = surface area of soil thin-layer [cm^2]

[7] For determination of water holding capacities, see OECD Guideline 307.

34. All soil thin-layers are continuously incubated in the described test system for a period of 240 hours (=10 days) which corresponds to approx. 30 days of natural midsummer sunlight (calculated by the equation given in paragraph 17 using intensity values of Annex 3).

Performance of the test

35. After treatment with the test substance, the soil thin-layers are placed in the stainless steel cooling tank illustrated in Figure 3. The tank is then sealed with the quartz lid as described in paragraph 17 and placed beneath the Xenon light source. The soil thin-layers are then irradiated for appropriate time intervals under the test conditions described above (see paragraphs 31 – 34).

36. Duplicate soil thin-layers are removed at appropriate time intervals and the soil samples are extracted with solvents of different polarity and analysed for the test substance and/or phototransformation products. Also, absorption solutions are removed at the same time intervals and analysed for volatile products. Besides a soil sample taken directly after application (0-hour 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 phototransformation products can be established (e.g. 0, 17, 24, 41, 65, 137 and 240 hours).

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

DATA AND REPORTING

Treatment of results

38. The amounts of test substance, phototransformation products, volatile products and non-extractables should be given as % of applied initial dose for each sampling interval. Also a mass balance should be given in percentage of the applied initial dose for each sampling interval. A graphical presentation of the test substance percentages against time will allow an estimation of its phototransformation half-life or DT_{50}. Major phototransformation products should be identified and their percentages should also be plotted against time to show their rates of formation and decline. A major phototransformation product is any product representing $\geq$ 10 % of the applied dose at any time during the study.

39. More accurate determinations of half-lives or DT_{50} values and, if appropriate, 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 correlation coefficient (r^2). If appropriate, the calculations should also be applied to the major phototransformation products. Examples of appropriate models are described in references (21) to (24).

Test report

40. The test report must include:

Test substance:

- common name, chemical name, CAS number, chemical structure (indicating position of label when radiolabelled material is used) and relevant physical-chemical properties (see paragraph 6);
- purity (impurities) of test substance;
- radiochemical purity of labelled chemical and specific activity (where appropriate).

Test soil:

- details of collection site;
- date and procedure of soil sampling;
- properties of soil, such as pH, organic carbon content, texture and water retention characteristics;
- length of soil storage and storage conditions

Xenon light source:

- manufacturer and model;
- filters and their purpose;
- measured spectrum of the light source between 295 and 800 nm at the beginning and the end of the test;
- comparison of the spectrum of the light with that of natural sunlight;
- intensity at sample level (Watt • m^{-2}) and area irradiated.

Test conditions:

- dates of the performance of the studies;
- amount of test substance applied;
- solvents used and method of application for the test substance;
- area of soil thin-layers treated;
- description of the incubation system used;
- air flow rates;
- temperature of experimental set-up;
- soil moisture content during incubation;
- method(s) of extraction;
- methods for quantification and identification of the test substance and major phototransformation products in soil and absorption solutions;
- number of replicates.

Test results:

- tables of results expressed as % of applied dose for the soil thin-layers;
- mass balance during and at the end of the studies;
- characterisation of non-extractable (bound) radioactivity or residues in soil;
- quantification of volatile products;
- plots of percentages in soil versus time for the test substance and, where appropriate, for the major phototransformation products;
- half-life or DT_{50} and DT_{90} for the test substance and, where appropriate, for major phototransformation products including confidence limits;
- an assessment of phototransformation kinetics for the test substance and, where appropriate, for major phototransformation products;

- proposed pathway of phototransformation, where appropriate;
- discussion and interpretation of results.

LITERATURE

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ANNEX 1

DEFINITIONS AND UNITS

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 product(s) 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 (1) [modified from IUPAC 1984 (2)].

Phototransformation: reactions occuring on soil thin-layers under the influence of light (> 290 nm).

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 guideline (3).

Xenon lamp: An intensive source of ultraviolet, visible and near-infrared light produced by electrical discharge in Xenon under high pressure.

Intensity: Traditional term for photo flux, irradiance or radiant power (radiant flux). In terms of an object exposed to radiation. The term should now be used only for qualitative descriptions.

Irradiance (E) [$W \cdot m^{-2}$]: The radiant flux or radiant power P of a all wavelengths incident on an infinitesimal element of surface containing the point under consideration divided by the area of the element (dP/dS; simplified expression: E = P/S when the radiant power is constant over the surface area considered).

Radiant Power (P), [$J \cdot s^{-1}$ or W]: Power emitted, transferred or received as radiation.

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

LITERATURE REFFERRED IN THE ANNEX 1

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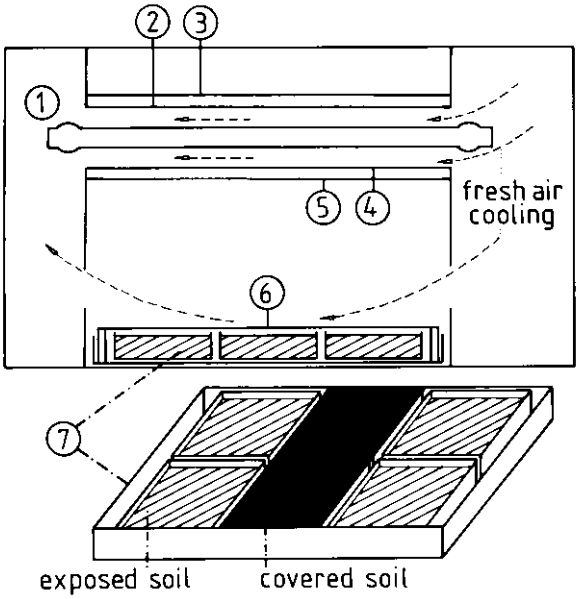
ANNEX 2

LIGHT SOURCE

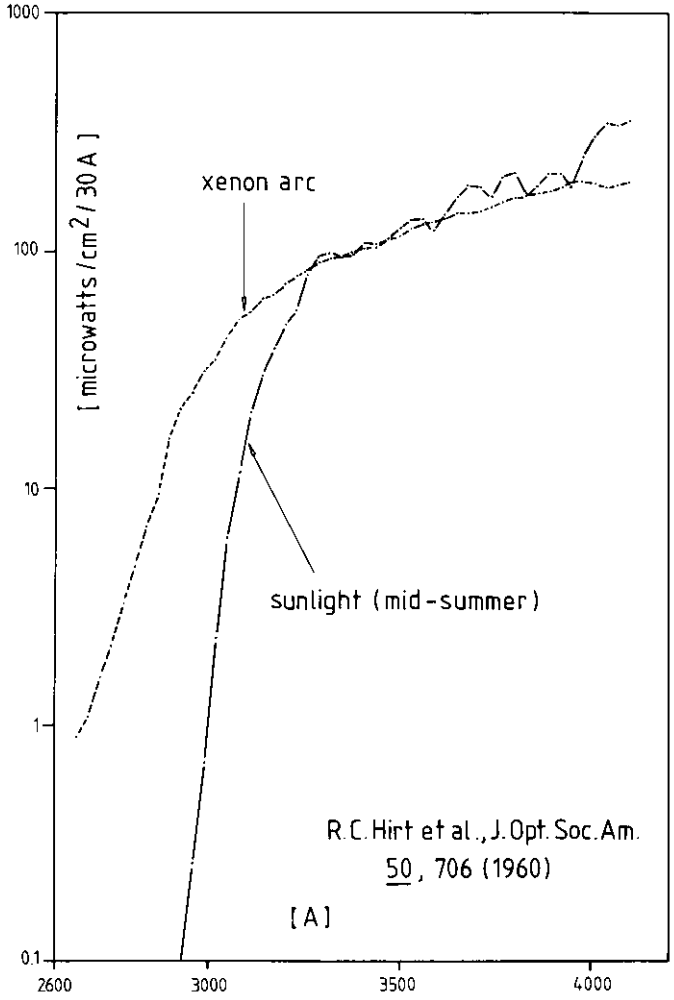
A. SCHEME OF THE HANAU SUNTEST APPARATUS (9)



1 Xenon lamp
2 UV reflecting mirror
3 Light reflecting mirror
} infrared transmissive
4 Quarz glass with infrared reflecting coating
5 UV filter with a radiation cut-off at 290 nm
6 Quartz glass
7 Metal boxes containing soil samples

ANNEX 2 (continued)

B. SPECTRUM OF XENON ARC COMPARED TO SUNLIGHT



ANNEX 3

LIGHT INTENSITIES OF A XENON LAMP AND OF NATURAL SUMMER SUNLIGHT

A. XENON LAMP

Position No.	Irradiance E (300 – 400 nm) [W/m^2]			r*
	Start	End	Mean	
1 +	69.2	69.7	69.4	1.036
2	78.0	78.9	78.4	1.171
3	79.6	80.7	80.1	1.196
4 ++	79.2	80.8	80.0	1.194
5	78.5	79.9	79.2	1.182
6	75.3	76.4	75.9	1.132
7 +	63.3	65.0	64.1	0.957
Mean	**74.7 ± 6.2**	**75.9 ±6.2**	**75.3**	**1.124 ± 0.09**
	75.3 ± 6.0			

\+ edge positions

++ central position

* r = ratio between artificial and natural light

B. NATURAL SUMMER SUNLIGHT

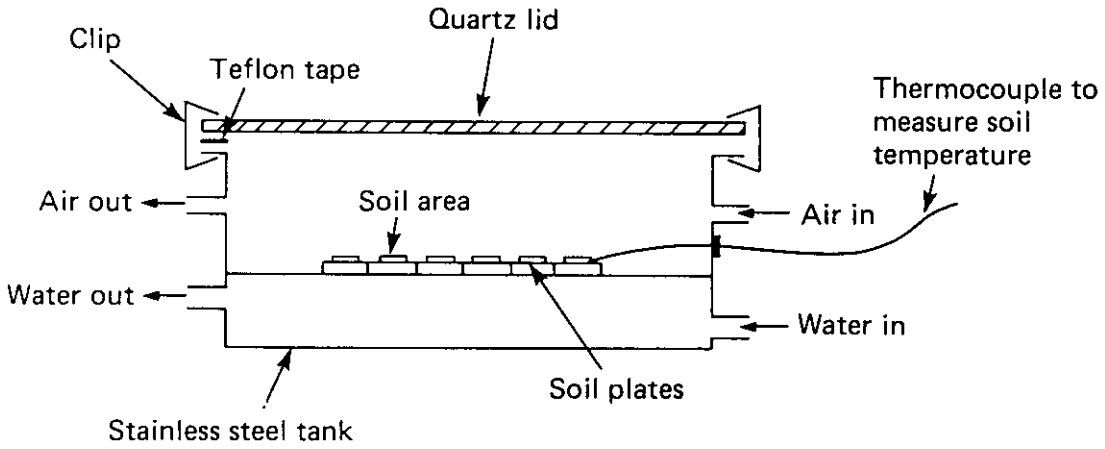
[Values taken from references (7) and (8)]

Region	latitude	Irradiance E (300 – 400 nm) [W/m^2]
Florida, USA	30°N	66.98
Greensboro, NC, USA	40°N	68.98
Basel, Switzerland	50°N	65.32
Mean	**30 – 50°N**	**67 ±3 %**

ANNEX 4

EXAMPLE OF A TEST SYSTEM FOR PHOTOTRANSFORMATION EXPERIMENTS ON SOIL SURFACES (8)

A. INCUBATION AND COOLING TANK



B. SOIL PHOTOLYSIS PLATE