

3 Commentary:

4 Promoting the 3Rs to enhance the OECD Fish Toxicity 5 Testing Framework.

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24 Fish testing has been used to understand chemical and effluent toxicity since the 1860's and
25 continues to play an important role towards defining safe levels of chemical contaminants in
26 lakes, rivers and coastal waters (Sprague 1971; Hunn 1989). Historically, many severe
27 chemical pollution problems led to fish kills giving rise to a focus on acute lethality testing of
28 chemicals and effluents. More recently, the focus of concern is on long term effects of
29 chemicals directly on fish and also indirectly via impacts on invertebrate prey species and
30 other taxa. Consequently, fish toxicity testing is embedded in most regulatory programmes
31 for prospective and retrospective assessment of individual chemical substances and effluents.
32 Current regulations implementing environment protection (e.g. REACH and Plant Protection
33 Products legislation) increasingly incorporate the wider societal view that vertebrate animal
34 use should be Replaced, Reduced and Refined (the 3Rs) where possible. Such a paradigm
35 shift also supports scientific and business needs to consider the 3Rs. The OECD Fish
36 Toxicity Testing Framework (OECD 2012) provides a useful structure with which to
37 simultaneously address the needs of high levels of environmental protection whilst
38 implementing the 3Rs. This commentary aims to encourage awareness of this activity and
39 promote the implementation of the recommendations of the OECD Fish Toxicity Testing
40 Framework.

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42 The historic need to be identify and prevent chemical impacts on fisheries and water quality
43 led in 1981 to the Organisation for Economic Co-operation and Development (OECD)
44 adopting the Fish Acute Toxicity Test Guideline 203 (updated in 1984 and 1992).
45 Subsequently the Fish Early-Life Stage Toxicity Test Guideline 210 was adopted in 1988
46 (since updated in 2013). As scientific knowledge of the environmental risks posed by diverse
47 chemicals has grown, the OECD has adopted a growing number of fish test guidelines to
48 address bioconcentration, development and reproduction in fish. Today's OECD 'toolbox' of

49 test guidelines plays a central role in supporting an internationally consistent approach to the
50 environmental safety assessment of chemicals. We define the environmental safety
51 assessment of chemicals as the evaluation of the predicted environmental exposure of a
52 chemical with the predicted *in vivo* biological effect of the chemical, supported by
53 mechanistic *in silico* and *in vitro* data describing the intrinsic (eco)toxicological properties or
54 mode-of-action (MOA) of the chemical. In contrast, the environmental risk assessment of
55 chemicals typically focusses solely on comparing the predicted exposure concentration (PEC)
56 to the predicted no effect concentration (PNEC) derived from *in vivo* experiments for relevant
57 taxonomic groups (fish, aquatic invertebrates and algae) in the absence of MOA data.
58 Though perhaps a subtle distinction, the safety assessment approach offers scope for a more
59 comprehensive use of all *in silico*, *in vitro* and *in vivo* information at multiple levels of
60 biological organisation. Balancing the need for high standards of environmental protection
61 with the demands and desire to implement the 3Rs is one of the key challenges for
62 environmental safety assessment today. As part of addressing this challenge, the OECD
63 (2012) developed the Fish Toxicity Testing Framework in order to provide guidance on how
64 best to deploy the various fish toxicity and bioconcentration test guidelines, including
65 consideration of the 3Rs. In our view, the OECD's Fish Toxicity Testing Framework
66 provides a logical and transparent approach to this complex aspect of environmental safety
67 assessment. A simplified version of the OECD Fish Toxicity Testing Framework is shown in
68 Figure 1 (see OECD (2012) for full details). The OECD (2012) also considered a number of
69 important outstanding questions of scientific and regulatory concerns, including for example,
70 what are the options for reducing animal numbers in fish toxicity tests and how can less
71 severe endpoints be given priority in decision making?

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73 More broadly, we believe there is reason to be optimistic that the 3Rs can be successfully
74 applied to the OECD Fish Toxicity Testing Framework to support environmental safety
75 assessment. Firstly, in terms of replacement, Figure 1 summarises some key opportunities
76 and gives priority to the replacement of *in vivo* fish testing, where feasible, through the use of
77 validated *in silico* and *in vitro* tools. However, these can only be applied with confidence
78 within the chemical domains of the data used for their validation. Replacement of fish acute
79 tests by the Fish Embryo Acute Toxicity Test Guideline 236 (adopted July 2013) may be
80 possible under some regulations. Replacement of fish toxicity testing with suitable
81 invertebrates may also be useful. Replacement may take the form of establishing targeted
82 threshold test levels for fish (determined by full invertebrate tests) or complete replacement
83 but this needs to be justified scientifically by an understanding of the exposure relevant MOA
84 of a chemical in order to derive robust environmental safety assessments. For example, a the
85 this mechanistic approach could offer a positive way forward to address the replacement of
86 fish with arthropod toxicity tests where there is a shared MOA (e.g. ion channel mediated
87 neurotoxicity of pyrethroids) or other *a priori* knowledge of a particularly more sensitive
88 taxonomic group of invertebrates. This would be in contrast to a very different mode-of-
89 action specific to vertebrates (e.g. receptor-mediated feminization of fish by steroidal
90 oestrogens) (ECETOC 2007). The development of adverse outcome pathways, as strongly
91 supported by the OECD, could in the future help to identify where cross-species extrapolation
92 is appropriate based upon a common Molecular Initiating Event (Ankley et al., 2010; Burden
93 et al. 2015a; OECD (2015)). Invertebrates may also provide an environmentally relevant
94 alternative bioconcentration test guideline given the potential for chemical uptake into lipid
95 rich invertebrates (ECETOC 2007).

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97 Secondly refinement of the severity of the experimental endpoints (i.e. degree of suffering
98 induced) is another aspect of the OECD Fish Toxicity Testing Framework that warrants
99 attention. For instance, minimising the assessment of lethality in fish and optimising the
100 experimental design to focus on sublethal endpoints via the Maximum Tolerated
101 Concentration (MTC) approach (Hutchinson *et al.*, 2009) is one aspect of refinement
102 considered by the OECD (2012). The OECD (2012) also recommended introducing the term
103 ‘moribund’ in the fish acute toxicity Test Guideline 203, which would represent a significant
104 refinement. Discussions are currently ongoing regarding this guideline revision. Test
105 Guideline 204 (Fish, Prolonged Toxicity Test: 14-Day Study) was deleted after it was
106 deemed as ‘ethically indefensible’ and concerns have also been raised about the lack of
107 feeding in the Test Guideline 212 (Fish, Short-term Toxicity Test on Embryo and Sac-Fry
108 Stages).

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110 Thirdly, further effort is needed to reduce the numbers of fish toxicity tests through a variety
111 of approaches. As summarized in OECD (2012), these approaches include moving away
112 from automatic ‘tick box’ testing to more efficient tiered testing frameworks and ‘intelligent’
113 or ‘integrated testing strategies’ which make better use of *in silico*, *in vitro* and *in vivo*
114 information. However, operating such a flexible approach will undoubtedly result in greater
115 regulatory complexity. Further, work to explore the application of test guideline validity
116 criteria was also recommended. This could determine which deviation(s) (or magnitude of the
117 deviation) from criteria fundamentally undermines study outcomes and overall test
118 performance (hence necessitating repeat studies), and conversely which do not impact on the
119 scientific quality of studies (thus negating the need for their repetition).

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121 The OECD framework is not comprehensive of all opportunities to address the 3Rs as
122 described elsewhere. However, it offers tangible opportunities to address the issues with what
123 constitutes the building blocks of the current regulatory data requirements (i.e. test guideline
124 studies mandated by the various chemical legislations). As such it fits with the current legal
125 frameworks and so offers an ability to improve 3Rs application in the short and medium
126 terms whilst fundamentally different approaches are developed and mature sufficiently for
127 regulatory implementation (Burden et al. 2015b). A number of the OECD Fish Toxicity
128 Testing Framework recommendations have already developed as projects and made it on to
129 the OECD's work plan (see Table 1).

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131 In conclusion, the OECD (2012) generic framework provides a highly valuable opportunity
132 to improve fish toxicity and bioconcentration testing. The OECD framework highlights key
133 3Rs opportunities that are consistent with the scientific and ethical principles increasingly
134 required by regulations, industry and society for chemical safety assessment. We encourage
135 international stakeholders to take up the recommendations from the OECD framework in
136 order to further promote the reduction, replacement and refinement of fish toxicity testing
137 within the environmental safety assessment context.

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178 *The author’s declare no competing financial interests.*

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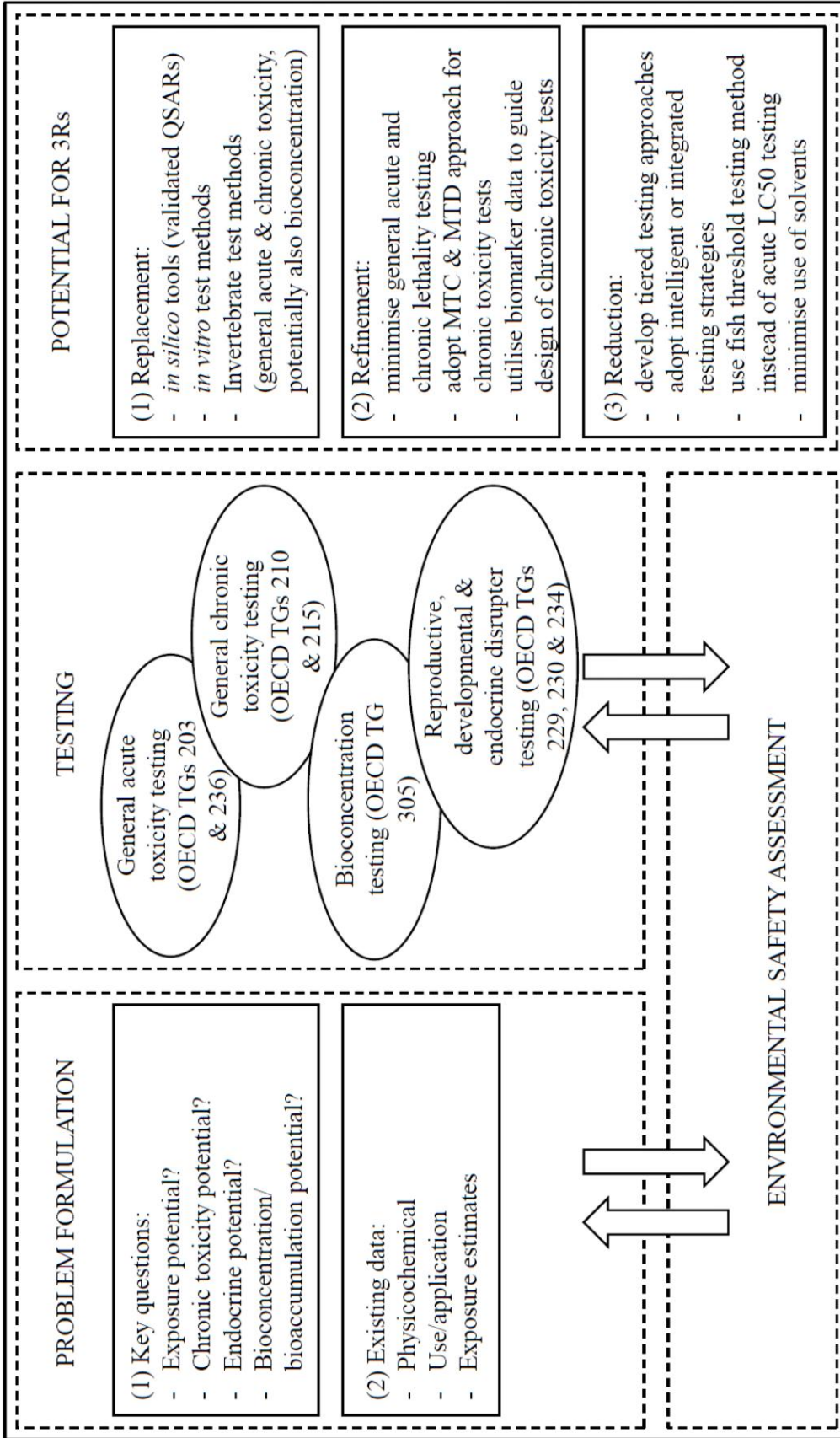


Figure 1. Simplified OECD generic testing strategy for fish toxicity and bioconcentration assessment with priorities for promoting the 3Rs (see OECD (2012) for the detailed flow-chart of the testing strategy).

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187 Table 1. Summary of OECD projects on (or proposed) the work plan associated with the 3Rs
 188 in guideline ecotoxicity tests.

Project Number	Title	Date included	Lead country	Issue
2.50	Revision of TG 203 Fish Acute Toxicity Test	2014	Switzerland/ United Kingdom	Definition and implementation of moribund to allow early of termination of individuals to prevent suffering (reliable prediction of death)
2.54	Guidance Document on IATA for Fish Acute Toxicity Testing	2015	Austria	Integrated Approaches to Testing and Assessment for acute fish toxicity testing
2.55	Use and analysis of control fish in toxicity studies	2015	European Commission	Review and update of poorly soluble substance guidance. Detailed Review Paper of use of controls in ecotoxicity tests
Proposed	Critical assessment of deviations from OECD Vertebrate Ecotoxicology Test Guidelines	2016	United Kingdom	Review of test guideline validity criteria. Update of test guidelines and guidance on interpretation to avoid unnecessary repeats.

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