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Evaluation of pollutant exposure by chemical and biological markers in a Mediterranean French urban stream: A step for *in situ* calibration of multixenobiotic resistance transporter expression as biomarker in Chironomidae larvae

G. Saez[a], L. De Jong[a,*], X. Moreau[a], L. Sarrazin[b], E. Wafo[b], T. Schembri[b], V. Lagadec[b], C. Diana[b], J.-L. Monod[b], A. Thiéry[a]

[a]*UMR-CNRS 6116 IMEP, Équipe "Biomarqueurs & Bioindicateurs Environnementaux", Université de Provence, Case 17, 3 Place Victor Hugo, 13331 Marseille cedex 03, France*

[b]*Faculté de Pharmacie, Laboratoire "d'Hydrologie et Molysmologie Aquatique", Université de la Méditerranée, Marseille, France*

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Abstract

This study was aimed at semi-quantifying the membrane density of multixenobiotic resistance (MXR) transporters in Chironomidae Orthocladiinae larvae from an urban stream by ELISA assay. The relationships between the MXR transporter membrane density and limnological parameters and pollutant concentrations, 16 priority polycyclic aromatic hydrocarbons (PAHs), as per the US Environmental Protection Agency (EPA) and seven polychlorobiphenyl congeners (PCBs), were assessed. Midge larvae were collected, and limnological parameters and pollutant concentrations were measured in three sites of a French Mediterranean urban stream, two located after sewage treatment plants, and one closed to the river mouth, and in two additional sites, one on the stream tributary, and one in a non-urbanized stream located in the same region. Results show that the PAH and PCB contamination levels are different between sites and that some congener concentrations are above their threshold toxic effect level (TEL). The MXR transporter membrane density was significantly higher in larvae from the tributary, the most polluted site, as compared with larvae from the non-urbanized stream. The MXR transporter density was positively correlated with 10 of the 16 US-EPA PAH concentrations and the increase in the MXR transporter density seems to be due to the US-EPA PAH concentrations that were above their TEL. No relations with PCB concentrations or limnological parameters were found. The results suggest that the MXR transporter membrane density in Chironomidae larvae could be an interesting biological marker of PAH exposure in freshwater ecosystems.

Keywords: Freshwater; Midge larvae; Multixenobiotic resistance (MXR) transporter; Polychlorobiphenyls; Polycyclic aromatic hydrocarbons

1. Introduction

Small rivers are ecologically important systems that provide diverse habitats for aquatic biota and are particularly susceptible to human pressure and activities, especially in Mediterranean areas because of the lack of natural flow due to climatic constraints (Fernandes et al., 2002). The present study focused on such overexploited Mediterranean small urban stream, the Cadière, that receives extensive urban and industrial wastewaters. To detect the impact of anthropogenic contaminants in aquatic systems, the integrated use of both chemical and biological markers is actually recommended (Walker and Livingstone, 1992; Porte et al., 2001; Fernandes et al., 2002; Bodin et al., 2004). In this context, limnological and chemical analyses and biological investigations (biomarkers) were conducted along a longitudinal transect in the

*Corresponding author.
E-mail address: laetitia.moreau@univ-provence.fr (L. De Jong).

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Cadière stream and in its tributary, the Raumartin. Same investigations were also conducted in another southern France Mediterranean stream, the Bayeux, remote from any industrial or urban location. As it is well known that the concentration of contaminants in the sediments may be hundreds of times greater than those in the water (Simkiss et al., 2001), chemical analyses of organic pollutants, i.e. polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyl congeners (PCBs) were measured in this compartment. In order to understand the impact of these pollutants on the aquatic fauna, macroinvertebrates living in the sediment have been retained. Among them, Chironomidae larvae were chosen according to their abundant distribution in freshwater ecosystems (Pinder, 1986). The multixenobiotic resistance (MXR) transporters semi-quantification has been selected as a biomarker response (Moreau et al., 2008). The MXR transporter is one of the cellular defense mechanisms that may influence the intracellular concentration and consequently the toxicity of xenobiotics. MXR transporter acts as an energy-dependent pump to translocate a wide variety of structurally and functionally diverse substrates, thus preventing their intracellular accumulation (Bard, 2000). The use of MXR transporter as a tool to biomonitor the aquatic environment, especially marine ones, has been previously documented (Minier et al., 1999; Bard, 2000). However, only few studies have investigated the expression of this MXR phenotype in larvae of the freshwater aquatic non-biting midges (Chironomidae, Diptera). Historically, first detection of MXR transporter in *Chironomus* larvae has been evidenced by Podsiadlowski et al. (1998). Recently, Moreau et al. (2008) have proposed an adaptation of the ELISA assay for the semi-quantification of MXR transporter in *Chironomus* gr *thumni* larvae. This latter study, which provided the first method to utilize MXR transporter semi-quantification in midge larvae, has answered to first calibration criteria for the use of MXR transporter expression in indigenous *C.* gr *thumni* larvae as biomarker environmental tool, i.e. (1) a simple and sensitive detection method and (2) the ability to distinguish complex polluted natural situation from experimentally unpolluted situation (Moreau et al., 2008). In the present work, the relationship between the biological response, i.e. variations in MXR transporter membrane density measured by ELISA assay according to Moreau et al. (2008), and physico-chemical data is discussed. Concentrations of the 16 Environmental Protection Agency (EPA) priority PAHs (US-EPA, 1976) and of total PCB concentrations were confronted to their defined sediment concentrations for quality guidelines in freshwater ecosystems (MacDonald et al., 2000). This work is also the first attempt to understand the variation of this cellular defense system in aquatic insect larvae that were exposed, in the field, to a complex multifactor pollution. Finally, this study is another step in the calibration of MXR transporter semi-quantification as a potential biomarker of freshwater environment.

2. Materials and methods

2.1. Sampling sites and sampling

Limnological, chemical and biological analyses were conducted along a longitudinal transect in the Cadière stream, a French Mediterranean urban stream. Three sites of sampling were chosen according to potential sources of pollutants (Fig. 1). Two sites, STP1 and STP2, are both located downstream a sewage treatment plant (STP; 10,000 and 60,000 inhabitant equivalents). The RM site is close to the river mouth and also collects the waters from an urban tributary, the Raumartin stream. Two sampling sites were added: one in the Raumartin (RAU) and one in the Bayeux stream (BAY) (Fig. 1). The Bayeux stream is located in the same Mediterranean region but in a non-urbanized area near the Sainte Victoire mountain. All sediment and biological samplings as well as limnological measures were done simultaneously in a 2-day period (20 and 21 December 2005). For chemical analyses, sediments were collected in triplicate at each site and designed as A, B, and C samples. They were stored in clean polyethylene bags and transported to the laboratory at 4 °C. Biological materials collected with a surber (125 μm mesh) were immediately fixed in the field in 0.1 M phosphate buffer (PB, pH 7.2) containing paraformaldehyde 1% and then kept at 4 °C before use.

2.2. Limnological measurements

In the field, pH was measured with a portable pH meter (Hanna Instruments®), and electrical conductivity (C_{20}), oxygen level (in $mg \cdot L^{-1}$ and in percent of saturation), and temperature were measured with WTW® material in the water column.

2.3. Sediment analyses

For a given site, equal fractions of A, B, and C sediment sample were mixed. Two subsamples of each mixture were done: the first was stored at 7 °C for grain size determination, which was performed with a Mastersizer E laser particle sizer (Malvern Instrument® Ltd., UK), the second was freeze-dried at −20 °C before PAH and PCB determination. Before analyses of organic pollutants, samples were dried at 40 °C. Dried sediments (particle size <2 mm) were ground and analyzed for PAHs and PCBs. The concentrations of the 16 US-EPA priority PAH pollutants (naphthalene (NAP), acenaphtylene (ACY), acenaphtene (ACE), fluorene (FL), phenanthrene (PHEN), anthracene (ANT), fluoranthene (FLR), pyrene (Pyr), benzo(*a*)anthracene (BaA), chrysene (ChY), benzo(*b*)fluoranthene (BbF), benzo(*k*)fluoranthene (BkF), benzo(*a*)pyrene (BaP), benzo(*ghi*)perylene (BP), dibenzo(*ah*)anthracene (DahA), and indeno(1,2,3-*cd*)pyrene (IP)) and seven PCB congeners (CB28, CB52, CB101, CB118, CB138, CB153, CB180) have been quantified. These seven congeners cover the major levels of chlorination and reflect the most recalcitrant chlorobiphenyls in the environment (Griepink et al., 1990). Analytical determinations of the16 EPA PAHs were done by RP-HPLC after extraction of sediments by ultrasonication and purification of extracts with C18-mini-column cartridges (Sarrazin et al., 2006). Quantitative analyses of PCBs were performed by the method previously described by Wafo et al. (2006). Briefly, organic compounds were extracted using a soxhlet apparatus. After cleanup with concentrated sulfuric acid, and desulfuration, the extracts were quantitatively determinated by capillary gas chromatography with electron capture detector (GC/ECD).

2.4. Biological material and biomarker

Fixed (1% paraformaldehyde in PB buffer) chironomidae larvae were rinsed in PB and isolated under stereomicroscope according to their subfamily (Armitage et al., 1995; Tachet et al., 2000). For each sampling site, 111 ± 37 midge larvae were isolated.