European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

Efficacy evaluation of plant protection products

Effects on succeeding crops

Specific scope

This standard describes methods used to examine whether the active substance of a plant protection product, in particular a herbicide, causes negative effects on crops grown as rotational or replacement crops after a crop treated with that product.

Specific approval and amendment

First approved in 1998-09.
Revision approved in 2007-09.

1. Introduction

This standard is intended as a general standard on the methods used to examine whether the active substance of a plant protection product can cause negative effects on crops grown after a crop treated with that product. These crops can be grown as normal rotational crops as well as replacement crops in case of crop failure.

The standard is not only intended to give information on the design of particular trials. It is also intended as a stepwise guide to the different types of examination that can be carried out, taking into account information from trials conducted for other purposes, such as information on the persistence of the active substance. Whenever efficacy and selectivity trials are carried out, effects on the succeeding crop can usefully be noted if the trial site can remain marked out until the following year, and/or be accurately re-marked out in the succeeding crop.

Results from the representative product tested are normally applicable to different formulations of the active substance. However, where a particular product has been formulated in a special way to affect the persistence of an active substance, e.g. slow-release granules, the specific product should be examined.

The extent and type of field tests that need to be conducted depend on the basic fate and behaviour in soil of the active substance and on the nature of its biological activity. These properties can be investigated in preliminary laboratory, glasshouse or field trials, and the results will allow one to judge, according to the interval between applications of the active substance and planting of any sensitive succeeding crop, whether field trials are required. The information gained from the preliminary trials can be used to design the field trials. For many active substances, further testing will not be required.

Field trials can also be conducted in stages. Where effects are predicted from preliminary laboratory, glasshouse or field trials, observational trials on large plots can be carried out to examine how much residual activity can be expected under field conditions. If effects are seen on succeeding crops in field trials, a risk management strategy will be required to minimize risks. This may include label restrictions on the intervals between the use of the active substance and the planting of certain crops as succeeding crops, or specific cultivation recommendations (e.g. ploughing after crop failure). If effects on the succeeding crops are more marginal and label restrictions on succeeding crops can be avoided, more intensive small plot trials should be conducted to examine yield effects on any particular crops that are at risk. Appendix 1 presents an appropriate decision-support scheme.

2. Initial examination of the properties of the active substance

2.1 Studies on fate and behaviour in soil

The persistence and availability of the active substance in soil should be examined in a specifically designed set of trials, the conduct of which is outside the scope of this standard. Information on these studies is given in EPPO Standard PP 3/3 *Environmental risk assessment scheme for plant protection products*, Chapter 4: Soil.

The calculation of the Predicted Environmental Concentrations (PEC) for the active substance and their relevant metabolites in the compartment soil can be performed with equations (1) and (2) (Kloskowski *et al.* 1999). These equations are presented here for reference, however, expert judgement is required in selecting the relevant values to use and other issues to be considered.

PEC $_{initial}$

$$PEC_{ini} = \frac{A \cdot (1 - f_{int})}{100 \cdot d \cdot bd} \qquad (1)$$

where
A = application rate [g/ha]
f_{int} = fraction intercepted by plant cover
d = depth of the soil layer [cm]
bd = bulk soil density [g/cm³]

Initial PEC values represent actual concentrations of the active substance [mg/kg], where bulk density of soil is 1.5 g/cm³ dry weight and thickness of the soil layer is 2.5 to 5 cm for applications at the soil surface. Interception by plants covering the ground is assumed to be 0% for both pre-emergence and early post-emergence applications (see Appendix 2).

For calculating the concentration in soil at the time relevant for growing the succeeding crop, a DT50-value should be used, representing a justified, realistic worst case and the interval after spraying in days.

PEC $_{actual}$

$$PEC_{act}(t) = PEC_{ini} \cdot e^{-kt} = PEC_{ini} \cdot e^{-\frac{t \cdot \ln 2}{DT50}} \qquad (2)$$

If available, DT50 from field studies should be used, otherwise DT50-values from laboratory experiments. The DT50-values should represent first order kinetics providing that the χ^2 (Chi-square test) is passed with a percentage error of less than 15% and a good visual fit is observed (FOCUS, 2006).

2.2 Biological activity of the active substance

A bioassay on a range of representative rotational or replacement crop types should be made to examine whether the active substance affects germination in or growth through soil in which it is present. A simple study for non-herbicides considering biological data may be all that is required. These data may come from environmental risk assessments or other pot tests.

The bioassay should be done even if the active substance is mainly effective via the foliage of plants, as some uptake from the soil may take place. This test is best conducted in controlled conditions (in a growth chamber or glasshouse, with plants grown in pots). Further tests may be performed on a wider range of species in small-scale field trials. The range of crop species tested should take into account the intended use of the active substance and the usual succeeding crops in the rotation or likely replacement in the region of use. At least 5 representative species should be tested and the EC_{10} values determined. If the substance is known to be selective in its biological activity, the crops tested should be relevant to this activity, e.g. mostly dicotyledonous crops if the substance is active against dicotyledonous plants. The plant protection product should be incorporated into the soil in which seeds will be sown. The dose applied should be equivalent to what would be expected in soil after application of the dosage specified for the intended use. In addition, a range of decreasing doses may be applied, so as to determine the dose that gives no significant effects on the most sensitive plant species tested. This may in practice mean that more than one trial should be conducted for very active substances, if the 'no effect' level is not found in the first trial. It may also be useful to screen higher doses, if the active substance is persistent and likely to accumulate in the soil. Suitable methods for carrying out these tests are indicated in Appendix 3.

2.3 Deciding on the need for field testing

The results of soil behaviour tests and screens of biological activity should be taken together to determine whether the active substance poses a risk to succeeding crops (Appendix 1). The nature of succeeding crops and the likely interval between application and planting of these crops should be considered. Therefore Toxicity-Exposure Ratio (TER) values are calculated. The TER values are calculated using the PEC $_{actual}$ values, i.e. the assessment is based on the likely level of active substance at the time of planting the following crop, the cultivation methods recommended should be considered and not the initial level of active substance.

If the active substance has no activity against plants in soil at the highest doses tested in the bioassays, then field trials are unnecessary.

If the TER values are >1 (or the specific national level, if higher), then no further testing is necessary.

If the TER values are ≤1 (or the specific national level, if higher), damage to the relevant succeeding crops is possible and further field-testing is necessary as described under point 3.

If it is intended to examine the effects on crops that might be planted in the case of failure of the treated crop, the same general principles apply. The level of the active substance likely to be present at intervals after application should be compared with the sensitivity in soil of likely replacement crops to determine whether a trial is required.

All usual succeeding crops should be considered. The following factors may need to be taken into account:

- Not only crops that are planted soonest after harvest should be considered. Very sensitive crops planted some time after harvest may be at greater risk
- In horticulture, short rotations may be practised
- Active substances applied immediately pre or post-harvest pose a particular risk if they are active in soil, as the interval before planting the succeeding crop is shorter
- Persistence may vary with certain soil types, which may or may not be suitable for growing sensitive following crops
- Green manure crops, or crops grown between or under another crop may be sensitive and should be considered if they represent normal field practice
- A crop may be grown after failure of the treated crop.

3. Field trials

Treatments are applied to plots of the initial crop for which the plant protection product is authorized, and this is referred to as the "treated crop". After harvest of the treated crop, rotational test crops are sown into these plots to examine whether their growth is affected. For the replacement trials, treatments are applied to plots on bare soil after a normal seed bed preparation. The most sensitive replacement crops are sown at defined intervals after the application. The first sowing date should be immediately after the treatment. Sequential planting of sensitive crops over a period of time can provide information on how long it takes for an active substance to decline to a non-damaging level. Trials may also test different cultivation techniques, for example minimum tillage versus ploughing based cultivation, as ploughing can dilute the residue of active substance.

3.1 Experimental conditions

3.1.1 Selection of treated crop and cultivar

The trial should be performed on the crop(s) specified for the intended use or on bare soil in the same way as for the intended use.

3.1.2 Trial conditions

The trial should be set up in the field. Cultural conditions (e.g. soil type, fertilization, tillage) should be uniform for all plots of the trial and should conform with local agricultural/horticultural practice. The crop treatments should be recorded as well as any plant protection products used on or after it. Sites treated with plant protection products known to have phytotoxic effects on the succeeding crop should be avoided.

The trial should form part of a series carried out in different regions with distinct environmental conditions and preferably in different growing seasons (see EPPO Standard PP 1/181 *Conduct and reporting of efficacy evaluation trials, including good experimental practice*). The trial sites should be selected to cover the range of soil types to which the product could be applied. If data examined under section 2.1 suggests that carry-over may be greater on certain soil types, trial(s) should include site(s) with these soil types (unless application on these soil types is not permitted for the intended use).

3.1.3 Design and lay-out of the trial

3.1.3.1 Rotational crop trials

Treatments: test product(s), untreated control and reference product (if available), arranged in a suitable statistical design. It may be useful to have a positive and a negative reference, i.e. a standard well-known for its long residual activity, and the opposite. Two types of trials may be carried out with large or small plots. Large-plot trials are conducted to examine visual effects on a range of crops. Plots should be at least 40 m^2. Replication may be reduced or non-replicated trials may be conducted on several sites. Small-plot trials are conducted to examine the effects on yield of specific succeeding crop(s). In this case plots at least 20 m^2 (net) are required, with at least 4 replicates. Depending on the crop, plots may need to be larger to allow yield assessment. The assessments described in section 3.4.2 are carried out in both types of trials; qualitative and quantitative recording of yield (section 3.4.5) is only conducted in the second type of trial.

Markers should be placed in the ground in the treated crop so that plots can be distinguished before the succeeding test crops are planted. The initial plots should be sufficiently large for the required soil cultivation to be carried out after harvest of the treated crop. Remains from the treated crop, e.g. straw, should be evenly distributed over the plot or removed from the trial before planting the test crops, depending on local practice. If there is a risk to succeeding crops from residues in the crop debris, these should be managed so that the following crop is subject to the maximum exposure likely to arise under local practice.

3.1.3.2 Replacement trials

A special design was developed to test and assess the effects of herbicide residues in the soil under conditions in the field simulating crop failure (Krauskopf *et al.,* 1991; Callens *et al.,* 1997; Eelen *et al.,* 2001). The herbicide in question and a reference product are applied to bare soil following seedbed preparation in strips. Untreated strips are included nearby. Plots should be at least 20 m^2 with at least 4 replicates, depending on the available facilities.

At given intervals after applications over a period of time, likely replacement crops are planted across treated and untreated strips. Usually, seedbed preparation consists of shallow non-inversion tillage operations but the effect of inversion tillage (= ploughing) on replacement crops can be studied too. Sequential planting of crops should be carried out until the decline of the active substance reaches a non-damaging level.

For further information on trial design, see EPPO Standard PP 1/152 *Design and analysis of efficacy evaluation trials*.

3.2 Application of treatments

3.2.1 Test product(s)

The product(s) containing the active substance under investigation, should be the named formulated product(s). The product with the highest total recommended dose of the active substance should be used., Products that have been formulated in ways that are likely to affect the persistence of an active

substance (e.g. slow-release granules), should also be examined.

3.2.2 Reference product(s)

If available, a product known to have similar persistence in the soil and similar activity against the same crops as the test product should be applied. In general, mode of action, route of uptake, and fate and behaviour should be similar to that of the test product. In these trials, the primary purpose of the reference product is to determine whether conditions during the trial were generally conducive to the carry-over of active substances.

3.2.3 Mode of application

Applications should comply with good standard practice.

3.2.3.1 Type of application

The type of application should be as specified for the intended use.

3.2.3.2 Type of equipment

Application(s) should be made with suitable equipment, which provides an even distribution of product on the whole plot or accurate directional application where appropriate.

3.2.3.3 Time and frequency of application

The number of applications and the date of each application should be as specified for the intended use. The state (emergence, growth stage) of the crop and the number and date of the applications should be recorded. If crop types or cultivars can be treated at a range of timings in the year, then application on the treated crop(s) should be done at representative timings, including those which leave the shortest time between treatment and harvest and the least microbial activity in the soil, i.e. autumn/winter in northern Europe.

3.2.3.4 Doses and volumes

The product should normally be applied at the maximum dosage specified for the intended use and, where appropriate (particularly for herbicides), at least one higher dose (normally the double dose) is tested. If an active substance may accumulate to a high level when applied in successive years, the dose applied should be chosen to represent the level likely to be reached. Similarly, spray interception by the crop should be considered.

The dosage applied should normally be expressed in kg (or L) of formulated product per ha and the volume of water per ha should also be recorded for sprays. It may also be useful to record the dose in g of active substance per ha. In certain circumstances, the dose may be expressed as a concentration (e.g. % or g hL^{-1}), if possible combined with a volume (L ha^{-1}) appropriate to specific use. It may be useful to record information on water quality (e.g. pH, hardness).

Deviations from the intended dosage should be noted.

3.2.3.5 Data on other plant protection products

If other plant protection products (or any biocontrol agents) have to be used, they should be applied uniformly to all plots separately from the test product and reference product. Possible interference with these should be avoided. Other residual active substances that might affect following crops should not be used.

3.3 Test crops

3.3.1 Selection of test crops and cultivar

A selection of succeeding test crops with at least three of the most sensitive rotational crops or most sensitive replacement crops should be planted or sown into the previously treated plots. These should include at least three representative crops shown to be most sensitive to the active substance in tests described in 2.2 and which are likely to be grown after the treated crop according to local practice or after crop failure.

Rotational crop trials should be planted after harvest of the treated crop, at the shortest interval likely under normal practice. If crops planted in the spring following an autumn harvest are found to be sensitive to the active substance under investigation, then these should be included in the trial.

For the replacement trials, sequential planting of the crops at intervals over a period should be done until the decline of the active substance reaches a non-damaging level.

3.3.2 Trial conditions

The test crops for both trial types should be established using normal agricultural practice. If different cultural operations are used before planting the test crop, plots may be subdivided and these different cultivation regimes performed on the sub-plots.

3.4 Mode of assessment, recording and measurements

3.4.1 Meteorological and edaphic data

3.4.1.1 Meteorological data

Throughout the trial period, meteorological data should be recorded which is likely to affect the persistence of the active substance. Data on precipitation (type and amount in mm) and temperature (average, maximum, minimum in °C) should be recorded as fully as possible. Any significant change in weather should be noted, and in particular its time relative to the time of treatment. All data should preferably be recorded on

the trial site, but may be obtained from a nearby meteorological station. Its location and distance from the trial site should be noted. It is particularly important to have information on extremes of soil moisture or temperature occurring on the trial site. All data concerning irrigation should be recorded as appropriate. After the test crop has been planted, extreme weather conditions, such as severe or prolonged drought, heavy rain, late frosts, hail, etc., which are likely to influence the growth of the test crop, should also be reported.

3.4.1.2 Edaphic data

The following characteristics of the soil should be recorded: pH, organic matter content, soil type (according to a specified national or international standard), moisture (e.g. dry, wet, waterlogged), seed-bed quality (tilth) and fertilizer regime.

3.4.2 Type, time and frequency of assessment

No assessments are required on the treated crop.

3.4.2.1 Type

The test crops should be examined for phytotoxic effects. In addition, any positive effects should be noted. The type and extent of such effects should be recorded and, if there are no effects, this fact should also be recorded. Measurements of soil residues of the active substance under investigation may be useful. Phytotoxicity should be scored as follows:

(1) if the effect can be counted or measured, it should be expressed in absolute figures

(2) in other cases, the frequency and degree of damage should be estimated. This may be done in either of two ways: each plot is scored for phytotoxicity by reference to a scale, or each treated plot is compared with an untreated plot and % phytotoxicity estimated.

In all cases, unintended effects on the crop should be accurately described (stunting, chlorosis, deformation, delay in emergence, etc.). For further details, see EPPO Standard PP 1/135 *Phytotoxicity assessment* which contains sections on individual crops.

Depending on the findings of the visual estimations in the replacement trials, the biomass of aboveground and subterranean (for root and bulb crops) plant parts may additionally be determined,.

3.4.2.2 Time and frequency

Rotational crop trials

1st assessment: at emergence of the test crop. Special attention should be paid to delay of emergence or thinning, preferably determined by counting the plants.

2nd assessment: 3-4 weeks later. The number of test crop plants present should be estimated.

Further phytotoxicity assessments should be made during the life of the crop. These may be done for example, after the beginning of spring re-growth for autumn-sown crops, at flowering, or at the time of appearance of the harvested part of the plant.

Replacement trials

Visual estimations of crop emergence and injury should be made at various intervals from emergence of the test crop onwards. Timing should be aligned with the sowing dates. Shorter intervals may provide extra information, if the effects are only temporary.

3.4.3 Effects on other pests

Any observed effects, positive or negative, on the incidence of other pests should be recorded, especially effects on weed species emerging in the test crop.

3.4.4 Effects on other non-target organisms

Any observed effects, positive or negative, on naturally occurring or introduced pollinators or natural pests should be recorded. Any other environmental effects should also be recorded, especially effects on wildlife.

3.4.5 Quantitative and qualitative recording of yield

Yield measurements are only made in the small-plot rotational crop trials (described in 3.1.3) if significant phytotoxicity levels are recorded in the large-plot rotational trials. The method of recording yield or components of yield should be those appropriate for the test crop. For some crops, this is described in EPPO Standard PP 1/135 *Phytotoxicity assessment*. In the replacement trials where yield assessments are not practical, biomass of above ground and subterranean (for root and bulb crops) plant parts may be determined in addition to the visual assessments.

3.5 Results

The results should be reported in a systematic form and the report should include an analysis and evaluation. Original (raw) data should be available. Statistical analysis should normally be used by appropriate methods which should be indicated. If statistical analysis is not used, this should be justified. See also EPPO Standard PP 1/152 *Design and analysis of efficacy evaluation trials*.

4. Further assessment

If in the field testing no significant effects were observed in the test crops, then no further testing is necessary. If there are negative effects, management practices (e.g. ploughing) to reduce the risk to rotational or replacement crops should be tested under field conditions. If management practices can reduce or negate the risk to successive crops then labelling

should include relevant restrictions. If effects still occur no further testing is necessary. The final decision should be made at country level as, depending on national requirements, various decisions are possible.

References

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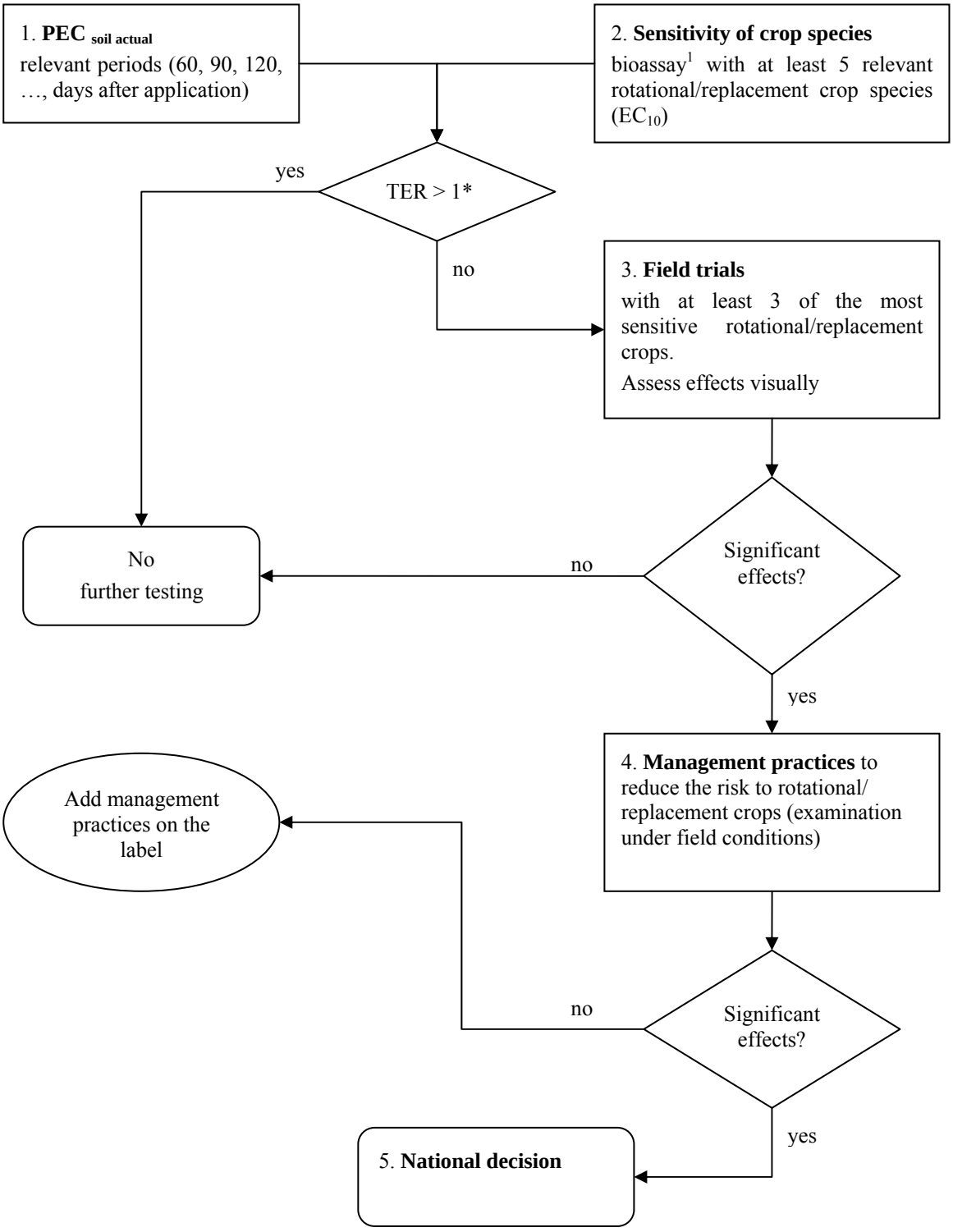
Appendix 1 Decision-support scheme on the extent of testing needed to examine effects on succeeding crops and on the consequent recommendations[1]



* or the specific national level, if higher

[1] A simple study for non-herbicides considering biological data may be all that is required. These data may come from environmental risk assessments or other pot tests

Appendix 2 Crop interception values for calculating Predicted Environmental Concentrations (PEC)

Table 1 gives interception data for specific growth stages of different crops. If the proposed crop is not present in the table a comparable crop should be used. Note that the interception data in Table 1 is only valid for applications made directly onto the crop.

Table 1. Interception (%) by crops and growth stage.

	BBCH[1]				
Crop	**Bare – emergence (00-09)**	**Leaf development (10-19)**	**Stem elongation (20-39)**	**Flowering (40-89)**	**Senescence Ripening (90-99)**
Bean (field + vegetable)	0	25	40	70	80
Cabbage	0	25	40	70	90
Carrot	0	25	60	80	80
Cereal (spring + winter)	0	25	50 (tillering) 70 (elongation)[3]	90	90
Cotton	0	30	60	75	90
Grass[2]	0	40	60	90	90
Linseed	0	30	60	70	90
Maize	0	25	50	75	90
Oilseed rape(summer+winter)	0	40	80	80	90
Onion	0	10	25	40	60
Pea	0	35	55	85	85
Potato	0	15	50	80	50
Soybean	0	35	55	85	65
Strawberry	0	30	50	60	60
Sugar beet	0	20	70 (rosette)	90	90
Sunflower	0	20	50	75	90
Tobacco	0	50	70	90	90
Tomato	0	50	70	80	50

[1] The BBCH code is indicative (BBCH, 1994).
[2] A value of 90 is used for applications to established turf
[3] BBCH code of 20-29 for tillering and 30-39 for elongation

These values and the table are taken from the "*FOCUS groundwater scenarios in the EU review of active substances*" Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference SANCO/321/2000 rev.2, 202pp (Table 1.6 in Version 1.1). http://viso.ei.jrc.it/focus/gw/index.html

Appendix 3 Method for screening the sensitivity of crop species to active substances present in soil

Test plants are sown in pots containing treated soil, into which the herbicide or active substance has been incorporated at known levels. Test species are chosen to be representative of the range of crops which could be sown as following crops. The bioassay should also include species already demonstrated to be very sensitive to the active substance. Enough test plants should be sown so that sufficient numbers of plants emerge for the purpose of the test. The test should be replicated and randomized, and plants should be grown in controlled conditions so that growing conditions are the same for all plants. An assessment should be made of emergence and all aspects of growth of the test plants in the treated soil compared with plants grown in untreated soil. Plant weight should be measured after an interval sufficiently long for effects of the active substance to be seen; this depends on the mode of action of the active substance.

For further information, see also OECD (2006).