Environmental Research 135 (2014) 156–164

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Optimizing the aquatic toxicity assessment under REACH through an integrated testing strategy (ITS)

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ARTICLE INFO

Article history:
Received 26 February 2014
Received in revised form
31 July 2014
Accepted 1 September 2014

Keywords:
ITS
REACH
Aquatic toxicity
Alternative methods
Hazard assessment

ABSTRACT

To satisfy REACH requirements a high number of data on chemical of interest should be supplied to the European Chemicals Agency. To organize the various kinds of information and help the registrants to choose the best strategy to obtain the needed information limiting at the minimum the use of animal testing, integrated testing strategies (ITSs) schemes can be used. The present work deals with regulatory data requirements for assessing the hazards of chemicals to the aquatic pelagic environment. We present an ITS scheme for organizing and using the complex existing data available for aquatic toxicity assessment. An ITS to optimize the choice of the correct prediction strategy for aquatic pelagic toxicity is described. All existing information (like physico-chemical information), and all the alternative methods (like *in silico*, *in vitro* or the acute-to-chronic ratio) are considered. Moreover the weight of evidence approach to combine the available data is included.

1. Introduction

To protect human health and environment, the European REACH (registration, evaluation, authorization and restriction of chemicals) regulation entered into force in June 2007. REACH requires that all the substances produced or imported in Europe above 1 t/y should be registered. The registrants need to supply physico-chemical, toxicological, ecotoxicological and environmental information about the substances depending on the tonnage level (REACH Annexes VII–X). Moreover, ECHA (European Chemical Agency) requests from the registrants to perform, depending on the tonnage level, a chemical safety assessment (CSA) to identify and describe the conditions under which the manufacturing and use of a substance is considered to be safe (ECHA, 2009). CSA goes through three steps: hazard assessment, exposure assessment and risk characterization. Our work is confined to the hazard assessment that requires the available information on the substance and its uses. Important aspects are (1) an evaluation of the potential of a substance to cause adverse effects to human health and the environment to derive threshold levels, for example the predicted no effect concentration (PNEC), and (2) an assessment of persistent, bioaccumulative and toxic (PBT) and very persistent, very bioaccumulative (vPvB) properties of the substances. The criteria for the PBT/vPvB assessment are reported in the new REACH Annex XIII (adopted by means of Commission Regulation (EU) No 253/2011 of 15 March 2011). In addition, the registrants should perform the classification and labeling (C&L) of the chemicals as dangerous (or not) on the basis of the criteria given in the CLP (classification, labeling and packaging) directive

Abbreviations: ACR, acute-to-chronic ratios; AF, assessment factor; BCF, bioconcentration factor; BOD5, 5-days biochemical oxygen demand; C&L, classification and labeling; CLP, classification, labeling and packaging; COD, chemical oxygen demand; CSA, chemical safety assessment; EBW, exposure-based waiving; EC10, 10% effect concentration; EC50, 50% effect concentration; ECHA, European Chemicals Agency; ErC50, EC50 in terms of reduction of growth rate; ITS, integrated testing strategy; LC50, 50% lethal concentration; LOEC, lowest observed effect concentration; NOEC, no observed effect concentration; PEC, predicted environmental concentration; PBT, persistent, bioaccumulative and toxic; PNEC, predicted no effect concentration; REACH, European regulation on registration, evaluation, authorization and restriction of chemicals; TD50, disappearance time of 50% of the initial amount of substance; vPvB, very persistent and very bioaccumulative; WoE, weight of evidence

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http://dx.doi.org/10.1016/j.envres.2014.09.002
0013-9351/

Table 1
Review of the cost and the animal needed in testing for REACH are reported. For fish also the number of estimated tests is given.

	Guideline	No. of tests (estimation)	Cost per test (€)	No. of animals per test	Source/Ref
Algae					
Short and long term	OECD 201		4510		Fleischer, (2007)
			4500		Deliverable 1 of Antares EU project, 2010 parts 4 and 5[a]
Invertebrates					
Short term	OECD 202		3742		Fleischer, (2007)
			2800		Deliverable 1 of Antares EU project, 2010 parts 4 and 5[a]
Long term	OECD 211		13,426		Fleischer, (2007)
			15,000		Deliverable 1 of Antares EU project, 2010 parts 4 and 5[a]
Fish					
Short term	OECD 203	79,707			Rovida and Hartung, (2009)
			4193	7–42 (14)[b]	van der Jagt et al., 2004
			3200	42	Deliverable 1 of Antares EU project, 2010 parts 4 and 5[a]
Long term	OECD 210	93,843			Rovida and Hartung, (2009)
			26,254	300–420 (400)[b]	van der Jagt et al., (2004)
			34,000	600	Deliverable 1 of Antares EU project, 2010 parts 4 and 5[a]
	OECD 212		10,238		Fleischer, (2007)
			7200	none	Deliverable 1 of Antares EU project, 2010 parts 4 and 5[a]
	OECD 215		16,426		Fleischer, (2007)
			15,500	120	Deliverable 1 of Antares EU project, 2010 parts 4 and 5[a]

[a] http://www.antares-life.eu/index.php?sec=home&pg=activitiesresults.
[b] min–max (normal).

(CLP, 2008). The information needed for C&L and CSA are partly overlapping, but the CSA is not required at the lower tonnage levels. The CLP directive entered into force in 2009 and intends to align the existing EU legislation with the United Nations Globally Harmonized System (GHS).

The REACH and CLP information requirements involve an intensive use of animal testing if conventional hazard testing methods are continued to be used. Authority (2010) counted that for a single substance, with no pre-existing data, and no attempt to minimize animal testing, registration could require over 5000 animals, assuming little or no avian testing. Without innovative testing strategies, REACH would cause prohibitive consumption of test animals and also excessive expenses and, furthermore, exceed the given time frame. To prevent animal testing on vertebrates as much as possible, REACH calls to use all available data and alternative methods (ECHA, 2010; REACH, 2006) also according to the Directive 2010/63/EU on protection of animals used for scientific purposes.

The ECHA guidance (ECHA, 2012a) defines aquatic pelagic toxicity as the property of a substance to be detrimental to freshwater and marine organisms living in the water column. It is assumed that the aquatic toxicity for pelagic organisms is mainly related to the exposure of a substance in water bodies. Generally, to represent the biota in this compartment, a simplified food chain including three trophic levels is employed for hazard assessment: algae or aquatic plants as primary producers, invertebrates (in particular *Daphnia* sp.) as primary consumers, and fish as secondary consumers. REACH annexes (VII–X) list the testing requirements from the three trophic levels for chemicals with different production volumes.

To satisfy the regulatory requirements, the registrants have to deal with a variety of endpoints, data and information requirements. This can be confusing and expensive. Indeed, in some cases the data necessary to satisfy the requirements of the REACH annexes are different from the data required by C&L or to perform the PBT/vPvB assessment and the CSA (*e.g.* for poorly water soluble substances REACH annexes require long term toxicity data whereas for C&L short term toxicity data are also required). Table 1 reports some estimation of costs, number of animals needed per test and also the total number of fish necessary to evaluate aquatic pelagic toxicity under the REACH registration. It becomes evident that if full testing is performed for all the compounds in the registration process, the expenses for the registrants and the number of animals used will be too high to be acceptable. The ways forward are integrated testing strategies (ITSs). A scientifically accepted definition of ITS still does not exist (Hartung et al., 2013). However, any ITS aims to optimize the testing of chemicals with regard to costs (money, time, animal welfare, *etc.*) in relation to the information gain to come to a robust conclusion about a particular hazard of a chemical. In this paper, we present an ITS for the aquatic pelagic toxicity in the form of a flow chart to combine different options of testing and non testing methods. Our ITS can guide registrants to pool information from different sources in a weight of evidence (WoE) approach to obtain an efficient hazard assessment as required by REACH. Some ITSs for aquatic toxicity already have been proposed in the ECHA guidance (ECHA, 2012a, 2012b). These schemes address the individual REACH requirements for C&L, CSA and PBT assessments separately, but they do not consider the complete spectrum of the assessments as a whole. It is our objective to build a combined ITS for the different aquatic toxicity assessment demands under REACH based on a wide range of building blocks in terms of available data and methods.

In this paper we present an ITS for aquatic pelagic toxicity, developed within the EU Integrated Project OSIRIS.[1] The ITS addresses the full range of REACH requirements (information necessary to satisfy the annexes and to perform C&L, PBT and CSA) using *in silico*, *in vitro* and *in vivo* methods. According to Annex XI of the REACH regulation (REACH, 2006), different methods to reduce animal testing are considered: the use of existing data (physico-chemical, human, ecotoxicological and environmental data), WoE evaluations, *in silico* methods (like (Q) SAR ((quantitative) structure-activity relationship) or read-across) and *in vitro* methods.

2. Material and methods

The ITS presented here is based on the ECHA Guidance for the implementation of REACH (ECHA, 2012a, 2012b), the ECHA Guidance on the Application of CLP

[1] OSIRIS. EU Project, contract no. GOCE-CT-2007-037017, 2007-2011. OSIRIS project website: http://www.osiris-reach.eu/. OSIRIS web tool website: http://osiris.simpple.com/OSIRIS-ITS/welcome.do.