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Review

# Conceptual Model for Improving the Link between Exposure and Effects in the Aquatic Risk Assessment of Pesticides

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## Abstract

Assessment of risks to aquatic organisms is important in the registration procedures for pesticides in industrialized countries. This risk assessment consists of two parts: (i) assessment of effects to these organisms derived from ecotoxicological experiments (= effect assessment), and (ii) assessment of concentration levels in relevant environmental compartments resulting from pesticide application (= exposure assessment). Current procedures lack a clear conceptual basis for the interface between the effect and exposure assessments which may lead to a low overall scientific quality of the risk assessment. This interface is defined here as the type of concentration that gives the best correlation to ecotoxicological effects and is called the ecotoxicologically relevant concentration (ERC). Definition of this ERC allows the design of tiered effect and exposure assessments that can interact flexibly and efficiently. There are two distinctly different exposure estimates required for pesticide risk assessment: that related to exposure in ecotoxicological experiments and that related to exposure in the field. The same type of ERC should be used consistently for both types of exposure estimates. Decisions are made by comparing a regulatory acceptable concentration (= RAC) level or curve (i.e., endpoint of the effect assessment) with predicted environmental concentration (= PEC) levels or curves (endpoint of the exposure assessment). For decision making based on ecotoxicological experiments with time-variable concentrations a tiered approach is proposed that compares (i) in a first step single RAC and PEC levels based on conservative assumptions, (ii) in a second step graphically RAC and PEC curves (describing the time courses of the RAC and PEC), and (iii) in a third step time-weighted average RAC and PEC levels.

Keywords: Aquatic ecotoxicology; Surface water; Exposure scenarios

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## 1. Introduction

Assessment of the risks to aquatic organisms is an important aspect of pesticide registration procedures in Europe, the USA and other industrialized countries. This risk assessment procedure consists of two parts: (i) assessment of effects to these organisms derived from ecotoxicological experiments (further called 'effect assessment'), and (ii) assessment of concentration levels to which organisms will be exposed in the field after pesticide application (further called 'exposure assessment'). Part (i) is the domain of ecotoxicology and part (ii) is the domain of environmental chemistry.

Until 2003, exposure of aquatic organisms to pesticides in surface water in the EU pesticide evaluation procedure was assessed using a very simple procedure taking into account only spray drift as a source of surface water contamination. FOCUS (2001) developed a tiered approach for surface water exposure assessment at the EU level. The approach is based on three steps that also take into account entry of pesticides into surface water via drainage and runoff (in addition to spray drift). Steps 1 and 2 are based on very simple models and scenarios (with a complexity level comparable to the procedure used before 2003). However, Step 3 (operational since 2003) is sophisticated; it consists of exposure assessments for ten scenarios using mechanistic models for describing leaching via drainage (MACRO), runoff (PRZM) and behavior in surface water (TOXSWA). The ten scenarios represent 'realistic worst case' exposure in the major agricultural areas across the EU by considering the main environmental driving factors (such as soil type, slope and rainfall intensity) for the three entry routes of the pesticide (spray drift deposition, drainage and runoff). In addition, FOCUS (2005) developed an extensive list of modelling refinements and mitigation measures that led to FOCUS Step 4 scenarios. So in the past years, aquatic exposure assessment at the EU level has become quite sophisticated. In the USA, the aquatic exposure assessment has reached a similar high level of sophistication. EPA (2004) has developed the Aquatic Level II Refined Risk Assessment (RRA) model which considers a range of surface water scenarios. Pesticide input is derived from runoff and erosion simulations with PRZM for a 36-year period. The concentrations in surface water are calculated with the VVWM model. The resulting 36 annual peak values are subsequently used as input to a probabilistic risk assessment procedure.

Also the effect assessment is at a high level of sophistication. Recently, detailed guidance on aquatic effect assessment (Campbell et al., 1999) and on risk assessment at EU level (European Commission, 2002a) has become available. The EU guidance document (European Commission, 2002a) describes the role of the FOCUS Step 1 to Step 3 surface water scenarios. For the lower effect tiers, this role is straightforward and consistent with the principles described before: the scenarios deliver the exposure concentrations (peaks or time-weighted averages) that are needed for the effect assessments. However, for higher-tier studies the EU guidance document offers as a complementary approach 'to simulate the fate dynamics experimentally in higher-tier studies' for substances that show a higher NOEC[1] in static studies (i.e., with a decreasing concentration) than in flow-through studies in which the concentration is kept constant (see p. 32 of guidance document). The document considers this as a complementary approach to using a time-weighted average concentration. Adding sediment to laboratory test systems to simulate adsorption or degradation or exposing the test system to natural light conditions to simulate photolysis are given as examples of this simulation of fate dynamics. Also micro/mesocosm test systems are often designed with the intention to mimic the time-variable exposure in the field more realistically. The EU guidance document states: 'In "fate simulation" studies the method used should be justified on the basis of its relevance to realistic environmental conditions.' No further guidance is provided on how this justification might be achieved. In regulatory practice, the notifier usually claims that such studies have a more realistic exposure because a spray drift event is simulated. However, the FOCUS Step 3 scenarios described above include also exposure from drainage and runoff and systems with short residence times of water which leads to a wide range of exposure patterns that cannot possibly all be simulated in one or a few higher-tier ecotoxicological experiments (FOCUS, 2001). Moreover, risk managers require usually realistic worst-case exposure patterns within the risk assessment and it is unlikely that e.g., a single mesocosm experiment available in the dossier shows a realistic worst-case exposure pattern. So the EU guidance document recommends two potentially conflicting approaches for exposure assessment in higher effect tiers and gives no guidance as to how the consistency between these two approaches should be ensured. This shows that the interaction between the assessments of exposure and of ecotoxicological effects in the higher tiers of risk assessment procedure for aquatic organisms is at a lower level of sophistication than either assessment of exposure or assessment of ecotoxicological effects. However, for an adequate risk assessment both aspects need to be combined in a sound way. Thus there seems to be a need for improvement of the aquatic risk assessment at the EU level by improving the interaction between exposure and ecotoxicological effects. The aim of this paper is to provide such an improvement by (i) defining more explicitly the interface between the assessment of exposure and of ecotoxicological effects, and (ii) providing adequate procedures for linking the exposure in higher-tier ecotoxicological experiments to that expected in surface water in the field.

We restrict ourselves as much as possible to the interaction between exposure and effects in the risk assessment because a review of assessment procedures of both exposure and ecotoxicological effects would divert

[1]NOEC is 'no observed effect concentration.'

attention from the interaction issue (see Brock et al., 2006, for a discussion of protection goals for aquatic risks in EU legislation). Although our discussion refers to the specific approach used for assessing risks of pesticides under current EU legislation, we believe that the principles we address are of much broader relevance with respect to other jurisdictions and other classes of chemicals.

## 2. General Principles of Exposure Assessment as Part of Risk Assessment for Aquatic Organisms

Any ecotoxicological risk assessment has to start with the question 'what has to be protected?' This protection aim will include usually a spatial component: e.g., protect aquatic and benthic organisms in watercourses neighbouring agricultural fields. It may also include a temporal component: e.g., consider only effects to be acceptable that show full recovery within a certain time period (see e.g., the 'class-2' and 'class-3 effects' described by European Commission, 2002a, p. 37). Once the protection aim is clear, a tiered risk assessment procedure can be designed to assess whether the aim will be met after introduction of a pesticide on the market. Such a procedure can be represented as an effect flow chart. The left part of Figure 1 shows an example of an effect flow chart consisting of four tiers. Each of the effect tiers of this chart needs estimates of field exposure concentrations for decision making. A crucial step is to define which type of field concentration is needed as the exposure input to the effect tiers. The choice should be based on ecotoxicological considerations because this should be the concentration that gives the best correlation to ecotoxicological effects. This type of concentration is defined here as the 'ecotoxicologically relevant concentration' (abbreviated to 'ERC'). The ecotoxicological considerations determining the ERC may include: (i) in which environmental compartment do the organisms live (e.g., in water and sediment)? (ii) what is the mode of action of the pesticide? (iii) what is bioavailable for the organism? (iv) what is the influence of the exposure pattern (e.g., short peaks or constant concentration over long periods) on the type- and degree of effects? and (v) Was the whole test duration of an ecotoxicological study necessary to cause the measured effects or would a shorter exposure period have given the same effect? It is of course necessary that the ERC is based on information available in the first tier of the effect assessment (i.e., box E-1 in Figure 1). Otherwise no adequate start of the risk assessment is possible. Several sources of information are already available in this first tier that can be used to define the type of ERC: data on the mode of action, acute and chronic toxicity to standard test species/taxa, time-to-event information and identification of the most sensitive lifestages as assessed from chronic standard tests (e.g., by a detailed evaluation of such tests on a time scale of days where possible).

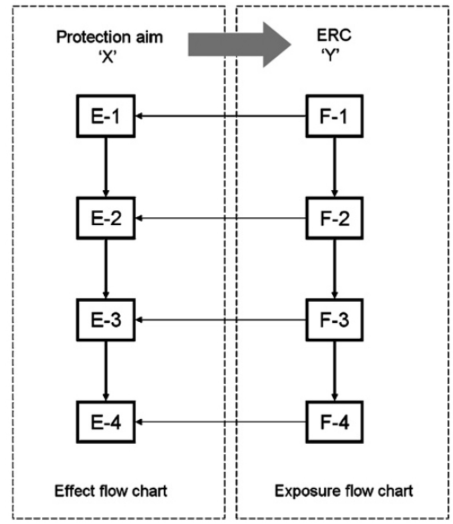


Figure 1. Tiered effect and exposure flow charts for a risk assessment addressing a protection aim 'X' which needs exposure estimates of an ecotoxicologically relevant concentration (ERC) 'Y' as indicated by the large arrow. The boxes E-1 to E-4 are four effect tiers and the boxes F-1 to F-4 are four tiers for assessment of exposure in the field ('F' from 'field'). Downward arrows indicate movement to a higher tier. Horizontal arrows from the exposure to the effect flow chart indicate delivery of field exposure estimates for comparison with effect concentrations in the effect flow chart.

For instance, for aquatic organisms the ERC could be e.g., the maximum over time or some time-weighted average of the concentration of dissolved pesticide in surface water. For sediment-dwelling organisms that live predominantly in the top centimetres of sediment, the ERC could be the maximum over time of the pore water concentration in the top 2 cm of the sediment (or as an alternative to the pore water concentration the bulk sediment concentration). After the ERC has been selected, an exposure flow chart can be designed as shown in the right part of Figure 1. The large arrow pointing to the right in the top of Figure 1 indicates that the effect flow chart determines the target for the exposure flow chart illustrating that this exposure flow chart is at a lower hierarchical level than the effect flow chart in the risk assessment. This is logical because the effect flow chart is more directly linked to the protection aim whereas the exposure flow chart becomes only meaningful after a relevant type of ERC has been selected.

To keep the example in Figure 1 simple, it was assumed that the effect flow chart needs the same type of ERC for all tiers. This is probable if this effect flow chart covers only one relevant taxonomic group. If an effect flow chart covers different relevant taxonomic groups (e.g., invertebrates, algae, macrophytes), then probably different time scales are to be considered for these different groups which may result in the need for different types of ERCs. This can be solved by designing different exposure flow charts for each type of ERC.

The concept of an ERC assumes implicitly that a concentration (i.e., mass per volume) gives the best correlation to an ecotoxicological effect. We use this concept because a concentration is the most common quantity used in the aquatic risk assessment. If another type of quantity such as a dosage (e.g., mass of pesticide per area of surface water) or a content (e.g., mass of pesticide per mass of sediment) would be considered more appropriate to characterize effects, this quantity can of course also be used as 'the ERC' in the system of effect and exposure flow charts of Figure 1.

The concept of tiered approaches is to start with simple conservative tiers and to do only more work if necessary (so providing an economic basis both for industry and regulatory agencies). The general principles of such tiered approaches are: (i) earlier tiers are more conservative than later tiers, (ii) later tiers are more realistic than earlier tiers, and (iii) earlier tiers usually require less effort than later tiers. A logical consequence is that jumping to later tiers (without considering all earlier tiers) is acceptable. An additional practical aspect is that there has to be some balance between the efforts and the filtering capacity of a tier. For instance, it does not make sense to define a tier that requires 50% of the efforts of the next higher tier but leads in 95% of the cases to the conclusion that this next tier is needed.

These general principles of tiered approaches imply that there need to be separate flow charts for each type of ERC or for each protection aim because different types of concentration may lead to different vulnerable scenarios, and different protection aims may lead to different types of ecotoxicological experiments. For example, exposure assessment based on the ERC 'total content of pesticide in the top 2 cm of sediment' will lead to surface water systems containing sediments with high organic matter being the most vulnerable scenarios because high organic matter leads to high pesticide contents in sediment. However, the ERC 'concentration of dissolved pesticide in surface water' will lead to sediments with low organic matter giving the most vulnerable scenarios because a low organic matter content of the sediment leads to high concentrations in surface water. As a consequence it is probably impossible to design one sequence of tiers that will assess these two different types of ERC appropriately. These examples show also that an adequate exposure assessment is only possible after the type of ERC has been defined: otherwise the vulnerability of field exposure estimates used in the risk assessment is undefinable. For types of ERC that differ only from each other with respect to the time aspect (e.g., peak concentration and 2-day average concentration in surface water), it is probably possible to use one single exposure flow chart. In general we recommend to first develop separate flow charts for each type of ERC or each protection aim and to consider thereafter whether flow charts can be merged to simplify the procedure as much as possible.

If the type of ERC would be difficult to define for some reason, it is advised to identify e.g., the two most likely ERCs and to perform the full risk assessment for each ERC hoping that the selected ERC options will lead to the same conclusion on the acceptability of the risk. If this is not the case, then a conservative approach would be to accept the worst result of the two risk assessments.

## 3. Clear Definition of the Ecotoxicologically Relevant Concentration (ERC)

A clear definition of the ERC is important because it provides the interface between the effect and exposure flow charts and thus an interface between two different fields of scientific expertise (ecotoxicology and environmental chemistry). Scientists from these two disciplines speak 'different languages' which may easily lead to confusion. To avoid this confusion, the definition of the ERC has to include the following aspects: (i) the definition of the quantity itself, (ii) the definition of the spatial scale of this quantity, and (iii) the definition of the temporal scale of this quantity. Defining the temporal and spatial scales is usually straightforward as shown by earlier examples of the ERC (e.g., 'maximum in time' and 'in top 2 cm of sediment'). The definition of the quantity is more complicated. It needs to include (i) the name of the quantity, (ii) the conceptual definition, (iii) the mathematical definition, and (iv) the operational definition. We consider the quantity with the name 'concentration of dissolved pesticide in surface water' as an example. Its conceptual definition is 'mass of dissolved pesticide per volume of surface water'. Its mathematical definition is

$$c = c^* - sX, \quad (1)$$

where $c$ is this quantity (mg/L), $c^*$ is total concentration of pesticide in water (mg/L), $s$ is mass of suspended solids per volume of water (kg/L) and $X$ is mass of pesticide adsorbed per mass of suspended solids (mg/kg). Its operational definition could be: (i) take a certain volume of surface water, (ii) filter this sample, (iii) extract the pesticide from the filtered water using some suitable extraction procedure (e.g., with acetone), (iv) measure the extracted mass by a suitable analytical method, and (v) divide this mass by the volume of surface water. In this operational definition it is assumed that filtering the water before extraction

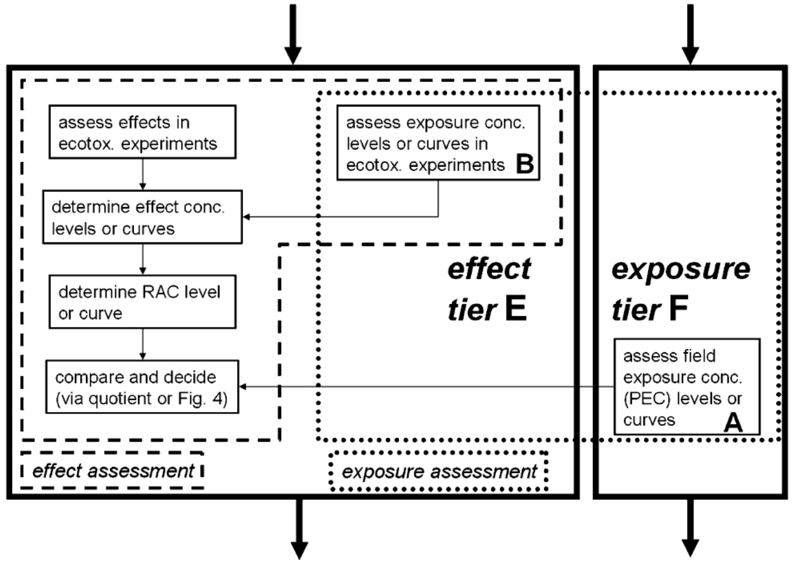


Figure 2. Schematic representation of activities in any combination of tiers of the effect and exposure flow chart. The dashed-line and dotted-line boxes indicate the division of the activities over the effect and exposure assessment illustrating that there are two distinctly different exposure assessments ('A' and 'B') in the risk assessment procedure (activity A being part of exposure tier F that delivers field exposure and activity B being part of the effect tier E).

removed the suspended solids and did not remove any of the dissolved pesticide due to sorption to the filter material.

## 4. Description of the Procedure for Integrating Exposure and Effect Tiers

We now analyze in more detail how the interaction between exposure in the field and in ecotoxicological experiments works. For that purpose, Figure 2 zooms in on an arbitrary combination of an E-box and an F-box of Figure 1. The standard procedure in ecotoxicological experiments is to use a range of concentration levels to derive a concentration–response relationship. It is obvious (but also crucial) that assessment endpoints within effect tiers such as the NOEC, the EC50[2] or the NOEAEC[3] have to be expressed in terms of the same type of ERC as the endpoints of exposure tiers. For instance, if the type of ERC was defined as the concentration in sediment pore water then this has to be used in the risk assessment both for evaluating the results of the ecotoxicological experiment and for estimating the exposure in the field (the requirement to use the same type of ERC does of course not include the spatial aspect of the definition of the type of ERC because many ecotoxicological experiments are carried out in the laboratory instead of the field). This implies that there are two equally important types of exposure assessments required for the risk assessment procedure. The first assessment (labelled 'A' in Figure 2) involves estimating the exposure (in terms of a certain type of ERC) that will occur in the field resulting from the use of the pesticide in agriculture. This is part of the exposure flow chart in Figure 1 and often referred to as PEC, i.e., Predicted Environmental Concentration (we use 'PEC' because this is the most common term but this does not exclude use of measured field concentrations in higher exposure tiers if these measured concentrations are more appropriate). The second exposure assessment (labeled 'B' in Figure 2) is a characterization of the exposure (defined in terms of the same type of ERC) to which the organisms were exposed in all ecotoxicological experiments (e.g., in simple static or flow-through laboratory experiments or in sophisticated outdoor mesocosm experiments). This exposure assessment B is part of all tiers in the effect flow chart. Figure 2 illustrates both exposure assessments and their interaction with the ecotoxicological activities. The figure also implies that fate experts and ecotoxicological experts have to co-operate closely in exposure assessment B.

Within an effect tier, the measured NOEC, EC50 or NOEAEC may not always be the assessment endpoint because it may have to be multiplied with a certain safety factor (see e.g., TER[4] values of 10 and 100 used by European Commission (2002a), and an example of EFSA, 2005) or extrapolated with a certain model (e.g., HC5[5] calculations). We assume here that the assessment

[2]EC50 is the concentration at which 50% of the test organisms show an effect.
[3]NOEAEC is No Observed Ecotoxicological Adverse Effect Concentration.

[4]TER is toxicity exposure ratio.
[5]HC5 is the concentration at which only 5% of the species show an effect for the selected endpoint. This value is derived from a Species Sensitivity Distribution curve (SSD) which may be constructed either with acute toxicity data (e.g., EC50s) or chronic toxicity data (e.g., NOECs).

endpoint of any effect tier can be simply called the 'regulatory acceptable concentration (RAC)' level thus including already any safety factors or extrapolation methods that are considered necessary. Once this RAC level has been determined, it has to be compared with the endpoint of an exposure tier (i.e., the field concentration level, called PEC level) after which it can be decided whether the risk according to this tier is acceptable. This activity takes place within the box 'compare and decide' in Figure 2. The simplest procedure is to calculate the quotient of the RAC level and the PEC level. If the concentration of the pesticide varies with time in the ecotoxicological experiment, also PEC and RAC curves (describing the time course) may have to be compared instead of PEC and RAC levels. This leads to a more complicated procedure which will be described later in Figure 4. Similarly the simplest procedure is to have deterministic RAC and PEC values (so a single RAC and a single PEC). However, the effect and exposure flow charts may produce probabilistic estimates of RAC and/or PEC (e.g., using RACs derived from SSD curves, using probabilistic PEC modelling tools or using field measured PECs that show considerable variability). In such a case the comparison between RAC and PEC will result in probabilistic risk assessment conclusions. E.g., the Aquatic Level II Refined Risk Assessment (RRA) model generates 36 annual peak surface water concentrations (EPA, 2004). If a single scenario run from this RRA model is used for estimating the PEC, the outcome of the 'compare and decide'-box could be that the risk is acceptable in 33 out of 36 years (so in 92% of the years). It is of course also possible to combine probabilistic PEC estimates with probabilistic RAC estimates using statistical techniques to estimate the probability that the PEC exceeds the RAC.

## 5. Routes through Combined Effect and Exposure Flow Charts

The route through combined effect and exposure flow charts is relevant because it influences the costs of the risk assessment (both in terms of conducting the risk assessment by industry and the subsequent review by regulatory authorities). One approach could be to link the level of sophistication in the ecotoxicological domain to that in the exposure domain. Figure 3A shows an example of this approach in which there is a one-to-one link between the effect and exposure tiers. We will call this the 'ladder' model. This is very restrictive and rigid. Moreover such a strong link between effect and exposure flow charts seems undesirable because changes in the exposure flow chart (e.g., incorporation of new emission routes) may then require changes in the effect flow chart. This seems not a cost-effective approach in the longer term.

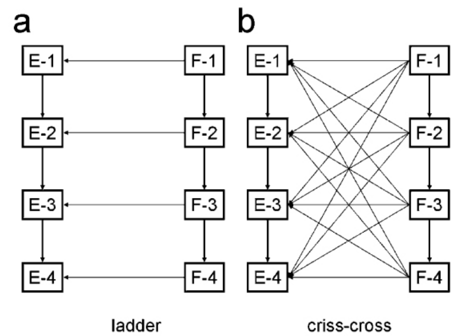


Figure 3. Diagrams of two different conceptual models of possible routes through combined effect and exposure flow charts. The boxes E-1 to E-4 are four effect tiers and the boxes F-1 to F-4 are four tiers for assessment of exposure in the field. Part A shows routes in which each effect tier is at the same level of sophistication as the exposure tier (called the 'ladder' model). Part B shows all possible routes (called the 'criss-cross' model). Downward arrows indicate movement to a higher tier. Arrows from right to left indicate delivery of field exposure estimates to the indicated effect tiers.

The principles of tiered approaches as described before imply that jumping to later tiers is always acceptable. So there is no need to restrict the route to the ladder model and any effect tier should be able to use results from any exposure tier. This approach is illustrated by Figure 3B. We will call this the 'criss-cross' model. In this figure any arrow implies a route through the flow chart. For instance, the arrow going from F-4 to E-2 implies that the risk assessor arrived in the tier F-4 for exposure and arrived in the tier E-2 for the effect assessment. In this criss-cross model the choice of the exposure tier is free and thus will be determined in practice by economic principles. For instance, if going to tier F-4 is much less expensive than going to tier E-4, then industry will first refine the exposure assessment via tier F-4 and compare this with e.g., tier E-1 of the effect flow chart (and of course the opposite if the effect tiers are much less expensive than the exposure tiers). In principle, there is no need for any restrictions, and therefore we recommend use of this criss-cross model. It implies a fully modular approach in which changes of elements of the exposure flow chart have no consequences for the effect flow chart. The criss-cross model is currently regulatory practice for aquatic risk assessment for the evaluation at the EU level, where recently exposure for a FOCUS Step 4 has been developed FOCUS (2005), because the first three tiers developed by FOCUS (2001) lead too frequently to the conclusion that risks cannot be excluded. FOCUS (2005) presented a diagram similar to the ladder diagram in Figure 3 (at p. 53 of this report) but added in the legend that in practice there can be flexibility as shown in the criss-cross diagram of Figure 3. So there seems to be consensus that the criss-cross model is better than the ladder model.

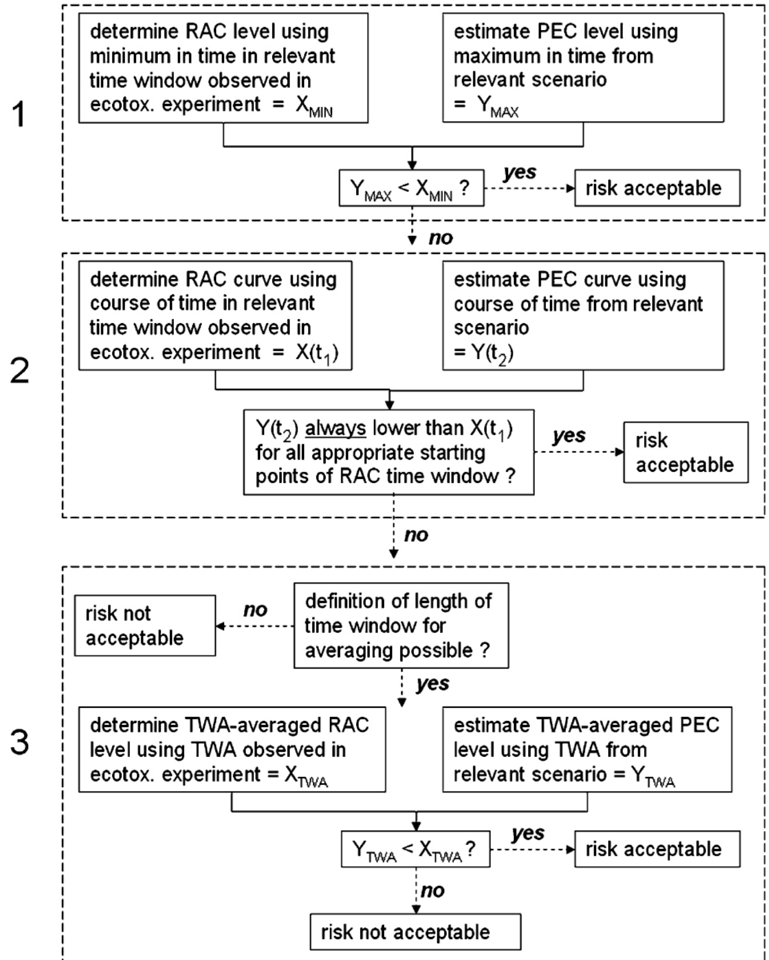


Figure 4. Flow chart for handling the procedure in the box 'compare and decide' of the effect tier shown in Figure 2 in case of a time-variable exposure concentration in the ecotoxicological experiment. The numbers 1, 2, and 3 indicate the numbers of the three steps. RAC is 'regulatory acceptable concentration', PEC is 'predicted environmental concentration', TWA is 'time-weighted average.'

## 6. Handling Time-Variable Exposure in Higher-Tier Ecotoxicological Experiments for Aquatic Risk Assessment

### 6.1. Proposal for a Stepped Approach for Handling Time-Variable Exposure

Higher-tier ecotoxicological experiments are the cornerstone of the aquatic risk assessment procedure. One of the most complex factors in the aquatic risk assessment with respect to the interaction of exposure and effects, is the handling of time-variable exposure concentrations in such higher-tier ecotoxicological experiments in relation to time-variable exposure concentrations in the field. Time-variable exposure concentrations are the rule rather than the exception for most pesticides under realistic field conditions. Also in sophisticated higher-tier ecotoxicological experiments such as mesocosms usually a pulsed exposure regime (i.e., based on repeated pesticide applications) is simulated. In fact time-variable exposure concentrations in complex test systems are inevitable in practice (e.g., it is difficult to keep a concentration of a non-persistent pesticide perfectly constant in a sophisticated outdoor mesocosm study). Considering the aspect of the time-variable exposure in the risk assessment is only relevant if the pesticide shows effects at lower initial concentrations when going from static studies (with single application and a decreasing concentration) to semi-static studies (with repeated refreshment of pesticide solution) to flow-through studies (with constant concentration). Otherwise the effect is obviously determined by the initial/maximum concentration and changes of the concentration over time do not matter for the risk assessment.

Until now, the standard procedure in most aquatic higher-tier ecotoxicological experiments (i.e., micro/mesocosm tests) has been as follows: (i) the study is conducted using a range of concentration levels (either static or semi-static; see previous paragraph), and (ii) the dynamics of the concentration in the water are measured for all or selected concentration levels. The

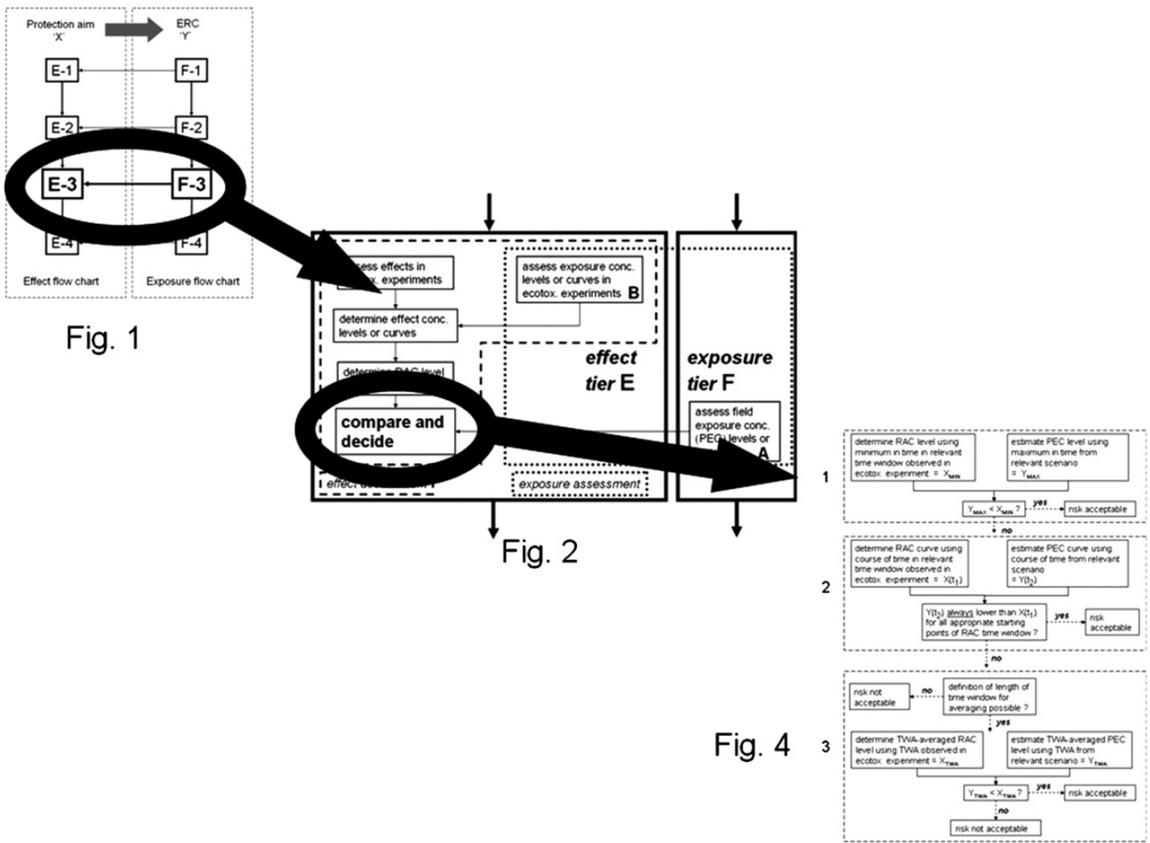


Figure 5. Schematic representation of the relationships between the flow charts of Figures 1, 2 and 4 illustrating that the flow chart of Figure 2 zooms in on a combined set of an effect tier and an exposure tier of Figure 1 and that the flow chart of Figure 4 zooms in on the 'compare and decide' box of Figure 2.

background for this procedure is that in the past the design of the exposure regime predominantly aimed at simulating the contamination by spray drift. EFSA (2005) analyzed the time aspect of one specific higher-tier ecotoxicological experiment in detail. Inspired by this case study, we developed the flow chart in Figure 4 for handling such cases. This flow chart zooms in on the box 'compare and decide' of Figure 2 for ecotoxicological experiments in which the exposure concentrations vary with time. Figure 5 illustrates that there are two zooming procedures: Figure 4 zooms in on Figure 2 while this Figure 2 itself zoomed in on Figure 1. The flow chart in Figure 4 consists of three steps. For all steps it first has to be decided which treatment level (characterized by its initial concentration level) in the experiment corresponds with the effect assessment endpoint derived from the experiment (e.g., LC50,[6] NOEC, NOEAEC). Then this treatment level is converted to an initial RAC level by applying an appropriate safety factor or an extrapolation model (if necessary). Linked to this initial RAC level, the time course of the concentration in the experiment may be available as well and we will call this 'the RAC curve.' This RAC curve may be needed in the risk assessment because the time course of the concentration in the experiment that determines the RAC, is an exposure characteristic that cannot be ignored in a consistent risk assessment if the observed effects (or their recovery) are not only influenced by the initial concentration level but also by this time course. If the effect assessment endpoint derived from the experiment is not one of the treatment levels (e.g., in case of the LC50 which may be determined by a statistical interpolation procedure), then the RAC curve may be estimated from the time courses at the two closest treatment levels.

Aquatic higher-tier ecotoxicological experiments (micro/mesocosms) may run for several months (see e.g., Crum et al., 1998). However, standard chronic toxicity tests in the first effect tier last usually much shorter; e.g., 7 days for vascular plants [*Lemna* test], 21 days for invertebrates [*Daphnia* test], 28-60 days for fish (European Commission, 2002a). It is not consistent in higher effect tiers to use time windows of the RAC curve that are longer than the experimental period of the chronic test in the first tier because the duration of this test should in principle be long enough to reveal possible effects that may occur during the whole life cycle of that species. If this assumption is

[6]LC50 is the concentration at which 50% of the organisms tested in an ecotoxicological experiment are killed.

frequently violated, the first-tier procedures should be adapted and made more conservative. Furthermore there is additional justification for this restriction to the time window of the RAC curve. Micro/mesocosm experiments are not only designed to assess threshold levels for effects but are also performed to study the potential for recovery of sensitive endpoints at higher exposure concentrations. In many micro/mesocosm experiments the 'post-application' period (i.e., after the last pesticide application to the system) is at least 8 weeks and often the exposure concentration has decreased to below the detection limit during part of this post-application period. So using the RAC curve of the full experimental period would imply that this 8-week period with low concentrations would become part of the RAC curve as well. This would lead in the risk assessment to 'punishment' of experimenters that continue their ecotoxicological observations for prolonged times. Such a punishment seems in principle undesirable in any pesticide risk assessment. However, if higher-tier tests would demonstrate unexpected effects resulting from long-term exposure to lower concentrations outside this 'first-tier' RAC time window, then it is advised (i) to analyze these effects critically, (ii) to assess their possible regulatory consequences, and (iii) to review the adequateness of the complete effect flow chart (which may e.g., lead to identifying the need for revising the experimental design of tests in the first tier).

So as an endpoint of the effect assessment we consider only time windows of the RAC curve that are equal to or shorter than the duration of the first-tier chronic test of the relevant taxonomic group. We will call this 'the relevant time window of the RAC curve.' This restriction to the time window applies only to the RAC curve (which is based on the exposure assessment in box B of Figure 2) and not to the effect assessment itself (i.e., the box 'assess effects in ecotox. experiment' in Figure 2). For this effect assessment it is of course desirable to consider the full experimental period of the micro/mesocosm experiment for the evaluation, e.g., to evaluate latency of effects, indirect effects and recovery.

The underlying principle for the flow chart in Figure 4 is a systematic comparison between the time course of the exposure concentration (PEC) in the field (further called the 'PEC curve') and the relevant time window of the RAC curve. The first step in Figure 4 is straightforward (and admittedly conservative). The RAC level is simply based on the minimum concentration of the RAC curve (within the relevant time window) and this is compared with the maximum PEC level (for all times considered, so global maximum), obtained from a relevant exposure scenario. The second step in Figure 4 is more sophisticated: here the relevant time window of the RAC curve is graphically compared with the PEC curve from a relevant exposure scenario. In the FOCUS Step 3 scenarios, the time of the PEC curve is available on an absolute scale (e.g., running from January 1, 1982 to May 1, 1983 using available meteorological time series). The RAC curve is on a relative time scale e.g., because it may be based on an ecotoxicological experiment in the laboratory. So it is most appropriate to start with the time scale of the PEC curve and to choose the starting point of the RAC curve freely in this graphical comparison. The concept of the comparison is that the PEC curve has to be below the RAC curve for all appropriate starting points of the RAC time window because this guarantees that the risk is acceptable in this Step 2 of Figure 4.

The definition of 'all appropriate starting points' of the relevant time window of the RAC curve is crucial as is illustrated by a few hypothetical examples

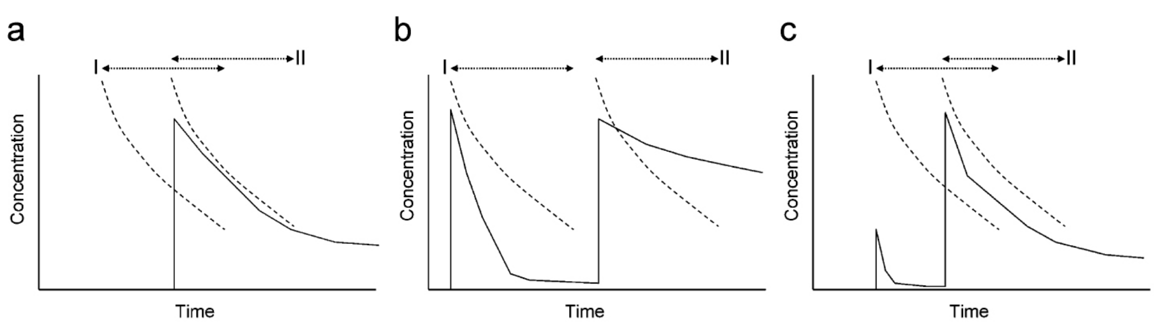


Figure 6. Different hypothetical PEC curves compared with the same hypothetical RAC curve using different starting points of the time window of this RAC curve (illustrating the procedure of Step 2 of Figure 4). Solid lines are PEC curves; dashed lines are relevant time windows of RAC curves (all dashed lines are identical except for a translation in time); the dotted line segments with the arrows (labelled 'I' and 'II') indicate the two time windows that are considered in each graph. Part A is an example demonstrating that RAC time windows have to start when a PEC maximum occurs. Part B is an example demonstrating that not only the time window starting at the time of the global maximum has to be checked but also time windows starting at the time of local maxima. Part C is an example demonstrating that a low local PEC maximum occurring just before the global PEC maximum should be ignored in the risk assessment procedure.

in Figure 6. In this figure, all RAC curves are identical (e.g., based on the same ecotoxicological experiment and using the same relevant time window). Figure 6A shows an example where the PEC curve has only one maximum. It is then only meaningful to consider a time window that starts at the time of this maximum (i.e., time window II) because this is the most critical period with respect to the effects. Figure 6A shows that the PEC curve is below the RAC curve for this time window II. So the conclusion in Figure 4 is 'risk acceptable.' It does not matter that the PEC curve is below the RAC curve for other starting points of the time window (such as time window I) because these are irrelevant to the risk assessment procedure. Figure 6B shows an example where the PEC curve has two maxima (one global and one local) and where the RAC time window that starts at the local maximum of the PEC curve (i.e., window II) leads to 'risk not acceptable' in Step 2 of Figure 4 whereas the RAC time window that starts at the global maximum of the PEC curve (i.e. window I) would have led to 'risk acceptable.' So Figure 6B demonstrates that not only the time window starting at the time of the global maximum has to be checked but also time windows that start at the time of local maxima. This leads to the following working hypothesis for the definition of appropriate starting points of RAC time windows: all windows starting at the time of occurrence of local maxima of the PEC curve. However, Figure 6C shows that this working hypothesis may be too conservative. Here a low local maximum of the PEC curve occurs shortly before the global maximum. According to the working hypothesis, time window I is appropriate and the PEC curve exceeds the RAC curve for part of the time. However, it seems unlikely that this would lead to an unacceptable risk. Thus we adopt the following revised working hypothesis for appropriate starting points of RAC time windows: starting at the times of all maxima (local or global) but excluding time windows such as window I in Figure 6C where the PEC curve remains initially below the RAC curve but later exceeds the tail of this RAC curve due to a new PEC maximum.

The examples in Figure 6 show that the definition of appropriate starting points of the RAC time window in Step 2 of Figure 4 is a complicated issue. Consideration of a range of other examples in the future may lead to other subtle refinements of this definition. In principle, this comparison of PEC and RAC curves can be easily automated via software in which all necessary refinements (based on expert judgement) are included via well-defined procedures (e.g., using output from the TOXSWA model which produces daily values of the pesticide concentration for the FOCUS surface water scenarios or using a time series of measured concentrations in the field).

The approach in Step 2 of Figure 4 (illustrated in Figure 6) implies that the PEC curve always has to be below the relevant time window of the RAC curve for all appropriate starting points of this time window. So if the PEC curve is above the RAC curve only for a few days in a time window of e.g., 21 d, this step cannot conclude an acceptable risk. This may be a serious restriction for such cases. An alternative is to define some time-weighted average (abbreviated to TWA) type of ERC as indicated in Step 3 of Figure 4. Use of TWA concentrations is a normal procedure in aquatic risk assessment at the EU level (European Commission, 2002a). However, the definition of the length of the TWA time window over which averaging is justifiable requires additional ecotoxicological (and possibly toxicokinetic) a priori knowledge (see Reinert et al., 2002, for a discussion of temporal aspects of effects of chemicals). If this knowledge is not sufficiently available, one could consider selecting one short TWA time window and one long TWA window (not exceeding the time frame of the relevant first-tier chronic test as discussed above) and performing the risk assessment twice, hoping that both time windows lead to the same conclusion. If this is not the case, the conservative choice would be to accept the result of Step 2.

Step 1 offers a very simple (but conservative) approach. Thus the more sophisticated Steps 2 and 3 are only necessary in the borderline cases in which the decline of the concentration during the higher-tier ecotoxicological experiment determines whether the risk is considered acceptable or not, thus leading the risk assessor to move to these more complex steps (or to stop, accepting the conclusion that the risk is not acceptable).

Once the length of the time window has been selected, carrying out Step 3 will be relatively easy. The standard output of the Step 3 FOCUS scenarios (i.e., surface water concentrations calculated by the TOXSWA model; note that Step 3 of Figure 4 has no relationship whatsoever to Step 3 of the FOCUS scenarios) contains time-weighted averages for periods of 1, 2, 4, 7, 14, 21, 28, 42, 50 and 100 days (Beltman et al., 2006). Calculation of time-weighted averages of RAC curves or of field-measured PEC curves usually requires little effort as well (e.g., if compared to the effort associated with performing and reporting higher-tier ecotoxicological experiments).

Until now the discussion of the stepped procedure of Figure 4 has been restricted to considerations on the time courses of the RAC and PEC curves. Ecotoxicological considerations can of course also restrict the comparison between these curves. E.g., consider an example where the RAC curve of an insecticide was based on an experiment with juvenile insects because this was considered the most sensitive part of the life cycle of this insect to this particular insecticide. Let us

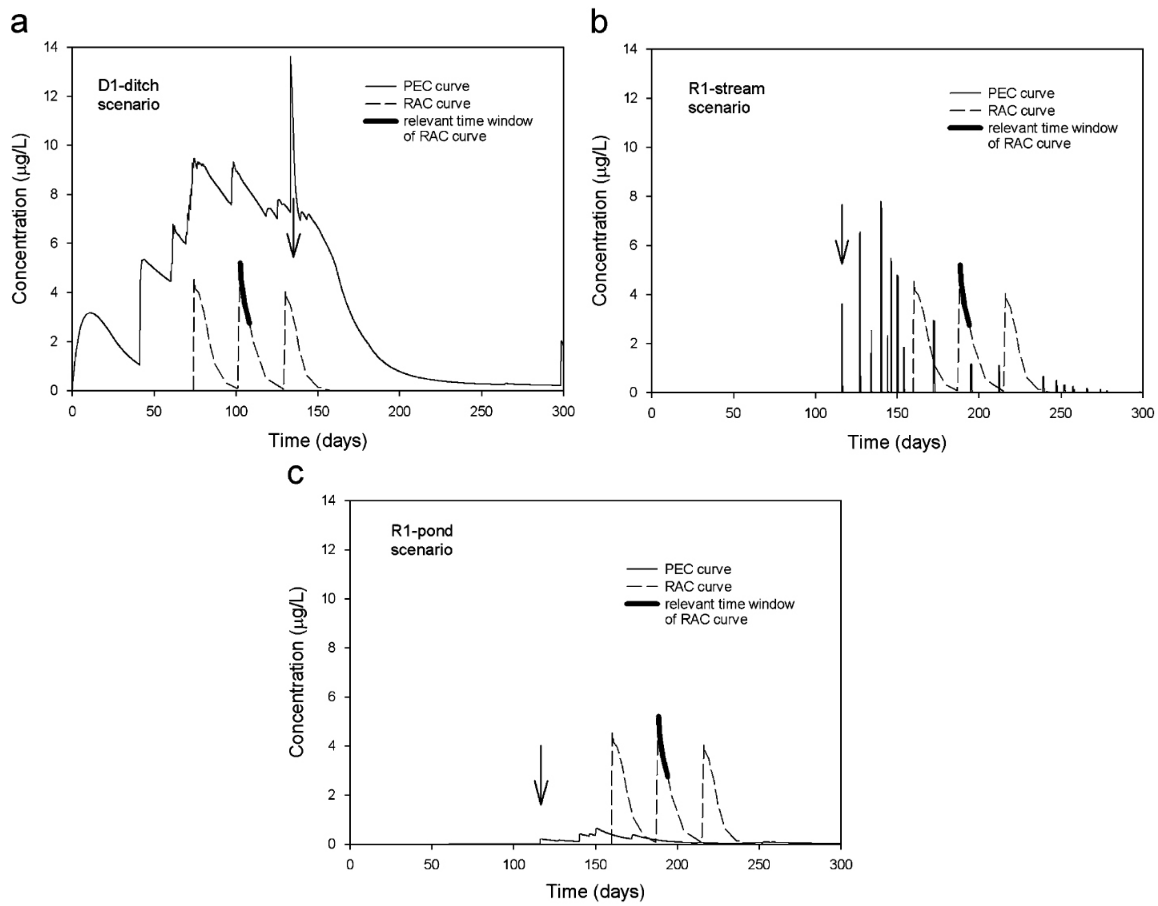


Figure 7. Linuron concentrations in surface water as a function of time as calculated with the TOXSWA model for three FOCUS Step 3 surface water scenarios compared with a RAC curve as derived from the mesocosm experiment by Van Geest et al. (1999) using effects of class 1 as a basis. Part A is scenario D1-ditch, part B is scenario R1-stream and part C is scenario R1-pond (see FOCUS, 2001, for definition of the scenarios). Time 0 is January 1, 1982 for the FOCUS PEC curve in part A and January 1, 1984 for the FOCUS PEC curves in parts B and C. The RAC curves start at arbitrary times. The arrow indicates the application time of linuron in the TOXSWA simulations. Note that the RAC curve is the same for all three graphs.

further assume that these juvenile insects occur in surface water only in spring. In such a case it could be justifiable to restrict the time window of the PEC curve to spring. It may also be justifiable e.g., to ignore PEC curves of winter periods if the organisms that have to be protected do not occur in surface waters in winter. Such ecotoxicological restrictions can be easily applied to the PEC curves before they are fed into the flow chart of Figure 4. However, ecotoxicologists have to justify and document any such restrictions appropriately as part of the risk assessment procedure.

## 6.2. Application of the Proposed Stepped Approach for Time-Variable Exposure to a Case Study

We illustrate now the use of the flow chart of Figure 4 with a case study for the herbicide linuron. The effect tier of the case study was based on a mesocosm experiment by Crum et al. (1998), Kersting and Van Wijngaarden (1999) and Van Geest et al. (1999). The mesocosm experiment lasted for about 90 days and linuron was applied three times at monthly intervals using nominal concentration levels of 0.5, 5, 15 and 50 mg/L. Between applications the concentration decreased by more than a factor 10 as the result of degradation in the water and of flushing of the systems with clean water. Based on the evaluation of the experiment (Kersting and Van Wijngaarden, 1999; Van Geest et al., 1999), we concluded that their repeated nominal treatment level of 5 µg/L can be considered as the RAC curve for the risk assessment. At this treatment level no effects ('effect class 1') could be demonstrated on ecosystem structure and only transient and slight effects ('effect class 2') on oxygen metabolism (Van den Brink et al., 2006). The RAC curve for this treatment level is shown in Figures 7A-C. For herbicides, the relevant time window is considered to be 7 days (length of the standard Lemna test) because linuron is a photosynthesis inhibitor that affects both algae and vascular plants. This relevant time window is assumed to start at the time of the maximum concentration of the RAC because this maximum is considered most relevant for the risk assessment (see the fat line segment in Figures 7A-C).

Table 1
Characteristics of the D1 and R1 FOCUS Step 3 scenarios (taken from FOCUS, 2001)

| Scenario characteristic | D1 scenario | R1 Scenario |
|---|---|---|
| Type of scenario | Drainage | Run-off |
| Representative field site | Lanna (Sweden) | Weiherbach (Germany) |
| Soil | Clay with shallow groundwater | Light silt with low organic matter content |
| Mean spring and autumn temperature (°C) | < 6.6 | 6.6-10 |
| Mean annual rainfall (mm) | 600-800 | 600-800 |
| Mean annual recharge (mm) | 100-200 | 100-200 |
| Slope (%) | 0–0.5 | 2–4 |

The exposure tier of the case study was based on calculations for three FOCUS surface water scenarios defined by FOCUS (2001). It was assumed that 1 kg/ha of linuron was applied to soil in spring just before emergence (i) of spring oilseed rape in the D1-ditch scenario, and (ii) of potatoes in the R1-stream and R1-pond scenarios. These are realistic applications for linuron. The half-life of linuron in soil (DT50) at 20°C and moisture content at field capacity was assumed to be 89 days; this is the average value of 18 soils as measured by Walker and Thompson (1977). The organic-matter/water adsorption coefficient ($K_{OM}$) of linuron was assumed to be 414 L/kg (based on average value of 18 soils; Walker and Thompson, 1977). The Freundlich exponent was assumed to be 0.9 (FOCUS, 2001). The half-life of linuron in water was estimated to be 10 days at 20°C based on dissipation rates in water observed in the mesocosm experiment Crum et al. (1998). In this experiment, the water was stagnant in the first week after each of the three applications. Crum et al. (1998) report dissipation half-lives in this set of three weeks ranging from 7 to 12 days at water temperatures ranging from 13 to 23°C. Only 6-7% of the doses was sorbed to the sediment and only about 1% was sorbed to the macrophytes. So the dissipation in the water was mostly the result of transformation in the water and a transformation half-life of 10 days at 20°C seems a reasonable estimate. The half-life in sediment was set at 1,000 days (i.e., a conservative value because reliable data on the transformation rate in sediment were not found in literature).

Figures 7A-C show the resulting FOCUS PEC curves as calculated with the TOXSWA model for the three scenarios. In the D1-scenario linuron enters the surface water by leaching through drain pipes (calculated with the MACRO model) and by spray drift. In the MACRO calculations for the FOCUS Step 3 scenarios the pesticide is applied each year over a period of six years before the exposure calculations with the TOXSWA model start (see p. 121 of FOCUS, 2001, for the background). This is of course a worst-case assumption for pesticides that are not applied every year (such as linuron) but this is part of the package of FOCUS Step 3 scenario assumptions (it would of course be possible to perform FOCUS Step 4 calculations with more realistic application schemes of linuron but this is beyond the scope of this example). In the R1-scenarios linuron enters the surface water by runoff (calculated with the PRZM model) and by spray drift. Table 1 gives general characteristics of the D1 and R1 scenarios. The D1-ditch scenario in Figure 7A shows a more or less gradual increase in the calculated linuron concentration between 0 and 100 days from zero to about 9 μg/L. This is the result of leaching through drain pipes of linuron that was applied in the six years before the start of the TOXSWA calculations. After about 140 days there is a sharp peak of about 14 μg/L caused by spray drift. So both drain flow and spray drift contributed to the linuron concentrations shown in Figure 7A. The R1-stream scenario in Figure 7B shows a number of very sharp concentration peaks. The first peak is caused by spray drift on the day of application of linuron and the other peaks are caused by runoff. The peaks are very sharp because the residence time of the water in this stream is very short due to high water flow rates. The R1-pond scenario in Figure 7C shows low calculated concentrations with a first low peak resulting from spray drift on the day of application and subsequent increases due to runoff events. The calculated concentrations show no sharp peaks such as the ones in Figure 7B because the residence time of water in the pond is much longer than the residence time of water in the stream.

So we have a number of PEC curves and the relevant time window of the RAC curve and can now perform 'compare and decide' as described in Figure 2. Note that the sources for the PEC curves and the RAC curve are completely different (e.g., the RAC curve was determined long before the FOCUS Step 3 scenarios became available). This will be the normal situation in current risk assessment procedures because the FOCUS Step 3 scenarios have become available only recently. However, this is not a problem for the 'compare and decide' activity. We follow the flow chart of Figure 4. In Step 1 we have to check whether the maximum of the PEC curve is always below the minimum of the relevant time window of the RAC curve. This is not the case in Figures 7A and B but it is true for Figure 7C. So for the R1-pond scenario we con-

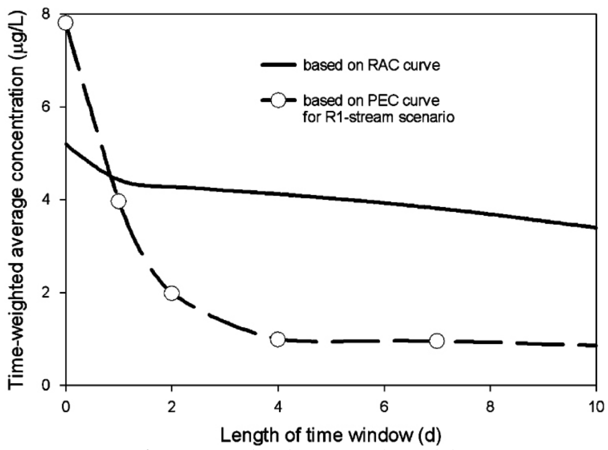


Figure 8. Maxima of time-weighted average (TWA) linuron concentrations in surface water calculated for the R1-stream FOCUS scenario as a function of the length of the time window compared with the TWA concentration derived from a RAC curve derived from a mesocosm study by Van Geest et al. (1999) using effects of class 1 as a basis. The scenario concentrations are output from the TOXSWA model and the line from the RAC curve was obtained by numerical integration of the time course of concentrations measured by Van Geest et al. (1999).

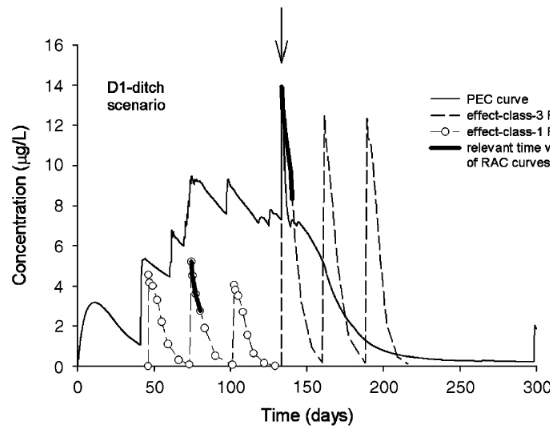


Figure 9. Linuron concentrations in surface water as a function of time as calculated with the TOXSWA model for the D1-ditch FOCUS Step 3 surface water scenario compared with an effect-class-1 and an effect-class-3 RAC curve as derived from the mesocosm experiment by Van Geest et al. (1999). Time 0 is January 1, 1982. The arrow indicates the application time of linuron in the TOXSWA simulations.

clude that the risk is acceptable and for the D1-ditch and R1stream scenario we have to go to Step 2. We now have to check whether a time window exists (including all relevant local maxima of the PEC curve) in which the PEC curve is always lower than the relevant time window of the RAC curve. This is clearly not the case in Figures 7A and B so we go to Step 3 for these scenarios. In Step 3 we have to decide firstly whether it is possible to decide on a length of a time window for averaging the exposure concentration. We consider it acceptable to use TWA-concentrations for linuron because effects of linuron have shown to be reversible (Snel et al., 1998). Figure 7A shows that averaging the concentration over a certain time window will not help for the D1-scenario because the PEC curve is for the full length of the time window of the mesocosm experiment above the RAC curve. So we will restrict ourselves to the R1-stream scenario for Step 3. Figure 8 shows the effect of the length of the time window on the TWA concentrations based on the RAC curve and taken from the calculations with TOXSWA for the R1-stream scenario. The TWA concentrations from the RAC curve were obtained via numerical integration of the RAC curve. Figure 8 shows that the TWA PEC curve for the R1-stream scenario decreases sharply. It is about 8 μg/L for a time window that is zero (i.e., simply the maximum value in Figure 7B). However, for a time window of 1 day, it has decreased to about 4 μg/L and is then already below the TWA RAC curve. So for any time window exceeding 1 day, the R1-stream scenario results in acceptable risk in Step 3.

As described above, the RAC curve used in Figures 7 and 8 was determined using an effect class 1 for ecosystem structure as a basis which implies that no treatment-related effects on the abundance of species should occur. For the D1-ditch scenario this led to the conclusion that the risk was unacceptable using all three steps of Figure 4 (which resulted in a case that was not very interesting). We perform now as an additional example the risk assessment using an 'effect class 3' as a basis. The EU guidance document on aquatic ecotoxicology (European Commission, 2002a) defined this effect class as giving a clear response to sensitive endpoints but showing full recovery of affected endpoints within 8 weeks post last application (Brock et al., 2000). At the regulatory level, there is no consensus between EU member states which effect class should be used, and using effect class 3 is a realistic regulatory option. When evaluating the possible ecological risks of a certain PEC curve (including the recovery potential), it is not only important to consider the rate of recovery (after exposure has dropped below a certain critical concentration level) but also the period in which possible effects can be expected (e.g., the period that the PEC is above the effect-class-1 RAC curve derived from a micro/mesocosm). Based on the evaluation of the mesocosm experiment by Van Geest et al. (1999), their treatment level of 15 μg/L might be considered as the effect-class-3 RAC curve for the risk assessment. We use the graphical comparison of PEC and RAC curves (so Step 2 of Figure 4). Figure 9 compares the PEC curve with effect-class-1 and effect-class-3 RAC curves. The effect-class-3 RAC curve has a maximum of about 14 μg/L based on measured values (Crum et al., 1998). The relevant time window of the RAC curve was selected to start at such a time that the maximum of this curve coincides with that of the PEC curve. We have to check whether the PEC curve is always below the effect-class-3 RAC curve for the relevant time window.

Figure 9 shows that this is indeed the case (admittedly, the maxima of the curves are difficult to compare in Figure 9 but the values are 14.0 µg/L for the RAC curve and 13.6 µg/L for the PEC curve). So the effect-class-3 RAC curve does not exclude recovery for this exposure scenario. However, Figure 9 shows also that the PEC curve is above the relevant time window of the effect-class-1 RAC curve for more than 100 days. Thus for a period of more than 100 days possible effects cannot be excluded. It is a matter for the risk manager to decide whether such long periods of possible effects are considered acceptable. This example shows that the graphical comparison of PEC and RAC curves may provide additional information that could be relevant to the risk manager.

In general, this case study shows that the approach of Figure 4 can be applied quite easily once PEC levels or curves and the RAC level or curve have become available.

### 6.3. Consequences for Designing the Exposure in Higher-Tier Ecotoxicological Experiments

We now consider the consequences of the stepped approach of Figure 4 for the exposure designs for higher-tier ecotoxicological experiments. When designing such an experiment, the RAC level is generally not known *a priori*. Performing such an experiment implies that lower tiers have already indicated risk. Therefore time course of the exposure concentration is likely to be a critical factor. We consider two alternative design approaches. The first approach is based on the desire to be able to use the results of this higher-tier ecotoxicological experiment for as many exposure scenarios as possible. The logical consequence for exposure in the ecotoxicological experiments is then to keep the concentration as constant as possible (i.e., as worst case as possible) within the time window that is relevant for the exposure assessment. This can be illustrated with Figure 6B: if the RAC curve (dashed line) is more or less constant over time, the PEC curve will be always below the RAC curve if the global peak of the PEC curve is below the RAC curve. If the RAC is constant, the approach in Figure 4 can be restricted to checking whether the exposure peak is below the RAC value (so to Step 1 in Figure 4).

Keeping the concentration as constant as possible has the disadvantage that it may lead to a too conservative risk assessment if the decline in all relevant exposure scenarios proceeds rapidly (as e.g., in the R1-stream scenario in Figure 7B). Therefore we consider here also a second approach in which first an analysis is made of the time course in the relevant exposure scenarios and subsequently this time course is simulated as closely as possible in the ecotoxicological experiments. However, this type of design has the disadvantage that the interpretation of the ecotoxicological experiment may become cumbersome if the range of exposure scenarios that has to be protected, changes after the ecotoxicological experiment has finished (or if, for some reason, the characteristics of the exposure scenario changes). We would like to stress that the design of the experiment in this second approach has to be based on adequate exposure scenarios and can never be based on the behavior of the pesticide in the ecotoxicological experiment itself because (i) the water in such experiments is usually stagnant, and (ii) the time course of the concentration in stagnant water is not necessarily a realistic worst case. This can be illustrated with the linuron concentrations calculated for the D1-ditch scenario as shown in Figure 9. As described before, it was assumed in these calculations that the half-life of linuron in water was 10 days at 20°C (based on the observed degradation rate in the mesocosm experiment; Crum et al., 1998). However, the D1-ditch scenario shows a linuron concentration that fluctuates within the narrow range of 7-9 µg/L between about 70 and 150 days (ignoring the sharp peak immediately after the application). The background of this more or less constant concentration is that the residence time of water in this ditch is only in the order of days as soon as significant drainage fluxes enter the ditch, and that these drainage fluxes are also the main source of pesticide input into the ditch. So when drain-flow events occur, there is a quick flow-through of water containing the pesticide. So this exposure scenario shows a more or less constant linuron concentration over a period of about 80 days whereas a stagnant mesocosm experiment at 20°C would have shown a decline corresponding with a half-life of 10 days (assuming that degradation was the only loss process from the water phase; other loss processes such as sorption to the sediment would lead to an even faster decline in the mesocosm experiment).

As described in the Introduction, the current technical guidance document on aquatic ecotoxicology for risk assessment at EU level (European Commission, 2002a) includes the suggestion to simulate the fate dynamics experimentally in higher-tier ecotoxicological experiments. According to EFSA (2005), risk assessors usually justify this methodology in the regulatory practice by checking whether all exposure-relevant properties of the system used in the experiment are in the range to be expected for relevant exposure scenarios and, based on this, assess whether the exposure was conservative enough. These relevant system properties may include (i) organic matter and clay content of the sediment (may influence sorption to sediment), (ii) redox potential in the sediment (may influence the degradation rate in the sediment), (iii) pH of the water (may influence the hydrolysis rate), (iv) light intensity (may influence photolytic degradation rate in water), (v) depth of the water layer (may influence the distribution of the pesticide over water and sediment), etc. (EFSA, 2005). We consider this justification unacceptable because it is only qualitative and because it ignores the concentration curves that are produced by the exposure flow chart (see linuron discussion in preceding paragraph). Instead we recommend the Step-2 approach of Figure 4 to justify that the measured course of the concentration with time in the ecotoxicological experiment is constant enough for the exposure scenarios that need to be assessed. This Step-2 approach is quantitative, considering the measured exposure in the ecotoxicological experiment as the only yardstick for comparison with exposure in the field. This is justifiable because this measured exposure is the only exposure characteristic that matters for the effect assessment conclusion.

It should be noted that this criticism of simulating fate dynamics in higher-tier ecotoxicological experiments is restricted to the exposure part of the risk assessment. Let us consider an example where the most relevant aquatic species in a certain effect tier is more sensitive to the pesticide if the pH is above 8 (e.g., because of toxicokinetics). Then it would be justifiable for ecotoxicological reasons to require that the pH in the higher-tier ecotoxicological experiment is above 8. Another example is a case where the most relevant aquatic species has a preference for a certain pH range. Then it would be justifiable for ecotoxicological reasons that the pH in the higher-tier ecotoxicological experiment is within the range of the pH values of the type of water body to be protected (e.g., a small stream with a pH below 7 in the case of an insecticide for forest application). Another example relates to phototoxic pesticides where the test species are likely to be more sensitive if exposed to such pesticides under natural light.

A completely different solution for matching the time course of the concentration in ecotoxicological experiments with that in the field would be to develop methods and models for extrapolating ecotoxicological responses from one exposure regime to other exposure regimes (Reinert et al., 2002). However, it will need considerable research efforts to develop methods and models that can be generally applied as extrapolation tools in the aquatic effect assessment because they will differ probably between pesticide groups that have different mode of actions and between different types of aquatic organisms. A disadvantage of this approach could be that an extrapolation method will introduce additional uncertainty in the risk assessment while such a method is mainly needed in borderline cases for decision making. However, an advantage could be that this approach simplifies the risk assessment procedure because it enables extrapolation of effects observed in one higher-tier ecotoxicological experiment to a range of different exposure scenarios.

## 7. Role of Exposure Information from Ecotoxicological Experiments in the Exposure Assessment for the Field

In the practice of aquatic ecotoxicological risk assessment there is regular discussion over the role and significance that the exposure part of higher-tier ecotoxicological experiments should play in the exposure assessment. It goes almost without saying that a higher-tier exposure assessment should take into account all relevant information. Usually lower-tier exposure assessments are based on input parameters for pesticide fate that have been derived from laboratory experiments. Higher-tier ecotoxicological experiments are often conducted outdoors. Therefore, higher-tier ecotoxicological experiments will often also deliver higher-tier fate information as a spin-off. However, as described above, there are two distinctly different exposure assessments needed in the risk assessment procedure: (A) exposure assessment in the field, and (B) exposure assessment in higher-tier ecotoxicological experiments (see Figure 2). The fate information derived from a higher-tier ecotoxicological experiment is crucial and unique information for assessment B and, in this context, overrules fate information from any other source. However, for assessment A this is different. The purpose of the exposure flow chart in Figure 1 is to estimate concentrations in the field for situations that are vulnerable with respect to exposure. Therefore, within the context of this flow chart, fate information derived from higher-tier ecotoxicological experiments is not to be preferred over fate information derived from other higher-tier fate experiments: both types of information are in principle equally important for the exposure assessment in the exposure flow chart. Let us for instance consider a substance which has a transformation half-life in water of 100 days derived from a water-sediment study conducted in the dark. A first exposure tier could then be to run FOCUS Step 3 scenarios using this half-life in water of 100 days. However, if a higher-tier ecotoxicological outdoor experiment would demonstrate a transformation half-life in water of 15 days (caused by photochemical transformation), the next exposure tier could be to run these FOCUS Step 3 scenarios with this half-life of 15 days. If there were two additional outdoor fate studies showing transformation half-lives in water of 20 and 65 days, it would be more appropriate to use the average of these three half-lives in runs of the FOCUS Step 3 scenarios or to run FOCUS Step 3 scenarios with all three half-lives to analyze the uncertainty resulting from this range in half-lives.

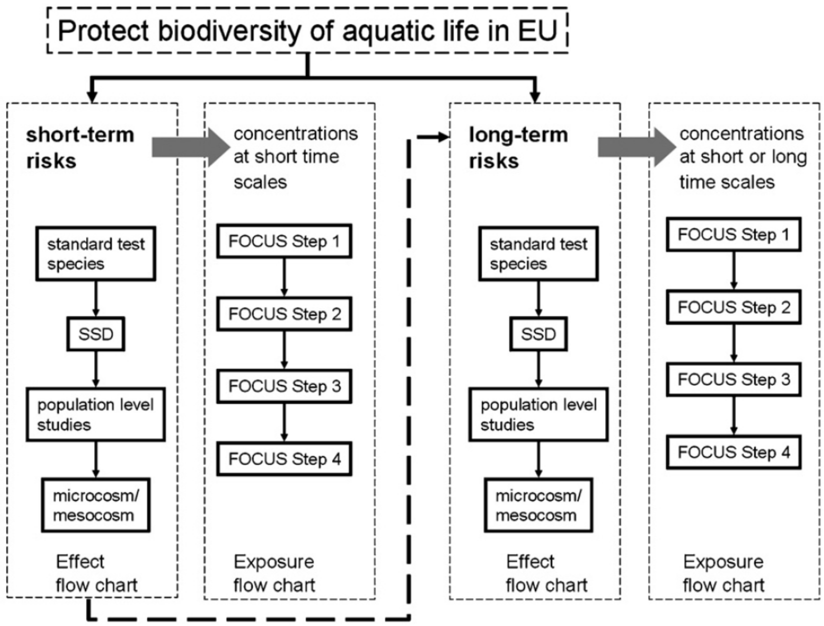


Figure 10. Proposed system of effect and exposure flow charts for aquatic risk assessment at EU level. The two solid arrows from the box 'Protect biodiversity of aquatic life in EU' indicate the need to assess always both short-term and long-term risks. The dashed arrow indicates the possibility for the risk manager to ignore short-term risks if long-term risks are absent.

## 8. Use of the Proposed Approach in Current Aquatic Risk Assessment at EU Level

We now will illustrate how the proposed approach of the interacting effect and fate flow charts shown in Figures 1, 2 and 4 could be applied within the current aquatic risk assessment procedure at the EU level (European Commission, 2002a). This procedure distinguishes between standard and higher-tier risk assessments (European Commission, 2002a). The standard assessment consists of acute and chronic testing and of comparing exposure endpoints to acute and chronic effect endpoints including safety factors of 10–100 (European Commission, 2002a). A range of options is described for the higher-tier assessment but no hierarchy of these options is given. Brock et al. (2006) propose a tiered risk assessment approach with a clear hierarchy considering both acute and chronic toxicity. Based on the EU guidance document (European Commission, 2002a) and Brock et al. (2006) we propose in Figure 10 a system of effect flow charts that is consistent with (i) current aquatic risk assessment practice, and (ii) the system of flow charts shown in Figures 1, 2 and 4. The system of effect flow charts of Figure 10 branches into (i) short-term risks, and (ii) long-term risks. As indicated, short-term risks are always linked to ERCs at short time scales whereas long-term risks may be linked to ERCs at either short or long time scales. For instance, an effect on a sublethal endpoint like reproduction may have been caused by some short-term peak concentration (latency of effect) or by a long-term exposure. In the example, each of the two effect flow charts has four tiers (standard test species, SSDs, population level studies and microcosm/mesocosms) largely in accordance to Brock et al. (2006). The exposure flow charts have also four tiers based on FOCUS (2001, 2005). All effect and exposure tiers are given here only for illustrative purposes, and their contents are not further discussed.

Figure 10 shows a dashed arrow going from the short-term to the long-term risk assessment. This arrow is necessary because a risk manager may consider short-term effects not to be a regulatory problem if long-term effects are absent. This may happen already in the first tiers of the EU risk assessment procedure (European Commission, 2002a). In this procedure the first-tier acute trigger concentration could be for example the 48-h EC50 *Daphnia magna* divided by 100 while the corresponding first-tier chronic trigger concentration would be the 21-d NOEC *Daphnia magna* divided by 10 (European Commission, 2002a). So if this EC50 is less than 10 times higher than this NOEC (which is not exceptional), then the first-tier acute trigger concentration is lower than the first-tier chronic trigger concentration. Another example of this dashed arrow is that risk managers may accept effect-class-3 concentrations due to short-term exposure as demonstrated in micro/mesocosm tests (effect class 3 implies clear short-term effects but recovery within 8 weeks). There is no arrow similar to the dashed arrow in the opposite direction in Figure 10: regulatory concerns on long-term risks will be difficult to remove by absence of short-term risks. Note that this dashed arrow in Figure 10 is not meant to describe the flow of information for cases where chronic toxicity is estimated from acute toxicity by extrapolation methods: use of such methods is simply part of the box 'standard test species' of the effect flow chart for long-term risks. Figure 10 is at a general level and therefore cannot prescribe exactly which type of ERC should be used. However, the branching into short-term and long-term risks implies the following restrictions to the time scales: (i) for short-term risks the ERC may be a peak concentration or e.g., a time-weighted average over a period of a few days; and (ii) for long-term risks the ERC may vary from a peak concentration to a time-weighted average over a period not exceeding the duration of the standard chronic test with the most relevant test species as discussed before.

## 9. Recommendations for Future Activities to Improve the Aquatic Risk Assessment Procedure

1. For the exposure assessment at the EU level, it is recommended to check via a number of well-defined experiments across the EU whether the FOCUS surface water scenarios are close enough to reality for realistic worst-case exposure conditions. Until now, these have been based only on calculations with sets of complex simulation models (FOCUS, 2001). It would be unfortunate if ecotoxicologists would start new research lines based on the correctness of these FOCUS scenarios to discover five years later that an important part of the scenarios lack realism. It is recommended to include ecotoxicological expertise in this checking procedure to ensure that the experiments are performed at locations that are also most appropriate for the risk assessment from an ecological point of view.
2. To facilitate the exposure assessment of the ecotoxicological experiments (activity B in Figure 2) it would be useful to review available methods and software for the calculation of time-weighted averages from measured time courses of the concentration and, based on this, to provide guidance on the most appropriate methods and software.
3. The temporal scale of the ERC plays an important role in the proposed approach. Therefore, it is recommended to perform measurements of effects in ecotoxicological experiments as often as possible (e.g., in daily increments) to gather time-to-event information necessary for the determination of the appropriate temporal scale of the ERC.

## 10. Applicability to Risk Assessment for Soil Organisms

All considerations so far have been restricted to the risk assessment for aquatic organisms. However, the principles of the interaction between exposure and ecotoxicological effect are exactly the same for aquatic and soil organisms. The flow charts and procedures described in Figures 1-4 are based on general principles and do not imply any assumptions that are specific for aquatic risk assessment. So they apply equally well to risk assessment for soil organisms. However, the risk assessment for soil organisms is especially relevant for persistent pesticides in soil. Concentrations of persistent pesticides in soil are expected to fluctuate less than concentrations of pesticides in surface water. As a consequence, the handling of time-variable exposure as described in Figure 4 may be less an issue in risk assessment for soil organisms.

In 2002, the European Commission presented a detailed risk assessment procedure for soil organisms including a test for earthworms (European Commission, 2002b). Comparatively little attention was paid to exposure in this guidance. Let us consider as an example the exposure assessment for earthworms. The guidance document states 'PEC values for the various use scenarios are supplied by the environmental fate section' (at its p. 29). However, no such section can be found in this document. The guidance given for earthworms implies that the pore water concentration in soil is considered as the yardstick for assessing effects (also recommended by Van Gestel, 1992). However, nowhere is any guidance given on how to estimate reliable realistic worst-case exposure concentrations for soil pore water. Instead it is recommended to divide the LC50 by a factor of 2 for compounds whose sorption is correlated to organic matter. The justification for this is that the standard soil used in earthworm tests has an organic matter content of about 10%, whereas the EU guidance document considers 5% organic matter more representative for agricultural soils. However, this 5% is nowhere justified and is certainly not justifiable as a realistic worst-case assumption for the EU (see organic carbon map of the EU; Jones et al., 2004). It seems thus not acceptable to use this 5% in the first (and also final) exposure tier. So it seems that also the interaction between effects and exposure in the risk assessment of soil organisms could be improved by using the procedure that we propose.

## 11. Conclusions

1. Within the risk assessment for aquatic organisms, the interface between ecotoxicological effects and exposure should be based on the type of exposure concentration that gives the best correlation to ecotoxicological effects (i.e., the ERC).
2. Definition of this type of ERC is necessary for a consistent handling of exposure in pesticide risk assessment.
3. Once this type of ERC has been defined, effect and exposure flow charts for risk assessment can be established that are flexible with respect to the route to be followed through the combined system of effect and exposure flow charts.

4. Within the risk assessment procedure, there are two equally important exposure assessments required: (A) exposure in the field, and (B) exposure in ecotoxicological experiments. Within a certain effect tier, the same type of ERC should be used consistently for both types of assessments.

5. Risk assessment conclusions that are based on higher-tier ecotoxicological experiments in which the ERC varies with time, should be based on a systematic comparison between (i) the time course of the ERC in these experiments, and (ii) the time course of the ERC in relevant exposure scenarios. In this comparison the time course in the experiments should in general be restricted to a time window with a length that does not exceed the duration of the standard chronic test in the first tier for the relevant taxonomic group considered.

6. The issues raised here, though focusing on aquatic risk assessment, are of more general relevance and may e.g., also improve risk assessment of soil organisms.

7. Application of the proposed approach is expected to lead to a better communication between ecotoxicological experts and fate experts within the risk assessment.

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This paper uses published data (references Kersting & Van Wijngaarden and Van Geest et al.) on the effects of linuron in a mesocosm study. These were conducted in accordance with national and institutional guidelines for the protection of animal welfare (Van Wijngaarden is coauthor of both papers and is member of the same research team as T. Brock).

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