

HEPATIC BIOMARKER RESPONSES TO ORGANIC CONTAMINANTS IN FERAL CHUB (*LEUCISCUS CEPHALUS*)—LABORATORY CHARACTERIZATION AND FIELD STUDY IN THE SAVA RIVER, CROATIA

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Abstract—As a widely spread cyprinid fish species, the European chub (*Leuciscus cephalus*) has been used extensively in bio-monitoring programs. However, no laboratory dose-response and/or time course studies related to applied biomarkers have been reported on chub yet. In order to address this issue, specimens of juvenile chub caught in September 2005 in the Sava River, Croatia, were laboratory exposed to various (0.25–50 mg/kg) doses of either model polycyclic aromatic hydrocarbons (PAH) premutagen benzo[*a*]pyrene (BaP), or β -naphthoflavone (β -NF), a well-known model cytochrome 1A (CYP1A) inducer, for 3 (BaP) or 5 d (β -NF). The responses of several hepatic biomarkers were determined in the exposed fish: The hepatic 7-ethoxyresorufin-*O*-deethylase activity, CYP1A content, glutathione *S*-transferase (GST) activity, liver bioactivation potential, and the amount of hydroxylated polycyclic aromatic hydrocarbon bile metabolites determined by the fixed wavelength fluorescence and the high-performance liquid chromatography technique. The relevance of determined biomarker responses has been analyzed further and crosscorrelated with the same set of biomarkers, as well as with tissue concentrations of polycyclic aromatic hydrocarbons and polychlorinated biphenyls, determined in chub specimens collected in September 2005 at five different polluted locations along the Sava River. The species-specific upper and lower limits in responses of studied biomarkers were determined and the obtained ranges successfully evaluated in real field situation. With the exception of the GST activity, all other biomarkers determined in chub proved to be valuable indicators of environmental pollution. Finally, the results of the present study demonstrated that the same strategy of laboratory characterization in combination with field evaluation should be used regularly in the selection of optimal biomarkers and indicator species.

Keywords—European chub Laboratory exposure Hepatic biomarkers Field evaluation

INTRODUCTION

The determinations of specific hepatic biomarker responses in feral or, more recently, caged fish, largely have been introduced into biomonitoring and management of the freshwater ecosystems to complement traditional chemical analyses of contaminants tissue levels [1]. However, despite significant improvements in methodology and promising field results obtained during the last 20 years, in numerous cases, biomarkers failed to offer reliable information critical for their affirmation as predictors of ecologically relevant effects [2,3]. In most cases the primary obstacle was the fact that many factors could complicate the interpretation of field evaluations: Species-specific differences, complexity of contaminants mixtures in water, seasonality, migration, habitat changes, and more. Accordingly, the selection of suitable bioindicator fish species and characterization of its species-specific pollutant responses is certainly one of the critical prerequisites for scientifically reliable and meaningful interpretation of field data.

European chub (*Leuciscus cephalus*) is a common cyprinid fish in European freshwaters. Due to its availability and reported sensitivity in response of several hepatic biomarkers, it has been proven as a suitable feral species for biomonitoring purposes [4–12]. Following that positive experience, chub was chosen as indicator species within the framework of the European Union (EU) FP6 SARIB project (Sava River Basin:

Sustainable Use, Management and Protection of Resources) dedicated to the development of integrated tools for the assessment of ecological quality of the Sava River basin in Croatia (<http://www.sarib.net/index.php?id=info>). The laboratory studies on responses of fish hepatic phase I and II enzymes to the presence of organic pollutants, were performed on several cyprinid fish species, such as carp, barbell, crucian carp, and roach [13–16]. However, there are no similar literature data available for chub, making the interpretation of field results difficult, especially in determining whether a particular biomarker response is an indicator of pollution-caused impairment or a part of homeostatic response indicating that an organism is successfully dealing with the exposure stress falling within its physiological range of cellular defense [17].

The main objective of the present study was to determine species-specific upper and lower physiological limits of response of a suite of hepatic biomarkers in chub. These were the hepatic 7-ethoxyresorufin-*O*-deethylase (EROD) activity, cytochrome 1A (CYP1A) content, glutathione-*S*-transferase (GST) activity, liver bioactivation potential, and the amount of hydroxylated-polycyclic aromatic hydrocarbon (OH-PAH) bile metabolites. Among biochemical biomarkers the induction of the fish EROD activity, a CYP1A monooxygenase has become a widespread tool for biomonitoring of marine and continental waters since the 1980s and is one of the best documented and widely used responses to dioxins, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) [18]. Because an increasing hepatic GST activity has

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