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Technical Note

Application of the threshold approach for acute fish toxicity testing to plant protection products: A proposed framework

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HIGHLIGHTS

- Feasibility of the threshold approach for formulated PPPs fish acute toxicity tests.
- Database analysis demonstrates that a reduction of approximately 38% of the fish might be achieved.
- Proposal for the threshold approach in the development of a regulatory dataset.

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ABSTRACT

In order to minimise animal testing, this paper explores the feasibility of the "threshold approach" that has been recently developed by the Organisation for Economic Cooperation and Development (OECD). Essentially the approach uses a limit test at a single threshold concentration determined by the results of *Daphnia* and algae tests. If no mortality is observed in the limit test the fish acute value can be expressed as greater than the threshold value. However, if mortality is observed a full concentration-response test is triggered. In order to assess the applicability of the approach to plant protection products (PPP), a database of 185 products (fish, *Daphnia* and algae endpoints) was constructed and the threshold approach retrospectively applied. However, this analysis did not take into account the use of the data in the regulatory process. To assess whether the "threshold approach" could be used for PPPs the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) held a workshop in December 2010. This meeting brought together representatives from a number of European regulators and researchers as well as industry to discuss the applicability of the approach. The outcome of this discussion is presented in the paper.

1. Introduction

Within the EU, requirements for ecotoxicity testing of pesticide active substances (AS) and plant protection products (PPP) (i.e. the formulated product that is applied) are set out in EC Regulation 1107/2009 (EC, 2009). Information on the potential toxicity to fish is an integral component of these requirements. Data from these studies are used to ensure that the AS and PPP are classified and labelled correctly (EC, 2008). The data are also used in the environmental risk assessment required for authorisation of PPPs to ensure that the product does not pose an unacceptable risk to fish. However, the regulation also requires the use of non-animal test methods, that other risk assessment strategies should be promoted and tests on vertebrate animals must be replaced, restricted or refined (Article 1; paragraph 40). The recently published EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013) mentions that a threshold approach should be considered for testing acute toxicity in fish. However, there is no recommendation on how the approach could be integrated into testing and assessment procedures.

Each acute fish study conducted under Organisation for Economic Co-operation and Development (OECD) guideline 203 (OECD, 1992) uses at least 14 individuals if it is a limit test (7 fish at both the limit concentration and an untreated control) or 42 (5 treatments of 7 fish and an untreated control) if it is a full concentration response study (in addition to any range finding studies that may be required). Guidance has been produced to ensure that the number of studies required on the PPP for both classification and labelling or risk assessment purposes is kept to a minimum.

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For example the UK regulatory Authority, the Chemicals Regulation Directorate (CRD), refers to an approach that enables a PPP to be classified on the basis of the information on the toxicity of the AS and the proportion contained in the PPP[2]. However, this approach does not provide a specific endpoint that can be employed in risk assessment.

Currently guidance produced under the previous EC PPP regulation 91/414/EC (see below) is used to assess the risk to aquatic life. This guidance document indicates that:

> "*Acute toxicity studies should not be required for every formulation. . ..Where the available information on the active substance indicates that one group is clearly more sensitive, then tests on the most sensitive species of the relevant group should be carried out. In this context, the most sensitive group is defined as being at least 100 times more sensitive than the next most sensitive. If the least sensitive group is at least 100 times less sensitive than the most sensitive, then formulation data are not required on the least sensitive group. . ..There is some scope for extrapolation of toxicity data between similar formulations. In addition, in some cases it may be possible to reliably predict the toxicity of a "simple" formulation from data on the active substance and information on the co-formulants*"

Additional guidance on when data on the PPP are required in the UK is also provided in Anon (2009) and this re-iterates the points made above as well as clarifies what constitutes a minor formulation change. However, there will be new impetus for the threshold approach following its reference in recent EFSA guidance (EFSA, 2013).

Via the new EU data requirements under the Plant Protection Products Regulation and in order to reduce the number of animals being tested, Member States (MSs), the European Food Safety Authority (EFSA) and the EU Commission (COM) agreed to require only one fish species (*Oncorhynchus mykiss*; as it is regularly the most sensitive species) and make reference to the "threshold approach", a testing strategy for acute fish toxicity that has the potential to substantially reduce the number of fish needed (OECD, 2010), signalling the desire to apply some form of the threshold approach in the regulation of ASs and PPPs in the EU:

> *The acute toxicity of the substance to fish shall be determined. In order to minimise fish testing, a threshold approach to acute fish testing should be considered. An acute fish limit test should be conducted at 100 mg substance/L or at an appropriate concentration selected from aquatic endpoints (points 8.2.4, 8.2.6 or 8.2.7) following consideration of the threshold exposure* (EFSA, 2005).

The OECD has produced guidance on the "threshold approach" for acute fish toxicity (OECD, 2010), a testing strategy based on the observation that fish are not always the most sensitive species tested for aquatic toxicity. In this approach, fish are initially tested using a limit test at a single threshold concentration determined by the results of tests with *Daphnia magna* and algae (OECD Test Guidelines 202 and 201). If no mortality is observed in the limit test the fish acute value can be expressed as greater than the threshold value. However, if mortality is observed a full concentration-response test is triggered.

In order to explore how the threshold approach could be used for agrochemicals a workshop was held in December 2010. This workshop was attended by representatives from regulators from several MSs, industry as well as researchers.

[2] HSE. Brief guide to chip 3 aquatic hazard classifications for plant protection products [Internet]. Health and Safety Executive. [Accessed 2012-10-29]. Available from: http://www.pesticides.gov.uk/Resources/CRD/Migrated-Resources/Documents/E/EcotoxChip3BriefGuidance.pdf.

At this workshop it was concluded that the "threshold approach" is unlikely to be appropriate for the majority of ASs. This was due to the fact that acute AS data are truly global data that are used for several purposes, for example risk assessment, classification, range-finding prior to conducting chronic studies, as part of the trigger for fish full lifecycle tests and to calculate acute to chronic ratios. This also reflects the fact that threshold tests are unlikely to be acceptable in all regions where the AS fish acute toxicity data are required.

It was considered that there is more scope for its use for PPPs, but participants highlighted that there were significant obstacles that needed to be addressed prior to it being widely accepted for use in regulatory risk assessments. The major issue highlighted related to the different assessment factors (AF) applied to different endpoints. For example, under 1107/2009 acute fish and *Daphnia* endpoints have an assessment factor of 100 whilst a factor of 10 is applied to the algal endpoint. This issue is considered further below, however, in the first instance work was carried out to explore the feasibility of the "threshold approach" for PPPs.

2. Feasibility of the threshold approach

A retrospective analysis of toxicity data for fish, *D. magna* and algae of 185 products was performed by Syngenta. A database of 185 PPPs and the relevant AS data for fish (*Oncorhynchus mykiss*), *D. magna* and algae (*Pseudokirchneriella subcapitata*) endpoints was constructed.

All data were derived from valid studies conducted to the relevant OECD test guideline (i.e. OECD TGs 203, 202 and 201 (OECD, 1992, 2004; Jeram et al., 2005; OECD, 2006). The database is representative of typical product types covering intended uses, formulation types and number of component ASs. The database contained fungicidal (44.9%), herbicidal (31.4%), insecticidal (20.5%) and plant growth regulator (3.2%) products. Formulations containing one (45.4%), two (51.4%) and three (3.2%) ASs were included. Formulation types were dominated by Emulsifiable Concentrates (29.7%), Soluble Concentrates (21.1%) and Water Dispersible Granules (20%), but included Capsule Suspension, Dispersible Concentrate, Emulsion oil in water, Smoke Pellet, Emulsifiable Gel, Granule, Micro-Emulsion, Oil Dispersion, Suspo Emulsion, Water Soluble Granule, and Wettable Powder formulations.

In agreement with similar analyses for general chemicals (Weyers et al., 2000; Weyers and Vollmer, 2000; Hutchinson et al., 2003; Jeram et al., 2005; EC, 2006) this database for PPPs demonstrated that fish are generally not more sensitive than *Daphnia* and algae (see Table 1): fish was the most sensitive species in only 14.6% of cases. This demonstrates a high potential for the threshold approach to be of value in the assessment of PPPs.

The Threshold Concentration (TC) for PPP could be determined according to the OECD Guidance Document (OECD, 2010) (see Fig. 1) or potentially from existing fish data on the AS. Both potential approaches are investigated below:

2.1. Threshold determined from Daphnia and algae endpoints

The database was interrogated as described in Fig. 1 to determine the TC as the lowest value from either the *Daphnia* (48 h EC_{50} immobilisation) or algae (72 h $E_{biomass}C_{50}$) study.

The TC value was then compared to the fish PPP study to determine what the likely outcome of a limit test at that concentration would be. Principally, the TC was compared to the fish 96 h $NOEC_{mortality}$ to determine whether testing would stop at the limit test or if a full fish acute study would have been triggered due to the occurrence of mortality. The 96 h $NOEC_{mortality}$ was defined as the highest concentration tested where no mortality was observed