Interest of a multibiomarker approach in the assessment of freshwater ecosystem quality: laboratory and field studies

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Abstract The aims of this study were to validate several biochemical parameters as biomarkers of pollution in the freshwater bivalve *Corbicula fluminea* and to underline the interest of a multibiomarker approach in environmental biomonitoring. The study was divided into a laboratory exposure to 4 doses of trichloroethylene, toluene, cadmium chloride or a coal tar fraction for 5 days and a field exposure for one week in 5 sites surrounding an industrial effluent outlet. Whatever the product was, parameters that exhibited significant responses were mainly parameters related to oxidative stress and components of phase I metabolism. As a result of discriminant analysis, doses were clearly discriminated from the control and from each other. Likewise, products were discriminated from each other, based on results of the whole parameter responses obtained for the first dose. Concerning the field experiment, all biochemical parameters assayed exhibited significant responses for sites located downstream of the effluent outlet, compared to the upstream reference site. Through a discriminant analysis, sites could be distinguished from each other in terms of pollution intensity. In order to characterise pollution at a qualitative level, further laboratory and field studies are needed to obtain typical profiles for the main pollutants present in freshwater ecosystems.
Keywords Biomarkers; biomonitoring; caging; *Corbicula fluminea*; freshwater ecosystems; pollutants

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Introduction

To assess the impact of pollutants on freshwater ecosystems, biomarkers make up a predictive method that concerns every molecular, biochemical, cellular, physiological or behavioural change occurring in an organism after an exposure to pollution. *Corbicula fluminea* is a freshwater bivalve originating from Asia that has become a major component of many benthic communities in Europe (Araujo *et al*., 1993). As it is known to accumulate pollutants (Narbonne *et al*., 1999) we study the possible use of this species as a sentinel organism of freshwater ecosystems. Thus, the aim of this study was to assess the potential use of several biochemical parameters as biomarkers in *C. fluminea* and to underline the interest of a multibiomarker approach in the assessment of freshwater ecosystem quality. Components of phase I and phase II (de)toxification metabolism, that is to say: cytochromes P450 (P450), P418 (P418), *b*5 (b5), NADPH and NADH-cytochrome *c* reductases (NADPH-red and NADH-red), NADPH-independent ethoxyresorufin *O*-deethylase (EROD), glutathione *S*-transferase using 1-chloro-2,4-dinitrobenzene or ethacrynic acid as a substrate (GST/CDNB and GST/EA), as well as parameters related to oxidative stress such as catalase (CAT), peroxided and peroxidizable lipids (PL and PLI), net peroxidation (NP) were measured. Propionylcholinesterase (PChE) was also considered. The first part of this study consisted of a laboratory experiment in which *C. fluminea* were exposed to 4 doses of 4 contaminants: trichloroethylene (TCE), toluene (TOL), cadmium chloride ($CdCl_2$) and a coal tar fraction (CT) with a high Polycyclic Aromatic Hydrocarbons content (PAHs). The second part of the study was a field experiment in which *C. fluminea* were transplanted by caging into 5 sites located around an industrial effluent outlet. After several

days of exposure, responses of biochemical parameters were studied and appropriate statistical analysis was performed, especially to discriminate doses, products (laboratory experiment) and sites (field experiment) from each other.

Methods

Chemicals

Trichloroethylene (TCE), toluene (TOL), cadmium chloride ($CdCl_2$) and the coal tar fraction (CT) were obtained from SDS (Peypin, France), Carlo Erba (Rueil Malmaison, France), Interchim (Montluçon, France) and Chemco (Poissy, France), respectively. Other chemicals were of the best technical grade available.

Animals

Adult *C. fluminea*, the anterior-posterior length of which ranged from 15 to 20 mm, were collected from the banks of the non-polluted freshwater Cazaux-Sanguinet lake (Aquitaine, France). They were placed in a plastic tank containing lake water for the transport back to the laboratory. They were maintained in stabilation for 8 days before the experiment in glass aquariums containing lake water at 20°C (laboratory experiment) or dechlorinated tap water at 18.5°C (field experiment). Aeration was provided by air bubbling. Clams were not fed during this period and were held under a natural light cycle. No sexual differences were taken into account for *C. fluminea* are hermaphroditic.

Laboratory experiment

Exposure studies were performed for 5 days in glass aquariums containing 10 l of lake water (contaminated or not) previously filtered through 50 µm and then 5 µm cotton cartridges (Cole-Parmer Masterflex® pump, model 7019-20, Cole-Parmer Instrument Co.). Ranges of doses assayed were of 1.56, 6.25, 25 and 100 mg/l for TCE; 7.5, 15, 30 and 60 mg/l for TOL; 0.31, 0.625, 1.25 and 2.5 mg/l for $CdCl_2$ (that is to say 0.154, 0.308, 0.615 and 1.23 mg/l expressed as Cd content) and 0.75, 1.5, 3 and 6 mg/l for CT. As $CdCl_2$, TCE and TOL were hydrosoluble in the range of doses assayed, they were directly mixed with filtered lake water. CT was previously mixed with dimethylsulfoxide (DMSO), the final concentration of which was 0.5 g/l. Appropriate controls consisting of filtered lake water (plus DMSO in the case of CT) were prepared. 50 *C. fluminea* were placed in each aquarium. They were not fed and were held under a natural light cycle at 20°C during the exposure. Aeration was provided by intermittent air bubbling (15 min every 6 hr for $CdCl_2$ and CT; 15 min every 12 hr for TCE and TOL).

Field experiment

60 *C. fluminea* were transplanted by caging into different points of a river in the vicinity of a factory. 5 sites were selected around the industrial effluent outlet. The first one was located just upstream of the effluent outlet and was considered as the reference site (UPS). The downstream sites were located 10 metres (DS1), one hundred metres (DS2), several hundred metres (DS3) and several kilometres (DS4) downstream of the effluent outlet. The exposure time was of 7 days.

Chemical analysis

Concentrations of TCE, TOL , $CdCl_2$ and CT were determined at the beginning (T0) and the end (T5) of the laboratory experiment. Analysis was performed by the Laboratory of Control and Environment of Elf Aquitaine (Lacq, France). Quantification of TCE, TOL, $CdCl_2$ (Cd content) and CT was performed by gas chromatography coupled to mass

spectrometry, High Performance Liquid Chromatography (HPLC), atomic absorption spectrophotometry and HPLC, respectively.

Preparation of subcellular fractions

All steps of homogenisation were achieved at 4°C. After the shell and the crystalline style were removed, *C. fluminea* were dissected-out to obtain entire soft bodies or visceral masses. For each experimental condition, 5 pools of 5 animals were prepared. Body samples were rinsed in 100 mM phosphate buffer, pH 7.4, dried on absorbent paper sheets, weighed and homogenised in the same phosphate buffer (1:4 weight:volume ratio) using an Ultra-Turrax® Antrieb T25 (IKA® Labortechnik). Homogenates were then centrifuged at 9,000 g for 30 min in a Sigma 3 MK centrifuge. In the case of soft entire bodies, supernatants consisting of the submitochondrial fractions (S9) were collected and stored at –80°C. For visceral masses, they were centrifuged at 105,000 g for 1 hr in a Beckman LE-80 ultracentrifuge. Supernatants (cytosolic fractions) were collected and stored at –80°C. Microsomal pellets were resuspended in a 100 mM phosphate buffer, pH 7.4, containing 1 mM EDTA and 20% glycerol, then collected and stored at –80°C.

Biochemical assays

Enzymatic activities were measured on a dual-beam temperature-controlled Kontron Uvikon 932 spectrophotometer except for EROD activity which was carried out on a Shimadzu RF-540 spectrofluorophotometer. Assays were run in triplicate for each pool. CAT, PChE, GST (CDNB/EA), EROD and NAD(P)H-red activities were assayed as described in Clairborne (1985), Ellman *et al*. (1961), Habig *et al*. (1974), Burke and Mayer – without NADPH – (1974) and Guengerich (1994), respectively. P450, P418 (denatured form of cytochrome P450) and b5 levels were measured as described in Estabrook and Werringloer (1978). PL and PLI levels were quantified as Thiobarbituric Acid Reactive Species (TBARs) as described in Buege and Aust (1978). NP level was calculated as the difference between PLI level and PL level. CAT, PChE, GST/CDNB (field experiment only), EROD activities and P450, P418, b5, PL and PLI levels were measured in S9 of entire soft bodies. GST (CDNB/EA) (laboratory experiment only) and NAD(P)H-red were assayed in cytosol and microsomes of visceral masses, respectively. Protein concentrations were determined by the method of Lowry *et al*. (1951) with bovine serum albumin as a standard.

Statistical analysis

Statistical analysis of data was performed with STATISTICA™ software (5.1 release, Statsoft®). Data fulfilling variance homogeneity conditions were submitted to a one-way analysis of variance (ANOVA) to estimate the effect of the contaminant dose or the site on biochemical parameter responses. Whenever a significant ($p \leq 0.05$) dose or site effect was observed on a biochemical parameter response, a Tukey HSD multiple comparison test was performed to check significant ($p \leq 0.05$) differences between doses or sites. Correlations between dose and biochemical parameter responses were determined with the Pearson correlation coefficient. For each contaminant, a discriminant analysis was carried out to distinguish doses from each other and to determine biochemical parameters allowing the best discrimination. Such an analysis was also performed to distinguish contaminants or sites from each other.

Results and discussion

Chemical analysis

Concerning TCE and TOL which are highly volatile solvents, the loss during preparation of contaminated water was very important (23 to 44% for TCE and 6 to 45% for TOL). Actual

concentrations at the beginning of the laboratory experiment (T0) were: 1.20, 3.58, 13.99 and 69.44 mg/l for TCE; 4.31, 8.19, 28.19 and 36.43 mg/l for TOL; 0.118, 0.257, 0.543 and 1.141 mg/l for $CdCl_2$ (quantified as Cd); 1.17, 2.24, 3.58 and 7.84 mg/l for CT. At the end of the experiment (T5), the loss was complete for TOL. Final concentrations were: 0.10, 0, 0.22 and 4.40 mg/l for TCE; 0.036, 0.140, 0.372 and 0.869 mg/l for $CdCl_2$ (quantified as Cd); 0, 0.0033, 0.043 and 0.247 mg/l for CT. For the following results, actual concentrations of products at T0 will be considered.

Laboratory experiment

Whatever the product was, biochemical parameters that exhibited significant responses compared to control were mainly parameters related to oxidative stress (CAT activity, PL, PLI and NP levels) and components of phase I metabolism (P450 and P418 levels, NAD(P)H-red and EROD activities). In the case of TCE, an increase of CAT activity (+70% and +40% at 1.2 mg/l and 3.6 mg/l respectively) and PL (+29% at 1.2 mg/l) could be indicative of an oxidative stress as it has been observed already for mice (Goel *et al.*, 1992). P418 and especially P450 exhibited significantly high responses at 3.6 mg/l (+249%) and from 3.6 mg/l up to 69.4 mg/l (from +835% to +649%) respectively. On the other hand, NADH-red activity significantly decreased by 21% and 20% at 14 mg/l and 69 mg/l, respectively. Regarding TOL, all parameters related to oxidative stress exhibited a significant increase at the two lowest doses (+26%, +33%, +26% and +26% for CAT activity, PL, PLI and NP levels respectively at 4.3 mg/l). Among components of phase I metabolism, P450 level and to a lesser extent P418 level, exhibited a significant response. Their increase (+1093% and +240% at 28 mg/l for P450 level and P418 level, respectively) was almost similar to that observed in the case of TCE. An increase of P450 level has already been described in mammals for both products (Kawamoto *et al.*, 1988; Nakajima and Wang, 1994). For $CdCl_2$, CAT activity exhibited a significant decrease at the lowest dose (–39%) followed by a significant increase at the two highest doses (+31%). PLI and NP responses were correlated with the dose ($r = 0.9312$ and $r = 0.9309$, respectively). They significantly increased at 1.1 mg/l of Cd (+29%). Indeed, cadmium is known to induce oxidative stress and to alter the antioxidant system (Sarkar *et al.*, 1998). EROD activity was annulled at the three highest doses. Such a dramatic decrease was previously observed in rats (Rosenberg and Kappas, 1991). GST/CDNB was significantly reduced but only at the lowest dose (–29%). Such an inhibition of GST activity by cadmium has been reported by Sidhu *et al.* (1997). For CT, NADPH-red activity as well as P450 and PL levels were correlated with the dose ($r = 0.9548$, $r = 0.9491$ and $r = –0.9258$ respectively). They were significant at the highest dose (+86%, +317% and –41% respectively compared to control). P418 level increased significantly at 2.2 mg/l (+26%) and 7.8 mg/l (+19%). Gilewicz *et al.* (1984) have already observed such an induction of P450 following exposure of *Mytilus galloprovincialis* to petroleum hydrocarbons. NADH-red activity and NP level also increased significantly but only at the lowest doses (+32% and +41% at 1.2 mg/l and 2.2 mg/l respectively for NADH-red activity; +20% at 2.2 mg/l for NP level).

Results of discriminant analysis are presented in Tables 1 and 2. For each product, control was discriminated from other doses. Generally, doses were discriminated from each other, except for the lowest dose and the highest one for TOL, the second dose and the third and the fourth ones for $CdCl_2$ and the two lowest doses for CT. For TCE and TOL, doses were discriminated thanks to parameters related to oxidative stress (CAT activity, PL and NP levels) and to components of phase I (P450 level and NADH-red activity). More biochemical parameters contributed to discrimination in the case of $CdCl_2$ and CT. However, some parameters exhibiting no significant response compared to control in the ANOVA analysis contributed to discrimination, especially PChE activity for CT. To try to

Table 1 Discriminant analysis: distances between doses of each product expressed as square of Mahalanobis distances between group centroids. Discriminated doses are notified * ($p \leq 0.05$), ** ($p \leq 0.01$) or *** ($p \leq 0.001$). Non-discriminated doses are notified ns

Products		Dose 1	Dose 2	Dose 3	Dose 4
TCE	Control	47.7 ***	63.9 ***	35.9 ***	30.1 ***
	Dose 1	–	41.3 ***	41.4 ***	79.9 ***
	Dose 2		–	8.4 *	35.5 ***
	Dose 3			–	15.1 **
TOL	Control	27.0 ***	24.6 ***	55.3 ***	16.7 ***
	Dose 1	–	22.9 ***	45.1 ***	5.6 ns
	Dose 2		–	29.7 ***	15.8 ***
	Dose 3			–	23.6 ***
$CdCl_2$	Control	62.0 ***	24.1 ***	23.1 ***	33.8 ***
	Dose 1	–	73.2 ***	102.6 ***	106.3 ***
	Dose 2		–	5.3 ns	7.9 ns
	Dose 3			–	9.9 *
CT	Control	26.3 ***	23.9 ***	12.3 **	138.2 ***
	Dose 1	–	3.4 ns	35.6 ***	234.3 ***
	Dose 2		–	33.4 ***	204.9 ***
	Dose 3			–	101.5 ***

Table 2 Discriminant analysis: biochemical parameters contributing significantly to discrimination between doses of each product. F of exclusion and corresponding degree of significance are in brackets (*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$); n=30

Products	Biochemical parameters					
TCE	P450	PL	CAT	NADH-red		
	(25.2 ***)	(15.6 ***)	(12.9 ***)	(6.1 **)		
TOL	P450	NP	CAT	NADH-red		
	(25.6 ***)	(11.4 ***)	(7.4 ***)	(4.8 **)		
$CdCl_2$	CAT	GST/CDNB	NP	NADPH-red	GST/EA	
	(19.9 ***)	(19.5 ***)	(10.1 ***)	(3.9 *)	(3.5 *)	
CT	NADPH-red	P450	NADH-red	P418	PChE	NP
	(41.4 ***)	(14.1 ***)	(6.6 ***)	(5.9 **)	(3.2 *)	(2.9 *)

discriminate products from each other a discriminant analysis was performed based on the responses of biochemical parameters at the lowest dose. As presented in Table 3, products could be discriminated from each other. Biochemical parameters contributing to this discrimination are presented in Table 4. As observed previously for dose discrimination, parameters allowing a good discrimination between products were mainly parameters related to oxidative stress and components of phase I metabolism.

At the end of this laboratory study, typical profiles of biochemical parameter responses could be obtained for each product as described in Table 5. For the considered products, b5 level, GST/EA and PChE activities never exhibited a significant response compared to control.

Table 3 Discriminant analysis: distances between each product expressed as square of Mahalanobis distances between group centroids. Discriminated products are notified *** ($p \leq 0.001$)

Products	TCE	TOL	$CdCl_2$	CT
TCE	–	128.1 ***	184.2 ***	282.4 ***
TOL		–	348.2 ***	507.3 ***
$CdCl_2$			–	54.9 ***

Table 4 Discriminant analysis: biochemical parameters contributing significantly to discrimination between products. F of exclusion and corresponding degree of significance are in brackets (*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$); n=20

Products	Biochemical parameters						
All	PL (27.4 ***)	CAT (19.6 ***)	NP (15.1 ***)	PChE (13.6 ***)	P450 (12.3 **)	NADH-red (11.6 **)	NADPH-red (4.6 *)

Table 5 Typical profiles of biochemical parameter responses obtained for each product (no consideration of dose). Significant increase or decrease of responses is notified + or –. Non-significant responses are notified ns

Biochemical parameters	Signification	Products			
		$CdCl_2$	TCE	TOL	CT
CAT	Oxidative stress	–/+	+	+	ns
PL		ns	+	+	–
PLI		+	ns	+	ns
NP		+	ns	+	+
P450	Phase I metabolism	ns	+	+	+
P418		ns	+	+	+
b5		ns	ns	ns	ns
EROD		–	ns	ns	ns
NADPH-red		ns	ns	ns	+
NADH-red		ns	–	ns	+
GST/CDNB	Phase II metabolism	–	ns	ns	ns
GST/EA		ns	ns	ns	ns
PChE	Nervous system	ns	ns	ns	ns

Field experiment

For downstream sites, all biochemical parameters exhibited significant responses compared to those observed for the reference site. Results observed for CAT activity were contradictory. It increased significantly for DS1 (+43% compared to the reference site) and then began to decrease until it was significantly lower for DS3 and DS4 compared to the reference site (–71%). Both PL and PLI levels decreased for all downstream sites (–50% and –78% respectively for DS1). This tendency was slightly attenuated along with the pollution gradient for only PLI level. Concerning GST/CDNB activity, the highest increase was observed for the two sites nearest to the effluent outlet (+25% for DS1 and DS2). Then activity decreased along with distance and pollution gradient (+16% and +11% for DS3 and DS4, respectively). PChE activity was not significantly affected for the first downstream site (DS1) whereas it was for the next two (–24% and –23% for DS2 and DS3, respectively). This effect tended to attenuate along with the pollution gradient (no significant response was observed for DS4).

Results of discriminant analysis are presented in Tables 6 and 7. All downstream sites were discriminated from the reference site. DS1 and DS2 as well as DS3 and DS4 were not discriminated from each other. Biochemical parameters accounting for site discrimination were the only parameters related to oxidative stress.

Among downstream sites, we could distinguish two categories of sites as presented in Figure 1 (obtained from discriminant analysis): 1) near the effluent outlet, highly polluted sites (DS1 and DS2); 2) far from the effluent outlet, moderately polluted sites (DS3 and DS4). Thus, discriminant analysis based on biochemical parameter responses was suited to classifying the pollution status of different sites as previously described by Van der Oost *et al*. (1997). As the set of biochemical parameters was too restricted and because of the non-specificity of parameters related to oxidative stress, we were unable to express a hypothesis

Table 6 Discriminant analysis: distances between sites expressed as square of Mahalanobis distances between group centroids. Discriminated sites are notified *** ($p \leq 0.001$). Non-discriminated sites are notified ns

Sites	DS1	DS2	DS3	DS4
UPS	224.5 ***	216.5 ***	259.8 ***	244.7 ***
DS1	–	3.2 ns	156.3 ***	158.8 ***
DS2		–	123.9 ***	126.8 ***
DS3			–	0.4 ns

Table 7 Discriminant analysis: biochemical parameters contributing significantly to discrimination between sites. F of exclusion and corresponding degree of significance are in brackets (*: $p \leq 0.05$; ***: $p \leq 0.001$); n = 29

Sites	Biochemical parameters		
All	CAT (147.8 ***)	PLI (30.2 ***)	PL (3.1 *)

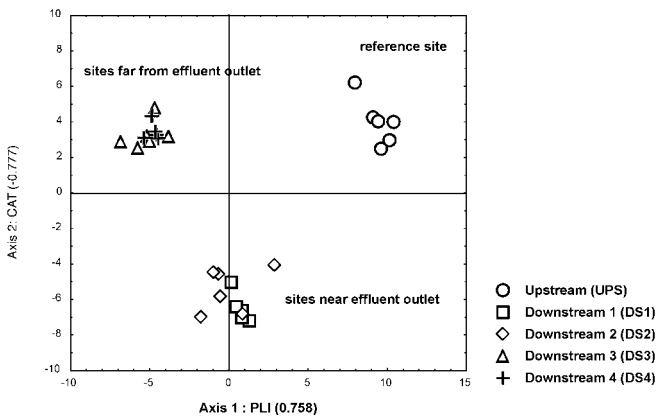

Figure 1 Representation of sites (field experiment) in the first factorial plane obtained from discriminant analysis. The biochemical parameter that is the highest correlated with each axis is specified (correlation coefficient bracketed)

concerning the nature of the effluent. Further studies to obtain typical profiles of more pollutants and the use of a larger set of biochemical parameters in field studies are needed.

Conclusions

In the laboratory experiment, most of the biochemical parameters studied could be considered as potential biomarkers in *C. fluminea*. Whereas product specificity of each parameter was limited, profiles including the whole parameter responses were typical of each product thus emphasising the interest of a multibiomarker approach. In the field experiment, biochemical parameters studied were of great interest for the detection of polluted effluents and it would now be interesting to include more than five parameters in further studies to obtain more accurate interpretations. A multibiomarker approach combined with appropriate statistics such as discriminant analysis could be a useful tool in the biomonitoring of freshwater ecosystem quality. In particular, sites could be distinguished in terms of pollution intensity. It would now be interesting to acquire data on other pollutants to generate typical profiles such as those obtained in the laboratory study and thus to be able to characterise pollution in field experiments.

References

Araujo, R., Moreno, D. and Ramos, M.A. (1993). The Asiatic clam *Corbicula fluminea* (Müller, 1774) (Bivalvia: Corbiculidae) in Europe. *American Malacol. Bull.*, **10**(1), 39–49.

Buege, J.A. and Aust, S.D. (1978). Microsomal lipid peroxidation. *Methods Enzymol.*, **50**, 302–310.

Burke, M.D. and Mayer, R.T. (1974). Ethoxyresorufin: direct fluorimetric assay of a microsomal *O*-dealkylation which is preferentially inducible by 3-methylcholanthrene. *Drug Metab. Dispos.*, **2**(6), 583–588.

Clairborne, A. (1985). Catalase activity. In: *Handbook of Methods for Oxygen Radical Research*, R.A. Greenwald (ed.), CRC Press, Boca Raton, pp. 283–284.

Ellman, G.L., Courtney, K.D., Andres, V. and Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **7**, 88–95.

Estabrook, R.W. and Werringloer, J. (1978). The measurement of difference spectra : application to the cytochromes of microsomes. *Methods Enzymol.*, **52**, 212–220.

Gilewicz, M., Guillaume, J.R., Carles, D., Leveau, M. and Bertrand, J.C. (1984). Effects of petroleum hydrocarbons on the cytochrome P450 content of the mollusc bivalve *Mytilus Galloprovincialis*. *Mar. Biol.*, **80**, 155–159.

Goel, S.K., Rao, G.S., Pandya, K.P. and Shanker, R. (1992). Trichloroethylene toxicity in mice: a biochemical, hematological and pathological assessment. *Indian J. Exp. Biol.*, **30**, 402–406.

Guengerich, F.P. (1994). Analysis and characterization of enzymes. In: *Principles and Methods of Toxicology*, A.W. Hayes (ed.), 3rd edn, Raven Press, New York, pp. 1269–1270.

Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974). The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, **249**(22), 7130–7139.

Kawamoto, T., Hobara, T., Nakamura, K., Imamura, A., Ogino, K., Kobayashi, H., Iwamoto, S. and Sakai, T. (1988). Induction of cytochrome *P*-450, cytochrome *b*-5, NADPH-cytochrome *c* reductase and change of cytochrome *P*-450 isozymes with long-term trichloroethylene treatment. *Toxicology*, **53**, 239–249.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

Nakajima, T. and Wang, R.S. (1994). Induction of cytochrome *P*450 by toluene. *Int. J. Biochem.*, **26**(12), 1333–1340.

Narbonne, J.F., Djomo, J.E., Ribera, D., Ferrier, V. and Garrigues, P. (1999). Accumulation kinetics of polycyclic aromatic hydrocarbons adsorbed to sediment by the mollusk *Corbicula fluminea*. *Ecotoxicol. Environ. Saf.*, **42**, 1–8.

Rosenberg, D.W. and Kappas, A. (1991). Induction of heme oxygenase in the small intestinal epithelium: a response to oral cadmium exposure. *Toxicology*, **67**, 199–210.

Sarkar, S., Yadav, P. and Bhatnagar, D. (1998). Lipid peroxidative damage on cadmium exposure and alterations in antioxidant system in rat erythrocytes: a study with relation to time. *BioMetals*, **11**(2), 153–157.

Sidhu, M., Prasad, R., Gill, K.D. and Nath, R. (1997). Alterations in isoforms of glutathione S-transferase in liver and kidney of cadmium exposed rhesus monkeys: purification and kinetic characterization. *Mol. Cell. Biochem.*, **166**, 55–63.

Van der Oost, R., Vindimian, E., Van den Brink, P.J., Satumalay, K., Heida, H. and Vermeulen, N.P.E. (1997). Biomonitoring aquatic pollution with feral eel (*Anguilla anguilla*). III. Statistical analyses of relationships between contaminant exposure and biomarkers. *Aquat. Toxicol.*, **39**, 45–75.