



Short communication

# Use of fish in vitro hepatocyte assays to detect multi-endpoint toxicity in Slovenian river sediments

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## Abstract

There is an increasing demand for rapid, sensitive and robust methods for toxicity testing of single chemicals, complex mixtures and environmental samples. The objective of this work was to validate and use a primary culture of rainbow trout (*Oncorhynchus mykiss*) hepatocytes as a multi-endpoint in vitro bioassay for toxicity characterisation of river sediments from four areas of the Sava and Krupa Rivers (Slovenia). The endpoints were chosen to encompass acute toxicity (cytotoxicity) as well as sub-lethal biomarker and effect endpoints such as metabolic inhibition, DNA damage (Fast Micromethod), endocrine disruption (estrogenicity), and 7-ethoxyresorufin *O*-deethylase (EROD) activity. Results from these studies show that the primary hepatocyte culture was able to successfully detect effects of single model chemicals in all endpoints analysed. Furthermore, the bioassays were also able to discriminate between contaminated and less contaminated sediments for a number of endpoints such as cytotoxicity, metabolic inhibition and induction of EROD activity, although no increase in DNA damage and estrogenicity was observed above background at any site. The present study shows that primary fish hepatocytes may be used to determine multiple mechanisms of toxic action and that a holistic assessment of effects may improve our understanding of cellular toxicity of complex mixtures such as sediments.

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## 1. Introduction

Sediments are considered to act both as a sink and a source for organic chemicals in the aquatic environment. Some of these compounds may adversely affect aquatic organisms, either through direct contact with sediments or remobilisation into pore water or the water column by natural or anthropogenic processes. It is well documented that contaminated sediments contain chemicals that may cause acute toxicity as well as sub-lethal effects such as mutagenicity, teratogenicity, embryotoxicity, carcinogenesis and estrogenicity (Nebert et al., 1993; Thomas et al., 2001; Klamer et al., 2005), but it is still a challenge to assess the toxicity of complex environmental samples such as water samples and sediments. Several in vitro bioassays from various species including fish are currently used to characterize the toxicological properties of chemicals and environmental samples (see Castaño et al., 2003, for details).

The objective of this work was to validate and use a primary culture of rainbow trout (*Oncorhynchus mykiss*) hepatocytes as a multi-endpoint in vitro bioassay for toxicity characterization of sediment extracts from four areas of the Sava and Krupa Rivers (Slovenia). The bioassay endpoints were chosen to include acute toxicity (cytotoxicity) as well as sub-lethal biomarker and effect endpoints such as metabolic inhibition, DNA damage, endocrine disruption (estrogenicity) and cytochrome P450 1A-catalysed 7-ethoxyresorufin *O*-deethylase (EROD) activity.

## 2. Materials and methods

Sediments were sampled by a piston corer at two locations of the Sava (Moste and Mojstrana) and two locations of the Krupa River (Mlin and Krupa River source) in Slovenia, stored cool and dark, and subjected to Accelerated Solvent Extraction (ASE) within 72 h of sampling, essentially as described by Giergielewicz-Mozajska et al. (2001). Resulting organic extracts and the model compounds 17 $\beta$ -estradiol (E2), 2,3,7,8-tetrachlorodibenzodioxin (TCDD), 4-*n*-nonylphenol (NP), and 4-nitroquinoline 1-oxide (4-NQO) were all dissolved in DMSO and subjected to bioassay testing in a primary culture of male rainbow trout (*Oncorhynchus mykiss*) hepatocytes (Tollefsen et al., 2003). Cell media was assayed for the estrogenic biomarker vitellogenin (Tollefsen et al., 2003; Tollefsen et al., 2006), whereas the cells were analyzed for EROD activity, DNA damage by the Fast Micromethod, metabolic inhibition and cytotoxicity (membrane integrity) as described elsewhere (Sanchez-Fortun et al., 2005; Tollefsen et al., 2006).

## 3. Results and discussion

The present work demonstrates that the primary culture of fish hepatocytes may be used as a multi-endpoint screening assay for CYP1A inducing chemicals (Fig. 1A), estrogenic chemicals (Fig. 1B), DNA damaging compounds (Fig. 1C), cytotoxic and metabolic inhibiting chemicals (Fig. 1D) when exposed to various model chemicals. A significant and reproducible response was obtained for all chemicals, although individual variation in responsiveness was observed for some endpoints such as DNA damage (results not shown). Nevertheless, the dose–response relationships obtained were comparable to that obtained for similar compounds elsewhere (Tollefsen et al., 2003; Sanchez-Fortun et al., 2005; Tollefsen et al., 2006).

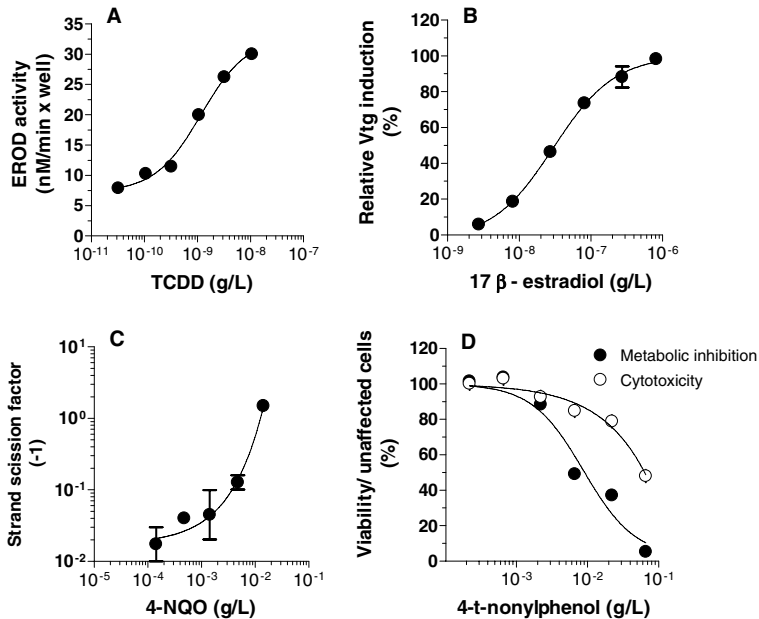


Fig. 1. Assessment of various biological responses in a primary culture of rainbow trout (*Oncorhynchus mykiss*) hepatocytes: (A) EROD activity after exposure to 2,3,7,8-tetrachlorodibenzodioxin (TCDD) for 48 h, (B) Vitellogenin (Vtg) induction after exposure to 17 $\beta$ -estradiol (E2) for 96 h, (C) DNA-damage (strand scission factor) after exposure to 4-nitroquinoline 1-oxide (4-NQO) for 2 h, and (D) metabolic inhibition and cytotoxicity (membrane integrity) after exposure to 4-*n*-nonylphenol (NP) for 96 h. The results (Mean  $\pm$  SD) depict the data from 3 individual determinations.

Toxicity characterisation of organic extracts of sediments of the Sava and Krupa Rivers showed that the bioassays were able to discriminate between contaminated and less contaminated sediments for a number of endpoints such as CYP1A induction (EROD activity), metabolic inhibition and cytotoxicity, although no increase in DNA damage and estrogenicity was observed at any site compared to solvent controls (Table 1). The total

Table 1

Assessment of CYP1A inducing activity (TCDD equivalents, TCDD-EQ), estrogenicity (E2 equivalents, E2-EQ), DNA damage (4-NQO equivalents, NQO-EQ), metabolic inhibition (EC<sub>50</sub>) and cytotoxicity (EC<sub>50</sub>) after exposure to organic extracts of sediments from the Sava and Krupa Rivers

Endpoint	Location			
	Sava polluted (Moste)	Sava reference (Mojsstrana)	Krupa polluted (Mlin)	Krupa reference (River source)
EROD activity (ng TCDD-EQ/g dry sediment)	9.3 $\pm$ 4.9	0.86 $\pm$ 0.83	15.7 $\pm$ 6.0	6.4 $\pm$ 5.0
Estrogenicity (ng E2-EQ/g dry sediment)	n.d.	n.d.	n.d.	n.d.
DNA damage ( $\mu$ g NQO-EQ/g dry sediment)	n.d.	n.d.	n.d.	n.d.
Metabolic inhibition (EC <sub>50</sub> , g dry sediment/L)	31.9 $\pm$ 4.8	279 $\pm$ 307.7	17.9 $\pm$ 5.9	21.7 $\pm$ 18.7
Cytotoxicity (EC <sub>50</sub> , g dry sediment/L)	64.7 $\pm$ 32.9	n.d.	49.8 $\pm$ 23.0	52.0 $\pm$ 18.4

The results (Mean  $\pm$  SD) depict data from three individual determinations.

n.d. not detected.

toxic potential varied between the selected sites, however. Organic extracts of sediments from the PCB polluted site at Mlin, Krupa River (Polic et al., 2000) contained the highest levels of CYP1A inducers, metabolic inhibitors and compounds causing cytotoxicity. Interestingly, exposure to extracts from the assumed reference site located 2 km up-stream (Krupa River source) led to comparable cytotoxicity and metabolic inhibition, but with a 2-fold lower induction of EROD activity. Sediments from the metal polluted hydroelectric reservoir at Moste, Sava River (Scancar et al., 1999) also contained high levels of CYP1A inducers, although the response to cytotoxic chemicals and metabolic inhibitors were lower than that observed for the Krupa sites and considerably higher than that obtained for the assumed reference site at Mojstrana located 12 km up-stream. In general, the toxic responses of sediment extracts in the multi-endpoint bioassay from the two areas differed thus suggesting that the composition of pollutants in sediments from the two rivers were quite different.

In summary, the multi-endpoint bioassays based on primary hepatocytes from rainbow trout were capable of detecting multiple toxic endpoints after exposure to single model chemicals as well as complex environmental samples. The present results suggest that determination of multiple mechanisms of toxic action may improve our understanding of cellular toxicity of complex mixtures and lead to a more holistic approach to environmental monitoring of contaminated sediments.

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