# 4 Background document: cytochrome P450 1A activity (EROD)

**Ketil Hylland, Thomas Maes, Concepción Martínez-Gómez, Ulrike Kamman, Matt Gubbins, and Ian M. Davies**

## 4.1 Introduction

The cytochrome P450 1A family of enzymes is responsible for the primary metabolism of planar polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) and the activation of several procarcinogens, such as benzo[*a*]pyrene. 7-Ethoxyresorufin is a convenient artificial substrate which was developed as a safe, sensitive assay by Burke and Mayer (1974). Thus, the term "EROD" has been adopted as a measure of CYP1A activity in aquatic organisms (Stagg and McIntosh, 1998).

In addition to being substrates for biotransformation, planar compounds such as PAHs, PCBs, and dioxins also induce synthesis of cytochrome P450 1A by binding to the cytosolic aryl hydrocarbon (Ah) receptor/ARNT complex. Measurement of EROD activity is the tool used currently to quantify this induction. The induction of cytochrome P450 enzymes in fish liver was first suggested as an indicator of environmental contamination in the 1970s by Payne (1976), and has now gained widespread use (see, e.g., Förlin and Haux, 1990; Goksøyr *et al.*, 1991a; George *et al.*, 1995a; Whyte *et al.*, 2000) and been standardized by ring-testing (BEQUALM, 2000).

## 4.2 Dose–response

In a review, Whyte *et al.* (2000) rank chemicals according to the level of EROD activity they induce in treated or exposed fish when compared with untreated or control fish. Contaminants that induce EROD less than tenfold above control levels are considered "weak" inducers, 10- to 100-fold are "moderate" inducers, and chemicals that elicit >100-fold induction are considered "strong" inducers. Dioxins, planar PCBs, and PAHs (benzo[*a*]pyrene) are categorized as "strong" inducers. Over 25 studies have observed induction of hepatic EROD by benzo[*a*]pyrene in 15 species of fish (Whyte *et al.*, 2000).

## 4.3 Relevance of other factors

Several endogenous and exogenous factors have been shown to affect hepatic EROD. The most important endogenous factors for most fish species are gender, reproductive status, and season, all of which can be controlled through sampling design. In addition, environmental temperature has been shown to affect EROD (Sleiderink *et al.*, 1995a; Lange *et al.*, 1999). Seasonal cycles in EROD induction have been observed for rainbow trout (*Oncorhynchus mykiss*; Förlin and Haux, 1990), flounder (*Platichthys flesus*; von Westernhagen *et al.*, 1981; Hylland *et al.*, 1996), plaice (*Pleuronectes platessa*; George and Young, 1986) and salmon (*Salmo salar;* Larsen *et al.*, 1992), most likely owing to changes in both water temperature and reproductive cycles (which it is not really possible to separate in the field). The main age-related factors are time of exposure/accumulation, food selection, and reproductive stage.

Several species have baseline EROD activities within the same order of magnitude among different studies/measurements and also show greater than tenfold EROD

induction after contaminant exposure (Whyte *et al.*, 2000). These are, however, mostly freshwater species.

CYP1A expression is suppressed in spawning females because of interference of oestrogens (e.g. 17β-oestradiol, E2, or xenoestrogen) with transcription of the gene. This may also lead to an underestimation of a PAH-type response of EROD activity; however, this hormone also controls the induction of vitellogenin (Vtg; egg-yolk protein), which is produced by the liver during gonadal recrudescence. Therefore, interference of CYP1A induction by environmental oestrogens can be assessed.

Dietary factors may be potentially important for the induction of CYP1A. First, aryl hydrocarbon receptor (AhR) ligands may be ingested by the organism in the food. Second, proper nutrition is a prerequisite for enzyme systems to function properly. Hylland *et al.* (1996) reported an elimination of the EROD response (i.e. to control levels) in benzo[*a*]pyrene-treated flounder deprived of food for one month.

## 4.4 Background responses

Baseline levels of EROD in seven marine species have been estimated from results derived from the joint ICES/OSPAR WKIMON III meeting (ICES, 2007a) and recent data submitted to the ICES database (Table 4.1). The fish were from sites that the contracting parties consider to be reference stations (i.e. no known local sources of contamination) or those areas not considered unequivocally as reference sites but considered to be less affected by human and industrial activity. The datasets from which these values have been derived are described in Table 4.2. Further information on the baseline levels and dose–response of EROD activity in experimental systems and field studies is given in Tables 4.3 and 4.4.

## 4.5 Assessment criteria

Background response ranges have been developed as described above, and 90th percentiles of values from reference sites can be used to distinguish between "background" and "elevated" responses. Because many factors are known to influence EROD activity (see above), and because it is difficult to correct for all in the assessment of data, it is advisable to include an appropriate reference group in studies that include EROD as an endpoint. The information provided in Table 4.2 will also allow data to be assessed against the appropriate assessment criteria for fish species, gender, size, sampling season, and bottom-water temperature.

## 4.6 Quality assurance

Cytochrome P450 1A is possibly the most widely used biomarker. There have been three international intercalibrations for the method, all within BEQUALM. The intercalibrations have pinpointed variability relating to most steps in the analytical process, except possibly the enzyme kinetic analysis itself. It is imperative that laboratories have internal quality assurance procedures (e.g. use internal references samples with all batches of analyses).

## 4.7 Acknowledgement

The current review has been derived from an overview prepared for the Norwegian offshore companies through OLF (Hylland *et al.*, 2006a), the joint workshop ICES/OSPAR WKIMON III (ICES, 2007a), and the workshop SGIMC (ICES, 2009a).

Table 4.1. EROD assessment criteria in fish target species used in biomonitoring programmes around European waters. EROD background responses established are restricted to the sampling conditions and the length of the specimens used. The values of the assessment criteria must be considered as provisional and should be updated and revised when more data become available

| EROD assessment criteria S9 fraction | Sampling season | Bottom-water temperature range (°C) | Length (cm) | Sex | Background response range EROD activity (pmol min$^{-1}$ mg$^{-1}$ protein) 90P | Elevated response range EROD activity (pmol min$^{-1}$ mg$^{-1}$ protein) 90P | *N* |
|---|---|---|---|---|---|---|---|
| Dab (*Limanda limanda*) | August–November | 10–18 | 12–25 | Females | ≤178 | >178 | 556 |
| | | | | Males | ≤147 | >147 | 571 |
| European flounder (*Platichthys flesus*) | August–November | 10–18 | 20–25 | Females and/or males | ≤24 | >24 | 65 |
| Plaice (*Pleuronectes piatessa*) | January | 5–10 | 18.5–22.5 | Males | ≤10 | >10 | 116 |
| **EROD assessment criteria microsomal fraction** | | | | | | **Elevated Response Range EROD activity (pmol min$^{-1}$ mg$^{-1}$ prot) 90P** | ***n*** |
| Dab (*Limanda limanda*) | August–November | 10–18 | 20–30 | Females and/or males | ≤780 | >780 | 53 |
| Cod (*Gadus morhua*) | August–November | 10–18 | 30–45 | Females and/or males | ≤145 | >145 | 198 |
| Plaice (*Pleuronectes platessa*) | September | 7–10 | 40–60 | Females and/or males | ≤255 | >255 | 64 |
| Four spotted megrim (*Lepidorhombus boscii*) | September–October | 11.7–12.7 | 18–22 | Females and/or males | ≤13 | >13 | 317 |
| Dragonet (*Callionymus lyra*) | September–October | 12.0–12.8 | 15–22 | Females and/or males | ≤202 | >202 | 159 |
| Red mullet (*Mullus barbatus*) | April | 13.3–15.3 | 12–18 | Males | ≤208 | >208 | 40 |

Table 4.2. Description of data used in setting background and elevated response ranges

| EROD assessment criteria | Sampling season | Bottom-water temperature range (°C) | Length (cm) | Sex | EROD background response activity median (pmol min$^{-1}$ mg$^{-1}$ protein) | Upper limit of EROD background response activity P90 (pmol min$^{-1}$ mg$^{-1}$ protein) | *N* |
|---|---|---|---|---|---|---|---|
| Dab (*Limanda limanda*) | August–November | 10–18 | 12–25 | Females and/or males | <30 † | <152 † | 1 034 |
| European flounder (*Platichthys flesus*) | August–November | 10–18 | 20–25 | Females and/or males | <14 † | <24 † | 30 |
| Cod (*Gadus morhua*) | August–November | 10–18 | 30–45 | Females and/or males | <78 * | <151 * | 74 |
| Four spotted megrim (*Lepidorhombus boscii*) | September–October | 11.7–12.7 | 18–22 | Females and/or males | <12 * | <13 * | 317 |
| Dragonet (*Callionymus iyra*) | September–October | 12.0–12.8 | 15–22 | Females and/or males | <144 * | <202 * | 159 |
| Red mullet (*Mullus barbatus*) | April | 13.3–15.3 | 12–18 | Males | <85 * | <208 * | 40 |
| Plaice (*Pleuronectes piatessa*) | January | 18.5–22.5 | 7–10 | Males | <71 † | <9.49 † | 116 |
| Haddock (*Melanogrammus aeglefinus*) | August | 5–10 | 33–55 | Females and/or males | <72 †/<215 * | <162 †/<421 * | 20/23 |
| Saithe (*Pollachius virens*) | September | 5–10 | 40–100 | Females and/or males | <57 † | <142 † | 21 |
| Herring (*Clupea harengus*) | November | 5–10 | 22–33 | Females and/or males | <10 † | <23 † | 24 |

Table 4.3. Dose–response, background response, and sensitivity in experimental studies with gadoid fish

| Species | Substance(s) | Lowest–highest concentrations | Exposure time | Baseline/control (level/activity) | Induction (fold) | Reference |
|---|---|---|---|---|---|---|
| Polar cod (*Boreogadus saida*) juvenile | Crude oil (Oseberg C) | ~200 mg $kg^{-1}$ (i.p. inj.) | 10 and 21 d post inj. | ~30 pmol $min^{-1}$ $mg^{-1}$ | ~8 and ~2.5 (245 and 80 pmol $min^{-1}$ $mg^{-1}$) | George *et al.* (1995a) |
| Polar cod (*Boreogadus saida*) male | Crude oil (Oseberg C) | ~200 mg $kg^{-1}$ (oral) | 21 d post exposure | 28 pmol $min^{-1}$ $mg^{-1}$ ±6 ($n$=12) | ~5 (132 ± 14 pmol $min^{-1}$ $mg^{-1}$) | George *et al.* (1995a) |
| Polar cod (*Boreogadus saida*) female | Crude oil (Oseberg C) | ~200 mg $kg^{-1}$ (oral) | 21 d post exposure | 8 pmol $min^{-1}$ $mg^{-1}$ ±2 ($n$=14) | ~5 (42 ± 6 pmol $min^{-1}$ $mg^{-1}$) | George *et al.* (1995a) |
| Polar cod (*Boreogadus saida*) juvenile | β-Naphthoflavone | 50 mg $kg^{-1}$ (i.p. inj.) | 21 d post inj. | ~30 pmol $min^{-1}$ $mg^{-1}$ | ~12.5 (380 pmol $min^{-1}$ $mg^{-1}$) | George *et al.* (1995a) |
| Cod (*Gadus morhua*) juvenile | 2,3,7,8-TCDD | 0.008 mg $kg^{-1}$ oral dose twice, d 0 and d 4 | 9 and 17 d post exposure | 55.4 (d 9) and 91.4 (d 17) pmol $min^{-1}$ $mg^{-1}$ | ~4 and ~3 (230 and 277 pmol $min^{-1}$ $mg^{-1}$) | Hektoen *et al.* (1994) |
| Cod (*Gadus morhua*) juvenile | PCB105 | 10 mg $kg^{-1}$ oral dose twice, d 0 and d 4 | measure at d 9 and d 17 | 55.4 (d 9) and 91.4 (d 17) pmol $min^{-1}$ $mg^{-1}$ | 1.5 and 1.2 pmol $min^{-1}$ $mg^{-1}$ | Bernhoft *et al.* (1994) |
| Cod (*Gadus morhua*) juvenile | β-Naphthoflavone | 100 mg $kg^{-1}$ (i.p. inj. at d 0 and d 4) | measure at d 7 | 84 pmol $min^{-1}$ $mg^{-1}$ ±8 ($n$=5) | ~13 (1 074 ± 340 pmol $min^{-1}$ $mg^{-1}$) | Goksøyr *et al.* (1987) |
| Cod (*Gadus morhua*) | β-Naphthoflavone | 100 mg $kg^{-1}$ (2 i.p. inj.) | measure 3–4 d after last injection | 40 pmol $min^{-1}$ $mg^{-1}$ | ~72 (2 870 pmol $min^{-1}$ $mg^{-1}$) | Goksøyr *et al.* (1991b) |
| Cod (*Gadus morhua*) juvenile | Crude oil (North Sea) | 0.06–1 ppm | 30 d | ~2 pmol $min^{-1}$ $mg^{-1}$ | ~2–5.5 (~ 4–11 pmol $min^{-1}$ $mg^{-1}$) | Aas *et al.* (2000a) |

†Subfraction S9. *Microsomes subfraction.

Table 4.4. Dose–response, background response, and sensitivity in field studies with gadoid fish

| Species | Substance(s) | Lowest–highest concentrations | Exposure time | Baseline/control (level/activity) | Induction (fold) | Reference |
|---|---|---|---|---|---|---|
| Rockling (*Ciliata mustella*) | Crude oil (Gullfaks; MV "Braer" spill, Shetland) | 85 000 tons spill<br>129 ± 38 ng $g^{-1}$ dry wt. of PAHs (selected 2- and 3-ring) detected in muscle | 3 months after spill | ~160 ± 50 pmol $min^{-1}$ $mg^{-1}$ | ~9 (1 480 pmol $min^{-1}$ $mg^{-1}$) | George *et al.* (1995b) |
| Roundnose grenadier (*Coryphaenoides rupestris*) | i.a. PAHs and PCBs | | | 260 ± 20 (male) and ~170 (female) pmol $min^{-1}$ $mg^{-1}$ | ~2 (530 ± 70 (male) and ~350 (female) pmol $min^{-1}$ $mg^{-1}$) | Lindesjoo *et al.* (1996) |
| Hake (*Urophycis* spp.) | Pollution (PAH) from oil platforms (Gulf of Mexico)<br><100 m from platforms | | | 10.9 ± 6.4 and 11.7 ± 10.5 pmol $min^{-1}$ $mg^{-1}$ (>3 000 m from platforms) | <1 (10.6 ± 3.8 and 10.5 ± 7.1 pmol $min^{-1}$ $mg^{-1}$) | McDonald *et al.* (1996) |