

# **Fish Bioconcentration Data Requirement: Guidance for Selection of Number of Treatment Concentrations**

[Supplement to OCSPP Test Guideline 850.1730]

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**Office of Pesticide Programs  
Office of Chemical Safety and Pollution Prevention  
U.S. Environmental Protection Agency**



## I. Purpose

The purpose of this document is to clarify EPA recommendations for the number of treatment concentrations needed to result in acceptable fish bioconcentration factor (BCF) studies for pesticide registration. EPA routinely requires BCF studies to determine whether pesticide active ingredients have the potential to accumulate in fish, enter the food chain, and cause adverse effects in fish-eating predators such as aquatic mammals and birds of prey.

In April 2017, EPA was approached by an outside party, the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3R), with a suggestion to modify the test guideline for the BCF study to reduce the number of animals used in BCF testing, by reducing the number of concentration levels used from three (two positive doses and one control) to two (one positive level and one control).

NC3R stated that this would be “[i]n the interest of international harmonization and reducing unnecessary animal testing” because “[a]t the moment the Japanese and US EPA guideline require that two concentrations are always tested, which is in contrast to the OECD Test Guideline<sup>[1]</sup>; therefore, many companies are understandably continuing to test two concentrations to ensure acceptance within these regions.”

This modification has the potential to reduce the number of fish used by one-third. EPA has considered NC3R’s suggestion and has considered/evaluated data in its own holdings as part of that consideration. This paper documents this analysis and results of EPA’s analysis of this proposal.

## II. Issue

According to pesticide data requirements set forth in Part 158 of Title 40 in the Code of Federal Regulations (40 CFR part 158)<sup>2</sup>, Fish Bioconcentration Factor (BCF) data (OCSP Guideline 850.1730; USEPA 2016<sup>3</sup>) are required to support applications for pesticide registrations under the following conditions: the octanol-water partition coefficient ( $K_{ow}$ ) of the chemical is  $>1000$  ( $\log K_{ow} > 3.0$ ); the use pattern results in potential exposure to fish and other nontarget aquatic organisms; or, the hydrolytic half-life of the chemical is  $>5$  days at pH 5, 7 and 9. As part of the exposure phase of the study, the guideline specifies the use of at least two test concentrations which are a factor of 10 apart, plus the appropriate control(s), in order to document that the potential to bioconcentrate is independent of the concentration of the test substance. The 850.1730 guidance states:

“(iii) **Treatment concentrations.** To document that the potential to bioconcentrate is independent of the concentration of the test substance,

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<sup>1</sup> Test Guideline issued by the Organisation for Economic Co-operation and Development (OECD) are available online at <http://www.oecd.org/chemicalsafety/testing/>.

<sup>2</sup> Information about the EPA data requirements for pesticide registration and related test guidelines is available online at <https://www.epa.gov/pesticide-registration/data-requirements-pesticide-registration>.

<sup>3</sup> <https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0154-0031>.

bioconcentration values for the test substance should be determined using at least two concentrations during the exposure phase which are a factor of 10 apart, plus the appropriate control(s). Preliminary toxicity tests or a range-finding test can be used to establish the appropriate test solution concentrations for the definitive test. The two concentrations selected should not stress or adversely affect the fish.”

Analysis presented to EPA’s Office of Pesticide Programs (OPP) by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (Creton *et al.* 2013; Burden *et al.* 2014) documents that for a wide range of pesticides and industrial chemicals, the BCF factors obtained from high and low doses do not differ by a factor that would lead to a difference in conclusions regarding the potential for the tested chemical to exceed regulatory criteria for bioconcentrating substances. Using this information, the OECD (2017) has proposed to increase acceptance of studies across international authorities by specifying the conditions under which one test concentration is acceptable. Section III of this document describes the results of the OECD’s analysis.

However, there are well-documented cases of concentration-dependent BCF factors, such as for essential metals (McGreer *et al.* 2003). Also, Beek *et al.* 2000 provides examples of compounds with demonstrated bioconcentration behavior despite high water solubility or low lipophilicity (*i.e.*,  $\log K_{ow} < 3$ ), such as quaternary ammonium compounds, and anionic surfactants. These data indicate that BCF studies should be designed carefully for chemicals whose bioconcentration behavior is not expected to correlate with their  $\log K_{ow}$ . This is addressed in TG305, section 2.4 (Ionising chemicals).

### **III. Analysis and Proposed Resolution**

OECD analyzed a data set for precision and accuracy of BCF estimations based on one or two test concentrations. OECD conducted an analysis (TG305, section 2.5.1) of a data set of 40 anonymized compounds provided by the German environmental agency (UBA) that were tested at two concentrations. This analysis showed that the mean percent difference in BCF between high and low test levels was about 2.2%, with a standard deviation of 32% (TG305, Table 2-1). This analysis further showed that an allowable Maximum Percent Difference (MPD) of 50% in the BCF between low and high test concentrations reduces “discordance,” or disagreement as to whether the test signifies that the tested chemical qualifies as “B,” or bioconcentrating, to a minimum (TG305, section 2.5.1, paragraph 72). OPP reviewed, and agrees with, OECD’s analysis.

Using the approach described above, OPP conducted an analysis to determine when a single concentration would be adequate for OPP regulatory purposes. To make an adequacy determination, OPP needs a BCF point of departure. While OPP has not established a BCF value of regulatory concern per se, it has used a BCF of 1,000 as a point of departure for devoting additional resources to evaluate bioaccumulation risks for a pesticide. The initial basis for the consideration of a BCF value of approximately 1,000 as a point of departure for bioaccumulation evaluation is linked to information developed at a meeting sponsored by the American Society for Testing and Materials held in 1976 which was published in the open literature two years later

(Cairns et al. 1978)) and which was reaffirmed in Cairns and Dickson (1995). Furthermore, a BCF value of 1,000 has been suggested by a variety of sources to denote chemicals warranting consideration for bioaccumulation potential (Cairns et al. 1978, Akerman and Coppage 1979, AIBS 1978, ASTM 1978, Kimerle et al. 1978, Maki and Duthie 1978, and Stern and Walker 1978). More recent evaluations have similarly supported a BCF of 1,000 as a point of departure (USEPA, 1998, 1999a, 1999b). Considering the consistency in the historical application of a BCF value of 1,000 as a point of departure triggering evaluation of bioaccumulation, OPP decided to use 1,000 as the point of departure for this evaluation of the adequacy of single concentration BCF studies in the context of pesticide risk assessment evaluation.

Using OECD's approach described above, applying a MPD of 50% to a BCF value of 1,000 as a consistent and reasonable point of departure for bioaccumulation evaluation efforts, EPA determined that a BCF test using one concentration level with results equal to or less than 667 would not require a second concentration level. However, those exceeding a value of 667 would require testing at a second concentration level to determine whether the active ingredient meets the "B" criterion of BCF = 1,000.

#### **IV. Conclusion**

Based on the information described above, OPP will accept a single treatment level concentration if the BCF value is less than or equal to 667. Based on historical data submission, OPP expects that most submitted Fish Bioconcentration Factor (BCF) studies will only need one treatment concentration. As always, companies must ensure that the study results meet the other guideline specifications and the raw data are determined to be scientifically sound. OPP recommends that the selected concentration avoid toxic or metabolic effects in the fish that might affect the outcome of the study yet also be high enough to provide quantifiable concentrations of residues of concern in both fish tissue and water. OPP expects companies to describe in their study reports their rationale for any selected test concentration and how the results for the BCF study using that concentration align with the acceptance criteria outlined in Section III of this document.

EPA expects that this policy change will result in savings of approximately 240 test animals per year as well as EPA, industry, and laboratory resources in conducting and reviewing BCF studies.

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