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Review

Bioindicator species for EROD activity measurements: A review with Australian fish as a case study

M. Monique Gagnon*, Christopher A. Rawson

Department of Environment and Agriculture, Curtin University, Kent Street, Bentley, WA, 6102, Australia

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ABSTRACT

The conversion of ethoxyresorufin to the fluorescent product resorufin by the enzyme ethoxyresorufin-*O*-deethylase (EROD) enables researchers to rapidly measure the upregulation of this protein in response to exposure to a range of organic contaminants e.g. PAH, PCBs. The EROD activity assay has been widely used to examine the effects of such pollutants on fish taxa in both laboratory and field studies. This review is intended to provide fundamental information for researchers using this EROD activity as an endpoint, including methods used in the assay, the species studied to date, the background EROD levels which would be expected for these species and when available, the EROD activity induction potential for these species. While the focus in on Australian studies, many species listed in this review have a worldwide distribution and the information presented may be extend to other bioindicator fish species. Common shortfalls in the published literature leads to recommendations: it is recommended to have multiple laboratory control or field reference groups as basal EROD activity might vary with biotic and/or abiotic factors. The use of native species over introduced species offers no advantages, with EROD activity induction potential (x-fold) being similar for native or introduced fish species. Similarly, in laboratory studies, EROD activity induction potential is comparable for lab/hatchery-reared fish to the activity observed in field-caught animals. Because EROD activity is often reported to reduce at high concentrations of toxicants, laboratory studies should use a carefully considered range of contaminant concentrations rather than a single exposure concentration, as a single exposure concentration may be on the decreasing side of the response curve. The measurement of serum sorbitol dehydrogenase (SDH) activity in conjunction with EROD activity is recommended to insure liver functions are not jeopardized by high contaminant levels. In field studies, and depending on the variability observed in EROD activity within a given fish species, a number of 8–13 non-reproductively active fish, preferably of similar age and sex, per site is recommended to improve the chances of detecting a significant difference in EROD activity, if one does exist. Finally, an inter-continental comparison of EROD activity induction potential (expressed as x-fold relative to a control or reference group) suggests that highly inducible species can be found on all continents, with reported EROD activity induction as high as 135-fold in exposed groups but more commonly less than 5-fold relative to laboratory control or field reference groups.

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* Corresponding author at: Department of Environment and Agriculture, Curtin University, GPO Box U1987, Perth, WA 6845, Australia.
E-mail address: M.Gagnon@curtin.edu.au (M.M. Gagnon).

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1. Introduction

Aquatic ecosystems are a sink for many organic chemicals such as polycyclic aromatic hydrocarbons (PCBs), organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs) and polychlrorinated dibenzo-dioxin/furan (PCDDs/PCDFs) (Perelo, 2010). The impacts of such contaminants are often only identified once organism or population level effects are evident (Adams, 2001). Biochemical markers (biomarkers) representing the initial, sub-organism, biological response to contaminants have been developed to aid in the detection of early indications of environmental impact (Van der Oost et al., 2003). One of the most widely used biomarkers of exposure to organic pollution is the measurement of hepatic ethoxyresorufin-*O*-deethylase (EROD) activity in fishes (Whyte et al., 2000). Increased levels of EROD are indicative of elevated detoxification activity induced upon exposure to organic pollution (Van der Oost et al., 2003). Induction of EROD activity above basal levels is not equivalent across fishes (Whyte et al., 2000) and, the selection of species with an appropriate potential for induced EROD activity in response to contamination is crucial to the early assessment of environmental impacts (Adams, 2001).

This paper reviews the literature describing studies on EROD activities in marine, freshwater, and estuarine fish species under laboratory and field settings in an Australian context. It focuses on providing guidance for species selection in future studies through an evaluation of differences in basal EROD activity and induction potential across species from different provenances (geographic, wild vs lab/hatchery reared, native vs introduced). The review uses Australia as a case study as the fish fauna from that country could provide insights on the role that endemism and evolutionary isolation might play in explaining EROD induction potentials. The information provided may, however, be extended to other bioindicator fish species occurring worldwide. Whyte et al. (2000) extensively reviewed literature on EROD induction and described many of the factors that influence the induction, conduct of the assay, and interpretation of results. Here we build on that review using recent research and describe a different set of species and population characteristics to specifically answer the following questions:

(1) In laboratory studies, is there a difference in the EROD activity induction potential between lab/hatchery-reared and wild-caught fish?
(2) In field studies, is there a difference in the EROD activity induction potential between Australian native and introduced species?
(3) Is there a geographic difference in EROD activity induction potential at the continental scale?

1.1. Mechanism of CYP1A induction

The bioactivity of many xenobiotics and endogenous ligands is mediated via high affinity binding to the aryl hydrocarbon receptor (AhR). While the AhR promiscuously binds many xenobiotic compounds (Denison and Heath-Pagliuso, 1998), high affinity ligands are generally planar, aromatic and hydrophobic (Denison, 1991) and include amongst others tetrachlorinated dibenzo-*p*-dioxins (TCDDs), some polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and some organochlorine pesticides (OCPs) (Fent, 2001; Oropeza-Hernandez et al., 2003; Sierra-Santoyo et al., 2000). Limited numbers of endogenous AhR ligands have been identified (Andreola et al., 1997; Rannug et al., 1987). The AhR/ARNT (aryl hydrocarbon nuclear translocator) dimer activates members of the Ah-gene battery, resulting in a pleiotropic response including the production of cytochrome P450-1A (CYP1A), immuno-toxicity, hepatic damage, carcinogenesis, reproductive toxicity, and neurotoxicity (see Mandal, 2005 for review).

The adverse effects generated by AhR ligands can occur via Phase I metabolism of the ligand to toxic intermediates (Huff et al., 1994; Machala et al., 2001). The best-studied of these is the CYP1A and epoxide hydrolase catalysed metabolism of benzo(a)pyrene to DNA adduct forming BaP-7,8-dihydrodiol-9,10-epoxide (BPDE) (Hecht, 2003; Varanasi et al., 1986). AhR binding by other ligands, may result amongst other effects in the activation of genes associated with the formation of tumours (oncogenes, Puga et al., 2000) or suppression of immune responses (Camacho et al., 2001).

1.2. CYP1A induction as a biomarker of exposure

The environmental concentration of some contaminants can correlate with measured body burdens in animals in intimate contact with the environmental matrix but, in cases where animals rapidly metabolise rather than accumulate certain contaminants, the measurement of body burden concentrations becomes irrelevant. Further, the mere presence of a contaminant in the body of an organism does not necessarily translate to toxicity (Van der Oost et al., 2003) and the concentration at which this occurs is dependent on a range of variables. Hence simply measuring environmental concentrations and/or body burdens is insufficient to infer toxicity (Van der Oost et al., 2003). Inherent spatial and temporal variability in contaminant concentrations merely suggests that toxicity might be under- or over-estimated.

In ecotoxicological studies, such chemical analyses are used as supporting tools to the measurements of biomarkers of *exposure* and *effect*. The induction of CYP1A is a biomarker of *exposure* of (primarily) vertebrates to organic contaminants, allowing an assessment of the contaminant-driven activation of the AhR gene battery (Whyte et al., 2000). Joined with contaminant burden in