Environment International 92–93 (2016) 422–441

Contents lists available at ScienceDirect

Environment International

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

Review

Interpretation of fish biomarker data for identification, classification, risk assessment and testing of endocrine disrupting chemicals

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ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form 3 April 2016
Accepted 3 April 2016
Available online xxxx

Keywords:
Vitellogenin (VTG)
Sex ratio
Secondary sex characteristics (SSC)
Endocrine disruptor
Testing strategy
Risk assessment

ABSTRACT

Chemical induced changes in fish biomarkers vitellogenin (VTG), secondary sex characteristics (SSC), and sex ratio indicate modes/mechanisms of action (MOAs) of EAS (estrogen, androgen and steroidogenesis) pathways. These biomarkers could be used for defining MOAs and the causal link between MOAs and adverse effects in fish for the identification of endocrine disrupting chemicals (EDCs). This paper compiled data sets of 150 chemicals for VTG, 57 chemicals for SSC and 38 chemicals for sex ratio in fathead minnow, medaka and zebrafish. It showed 1) changes in fish biomarkers can indicate the MOAs as anticipated; 2) in addition to EAS pathways, chemicals with non-EAS pathways induced changes in fish biomarkers; 3) responses of fish biomarkers did not always follow the anticipated patterns of EAS pathways. These responses may result from the interaction of chemical-induced multiple MOAs and confounding factors like fish diet, infection, culture conditions, general toxicity and stress response. The complex response of fish biomarkers to a chemical of interest requires EDC testing at multiple biological levels. Interpretation of fish biomarker data should be combined with relevant information at different biological levels, which is critical for defining chemical specific MOAs. The utility of fish biomarker data for identification, classification, PBT assessment, risk assessment, and testing of EDCs in the regulatory context was discussed. This paper emphasizes the importance of fish biomarker data in the regulatory context, a weight of evidence approach for the interpretation of fish biomarker data and the need for defining levels of evidence for the identification of EDCs.

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http://dx.doi.org/10.1016/j.envint.2016.04.003
0160-4120/

1. Introduction

Identification of Endocrine Disrupting Chemicals (EDCs) is considered as an important issue under several pieces of European Union (EU) legislation, including the regulation on industrial chemicals (Registration, Evaluation, Authorization and restriction of Chemicals, EC 1907/2006, REACH), the Plant Protection Products Regulation (EC 1107/2009, PPPR), and the Biocides Products Regulation (528/2012, BPR). Until now, criteria for identifying EDCs have not yet been agreed in the EU. But there is a general consensus on the WHO/IPCS definition of EDCs (IPCS, 2002). Current identification of EDCs under REACH is mainly based on this definition by considering three essential elements, *i.e.* chemical-induced adverse effects (adversity), chemical specific endocrine modes/mechanisms of action (MOAs) and the causal relationship (causality) between adverse effects and endocrine MOAs (Munn and Goumenou, 2013).

Chemical specific endocrine MOAs in fish are often showed by changes in a suite of biomarkers like vitellogenin (VTG), secondary sex characteristics (SSC), and sex ratio. VTG is normally produced by the liver of mature female fish in response to circulating endogenous estrogens. It is almost undetectable in the plasma of male and immature female fish. In the presence of estrogens including estrogenic EDCs, however, the liver in both male and female fish is stimulated to synthesize and secrete VTG. VTG in male fish is considered as a sensitive fish biomarker indicating exposure to agonists of estrogen receptors (ERs). Similarly, SSC in females of fathead minnow (*Pimephales promelas*) and medaka (*Oryzias latipes*) is considered as an important fish biomarker indicating exposure to agonists of androgen receptors (ARs). Another endpoint sex ratio is not only an important biomarker for EAS (estrogen, androgen and steroidogenesis) pathways but also a population relevant apical endpoint for adversity. Until now, fish biomarkers VTG, SSC, sex ratio have been included in different OECD test guidelines to show the effects of a chemical on EAS pathways (OECD, 2012a, 2012b).

The initial testing in fish has been focused on many chemicals known to interfere with EAS pathways. It has turned out that these chemicals of known MOAs induced responses of fish biomarkers in anticipated patterns (OECD, 2012a, 2012b). In the past two decades, more and more chemicals with both known and unknown MOAs have been tested. Some chemicals appear to influence fish biomarkers in a way that differs from the anticipated patterns of EAS pathways (Dang, 2014). This is especially true for chemicals with multiple MOAs or with unknown MOAs. For example, when zebrafish were exposed to dibutylphthalate, VTG expression was inhibited at 4 and 6 days post fertilization (dpf) but increased at 21 dpf and had no influence at 35 dpf. These changes in VTG were different from those of fish exposed to the typical ER agonist 17α-ethynylestradiol at all sampling days (Ortiz-Zarragoitia et al., 2006). Another example is microcystin-LR, which increased the whole body VTG protein levels in zebrafish females but led to decreased levels in males; whereas a reduction of mRNA expression of VTG1 in the liver of both female and male zebrafish was observed (Qiao et al., 2013). Besides, melatonin, progesterone, and dexamethasone may not directly interfere with ERs but still could induce changes in VTG in zebrafish and in fathead minnow (see the review of Dang, 2014). Clearly, chemical induced changes in fish biomarkers are rather complex and may differ from the anticipated patterns of typical chemicals with known MOAs of EAS pathways.

OECD revised the conceptual framework for testing and assessment of EDCs in which *in vitro* and *in vivo* tests are listed. OECD also developed a guidance document for data interpretation of these tests (OECD, 2012a, 2012b). This document provides a good basis for interpreting fish biomarker data too. However, it is not bound to any legal framework. It focuses only on EATS pathways and there is limited information on the effects of chemicals with different MOAs. In the EU discussions on regulating EDCs, many questions have been raised over the interpretation and the use of fish biomarker data that are related to different EU legal frameworks. For a majority of chemicals regulated by REACH, *a priori* knowledge related to possible MOAs usually would not be available. For pesticides, information on the MOA is usually available for target organisms, but does not necessarily relate to EAS pathways in fish. Responses of fish biomarkers to these chemicals may not follow the anticipated patterns of chemicals with known MOAs. Interpretation of fish biomarker data would be a great challenge in terms of the identification of EDCs in the EU legal frameworks. Many chemicals with potential to interfere with EAS pathways, with multiple MOAs or with unknown MOAs have been tested in fish in the past two decades. Extensive fish biomarker data are available in the open literature. This paper aims to compile laboratory fish biomarker data that are influenced by chemicals with the potential to interfere with both EAS and non-EAS pathways. It does not intend to go deep into the interpretation of individual results and elucidate the relationship between fish biomarker changes and MOAs. Rather, it is to show the facts of changes in fish biomarkers and the related MOAs in order to answer some questions discussed in the regulatory field on the use of fish biomarker data for the identification, classification, PBT (persistence, bioaccumulation, and toxicity) assessment, risk assessment, and testing of EDCs. Results drawn from such an analysis could form a basis for the development of guidance on how to use fish biomarker data for the identification, classification, risk assessment and testing of EDCs.

2. Fish biomarkers

This paper focuses on the most studied biomarkers: VTG, SSC and sex ratio, in three small model fish species: fathead minnow (*P. promelas*), medaka (*O. latipes*), and zebrafish (*Danio rerio*). These fish are the main species recommended in the OECD fish test guidelines for screening and testing of EDCs (OECD, 2012a, 2012b). The biomarker endpoint spiggin in fish species stickleback (*Gasterosteus aculeatus*) has also been recommended in the OECD guidance document. This endpoint is not included in our data compilation because it has been extensively reviewed during the development of the test method for the OECD (Katsiadaki and Sebire, 2011).

2.1. Vitellogenin

Vitellogenin (VTG), an egg yolk precursor protein, is encoded by a multigene family. In zebrafish, for example, VTGs are derived from seven VTG genes. VTG is normally produced by the liver of female oviparous animals in response to circulating endogenous estrogens. It is also detected in heart, spleen, kidney, skin, muscle, gill, eye and brain tissues (Zhong et al., 2014). Once released from the liver, VTG is transported through the blood to the ovary and then is taken up and modified by developing eggs to form the egg yolk. In addition to oogenesis, VTG plays an important role in embryogenesis by providing the embryo with essential nutrients including amino acids, lipids, metal ions, phosphates and carbohydrates. VTG synthesis is tissue-, stage- and sex-dependent under hormonal regulation. A low level of VTG can be detected in the plasma of male and immature fish because of low circulating estrogen stimulation. In the presence of estrogens or EDCs, however, the liver is induced to synthesize and secrete VTG (OECD, 2012a, 2012b). Early