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Toxicology and ecotoxicology of persistent organic microcontaminants in aquatic systems

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Summary. - Persistent organic pollutants (POP) are a heterogeneous group of chemicals widely distributed in the aquatic environment. Some classes cause adverse effects in the biota at minute concentrations, persist in the environment for a long time, and bioaccumulate in animal tissues. Several strategies exist today to monitor their levels and evaluate their effects in the environment. Particularly, some interesting approaches have recently been developed in the field of biomarkers, although much work seems to be required to study the relationships between toxic responses at different levels of the biological organization of interest and concentration levels in tissues.

Key words: organic microcontaminants, persistent organic pollutants, aquatic systems.

Riassunto *(Tossicologia ed ecotossicologia dei microcontaminanti organici persistenti nei sistemi acquatici)*. - I microcontaminanti organici persistenti sono un gruppo eterogeneo di sostanze chimiche ampiamente distribuite nell'ambiente acquatico. Alcune classi di queste sostanze producono effetti tossici a dosi molto basse, persistono nell'ambiente per lungo tempo, e bioaccumulano nei tessuti animali. Sono state messe a punto numerose strategie per monitorare i loro livelli e valutarne gli effetti nell'ambiente. In particolare, recentemente sono stati sviluppati alcuni approcci nell'ambito dei biomarcatori. Tuttavia molto lavoro risulta ancora necessario per studiare le relazioni tra la risposta tossica del livello dell'organizzazione biologica d'interesse e le concentrazioni di sostanza chimica nei tessuti.

Parole chiave: microinquinanti organici, inquinanti organici persistenti, sistemi acquatici.

Introduction

Persistent organic pollutants (POP) are toxic organic compounds generally resistant to different degradation pathways. Over the past several years they have been the object of strict international regulations in order to reduce their potential impact on human health and the environment. In 1995, at the request of the Council of the United Nations Environment Program (UNEP), an international working group conducted an assessment on POP and identified the 12 most relevant. These are characterized by high resistance to degradation, high bioaccumulation potential, (eco)toxicity, availability to long-range environmental transport, and hazard for human health and the environment. The working group developed a report and recommendations which were unanimously supported by all parties. They were submitted to the UNEP Council and the World Health Assembly (WHA, the policy body for the World Health Organization) for consideration at their respective 1997 meetings. The report and its recommendations were approved by both bodies, that reached the following conclusions: *a)* immediate international action should be initiated to protect human health and the environment from the 12 chemicals, and *b)* an intergovernmental negotiating committee (INC) was to be established in order to investigate different approaches with regard to the proposed action programs for each category of POPs. The INC was convened and met several times over the 1998-2001 period to fulfil its mandate to prepare an international legally binding instrument for implementing international action [1]. In the meantime, intergovernamental treaties (Oslo and Paris Convention, Barcelona Convention) identified these contaminants as an absolute priority in defining protective measures for the marine environment.

In this short review, we have focused on the problems related to the presence of these xenobiotics in the aquatic environment, on their potential effects, and recent developments on environmental monitoring.

Contamination sources and physico-chemical properties

The term "dioxins" refers to a large group of chloro-substituted, heteroaromatic tricyclic pseudo-planar compounds, with similar structures and chemical and

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physical properties that make them ubiquitous contaminants of great toxicological relevance. The group consists of 75 polychlorinated dibenzo-*p*-dioxins (PCDD) and 135 dibenzofurans (PCDF). Dioxins are and were not produced commercially; they unintentionally form during various human activities such as combustion processes or industrial processes that involve chlorine. The incineration of municipal, industrial, or hazardous waste is generally considered as the major contributor of dioxin emissions into the air. Release from chlorophenol wood preservatives, emission by sinter industries, the use of defoliants, the preparation of herbicides, vehicular traffic, and paper pulp bleaching using chlorine are also known to contribute to environmental contamination with dioxins. Congeners with four or more chlorine atoms are characterized by very low water solubility, high lipophilicity, very low vapour pressure, high melting point, and high physico-chemical stability. Among the 210 compounds, only the 17 2,3,7,8-chloro-substituted congeners have toxicological interest especially due to their potential carcinogenicity and reproductive and immunotoxic effects. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-T_4CDD) is the most active and most studied congener [2].

The group of polychlorinated biphenyls (PCB) consists of 209 congeners that differ from each other because of the number or the position of chlorine atoms. No longer industrially produced in western countries, PCB have found widespread applications because of their general stability and inertness as well as their excellent dielectric properties. They were produced commercially in mixtures (e.g., Aroclor) containing a large number of congeners (up to 130); this large usage - often not matching an adequate disposal - together with their environmental persistence, have made them ubiquitous contaminants. PCB have several toxic effects on living organisms, such as: liver damage, endocrine and reproductive dysfunctions, carcinogenicity, etc. Some PCB congeners may adopt a pseudo-planar "dioxin-like" chemical structure; these dioxin-like PCB have indeed been found to resemble the biochemical and toxicological properties of 2,3,7,8-T_4CDD [3].

Although they may be viewed as POP, polycyclic aromatic hydrocarbons (PAH) are not classified as priority pollutants at present [1]. These chemicals, including carcinogens, mutagens, and teratogens, are indeed of great toxicological concern even if their environmental persistence is shorter than that of PCDD, PCDF and PCB. Major anthropogenic sources of PAH include incomplete combustion of organic materials during industrial processes and human activities. Benzo[*a*]pyrene is a renown representative of this group [4].

Chlorinated pesticides such as DDT (1,1,1-trichloro-2,2-*bis*(chlorophenyl)-ethane) and DDD (1,1-dichloro-2,2-*bis*(chlorophenyl)-ethane) are no longer manufactured in Europe, but they are still present in the environment as a result of their previous usage and of long-range transport [5]. Hexachlorobenzene is also widely diffused due to its past use as a pesticide and because it is an intermediate in several chemical syntheses. Moreover, it is an unwanted by-product of the production of chlorinated organic solvents, the chemistry of chlorine, and the incineration of municipal waste [6].

Persistent organic pollutants and aquatic systems

Because of their physico-chemical properties and their ability to persist in the environment, POP easily migrate through biological membranes, bioaccumulate in fatty tissues of living organisms, and concentrate in the food chain. Bioaccumulation levels in aquatic organisms detected in laboratory analysis are generally quite high; in particular, bioconcentration factors (BCF) as high as 3×10^5 have been reported in the literature for PCB (Aroclor 1260) [3]. BCF considerably vary according to the chlorination level of the substance, the exposure level (the smaller the exposure, the greater the BCF is), the lipid amount of the particular organism. All these factors contribute to determine the half-life of POP.

Dietary consumption is the main pathway of exposure as POPs are very lipophilic compounds. Therefore, these contaminants are easily transferred to higher trophic levels and their concentration increases through successive levels of food chains, a phenomenon called biomagnification. On this subject, an interesting in-field study was conducted on two Baltic sea food chains: one constituted of typical coastal organisms, the other of open sea organisms. It was observed that biomagnification of POPs is congener-dependent: in both food chains, the most toxic congeners (tetra- and pentachlorinated) reached higher concentrations in the highest trophic levels (e.g., herring, cod), while concentrations of the less toxic congeners (such as O_8CDD) appeared to decrease as the trophic level rose. In particular, in cod and herring (belonging to the pelagic food chain) the levels of tetra- and pentachlorinated compounds were five times higher than in phytoplankton, while the levels of fully chlorinated congeners fell when moving from open sea to coastal food chains. An explanation is that, because of their molecular and physico-chemical characteristics, the more chlorinated congeners have limited bioavailability, this hindering their passage from one link to another in the food chain [7].

A recent study on Adriatic sea fishing products apparently showed an increase in concentration related to the trophic level of the organism considered and to the geographic area of catch (i.e., North, centre, and South). Particularly, PCB, PCDD, and PCDF levels in mackerel, belonging to the highest trophic level, resulted higher than in organisms belonging to lower trophic levels [8].

From a quantitative point of view, the contamination levels of benthic fauna appear to be related to those detected in sediments [9]. However, other factors seem to be responsible of the variation usually found in animals; in particular some underestimated characteristics - such as the polarity of the lipids of the animals [7], the filtration rate, and the physiological state of the organism - can deeply influence the contamination levels observed in animal tissues [10].

Biomagnification through an aquatic food chain shows some peculiar aspects. Indeed, when comparing PCB, PCDD, and PCDF concentrations in different trophic levels of the aquatic environment, such a phenomenon does not appear to be so marked. In fact, species live in an intimate contact with the physical environment, to the extent that any such tendency for pollutant concentrations to increase is counteracted by a constant tendency to achieve equilibrium with the pollutant levels in the surrounding water. Through their food, animals can acquire quantities of a POP larger than the equilibrium really permits, but as soon as this happens, net uptake of the substance through different pathways ceases and is replaced by an outward flow. This limits the levels of toxic pollutants that can occur in aquatic animals, although restoring the balance can take time, especially for larger animals. Large fish may ingest more pollutants with their food than they will ever be able to shed in their environment, and their concentrations of persistent substances may thus be constantly in a state of imbalance with those in the water [7]. When an aquatic food chain involves piscivorous birds, final biomagnification turns out to be very large because the mechanisms responsible for clearance of the chemicals dealt with in the atmospheric environment are physiologically different [10]. Several authors have showed that, in this case, tissue levels can be remarkably high [11, 12].

Even if concentrations are expressed on a lipid basis, POP levels in animal tissues may vary considerably. One explanation is that exposure levels may be different at different times and places. However, other factors must be taken into account as well, such as: the physiological state of the organism (because lipid quantities are related to seasons and reproductive periods), the individual size, and environment characteristics (e.g., lagoon areas are rich in suspended organic matter while open sea areas are quite oligotrophic).

A broad spectrum of toxic and biochemical effects has been reported for 2,3,7,8-T_4CDD: most of them can be ascribed to the same mechanism of action that involves the binding to specific intracellular receptors. This mechanism was observed in mammals, birds, and fish: dioxin-like POPs, owing to their similar structures and conformations, induce similar toxic effects. As environmental exposure to these substances is generally low and protracted in time and POP have a tendency to accumulate in lipid tissues, we normally observe chronic effects. One of the most serious is the potential for reproductive impairment, widely proven in aquatic piscivorous birds and mammals but not enough investigated in fish. In fish, 2,3,7,8-T_4CDD seems to act in the first developmental stages: some studies indeed showed that embryos may result as much as 25 times more sensitive than young fish. Among vertebrates, young fish are the most sensitive to 2,3,7,8-T_4CDD lethal effects (due to their susceptibility, embryo body burden is generally in the order of a few pg/g, fresh weight) [13, 14].

In the Mediterranean sea waters and surface microlayer, PCB levels were found to reach 550 and 600 mg/l, respectively; in bottom sediments, PCB levels were measured from ca. 0.0001 mg/kg, dry weight (*dw*), in unexposed areas up to 16 mg/kg *dw* in severely polluted waters [15]. Recent studies on the Venice lagoon showed that total PCB levels were comprised between ca. 0.0003 and 5.5 mg/kg *dw*, depending on the prevailing anthropic impact. Concurrently, PCDD and PCDF levels appeared to range from 1.0 to 29000 ng/kg *dw*, while PAH concentrations fell between 0.0039 and 48 mg/kg *dw* [16].

Monitoring

Over the last few years, in addition to the traditional chemical assessment of sediment and biota, some interesting diagnostic techniques have come out aiming at identifying sublethal levels for natural populations. Several reasons may be considered as an explanation of the development of these new techniques: a) the canonical determination of the microcontaminants of interest is time-consuming and expensive; b) POP are individual substances (e.g., Aldrin, hexachlorobenzene), or groups of chemicals (e.g., PCDD, PCDF, PCB), capable of interacting in a synergical, additive, or antagonistic way (a fact that makes the toxicological interpretation of the levels observed in animal tissues and the environment much more difficult); c) lastly, in order to keep the size of the population unmodified and allow measurements to be repeated several times, an analytical method should not be harmful towards the organisms under study. As a matter of fact, with such a technique it would be possible to cut down on the temporal and spatial variability of measures and, consequently, experimental data would be more reliable and less expensive.

Other considerations are correlated to POP physico-chemical properties and their environmental behaviour: a) with regard to the routine monitoring of POP, aquatic sediments are the most suitable matrices as they are the final destination of such contaminants; b) the number of factors determining analytical variability is smaller in sediments than in organisms; c) if there is no direct source of exposure, sediments generally act as a “chronic” source of exposure for the water column through processes such as re-suspension, bioturbation, and release due to chemical equilibrium.

With regard to the assessment of dioxin-like POP, at present the most known technique, considered in some countries for routine monitoring, entails the determination of the cytochrome P-450 1A1 amount in tissues by adding a chemical substance, the 7-ethoxiresorufine, easily converted to resorufine by the enzyme: resorufine concentration measures the sample 7-ethoxiresorufine-*o*-deetilasic (EROD) activity. The rationale of these technique is that dioxin-like POP induce a specific enzymatic activity mediated through the *Ah* receptor mechanism, so that the higher the exposure, the more EROD activity there is.

Other effects can also be monitored, such as vitamin A amount, thyroidal and steroidal hormone concentrations, T-cell number, etc. All these measurements reflect the typical responses of organisms exposed to the chemicals of interest. However, other tests may be employed to monitor the "non-dioxin-like" POP, such as: the ability to induce mutations *in vitro* (Ames' test) or determine DNA adducts, the measure of enzymatic activities induced by non-dioxin-like substances (PROD activity), etc. These tests are attractive because they measure specific effects, but actually they cannot be easily managed in risk assessment as: *a)* the effects measured are not congener-specific, so that different congeners of the same chemical group may have different toxicological activity, and *b)* tests have not been adapted for wild fauna nor for testing of complex exposure matrices like sediments, interstitial water, or elutriates. Before they can be regularly used, they should be subjected to adequate validation with regard to the influencing/interfering factors and their reliability compared with the canonical chemical assessment procedures.

Conclusions

At this time, there is no consensus on the correct approach for the assessment of the hazard of sediment-associated POP. Numerous protocols exist, and a number of species and other biological systems at cellular and subcellular level have been utilized as test systems, but it seems that no comprehensive data set exists for a specific bioassay, in particular for non-dioxin-like POP. Questions also remain concerning the choice of the exposure matrices and their handling for test purposes. Probably, the most correct approach at this development stage would be to integrate chemical analyses and bioassays in order to interpret the chemical levels measured in biological terms.

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