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Effects of Pesticides on Marine Bivalves: What Do We Know and What Do We Need to Know?

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1. Introduction

Estuaries are among the most productive environments in the world, by serving as feeding grounds, as nurseries for juvenile economically important fish and invertebrate larvae, and by providing shelter for many types of benthic organisms. However, they also rank among the most contaminated areas.

Among pollutants, pesticides have become more common in estuarine areas. They are mainly introduced into rivers via run-off and then may enter marine areas, particularly estuarine and coastal zones. These pollutants may have major ecological consequences and could endanger organismal growth, reproduction or survival (Banerjee et al., 1996).

Among important organisms inhabiting estuarine zones, bivalves are sessile and filter-feeder species, able to accumulate contaminants in their tissues. Moreover, bivalve farming is an ancestral activity all around the world. It has been expanded and intensified in the last century and represents a major economic activity in various countries. In the majority of cases, bivalve species are reared in estuaryine zones, continually impacted by pollutants including pesticides. Natural and man-made toxicants enter marine ecosystems by various routes, including direct discharge, land run-off, atmospheric deposition, *in situ* production, abiotic and biotic movements and food-chain transfer.

Pollutant run-off into the ocean represents a potential threat to marine organisms, especially bivalves living in coastal environments. In this context, bivalve molluscs such as mussels and oysters have been postulated as ideal indicator organisms because of their wide geographical distribution, and sensitivity to environmental pollutants. They filter large volumes of seawater and may therefore accumulate and concentrate contaminants within their tissues (Ramu et al., 2007; Bernal-Hernandez et al., 2010). As an example, the level and extent of organic contaminants along the Korean coast has been estimated through a mussel watch program (Choi et al., 2010). Moreover, development of techniques allowing effect analysis of pollutant on bivalve biology may lead to the development of diagnosis tools adapted to analyze pollutant transfer towards estuarine areas.

A pesticide is defined as a chemical substance used for killing pests, as insects, weeds or rodents. Pesticids are often classified by the type of organism: fungicides, herbicides, insecticides, nematocides and rodenticides. They are used especially in agriculture and around areas where humans live. Some are harmful to humans, either from direct contact or

as residue on food, or are harmful to the environment because of their high toxicity, such as DDT (which is now banned in many countries). All pesticides act by interfering with the target species normal metabolism. Some inadvertently may affect other organisms in the environment, either directly by their toxic effects or via elimination of the target organism.
By World War II, only about 30 pesticides existed. Dichloro-Diphenyl-Trichloro-ethane (DDT) was recognized as an insecticide until 1942. Other pesticides soon followed, such as chlordane and endrin. Poison gas research in Germany yielded the organophosphorus compounds, the best known of which is parathion. Further research yielded hundreds of organophosphorus compounds including malathion. The Environmental Protection Agency (EPA) estimates that the use of pesticides doubled between 1960 and 1980 with over 1.8 billion kilograms a year used today worldwide. In most countries, pesticides must be approved for sale and use by a government agency. However pesticide regulations differ from country to country. To deal with inconsistencies in regulations among countries, an International Code of Conduct on the Distribution and Use of Pesticides has been adopted in 1985 under the umbrella of the United Nations Food and Agriculture Organization and then updated several times.
Bivalves in culture may be affected by the presence of pesticides, potentially increasing their susceptibility to a wide range of infectious diseases. The effects of environmental contaminants may result from direct toxic actions on tissues or cells or from alterations of the homeostatic mechanisms including the immune system (Coles and Pipe, 1994; Carajaville et al., 1996). It has been suggested that bivalves may be weakened in relation to the presence of these pollutants. It has been shown in several vertebrates and invertebrates that pesticides are capable of diminishing immune defenses and/or of modifying genomes. They may render animals more vulnerable to infectious diseases (Ross et al., 1996; Gagnaire et al., 2007).
Although pesticide effects on marine bivalves have been already studied in bivalves, a few of reviews summarizing their different effects are available. In this context, one of the major objectives of this chapter relies on summarizing existing body of data on pesticide detection in marine environments and their effects on bivalve physiology including genotoxicity and immunotoxicity. Moreover, another aim of the present chapter is to identify the topics on which scientific data are needed in order to better understand the complex interactions between pesticides, environment, marine bivalves and their infectious agents.

2. Pesticides in the marine environment

Aquatic habitats are particularly subjected to contamination by pesticides, via run-off, leaching, spray drift or accidental spills. Pestices contamination of the marine environment has been and is monitored worldwide through analysis of water, sediment and marine species samples in order to elucidate the contamination status, distribution and possible pollution sources and to assess the risks on aquatic organisms and human.
Levels of pesticides measured in superficial waters generally range below lethal concentrations for aquatic species. However, sublethal adverse effects may result from exposure to these products at environmentally relevant concentrations.
Buisson et al. (2008) reported recently results about the monitoring of contamination levels in the Pacific cupped oyster, *Crassostrea gigas*, reared in Normandy (France). Six herbicides were detected in seawater for a total of 15 herbicides. Although the most estuarine sites showed

relatively high values in sea water samples, no pesticides were detected in the flesh of collected oysters (Buisson et al. 2008). At the contrary, Monirith et al. (2003) reported that all samples collected from all the sampling sites demonstrated the detection of organochlorines (OCs) with considerable residue levels of p,p(')-DDT and alpha-hexachlorocyclohexane (HCH) in mussels collected from coastal waters in the Asia-Pacific region.

Pandit et al. (2006) conducted a multi-compartment monitoring (sediment, water and marine species) of residue levels of pesticides in coastal marine environment of Mumbai in India. The total HCH concentration in sediment samples varied from 3.8 to 16.2 ng g^{-1} lindane (gamma-HCH) contributing almost 55% to the total HCH. The concentration of total HCHs in seawater ranged from 0.16 to 15.92 ng L^{-1} and concentrations of total DDT varied from 3.01 to 33.21 ng L^{-1}.

The presence of herbicides, such as diuron, has been also detected in many aquatic ecosystems worldwide. For instance, in France, diuron has been detected in surface waters with concentrations ranging from 0.05 µg L^{-1} to 20.3 µg L^{-1} (Léonard, 2002). In Atlantic bays and estuaries, concentrations up to 0.7 and 1 µg L^{-1} have been reported (Munaron et al., 2006).

Due to its toxicity, the use of diuron has been forbidden by French policies since 2008. Diuron and isoproturon are also included in the list of priority to contaminants of the EU Water Framework Directive (European Comission, 2000). However, it is well-known that some herbicides may persist in the environment even if their use has been banned, e.g. atrazine (EEA, 2000). A recent study reported the presence of diuron on French aquatic environments, confirming its persistence despite restriction policies (Pesce et al., 2010).

Diuron metabolites such as DCPU (N-(3,4 dichlorophenyl)-urea), DCPMU (N-(3,4 dichlorophenyl)-N-(methyl)-urea) and DCA (3,4-dichloroaniline) have also been detected in aquatic environments (Munaron et al., 2006). Studies on biofilms have reported DCPMU to be more toxic than DCA (Pesce et al., 2010). However, the principal product of degradation of diuron reported in the literature is DCA, which has shown to be more toxic for various organisms of higher trophic levels, such as crustacean, insects and fish (Giacomazzi & Cochet, 2004). This product exhibits higher toxic effects than the parent diuron, and can affect organisms, such as crustacean with low concentrations (1 µg.$^{-1}$, Giacomazzi and Cochet, 2004).

Different compounds including herbicides and their metabolites (Lanyi & Dinya, 2003; Sorensen et al., 2003; Vargha et al., 2005) are detected simultaneously in aquatic environments, suggesting that experimental approaches with toxicant mixtures are needed. Studies with diuron and its metabolites have shown additive, enhanced, antagonistic or independent effects (Knauert et al., 2008; Pesce et al., 2010; Neuwoehner et al., 2010). Thus, there is still a lack of data concerning the toxicity and effects of pesticide metabolites on bivalves, whether individually or in mixture with their parent compounds.

3. Lethal effect of pesticides on marine bivalves

Several studies have been conducted in various marine bivalve species in order to define LC_{50} for different pesticides including DDT, diuron, atrazine or lindane.

Chung et al. (2007) evaluated the sensitivity of the juvenile hard clam, *Mercenaria mercenaria*, to DDT (organochlorine pesticide) by exposure to contaminated sediments (10 day) and seawater (24-h). The aqueous LC_{50} (24h) value was defined at 0.61 mg L^{-1} DDT. and the LC_{50} (10 day) value for sediment toxicity tests was 5.8 mg kg^{-1} DDT. The authors concluded that

based on comparisons to toxicity data for other marine species, the hard clam, *Mercenaria mercenaria*, is one of the more sensitive species to contaminants.
No significant mortalities were reported after two months of exposition to 100 mg L^{-1} of diuron while with 100 µg $L^{-1}$of isoproturon, 60% of mortalitites were observed (Moraga & Tanguy, 2000). Isoproturon has shown to be present at lower concentrations than diuron on aquatic environments (Munaron, 2006).
Lawton et al. (2010) studied the effects of atrazine on the hard clam, *Mercenaria mercenaria,* in aqueous and sediment laboratory assays. Through an acute aqueous bio-assay, these authors determined a 96h LC_{50} for the juvenile clams at 5608 µg L^{-1}. They conducted also a chronic aqueous bio-assay at low atrazine concentrations and a chronic sediment bioassay over a 10 day exposure period to examine both lethal and sublethal (dry mass, shell size, and condition index) endpoints (Lawton et al., 2010). On the basis of their results, the authors suggested that atrazine is not directly toxic to *M. mercenaria* at environmentally relevant concentrations.
Bouilly et al. (2003) reported similar results for the Pacific cupped oyster, *Crassostrea gigas*:, in adult and juvenile animals subjected to 2 different concentrations of atrazine (46.5 nM and 465 nM). These authors did not observed any effect on mortality.
In vivo in laboratory assays testing 10 different concentrations (0 to 10 mg L^{-1}) of lindane (gamma-hexachlorocyclohexane [gamma-HCH]) allowed to define the median lethal concentration (LC_{50}) after a 12 day period as 2.22 mg L^{-1} in the Pacific cupped oyster, *Crassostrea gigas* (Anguiano et al., 2006). Lindane and isoproturon tested at concentrations of up to 10 mg L^{-1} for a 9 day esposure period showed negative effects on survival and growth of Pacific cupped oyster, *Crassostrea gigas,* larvae (Hiss & Seaman, 1993).
Domart-Coulon et al. (2002) assessed the acute cytotoxicity of an organic molluscicide, Mexel-432, used in antibiofouling treatments in industrial cooling water systems on primary cell cultures derived from 2 marine bivalve species, the Pacific cupped oyster, *Crassostrea gigas,* and the carpet clam, *Ruditapes decussatus*.

4. Genotoxicity in marine bivalves

Results reported by Jha et al. (2002) suggested that tributyltin oxide is both cytotoxic (proliferation rate index) and genotoxic (sister chromatid exchanges and chromosomal aberrations) to embryo-larval stages in the blue mussel, *Mytilus edulis*.
Bouilly et al. (2003) researched potential genotoxic effects of atrazine in the Pacific cupped oyster, *Crassostrea gigas*. Adult and juvenile oysters were subjected to 2 concentrations of atrazine: 46.5 nM, representing a realistic potential exposure (peak value found in polluted environment) and 465 nM. These authors reported significant differences in aneuploidy after atrazine treatments in comparion to control: 9% in control oysters, 16% at 46.5 nM and 20% at 465 nM atrazine. Similar aneuploidy levels were observed in adults and juveniles.
Bouilly et al. (2007) showed that the herbicide diuron induced also aneuploidy in adult Pacific cupped oysters after a 11 week exposure period at 300 ng L^{-1} and 3 µg L^{-1}. The induced aneuploidy observed appeared to be transmitted to the next generation as offspring exhibited significantly higher aneuploidy levels when their parents had been exposed to diuron (Bouilly et al., 2007).
Genotoxicity induced by lindane at 0.7 mg L^{-1} was also demonstrated in Pacific oyster, *Crassostrea gigas*, hemocytes after a 12 day contamination period (Anguiano et al., 2006).

Wessel et al. (2007) investigatied embryotoxic and genotoxic effects of the organochlorine pesticide, endosulfan, on *Crassostrea gigas* embryos. Embryotoxicity and genotoxicity in terms of DNA strand breaks were observed for 300 nM and 150 nM.
Siu et al. (2008) used green-lipped mussels (*Perna viridis*) in order to study the bioaccumulation of organic pollutants, including organochlorine pesticides. Micronuclei and DNA strand breaks were observed in mussels transplanted in different sites and collected after 4, 8, 12, 16 and 30 days.
Revankar and Shyama (2009) explored genotoxic effects of monocrotophos, an organophosphorous pesticide, at different time periods, 2, 3, 7 and 14 days. A significant increase of micronuclei in a dose dependant manner was observed indicaring possible chromosomal damages induced by monocrotophos.

5. Immunotoxicity in marine bivalves and susceptibilty to infectious diseases

The impact of contaminants and other environmental factors on the immune system of bivalves is an issue of ecological and economical concern, because it may result in clinical pathology and disease, by increasing the susceptibility of affected organisms to pathogens.
Contaminants known to induce alterations of immune functions including pesticides (Vial et al., 1996; Banerjee et al., 1996; Banerjee et al., 2001) are present in almost all coastal areas. Among physiological processes possibly disturbed by pollutants, the immune system is likely to be one of the more sensitive (Baier-Anderson & Anderson, 2000; Fournier et al., 2000).
In contrast to the vertebrate immune system which consists of innate and acquired mechanisms, invertebrate immunity relies only on innate defence mechanisms. The fact that invertebrates represent more than 90% of the total number of species living on earth demonstrates the efficiency of their «primitive» host defence systems. It becomes more and more obvious that some of these innate mechanisms are conserved in invertebrates and vertebrates (Medzhitov et al., 1997; Means et al., 2000). Thus, the fundamental importance of the toxically-induced modulation of non-specific immune functions has increasingly been perceived.
Bivalve immunity is mainly supported by hemocytes and participate directly in eliminating pathogens by phagocytosis (Cheng, 1981; Feng, 1988). In addition, hemocytes produce compounds including lysosomal enzymes and antimicrobial molecules which contribute to the destruction of pathogens (Coles & Pipe, 1994).
Investigating the effects of pesticides on hemocyte functions and immunity in bivalves has been based on the monitoring of several biomarkers (Pipe & Coles, 1995). As an example, Gagnaire et al. (2006) tested the effect of 23 pollutants on Pacific cupped oyster haemocytes by flow cytometry monitoring different cell parameters and demonstrated that 3 pesticides (2,4D, paraoxon, and chlorothalonil) induced a modulation of hemocyte activities. However, biomarkers used differ very often between published studies.
Triforine, a fungicide, induced decreased hemocyte viability in the eastern oyster, *Crassostrea virginica* (Alvarez & Friedl, 1992). Cytotoxic effects were also observed in adult Pacific cupped oyster, *C. gigas*, hemocytes: the mean cell viability was significantly decreased at 1.0 mg L^{-1} of lindane (gamma-hexachlorocyclohexane) after 12 day exposure period (Anguiano et al., 2006). Alteration in cell viability was also reported in the blue mussel, *Mytilus edulis*, exposed to 0.1 mg L^{-1} azamethiphos, an organophosphate pesticide

(Cantry et al., 2007). Moreover, a mix of herbicides containing atrazine, diuron and isoproturon showed an effect on *C. gigas* hemocyte aggregation (Auffret et Oubella., 1997).
Chlordan, an insecticide, demonstrated effects on *C. virginica* hemocyte phagocytosis at 250 µM *in vitro* (Larson et al., 1989). A decreased phagocytosis activity was observed after a triforine exposure in the eastern oyster, *C. virginica* (Alvarez and Friedl, 1992). A pesticide mixture (alachor, metolachlor, terbutylazine, glyphosate, diuron, atrazine, carbaryl and fosteyl aluminium) representative for surface waters of the Marennes-Oleron Basin (Charente Maritime, France, 0.25 nM to 4 nM) induced a decrease of phagocytic activity (Gagnaire et al., 2007). Moreover, Cantry et al. (2007) reported a decrease in phagocytic index in the blue mussel, *Mytilus edulis*, after a short exposure to 0.1 mg L^{-1} azamethiphos. This result suggests that azamethiphos can modulate haemocyte function in mussels at environmentally relevant concentrations.
At the contrary, Gagnaire et al. (2003) reported no effect on cell viability, cell cycle and cellular activities except for peroxidase activity for Pacific cupped oyster haemocytes exposed to atrazine in *in vitro* and *in vivo* assays.
Pentachlorophenol decreased the production of ROS by the inhibition of NADPH production in the eastern oyster, *Crassostrea virginica* (Baier-Anderson & Anderson, 1996). Dieldrin, tested *in vitro* on *C. virginica* hemocytes induced a decrease of chemiluminescence at concentrations ranging from 3 to 300 µM (Larson et al., 1989). Hemocytes of *C. virginica* exposed to chlorothalonil (fungicide) for 20 h at concentrations between 4 nM and 2 µM showed no modification of cell mortality and phagocytosis, but a decrease of ROS production (Baier-Anderson & Anderson, 2000).
In the past decades, the emergence of infectious diseases has been reported in marine species and disease outbreaks have also increased (Harvell et al., 1999). According to Snieszko (Snieszko, 1974), the development of an infectious disease results from an unbalance between the host and the pathogen due to external factors (including pollutants) and/or internal factors of both protagonists (virulence of the pathogen, susceptibility of the host). Animals presenting impaired defence mechanisms may be more susceptible to infectious diseases.
Demonstration of the relationship between pollution and increase of susceptibility to infectious diseases exist in vertebrates (Fournier et al., 1988; Van Levoren et al., 2000; Jepson et al., 2005), a few of studies was carried out in invertebrates (Galloway & Depledge, 2001). Rare studies have attempted to link contaminant presence and susceptibility to infectious diseases in marine molluscs and demonstrated harmful effects of pollutants in bivalves.
Contamination of the eastern oyster, *Crassostrea virginica*, by polluted sediment and tributyltin increased the intensity of *Perkinsus marinus* infection, but no cellular or humoral parameters were modulated (Anderson et al., 1996; Chu et al., 2002). Anderson et al. (1981) demonstrated previously that the hard clam, *Mercenaria mercenaria,* exposed to PCP were unable to kill injected bacteria. Kim et al. (2008) studied the relationship of parasite detection to contaminant body burden in sentinel bivalves through a 'Mussel Watch' Program. These authors showed that correlations between parasites/pathologies and pesticides were frequent in mussels and oysters (Kim et al., 2008).
The Pacific cupped oyster, *Crassostrea gigas*, has been also used to evaluate the impact of a pesticide mixture (atrazine, glyphosate, alachlor, metolachlor, fosetyl-alumimium, terbuthylazine, diuron and carbaryl) on some immune-related parameters and to demonstrate a relationship between infectious diseases, defence capacities and pollutants.

Indeed, a mixture of 8 pesticides reduced phagocytosis on hemocytes and enhanced susceptibility to *Vibrio splendidus* (Gagnaire et al., 2007). Pacific cupped ysters were exposed over a 7 day period to the mixture of pesticides. The pesticides were selected on the basis of spread amounts in the Marennes-Oleron Basin (Charente-Maritime, France), one of the most important oyster producing areas in France (Léonard, 2002; Munaron et al., 2006). Moreover, a down-regulation of the LBPB/BPI, TIMP and lysozyme genes were reported in Pacific oysters exposed to the mixture of 8 pesticides (Gagnaire et al., 2007).

6. Other effects of pesticides on marine bivalves

The evaluation of acetylcholinesterase activity in marine organisms has been and is at present time extensively used as a biomarker of exposure to neurotoxic agents such as organophosphorus and carbamate pesticides. Indeed, organophosphorous compounds and carbamates including paraoxon and carbaryl are known to inhibit acetylcholinesterase (AChE) and carboxylesterase (CE) (Cooreman et al., 1993).

Paraoxon inhibited the activity of AChE in the hepatopancreas of the blue mussel, *Mytilus edulis,* in vitro at concentrations ranging from 1 µM to 1 mM (Ozretic and Krajnovic-Ozretic, 1992). Inhibition by carbaryl was less distinct. AChE from *M. edulis* hemocytes was inhibited in vitro by 0.1-3 mM paraoxon, eserine and DFP (Galloway et al., 2002). Cantry et al. (2007) showed that exposure of the blue mussel, *M. edulis*, to 0.1 mg L^{-1} azamethiphos, an organophosphate pesticide used to combat sea lice infestations in farmed salmonids, for periods of up to 24h caused a significant reduction in acetylcholinesterase activity in both the haemolymph and the gill. However, cholinesterases found in the Pacific cupped oyster, *Crassostrea gigas,* appeared to be insensitive to organophosphorous insecticides (Bocquene et al., 1997).

Anguiano et al. (2006) showed that after a 4 h exposure to lindane (gamma-hexachlorocyclohexane), filtration rates of adult Pacific cupped oysters, *Crassostrea gigas*, were significantly reduced compared with controls at concentrations of 0.3 and 0.7 mg L^{-1}. However, a short term exposure of the blue mussel, *Mytilus edulis*, to azamethiphos did not change the feeding rate (Chantry et al., 2007). Studies carried out in adult Pacific cupped oysters revealed that diuron induces partial spawning and atrophy of the digestive epithelium after 1 week of exposure at 1 µg L^{-1} (Buisson et al., 2008).

Greco et al. (2010) investigated effects of a mixture of herbicides on the physiological status of the soft clam, *Mya arenaria*. Clams were exposed for 28 days to 0.01 mg L^{-1} of a pesticide mixture: dichlorophenoxyacetic acid (2,4-D), 2-(2-methyl-4-chlorophenoxy) propionic acid (mecoprop), and 3,6-dichloro-2-methoxybenzoic acid (dicamba). Although a gradual sexual maturation was reported in both sexes during the course of the experiment, females demonstrated a higher sensitivity to pesticides compared to males.

Favret and Lynn (2010) during the course of a study monitoring sperm viability by flow cytometry in the eastern oyster, *Crassostrea virginica*, after exposure to a pesticide (Bayluscide) reported effects on mitochondrial membrane potential and plasma membrane in the sperm. Buisson et al. (2008) studied impact of pesticides in the cupped Pacific oyster, *C. gigas,* and reported partial spawning and atrophy of the digestive tubule epithelium in relation to pesticides.

A study with a mix of herbicides containing atrazine, diuron and isoproturon revealed effects on gene expression in the Pacific cupped oyster, *Crassostrea gigas* (Tanguy et al., 2005). Gagnaire et al. (2007) studied also the impact of pesticides on *C. gigas*, monitoring

gene expression in hemocytes by real-time PCR. The expression of genes involved in *C. gigas* hemocyte functions was up-regulated in pesticide-treated oysters compared to untreated oysters after a bacterial challenge. The authors hypothesized that gene over-expression could lead to an injury of host tissues, resulting in higher mortality rates.
Collin et al. (2011) explored under experimental conditions the effects of a cocktail of three pesticides (lindane, metolachlor and carbofuran) on physiological functions of the Pacific cupped oyster, *C. gigas,* using the suppression subtractive hybridisation technique. The authors reported a site and organ-specific response to the pesticides. Effects of imidacloprid and thiacloprid, 2 neonicotinoid insecticides, at transcriptomic and proteomic levels in the marine mussel, *Mytilus galloprovincialis*, were also reported by Dondero et al. (2010).
Tlili et al. (2010) compared the size-distribution of the intra-sedimentary bivalve *Donax trunculus* collected in two sites in Tunisia, a polluted site and a comparatively reference site The auhors showed that the size-distribution from the polluted site consisted of 4 cohorts.whereras 5 cohorts were observed in the comparatively reference site. Moreover, the mean total length size and the growth rate of cohorts were significantly reduced in the impacted site compared to the reference site. These results suggest effects of pollutants on marine bivalves at a population level with an ecological relevance.
Pariseau et al. (2010) studied haemic neoplasia in the soft-shell clam *Mya arenaria,* in relation to exposure to fungicides, chlorothalonil and mancozeb, without demonstrating a link.

7. Conclusions and perspectives

The results obtained through the cited studies alerts to the negative effects of pesticides in bivalves and may be important to initiate and implement programs to protect the bivalve estuaries. These studies bring scientific evidence regarding the biological effects of pesticides on the animals inhabiting contaminated estuaries and the potential effect of the contaminates on shellfish and on human health if the seafood is consumed.
The great variability of response (depending on duration of exposure, toxicant concentration, test species or experimental conditions) is a reminder that the effects of pollutants on the marine environment cannot be assessed by simple methods (e.g. short-term bioassays with one or two test species). As an example, Greco et al. (2010) investigating effects of a mixture of herbicides on the physiological status of the soft clam, *Mya arenaria*, showed that in clams kept at 18°C, pesticides appeared to induce minor effects compared with animals kept at 7°C. They concluded that increased temperature may modify the response of *Mya arenaria* to pesticides.
It is recognized that bivalve habitats may differ in environmental parameters. Thus, animals may be exposed to numerous variables that include other pollutants, different temperatures, salinities, amounts of dissolved oxygen, and changes in pH. In this context, a better understanding of the possible interactions between pesticides and other abiotic environmental factors (temperature, salinity,) and biotic factors associated with the physiological status of bivalves is necessary.
As it is possible to evaluate only a limited number of environmental factors in laboratory assays, it appears difficult to investigate all of the potential environmental factors that also may affect bivalve physiology.
A lot of studies concerning effects of pesticides in bivalves have been carried out using high pollutant concentrations. However, levels of pesticides measured in superficial waters generally range below lethal concentrations for aquatic species. In this context, sub-lethal

adverse effects need to be more documented through experiments carried out using pesticides at environmentally relevant concentrations.

Among studies focused on pesticides, most of them have been carried out by exposing animals to relatively long periods of time (Auffret et Oubella., 1997; Tanguy et al., 2005; Bouilly et al., 2007, Buisson et al., 2008) giving an insight on the effects of chronic exposures on physiological functions of the organism.

Nevertheless, it is well known that in natural waters, uneven concentrations of pesticides are found in the water mass because of different factors such as seasonal agricultural practices, weathering processes and peak concentrations are often found in the aquatic environment for short periods of time (Munaron, 2006; Hyne et al., 2008). Thus, long-term studies may be not so predictive of what could happen on a natural environment. Short-term exposures of herbicides under laboratory controlled conditions have shown to exert an effect on aquatic organisms (Bretaud et al., 2000; Saglio et al., 2002). They can give an insight of the potential effect of contaminants in organisms in the natural environment.

In order to assess the impact of persistent pollutants on the marine ecosystem a suite of biomarkers are being extensively used worldwide (Ozretic & Krajnovic-Ozretic, 1992; Lowe & Fossato, 2000). These biomarkers are being used to evaluate exposure of various species of sentinel marine organisms (e.g. mussels, clams, oysters.) to and the effect of various pesticides using different molecular approaches (Wong et al., 1992; Cajaraville et al., 1996; Galloway et al., 2002).

As an example, Matozzo et al. (2010) developed a multibiomarker approach in order to assess effects of environmental contaminants in the Manila clam, *Ruditapes philippinarum*, collected in 8 sites of the Lagoon of Venice (Italy). The authors used several biomarkers includig total haemocyte count and lysozyme activity, acetylcholinesterase activity in gills, vitellogenin-like protein levels in both digestive gland and cell-free haemolymph, and survival-in-air widely used to evaluate general stress conditions. In addition, different pollutants were also measured in collected animals. Results showed that the selected integrated approach between biomarkers and chemical analyses is a useful tool in biomonitoring (Matozzo et al., 2010).

Different compounds including different pesticides have been found simultaneously in aquatic environments, underlining that experimental approaches with toxicant mixtures are needed. Most of the studies evoked before, have been carried out in adults, but juvenile organisms are known to be generally more sensitive to environmental stress than adults (Perdue et al., 1981). Additional residue-effects data on sublethal endpoints, early life stages, and a wider range of legacy and emergent contaminants will be needed.

Finally, research in ecotoxicology needs also to fill the gap existing between sub-organismal responses to toxicants and effects occurring at higher levels of biological organisation (e.g. population) (Tlili et al., 2010).

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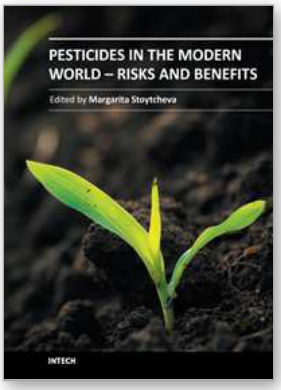

Pesticides in the Modern World - Risks and Benefits

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This book is a compilation of 29 chapters focused on: pesticides and food production, environmental effects of pesticides, and pesticides mobility, transport and fate. The first book section addresses the benefits of the pest control for crop protection and food supply increasing, and the associated risks of food contamination. The second book section is dedicated to the effects of pesticides on the non-target organisms and the environment such as: effects involving pollinators, effects on nutrient cycling in ecosystems, effects on soil erosion, structure and fertility, effects on water quality, and pesticides resistance development. The third book section furnishes numerous data contributing to the better understanding of the pesticides mobility, transport and fate. The addressed in this book issues should attract the public concern to support rational decisions to pesticides use.

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