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Short Communication

Reducing the number of fish in bioconcentration studies for plant protection products by reducing the number of test concentrations

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HIGHLIGHTS

- We reviewed bioconcentration data for 55 plant protection product active substances.
- Bioconcentration factors did not differ between low and high exposure concentrations.
- This evidence supports the use of one concentration for plant protection products.
- This would reduce fish use in bioconcentration factor testing by one third.

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ABSTRACT

Fish bioconcentration tests are time consuming, expensive, and use many animals. Alternative methods that replace, reduce or refine the use of fish for BCF testing would therefore be of value. Test guidelines generally require that bioconcentration factors (BCFs) are determined at two exposure concentrations. However, recent revisions to the OECD Test Guideline for BCF testing (TG 305) provide the option to use only one exposure concentration, when justification is provided, although two concentrations may still be required for some regulatory purposes. Analysis of 55 studies on plant protection products demonstrates that BCF values do not significantly differ between the two exposure concentrations. This analysis therefore provides evidence to support the revision of OECD TG 305, and in particular provides justification for using the one test concentration approach for plant protection product active substances.

1. Introduction

Fish bioconcentration studies assist in determining the potential for substances to bioaccumulate. This is used for Persistence, Bioaccumulation and Toxicity (PBT) and secondary poisoning assessments, and classification and labelling. International data requirements for the active substances in plant protection products include triggers for fish bioconcentration testing, generally where bioconcentration might be expected, e.g. for substances with an octanol–water partition coefficient ($\log K_{ow}$) > 3 and that are stable in water (EC, 2002; EPA, 2004).

The Organisation for Economic Cooperation and Development (OECD), US Environmental Protection Agency (US EPA) Office of Chemical Safety and Pollution Prevention (OCSPP) and Japanese Ministry for Agriculture, Forestry and Fisheries (JMAFF) provide test guidelines for fish bioconcentration factor (BCF) assessment (EPA, 1996; OECD, 1996; JMAFF, 2005). Testing involves two phases: an exposure (uptake) phase where fish are exposed to the test substance in water, followed immediately by a clean water (depuration) phase where the fish are transferred to a medium free of the test substance. The concentration of test substance in the whole fish (or a specified tissue) is determined at various time points throughout both phases. Thereafter the BCF is determined via kinetic modelling of all data (kinetic BCF or BCF_k) and, if data allow, by dividing the measured plateau fish concentration at equilibrium during the uptake phase by the mean measured water concentration (steady state BCF or BCF_{ss}). In most cases the bioconcentration study is conducted with ^{14}C-labelled test compound and the resulting BCF is then based on total radioactive residue (TRR), which does not differentiate between test compound and potential metabolites. Alternatively, the BCF can be based on the actual test compound as measured in water and fish.

BCF tests are time and resource intensive and require the use of large numbers of animals. The OECD flow-through fish test (test

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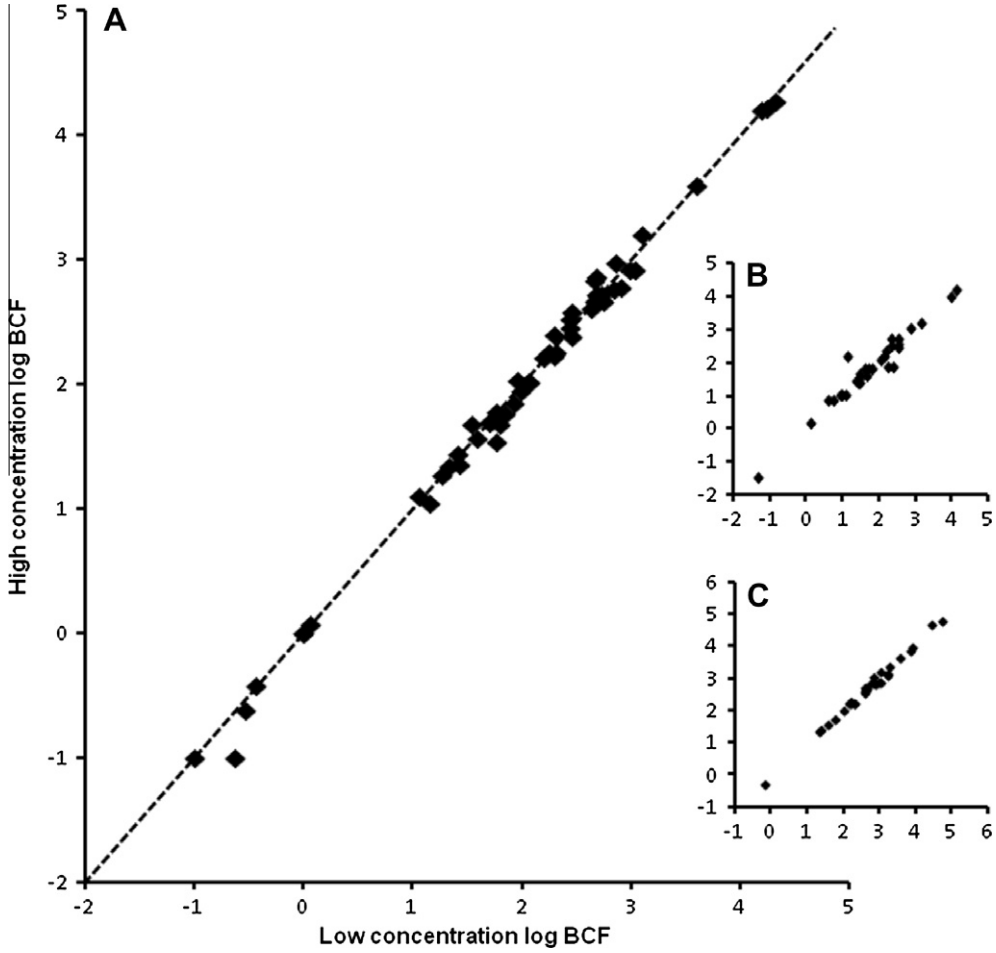

Fig. 1. Relationship between log BCF values from low and high exposure concentrations for (A) whole body (n = 55), (B) edible (n = 32) and (C) non-edible tissues (n = 31). Line represents $y = x$.

guideline [TG] 305) requires the use of at least three experimental groups (a control plus a low and high concentration exposure group), with a minimum of four fish per group sampled on at least five occasions during the uptake phase and on at least four occasions during the elimination phase – i.e. a minimum of 108 animals, but in practice larger numbers are used to allow for a potentially longer exposure phase and to cover for background mortality. The US EPA and JMAFF test guidelines (EPA, 1996; JMAFF, 2005) also require a control and at least two concentration groups. Alternative methods that replace, reduce or refine the use of fish for BCF testing would therefore be of value in improving efficiency, reducing costs and supporting animal welfare considerations.

Research is ongoing to develop *in vitro* assays to predict fish metabolism and predictive models for BCF assessment (Arnot et al., 2009; Weisbrod et al., 2009; Lombardo et al., 2010; Escher et al., 2011). Already various regulatory frameworks allow for the use of quantitative structure activity relationships (QSARs) for predicting the BCF from a molecule's structure and, if available, the experimental logK_{ow} value (e.g. EC, 2003). In addition, a number of recent publications have proposed test strategies to reduce and refine *in vivo* BCF testing (de Wolf et al., 2007; Springer et al., 2008).

Full replacement remains some way off, but OECD TG 305 has recently been revised to include the possibility of reducing the cost and number of fish used, when this can be done without compromising the BCF determination.[1] This revision was endorsed at a recent meeting of the OECD WNT in April 2012. One modification that could substantially reduce animal numbers is to use only one test concentration instead of two (de Wolf et al., 2007). This option is included in the revised version of OECD TG 305, provided a justification for the use of one exposure concentration is given. However, the US and Japanese TGs (EPA, 1996; JMAFF, 2005) are not currently being revised, and in general two concentrations may still be required under some regulatory frameworks. Experience with the test suggests that in the majority of cases a single exposure concentration may be sufficient as approximate first order kinetics are usually followed, although the BCF may be concentration dependent if the substance's tissue concentrations are regulated such that (first order) uptake rate constants differ between concentrations (e.g. some metals) or if a substance must be metabolised to be readily excreted. However, to date a formal data review has not been reported. To provide supporting evidence for the use of one test concentration we present an analysis of BCF data for 55 studies on plant protection product active substances.

2. Materials and methods

Draft assessment reports submitted for the EU peer review of active substances used in plant protection products were retrieved from the European Food Safety Authority (EFSA) website[2] (May–June 2011). Where available, fish BCF values (whole body, edible and non-edible tissue) were extracted from study summaries and tabulated. BCF values provided in the study summaries were taken

[1] http://www.oecd.org/dataoecd/10/48/50309198.pdf.

[2] http://dar.efsa.europa.eu/dar-web/provision.