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SCIENTIFIC OPINION

Updating the opinion related to the revision of Annexes II and III to Council Directive 91/414/EEC concerning the placing of plant protection products on the market – Ecotoxicological studies[1]

Scientific Opinion of the Panel on Plant Protection Products and their Residues

(Question No EFSA-Q-2009-00556)

Adopted on 18 June 2009

PANEL MEMBERS

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SUMMARY

The European Food Safety Authority (EFSA) asked its Panel on Plant Protection Products and their Residues to review the Opinions of the PPR Panel issued in 2006 and 2007 related to the revision of Annexes II and III to Council Directive 91/414/EEC (data requirements) concerning the placing of plant protection products on the market.

The present opinion updates the existing opinion on Annex II & III: Ecotoxicological studies (EFSA, 2007c), revising certain aspects and adding further comments and recommendations. If not otherwise stated, the findings and recommendations given in EFSA 2007c remain valid and applicable. Therefore, the present opinion should be read together with the opinion issued in 2007 (EFSA, 2007c). The Panel reemphasises that the data requirements should be flexible enough to allow new risk assessment developments to be considered when available.

The present opinion focuses in particular on the number of animals (vertebrates) used in testing, the harmonisation of risk assessment quotients, ECx versus NOEC, risk assessment for honeybees and aquatic plants, and a number of other issues that should be considered in future risk assessment.

Key words: ecotoxicological studies, plant protection product, pesticides, active substance, risk assessment, data requirements, Annex II and III, Directive 91/414/EEC, triggers, honeybees, aquatic plants, NOEC, ECx, animal tests.

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BACKGROUND AS PROVIDED BY EFSA

In November 2005, the Commission initially informed the European Food Safety Authority (EFSA)[2] that they were revising the data requirements for authorisation of active substances and plant protection products in the framework of Council Directive 91/414/EEC. The revision process involved Part A of Annexes II and III and had been organised in order to amend the directives[3] laying down the data requirements for active substances and plant protection products. The Commission had prepared SANCO Working Documents[4] containing the proposed data requirements to revise Annexes II and III to Directive 91/414/EEC and asked the PPR Panel to provide observations and/or possible recommendations, and in particular to verify that the methodology and the approaches presented in the draft data requirements were in line with the scientific state of the art in the relevant field and the extent of its applicability with respect to the risk assessment of plant protection products.

Between May 2006 and March 2007, upon request of the Commission, the PPR Panel issued six opinions on the SANCO working documents related to the revision of Annexes II and III to Council Directive 91/414/EEC concerning the placing of plant protection products on the market (EFSA, 2006a, b, c; EFSA, 2007a, b, c).

Until now the Annexes II and III to Council Directive 91/414/EEC have not been finally amended, but meanwhile a new regulation of the European Parliament and of the Council on the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC has been elaborated and will enter into force in 2009.

Following Art 8(4) of this new regulation, the data requirements shall contain the requirements for active substances and plant protection products as set out in Annexes II and III to Directive 91/414/EC and laid down in further regulations to be adopted.

Therefore, the PPR Panel would like to revisit their opinions issued in 2006 and 2007 to make sure, that the data requirements for active substances and plant protection products are up to date at the time of their adoption under the relevant regulation.

TERMS OF REFERENCE AS PROVIDED BY EFSA

The Scientific Panel on Plant protection products and their Residues is asked by EFSA to review the Opinions of the PPR Panel issued in 2006 and 2007 related to the revision of Annexes II and III to Council Directive 91/414/EEC concerning the placing of plant protection products on the market.

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[2] Letters P. Testori Coggi 21 Nov 2005 (requesting opinions on phys-chem. properties, analytical methods, residues); 03 Aug 2006 (fate and behaviour, toxicological and metabolism studies); 29 Sept 2006 (ecotoxicological studies).
[3] 94/37/EC physical and chemical properties; 96/46/EC analytical methods; 94/79/EC toxicological and metabolism studies; 96/68/EC residues; 95/36/EC fate and behaviour in the environment; 96/12/EC ecotoxicological studies.
[4] SANCO 10438, 10439, 10440, 10481, 10482, 10483

ASSESSMENT

1. Introduction

In March 2007, the PPR Panel adopted upon request of the Commission its opinion on the SANCO working document related to the revision of Annexes II and III to Council Directive 91/414/EEC concerning the placing of plant protection products on the market: Ecotoxicological studies (EFSA, 2007c).

Annexes II and III to Council Directive 91/414/EEC have not yet been finally amended, but meanwhile a new Regulation of the European Parliament and of the Council on the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC was adopted by the European Parliament in January 2009 and is expected to enter into force in 2009.

Therefore, the PPR Panel is reviewing its opinion issued in 2007 to ensure that the data requirements for active substances and plant protection products are up to date at the time of their adoption under the relevant regulation.

It is not the intention to replace the existing opinion (EFSA, 2007c) but to review certain aspects and to add some further comments and recommendations. If not otherwise stated, the findings and recommendations given in EFSA, 2007c remain valid and applicable.

2. General remarks and recommendations

The PPR Panel reiterates its recommendation that the revised Annex II and III data requirements should be flexible enough to allow new risk assessment developments to be applied when available, in particular in relation to the EFSA mandates received at the end of 2008 concerning the revisions of the Guidance Documents on Aquatic Ecotoxicology and on Terrestrial Ecotoxicology (EC, 2002a,b), as well as other ongoing initiatives (ICPBR/ EPPO, etc.).

Additionally, considering proactively the imminent adoption of a new regulation concerning the placing of plant protection products on the market[5], the Panel anticipates that additional data may be needed in order to perform the requested risk assessments. Reporting of additional toxicological effects from existing studies should be encouraged where this could enhance ecological models and is possible without unreasonable additional effort or resources.

3. Specific remarks

3.1. Reduction of number of animals (vertebrates) in testing

In view of the generally accepted policy to minimise testing in animals,, and in line with EFSA's Opinion on replacement, reduction and refinement of animal testing (EFSA, 2009a), the PPR Panel recommends that additional animal (vertebrates) testing, with standard or non-standardised tests should be carried out only if appropriate and properly justified.

[5] Regulation *(EC) No .../2009* of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. To be published in 2009.

Replacement of testing implies the possibility of alternative methods such as *in vitro* tests, QSARs (see OECD, 2008a for further guidance) or other predictive methods, or the use of existing equivalent data as a surrogate for a test that is in principle required. Reduction may concern the number of animals used in a required test or the number of tests required. Refinement means the implementation of tests that offer the best preservation of animal welfare whilst still providing satisfactory results. Further detailed recommendations as regards testing procedures and integrated testing and risk assessment strategies may be found in the EFSA opinion already cited above (EFSA, 2009a). The following text proposes recommendations in areas in which some improvements may now be implemented based on the outcome of previous work, i.e. testing with birds and fish.

3.1.1 Reducing the number of species to be tested

Acute oral toxicity to birds

The current text of Annex II, 8.1.1 for birds implies that testing a single species is normally sufficient (except when regurgitation occurs at relevant doses). The PPR Panel understands that there has been discussion about adding a requirement for a second species to be tested, in cases where the active substance has a novel chemistry or mode of action. This would presumably be intended to investigate whether the new chemistry might increase the variation between species seen with existing active substances. However, conducting one additional test would provide very little statistical power for detecting a wider variation. Also, if indeed a wider variation is found, the logical consequence would be to consider increasing the Toxicity-Exposure-Ratio (TER) value, i.e. the uncertainty factor used in the risk assessment, and it is unclear whether this would be compatible with the provisions of Annex VI. Before adding a requirement for testing a second species, an assessment should be made of the value of the additional information and how it would be used in practice.

In the case of birds, the PPR recommends that either the bobwhite quail or Japanese quail is tested since regurgitation is quite rare in these species, instead of the mallard duck, which is known to regurgitate in a higher proportion of studies than the other species.

The PPR Panel recommends the following wording:

> 8.1 Effects on birds and other terrestrial vertebrates
>
> 8.1.1 Acute oral toxicity to birds
>
> Test conditions
>
> "The acute oral toxicity of the active substance must be determined. The test should preferably be performed with a quail species (Japanese quail (*Coturnix coturnix japonica*) or Bobwhite quail (*Colinus virginianus*)), since regurgitation is rare in these species. The highest dose used in tests need not normally exceed 2000 mg/kg body weight".

Short term dietary toxicity to birds

The PPR Panel would like to emphasise its previous opinion about the short-term toxicity test with birds (EFSA, 2007c; p. 11):

"The short-term dietary study was originally designed to assess risks of bioaccumulating chemicals such as organochlorines, to test whether dietary exposure over several days may be more hazardous than a single gavage dose. However, for many pesticides the results are confounded by reduced food consumption, which makes interpretation and use of the test endpoints very difficult. Consumption and dose cannot be determined for individual birds because they are tested in groups. These difficulties have been recognised for some time (Mineau et al., 1994; OECD, 1996)."

(…)

"The short-term dietary study is also unattractive from an animal welfare perspective: it is a lethal study which exposes 50 birds to the pesticide without access to untreated food, over a period of five days. Affected individuals may suffer adverse symptoms over several days, and some pesticides cause such severe food avoidance that animals have to be sacrificed to avoid death by starvation (Luttik, 1998).

Given the scientific limitations and welfare costs of the short-term dietary study, the PPR Panel recommends restricting its use as far as possible. The Panel therefore considers that, by making its use conditional, the current draft of 8.1.2 is moving in the right direction. However, the criteria stated in the current draft would result in the test continuing to be triggered for acutely toxic pesticides, many of which will result in interpretational difficulties and welfare concerns outlined above. The PPR Panel recommends instead that the short term dietary study be conducted only for those pesticides where the mode of action and/or results from mammalian studies indicate a potential for the dietary LD50 measured by the short term study to be lower than the LD50 based on an acute oral study. This would apply, for instance, to many of the organochlorine compounds and anticoagulants like flocoumafen.

The short-term dietary test should not be conducted for any other purpose unless it can be clearly justified. When the study is justified, the PPR Panel recommends that it should be conducted with one species only. The short-term dietary test should not be used simply to demonstrate the potential for food avoidance, as this can be achieved satisfactorily with fewer birds in a shorter (1 day) study."

The PPR Panel thus recommends the following wording:

> 8.1.2 Short-term dietary toxicity to birds
>
> Circumstances in which required
>
> "A study on the dietary (five-day) toxicity of the active substance to birds should be required for compounds where the mode of action and/or results from mammalian studies indicate a potential for the dietary LD_{50} measured by the short term study to be lower than the LD_{50} based on an acute oral study. The short-term dietary test should not be conducted for any other purpose than to determine intrinsic toxicity through dietary exposure, unless the necessity can be clearly justified.
>
> Test conditions
>
> The dietary (five-day) toxicity of the active substance to birds must be conducted only in one species, being the same as the species tested under 8.1.1."

Acute toxicity to fish

For fish, acute lethality tests with rainbow trout and a warm water fish species are required by Annex II. In the Guidance on PNEC derivation under REACH, requirements for fish testing are minimized (ECHA, 2008, pp 49-52). Whether a similar procedure is also applicable to pesticides should be further investigated in the context of the relevant risk assessment schemes. In particular the possibility of waiving testing on a second fish species should be investigated if the sensitivity of e.g. rainbow trout is at least an order of magnitude less than that of the other standard test species.

3.1.2. Reducing the number of animals in a test

Acute bird testing

The avian acute oral LD_{50} test is generally conducted with a minimum of 50 birds. A new draft guideline (OECD, 2007), which is currently under development, appears likely to deliver the same endpoints with similar precision using fewer birds (e.g. 12-24). In view of the policy goal of minimising animal testing, the PPR Panel recommends that support should be given to completing the development and evaluation of this guideline, and to ensuring that when available it can be readily taken up in risk assessment.

The PPR Panel recommends the following wording:

> 8.1.1 Acute oral toxicity to birds
>
> Test guideline
>
> "OECD Acute oral toxicity study TG 223 or US EPA OPPTS 850.2100: Acute Oral Toxicity Test should be used".

Acute mammal testing used for mammalian wildlife risk assessment

For acute mammal testing, a number of tests are available. Two of the tests (the Fixed Dose Procedure EU method B.1bis /OECD TG 420) and the Acute Toxic Class Method (EU method B. 1tris/OECD TG 423) are range estimators and hence are not the ideal tests for mammalian wildlife risk assessment but:

- they can be used as limit tests (e.g. > 2000 mg/kg bw), or
- they can be used as conservative surrogates for the LD_{50} (i.e. lowest value of range).

If this lower value initiates a high tier risk assessment, then the estimate of toxicity could be assessed in a more precise way with the Up and Down test (OECD, 2008b).

However, in line with the policy goal of minimising animal testing, the PPR Panel recommends that the OECD 425 Acute Oral Toxicity - Up and Down Procedure (OECD, 2008b) should be used in preference to prevent additional testing.

The PPR Panel then recommends the following text in the section on toxicology:

> 5.2.1. Oral (Sanco/10482/2006)
>
> Circumstances in which required

"The acute oral toxicity of the active substance must always be reported. If it is anticipated in advance that the LD50 is likely to be less than 2000 mg/kg body weight, the OECD 425 study is required without doing a limit test.."

Acute fish testing (oral)

For fish, the draft revised OECD guideline recommends reducing the number of test animals in the limit test (OECD, 2008c). It is proposed to perform the limit test with a minimum of 7 fish including for the control, as when zero mortality is recorded in 7 to 9 fish there is a 99% confidence that the LC_{50} is above 100 mg/L. In the main test of OECD no. 203, there should be seven fish per concentration tested (OECD, 1992).

The PPR Panel recommends the following wording:

8.2.1. Acute toxicity to fish

Test guideline

"The test must be carried out in accordance with OECD 203. Efforts to reduce the number of animals in the limit and main tests should be made as far as possible, according to the recommendations of the draft revised guideline for OECD 203, when adopted."

Chronic fish testing

For chronic effects, the PPR Panel recommends that a fish full life cycle test is performed rather than separate tests on different life stages since exposure in early life stages may trigger effects only in later phases which would not be detected in an early life stage test (EFSA, 2007c) unless it can be demonstrated from e.g. the mode of action of the substance, that a specific life stage is more sensitive.

The PPR Panel recommends the following sentence under 8.2.2:

8.2.2. Chronic toxicity to fish

Circumstances in which required

"A fish full life cycle test is preferable to separate tests on different life stages, since exposure in early life stages may trigger effects only in later phases which would not be detected in an early life stage test unless it can be demonstrated from e.g. the mode of action of the substance, that a specific life stage is more sensitive."

3.2. Harmonizing the risk assessment quotients

The PPR Panel noted in 2007 that, under the EU legislative requirements, the risks from chemical substances are not assessed consistently/uniformly for different groups of organisms. For pesticides, regulated under Council Directive 91/414/EEC, the following risk assessment approaches are used:

Toxicity/Exposure Ratios (TERs) for birds, mammals, aquatic organisms, soil macro-organisms and non target terrestrial plants, i.e the inverse of the hazard quotient (where the larger the ratio, the smaller the risk);

Hazard Quotients (HQs) for honeybees and non-target arthropods (the larger the ratio, the larger the risk).

All other EU legislations (e.g. REACH; EC, 2006) use a Hazard Quotient (HQ) approach to assess risk, in which predicted or measured exposure is divided by a predicted no-effect concentration (PNEC). As the value of this quotient increases, the degree of risk is expected to increase. However, in the case of pesticides there appears to be no justifiable explanation for the difference in approach (see section 3.2.2).

3.2.1. Level of protection

Acute and chronic TERs are compared with trigger values in accordance with the Annex VI of 91/414/EEC Directive. Acute TERs are compared to a trigger value of 10 for birds, mammals, soil macro-organisms and non-target terrestrial plants, and with a trigger value of 100 for aquatic organisms. Similarly, chronic TERs are compared to a trigger value of 5 for birds, mammals, soil macro organisms and non target terrestrial plants, and with a trigger value of 10 for aquatic organisms.

For aquatic organisms, a review of historical sources (OECD, European Commission and US-EPA) was performed by the PPR Panel in its opinion on the acute and chronic risk to aquatic organisms and related uncertainty factors (EFSA, 2005). In summary, the triggers of 100 for the acute and 10 for the chronic were not found to be based on a strong and transparent scientific justification. Some work has been done to compare the trigger value of 100 used to extrapolate aquatic acute toxicity data with results of aquatic micro/mesocosms (Van Wijngaarden et al., 2005; Brock et al., 2006). This comparison revealed that the application of the trigger of 100 to the acute toxicity value of the most sensitive standard test species suffices to avoid ecological effects in micro/mesocosms treated once or repeatedly with organophosphorous and pyrethroid insecticides, photosynthesis inhibiting herbicides and several fungicides. Further work is still needed to support the trigger for chronic risks and for compounds with other modes of action.

For birds, a comparison was conducted of the TER value achieved for acute risks posed by an active substance with observations from field studies, assessed as a subjective probability of lethal effects, as judged by evaluators of the studies (EFSA, 2008). The PPR Panel concluded that the use of substances displaying a TER above the trigger of 10 would rarely cause visible mortality. More detail including an analysis of uncertainty may be found in this opinion (EFSA, 2008). The PPR Panel concluded that “the historical record of incidents provides good evidence regarding the actual protection goal of preventing visible mortality, weaker evidence regarding the actual protection goal of preventing long-term population effects, and very weak evidence regarding the surrogate protection goal of making any mortality unlikely.” Thus further validation is also deemed necessary for long term risks and related long-term trigger value.

To our knowledge, information on which data sources provide the basis for TER trigger values used for mammals, soil macro-organisms and terrestrial plants is not available in the public domain. In the PPR Opinon on the usefulness of total concentrations and pore water concentrations of pesticides in soil as metrics for the assessment of ecotoxicological effects (EFSA, 2009b), an analysis of the level of protection achieved by the earthworm risk

assessment for the terrestrial environment was given. It led to the conclusion that the current methodology allows for effects of up to 50% difference in the abundance of earthworms under field conditions (with recovery within one year), for every plant protection product. This assessment is however not protective at the same level of effect (50%) for other soil macrofauna than earthworms, and hence does not support the general principles on decision making for non-target soil macro-organisms as stated in the Annex VI of Directive 91/414/EEC.

As far as TERs are concerned, the level of protection that is actually reached when these values are applied to an endpoint is not directly comparable between different groups of organisms. As a consequence, the degree of harmonization among the levels of protection that these trigger values achieve remains unclear. The PPR Panel thus identifies the need for further validation of these trigger values, which could be done through a proper comparison of the TER values that are estimated for particular substances with the level of impact that these substances may exert under field studies.

The threshold of 50 to which Hazard Quotients calculated for bees are compared was derived from a comparison with the outcome of field studies performed at similar application rates as the one used in the HQ calculation (Smart and Stevenson, 1982). The level of protection corresponding to the HQ values of 50 then corresponds, according to the literature, to non significant impacts on bee colonies, as assessed at the scale of the fields in which the colonies were distributed, and at the time (before 1982) the experiments were performed. It may then be supposed, despite not being explicitly demonstrated, that this value of 50 is protective for lethal, sublethal and reproductive effects. A further validation has been undertaken recently by Mineau et al. (2008), based on a database containing more than two decades of honey bee hive poisoning incidents from the United Kingdom (Wildlife Incident Investigation Scheme (WIIS)) and corresponding surveys of pesticide use. The results seem to confirm that an HQ of 50 is not likely to result in impacts in the field, but also highlights the influence of the area treated in predicting and reporting incidents.

The threshold of 2 to which Hazard Quotients calculated in the first tier of the risk assessment for other non-target arthropods and more specifically for the two standard species *Aphidius rhopalosiphi* and *Typhlodromus pyri* are compared, is also derived from a comparison of the outcome of laboratory derived LR_{50} with semi-field and field studies (SETAC, 2001). As for bees, the level of protection corresponding to these HQ values is proposed to cover lethal, sublethal and reproductive effects. It was recognised by the ESCORT working group that the database on which this validation had been done was limited. Additionally, the extent to which these values could be used for the off-field communities was uncertain. An update of the database and the calculations is thus needed, in order to address both in- and off-field risk assessment issues.

The PPR Panel thus identifies a need for further validation in particular of TER trigger values and HQ trigger values for non-target arthropods, which could be done through a proper comparison of the TER/HQ values that are estimated for specific substances with the level of impact that these substances may exert under field studies. In this context, the PPR Panel reiterates its recommendation that the revised data requirements should be flexible enough to allow new risk assessment developments to be applied when available.

3.2.2. Communicating on a level of risk

Hazard quotients are calculated by dividing an exposure level by an ecotoxicological endpoint. In contrast, for most organisms, TERs are calculated by dividing the ecotoxicological endpoint by the level of exposure. The PPR Panel strongly recommends that risk is characterised only by using quotients that divide exposure by effect (e.g. HQ), in order to harmonise risk communication across regulatory frameworks. In the context of harmonisation, the current initiatives underway in EFSA and other European entities should be considered in future validation work to be performed for decision-making trigger values.

3.3. Using ECx instead of NOEC

In its scientific opinion of 2007, EFSA's PPR Panel referred to the scientific developments in support of the use of ECx (concentration where x% effect was observed/calculated) as an alternative and preferable ecotoxicological endpoint to the commonly used No Observed Effect Concentration (NOEC) (EFSA, 2007c). The PPR Panel wishes to emphasize its earlier recommendation regarding the use of ECx in relation to all environmental areas. It is important to note that using ECx instead of NOEC is considered particularly relevant for laboratory single species toxicity data.

Further recommendations as regards the advantages of ECx over NOEC are given below, together with information on the methods to derive this ecotoxicological endpoint (see also EFSA, 2009c).

Traditionally, the responses measured in chronic ecotoxicity tests have been expressed as the no observed effect concentration (NOEC) and/or Lowest Observed Effect Concentration (LOEC). The NOEC is defined as the highest concentration that has no statistically significant adverse effects on the exposed population compared with the control, whereas the LOEC is defined as the lowest concentration that has a statistically significant adverse effect compared with the control (van Leeuwen and Vermeire, 2007). Laskowski (1995) was among the first to draw attention to the disadvantages of the NOEC and LOEC as ecotoxicological endpoints. However the debate has been ongoing and continues today (e.g. Kooijman, 2006, Fox, 2009, and others). For example, Environment Canada's guidance document on statistical methods for toxicity tests, although presenting guidance on the NOEC approach, does not encourage its use to derive ecotoxicological endpoints in multiple concentration tests (Environment Canada, 2007).

Disadvantages of using a NOEC/LOEC as an ecotoxicological endpoint include:

1) The NOEC/LOEC depends on the choice of concentrations tested (indeed it has to be one of the tested concentrations). The wider the interval of concentrations tested, the less accurate the NOEC/LOEC will be as an indicator of actual effects;

2) The NOEC/LOEC increases (i.e., the chemical appears less toxic) as the number of replicates per test concentration decreases - generally the statistical power of the ANOVA and of the multiple-comparison test use to derive NOEC/LOECs is low due to the generally low number of replicates);

3) The NOEC/LOEC increases as the variability among replicates increases; in some cases NOEC/LOECs cannot be determined due to a large variability among replicates within treatments. This is also true in deriving an ECx (in particular for EC_{50} values).

However, such problems in deriving a NOEC may occur even if a decrease in the response parameter is observed, due to large variability within treatments;

4) The NOEC/LOEC is a function of the choice of statistical test for the multiple comparison and level of significance. In general, the rather arbitrary Type I error[6] of 0.05 is used, and rarely is the Type II error[7] reported (thus if no statistical difference is reported we rarely have a measure of the likelihood that a difference could have been detected)

5) Since no confidence limits are calculated for NOEC/LOECs, statements of precision/uncertainty are not possible.

In contrast to the NOEC/LOEC approach, using regression models to calculate the results of chronic toxicity tests, from which toxicity can be expressed as ECx (where x is some percentile), shows a number of advantages as described for example by Stephan and Rogers (1985):

1) The ECx is robust to variability in experimental design, including concentration interval, number of replicates, replicate variability;

2) Problems related to the sizes of Type I and Type II error are reduced because the focus is not on trying to disprove the null hypothesis of no effect (but note that there are still several statistical issues, such as whether there is a significant trend in the data; goodness of fit tests; choice of models to test);

3) The percentage effect is clearly defined and confidence intervals can be derived (which involves a number of statistical choices).

Detailed methods for deriving point estimates of inhibitory concentrations from ecotoxicological tests are available, e.g., in the Guidance Document on Statistical Methods for Environmental Toxicity Tests from Environment Canada (2007). This guidance document contains information on the models for the most common response trends observed in ecotoxicological tests and includes the EC_{50} and EC_{25} scripts for each model in a "ready to use form" that can be used in normal statistical software (e.g., SPSS, SAS, Statistica, SYSTAT, etc). Moreover the formulae allow the parameters to be changed and thus, the derivation of an ECx value for any value of "x".

The PPR Panel recommends that risk assessment for all groups of organisms should use the ECx approach in preference to NOEC/LOEC approach. To implement this will require agreement on the appropriate ECx to use for each group of organisms, so that this can be included in the anticipated revision of Annex VI and in future guidance documents. The PPR Panel therefore proposes that the revision of Annexes II and III should leave open the final choice of ECx, and that further work to develop recommendations on that choice should be conducted (e.g. through a self-tasking by the PPR Panel) in time for the revision of Annex VI and the guidance documents. The recommendations for the choice of ECx for each group of organisms should be based on an analysis of existing study data for each of these groups and take into consideration the following issues:

[6] occurs when the null hypothesis is rejected while it is true
[7] occurs when the null hypothesis is accepted while it is wrong

- The ECx for each group of organisms should be chosen so as to ensure a level of protection consistent with the aims of the regulations, taking into account the conservatism of other parts of the risk assessment.

- The choice of ECx should take into account the reliability of the estimates that can be provided by standard test methods.

- The procedure for using the chosen ECx in the risk assessment should take account of the quality of the estimates available for each substance, e.g. by examining confidence intervals for the ECx and possibly using these in the risk assessment.

- As existing study methods were not designed to estimate ECx, it is expected that a proportion of existing studies will not provide a usable estimate for the preferred (or even any) ECx. For reasons of cost and animal welfare the procedure should be designed to minimize or if possible prevent any need for retesting in such cases, e.g. the procedure could include provision to use alternative ECx or even the NOEC in such cases, together with appropriate adjustments to the risk assessment (e.g. different assessment factor) to provide the required level of protection.

- The desirability of harmonising with ECx approaches used under other EU legislation (e.g. REACH; EC, 2006) should be considered, unless there are good reasons to differ.

In order to provide the flexibility to accommodate these issues in the final procedures, the revision of Annexes II and III should leave open the choice of ECx, and should also make a recommendation on whether or not to use confidence intervals on the ECx. It should also leave open the possibility to use the NOEC where necessary. Therefore the PPR Panel recommends that, for every group of organisms where a NOEC is mentioned in the Annexes, the text should also require provision of estimates for the EC_{10}, EC_{20} and EC_{50} together with their 95% confidence intervals (or an explanation if they cannot be estimated in a particular case). This will have the added benefits of (a) providing an indication of the slope of the dose-response (by comparing the EC_{10}, $_{20}$ and $_{50}$) and (b) helping the transition from NOEC to ECx by presenting both together.

The PPR Panel recommends the following changes:

8.1. Effects on birds

8.1.3. Subchronic toxicity and reproduction

Aim of the test

"The test should determine effects on the subchronic toxicity and reproductive toxicity of the active substance to birds. The EC_{10}, EC_{20} and EC_{50} together with their 95% confidence intervals should be reported (or an explanation if they cannot be estimated) together with the NOEC."

8.2.1 Effects on aquatic organisms

8.2.2.1. Chronic toxicity test on juvenile fish

Aim of the test

"The test should determine effects on growth, the threshold level for lethal effects. Details of observed effects should be reported together with the EC_{10}, EC_{20} and EC_{50} and their 95% confidence intervals (or an explanation if they cannot be estimated) and the NOEC."

8.2.2.2. Fish early life stage toxicity test

Aim of the test

"The test should determine effects on development, growth and behaviour, and details of observed effects on fish early life stages. The EC_{10}, EC_{20} and EC_{50} together with their 95% confidence intervals should be reported (or an explanation if they cannot be estimated) together with the NOEC."

8.2.2.3. Fish life cycle test

Aim of the test

"The test will provide effects on reproduction of the parental and the viability of the filial generation. The EC_{10}, EC_{20} and EC_{50} together with their 95% confidence intervals should be reported (or an explanation if they cannot be estimated) together with the NOEC."

8.2.5. Chronic toxicity to aquatic invertebrates

Aim of the test

"The test should measure adverse effects such as immobilization and reproduction and provide details of observed effects. The EC_{10}, EC_{20} and EC_{50} together with their 95% confidence intervals should be reported (or an explanation if they cannot be estimated) together with the NOEC."

8.2.6. Effects on algal growth

Aim of the test

"The test should measure growth and growth rate, and provide details of observed effects. The EC_{10}, EC_{20} and EC_{50} together with their 95% confidence intervals should be reported (or an explanation if they cannot be estimated) together with the NOEC."

8.2.7. Effects on sediment dwelling organisms

Aim of the test

"The test will measure effects on survival and development (including effects on emergence of adults for Chironomus). The EC_{10}, EC_{20} and EC_{50} together with their 95% confidence intervals should be reported (or an explanation if they cannot be estimated) together with the NOEC."

8.4 Effects on earthworms

8.4.2. Sublethal effects

Aim of the test

"The test should measure effects on growth, reproduction and behaviour. The EC_{10}, EC_{20} and EC_{50} together with their 95% confidence intervals should be reported (or an explanation if they cannot be estimated) together with the NOEC."

10.6. Effects on earthworms and other soil non-target macro-organisms, believed to be at risk

10.6.1.2. Tests for sublethal effects

"The test should measure effects on growth, reproduction and behaviour. The EC_{10}, EC_{20} and EC_{50} together with their 95% confidence intervals should be reported (or an explanation if they cannot be estimated) together with the NOEC."

Finally, the PPR Panel has the same recommendations for aquatic plants and any other organism on which testing is required.

3.4. Ecotoxicity tests with aquatic plants

For herbicides, Annex II requires a test with an aquatic plant species, but makes no recommendation as regards the species to be tested or the test system. In most cases the test is performed with *Lemna* sp. based on an OECD guideline. However, there might be cases where due to the mode of action, or due to the route of exposure (substance partitions to sediment) *Lemna* is not an appropriate species and dicotyledonous macrophytes growing in sediments (e.g. *Myriophyllum* sp.) would be more appropriate for testing (Belgers et al, 2007; Maltby et al., in press).

The Panel therefore recommends the following text to be included:

Circumstances in which required

"A laboratory test with *Lemna* sp.should be performed for herbicides and plant growth regulators and for fungicides where there is evidence from data submitted under Annex II 8.6 and Annex III 10.6 that the test compound has herbicidal activity. If, due to the substance's mode of action, or due to the route of exposure (substance partitions to sediment) *Lemna* sp. might not be an appropriate species, or if the substance is an auxin inhibitor, or if there are clear indications from efficacy data or from testing with terrestrial non-target plants (Annex II 8.6 or Annex 10.6) for higher toxicity to dicotyledonous plant species, then in addition a test should be carried out using a dicotyledonous species growing in the sediment (e.g. *Myriophyllum* sp.)."

3.5. Updating the risk assessment for the honey bee

The EFSA opinion on Annexes II and III already mentioned the difficulties in clearly identifying the data requirements to be used in evaluating the risks from products which are to

be used as soil or seed treatments and the related risk assessment recommendations (EFSA, 2007c). The PPR Panel pointed out that recent development in the International Commission on Plant-Bee Relationship (ICPBR) should be taken into account. The recommendations of the ICPBR working group are expected to be available mid 2010 as an EPPO guidance document. The outcome of this working group should be considered in the revision of the risk assessment schemes and if applicable the data requirements, in particular with regard to the issues that needed to be clarified; i.e. decision-making criterion for "very low LD_{50}", the nature of residue data to be considered in the risk assessment and the trigger to be used for the first tier risk assessment (EFSA, 2007c).

Due to the timing mentioned above on current activities of international bodies, which should be considered at EU level, the PPR Panel reiterates its recommendation that the revised data requirements should be flexible enough to allow new risk assessment developments to be applied when available.

The following text is proposed at the end of the relevant paragraph:

10.4. Effects on bees

"The hazard quotients of 50 for oral and contact exposure do not apply to exposure of bees to residues in nectar and/or pollen that would result from systemic properties of a soil/seed treatment. In these cases the risk has to be based on a comparison of LD_{50} with residue concentrations in these matrixes. If this comparison indicates that toxic levels cannot be excluded, effects should be investigated with higher tier tests."

3.6. Other issues that should be considered in risk assessment in the future

3.6.1. Indirect effects

Indirect effects occur when direct toxic effects on one group of organisms have consequences (not mediated by toxicity) for other organisms, e.g. toxic effects of insecticides on insect populations may reduce the food supply for insectivorous birds. There is evidence that indirect effects can be important, e.g. that they have contributed to declines in farmland birds (Burn, 2000). Such effects fall within the scope of both Directive 91/414/EEC and the new regulation, as both aim at a high level of protection for the environment, which is defined as including "wild species of fauna and flora, and any interrelationship between them, and any relationship with other living organisms". Currently, neither the existing Annexes and guidance documents nor their proposed replacements contain any specific provisions or guidance for assessing indirect effects. Indirect effects may occur – and thus be taken into account – in some types of regulatory studies (e.g. aquatic mesocosm studies) but not others (e.g. avian field studies, which are usually focused on acute toxic effects). In order to address indirect effects more fully, substantial work is required to develop appropriate scientific approaches, and to establish data requirements, risk assessment procedures and decision criteria for inclusion in future revisions of the legislation and/or guidance documents.

3.6.2. Risk assessment to soil organisms

The PPR Panel issued an opinion on the usefulness of total concentrations and pore water concentrations of pesticides in soil as metrics for the assessment of ecotoxicological effects

(EFSA, 2009b). In that opinion, the PPR Panel stated that for plant protection products, in view of the current scientific evidence, free pore water concentration would be the relevant metric for effects assessment, and consequently also for exposure assessment for soft-bodied organisms and plants in close contact with soil solution. For other in-soil organisms additional routes of uptake (e.g. feed, contact with substrates in soil and litter) may need to be considered for terrestrial risk assessment. However, the PPR Panel recognises that exposure assessments based on pore water could be more complicated than the current approach. It is therefore pertinent to consider whether it would materially alter the outcome of the risk assessment. The Panel examined the only three available examples and concluded that these were insufficient to reach a general conclusion on whether using pore water concentrations would make a difference.

Additionally, the PPR Panel has identified a series of issues which need further consideration for the risk assessment of pesticides to soil organisms, such as the desired level of protection goals which need to be defined by risk managers, the need to consider all exposure routes (pore water, food, contact), considering the habitat structure (soil depth, litter layer), as well as the organism's vertical, horizontal, and temporal distribution in soil.

Further work needs to be carried out to clarify the level of effects that should be considered as acceptable in field studies with terrestrial organisms and this should be harmonized across groups of organisms.

With regard to the number of issues which need to be defined for the soil compartment in order to update the corresponding risk assessment guidance, the revised data requirements should be flexible enough to allow new risk assessment developments to be applied when available.

3.6.3. Endocrine disruption

In the previous opinion, the PPR Panel recommended that new and enhanced guidelines for the detection and characterization of endocrine disrupting chemicals should be developed, together with a harmonized testing strategy for the screening and testing of endocrine disrupters, in order to propose draft data requirements in this area.

The PPR Panel reiterates its recommendations, but wishes to emphasise that endocrine disruption remains one expression of toxic effects among others, where changes in growth and development may be observed at various concentration thresholds (i.e. from maternotoxic concentrations for the least potent disruptors to very low concentrations for the most potent disruptors).

In this respect, the new Regulation *(EC) No .../2009* of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC states that the *"Com shall present (…) a draft of the measures concerning specific scientific criteria for the determination of endocrine disrupting properties (…)."* The Commission will have four years time to work on this and might come back to the PPR Panel in due time to request a separate opinion on their proposal. For the time being, the PPR Panel does not suggest any changes to the text of EFSA, 2007c but recommends that future developments in that area need to be considered and appropriately taken into account.

3.6.4. Nanomaterials used as/in pesticides

Although so far there are no registered pesticides on the market which contain nanomaterials, progress of science indicates that this might be the case in the future, in particular since there are already biocides with these characteristics. Regarding the assessment of risks in nanotechnology, the Commission's independent Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has already published several opinions (SCENIHR, 2006, 2007, 2009). The latest one, published in January 2009, indicates that methodologies to assess exposure to manufactured nanomaterials of humans and the environment and the identification of potential hazards require further development, that more research is needed and that risk assessment should be performed case-by-case for each nanomaterial.

Also EFSA's Scientific Committee (SC) has already published a scientific opinion on nanoscience and nanotechnologies in relation to food and feed safety (EFSA, 2009d). The SC concluded that established international approaches to risk assessment can also be applied to engineered nanomaterials (ENM). A case-by-case approach would be necessary and that, in practice, current data limitations and a lack of validated test methodologies could make risk assessment of specific nano products very difficult and subject to a high degree of uncertainty.

The PPR Panel recommends that future developments in that area need to be considered and appropriately taken into account when risk assessments of these kinds of material are needed. Until then, these risk assessments will need to be performed on a case by case basis until appropriate guidance is available.

3.6.5. New issues relevant to the draft new regulation

The draft regulation text considers marine, estuarine, coastal, transitional and groundwater in the definition of areas to be protected from effects. The current Annex II and III have no data requirements for effect testing of marine and estuarine species, nor for groundwater species. Whether these should be developed may depend on observed differences in sensitivity between marine and freshwater species. Based on the most sensitive taxonomic group (arthropods; crustaceans) Maltby et al. (2005) could not demonstrate significant differences between insecticide Species Sensitivity Distribution models (SSDs) constructed with freshwater and marine species. It is possible, however, that taxonomic groups that exclusively/predominantly occur in the marine environment are more sensitive to plant protection products than the taxonomic groups currently tested. In SSDs constructed with toxicity data from all available taxonomic groups Wheeler et al (2002) observed that in the case of pesticides, saltwater species tended to be more sensitive than freshwater species.

The PPR Panel thus identifies further work aiming at developing guidance as necessary in order to fulfil the requirement of the new regulation.

CONCLUSIONS AND RECOMMENDATIONS

The PPR Panel recommends:

- That the Annexes II and III should be revised taking into consideration the comments provided by the PPR Panel in all its relevant opinions (EFSA 2006a,b,c, 2007a,b,c and 2009a,b,c,d);

- That the revised Annex II and III data requirements should be flexible enough to allow new risk assessment developments to be applied when available, in particular in relation to the EFSA mandates received at the end of 2008 concerning the revisions of the Guidance Documents on Aquatic and on Terrestrial Ecotoxicology (EC, 2002a,b), as well as other ongoing initiatives (ICPBR/ EPPO, etc.);
- Reporting of additional toxicological effects from existing studies should be encouraged where this could enhance ecological models and is possible without unreasonable additional effort or resources;
- That additional animal (vertebrates) testing, with standard or non-standardised tests should be carried out only if appropriate and properly justified;
- That an acute toxicity test with birds should be performed on one species only, preferably a quail species, since regurgitation is quite rare in these species;
- That the short-term dietary study with birds be conducted only for those pesticides where the mode of action and/or results from mammalian studies indicate a potential for the dietary LD_{50} measured by the short term study to be lower than the LD_{50} based on an acute oral study, that it should not be conducted for any other purpose unless it can be clearly justified, and that this study be performed only on one species, preferably the same as the species tested for acute toxicity.
- That efforts should be made to limit the number of birds used in acute toxicity test and short-term dietary test. The Panel therefore recommends that support should be given to completing the development and evaluation of the draft guideline no. 223 (OECD, 2007), and to ensuring that when available it can be readily taken up under Directive 91/414/EEC.
- That for acute mammal testing the OECD 425 Acute Oral Toxicity - Up and Down Procedure (OECD, 2006) should be used in preference to e.g. OECD 420 and 423 to avoid additional testing.
- That efforts to reduce the number of fish used in acute toxicity tests should be made as far as possible according to the recommendations of the draft revised guideline for OECD 203, when adopted.
- That for detecting chronic effect a fish full life cycle test rather than separate tests on different life stages should be performed unless it can be demonstrated from e.g. the mode of action of the substance, that a specific life stage is more sensitive.
- That risk is characterised only by using quotients that divide exposure by effect, in order to harmonise risk assessment and communication within the scope of Directive 91/414/EEC and across regulatory frameworks.
- That further validation of trigger values (HQ and TER if retained) is carried out in order to make the level of protection that is actually reached when these values are applied to an endpoint directly comparable between different groups of organisms.
- That risk assessment for all groups of organisms should use the ECx approach in preference to NOEC/LOEC approach and that for every group of organisms where a NOEC is mentioned in the Annexes, the text should also require provision of estimates for the EC_{10}, EC_{20} and EC_{50} together with their 95% confidence intervals.
- To include the following text into the revised Annexes: “The Hazard Quotient of 50 for oral and contact exposure does not apply to exposure of bees to residues in nectar

and/or pollen that would result from systemic properties of a soil/seed treatment. In these cases the risk has to be based on a comparison of LD_{50} with residue concentrations in these matrices. If this comparison indicates that toxic levels cannot be excluded, effects should be investigated with higher tier tests."

- That substantial work is required to develop appropriate scientific approaches to address indirect effects and to establish data requirements, risk assessment procedures and decision criteria for their inclusion in future revisions of the legislation and/or guidance documents.
- That further work needs to be carried out to clarify the level of effects that should be considered as acceptable in field studies with terrestrial organisms and further to harmonize this across groups of organisms (arthropods, soil organisms…);
- That future developments in that area of endocrine disruption need to be considered and appropriately taken into account;
- That future developments in the area of nanomaterials used as/in pesticides need to be considered and appropriately taken into account when risk assessments of these kinds of material are needed. Until then, these risk assessments will need to be performed on a case by case basis until appropriate guidance is available.

REFERENCES

Belgers JDM, Van Lieverloo RJ, Van der Pas LJT and Van den Brink PJ, 2007. Effects of the herbicide 2,4-D on the growth of nine aquatic macrophytes. *Aquatic Botany* 86: 260-268.

Brock TCM, Arts GHP, Maltby L, and Van Den Brink PJl, 2006. Aquatic risks of pesticides, ecological protection goals, and common aims in European Union legislation. Integr Environ Assess Manag 2:e20-e46

Burn A, 2000. Pesticides and their effects on lowland farmland birds. In: Aebischer, N.J., Evans, A.D., Grice, P.V., and Vickery, J.A. (eds.). Ecology and Conservation of Lowland Farmland Birds, pp 89-104. British Ornithologists' Union, London.

EC (European Commission), 1991. Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Official Journal L 230, 19/08/1991; 1-32.

EC (European Commission), 2002a. Guidance Document on aquatic ecotoxicology under Council Directive 91/414/EEC. DG SANCO (Directorate General for Health and Consumer Affairs); SANCO/3268/2001 rev.4 (final) - 17 October 2002.

EC (European Commission), 2002b. Guidance Document on terrestrial ecotoxicology under Council Directive 91/414/EEC. DG SANCO (Directorate General for Health and Consumer Affairs); SANCO/10329/2002 rev 2 final, 17 October 2002.

EC (European Commission), 2006. Regulatioon (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

ECHA (European Chemicals Agency), 2008. Guidance on information requirements and chemical safety assessment.[8]

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Plant health, Plant protection products and their Residues on a request from EFSA related to the assessment of the acute and chronic risk to aquatic organisms with regard to the possibility of lowering the uncertainty factor if additional species were tested. *The EFSA Journal* (2005) 301, 1-45.

EFSA (European Food Safety Authority), 2006a. Opinion of the Scientific Panel on Plant health, Plant protection products and their Residues on a request from the Commission related to the revision of Annexes II and III to Council Directive 91/414/EEC concerning the placing of plant protection products on the market - Physical and Chemical Properties. 17 May 2006. *The EFSA Journal* (2006) 361, 1-17.

EFSA (European Food Safety Authority), 2006b. Opinion of the Scientific Panel on Plant health, Plant protection products and their Residues on a request from the Commission related to the revision of Annexes II and III to Council Directive 91/414/EEC concerning the placing of plant protection products on the market – Analytical methods. 17 May 2006. *The EFSA Journal* (2006) 363, 1-18.

EFSA(European Food Safety Authority), 2006c. Opinion of the Scientific Panel on Plant health, Plant protection products and their Residues on a request from the Commission related to the revision of Annexes II and III to Council Directive 91/414/EEC concerning the placing of plant protection products on the market – Residues. 17 May 2006. *The EFSA Journal* (2006) 364, 1-27.

EFSA (European Food Safety Authority), 2007a. Opinion of the Scientific Panel on Plant protection products and their Residues on a request from the Commission related to the revision of Annexes II and III to Council Directive 91/414/EEC concerning the placing of plant protection products on the market – Toxicological and metabolism studies. 31 January 2007. *The EFSA Journal* (2007) 449, 1-60.

EFSA (European Food Safety Authority), 2007b. Opinion of the Scientific Panel on Plant protection products and their Residues on a request from the Commission related to the revision of Annexes II and III to Council Directive 91/414/EEC concerning the placing of plant protection products on the market – Fate and Behaviour in the Environment. 31 January 2007. *The EFSA Journal (2007) 448, 1-17.*

EFSA (European Food Safety Authority), 2007c. Opinion of the Scientific Panel on Plant protection products and their Residues on a request from the Commission related to the revision of Annexes II and III to Council Directive 91/414/EEC concerning the placing of plant protection products on the market – Ecotoxicological studies. 7 March 2007. *The EFSA Journal (2007) 461, 1-44.*

EFSA (European Food Safety Authority), 2008. Opinion of the Scientific Panel on Plant protection products and their Residues on the science behind the proposed new GD on risk assessment for birds and mammals. *The EFSA Journal* (2008) 734: 1-181).

EFSA (European Food Safety Authority), 2009a. Scientific Opinion of the Scientific Committee on existing approaches incorporating replacement, reduction and refinement of

[8] Available at: http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r7b_en.pdf?vers=20_08_08

animal testing: applicability in food and feed risk assessment. *The EFSA Journal* (2009), 1052, 1-77.

EFSA (European Food Safety Authority), 2009b. Scientific Opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA on the usefulness of total concentrations and pore water concentrations of pesticides in soil as metrics for the assessment of ecotoxicological effects. *The EFSA Journal* (2009) 922, 1-14.

EFSA (European Food Safety Authority), 2009c. Scientific Opinion of the Scientific Committee on the Use of the Benchmark Dose Approach in Risk Assessment. *The EFSA Journal* (2009) 1150, 1-72.

EFSA (European Food Safety Authority), 2009d. Scientific Opinion of the Scientific Committee on a request from the European Commission on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety. *The EFSA Journal* (2009) 958, 1-39.

Environment Canada, 2007. Guidance document on statistical methods for ecotoxicity tests/Method development and application section. Appendix O. Report EPS/1/RM/46, March 2005, amended June 2007.

Fox DR, 2009. Is the ECx a legitimate surrogate for a NOEC? Integr Environ Asses Manag 5: 351-353.

Jager T, Heuges EHW and Kooijman SALM, 2006. Making sense of ecotoxicological test results: towards application of process-based models. Ecotoxicology 15: 305-314.

Kooijman SALM, 2006. An alternative for NOEC exists, but the standard model has to be abandoned first. Oikos 75: 310-316.

Laskowski R, 1995. Some good reasons to ban the use of NOEC, LOEC and related concepts in ecotoxicology. Oikos 73:140-144.

Luttik R, 1998. Assessing repellency in a modified avian LC_{50} procedure removes the need for additional tests. Ecotoxicol Env Safety 40, 201-205.

Maltby L, Blake N, Borck TCM and Van Den Brink P, 2005. Insecticide species sensitivity distributions: the importance of test species selection and relevance to aquatic ecosystems. Environ Toxicol Chem 24:379-388.

Maltby L, Arnold D, Arts G, Davies J, Heimbach F, Pickl C and Poulsen V (eds.), (in press). Aquatic Macrophyte Risk Assessment for Pesticides. Guidance from the AMRAP workshop in Wageningen (NL), 14-16 January 2008).

Mineau P, Boersma DC and Collins B, 1994. An analysis of avian reproduction studies submitted for pesticide registration. Ecotoxicology and Environmental Safety, Vol. 29, p. 304-329.

Mineau P, Harding KM, Whiteside M, Fletcher MR, Garthwaite D and Knopper LD, 2008. Using Reports of Bee Mortality in the Field to Calibrate Laboratory-Derived Pesticide Risk Indices. Environmental Entomology. 37, 2, 546-554.

OECD (Organization for Economic Cooperation and Development), 1992. OECD guideline for testing of chemicals. Guideline 203: fish, acute toxicity test.[9]

[9] Available at: http://www.oecd.org/dataoecd/17/20/1948241.pdf

OECD (Organization for Economic Cooperation and Development), 1996. Report of the SETAC/OECD Workshop on Avian Toxicity Testing/ OECD/GD (96) 166.

OECD (Organization for Economic Cooperation and Development), 2007. Draft OECD guideline for the testing of chemicals no. 223: Proposal for a new guideline 223. Avian acute oral toxicity test. March 2007.[10]

OECD (Organization for Economic Cooperation and Development), 2008a. OECD Quantitative Structure-Activity Relationships [(Q)SARs] Project.[11]

OECD (Organization for Economic Cooperation and Development), 2008b. OECD guideline for the testing of chemical. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. Adopted 3 Oct 2008.[12]

OECD (Organization for Economic Cooperation and Development), 2008c. OECD guideline for the testing of chemicals. Draft revised guideline 203: fish, acute toxicity test.[13]

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), 2006. Opinion on the appropriateness of exissting methodologies to assess the potential risk associated with engineered and adventitious products of nanotechnologies. Adopted by the SCENIHR during the 10th plenary meeting of 10 March 2006 (after public consultation).[14]

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), 2007. Opinion on the scientific aspects of the the existing and proposed definitions relating to products of nanoscience and nanotechnologies.[15] Adopted by the SCENIHR at the 21st plenary meeting on 29 November 2007. And accompanying information by Commission services.[16]

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), 2009. Opinion on risk assessment of products of nanotechnologies. Adopted by the SCENIHR during the 28th plenary meeting of 19 January 2009.[17]

SETAC (Society of Environmental Toxicology and Chemistry), 2001. ESCORT 2 "Guidance Document on Regulatory Testing and Risk Assessment for Plant Protection Products with Non-target Arthropods.

Smart L and Stevenson JH, 1982. Laboratory estimation of toxicity of pyrethroid insecticides to honeybees: relevance to hazard in the field. Bee World, 63,150–152.

Stephan CE and Rogers JW, 1985. Advantages of using regression analysis to calculate results of chronic toxicity tests. In: Aquatic Toxicology and Hazard Assessment: Eighth Symposium, ASTM STP 891, RC Bahner, DJ Hansen, eds., pp. 328-8. American Society for Testing and Materials, Philadelphia, USA.

Van Leeuwen CJ, and Vermeire TG, 2007. Risk Assessment of Chemicals – An Introduction, 2nd edn., Springer, Dordrecht, The Netherlands.

Van Wijngaarden RP, Brock TCM and Van Den Brink PJ, 2005. Threshold levels of insecticides in freshwater ecosytems: a review. Ecotoxicology 14:353-378.

[10] Available at: http://www.oecd.org/dataoecd/58/15/38390360.pdf
[11] Available at: http://www.oecd.org/document/23/0,3343,en_2649_34379_33957015_1_1_1_1,00.html
[12] Available at: http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html
[13] Available at: http://www.oecd.org/dataoecd/45/30/41888165.pdf
[14] Available at: http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_003b.pdf
[15] Available at: http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_012.pdf
[16] Available at: http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_oc_012.pdf
[17] Available at: http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_023.pdf

Wheeler JR, Leung KMY, Morritt D, Sorokin N, Rogers Toy R, Holt M, Whitehouse P, and Crane M, 2002. Freshwater to saltwater toxicity extrapolation using species sensitivity distributions. Environ Toxicol Chem 21:2459-2467.

LIST OF ACRONYMS

ANOVA	Analysis of variance
DG SANCO	Directorate General for Health and Consumer Affairs
ECHA	European Chemicals Agency
ECx	Concentration where x% effect was observed/calculated
EEC	European Economic Community
EFSA	European Food Safety Authority
ENM	Engineered nano materials
EPPO	European and Mediterranean Plant Protection Organisation
ESCORT	European Standard Characteristics Of non-target arthropod Regulatory Testing
HQ	Hazard Quotient
ICPBR	International Commission on Plant-Bee Relations
LD_{50}	Lethal dose killing 50% of the exposed organisms
LOEC	Lowest Observed Effect Concentration
LR_{50}	Lethal rate; application rate at which 50% mortality occurs
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
OECD	Organization for Economic Cooperation and Development
PNEC	Predicted no-effect concentration
PPR	Plant Protection Products and their Residues
QSAR	Quantitative structure-activity relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemical substances
SC	Scientific Committee
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SETAC	Society of Environmental Toxicology and Chemistry
SSD	Species Sensitivity Distribution
TER	Toxicity-Exposure-Rate
US EPA	United States Environmental Protection Agency