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CHEMICAL CONTAMINATION IN THE MEDITERRANEAN: THE CASE OF SWORDFISH

Chemical Contamination in the Mediterranean: the case of swordfish

Text: Eva Alessi, Gianluca Tognon, Michela Sinesi, Cristiana Guerranti, Guido Perra and Silvano Focardi

Editing: Eva Alessi and Brettania L. Walker

Layout: Brettania L. Walker and Patrizia Zaratti

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EXECUTIVE SUMMARY

Recent years have seen increasing awareness of the multiple threats to the environment. The potential dangers associated with the lack of a sound system for the safe management of hazardous chemicals is one of them. Chemicals enter the environment in a number of ways, including release during production, from industrial effluents, direct applications, disposal, transport and a wide variety of uses in products. As a result, persistent organic pollutants (POPs) have now reached all corners of the world, contaminating humans and wildlife alike, and marine ecosystems such as the Mediterranean Sea play a major role as a sink for chemical pollutants.

The Mediterranean region is a complex geographic, ecological, cultural and socio-political area. The sea has limited water exchange with the open seas, and is thus sensitive to the build-up of pollutants (UNEP, 1996, 2002) that may cause a progressive degradation of the marine ecosystem. In fact, chemical levels of many substances such as some brominated flame retardants and perfluorinated chemicals are increasing in the marine food chain and are expected to continue to increase.

This new WWF report, divided into two parts, aims at showing how chemicals are building up in the Mediterranean Sea and its wildlife. The first part presents the results of new analytical investigations on Mediterranean swordfish (*Xiphias gladius*), carried out by the research group of Professor Focardi of the University of Siena, Italy. By analysing the presence of older (those already banned for decades such as DDT) but also newer chemical compounds in swordfish tissues, the present study makes a significant contribution to the existing scientific literature, which until now has mainly focused on already restricted compounds -such as polychlorinated pesticides or dioxins and dioxin-like compounds. The second part reviews the most interesting scientific studies, concluding that contamination of Mediterranean wildlife is already a serious cause for concern. In fact, many persistent and bioaccumulative chemicals have been linked to possible serious health effects on wildlife, people and entire ecosystems, e.g. by altering sexual and neurological development, reproduction and immune systems.

For this study, 29 swordfish tissues samples (liver and muscle) were removed from 17 adult swordfish captured in 2005 in the Mediterranean (Eastern South Tyrrhenian Sea). The samples were analysed for 28 endocrine disrupting chemicals (EDCs)[1]: 7 organochlorine pesticide residues (the fungicide hexachlorobenzene [HCB] and 6 isomers and metabolites of the insecticide DDT); 19 brominated flame retardants (polybrominated diphenyl ethers [PBDEs] congeners), and 2 perfluorinated compounds (perfluoroctanesulfonate [PFOS] and perfluorooctanoate [PFOA]).

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[1] The Endocrine System is a complex, multi-component system whose purpose is to assist in major physical functions including reproduction, growth, development, maintenance of normal glucose levels and blood pressure, and metabolism. Major endocrine glands include pituitary gland, thyroid, pancreas, adrenal, hypothalamus, testes, and ovaries. These glands produce hormones that travel through the bloodstream to organs and tissues, telling them how to function. The hormones bind to protein molecules, called receptors, which decode and respond to hormone signals. This binding, often compared to a lock and key system, causes responses in tissues according to their function.

To the authors' knowledge, this study detected for the first time the presence in Mediterranean swordfish of one of the most frequently used groups of brominated flame-retardants: polybrominated diphenyl ethers (PBDEs). These are highly persistent and bio accumulative compounds, but there is currently only scarce data available on their occurrence in Mediterranean species. The commercially used mixtures penta- and octa BDE were banned in the European Union in 2004, but continue to be used in other parts of the world and will persist for many years in the marine environment.

Brominated flame retardants were detected in all swordfish tested but one. In liver samples, PBDEs were found in concentration ranges of 189-11184 pg/g w.w., while in muscle samples they were found between <0.04-1882 pg/g w.w. The prevalent PBDE congeners found were 47 and 100, two of the most common congeners found in marine organisms.

While organochlorines were found in all swordfish samples, PFOS and PFOA were not detected at this time. Data on contamination of swordfish with persistent organohalogens were, in general, comparable with the levels observed in previous studies, even though pp' DDE (a DDT metabolite) showed slightly higher concentrations compared to other studies around the Italian coast. In general, organochlorine concentrations have decreased over the last 15 years, but despite the strong restrictions and/or bans DDT, its homologues and metabolites (especially *pp'*-DDE), still occur at average levels of 173 ng/g w.w. (muscle) and 309 ng/g w.w. (liver) in the swordfish analysed. Levels of the banned pesticide HCB were low, ranging from <0.01-0.53 ng/g w.w. and <0.01-0.84 ng/g w.w. in muscle and liver respectively.

In addition to being an important ecosystem component, swordfish is an edible fish species of high commercial interest and constitutes an important nutritional contribution to the human diet, providing proteins and fatty acids. But at the same time, fish consumption is one of the major pathways for human exposure to contaminants. While this study does not engage on the pros and cons of fish consumption, its purpose is to raise awareness about the widespread chemical contamination of wildlife and to highlight the need for better chemical management. The top priorities in Mediterranean chemical management are the adoption of a strengthened REACH (the current draft European chemicals legislation) and the ratification and implementation of Barcelona Convention protocols for the Mediterranean.

INTRODUCTION

Based around the Mediterranean Sea basin, the Mediterranean region is a complex geographic, climatic, hydrological, ecological, cultural and socio-political area. The Mediterranean is characterised by high biodiversity: it is one of the richest seas in the world for flora and fauna, particularly in the coastal zone, with a high rate of endemism (UNEP/MAP/WHO, 1999; EEA, 2006). A rough estimate has been made that more than 8,500 species of macroscopic marine animals live in the Mediterranean Sea (Bianchi and Morri, 2000). This rich biodiversity represents 8 to 9% of the total number of species in the world's seas and new species are still being found, especially in areas of deep water (EEA, 2006). This is remarkable when considering that the Mediterranean's area is only 0.82% and its volume 0.32% of the total area and volume of the world's oceans. The Mediterranean hosts, moreover, several endangered marine species: the monk seal (*Monachus monachus*), of which about 350 - 400 now survive in the world, the green turtle (*Chelonia mydas*) and the 100-million year old loggerhead turtle (*Caretta caretta*), which nests on Mediterranean beaches, 18 cetacean species, of which 7 can be observed throughout the year: the pilot whale, fin whale, sperm whale, common dolphin, striped dolphin, bottlenose dolphin and the Risso's dolphin, and the endemic sea-grass *Posidonia oceanica*, which plays a crucial role in coast protection by acting as a buffer against currents and waves (Holmer *et al*., 2003). The Mediterranean Sea is also an important commercial fishing ground. Of the 900 fish species found in the Mediterranean, 100 are commercially exploited. Some of these species have a high market value. Thus, the Mediterranean Sea intricately links humans and nature.

Chemical contamination is a serious global threat and the Mediterranean Sea is particularly vulnerable to this threat. The Mediterranean Sea is the largest semi-enclosed sea and is surrounded by heavily industrialized countries in the north and by highly developed agricultural countries all around its basin. Many human activities are degrading the Mediterranean Sea, which plays a key role in the accumulation, diffusion and impact of pollutants in the entire region. The main characteristics (e.g. high temperature, high salinity, microtidal regime[2]) of the Mediterranean Sea determine the fate of physicochemical and biological cycles affecting all aspects of Mediterranean ecological processes (EEA, 2006). Throughout the last decades, industrial, agricultural and urban wastes have been discharged into the Mediterranean via coastal outfalls, rivers and the atmosphere, causing a considerable increase in pollution and a progressive degradation of the marine ecosystem (UNEP, 1996, 2002). In the marine food chains, the bioaccumulation of chemical compounds is favoured due to the physical-chemical properties of the polluting substances and due to oceanographic characteristics:

1. many chemical pollutants are persistent, i.e. they do not or only hardly degrade in the environment. Due to their capacity to bind to organic matter and in particular to fat, they can accumulate in food chains,

particularly in the species occupying the highest levels (Aguilar and Borrell, 1994a; 1994b; Borrell *et al.*, 1996), representing a potential risk factor also for human health;

2. the Mediterranean Sea is connected to the Atlantic Ocean through the strait of Gibraltar. The flux[3] ($5 \cdot 10^{13}$ m^3 per year and 4% less for the outflowing flux [Ferrara and Maserti, 1986]) from the strait does not permit a significant water exchange. This increases the chemicals' residence time, favouring accumulation.

Persistent Organic Pollutants (POPs) are chemical products and by-products of human industry and are semi-volatile and mobile. They are widely distributed through the environment, travelling great distances on wind and water currents. The potential of a chemical to bioconcentrate, bioaccumulate, or biomagnify in organisms and food webs depends on the properties of the substance (e.g. hydrophobicity, lipophilicity, and resistance to degradation), environmental factors (e.g. salinity, temperature, concentration of other organic chemicals, and redox potential), biotic factors (e.g. organism feeding modality, trophic position, lipid concentration and metabolism) and bioavailability (e.g. current chemical inputs, transport mechanisms and the degree of contamination).

This report does not attempt to give an overall analysis of the state of the Mediterranean marine environment but addresses in detail some emerging issues about the widespread contamination of the Mediterranean Sea with a range of toxic substances. The focus is on man-made industrial chemicals and does not include other chemical groups such as heavy metals or pollutants caused by combustion processes such as PAHs or dioxins. The interested reader is referred to EEA and UNEP reports (see references), which provide a more complete analysis of the emerging issues of the Mediterranean marine environment. While there are several treaties or conventions (the Barcelona Convention, see box 1, and the POPs Convention, see box 2) to protect the basin, the Mediterranean Sea still suffers from the effects of increasing pollution. The existing legislation on chemicals is neither comprehensive nor effective enough in protecting wildlife and human health.

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[2] Regime with a tidal range typically less than 50 cm which, therefore, reduces the potential for dilution and dispersion of dissolved and particulate wastes (EEA, 2006)
[3] The rate of flow of fluid through a given surface

BOX 1 – THE BARCELONA CONVENTION

The Barcelona Convention for the Protection of The Mediterranean Sea Against Pollution, signed 16 February 1976, in force 12 February 1978, is the legal framework of the Mediterranean Action Plan (MAP), an action-oriented co-operative effort adopted by the Mediterranean States and the EC under the auspices of the United Nations Environment Programme (UNEP).

The Barcelona Convention was then modified in 1995 to give a legal status to the commitments made by the countries attending the 1992 Earth Summit in Rio. So far, only 8 countries (Croatia, Egypt, France, Italy, Malta, Monaco, Spain and Tunisia) and the European Union have ratified the amendments adopted in 1995. Ratification from another 7 Mediterranean countries is needed for the amendments to enter into force. Since 1975, the so called Barcelona system has given rise to six Protocols that are legally binding instruments addressing specific aspects of environmental protection. The Land-based Source of Pollution (LBS), a protocol related to the protection of Mediterranean Sea, is one of the six Protocols not yet ratified.

The implementation of the LBS Protocol, which has been ready to be put in place since 1996, would prevent high levels of pollutants being released. In a joint petition presented in November 2003 to the 21 Mediterranean Ministers and delegates, WWF and 15 environmental NGOs stressed that although the efforts of most Mediterranean countries to implement the LBS Protocol are appreciated, Algeria, Bosnia & Herzegovina, Croatia, Egypt, Israel, Lebanon, Libya, Serbia Montenegro and Syria still have to ratify the Protocol. Of these countries, only 3 signatures are needed to allow the LBS Protocol to be legally binding for the Mediterranean countries, which have adopted the Barcelona Convention. WWF and other NGOs urge these countries to proceed through the full ratification of the LBS Protocol.

BOX 2 – THE “DIRTY DOZEN” – POPs CONVENTION

The “Dirty Dozen” is the nickname for the 12 POPs - identified by the United Nations Environment Program (UNEP) that aims to ban- under the Stockholm Convention- PCBs, DDT, hexachlorobenzene, dioxin and furans, dieldrin, aldrin, endrin, chlordane, heptachlor, toxaphene, mirex. These chemicals pose a significant risk to human health and the environment and they are particularly hazardous because of their characteristics. They are toxic to humans and wildlife. They are persistent and remain intact for long periods of time, resisting break down. Having a high 'lipid' or fat solubility, they accumulate in the bodies of humans, marine mammals and other wildlife. They are found at higher concentrations at the top of the food chain. They pass from mother to the foetus in the womb and to the child through breast milk.

POPs can cause nervous system damage, diseases of the immune system, reproductive and developmental disorders, as well as cancers. Chemicals, moreover, occur in the environment and in our bodies not as single entities but as complex mixtures. We are exposed to unlimited combinations. This synergism between chemicals makes it very difficult to pin down the likely effects on human and wildlife health. The effects of multiple exposures are poorly understood: combined effects may be worse than the effect of each chemical alone.

Concerning Persistent Organic Pollutants (POPs), a series of inter-governmental agreements, started with the 1992 Earth Summit in Rio and the adoption of Agenda 21, and culminated with the Stockholm Convention (May 22, 2001), aimed to undertake all the necessary measures to eliminate or to reduce the emissions of toxic compounds in the atmosphere to non-dangerous levels (UNEP, 2001). The Convention seeks also to substitute existing POPs with more environmentally-friendly alternatives and to set up precautionary measures to ensure that fewer POPs are released into the environment. Envisioned by the international community to be a dynamic, living treaty that responds to current realities, the Stockholm Convention provides a rigorous scientific process through which new chemicals that meet POPs criteria can be added to the treaty.

Signed by more than 100 countries, the convention entered into force on May 17, 2004, after France became the 50th country to ratify it (February 2004). As of May 2005, 98 countries have ratified the convention. WWF is promoting additional ratifications and effective implementation of the Stockholm POPs Convention. In fact, among the Mediterranean countries, Italy, Greece and Turkey have not yet ratified the Convention and WWF urges them to do so.

POPs’ endocrine disruption properties have been documented in many studies, including the following recent studies: Tabuchi *et al.* (2006), Fonnum *et al.* (2006), Ropstad *et al.* (2006), Debier *et al.* (2005).

Despite the widespread contamination of wildlife and people, and the discovery of harmful chemical health effects, there is an astonishing lack of publicly available safety data on chemicals currently in use. However, a European Union (EU) reform is underway that could fundamentally change the way chemicals are managed in Europe, and which will also have global implications. A priority for WWF is to ensure that the proposed reform known as REACH (Registration, Evaluation and Authorization of Chemicals; see box 3), delivers on its aim of identifying and phasing out the most hazardous chemicals. Chemicals of very high concern including hormone disruptors should be replaced with safer alternatives whenever available. The evidence presented in this report shows how necessary it is to have a strong new EU law on chemicals that can effectively protect humans, wildlife and the environment from harmful chemicals.

This report is divided into 2 parts: the first part presents the results of the analysis of the 29 swordfish samples taken from the Mediterranean Sea conducted by Professor Silvano Focardi of the University of Siena. The second part contains a bibliographic review of scientific literature about chemical contamination of Mediterranean marine wildlife with the aim of summarizing the available data on the widespread occurrence of hazardous man-made chemicals and bioaccumulation in the Mediterranean food chain.

BOX 3 - THE NEW EU CHEMICALS LEGISLATION – REACH AND WHY IT IS NEEDED

REACH is a draft regulation that should lead to the identification and phasing out of the most harmful chemicals. When approved, it will be enforced in all countries in the European Union. REACH stands for the **Registration**, **Evaluation** and **Authorisation** of chemicals.

Current chemical regulation in Europe makes a distinction between "new" chemicals (about 3000 chemicals that came on the market after September 1981) and "existing" chemicals (over 100 000 of chemicals that were on the market and registered by 1981). While all post-1981 "new" chemicals are required to undergo basic safety testing, the same is not required for the "existing" chemicals, which make up the majority of chemicals in current use. As a result, thousands of chemicals -more than 90% of those on the market today - have not been evaluated for basic safety. In addition, the current system discourages industry innovation and the development of new, safer alternatives, since the testing requirements to bring a chemical to the market are stricter today when compared to a continued use of pre-1981 chemicals, for which safety testing is not required.

The aims of the proposed new Regulation are to improve the protection of human health and the environment while maintaining competitiveness and enhancing the innovative capability of the EU chemicals industry. REACH would furthermore give greater responsibility to industry to manage risks from chemicals and to provide safety information on substances. This information would be passed down the supply chain to enable downstream users to handle chemicals safely.

The decision on REACH is shared between two bodies: The European Parliament and the EU Council of Ministers (represented by the Environment and Industry Ministers from each EU country). The first reading in the European Parliament took place on November 17, 2005 and the political agreement in the European Council occurred on December 13, 2005. The Council rejected the crucial principle adopted the previous month by the European Parliament: the requirement to substitute hazardous chemicals with safer alternatives whenever possible. Although chemical producers would be required to 'assess' substitutes for a hazardous chemical, decision-makers will still have to grant an authorisation under an 'adequate control' procedure, even if safer alternatives are available. This loophole represents little change from the current, flawed system, which has failed to control the most dangerous chemicals and hinders safe, innovative products from entering the market. The Council also voted to drastically reduce safety data that chemical producers would be obliged to supply, particularly for substances produced in low quantities. Thousands of chemicals could thus stay on the market, despite no health information being available. This, too, undermines the likelihood of identifying safer alternatives.

The Second Reading on the REACH legislation is expected by the end of 2006 and it is essential that the Parliament reaffirms its support for stronger substitution requirements, for a legally binding 'Duty of care and improved access to information'. Through the **DetoX campaign, WWF** is working to raise greater public awareness and understanding about the failures of the current chemical regulation system and the need for improved chemical legislation. WWF warns that the opportunity that REACH provides, to ensure a safer future for people and wildlife, should not be missed.

PART I

CHEMICAL THREAT IN THE MEDITERRANEAN SEA (ITALY): ENDOCRINE DISRUPTORS IN SWORDFISH

Swordfish as an indicator for environmental contamination

Swordfish (see box 4 to learn more about swordfish biology) is a fish species of high commercial and ecological interest. Swordfish are taken either as a commercial target, as a by-catch in long line and trawl fisheries, or as a recreational species. Globally, 66 countries reported fishing commercially for swordfish in 1999. The total weight landed worldwide in 1999 was estimated at 97.110 mt (FAO, 1999), although this amount is an underestimate, as several countries with developing fisheries do not report their catches to the FAO.

Since 1996, North Atlantic swordfish has been listed as endangered on the World Conservation Union's (IUCN) "Red Data List" of threatened animals (http://www.redlist.org). This is principally due to the overexploited status of the North Atlantic swordfish stock, a result of poor management that was ineffective in preventing over-fishing. The status of swordfish in other areas is largely unknown because of a lack of information and data, although it is believed that the Mediterranean stock is also over-exploited.

Today, swordfish is consumed and marketed either fresh or frozen, as a luxury table food in many countries and consumer demand is increasing. Because it is so firm, swordfish can be prepared in almost any manner including sautéing, grilling, broiling, baking and poaching. In addition, swordfish are good indicators of the quality of the environment in which they live and a useful sentinel species for estimating environmental contaminants levels.

BOX 4 - SWORDFISH: BIOLOGY AND DISTRIBUTION

The swordfish or broadbill, *Xiphias gladius L.,* is the only living species of the family *Xiphiidae*. Like all billfish (the collective name given to fish that have a sword-like extension to their upper jaw, including swordfish, marlins, sailfish and spearfish), swordfish have a large sword (or *rostrum*), which makes up a third of their total length. This large oceanic fish can reach a maximum total length of 445 cm and can weigh up to 540 kg, it can live for 25 years and is highly fecund (Govender *et al*., 2003). Swordfish are the most widely distributed of billfish and are found worldwide in tropical, subtropical and temperate seas. Swordfish display a wide temperature tolerance (5 to 27°C) and are able to undertake extensive vertical migrations to depths of up to 1000 m (Govender *et al*., 2003). Swordfish, and other billfish, have a "brain heater" which allows them to elevate the temperature of their brain and eyes, while diving to great depths.

Evidence of the harvesting of swordfish can be traced back over centuries, for example to the Mediterranean in the time of Aristotle (384-322 BC). The swordfish is a marine predator having a world-wide distribution (Fossi *et al*., 2001). Adults are opportunistic diurnal feeders, feeding throughout the water column as they follow the migration of small shrimp, fish and squid. Information on swordfish growth is limited and somewhat contradictory. There is good evidence that males and females have different growth patterns, females attaining the largest size. Swordfish commonly live to at least 9 years of age, migrating toward temperate or cold waters in the summer and back to warm waters in the fall.

Pollutants in swordfish

The aim of the investigations presented in this report was to assess the pollution by organohalogens (OCPs, PCBs, BFRs) and perfluorinated alkylated (PFCs) compounds with suspected or proven endocrine disrupting properties (see boxes 5-8) in a Mediterranean specie, the swordfish.

Endocrine disrupting chemicals (EDCs) have recently attracted great public and scientific attention. EDCs are a structurally diverse group of compounds that may adversely affect the health of humans, wildlife and fish, or their progeny, by interacting with the endocrine system, and particularly influencing reproductive function.

Endocrine disrupting chemicals can mimic endogenous hormones, disrupt reproductive functions and cause developmental abnormalities (such as intersexes) in wild animal populations. EDCs include chemicals heavily used in the past, in industry and agriculture, such as polychlorinated biphenyls and organochlorine pesticides, and chemicals currently used as plasticizers and surfactants.

Even though production and use of some of these substances (as organochlorine pesticides, polychlorinated biphenyls and some brominated flame retardants), are prohibited (compare boxes 5, 6 and 7), these substances continue to be among the most common pollutants found in the Mediterranean. In addition, the Mediterranean suffers from other xenobiotic compounds such as the perfluorochemicals, which are still in widespread use and found in biotic (flora and fauna) and abiotic (water, soil and air) elements of the marine ecosystem.

In particular, new evidence indicates that swordfish is a species potentially "at risk", based on monitoring hormone and vitellogenin levels, together with gonad histology. Potential reproductive alterations, such as undergoing sex inversion (14%) (Fossi *et al*., 2001; Fossi *et al*., 2006), have been found in the central Mediterranean male swordfish, showing the presence of female germ cells, in macroscopically classified males fish (De Metrio *et al*., 2003). This researches represent a warning signal of the potential reproductive alterations in marine top predators and suggest the need for continuous monitoring to avoid reductions in population and biodiversity in the Mediterranean Sea.

The EDCs investigated in this study are well-known to bioaccumulate in the aquatic food-chain and fish have relatively high levels of these compounds compared to other food species. The importance of this study is that it shows that new chemicals are a cause of concern because there is still a lack of monitoring data about the occurrence and effects in the marine environment. This is particularly true for toxic contaminants with endocrine disrupting properties, such as PBDE and perfluorinated chemicals, for which only scarce data exist for Italy in the scientific literature. As far as we know, no other studies are available on swordfish PBDE contamination for Italy, demonstrating the great scientific importance of this report in order to assess the chemical contamination of a Mediterranean edible species.

This research was performed with the collaboration of Silvano Focardi (Department of Environmental Science, Siena University, Italy) and part of his team (Dr. Cristiana Guerranti, Dr. Guido Perra, Dr. Ilaria Bisogno, Dr. Ilaria Nesi).

BOX 5 - ORGANOCHLORINE PESTICIDES (OCPs)

Organochlorine pesticides include substances with high toxicity and environmental persistence. Extensively used in agriculture during the 1960s, OCPs were banned in the 1970s in Europe: nonetheless they are still detected in Mediterranean aquatic habitats and biota (Goutner *et al*., 2001). Several studies indicate that some OCP compounds result in reproductive damage and impairment to wildlife (Konstantinou *et al*., 2000). They also pose threats to humans at high and low concentrations (Longnecker *et al*., 1997). In fact, due to their lipophilic structure, OCPs tend to accumulate in food chains, especially in higher organisms, where they are found at elevated levels (Guruge *et al*., 1997; Harding *et al*., 1997). Hormone disruption is also a proven effect of many OCPs (Ropstad *et al*., 2006; Asawasingsopon *et al*., 2006; Sormo *et al*., 2005).

Examples of the most common OCPs are:

DDT (dichlorodiphenyltrichloroethane), a man-made chemical developed in the 1940s and used as an insecticide against a very wide range of insect pests, particularly malarial mosquitoes and as an agricultural insecticide. DDT is a long-lasting toxic chemical, which builds up in the tissue of living organisms. DDT sticks strongly to soil where it is broken down slowly to DDE (dichlorodiphenyldichloroethylene) and DDD (dichlorodiphenyldichloroethane) by microorganisms; they can contaminate surface water from soil run-off. Because of their chemical characteristics, DDT and its breakdown products can travel long distances and this results in widespread global dispersion. DDT, and especially DDE, build up in plants and in fatty tissues of fish, birds and other animals. DDT has been banned in many countries, including the EU. It is, however, still used in some developing countries. It is regulated under the international treaty as a “POP” – a persistent organic pollutant. The International Agency for Research on Cancer (IARC) determined that DDT may cause cancer in humans. The Environment Protection Agency (EPA) determined that DDT, DDE, and DDD are probable human carcinogens. DDT and its metabolites are proven endocrine disruptors; see for example Debier *et al.* (2005) and Dickerson *et al.* (1999).

HCB (hexachlorobenzene) is a fully chlorinated hydrocarbon industrial chemical used for various applications, ranging from an active ingredient in fungicides to the production of chlorinated chemicals; it is also a by-product of incineration of municipal waste. Though usage of HCB as a fungicide in agriculture was banned in several countries, officially reported data from Europe relatively to the 1990s, showed that agricultural application was still the major source of HCB release (Storelli *et al*., 2004). HCB is one of the most persistent environmental pollutants due to its chemical stability and resistance to biodegradation. Its persistence and tendency to bioaccumulate means that HCB can travel around the globe. The IARC has determined that HCB is a possible human carcinogen. HCB is also a proven endocrine disruptor; see for example the study by Ralph (2003).

BOX 6 - POLYCHLORINATED BIPHENYLS (PCBs)

Polychlorinated biphenyls (PCBs) are a group of halogenated organic substances that are frequently assigned to the larger group of persistent organic pollutants (POPs); they are a group of 209 synthetic chemical compounds that occur as mixtures of individual components, known as congeners, which differ in the number and position of chlorine atoms. PCBs have been commercially produced for various applications since the 1930s. In 1966 attention focused on PCB poisoning of birds and people. By the late 1970s, evidence of the extreme persistence and adverse health effects of PCBs had resulted in bans on their manufacture in some industrialised countries. Many industrialised countries have now taken steps to control and restrict the flow of PCBs into the environment. PCBs have been used for a variety of applications, including dielectric fluids for capacitors and transformers, heat transfer fluids, hydraulic fluids, lubricating and cutting oils, and as additives in pesticides, paints, plastics, etc. The current environmental occurrence of these compounds is thus believed to be a direct result of the historical intentional production, consecutive use and disposal or accidental release from products or materials containing PCBs. Their resistance to physical-chemical or biological degradation processes makes these contaminants extremely persistent in the environment.

PCBs are exclusively of anthropogenic origin and are not readily water soluble; their solubility diminishes with increasing chlorine atoms. Their hydrophobic quality renders them, on the other hand, easily soluble in lipid-rich biological tissues. The environmental concerns caused by PCBs in a water environment are primarily a result of the affinity for PCBs to partition into the organic phases of suspended particles and bottom sediments as well as into the lipids of aquatic biota. This is problematic for several reasons. A primary issue of concern is that human exposure to PCBs tends to be significantly controlled by consumption of seafood, such as fatty fish. A second issue of long-term concern is the potential remobilisation of PCBs from contaminated sediments (Larsson, 1985), representing a potential threat for further uptake in biota for decades to come because of the long response time of sediments to loading reductions (Perez *et al*., 2003).

In humans, these compounds, especially the so-called coplanar or dioxin-like PCB congeners (the most toxic members of the PCB family), can cause a variety of adverse effects, including chloracne, thymic atrophy, liver damage, birth defects, immunotoxicity and cancer (Birnbaum and DeVito, 1995). There is also an accumulating body of experimental evidence that children exposed to dioxin-like PCBs prenatally or through mother's milk show transient developmental neurological deficits (Brouwer *et al*., 1995, Huisman *et al*., 1995, Koopman-Esseboom *et al*., 1994). Surveys measuring the daily exposure to PCBs in humans have shown that over 90% occurs through the diet (Beck *et al*., 1992, Birmingham *et al*., 1989, Schaum *et al*., 1994), with seafood usually being the predominant source (Alcock *et al*., 1998, Anderson *et al*., 1998, Kannan *et al*., 1994). The IARC and EPA have classified PCBs in Group 2A: probably carcinogenic to humans.

Many studies have investigated the endocrine disrupting properties of PCBs (Tabuchi *et al*., 2006; Fonnum *et al*., 2006; Brevini *et al*., 2005; Debier *et al*., 2005; Braathen *et al*., 2004)

BOX 7 - BROMINATED FLAME RETARDANTS (BFRs)

Brominated flame retardants (BFRs) are a group of ubiquitous industrial chemicals, and many of them are produced in large volumes. Currently BRFs are the largest market group of all chemicals because of their low cost and high performance efficiency (Birnbaum and Staskal, 2004). In fact, there are more than 75 different BFRs recognized commercially. The estimated global consumption of BFRs shows that their usage is on the rise: during the period from 1990 to 2000 their use doubled from approximately 145 to 310 kilotons (Arias, 2001; Eljarrat *et al*., 2005). They are used in plastics, textiles, electronic circuitry and other materials to prevent fires. The five major BFRs are tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD), and three commercial mixtures of polybrominated diphenyl ethers (PBDEs), which are known as deca-bromodiphenyl ether (DBDE), octa-bromodiphenyl ether (OBDE), and penta-bromodiphenyl ether (PentaBDE). Despite their large usage, little toxicity information is present for nearly half of the existing BFRs (Birnbaum and Staskal, 2004).

BFRs have recently received much attention due to their similarity with "old" classes of organohalogenated compounds such as PCBs, in terms of their fate, stability and environment accumulation. They are persistent and lipophilic and many are known to bioaccumulate in humans and wildlife. Recent reports have demonstrated that BFRs exist in the environment far from the locations where they are produced and/or used, and that the concentrations of some of the BFRs, both in the environment and in humans, are rapidly increasing (Birnbaum and Staskal, 2004). The PBDEs are reported to be extremely stable. However, several studies have investigated the stability of commercial PBDE mixtures (Penta-BDE, Octa-BDE and Deca-BDE) and there is evidence for PDBEs' transformation in biota and in the environment. The Deca-BDE commercial mixture is considerably less toxic in its original state, with respect to the other two mixtures, but has been shown in studies to break down into furans and harmful chemical forms similar to those of Penta-BDE and Octa-BDE (Birnbaum and Staskal, 2004).

The PBDEs potentially involve 209 different congeners, present in flame-retarded products in a range from 5% to 30%, extensively used in electronic equipment such as computers and televisions and also in textiles and polyurethane foam in furniture and cars. The last 15 years of studies focused mainly on PBDEs: DecaBDE is the most widely used PBDE globally. PentaBDE is essentially only used in America, whereas OctaBDE remains a minor product worldwide. Some of these differences may be due to the voluntary ban on PentaBDE in Europe (formalized as of July 2003), which was followed by a European Union directive restricting the use of PentaBDE and OctaBDE in electrical and electronic equipment by July 1, 2006 (Birnbaum and Staskal, 2004). Still, DecaBDE is in use, and it represents the major product in PBDE markets, accounting for approximately 80% of the total PBDE production worldwide, although studies have shown it can degrade in the environment to lower brominated PBDEs.

The major PBDE congeners are n° 47 (TBDE), 99 and 100 (PentaBDEs), and 153 and 154 (HxBDEs). BDEs 47 and 99 are the major congeners in the pentaBDE mixture, accounting for approximately 75% of the total mass. There is roughly twice as much BDE 99 as BDE 47 in the commercial mixtures, and approximately equal amounts of BDEs 153 and 154 (Birnbaum and Staskal, 2004)

The use of PentaBDE has decreased, in response to its ban in Europe, in 2004. The critical effects of PentaBDE are those on neurobehavioural development (from 0.6 mg/kg body weight) and, at somewhat higher doses, on thyroid hormone levels in rats and mice, OctaBDE affects foetal toxicity/teratogenicity in rats and rabbits (from 2 mg/kg body weight), and DecaBDE affects thyroid, liver and kidney morphology in adult animals (from 80 mg/kg body weight) (Darnerud, 2003). Carcinogenicity studies, only performed for DecaBDE, show some effects at very high levels, and IARC (1990) evaluates DecaBDE as not classifiable as to its human carcinogenicity.

The environmental fate of PBDEs appears thus to be analogous to the fate of other structurally similar environmental pollutants such as PCBs, for which the main route of human exposure is via food (Darnerud *et al*., 2001). Although sources other than food may also influence total PBDE intake in humans, to date, there is an evident lack of data on these hypothetical sources of human exposure (Bocio *et al*., 2003). The chemical properties of PBDEs have made them ubiquitous environmental contaminants and there is scientific evidence that they can subsequently produce a wide range of adverse effects (McDonald, 2002).

Our toxicology database is inadequate to truly understand the risk. Many of the studies that do exist involve commercial mixtures, but we need studies that focus on the congeners, and potentially their metabolites and/or breakdown products, present in people and wildlife in order to understand the risk from exposure to BFRs (Birnbaum and Staskal, 2004).

Many studies link BFRs to endocrine disruption (Fonnum *et al*., 2006; Hamers *et al*., 2006; Gill *et al*., 2004; Brown *et al*., 2004; Legler *et al*., 2003; Branchi *et al*., 2003; Darnerud, 2003).

BOX 8 - PERFLUORINATED COMPOUNDS (PFCs)

Perfluorinated compounds are man-made chemicals, commercially produced by an electrochemical fluorination process for over 40 years (Kissa, 2001). PFCs have become the focus of public health concern over the past 5 years as studies have revealed their extreme persistence due to the very strong carbon-fluorine bonds in the chain of the PFC molecule. They resist natural breakdown processes; nevertheless, some PFCs can be transformed in the environment or in living organisms to form other, more stable PFCs. Research has revealed that they have the potential to bioaccumulate in the blood and liver of living organisms and data have emerged about their toxicity. PFCs are used in a wide diversity of products not just the name-brand products such as Teflon, Scotchgard and related products, but also an array of industrial and consumer products, including various protective, stain-resistant coatings for carpets and apparel, paper coatings, insecticides, and surfactants (Kannan *et al*., 2004). The properties that make PFCs so effective in these products are also the reasons why they tend to persist in the environment. PFC compounds are typically derived from perfluorooctanesulfonyl fluoride (POSF). Using this fluorinated compound as a building block, further reactions produce several other fluorinated compounds, including perfluorooctane sulfonate (PFOS) (Kissa, 2001; Olsen *et al*., 1999). POSF-based compounds may degrade or metabolise to PFOS (Olsen *et al*., 1999). PFOS is stable, chemically inert, and non-reactive and has the potential to bioaccumulate (Key *et al*., 1997; Moody and Field, 2000; Giesy and Kannan, 2001).

FOSA (Perfluorooctanesulfonamide), PFOA (Perfluorooctanoic acid) and PFHxS (Perfluorohexane sulfonate) are intermediates in the production of several perfluorinated compounds, FOSA and PFOA are also used in various applications. FOSA is used as an insecticide (Sulfluramid) to control cockroaches, termites and ants (Vitayavirasuk and Bowen; 1999). PFOA is an impurity in various formulations of perfluorochemicals including aqueous film fire-fighting foams (Moody and Field, 2000).

PFCs have also been found in the body tissues of many different living organisms throughout the world including humans. PFOS has been identified in serum samples from both occupationally and non-occupationally exposed human populations and in various species of wildlife (Olsen *et al*., 1999; Giesy and Kannan, 2001; Hansen *et al*., 2001; Kannan *et al*., 2001a; 2001b; 2002b; 2002c) while FOSA, PFHxS and PFOA have been detected in human blood (Hansen *et al*., 2001). However, studies describing the occurrence of FOSA, PFHxS and PFOA in wildlife are scarce.

PFOS and PFOA have been shown to have harmful effects on cell membranes and on communication between cells (Hu *et al*., 2003). Effects including memory decline; impaired learning; decreased reflex response time and neonatal deaths have been demonstrated in laboratory rats (Austin *et al*., 2003; Grasty *et al*., 2003; Lau *et al*., 2003; Thibodeaux *et al*., 2003). Harmful liver effects were observed in wood mice living near a fluorochemical plant (Hoff *et al*., 2004).

PFCs are now ubiquitous global contaminants. These chemicals have been detected in indoor and outdoor air, in rivers, lakes and groundwater, in wastewater treatment effluent, in landfills and in the marine environment. During the past few years research has revealed that PFOS is the predominant PFC compound detectable in living organisms. Other PFCs, such as PFOA and long-chain perfluorocarboxylates, are also detectable but often at lower concentrations.

The EU is currently preparing some legal restrictions for many uses of PFOS. Studies have already shown that fluorinated chemicals are associated with endocrine disruption and other harmful health effects (Lau *et al*., 2006; Maras *et al*., 2006; Nakayama *et al*., 2005; Harada *et al*., 2005; Lau *et al*., 2004; Hekster *et al*., 2003; Austin *et al*., 2003).

The pros and cons of fish consumption: a human and environmental health problem

Some geographic areas are potentially more threatened by man-made persistent,, bioaccumulative and toxic chemicals than others: one of these is the Mediterranean Sea and all the wildlife dependent on it. (Ankley *et al*., 1998). Contaminants in fish derive predominantly from the water they live in and the food they eat (EFSA, 2005); how much of a chemical a fish will accumulate depends on the:

trophic position of the fish - higher concentrations are generally found in fish that are higher up in the food chain;

fat content of the fish - many chemicals accumulate in fat, so fish with more fat will have more chemical accumulation ;

age of the fish - older, larger fish usually have higher contamination levels than younger, smaller fish;

chemical contamination levels in the living environment - due to a combination of social, historical and natural factors, chemical concentrations can vary from one place to another.

Other than being an important ecosystem component that operates at several trophic levels in the sea, from primary consumers of plant material and detritus to carnivores and scavenger, fish constitute an important nutritional contribution to the human diet, providing proteins, fatty acids (such as long-chain n-3 polyunsaturated fatty acids: EPA [eicosapentaenoic acid] and DHA [docosahexaenoic acid]) and certain vitamins and minerals. Consumption of fish is beneficial to cardiovascular health and may also benefit development of the unborn child (ISSFAL, 1994). Several institutions provide dietary recommendation guidelines for fish consumption (the interested reader is advised to refer to the National Academy of Sciences, Dietary Guidelines Committee Report, and American Heart Association).

On the other hand, fish consumption can significantly contribute to the human dietary exposure to some contaminants, such as persistent organochlorine compounds, brominated flame retardants and organotin compounds. Intake of high levels of contaminants from fish is undesirable and may represent a risk to human health if exposure is chronic (EFSA, 2005).

Fish consumption recommendations, estimating the amount of substances that can be ingested daily without appreciable health risk, are based on only a few toxic compounds because data generally are lacking for many other known fish contaminants (such as pesticides and PBDEs). Since food is the main chemical exposure route, data on daily intake of EDCs are of major relevance and are a necessary basis to develop an adequate risk assessment. In addition, humans are exposed not to a single endocrine disrupter but to a ‘cocktail’ of such chemicals, and the possibility that such chemicals have combined and/or additive effects must be considered seriously. Moreover, the ability to metabolize and excrete contaminants varies from person to person. Another major complicating factor is that some endocrine disruptors may cause adverse effects at very low levels ("low-dose hypothesis" [EPA, 2002]).

For some recent overviews of the health benefits and risks related to fish consumption the reader is referred to the following references: Morrissey (2006), Foran *et al.,* (2005), Cohen *et al*., (2005), Gochfeld *et al*., (2005).

Swordfish study

To monitor the contamination levels of EDCs in swordfish, muscle and liver tissues were analysed. Liver, although not eaten by humans, is the most important accumulation organ of the fish.

The recent swordfish findings were presented at the 37th SIBM (Italian Society of Marine Biology) Congress on June 5-10 which was organized - in 2006 – by the University of Siena's "G. Sarfatti" Department of Environmental Science. Proceedings were published in the scientific journal *Biol. Mar. Medit*. (2006); 13 (2): 336-337 with the title "Emerging contaminants in *Xiphias gladius* from Tyrrhenian sea area" (C. Guerranti, G. Perra, I. Bisogno, S. Focardi). The interested reader is referred to the original work by Professor Focardi.

Seventeen adult swordfish (*Xiphias gladius*), 10 male and 7 female specimens, various ages with a weight range of 11-93 kg and length range of 100-205 cm, were captured between March-April 2005 in the Mediterranean (Eastern South Tyrrhenian Sea, see figure 1).

Figure 1. Sampling stations where swordfish were collected

As soon as the fish were captured, 29 samples of liver and muscle were collected. Muscles samples were obtained for all 17 fish and liver samples for 12 fish. Samples were analysed[4] to assess the concentrations of the following 28 chemical compounds:

7 organochlorine pesticide residues:
 the fungicide hexachlorobenzene (HCB);
 6 isomers and metabolites of the insecticide DDT;
19 brominated flame retardants: polybrominated diphenyl ethers (PBDE) congeners;
2 perfluorinated compounds: perfluoroctanesulfonate (PFOS) and perfluoroctanoate (PFOA)

What do the data show?

To the authors' knowledge, this study detected for the first time in Mediterranean swordfish the presence of one of the most frequently used groups of brominated flame-retardants: polybrominated diphenyl ethers (PBDEs). These are highly persistent and bio accumulative compounds, but there is currently only scarce data available on their occurrence in Mediterranean species. The commercially used mixtures penta- and octa BDE were banned in the European Union in 2004, but continue to be used in other parts of the world and will persist for many years in the marine environment.

Table 1: Selected compounds in the tested swordfish

	HCB	pp-DDE	PBDEs	BDE-47	BDE-100	BDE-99	PFOS/PFOA
# of fish positive for:	7/17	17/17	16/17	16/16	10/16	6/16	0/17
% of fish positive for:	41.1%	100%	94.1%	100%	62.5%	37.5%	0%

Brominated flame retardants were detected in all swordfish tested but one. In liver samples, PBDEs were found in concentration ranges of 189-11184 pg/g w.w., while in muscle samples they were found between <0.04-1882 pg/g w.w. The prevalent PBDE congeners found were 47 and 100, two of the most common congeners found in marine organisms.

While organochlorines were found in all swordfish samples, PFOS and PFOA were not detected. Data on contamination of swordfish with persistent organohalogens were, in general, comparable with the levels observed in previous studies, even though pp' DDE (a DDT metabolite) showed slightly higher concentrations compared to other studies around the Italian coast. In general, organochlorine concentrations have decreased over the last 15 years, but despite the strong restrictions and/or bans DDT, its homologues and metabolites (especially *pp'*-DDE), still occur at average levels of 173 ng/g w.w. (muscle) and 309 ng/g w.w. (liver) in the swordfish analysed. Levels of the banned pesticide HCB were low, ranging from <0.01-0.53 ng/g w.w. and <0.01-0.84 ng/g w.w. in muscle and liver respectively. As expected, the levels of all analysed compounds were always higher in liver than muscle.

A high variability in the contamination levels, a common phenomenon when working with biological samples, was observed. No statistical correlation between pollution levels and gender, weight or length of the swordfish was found.

[4] The organohalogen and perfluorinated compounds in swordfish samples were analysed with the analytical methodology described by Kannan *et al*. (2001a) and Hansen *et al*. (2001)/Kannan *et al*. (2001b) respectively.

a) Results for organohalogen compounds (HCB and DDT) found in this study

Pollution by organohalogen compounds has spread all over the world as evidenced by their detection both in humans and wildlife. Within the marine environment, the coastal areas deserve special consideration as primary receivers of urban, industrial and riverine chemical inputs. Little attention has, in contrast, been paid to open-sea waters of the marine ecosystem (Storelli and Marcotrigiano, 2006). POPs are distributed all over the marine ecosystem. Swordfish, occupying upper links in the pelagic food chain, may exhibit a high potential for the accumulation of these pollutants. For more information on the studied compounds please see box 5.

HCB results:

HCB concentrations detected in the 29 swordfish liver and muscle samples were low and often below detection limits (range values: muscle between <0.01 and 0.53 ng/g w.w.; liver between <0.01 and 0.84 ng/g w.w.). Eight samples were positive for HCB (5 muscle and 3 liver samples, from seven different fish). HCB values were generally higher in swordfish samples taken near the Calabria coast than those from the Eolie Islands (see table 2).

Table 2: Average HCB values in swordfish compared with data from another capture area

Swordfish capture area	Average HCB (ng/g w.w.)	
	Muscle	Liver
Eolie Islands	<0.01	0.01
Calabria	0.13	0.16

Corsolini *et al*. (2005), whose samples were collected from the North-Eastern coasts of Sicily, found a HCB average concentration below the detection limit in muscle and a concentration of 1.1 ng/g w.w. in liver. The comparison shows (see figure 2) that the liver contamination values found by Corsolini compared to liver samples analysed in the present study are lower whereas the average level in muscle samples is higher.

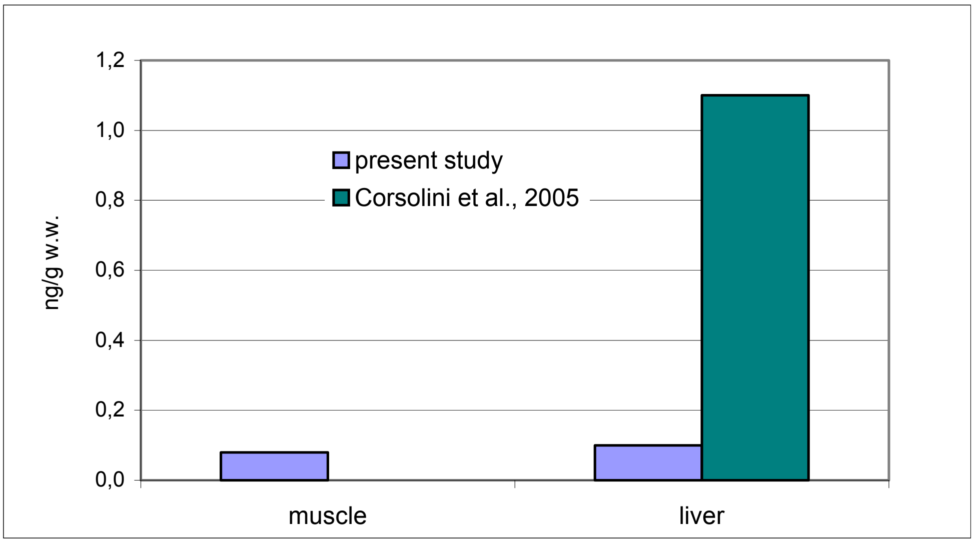

Figure 2. Comparison between HCB average concentrations in swordfish liver and muscle obtained in the current study compared to Corsolini *et al*. 2005

DDT results:

Regarding DDT and the respective metabolites, the DDT metabolite *pp′*-DDE was found in all samples. *Pp′*-DDE is the most abundant of the DDT group, representing 78% and 76% of the total DDTs determined in muscle and liver respectively, indicating the high stability of *pp′*-DDE in the environment.

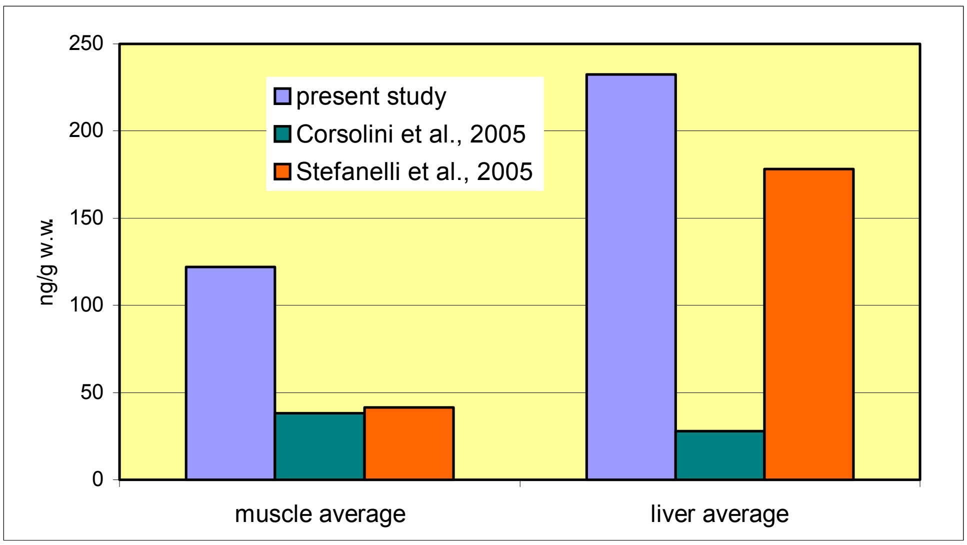

Figure 3. Average concentrations of *pp*'DDE found in swordfish liver and muscle obtained in the current study compared to data from other literature

As DDT is metabolised to DDE, the ratio of *pp'*-DDE to total DDTs can be used as an indicator of new sources of DDT entering the ecosystem. The value of 0.6 is reported as a threshold (Tsydenova *et al.,* 2004): a ratio greater than 0.6 is indicative of a lack of recent inputs of DDT (Stefanelli *et al*., 2004). In the analysed liver and muscle samples, this ratio was 0.79 and 0.75, respectively. According to these data, it appears that the Mediterranean ecosystem is not receiving significant new inputs of DDT.

Results of average DDT levels obtained in the present study have been compared with data from Corsolini *et al*., (2005) for swordfish collected off the North-Eastern coasts of Sicily and from Stefanelli *et al*., (2004) for swordfish caught in the Strait of Messina (Sicilian coast). Data are shown in figure 3 and indicate higher contamination levels in the present study compared to reference data for both tissues analyzed.

A recent study by Storelli and Marcotrignano (2006) of swordfish caught in the Ionian Sea showed, for liver samples, a pattern of DDT compounds in agreement with those reported by Stefanelli *et al*. (2004) but lower than contamination levels in the samples analysed in the present study. Storelli and Marcotrignano (2006) confirmed the data about *pp'*-DDE making up the largest percentage of the total DDT burden and affirmed that contamination levels in swordfish are comparable to those detected in other Mediterranean fish occupying similar trophic positions (such as shark and tuna).

b) Results for brominated flame retardants found in this study

There are different commercial formulations of PBDEs, made of several of the 209 different PBDEs congeners. There is growing concern about the possible harmful consequences of exposure to PBDEs because they are suspected to be capable of modulating or disrupting the endocrine system.

To the authors' knowledge, this study investigates, for the first time, the presence of one of the most prominent classes of brominated flame-retardants in Mediterranean swordfish: the persistent and bioaccumulative polybrominated diphenyl ethers (PBDEs).

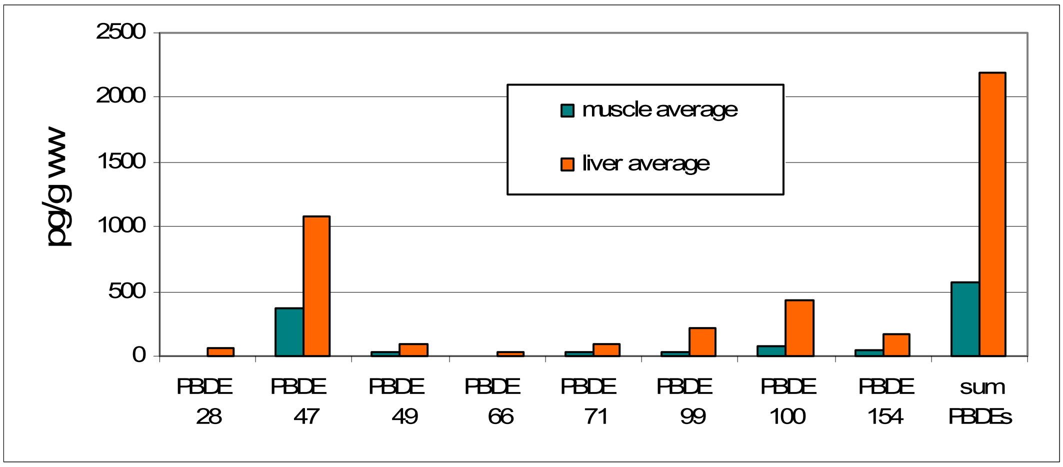

Figure 4. Average concentrations of PBDE congeners in swordfish muscle and liver and the sum of the 19 PBDEs analysed in the present study. PBDE congeners that were not found in any sample are not listed.

PBDEs were found in 16 of the 17 swordfish tested (in all liver samples and 13 muscle samples). The concentration pattern of PBDEs showed relatively lower contamination of muscle compared to liver samples, which were approximately an order of magnitude higher. The range values were 189 and 11184 pg/g w.w. and <0.04 and 1882 pg/g w.w for liver and muscle samples respectively (compared in figure 4).

The positive BFR results in this study are reported in figure 4, as the average concentrations in muscle and liver samples. Of the 19 PBDEs analysed, BDE-3, -7 -15, -17, -66, -77, -85, 119, 126, -138, -153, and -156 were not found in any of the samples. BDE-47, -99 and -100 seem to be predominant and the highest concentration for an individual PBDE was 5421.78 pg/g w.w. found for BDE-47 in a liver sample.

In this study the prevalent congeners were BDE-47, BDE-99 and BDE-100, used in the technical mixture "pentaBDE" (see box 5) found in the following percentage of samples:

Table 3: Percentage of the swordfish tissue prevalent congeners

Swordfish tissue	PBDE congeners		
	BDE-47 (%)	**BDE-99 (%)**	**BDE-100 (%)**
Liver	31	14	27
Muscle	45	7	14

Similar ranges have been confirmed in various studies conducted in sea organisms (Akutsu *et al.,* 2001; Christensen *et al.,* 2002; Law *et al.*, 2002) and, in fact, PBDE-47 has been the congener most often used in commercial mixtures of PBDEs (EU, 2001).

As for HCB values, average PBDE concentrations were higher in liver samples from Calabria swordfish than concentrations from the Eolie Islands while muscle samples showed an opposite trend.

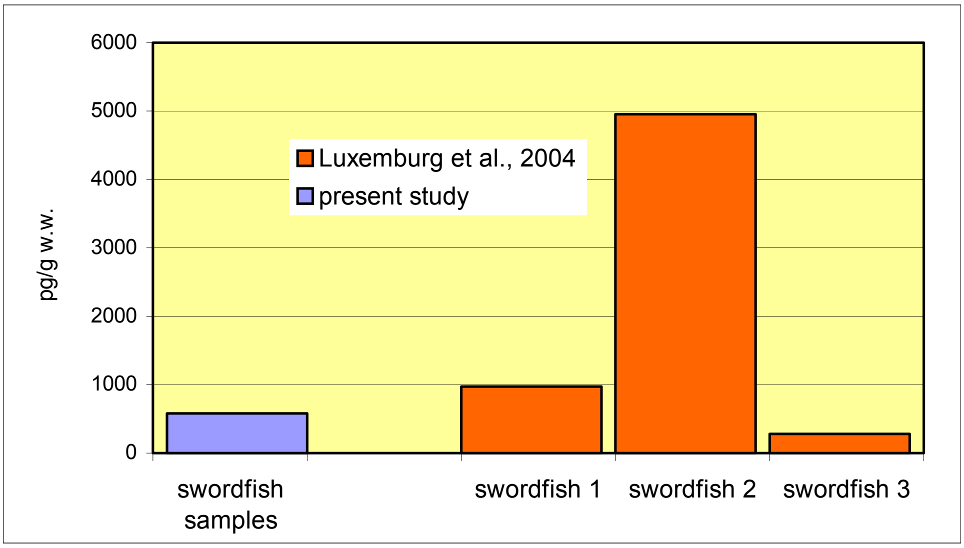

Figure 5. Average values of PBDEs in swordfish muscle of the present study compared with data from the literature.

To the authors' knowledge, no other studies are available on PBDE swordfish contamination for Italy; therefore, it is difficult to compare the results. However, a general consideration of the status of contamination of this Mediterranean fish can be drawn from comparison with average PBDE concentrations detected in this study versus concentrations found in the study by Luksemburg *et al*., (2004), designed to measure PBDE exposure through the consumption of fish products. In this study Luksemburg *et al* analysed fillets from several species of freshwater and ocean fish purchased from three food markets in northern California (USA). The authors reported PBDE concentrations relative to 3 wild swordfish samples. The data comparison in figure 5 shows that Mediterranean samples are in the same range as Luksemburg's samples, with the exception of the high level in swordfish sample number 2. In both studies, the 2 PBDE congeners PBDE-47 and PBDE-100 were found in the highest concentrations.

PBDEs represent a complex group of related chemicals and the pattern of PBDE congeners in food is not clearly defined by a single commercial mixture. The UK Committee on Toxicity of chemicals in food, Consumer Products and the Environment (COT) concluded that due to the limited data available, no Tolerable Daily intakes (TDI) can be set for PBDE (Committee on Toxicity, 2006).

c) Results for perfluorinated compounds found in this study

More and more scientific studies reveal global ecosystem contamination with perfluorinated compounds (for details compare box 6 and part II of this report) although the results of this investigation indicate that perfluorochemicals (PFOS and PFOA) were not major contaminants in the Mediterranean swordfish samples. PFOS and PFOA were found to be below the limit of quantification (5 ng/g wet weight [w.w.]).

Kannan *et al*. (2002) investigated levels in swordfish collected from the Strait of Messina (Ionian-Thyrrenian Sea): the mean concentration of PFOS in blood of swordfish was 10 ng/mL w.w. and, similarly, livers of swordfish contained quantifiable concentrations of PFOS (range 4 to 21 ng/mL; mean: 10); PFOA was not found at the quantification limit in any of the swordfish tissue samples (blood and liver).

Final considerations

Data from the present study on contamination of *Xiphias gladius* swordfish with persistent organohalogen and perfluorinated compounds are, in general, comparable with levels observed in previously reported similar studies. The increased level of the principal metabolite of DDT, *pp'*-DDE, found in the analysed samples may be attributed to a high level of previous use of insecticide in the local environment. Perfluorinated alkylated (PFOS and PFOA) compounds were, in all analysed samples, below the detection limits.

As far as we know, this study reports the first data on swordfish contamination by PBDEs for Italy. The widespread production and use of brominated flame retardants; strongly evidenced by the increasing contamination of the environment, wildlife, and people; and limited knowledge of potential effects heighten the importance of identifying emerging issues associated with the use of BFRs. In this context, the PBDE results are an important reference point for future studies, because they provide the first data in the Mediterranean Sea on contamination of swordfish, a species with high ecological and commercial interest. It is important to remember that low chemical levels do not necessarily mean low risk for the organism.

It would be interesting and worthwhile to expand on this analysis by using a larger sample size, to verify the significance of the differences in contamination levels for the 2 investigated areas. In addition, more data are needed on the occurrence of emerging contaminants in additional species and environmental matrices (water, marine sediments) to better characterize the state of Mediterranean Sea contamination with endocrine disrupting chemicals.

PART II

MEDITERRANEAN WILDLIFE AS AN INDICATOR OF CHEMICAL CONTAMINATION


Herring gull, (Larus argentatus) (c) WWF-Canon / Anton VORAUER

Introduction

This second part of the report focuses on Mediterranean Sea contamination, which affects not only swordfish but the entire ecosystem. This second section is a bibliographic review of scientific literature about the occurrence and possible impact of man-made industrial chemicals (organochlorine pesticides [OCPs], polychlorinated biphenyls [PCBs], brominated flame retardants [BFRs] and perfluorinated compounds) on different species living in the Mediterranean Sea. Scientific literature on Mediterranean contamination with heavy metals and current-use pesticides has not been included in the overview. The interested reader is also referred to the recent report "Priority issues in the Mediterranean environment" by UNEP and EEA (EEA Report No.4, 2006).

By looking at a range of species representing various trophic levels an overview of the occurrence and distribution of pollutants in the marine ecosystem is obtained. In particular, fish and invertebrates (many of which are edible), mammals and seabirds are useful bioindicators of contamination. It is very difficult for scientists to make direct cause-effect linkages because occurrence and severity of adverse effects depend not only on the biological and physiological traits of the species, but also on other factors. For example, prolonged exposure to many of the described pollutants can, even at low concentrations, interfere with the normal physiology of the organism (Storelli *et al*., 2006). This is particularly true for long living species that accumulate contaminants over many years Among marine mammals, cetaceans have a number of biological traits that make them particularly prone to accumulate high levels of pollutants: they feed on other species relatively high in the food chain; they are warm blooded, have high metabolic rates and, as a consequence, their food intake rate is high. Finally, because of thermoregulatory and energy storage needs, a large proportion of marine mammals' body mass is composed of lipid-rich blubber, a body compartment that efficiently retains and accumulates lipophilic contaminants. Cetaceans provide important information on how contaminants are transferred between trophic levels, showing the maximum capacities of chemical bioaccumulation. Invertebrates are also used as bioindicator organisms due to the fact that they constitute 95% of all animal species, are major components of all ecosystems and live in confined areas. Waterbirds, especially those which eat marine fish, are exposed to contaminants because of their position at the top of the food chain.

Mediterranean wildlife and organochlorine compounds

Many studies have documented the chemical stress related to organochlorine compounds (OCPs) in marine mammals and waterbirds in the Mediterranean Sea: substances such as DDT, and its metabolites, and PCBs that have been banned for decades continue to be found in dolphins, cormorants and gulls. Details on these substances can be found in boxes 5 and 6.

Organochlorines in Mediterranean marine mammals

Cetacean species are thought to have different sensitivities to environmental contaminants, especially organochlorines, and this may impinge on their vulnerability to these contaminants, many of which are endocrine disruptors (Marsili *et al*., 1996; 1998). The transfer of a significant portion of the organochlorine contaminant burden from a cetacean female to her offspring, particularly during sensitive portions of the foetal and neonatal life cycle phases, could result in serious health problems. The offspring of primiparous females, whose body burden of contaminants is particularly high, are especially vulnerable (Aguilar and Borrell, 1994a, 1994b). Substantial evidence points to organochlorine compounds having, at environmental levels, a number of adverse effects on marine mammal populations (e.g. common seals [*Phoca vitulina*], grey seals [*Halichoerus grypus*] and ringed seals [*Pusa hispida*]). These include depression of the immune system (De Swart *et al*., 1994, 1995, 1996) and the subsequent triggering of infectious diseases (Aguilar and Borrell, 1994a), reproductive impairment (Reijnders and Brasseur, 1992), lesions of the adrenal glands and other organs (Olsson *et al.*, 1994), cancers (Martineau *et al.*, 1994), alterations in skeletal growth and ontogenic development as well as the induction of bone lesions (Aguilar and Borrell, 1994a).

Marsili and colleagues (2004) measured HCB, DDTs and PCB congener concentrations in striped dolphin (*Stenella coeruleoalba*) making a comparison between free-ranging and stranded specimens. Levels of each organochlorine compound were higher in the stranded animal group than in the free-ranging group. The probable cause of death of the stranded dolphins has been attributed to a virus of the genus *Morbillivirus* that affected the animals following impairment of their immune systems by organochlorines (Aguilar and Borrell, 1994a).

Odontocetes (dolphins and porpoises) are incapable of metabolising certain PCB congeners, and therefore accumulate these compounds more readily than other mammals or birds with comparable biological traits. Through the analysis of blubber of 186 striped dolphins (*Stenella coeruleoalba*), Aguilar and Borrell (2005) showed that environmental levels of DDT and PCB in the oceanic waters of the Western Mediterranean have decreased significantly during the last 15 years. These concentrations are of the same order of magnitude as those reported in previous studies in the same species and region (Aguilar, 2000; Marsili *et al.*, 1997), but substantially higher than those typically found in striped dolphin populations in waters of Japan and the Atlantic coasts of Europe (O'Shea and Aguilar, 2001). Results are also consistent with findings in other organisms, which show that contaminant levels in the western Mediterranean Sea are exceptionally high, particularly in the north-western basin. This is a consequence of the basin's proximity to the highly populated, industrialized European coasts (Fowler, 1987) and of the scarce flux rate, a characteristic that reduces the potential for dilution and dispersion of contaminants.

In another interesting study, Borrell and colleagues (1996) compared organochlorine concentrations in common dolphins (*Delphinus delphis*) from the Mediterranean waters of Spain and the Atlantic Ocean. The study revealed a higher total DDT concentration in Mediterranean common dolphins than their Atlantic counterparts and similar concentrations of total PCBs. This result denotes a proportionally higher contribution of pollutant releases of agricultural origin than industrial origin in the Mediterranean Sea. The study also reveals different proportions of PCB congeners in specimens of the two populations suggesting differences in the pollution source of PCB or in the input time-scale (Atlantic input of PBC is older than that of the Mediterranean). Whatever the case, it appears clear that the two dolphin populations feed in different areas and do not mix, at least on a short or medium-term scale.

Pollutant concentrations in Mediterranean common dolphins (*Delphinus delphis*) are in the lowest range of those detected in other dolphin species from the same region and time period. Thus, total DDT and total PCB concentrations in striped dolphins (*Stenella coeruleoalba*), bottlenose dolphins (*Tursiops truncatus*), Risso's dolphins (*Grampus griseus*) and long finned pilot whales (*Globicephala melas*) were between 2-fold and 27-

fold of those detected in the common dolphins sampled for the present survey (Corsolini *et al*., 1995; Borrell *et al*., 1996; Marsili and Focardi, 1997). It is unclear why there was such a marked significant difference in pollutant concentrations in species that are so similar in biological and ecological characteristics. This variability may be the result of subtle, but meaningful, differences in diet composition, a dissimilar ability of the different species to handle pollutants or a combination of the two factors.

Organochlorines in cormorants and gulls

Waterbird species, especially pelecaniform (pelican, cormorant) and larid (gull, tern) are particularly useful as bioindicators of organochlorines in the aquatic environment (Goutner *et al.*, 2001). One of the negative effects of organochlorine contaminants is the thinning of bird eggshells. This has been found in several species of fish-eating waterbirds and raptors. In some cases it has caused severe reductions in reproductive success. In most cases eggshell thinning is caused by *pp'*-DDE, a persistent metabolite of DDT. Several authors have presented dose-effect relationships between levels of *pp'*-DDE in the egg and eggshell thickness. Moreover, pollutants in eggs can interfere with the proper development of chicks.

The levels of organochlorines in seabird eggs reflect the diet of the female (Furness, 1993). Cormorants (*Phalacrocorax* spp.) are fish feeders, and thus are top predators in aquatic environments and are affected by contamination (Scharenberg, 1991; Ryckman *et al*., 1998

The Greek wetland region is the home to internationally important populations of wildlife, especially birds. Goutner and colleagues (2001) investigated the occurrence, levels and potential effects of organochlorines on Audouin's gulls (*Larus audouinii*) in Aegean sea colonies. The authors measured the levels of PCBs and organochlorine pesticides in the eggs of the gulls and all eight PCBs congeners analyzed were detected. In regard to pesticides, all 13 organochlorine compounds analyzed (such as aldrin, dieldrin, DDTs, etc.) were detected. However, the effects on Audouin's gulls remain unclear.

Organochlorines in invertebrates

Among invertebrates, sponges have some of the fundamental characteristics of good biomonitors (Perez, 2000), because they are sessile filter feeders, constitute the most dominant phylum found in the marine hard substrates, are characterized by a sophisticated aquiferous system, a strong pumping activity and the ability to absorb a wide array of microscopic particles and dissolved material and have high lipid content that accumulates some very persistent xenobiotics (Perez *et al*., 2003). Their feeding habits make sponges particularly vulnerable to seawater quality. They are tolerant of physical-chemical fluctuations and may be abundant in conditions of extreme pollution. The concentration of pollutants in these organisms is directly dependent on the quality of the environment and does not result from amplification phenomena (Perez *et al*.,

2003) since they do not use other marine organisms for nutrition. Moreover sponges cannot contaminate higher trophic levels since they have few or no predators. Burnt sponge (*Spongia officinalis*) has been used by Perez and colleagues (2003) to assess its relevance as a biomonitor for PCBs. Twenty-four chlorobiphenyl congeners have been measured along a pollution gradient both in sponges and seawater. *S. officinalis* displays a capacity to accumulate all types of PCBs. Concentrations recorded in sponges agreed quite well with the PCB concentrations of study sites. Different ratios between PCB congeners were found in water samples and in sponges indicating that sponges are able to metabolise some persistent PCB congeners.

It is clear from the discussed scientific studies that, although highly-variable and not always at elevated levels, organochlorine pollutants still persist in the Mediterranean Sea, in spite of the fact that most of them were banned more than 30 years ago. This is a warning for other persistent and bioaccumulative chemicals that are still allowed in the EU despite their widespread occurrence in ecosystems and wildlife. Therefore, periodic monitoring of such persistent compounds is highly relevant and, in addition to compounds such as PCBs, new contaminants as described below should be monitored regularly.

Mediterranean wildlife and perfluorinated compounds

Perfluorinated chemicals used in daily consumer items such as carpets, clothing and kitchenware have made their way to the Mediterranean Sea and have in recent years been found in dolphins, swordfish, tuna and cormorants. In comparison with organochlorine compounds, data are still scarce, as only few studies have been carried out on Mediterranean wildlife concerning these substances. Details on these substances can be found in box 8.

In recent years, a new class of emerging contaminants, perfluorinated compounds (PFCs), have gained increasing attention. In contrast to most classes of persistent organic contaminants (e.g. polychlorinated biphenyls and pesticides), PFCs tend to accumulate in blood proteins rather than in body fat. For this reason traditional monitoring studies for persistent chemicals failed to identify this contaminant for a long time (Corsolini e Kannan, 2004). PFCs can now be found in wildlife and humans worldwide.

Kannan and colleagues (2002) carried out a study on the presence of concentrations of PFOS, FOSA (or PFOSA), PFHxS, and PFOA in marine mammals including bottlenose dolphins *(Tursiops truncatus*), striped dolphins *(Stenella coeruleoalb*a), common dolphins *(Delphinus delph*i), fin whales *(Balenoptera physalu*s), long-finned pilot whales *(Globicephala mela*s); fish such as northern bluefin tuna *(Thunnus thynnu*s), swordfish *(Xiphias gladiu*s); and common cormorants *(Phalacrocorax carbo*) collected from the Italian coast of the Mediterranean Sea. PFOS was the most predominant fluorochemical in the tissues analysed, it was found in bottlenose dolphins and common dolphins collected from the Adriatic and Thyrrenian Seas and often muscle tissue contained a PFOS concentration that was 12-fold less than that in liver. Livers of most of the cetaceans (except striped dolphin) contained quantifiable concentrations of FOSA and the greatest FOSA concentration was found in the liver of a common dolphin (878 ng/g wet wt). Occurrence of FOSA in marine mammals from the Mediterranean region indicates the presence of specific and current sources.

Concentrations of PFOS and PFOA were consistently also found in cormorant livers collected from Cabras Lagoon in Sardinia, contamination of juvenile birds was not significantly different from that of adults. The mean concentration of PFOA in cormorant livers was 1.7 fold greater than the PFOS concentrations (Kannan *et al*., 2002).

PCFs were also detected in bluefin tuna and swordfish liver and blood (Kannan *et al*., 2002). The average concentration of PFOS in livers of bluefin tuna was greater than that determined in swordfish. Concentrations of FOSA in bluefin tuna blood were 2-4 fold less than those of PFOS but FOSA was not detected in the livers. PFHxS was detected in one swordfish liver while PFOA was not found (Kannan *et al*., 2002).

Mediterranean wildlife and brominated flame retardants

Brominated flame retardants such as PBDEs (polybrominated diphenylethers) are ubiquitous compounds which have been found in Mediterranean dolphins. There are hardly any data on the occurrence and distribution of these chemicals in Mediterranean wildlife. This shows the huge lack of knowledge on chemicals and was one of the reasons for performing the analysis described in part 1. (for details on this substance class see box 7)

Brominated flame retardants have been found to be bioaccumulating in various whale and dolphin species. Moreover, these contaminants are still manufactured and tests show that many of them degrade very slowly. The long-term effects of these chemicals on top consumers are difficult, if not impossible, to predict from limited short-term toxicity tests on a few selected species. When effects do become evident, it will be impossible to reverse these effects in a short-term scale due to the persistent and bioaccumulative nature of these substances.

In a study by Petterson and colleagues (2004), eight brominated flame retardants were present at ppb (part per billion) levels in the livers of cetaceans found stranded on the beaches of the Mediterranean Sea, Italy. The highest PBDE concentration was found in striped dolphin and the lowest concentration in bottlenose dolphin.

Although contamination data for the Mediterranean are still insufficient, several studies have reported PBDEs in marine ecosystems around the world (Haglund *et al*., 1997; Olsson *et al*., 2000; Asplund *et al*., 2001; Marsh *et al*., 2001; Vetter, 2001; Vetter *et al*. 2001; Vetter *et al*., 2002; Marsh *et al*. 2004), demonstrating their global occurrence in wildlife.

Final considerations

Research over the last years has shown that a variety of persistent, bioaccumulative contaminants in the Mediterranean food chain are impacting marine species, some of which are food species used by humans. There is a growing body of literature supporting the use of wildlife populations as indicators of ecosystem health, suggesting that many species are sentinels for toxic chemical effects which may also affect humans.

Results from many studies have elucidated that concentrations of different organochlorine compounds have levelled off during the last years and are now more or less stagnant. However, contamination of the marine area by these older pollutants is still impacting marine ecosystems and, in addition, new contaminants are on the rise. The persistence of older chemicals, after 30 years, highlights the concern for "new" and currently allowed chemicals such as brominated flame retardants and perfluorochemicals. Semi-enclosed seas are, in fact, among the areas deserving particular attention for future investigations pertaining to persistent pollutants and their impact and effects on the environment and on humans, for which little or no knowledge is available.

If persistent and bioaccumulative chemicals are not severely restricted as soon as possible, their burden in the aquatic environment (and consequently in marine wildlife) will continue to increase, threatening both ecosystem and human health. In particular, regarding perfluorinated compounds, the results of a study by Kannan and colleagues (2002) are of particular concern because they highlight the problem of a considerable and widespread occurrence of PFOS in fishes, birds and marine mammals from the Italian seas. This fact should be considered alarming and should fully justify measures aimed at reducing the burden of perfluorinates by more restrictive policies. Without proper controls perfluorinate concentrations could increase and interact with "older" compounds in a synergic way, thus exacerbating their toxic and endocrine-disrupting effects. Many toxic substances posses an "endocrine disrupting" mechanism, which is a further matter of concern for the future and which should not be neglected.

WWF's call for a stronger chemicals legislation

Chemical contamination is a threat to wildlife as well as people. The chemicals found in the participants of WWF blood tests (see www.panda.org/detox for details) also contaminate birds, dolphins, fish and many other species even in the most remote environments. Although the majority of chemicals in commercial use today do not have enough safety information publicly available to do a basic safety assessment, research increasingly links chemicals to cancers, allergies, reproductive problems and defects in children's development.

Given the hazards of these chemicals and their persistence in the environment, governments should be seeking to eliminate them at the source. The current regulations to protect wildlife and people from these hazardous chemicals are ineffective.

In WWF's view, the proposed new EU chemical law, REACH, could provide a good framework to deal in a systematic way with controlling industrial chemicals. But after huge concessions to the industry and successive cuts in the health and safety provisions of REACH, it is not clear that the new EU chemicals law will be an improvement over the current legislation.

Thus WWF urges European legislators to ensure that the proposed new legislation on chemicals, REACH, delivers sufficient safety data on chemicals in order to identify the most hazardous ones. Chemicals of very high concern, including hormone disrupting chemicals, should be replaced with safer alternatives whenever available.

In the run-up to the final adoption of REACH, WWF urges European legislators to adopt the necessary measures to ensure that the new law will:

- Phase out all persistent, bioaccumulative and endocrine disrupting chemicals and substitute all hazardous chemicals with safer alternatives when they are available.
- Set strict requirements on chemical producers to provide safety information before a chemical can be sold or continue to be used.
- Ensure the chemical industry's responsibility for the safety of their products (Duty of Care).
- Allow consumers to easily find out what chemicals are in everyday products.

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Monk seal, (Monachus monachus) (c) WWF-Canon / Jacques TROTIGNON

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