Residues of pesticides in aquatic organisms

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SUMMARY

Pesticides are designed to kill or adversely affect living organisms, thus their use may cause harmful ecological effects. It is therefore important to define the chemical properties, environmental fate, and impact of pesticides on non-target species in order to protect wildlife and humans from pesticide risks. Among the different environments which are potentially under threat by the use of pesticides, this paper will focus on coastal and estuarine areas, and will distinguish between persistent and non-persistent pesticides. Several case studies and their toxicological implications are presented.

KEY-WORDS: pesticides - bioaccumulation - cholinesterases - mussels - fish.

Introduction

Pesticides are synthetic or natural substances intended for preventing, destroying, repelling, or mitigating any pest. The term applies then to insecticides, herbicides, defoliants, fungicides, antifouling agents, and various other substances designed to kill or adversely affect living organisms. Obviously, they are useful to society because of their ability to kill potential disease-causing organisms and control insects, weeds, and other pests. However, by their nature, they are also expected to cause harmful environmental effects.

Approximately 800 active substances are registered for pesticide use in Europe, although only a small fraction represents the major use, and it has been detected in environmental samples. Agriculture is the main user of pesticides, it accounts for more than three-fourths of the volume used annually. Other users are industry, public health, and home and gardening. Overall, pesticide use — measured by mass of active ingredient — appears to have been decreasing in most EU countries over the past two decades (THYSSEN, 1999). However, consumption in terms of mass, does not necessarily reflect environmental impact, as more active and more specific substances are being developed. Additionally, even though the production and use of some compounds (e.g. DDT, lindane, organometallic biocides), has been reduced or prohibited for decades, residues are still present in various environmental compartments.

Generally, the type of pesticide used varies with geographical area, viz. humid countries such as Mid-European and the Scandinavian countries use mainly herbicides for weed control problems, while Mediterranean countries are consuming pesticides for agricultural fungus control. Hence, different countries are facing to different environmental problems. Overall, during pesticide application, less than 5% of the pesticide applied can reach its intended target, because of losses from volatilisation or washing-out, which further reinforces the need of assessing the environmental impact of these compounds. To do this, it is important to know its che-
mical properties, environmental fate, and impact on non-target species in order to protect wildlife and humans from pesticide risks.

Among the different environments which are potentially under threat by the use of pesticides, this paper will focus on coastal and estuarine areas, where the co-existence of intense agriculture practices, fishing and often aquaculture facilities, makes necessary the development of effective monitoring tools.

**Persistent pesticides**

Organisms are generally facing chronic (long term) exposure to persistent pesticides, but health effects are varied and frequently difficult to attribute to exposure. Nonetheless, certain pathological observations including eggshell thinning in various bird species, skeleton malformation in seals and otters, hormonal (endocrine) or reproductive disturbances in various species, are found to coincide with pesticide residues in organism tissue. The most widely distributed group of persistent pesticides are the chlorinated ones, namely DDT and its main metabolites (DDE and DDD), hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs), aldrin, dieldrin, endrin, toxaphene, and heptachlor. These compounds were produced and used in vast quantities all over the world in 1940s. Twenty years latter there was an alarming decline in the population of several seabirds and marine mammals, which was linked to exposure to organochlorines, and this led many countries in the northern hemisphere to ban or strictly regulate the use of such compounds in the 1970s. In 1997, the Governing Council of UNEP took a major step against these persistent compounds with the preparation of an international legally binding instrument for implementing action on an initial list of 12, which is known as the Stockholm Convention.

Fish inhabiting coastal areas have often been proposed as sentinels for monitoring pollutants of land-based origin because they may concentrate indicative hydrophobic compounds in their tissues, directly from water through respiration and also through the diet. The red mullet (*Mullus barbatus*) is a benthic and territorial fish of commercial interest in the NW Mediterranean region, which has been used in several studies of coastal pollution monitoring (PORTE and ALBAIGÉS, 1993; UNEP, 1997). In the present work, fish were collected from 10 stations along the Spanish and Corsica and Sardinia coasts (Fig. 1), to grant a wide coverage of the area encompassing large pollution gradients. A piece of dorsal muscle tissue was homogenised with anhydrous Na$_2$SO$_4$, Soxhlet-extracted, cleaned-up with conc. H$_2$SO$_4$, and analyzed for chlorinated compounds by gas chromatography coupled with an electron capture detector (GC-ECD) (SANCHEZ et al., 1993).

The major pesticides identified in fish tissues were p,p'-DDT, their metabolites p,p'-DDE and p,p'-DDD, and hexachlorobenzene (HCB). Levels of total DDTs recorded in the muscle of red mullets are indicated in Figure 2. The highest concentrations (up to 230 ng/g w.w.) were found in the area of influence of Barcelona (stations 2 - 4), whereas the lowest (< 1 ng/g w.w.) were found in Corsica and Sardinia Islands (stations 9 and 10). Tissue concentration ranges may then be considered as representative of pristine, medium and highly contaminated areas, and they are consistent with those found in sediments (TOLOSA et al., 1995), supporting the close relationship of the red mullet uptake with the benthic environment.

The elevated DDTs concentration detected in stations 2-4 were mainly due to the isomer p,p'-DDE, which accounted for 80-90 % of total DDT along the Spanish coast, but it only represented the 30-42 % of total DDT in the French area. Considering that all organisms exhibit a similar metabolic activity, the large occurrence of DDE in fish tissue has been attributed to a specific input of this compound, which is a subproduct of the synthesis of the pesticide dicofol, that is produced in the area. It is interesting to notice that levels of DDTs detected in the Spanish coast are similar to those found 10 years earlier by PORTE (1990). At that time, DDTs residues in red mullet were in the range of 17 to 172 ng/g w.w. according to the proximity to the hot spots. This may well reflect the high persistence of DDT residues associated to sediments in the region. Nonetheless, maximum values reported for DDT are lower than the permissible limits (1 µg/g) for seafood consumption adopted in certain countries (WHO/UNEP, 1995).

Measurable amounts of HCB were also found in all samples (Figure 2), with levels along the Spanish coast slightly higher than those found in the French one. This pollution problem is probably related to the existence of an industrial source in the vicinity of station 1, upstream the Ebro River (GRIMALT et al., 1988).

Bioaccumulation of persistent pesticides in a well reported phenomena, and it will continue as a result of environmental redistribution processes for a long time after a substance has been banned. Figure 3 shows the concentration of total DDTs in muscle of different NW Mediterranean fish species: *Salmo trutta* from a high altitude mountain lake in the Pyrenees area (SANCHEZ et al., 1993), *Mullus barbatus, Serranus cabrilla,* and *Diplodus annularis* from the coastal area (30-50 m depth), *Micromesistius poutassou, Lepidrombus boscii,* and *Physicis bennedoes,* hemipelagic species (100-200 m depth), and *Lepidion lepidion* and *Alepocephalus rostratus* from the deep-sea basin (1500-2000 m depth) (GARCÍA, 1998). The figure clearly shows the widespread presence of DDTs in fish samples. Low tissue residues may be representative of basal or chronic pollution of the NW Mediterranean basin, whereas the high ones indicate hot-spots. Site pollution gradients were clearly observed in coastal fish, particularly in benthic species (e.g. *M. barbatus,* whereas no such gradients were detected in species living in more remote habitats (e.g. *L. lepidion*) (PORTE et al. 2000). It is apparent that tissue residues decrease with the distance from the source, but some benthic deep-sea fish exhibit levels in the range of those observed in fish from shallower waters, directly affected by anthropogenic activities. The long-range transport of persistent pesticides to sites where they may be accumulated constitutes a potential environmental threat that deserves consideration. Overall, there is a need to assess potential negative effects of pesticide exposure/accumulation, and the consequences for the global ecosystem.
FIG. 1. — Map of the Western Mediterranean Sea showing the sites where red mullets *Mullus barbatus* were sampled. Numbers correspond to: 1- Tarragona, 2- Llobregat river, 3- Barcelona, 4- Besós river, 5- Tordera river, 6- Rhone delta, 7- Couronne, 8- Cortiou (Marseille), 9- cap Feno, 10- Porto bay.

FIG. 1. — Carte des zones de l’ouest de la mer Méditerranée montrant les sites dans lesquels les muges (*Mullus barbatus*) ont été prélevés. Les nombres correspondent à: 1- Tarragone, 2- Llobregat (rivière), 3- Barcelone, 4- Besós (rivière), 5- Tordera (rivière), 6- delta du Rhône, 7- Couronne, 8- Cortiou, 9- cap Feno, 10- baie de Porto.

FIG. 2. — Concentration of (A) total DDTs (Σ p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDE) and (B) hexachlorobencene (HCB) in muscle of *Mullus barbatus*. All compounds determined in a pool of 4-6 organisms and expressed as ng/g w.w. Number of stations as in Figure 1.

FIG. 2. — Concentration de (A) DDT total (Σ p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDE) et (B) hexachlorobencene (HCB) dans le muscle de *Mullus barbatus*. Tous les composés sont déterminés sur un lot de 4-6 organismes et les valeurs exprimées en ng/g (w.w). Le code des stations est identique à celui de la Figure 1.
**Non-persistent pesticides**

Herbicides, fungicides and insecticides are among the most widely used pesticides in modern agricultural practices, and as most of their residues ends in surface waters, their behaviour and effects in rivers, ponds, and lakes are of major concern.

Among the living organisms in aquatic ecosystems, phytoplankton communities are key targets for herbicide contamination, because of their ecophysiological similarities with terrestrial plants and therefore, high sensitivity of the same metabolic processes, viz. atrazine acts inhibiting photosystem II of both terrestrial plants and algae. This will lead to changes in quality and quantity of primary producers and global ecosystem perturbation (LEBOULANGER et al., 2001).

The majority of insecticides currently in use are organophosphorus (OPs), carbamate, and synthetic pyrethroid compounds. Because of their relatively nonpersistent characteristics, OPs have become one of the most widely used classes of insecticides worldwide. Although these compounds offer the advantage of rapid environmental degradation, they generally lack specificity and have high acute toxicity towards many terrestrial and aquatic organisms. They exert their toxic action by inhibiting cholinesterase enzymes, these enzymes being responsible for the removal of the neurotransmitter acetylcholine from the synaptic cleft through hydrolysis (HABIG and DIGIULIO, 1991).

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Metabolic biotransformation by the cytochrome P450 monooxygenase system plays a key role in modulating the toxicity of OPs, as the parent compounds are usually poor inhibitors of cholinesterases. This system catalyzes the oxidation of OPs into the corresponding phosphate triesters or oxons, which are potent inhibitors of the enzyme acetylcholinesterase (AChE). Alternatively, OPs can degrade through a dearylation process, giving rise to non-toxic metabolites, and oxons may be hydrolyzed by serum oxonases, or detoxified by means of sequestration with B-esterases.

B-esterases (cholinesterases and carboxylesterases) have been successfully used in aquatic organisms to assess exposure to pesticides. Cholinesterases are typically subdivided into two major classes: (a) acetylcholinesterases, which preferentially hydrolyze acetylcholine over butyrylcholine as a substrate, they are extremely sensitive to eserine inhibition, and show substrate inhibition at high substrate concentrations, and (b) butyrylcholinesterases, which preferentially hydrolyze butyrylcholine, they are selectively inhibited by iso-OMPA (teraisopropyl pyrophosphoramide), and do not show substrate inhibition. Conversely, carboxylesterases or aliesterases are known to hydrolyse a wide range of exogenous and endogenous esters and they are assumed to provide protection against OPs poisoning, either through direct hydrolysis of ester bonds in OPs, or stoichiometric phosphorylation with resultant destruction of the oxon, which reduces the amount of OPs available for AChE inhibition (JOKANOVIC et al., 1996). Although the physiological role of carboxylesterases is not clear, their sensitivity and rapid inhibition rate by very low concentration of certain OPs and carbamates, makes them good candidates for use as biomarkers.

In a previous work, we evaluated the potential of using the mussel — *Mytilus galloprovincialis* — to monitor the effects of pesticide runoff in an agricultural area of the Ebro Delta (ESCARTÍN and PORTE, 1997). Laboratory-derived IC50 values were determined for gills and digestive gland after in vitro exposure to fenitrothion, fenitrooxon, and carbofuran for 15 min. Gills were more susceptible to inhibition by the compounds than digestive glands. In gills, the IC50 was 300 µM for fenitrothion, and much lower (5.8 µM) for fenitro...
trooxon, indicating that fenitrothion requires activation to its oxygen analogue in order to cause significant AChE inhibition. The IC50 for carbofuran was 3.7 µM, which indicates that this compound is a direct inhibitor of AChE. Carboxylesterases were more sensitive to inhibition than AChE; the IC50s were of 2.1 µM for fenitrothion, 0.6 nM for its metabolite fenitrooxon, and 3.8 µM for carbofuran.

In terms of field-collected mussels from the area, AChE and CbE were determined monthly in the gills of mussels collected from the breeding grounds in the Ebro Delta (Fig. 4). AChE suffered the greatest seasonal changes in terms of specific activity (13 to 54 nmol/min/mg protein), the maximum activity recorded in January, which could be interpreted as an indication of little or no exposure to OPs or carbamates. The minimum activity found in April coincided with maximum residues of OPs fenitrothion and vamidothion in the tissues. A certain correlation was seen between the concentration of fenitrothion in whole mussel tissue and gills AChE activity ($r = 0.62 ; n = 6$), indicating that fenitrothion might account in part for the observed AChE variation. Gills CbE activity showed a broader profile with two well differentiated periods: high specific activities (76 to 94 nmol/min/mg protein) from September to February, and low activities (43-59 nmol/min/mg protein) from April to August. During the second period, the irrigation channels are open, enabling fresh water to circulate from the rice crop fields to the mussel breeding grounds.

However, several other factors, such as the developmental stage and age of the organisms, but also water temperature or salinity are known to affect the level of esterase activity in marine organisms. In the summer period, and particularly in July-August, water temperature was extremely high (24-26°C) and this factor may increase the susceptibility of mussels to some pesticides (MOULTON et al., 1996). Increases of water temperature coincided with decreases of gills AChE activity and CbE activity, and this is in accordance with the fact that in poikilotherm organisms, high water temperature is associated with elevated metabolic rates, which would affect rates of activation of pesticides or even penetration to the active site of the enzyme and originate a general increase in toxicity of cholinergic poisons in the summer (MOULTON et al., 1996). Conversely, there is abundant evidence that endogenous AChE levels increase as a function of temperature (EDWARDS and FISHER, 1991), then an increase in cholinesterase activity with increasing temperature would be expected in a non-contaminated environment. Hence, these results further support the hypothesis that the observed seasonal profiles are most likely a reflection of the level of pollution of the area.

AChE monitoring in aquatic species can certainly play an important role as an early warning system of pesticide exposure. However, additional work is needed in order to facilitate the interpretation of depressed AChE in terms of mortality, negative effects for the physiology of the organisms, neurological disorders, changes on behavior, etc. Given the wide range of pesticides used, and the fact that chemical surveillance is not adequate to assess non persistent pesticide pollution, further research should be aimed to develop accurate simple tools to monitor the occurrence of pesticides and its degradation products in the aquatic environment, its toxic effects on non target species, and the final consequences for the ecosystem.

References


