A brief history of the fish embryo toxicity (FET) test

- Adam Lillicrap

Norwegian Institute for Water Research

3Rs

- Reduction
- Refinement
- Replacement

Additional Rs

- Relevance
- Reproducibility
- Regulatory Acceptance

6Rs

Chemical hazard and risk assessment


NIVA

Acute ecotoxicity tests

– Base Set

NORSK AKKREDITERING

BEKREFTELSE

på overholdelse av
OECD's prinsipper for God laboratoriepraksis (GLP)
i henhold til Eus Parlaments - og Rådsdirektiv 2004/10/E

Laboratorieinspeksjon og revisjon av forsøk er gjennomført hos:

Norsk institutt for vannforskning

Økotoksikologisk laboratorium

Produktgruppe	Ekspertiseområde
Industrikjemikalier	Fysisk - kjemisk testing
Industrikjemikalier	Økotoksikologiske undersøkelser på akvatiske og terrestriske organismer

Dato for besøket: 15.02.2016 -16.02.2016

Laboratoriet er innført i Norsk akkrediterings register over
GLP-inspiserte laboratorier og er
underlagt bestemmelsene i Norsk akkrediterings GLP-ordning

Registreringsnummer: GLP 007

Neste ordinære GLP-inspeksjon vil finne sted innen 2 år og 6 måneder.

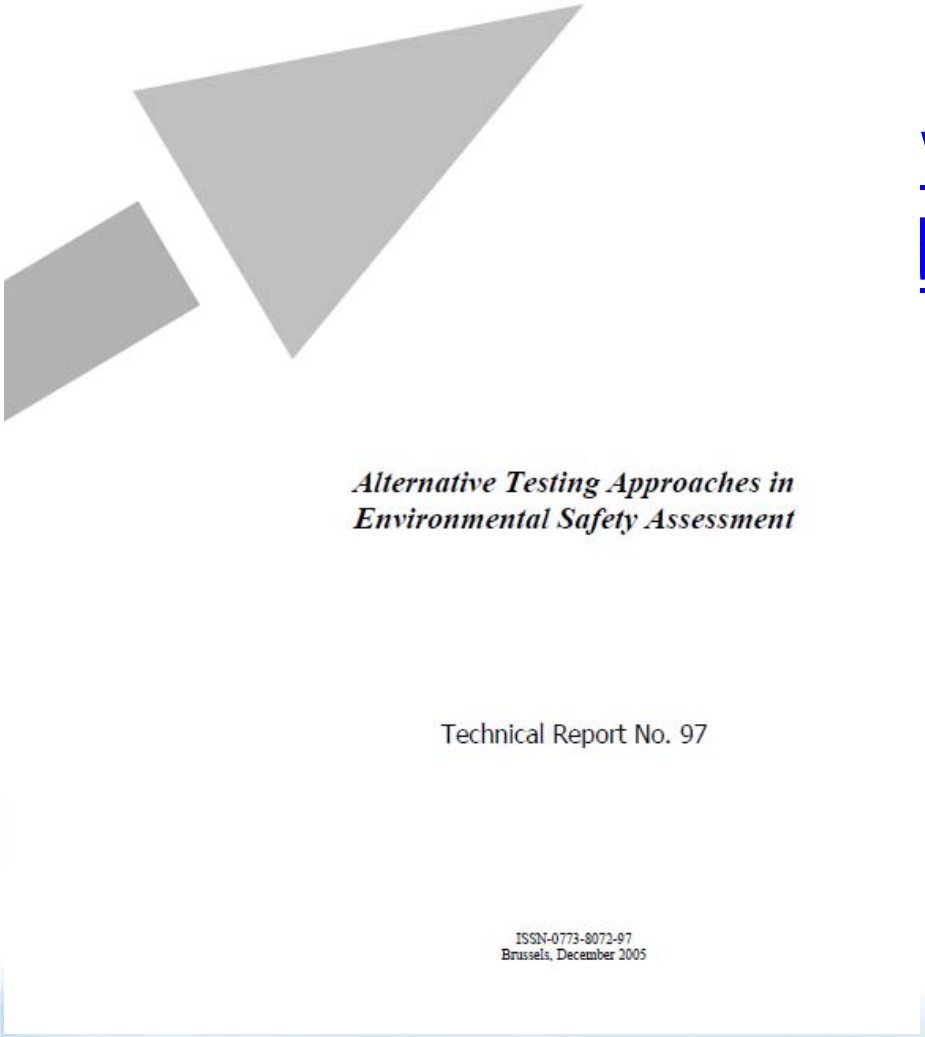
NORSK AKKREDITERING

20.12.2016
Dato

Norsk akkreditering

Historic perspective

www.ecetoc.org/wp-ls/2014/08/ECETOC-TR-097.pdf



Workshop on Alternative Testing Approaches in Environmental Risk Assessment
7-9 July 2004, Crécy-la-Chapelle

Workshop Report No. 5

Aims

- Identify methodology for generating information for environmental risk assessment, in line with the 3Rs;
- identify research to address knowledge gaps in the proposed methodology;
- draft research plans including potential funding opportunities, collaborations and timelines to develop alternative methodologies to address the environmental safety of chemicals.

Contact www.cefic.org Members portal

Advanced search

Long-Range Research Initiative

LRI research programme | Funding Opportunities | LRI Projects | LRI Toolbox | News & Events

Home About

All projects | Intelligent testing | Complex environments | Acceptance of technology

Home › Projects › ECO8: Development of a strategy to predict ac...

Share Share

SEARCH IN ...

LRI Projects

- All projects
- Intelligent testing
- Complex environments
- Acceptance of technology

ECO8: Development of a strategy to predict acute fish lethality using fish cell lines and fish embryos

Principal Investigator

PD Dr. habil Kristin Schirmer
Head of Department - Environmental Toxicology
Eawag
íœberlandstrasse 133
P.O. Box 611
8600 Dübendorf
Switzerland
email: kristin.schirmer@eawag.ch

PUBLICATIONS

Testing acute toxicity in the embryo of zebrafish, Brachydanio rerio, as an alternative to the acute fish test: preliminary results [1994]

Schulte, C.
Nagel, R.

Abstract

Acute toxicity testing in fish is a standard method used in estimating the influences of chemicals on aquatic vertebrates. The ecotoxicological data obtained from acute toxicity tests in fish are however, not sufficiently reliable to justify the continued use of this test. Fertilised eggs of zebrafish (Brachydanio rerio) were used to test the acute toxicity of chemicals. They were chosen because the development of B. rerio has been studied extensively and information already exists concerning the normal development of this species. The following parameters of the development of B. rerio were observed: coagulation of the egg, gastrulation, number of somites, movement, development of organs, pigmentation, heartbeat and circulation. Some of these toxicological endpoints indicate lethality. In addition, various interactions between the test chemical and the embryos can be measured by investigating these parameters. Six chemicals (2,4-dinitrophenol, 4,6-dinitro-o-cresol, malathion, carbaryl, phenol and 4-nitrophenol) were selected for testing in the embryos of zebrafish within the first 48 hours of their development. The toxicities of the test chemicals to zebrafish embryos were compared with their acute toxicities to adult fish. Further investigations with more chemicals are in progress. The effective concentrations (EC50) and the lowest effect concentrations (LOEC) investigated in this preliminary study were comparable to the LC50 values for adult fish. The u

DRAFT GUIDELINE
May 30, 2006 (1st Version)

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

DRAFT PROPOSAL FOR A NEW GUIDELINE

Fish Embryo Toxicity (FET) Test

INTRODUCTION

1. This Test Guideline describes a Fish Embryo Toxicity (FET) test mainly developed for use with the zebrafish (*Danio rerio*) but the test method can also be adapted to fathead minnow (*Pimephales promelas*), Japanese medaka (*Oryzias latipes*) and other relevant species of interest (1). This Guideline intends to define lethal effects of chemicals on embryonic stages of fish and constitute an alternative test method to the acute toxicity tests with juvenile and adult fish, *i.e.,* the OECD Test Guideline 203 (2), thus providing a reduction in fish usage. The FET-test is mainly developed from studies and validation activities performed on zebrafish (1)(3)(4)(5)(6)(7)(8)(9)(10)(11)(12)(13)(14)(15)(16)(17)(18), but also from studies on fathead minnow (1)(19)(20)(21)(22) and Japanese medaka (1)(23)(24)(25)(26)(27)(28)(29).

DRAFT GUIDELINE
May 30, 2006 (1st Version)

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

DRAFT PROPOSAL FOR A NEW GUIDELINE

Fish Embryo Toxicity (FET) Test

INTRODUCTION

1. This Test Guideline describes a Fish Embryo Toxicity (FET) test mainly developed for use with the zebrafish (*Danio rerio*) but the test method can also be adapted to fathead minnow (*Pimephales promelas*), Japanese medaka (*Oryzias latipes*) and other relevant species of interest (1). This Guideline intends to define lethal effects of chemicals on embryonic stages of fish and constitute an alternative test method to the acute toxicity tests with juvenile and adult fish, *i.e.*, the OECD Test Guideline 203 (2), thus providing a reduction in fish usage. The FET-test is mainly developed from studies and validation activities performed on zebrafish (1)(3)(4)(5)(6)(7)(8)(9)(10)(11)(12)(13)(14)(15)(16)(17)(18), but also from studies on fathead minnow (1)(19)(20)(21)(22) and Japanese medaka (1)(23)(24)(25)(26)(27)(28)(29).

Joint Research Centre

TU 138

www.jrc.ec.europa.eu

OECD validation study on the transferability, intra- and inter-laboratory reproducibility of the Zebrafish Embryo Toxicity Test (ZFET)

F. Busquet[1], S. Belanger[2], T. Braunbeck[3], G. Carr[2], M. Halder[1], A. Lillicrap[4], J. Rawlings[2], R. Strecker[3], S. Walter-Rohde[5] and P. Amcoff[6]

1 European Commission JRC/IHCP/ECVAM, Ispra, ITALY, 2 Procter & Gamble, Cincinnati, OH, U.S.A., 3 University of Heidelberg, Heidelberg, GERMANY, 4 NIVA, Oslo, NORWAY, 5 UBA, Dessau-Rosslau, GERMANY, 6 OECD, Environment, Health and Safety Division, Environment Directorate, Paris, FRANCE

"Disclaimer: The opinions expressed and the arguments employed herein are those of the authors and do not necessarily reflect the official views of the OECD or of the governments of its member countries"

Introduction

- One of the most promising alternative approaches to the 96h LC50 acute fish toxicity test (OECD TG 203) is based on the use of zebrafish embryos.
- In fall 2005, the German Federal Environment Agency (UBA) submitted the draft test guideline "Fish embryo toxicity (FET) test" to the OECD Test Guideline Program together with a Background Paper.
- OECD established the *ad hoc* Expert Group on the Fish Embryo Toxicity Test and based on the outcome of expert meetings, OECD decided to perform a validation study (coordinated by ECVAM and steered by a validation management group).
- The validation study involves several international laboratories in order to evaluate the transferability of the zebrafish embryo toxicity test (ZFET) and assess its intra- and inter-laboratory reproducibility.

Unclassified

ENV/JM/MONO(2011)37

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

25-Aug-2011

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Cancels & replaces the same document of 23 August 2011

VALIDATION REPORT (PHASE 1) FOR THE ZEBRAFISH EMBRYO TOXICITY TEST PART I

Series on Testing and Assessment

No. 157

ENV/JM/MONO(2011)37
Unclassified

Unclassified

ENV/JM/MONO(2012)25

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

10-Aug-2012

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

VALIDATION REPORT (PHASE 2) FOR THE ZEBRAFISH EMBRYO TOXICITY TEST

Series on Testing and Assessment

No. 179

ENV/JM/MONO(2012)25
Unclassified

Regulatory Toxicology and Pharmacology

Volume 69, Issue 3, August 2014, Pages 496–511

OECD validation study to assess intra- and inter-laboratory reproducibility of the zebrafish embryo toxicity test for acute aquatic toxicity testing

François Busquet[a, 1, 2], Ruben Strecker[b, 1, 3], Jane M. Rawlings[c, 1], Scott E. Belanger[c], Thomas Braunbeck[b], Gregory J. Carr[d], Peter Cenijn[e], Przemyslaw Fochtman[f], Anne Gourmelon[g], Nicole Hübler[h], André Kleensang[a, 4], Melanie Knöbel[i, 5], Carola Kussatz[j], Juliette Legler[e], Adam Lillicrap[k], Fernando Martínez-Jerónimo[l], Christian Polleichtner[j], Helena Rzodeczko[f], Edward Salinas[m], Katharina E. Schneider[m, 6], Stefan Scholz[i], Evert-Jan van den Brandhof[n], Leo T.M. van der Ven[o], Susanne Walter-Rohde[p], Stefan Weigt[h], Hilda Witters[q], Marlies Halder[a]

DRAFT GUIDELINE
May 30, 2006 (1st Version)

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

DRAFT PROPOSAL FOR A NEW GUIDELINE

Fish Embryo Toxicity (FET) Test

INTRODUCTION

1. This Test Guideline describes a Fish Embryo Toxicity (FET) test mainly developed for use with the zebrafish (*Danio rerio*) but the test method can also be adapted to fathead minnow (*Pimephales promelas*), Japanese medaka (*Oryzias latipes*) and other relevant species of interest (1). This Guideline intends to define lethal effects of chemicals on embryonic stages of fish and constitute an alternative test method to the acute toxicity tests with juvenile and adult fish, *i.e.*, the OECD Test Guideline 203 (2), thus providing a reduction in fish usage. The FET-test is mainly developed from studies and validation activities performed on zebrafish (1)(3)(4)(5)(6)(7)(8)(9)(10)(11)(12)(13)(14)(15)(16)(17)(18), but also from studies on fathead minnow (1)(19)(20)(21)(22) and Japanese medaka (1)(23)(24)(25)(26)(27)(28)(29).

OECD/OCDE

236

Adopted:
26 July 2013

OECD GUIDELINES FOR THE TESTING OF CHEMICALS

Fish Embryo Acute Toxicity (FET) Test

INTRODUCTION

1. This Test Guideline (TG) 236 describes a Fish Embryo Acute Toxicity (FET) test with the zebrafish (*Danio rerio*). This test is designed to determine acute toxicity of chemicals on embryonic stages of fish. The FET-test is based on studies and validation activities performed on zebrafish (1)(2)(3)(4)(5)(6)(7)(8)(9)(10)(11)(12)(13)(14). The FET-test has been successfully applied to a wide range of substances exhibiting diverse modes of action, solubilities, volatilities, and hydrophobicities (reviewed in 15 and 16).

Environ Sci Pollut Res Int. 2015 Nov;22(21):16247-61. doi: 10.1007/s11356-014-3814-7. Epub 2014 Nov 15.

The fish embryo test (FET): origin, applications, and future.

Braunbeck T[1], Kais B[2], Lammer E[2], Otte J[2], Schneider K[2], Stengel D[2], Strecker R[2].

Author information

Abstract

Originally designed as an alternative for the acute fish toxicity test according to, e.g., OECD TG 203, the fish embryo test (FET) with the zebrafish (Danio rerio) has been optimized, standardized, and validated during an OECD validation study and adopted as OECD TG 236 as a test to assess toxicity of embryonic forms of fish. Given its excellent correlation with the acute fish toxicity test and the fact that non-feeding developmental stages of fish are not categorized as protected stages according to the new European Directive 2010/63/EU on the protection of animals used for scientific purposes, the FET is ready for use not only for range-finding but also as a true alternative for the acute fish toxicity test, as required for a multitude of national and international regulations. If-for ethical reasons-not accepted as a full alternative, the FET represents at least a refinement in the sense of the 3Rs principle. Objections to the use of the FET have mainly been based on the putative lack of biotransformation capacity and the assumption that highly lipophilic and/or high molecular weight substances might not have access to the embryo due to the protective role of the chorion. With respect to bioactivation, the only substance identified so far as not being activated in the zebrafish embryo is allyl alcohol; all other biotransformation processes that have been studied in more detail so far were found to be present, albeit, in some cases, at lower levels than in adult fish. With respect to larger molecules, the extension of the test duration to 96 h (i.e., beyond hatch) has-at least for the substances tested so far-compensated for the reduced access to the embryo; however, more research is necessary to fully explore the applicability of the FET to substances with a molecular weight >3 kDa as well as substances with a neurotoxic mode of action. An extension of the endpoints to also cover sublethal endpoints makes the FET a powerful tool for the detection of teratogenicity, dioxin-like activity, genotoxicity and mutagenicity, neurotoxicity, as well as various forms of endocrine disruption.

Analysis of the Fish Embryo Acute Toxicity (FET) OECD TG 236 in the context of fulfilling the information requirements of the REACH Regulation

Marta Sobanska[1], Romanas Cesnaitis[1], Simon Gutierrez Alonso[1], Anna-Maija Nyman[1], Laurence Deydier[1], Nina Falk[1], Francesca Pellizzato[1], Stefan Scholtz[2], Nils Klüver[2], Ralph Kühne[2], Derek Knight[1], Wim de Coen[1]

1. ECHA, Annankatu 18, FI-00121 Helsinki, Finland
2. Helmholtz Centre for Environmental Research UFZ, Permoserstr. 15, 04318 Leipzig, Germany

Report ECHA-UFZ contract ECHA/2014/341

Analysis of the relevance and adequateness of using Fish Embryo Acute Toxicity (FET) Test Guidance (OECD 236) to fulfil the information requirements and addressing concerns under REACH

14.04.2016

Dr. Stefan Scholz

- https://echa.europa.eu/documents/10162/13639/fet_report_en.pdf

Generally, a lack of quality data makes it challenging to conclude on several aspects of the applicability domain of FET. However, as the OECD TG 236 was published in 2013, it could possibly lead to more data being generated in the near future, which can be used for comparative analysis. This might also give more information on a wider range of substances (multi-constituents and UVCBs) and result in more certainty for hydrophobic or volatile substances. It is recommended that whenever possible the FET studies (especially with hydrophobic or volatile substances) are accompanied by chemical analytics for the verification of exposure concentrations and the additional evidence that the substance would fall within the applicability domain of FET.

SETAC GLOBE
Environmental Quality Through Science®

11 August 2016
Volume 17 Issue 8

Is the Fish Embryo Acute Toxicity Test Under Threat? A Perspective from the SETAC Global Animal Alternatives Advisory Group

Adam Lillicrap, Norwegian Institute for Water Research (NIVA); Scott Belanger, Procter & Gamble; Natalie Burden, NC3Rs; Michelle Embry, ILSI-HESI; Lucy Lee, University of the Fraser Valley; and Marc Léonard, L'Oreal

Return to the Globe

After nearly eight years of formal development, a previous decade of investigational science and the most rigorous validation exercise for any new ecotoxicity test guideline to demonstrate reliability, robustness and repeatability, the Organization for Economic Co-operation and Development (OECD) fish embryo acute toxicity (FET) test guideline was officially adopted in 2013.[5, 4, 2] The test had already been previously adopted in Germany for assessing the acute toxicity of wastewater effluents in place of an acute fish

ECHA
EUROPEAN CHEMICALS AGENCY

Aquatic

UPDATE OF THE TEST GUIDELINES

- OECD 209: Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)
- OECD 210: Fish, Early-life Stage Toxicity Test
- OECD 211: Daphnia magna Reproduction Test

The following guidelines, directly relevant for REACH registration dossiers, have been updated improving different elements. The new updated guidelines represent a scientific enhancement and are directly relevant for addressing the REACH information requirements within the scope mentioned in the ECHA guidance documents for the previous versions:

NEWLY ADOPTED TEST GUIDELINES

OECD 236: Fish Embryo Acute Toxicity test (FET)

Title of the test guideline and the year of approval: OECD 236: Fish Embryo Acute Toxicity (FET) test, 2013

Keywords: acute fish toxicity, fish embryo toxicity, animal alternatives

Link to the OECD site: http://www.oecd-ilibrary.org/environment/test-no-236-fish-embryo-acute-toxicity-fet-test_9789264203709-en

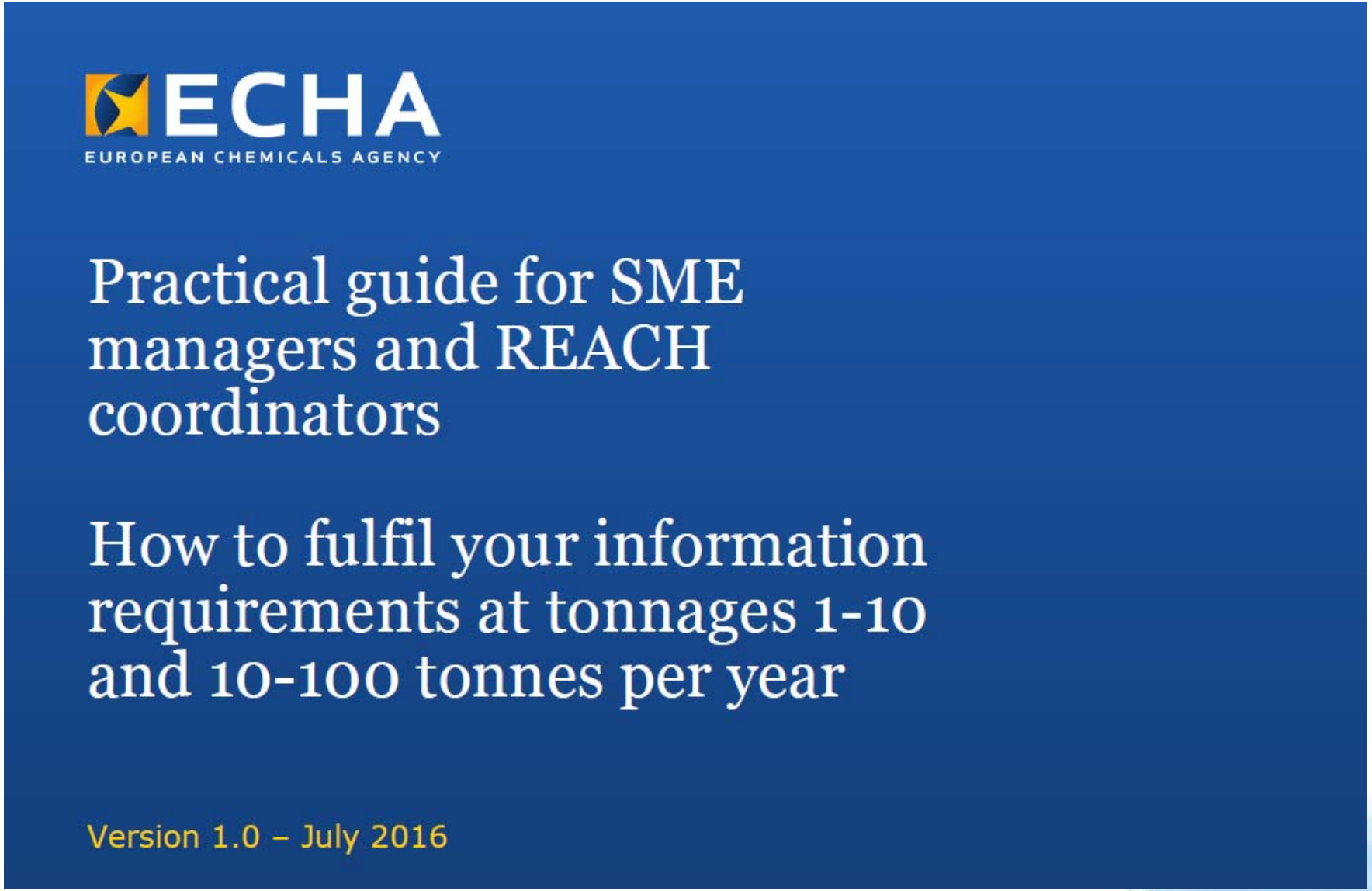
How to use this method under REACH:

The short-term toxicity test on fish is a standard information requirement under Annex VIII, 9.1.3. In ECHA's opinion, the results of the TG 236 would usually not be sufficient alone to meet the information requirement of Annex VIII, 9.1.3.

In the light of the analysis made by ECHA, there are certain limitations in the use of this test guideline and the registrant, who wants to adapt/waive the standard test needs to take these limitations into account.

Based on current knowledge, ECHA considers that OECD TG 236 might be used within a weight of evidence approach together with other independent, adequate, relevant and reliable sources of information leading to the conclusion that the substance has or does not have a particular dangerous property (for further information see Annex XI, 1.2 to the REACH Regulation and the considerations below).

https://echa.europa.eu/documents/10162/21650280/oecd_test_guidelines_aquatic_en.pdf/2548af92-ffe1-4e38-a42a-463103b1586f



- https://echa.europa.eu/documents/10162/13655/pg_sme_managers_reach_coordinators_en.pdf/1253d9f9-d1f0-4ca8-9e7a-c81e337e3a7d

Additional tips

Short-term toxicity tests with freshwater species are preferred but if a substance is released mainly directly into seawater, tests with marine species are more relevant.

Aquatic toxicity is 'unlikely to occur' when the substance is highly insoluble in water or when the substance is likely not to cross biological membranes.

Remember that to reduce the number of tests on animals, animal testing is the last option and you have to consider the possibilities to use alternative methods. The OECD TG 236 Fish Embryo Acute Toxicity (FET) Test is an alternative to the standard test and could be used within a weight-of-evidence approach together with other supporting information justifying the reliability and adequacy of the test.

OECD developed a fish testing strategy to avoid (reduce) testing (OECD Short Guidance on the Threshold Approach for Acute Fish Toxicity (No. 126, 2010) and OECD Guidance on Fish Toxicity Testing Framework (No. 171, 2012)).

Acute ecotoxicity tests

– Base Set

- Standardization is key to utilizing alternative approaches in a regulatory context
- Any method takes a significant amount of validation
- Use in a weight of evidence context is important
  - Relevance
  - Reproducibility/repeatability
  - Regulatory Acceptance
  - 6Rs

National Centre for the Replacement Refinement & Reduction of Animals in Research

Login | Register

Search this site

The 3Rs | Our science | 3Rs resources | Funding | News & blogs | Events | About us

Home > News & Blog > Using fewer animals to assess environmental safety

Using fewer animals to assess environmental safety

Friday 03 February 2017

A recent cross-sector review has highlighted several key approaches available for ecotoxicologists and risk assessors to evaluate potentially hazardous chemicals, while minimising the use of vertebrates. The Focus article has been published in *Environmental Toxicology and Chemistry* by the steering committee of the Society of Environmental Toxicology and Chemistry (SETAC) Animal Alternatives in Environmental Science Interest Group.

SETAC is the major society connecting environmental scientists, with over 6,000 members from almost 100 countries. The role of the Animal Alternatives Interest Group is to foster discussion of key technical challenges for the future of the 3Rs in environmental science. The team who put together this publication includes contributors from regulatory bodies, academia, industry and SMEs from Europe and North America. Dr Natalie Burden, one of our Programme Managers for toxicology and regulatory sciences, is a co-author of the paper.

Archive

https://www.nc3rs.org.uk/news/using-fewer-animals-assess-environmental-safety

Environmental
Toxicology and Chemistry

Explore this journal >

Focus Article

Alternative approaches to vertebrate ecotoxicity tests in the 21st century: A review of developments over the last 2 decades and current status

Adam Lillicrap ✉, Scott Belanger, Natalie Burden, David Du Pasquier, Michelle R. Embry, Marlies Halder, Mark A. Lampi, Lucy Lee, Teresa Norberg-King, Barnett A. Rattner, Kristin Schirmer, Paul Thomas

First published: 25 October 2016 Full publication history

DOI: 10.1002/etc.3603 View/save citation

Cited by (CrossRef): 1 article Check for updates | Citation tools ▾

View Issue TOC
Volume 35, Issue 11
November 2016
Pages 2637–2646

http://onlinelibrary.wiley.com/doi/10.1002/etc.3603/full

In This Issue:

ET&C FOCUS

Focus articles are part of a regular series intended to sharpen understanding of current and emerging topics of interest to the scientific community.

Alternative Approaches to Vertebrate Ecotoxicity Tests in the 21st Century: A Review of Developments Over the Last 2 Decades and Current Status

Adam Lillicrap,*† Scott Belanger,‡ Natalie Burden,§ David Du Pasquier,‖ Michelle R. Embry,# Marlies Halder,†† Mark A. Lampi,‡‡ Lucy Lee,§§ Teresa Norberg-King,‖‖ Barnett A. Rattner,## Kristin Schirmer,†††‡‡‡§§§ and Paul Thomas###

†Norwegian Institute for Water Research (NIVA), Oslo, Norway
‡Environmental Safety and Sustainability, Global Product Stewardship, Procter & Gamble, Mason, Ohio, USA
§National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), London, United Kingdom
‖WatchFrog, Evry, France
#ILSI Health and Environmental Sciences Institute, Washington, DC, USA
††European Commission Joint Research Centre, Ispra, Italy
‡‡ExxonMobil Biomedical Sciences, Annandale, New Jersey, USA
§§Faculty of Science, University of the Fraser Valley, Abbotsford, British Columbia, Canada
‖‖National Health and Environmental Effects Laboratory, Office of Research and Development, Mid-Continent Ecology Division-Duluth, US Environmental Protection Agency, Duluth, Minnesota, USA
##Patuxent Wildlife Research Center, US Geological Survey, Beltsville, Maryland, USA
†††Eawag-Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
‡‡‡Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Swiss Federal Institute of Technology, Zürich, Switzerland
§§§School of Architecture, Civil and Environmental Engineering, EPF Lausanne, Lausanne, Switzerland
###Consultancy for Environmental & Human Toxicology & Risk Assessment (Lyon Agency), L'Isle d'Abeau, France

Abstract—*The need for alternative approaches to the use of vertebrate animals for hazard assessment of chemicals and pollutants has become of increasing importance. It is now the first consideration when initiating a vertebrate ecotoxicity test, to ensure that unnecessary use of vertebrate organisms is minimized wherever possible. For some regulatory purposes, the use of vertebrate organisms for environmental risk assessments has been banned; in other situations, the number of organisms tested has been dramatically reduced or the severity of the procedure refined. However, there is still a long way to go to achieve a complete replacement of vertebrate organisms to generate environmental hazard data. The development of animal alternatives is based not just on ethical considerations but also on reducing the cost of performing vertebrate ecotoxicity tests and in some cases on providing better information aimed at improving environmental risk assessments. The present Focus article provides an overview of the considerable advances that have been made toward alternative approaches for ecotoxicity assessments over the last few decades. Environ Toxicol Chem 2016;35:2637–2646. © 2016 SETAC*

Keywords—Ecotoxicity; Vertebrate; In vitro; In silico; 3Rs

* Address correspondence to ali@niva.no

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/etc.3603

Introduction

The book on the principles of humane experimental techniques by Russell and Burch [1] is now more than half of a century old, and still it is considered the seminal writing for alternative approaches to animal testing. This is where the idea of the 3Rs (reduction, refinement, and replacement) was conceived: that any experimental technique should consider a reduction in numbers of animals used; refinement of any procedures to minimize pain, suffering, and distress; and replacement of the use of animals wherever possible. Yet the