This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier’s archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright
Detection of early effects of a single herbicide (diuron) and a mix of herbicides and pharmaceuticals (diuron, isoproturon, ibuprofen) on immunological parameters of Pacific oyster (Crassostrea gigas) spat

A. Luna-Acosta, T. Renault, H. Thomas-Guyon, N. Faury, D. Saulnier, H. Budzinski, K. Le Menach, P. Pardon, I. Fruitié-Arnaud, P. Bustamante

A. Luna-Acosta; Ifremer, Laboratoire de Génétique et Pathologie (LGP), Ronce-les-Bains, 17390 La Tremblade, France. Tel.: +33 (0)5 46 50 76 23; fax: +33 (0)5 46 50 76 63 (A. Luna-Acosta); Ifremer, Laboratoire de Génétique et Pathologie (LGP), Ronce-les-Bains, 17390 La Tremblade, France. Tel.: +33 5 46 76 26 10; fax: +33 5 46 76 26 11 (T. Renault).
E-mail addresses: aluna1508@yahoo.com (A. Luna-Acosta), trenault@ifremer.fr (T. Renault).

Abstract

In the context of massive summer mortality events of the Pacific oyster Crassostrea gigas, the aim of this study was to investigate the early effects on genes, enzymes and haemocyte parameters implicated in immune defence mechanisms in C. gigas oysters exposed to a potentially hostile environment, i.e. to an herbicide alone or within a mixture. Following 2 h of exposure to the herbicide diuron at 1 μg L⁻¹, the repression of different genes implicated in immune defence mechanisms in the haemocytes and the inhibition of enzyme activities, such as laccase-type phenoloxidase (PO) in the plasma, were observed. The inhibition of superoxide dismutase (SOD) activity in the plasma was also observed after 6 and 24 h of exposure. In the mixture with the herbicides diuron and isoproturon, and the pharmaceutical ibuprofen, catecholase-type PO activity in the plasma and the percentage of phagocytosis in the haemocytes were reduced after 6 h of exposure. Our results showed that early effects on molecular, biochemical and cellular parameters can be detected in the presence of diuron alone or within a mixture, giving an insight of its potential effect in situations that can be found in natural environments, i.e. relatively high concentrations for short periods of time.

1. Introduction

Estuaries are among the most ecologically and economically productive environments in the world, by serving as feeding grounds for migratory waterfowls, as nurseries for juvenile fish and invertebrate larvae, and by providing shelter for many types of benthic organisms (Johnston, 1981). Among important species inhabiting these zones, the Pacific oyster Crassostrea gigas is the leading aquaculture product at the worldwide level (FAO, 2010). However, high mortalities of this species have been observed in summer, in many regions of the world, and especially on the French coasts (Samain et al., 2007). It has been suggested that these mortality events are related to a weakening of C. gigas immune defences (Cheney et al., 2000), probably caused by multiple factors, including aquaculture practices, temperature variations, physiological stress, and the presence of pathogens and contaminants in the environment.

Aquatic coastal habitats are particularly subject to contamination by herbicides, via runoff, leaching, spray drift or accidental spills. The most widely used herbicides in agriculture are substituted urea herbicides, such as diuron or isoproturon (Barbash, 1999). These herbicides are also used as algicides in paints and coatings, and in the case of diuron, concentrations up to 6.7 μg L⁻¹ have been found in coastal waters (Thomas et al., 2001). Due to its toxicity, the use of diuron has been forbidden by French laws since 2008. However, a recent study reported the presence of diuron in French aquatic environments, confirming its persistence despite restriction policies (Pesce et al., 2010).

Diuron and other herbicides such as isoproturon and atrazine may exert an effect on immune parameters of C. gigas at the molecular, biochemical, and cellular levels (Tanguy et al., 2005; Bouilly et al., 2007; Gagnaire et al., 2007). Additionally, non-steroidal
anti-inflammatory drugs (NSAIDs), such as ibuprofen, have been detected at concentrations reaching up 1.3 μg L−1 in wastewater treatment plant (WWTP) effluents and in marine seawater samples (Rabiet et al., 2006; Togola and Budzinski, 2008) where herbicides have been found at high concentrations, suggesting that experimental approaches with toxicant mixtures are needed (ASCI ENCE, 2005). Nevertheless, very little is known on the toxic effects of pharmaceuticals such as ibuprofen on marine bivalves (Gagne et al., 2004, 2006; Ericson et al., 2010).

In this general context, the aim of this work was to study the short-term responses on immune parameters of C. gigas spat in experimental conditions at molecular (gene expression), biochemical (enzymatic levels) and cellular (cellular mortality and phagocytosis activity) levels, by exposing oysters (i) to diuron at nominal concentration (nc) of 1 μg L−1 for 24 h, and (ii) to a mixture of two herbicides and one pharmaceutical, i.e. diuron (nc = 5 μg L−1), isoproturon (nc = 5 μg L−1) and ibuprofen (nc = 5 μg L−1).

2. Materials and methods

2.1. Oysters

Pacific oyster (C. gigas) spat, less than 1 year old, known to be generally more sensitive to environmental stress than adults (Perdue et al., 1981), were purchased from commercial shellfish nursery located in Vendée (France). Oysters were acclimatized in the laboratory at 19 ± 1 °C during 2 weeks before starting experiments. This temperature was selected because, during summer mortality events, water temperatures are often over a value of 19 °C (Gagnaire et al., 2007). Animals were fed daily with 5.104 cell mL−1 of Heteroskeletonema sp. (Bacillariophyceae).

2.2. Contaminants

All chemicals were purchased from Sigma–Aldrich, France, unless specified. Stock solutions of diuron (1-[3,4-dichlorophenyl]-3,3-dimethyl urea) and isoproturon (3-[4-isopropylphenyl]-1,1-dimethylurea) were prepared in ethanol 80%. Stock solution of ibuprofen (2-methyl-4-[2-methylpropyl]-benzenacetic acid) was prepared in ultra pure water (Milli-Q). Diuron, isoproturon and ibuprofen were added to the tanks in the form of diluted solutions allowing spikes of small volumes. All the experiments were conducted under dark conditions to avoid photodegradation of the chemicals (Shankar et al., 2008).

2.3. Experimental design

2.3.1. Experiment conducted with diuron at 1 μg L−1

Two groups of 70 oysters were exposed in 35 L tanks to diuron (nc = 1 μg L−1) for a period of 24 h and under dark conditions. The concentration used was selected according to concentrations reported in the literature (Heberer, 2002). Two tanks containing 70 oysters maintained in 35 L clean aerated seawater were used as control experiment. The experiment was repeated three times.

2.3.2. Experiment conducted with diuron, isoproturon and ibuprofen

Two groups of 70 oysters were exposed in 35 L tanks to a cocktail of diuron (nc = 5 μg L−1), isoproturon (nc = 5 μg L−1) and ibuprofen (nc = 5 μg L−1). The concentrations used were selected based on the fact that concentrations up to 6.70 μg L−1 and 9.89 μg L−1 of phenylurea herbicides and NSAIDs have been detected in aquatic environments, respectively (Thomas et al., 2001; Rabiet et al., 2006; Togola and Budzinski, 2008). Two tanks containing 70 oysters maintained in 35 L clean aerated seawater were used as control experiment. Animals were exposed for 6 h under dark conditions. The experiment was repeated four times.

2.4. Sampling procedures

2.4.1. Seawater sampling

For each trial, 50 mL of seawater were collected in one of the two contaminated tanks, at 0 and 24 h in trials conducted with diuron, and at 0, 1, 3 and 6 h, in trials conducted with diuron, isoproturon and ibuprofen.

2.4.2. Haemolymph collection

After carving a small notch in the dorsal shell of the oyster, ~0.3–0.5 mL of haemolymph were withdrawn from the adductor muscle sinus with a 1 mL-syringe equipped with a needle (0.9 × 25 mm). Haemolymphs from 10 oysters from each of the two exposure tanks were pooled to obtain a sufficient volume. In this respect, one pool corresponds to the haemolymph of 20 oysters, with 10 oysters collected per tank. Haemolymph samples were filtered through a 60 μm mesh to eliminate aggregates and then kept on ice to avoid enzymatic degradation.

First, 400 μL of haemolymph from each pool was used immediately to study cell mortality and phagocytosis activity by flow cytometry. Then, 600 μL of haemolymph was centrifuged (260 × g, 10 min, 4 °C) and the acellular fraction (supernatant) was frozen at −80 °C until further enzymatic analysis by spectrophotometry. The remaining haemolymph was centrifuged (1500 × g, 10 min, 4 °C) and 1 mL of Trizol® was added to the cell pellets. The mix was frozen at −80 °C until further extraction for gene expression analysis.

2.5. Seawater and oyster contaminant concentrations

For trials carried out with diuron alone, concentrations of diuron and its metabolite (i.e. 1-[3,4-dichlorophenyl] urea [DCPU]) were determined in the seawater and in a pool of 5 oysters per treatment, at 0, 2 and 24 h of exposure for one experiment. Analyses were performed by the laboratory Idhesa (Brest, France). The detection limit was 0.02 μg L−1 for water samples, and 0.2 μg kg−1 for diuron and DCPU in oyster soft tissues.

For trials conducted with the mixture of contaminants (diuron, isoproturon and ibuprofen), analyses were carried out in two of the four experiment replicates conducted for this study. Contaminants were extracted via solid-phase extraction (SPE), using Oasis HBL (for herbicides) or MXX cartridges (for ibuprofen) and analyzed by LC/MS/MS. The analytical procedures were adapted from Alder et al. (2006) for herbicides and from Togola and Budzinski, (2008) for ibuprofen. Detection limits were 0.1 ng L−1 for pesticides and 0.5 ng L−1 for ibuprofen. Pesticides were analyzed using a UPLC/MS/MS system from Waters company (UPLC Acquity system from Waters coupled to a Quattro Premier XE mass spectrometer from Micromass Technologies, Manchester, UK; mobile phase: water (5 mM ammonium acetate + 0.1% acetic acid)/methanol (100%/0%–0%/100% within 17 min at 0.5 mL min−1); column: Kinetex C18 100 mm × 2.1 mm × 1.7 mm. The ionization mode was positive atmospheric pressure chemical ionization for all analytical compounds. Agilent (Palo Alto, CA, USA) supplied the 1100 Series HPLC system connected to a quadrupole 1100 mass spectrometer for ibuprofen analyses; mobile phase: water/acetonitrile (100%/0%–0%/100% within 30 min at 0.6 cc min−1); column: Zorbax SBC18 50 mm × 2.1 mm × 18 mm.

2.6. Flow cytometry analysis

Haemocyte mortality and phagocytosis percentage were analyzed with an EPCIS XL 4 (Beckman Coulter) flow cytometer as pre-
viously described (Gagnaire et al., 2006). For each sample, 3000 events were counted.

2.7. Enzymatic analysis

Catecholase-type and laccase-type PO activities were determined as described previously (Luna-Acosta et al., 2010), by using domapine and p-phenylenediamine (PPD) as substrates, respectively. One unit of catecholase or laccase activity corresponds to the amount of enzyme that catalyzes the formation of 1 μM of product per minute. SOD activity was determined by the method described by Theron et al. (1996). One unit of SOD is defined as the amount of enzyme that promotes a 50% decrease in the rate of INT reduction.

All enzyme activity assays were carried out in triplicate for each sample and were expressed in relation to protein concentration measured according to the Lowry method with slight modifications, by using bicinchoninic acid and copper sulphate 4% (Smith et al., 1985). Serum albumin was used as standard.

2.8. Oyster gene expression in haemocytes

Seven immune-related genes previously identified from C. gigas cDNA database were selected, i.e. multicopper oxidase (laccase), macrophage expressed protein 1-like protein (Mpeg1), myeloid differentiation factor 88 (MyD88), superoxide dismutase (SOD), lipopolysaccharide-binding protein /mammalian bactericidal/permeability increasing protein (LBP/BPI), tissue inhibitor of metalloproteinases (TIMP) and lyoyme. As a housekeeping gene, the Elongation factor I (EF I) gene was used as reference.

Samples were treated with the RTQ RNase-free DNase (Promega) to remove DNA. The quality of extracted RNA was checked on 1% agarose gel. RNA concentrations and purity were measured by absorption spectrophotometry (A260 nm).

Reverse transcription (RT) of extracted haemocyte RNA (500 ng) was carried out by using SuperScript™ III Reverse Transcriptase kit (Invitrogen), and following manufacturer’s recommendations. The relative levels of gene transcripts were investigated by real-time PCR using a MX3005P™ Stratagene. cDNA were diluted at 1/30 with sterile ultra pure quality water before use.

Real-time PCR was performed in triplicates and as described previously (Bado-Nilles et al., 2010). The cycling conditions consisted of Taq polymerase thermal activation (10 min, 95 °C) followed by 40 cycles of denaturation (30 s, 95 °C), annealing (1 min, 60 °C) and elongation (1 min, 72 °C). Finally, a melting curve of PCR product (60–95 °C) was performed to ensure the production of a single specific product.

The relative mRNA expression ratio for a considered gene was based on the PCR efficiency (E) and the cycle threshold (Ct) of the treated samples versus the untreated samples (control) expressed in comparison to the reference gene (EF I) as described previously (Pfaffl, 2001).

\[
\text{Ratio} = \frac{(E \text{ studied gene} \times 10^{Ct \text{ studied gene/untreated-treated}})}{(E \text{ reference gene} \times 10^{Ct \text{ reference gene/untreated-treated}})}
\]

2.9. Statistics

All values are reported as mean ± standard deviation (SD). For trials conducted with diuron alone, only two out of the three experiments were taken into account for statistical analysis, because for one of the experiments the temperature in the seawater was at 15 ± 1 °C and not at 19 ± 1 °C, the effects of herbicides differing depending on the seawater temperature (Gagnaire et al., 2007).

Statistical analysis was carried out with STATISTICA 7.0. Values were tested for normality (Shapiro test) and homogeneity of variances (Bartlett test). In some cases, values were normalized using logarithmic transformations (Log10) or an angular arcsin (\[\sqrt{\text{percentage}}\]) transformation (in the case of haemocyte parameters), before analysis. For normal values, a two-way nested ANOVA was used, with treatment and time as fixed factors, and pool as random factor. Pool was nested within each combination of treatment and time (Zar, 1984). When the null hypothesis (H0: no difference between treatments or within treatment at different time intervals) was rejected, significant differences were tested using Tukey’s HSD test. For non-normal values, a Kruskal–Wallis test was used, followed by a Dunn’s multiple comparisons test. Significant differences were considered at \( p < 0.05. \)

3. Results and discussion

It is well known that in natural waters, uneven concentrations of pesticides are found in the seawater mass because of different factors such as seasonal agricultural practices and weathering processes (rainfall, photodegradation, volatilization), and peak concentrations are often found in the coastal environment for short periods of time (Munaron, 2004; Samain et al., 2007). However, most of the exposure experiments in oysters have been carried out for relatively long periods of time, i.e. from 1 week to 1 month (Tanguy et al., 2005; Bouilly et al., 2007; Gagnaire et al., 2007), giving an insight on effects of chronic exposures on physiological functions, but not on the effects of short-term or pulse exposures (Burga and Wauchope, 1995; Buisson et al., 2008). Thus, the aim of this study was to investigate the early effects on genes, enzymes and haemocyte parameters implicated in immune defence mechanisms in C. gigas exposed to an herbicide alone or within a mixture, for a short time period.

3.1. Experiment conducted with diuron (1 μg L\(^{-1}\))

In the control tank, diuron was not detected either in seawater or in oyster soft tissues. In the tanks spiked with diuron, oysters were effectively exposed to 0.8 μg diuron L\(^{-1}\) and, by the end of the experiment, 21% of diuron was lost (Table 1). If we consider that, from the beginning to the end of the experiment, all oysters possessed \( -0.014 \ μg \) of diuron (9.2 μg kg\(^{-1}\), i.e. 1.3–10 kg), this means that 1 μg of waterborne diuron was present in all oyster soft tissues (n = 70), i.e. 2% of the initial quantity of dissolved diuron. This suggests that 19% of the initial quantity of dissolved diuron disappeared by volatilization or adsorption on the tank walls and oyster shells. However, diuron is a strongly hydrophilic molecule and is not volatile (Gramatica and Di Guardo, 2002). Some degradation products of diuron, such as DCPU, have been reported to be more toxic than diuron itself (Giacomazzi and Cochet, 2004). Nevertheless, no traces of DCPU were found in oyster soft tissues in the present study. Thus, the disappearance of diuron in the water column could be explained by a metabolization of diuron by oysters into other compounds than DCPU, such as DCPMU (1-[3,4-dichlorophenyl]-3-methyl urea) or DCA (3,4-dichloroaniline; Giacomazzi and Cochet, 2004), which were not measured in the present study.

As 28 μg of diuron (0.8 μg L\(^{-1}\), i.e. 35 L) was present at the beginning of the study in each contaminated tank, theoretically 0.4 μg (i.e. 28 μg/70 oysters) were available per oyster. Taking into account the theoretical oyster filtration rate is 1.4 L oyster\(^{-1}\) h\(^{-1}\) (Table 1), each animal can filtrate 0.4 μg = 1.4 L/35 L = 0.016 μg of diuron per hour. This result is similar to the quantity of diuron found in oysters at 2, 6 and 24 h of exposure, i.e. 0.014, 0.015 and 0.016 μg, respectively. In addition, the theoretical quantity of diuron that oysters were able to integrate in 1 h, was found in their soft tissues
after 2 h of exposure, demonstrating that diuron is very rapidly bioaccumulated in oyster soft tissues. Interestingly, no significant increase was observed after 24 h of exposure, suggesting that oysters present a very limited capacity to bioaccumulate diuron at high levels following an exposure from the dissolved phase.

In addition, in the present study, oysters presented a bioconcentration factor (BCF) for diuron of 17. In a previous study, experiments carried out with 21-month-old *Crassostrea gigas* oysters exposed to diuron (1 µg L⁻¹), led to an accumulation of diuron of 7.27 µg kg⁻¹ wet flesh (Buisson et al., 2008), and to a BCF of ~7, confirming that a 24 h-exposure is sufficient to detect diuron in oyster soft tissues and that oysters present a low potential to bioaccumulate diuron.

POs are a group of enzymes composed of tyrosinases, catecholases and laccases, the key enzymes of melanisation, known to be implicated in invertebrate immune responses (Söderhäll and Cerenius, 1998). Recently, the presence of catecholase and laccase, but not tyrosinase, activities were demonstrated in *C. gigas* (Luna-Acosta et al., 2010). In the present study, no significant effects were observed on catecholase activity in oysters exposed to diuron at 1 µg L⁻¹ for 24 h, while significantly lower activities were observed for laccase at 2 and 6 h, in comparison to control (decrease of ~40% and ~50%, respectively; Fig. 1a). The molecule 1-phenyl-2-thiourea (PTU) is known to be a specific inhibitor of all POs (Luna-Acosta et al., 2010). Therefore, diuron, a phenylurea herbicide, may act as an inhibitor of laccase activity. In addition, differential effects of diuron in catecholase and laccase activities may be due to differences for these enzymes on sensitivities to inhibitors, as it has been shown for acetylcholinesterases in *C. gigas*, and their capacity to be insensitive to organophosphate and carbamate inhibitors (Bocquene et al., 1997).

A significant decrease in the activity of the antioxidant enzyme SOD was also observed at 6 and 24 h of exposure, in comparison to control (decrease of ~15% and ~30%, respectively; Fig. 1b). In the present study, diuron may generate reactive oxygen species (ROS) and induce an overwhelming of the antioxidant system, as suggested previously (Geoffroy et al., 2002). In addition, these results are in agreement with studies carried out in adult oysters, which revealed that diuron can exert different effects in this marine bivalve, e.g. a transient effect on reproduction (partial spawning) and tissue structure (atrophy of the digestive epithelium) after 1 week of exposure at 1 µg L⁻¹ (Buisson et al., 2008), and on aneuploidy and immune parameters after 4 weeks of exposure at 300 ng L⁻¹ and 3 µg L⁻¹ (Bouilly et al., 2007).

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Parameter</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Seawater</td>
<td>Diuron concentration (µg L⁻¹)</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Quantity of diuron per tank (µg)</td>
<td>28.7</td>
</tr>
<tr>
<td>Oyster</td>
<td>Diuron concentration (µg kg⁻¹)</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td></td>
<td>DCPU concentration (µg kg⁻¹)</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td></td>
<td>Quantity of diuron per oyster (µg)</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td></td>
<td>BCF (L kg⁻¹)³</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Theoretical FR (L oyster⁻¹ h⁻¹)³</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Mean dry weight of soft tissue (g oyster⁻¹)</td>
<td>1.5 ± 0.2</td>
</tr>
</tbody>
</table>

³ BCF: The bioconcentration factor corresponds to the ratio: oyster diuron concentration/seawater diuron concentration.

² Theoretical FR: The theoretical filtration rate was calculated with the formula of Powell et al. (1992): FR = (SL⁻⁰.⁹₆ × T¹.⁹⁵)/2.⁹⁵, where FR is the filtration rate (ml filtered per oyster and per min), SL (in cm) is shell length and T is temperature (in °C). Then our result was expressed in L filtered per oyster and per hour.

---

![Fig. 1. Effect of diuron (1 µg L⁻¹) on enzymatic activities (U mg prot⁻¹) in the Pacific oyster *Crassostrea gigas*. (a) laccase-type phenoloxidase and (b) superoxide dismutase activities. White bars correspond to the control and black bars correspond to the treatment condition. Mean ± SD, *p < 0.05, **p < 0.01, ***p < 0.001.](image-url)
Laccase gene was significantly repressed at 2 h (0.40 ± 0.10; Fig. 2). The overexpression observed at 6 h (2.04 ± 0.29), could be an internal response to compensate the underexpression that occurred at 2 h. However, at 24 h the expression was repressed again (0.51 ± 0.33), suggesting that overcompensation observed at 6 h was not sufficient to abolish diuron effects on gene expression. A down-expression of Mpeg1 (0.23 ± 0.24), LBP/BPI (0.21 ± 0.22) and TIMP (0.63 ± 0.15) was also observed after 2 h of exposure. Since the products of these genes appear as important factors in innate immunity in different species and have been reported in marine molluscs (Mah et al., 2004; Mitta et al., 2005), a down-regulation of these genes may lead to a depletion of the defence activities against pathogens. Similarly to the present study, a down-regulation of the LBP/BPI and TIMP gene expression has been previously reported in C. gigas exposed to a mixture of eight pesticides (including diuron) at environmentally relevant concentrations over a 7-d period (Gagnaire et al., 2007). However, it is important to notice that in the present study, the effects on genes such as Mpeg1, LBP/BPI and lysozyme disappeared at 6 h of exposure. Similarly a down-regulation at 6 h was observed for MyD88 gene (0.36 ± 0.39), implicated on cell signalling (Wiens et al., 2005), but the effects disappeared at 24 h. Thus, the results of the present study suggest that diuron can exert rapid effects on the expression of genes implicated in immune defence mechanisms in C. gigas, but also that rapid acclimatization responses can take place in the organism.

Figure 3. Effect of a mixture of herbicides (diuron at 5 μg L⁻¹, isoproturon at 5 μg L⁻¹) and pharmaceuticals (ibuprofen at 5 μg L⁻¹) on (a) phagocytosis (% in) in the haemolymph (and b) catecholase-type phenoloxidase activity (in U mg prot⁻¹) in the plasma of the Pacific oyster Crassostrea gigas. White bars correspond to the control and black bars correspond to the treatment condition. Mean ± SD; n = 12 (3 replicates of 4 pools of 20 oysters), N = 4 experiment replicates. Statistical difference between control and treatment at each time with p < 0.05.

4. Conclusions

Early effects of a single herbicide (diuron) were observed in gene expression and enzyme activities of proteins implicated in immune responses in oysters, e.g. SOD and laccase. A mix of herbicides and pharmaceuticals (diuron, isoproturon, ibuprofen) also exerted early effects on plasmatic enzyme activities and haemocytic phagocytosis activity. These results are in agreement with previous studies where short-term exposures of herbicides exerted an effect on aquatic organisms (e.g. Saglio and Triassie, 1998; Bretaud et al., 2000), giving an insight of the potential effect of contaminants on organisms in the natural environment. An alteration in molecular, biochemical and cellular parameters could be related to the inability of the animal to manage other stressful conditions including the presence of pathogens. Therefore, coupling this type of short-term contamination approach to a pathogen challenge appears necessary (e.g. Gagnaire et al., 2007) in order to evaluate, at the physiological level, the effect of contaminants on immune defences in marine bivalves such as the Pacific oyster C. gigas.

Acknowledgments

This study was supported by a Ph.D grant from the Conseil Général de la Charente-Maritime for A. Luna-Acosta. The Conseil Régional de Poitou-Charantes is acknowledged for financial support through the research project ‘POLERON’ (Modifications chimiques de pollutants organiques dans le bassin de Marennes-Oléron, toxicité des produits de dégradation sur l’huitre creuse), N. Tapie for scientific advices on diuron and isoproturon analyses in water samples, and the laboratory IDHESA for diuron and DCPU analyses in water samples and oyster soft tissues.

References


