

BIBLIOGRAPHIC REVIEW ON THE IMPACT OF CHEMICAL POLLUTION ON CETACEANS, INCLUDING THE IDENTIFICATION OF *AD HOC* RESEARCH PROJECTS AIMED AT ASSESSING CHEMICAL POLLUTION ON CETACEANS IN THE ACCOBAMS AREA



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- DRAFT REPORT -

This report has been prepared by M. Cristina Fossi and Cristina Panti of the University of Siena. The version of this report presented to the Eighth Meeting of the Parties to ACCOBAMS (MOP8) is not yet reviewed by the Scientific Committee of ACCOBAMS. This review will be organized after MOP8 and the final version of the report will be published on the ACCOBAMS website as soon as it is available.

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Objectives of the document

The main objective of this document is to describe the results of a bibliographic review on the impact of chemical pollution on cetaceans in the last 30 years (including older study but with a priority importance for the field), including the identification of ad hoc research projects aimed at assessing chemical pollution on cetaceans in the ACCOBAMS area.

The analysis of the existing literature will help to identify priorities and knowledge gaps within the ACCOBAMS area related to pollution and chemical stress to cetacean populations.

1. An overview on the impact of chemical pollution on cetaceans in the Mediterranean Sea and the Contiguous area

The Mediterranean Sea and the Contiguous area represent biodiversity hotspots. Despite Mediterranean is among the most oligotrophic marine systems in the world, some areas, such as the Corso-Ligurian Basin and the Gulf of Lion, are characterized by high levels of primary productivity related to the upwelling of nutrients which allows the concentration of large marine vertebrates in the area.

The Mediterranean coasts support a high density of inhabitants; and it has been estimated by UNEP/MAP that the population will reach 572 million by 2030. Furthermore, this basin is economically important: fishing and oil and gas extraction are among the main activities, but it is also used for commercial shipping, recreational boating, and tourism (UNEP, 1996; Shaltout and Omstedt, 2014). Approximately 30% of all international maritime traffic passes through its waters, meaning that over 200,000 vessels cross the Mediterranean Sea annually (Vaes and Druon, 2013). These characteristics, combined with an increase in the coastal industrial economy, have a great impact on its waters, and sewage and industrial and incidental discharges are an important source of persistent organic pollutants (POPs). Furthermore, the invasion of alien species, most of them entering through the Suez Channel, is a crucial factor that will continue to change the biodiversity of the Mediterranean. In these waters, the other principal threats for marine life are overexploitation, habitat loss, and degradation, which are mostly due to human activities, such as fisheries, ship traffic, water pollution, and coastal anthropization.

A high percentage of the Mediterranean marine species are endemic (Bianchi and Morri, 2000). There are several unique and endangered habitats, including the endemic *Posidonia oceanica* and coralligenous assemblages (Green and Short, 2003; Ballesteros, 2006), as well as a set of emblematic species of conservation concern, such as sea turtles, several cetaceans, and the critically endangered Mediterranean monk seal (*Monachus monachus*). Regarding the cetaceans, a dozen species habitually inhabit the Mediterranean basin and the Black Sea, which are a status of conservation (according to the IUCN Red List) from “Critically endangered” (as for *Orcinus orca* from the strait of Gibraltar), “Endangered” (as for instance for *Physeter macrocephalus* in the entire Mediterranean basin and *Phocoena phocoena* in the Black Sea), or even “Data deficient” (as it happens for *Grampus griseus*). The most common species are the striped dolphin (*Stenella coeruleoalba*), common bottlenose dolphin (*Tursiops truncatus*), sperm whale (*Physeter macrocephalus*), and fin whale (*Balaenoptera physalus*) and harbour porpoise (*Phocoena phocoena relicta*) in the Black Sea.

Globally, as apex predators with long life spans, cetaceans are particularly sensitive to environmental contaminants, in particular to heavy metals, legacy organic pollutants as polychlorinated biphenyls (PCBs), Polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethane (DDTs), and emerging pollutants

such as plastic additives. They are considered as sentinels, their study provides a great source of information on the marine environment and can provide early warnings of acute perturbation.

Previous studies on cetaceans in the Mediterranean Sea and in particularly in the SPAMI Pelagos Sanctuary have shown that animals were highly contaminated by chemical pollutants (Marsili et al., 2018).

POPs are chemical compounds of global concern due to their persistence in the environment, their ability to be transported over long distances and their effects on natural populations. POPs are biologically active chemicals that have the ability to bioaccumulate and biomagnify within marine food webs and have the potential to negatively impact marine organisms.

Most research has been focused on the study of PCBs, PBDEs, DDT and some trace elements (Hg) concentration in cetacean tissues from the ACCOBAMS are. More recently, some studies are focussing their attention on PFAS (Sciancalepore et al. 2021) and plastic additives (Fossi et al., 2016; Baini et al., 2017). There is evidence indicating that some of these legacy contaminants are declining (PBDEs, DDT), while others are still at toxic levels PCBs can be considered as a general marker of human activity and because of their toxicity, the complete ban of these compounds as well as the elimination of all human sources in Europe, should result in a decrease in contamination in the blubber/liver of cetaceans in the Mediterranean area. Trace elements should also be considered as general indicators of the environmental contamination by human activities: mercury, cadmium and lead are mostly human sources and have well-known toxic effects in mammals. The studies on Black Sea cetaceans are scarce and scattered, some data are available on pesticides or heavy metals measured in harbour porpoise (Weijs et al 2010).

Given the impact and persistence of POPs on and within the environment, the use and manufacture of many of them have already been banned. Polychlorinated biphenyls (PCBs), man-made chlorinated organic chemicals, were banned globally in the 1970s and some polybrominated diphenyl ethers (PBDEs), a class of brominated flame retardants, started being banned around 20 years ago. By the beginning of the 21st century, environmental authorities (e.g., the European REACH regulation; the Stockholm Convention; the United States Environmental Protection Agency; Environment and Climate Change Canada) became aware of the harmful effects of PBDEs based on the available scientific evidence, and nations started to take measures. In 2004 the United States and Europe voluntarily phased out the production of penta- and octa-BDE mixtures (Hites, 2006), while in 2009 they were globally regulated with their introduction in the annex A (elimination) of the Stockholm Convention (UNEP, 2009). Accordingly, most reported PBDEs started to decline or level off in the early 2000s in environmental media around the world (Law et al., 2014), with notorious exceptions such as some top predators. Importantly, the ban on these two mixtures lead to an increased production of deca-BDE formulation, made up of 96.8% BDE-209, until its more recent inclusion in the annex A of the Stockholm Convention in 2019 (UNEP, 2022). The extensive use of deca-BDE shifted the environmental congener pattern distribution of PBDEs with increasing levels of higher brominated congeners in different abiotic and biotic matrices (Olofsson et al., 2012; Su et al., 2015; Verreault et al., 2018).

In the Mediterranean Sea, the environmental concentrations of PCBs do not seem to show any noticeable decline since 2014 (Sauvé, S. & Desrosiers, 2014) and it is also reasonable for PBDEs to be present in the marine environment in large concentrations. These chemicals are considered as legacy contaminants of re-emerging concern since it has been demonstrated to affect natural populations.

Exposure to xenobiotic compounds, i.e. POPs, can cause immune system suppression, and endocrine disruption in marine mammals (Desforger et al 2016, Murphy et al 2018; Schwacke et al 2012). Concentrations of PCBs in different Mediterranean odontocete species have been reported surpassing toxicity thresholds for marine mammals. Such concentrations can have population level consequences for marine mammals through reduced reproduction and/or survival (Hall et al., 2018).

Increased infectious disease susceptibility, immunosuppression, endocrine disruption, and neoplasia have been linked to increased levels of contaminants in marine mammals, highlighting the necessity of multidisciplinary collaborative research to assess the impact of pollution on cetaceans.

A recent project, supported by Pelagos Sanctuary Secretariat, emerging terrestrial pathogens, such as *Toxoplasma gondii*, *Sarcocystis neurona*, *Listeria monocytogenes*, *Salmonella spp.* and *Erysipelothrix rhusiopathiae*, have studied and recorded in stranded cetaceans in addition to contaminants data. Moreover, several studies have shown the association between extreme weather events and outbreaks of waterborne diseases, due to the presence of wildlife and livestock faeces that have been washed into the sea (Funari et al. 2012). Understanding the pathogenesis of a given disease, as well as its epidemiology, is paramount to understand the potential effects of such disease on cetaceans' populations.

At the same time, recent studies have drawn attention to the impact that climate change could have on POPs' cycling and distribution and consequently on exposure and accumulation in living organisms (Borgå et al., 2022; Hung et al., 2022; Nadal et al., 2015). Evidence suggested that climate change-driven processes could enhance long-range transport of POPs (Dalla Valle et al., 2007; Li et al., 2021) and their remobilization from secondary sources (Borgå et al., 2022; Chen et al., 2019; Ma et al., 2011; Potapowicz et al., 2019). Undoubtedly, this could translate into enhanced POP exposure and accumulation in cetaceans (Desforges et al., 2018; McGuire et al., 2020).

The examination of stranded marine mammals conveys a valuable source of biological and scientific data that can be used to assess the impact of human activities on the marine environment, and of pathogens on animal and human health (Giorda et al. 2017). Not only they can be sampled to quantify contaminants levels in tissues, but they can alert researchers on the circulation of pathogens in the living populations, in which they are challenging to be detected. In this context, long-term monitoring programs and epidemiological studies are recently carried out in the ACCOBAMS area to assess the impact of diseases and pollutants on marine mammal populations. Information on marine mammal health and environmental pollution are most efficiently and accurately acquired through collaborative, international and interdisciplinary baseline research.

On the other hand, the study of free-ranging organisms offers a valuable tool to understand the accumulation levels and impact of contaminants in natural populations and to measure also several endpoints to evaluate cetaceans health that could be otherwise not measured or undergo to bias related to the cause of stranding/death of an animal.

2. General methodology for bibliographic research

In order to compile the most up-to-date information (July 2022) on chemical pollution in cetaceans in the ACCOBAMS region, a complete search of peer-reviewed scientific literature, grey literature and reports was carried out. Search results were used to build an extensive database of studies.

The bibliographic research used general search engines such as Google scholar and Google and computerised databases such as ISI Web of Knowledge, Scopus and PubMed using the following keywords: cetaceans and Mediterranean sea/Black sea; cetaceans and contaminants; cetaceans and POPs; cetaceans and heavy metals/trace elements; cetaceans and OCs/PBDEs/PCBS/PFAS/Phthalates; cetaceans and pollution, dolphins/whales/porpoises and heavy metals/OCs/PBDEs/PCBS/PFAS/Phthalates; dolphins/whales/porpoise and dolphins and contaminants.

All the data were then analyzed for species and for area, including number of samples analyzed per study, year, number of males/females/juveniles, class of contaminants (and number of congeners), tissue.

3. Bibliographic review on the impact of chemical pollution on cetaceans in the ACCOBAMS area

The main papers published on the impact of chemical pollution on cetaceans in the ACCOBAMS area until July 2022 are reported in **Annex 1** as summary.

This paragraph will cover in detail the results of the bibliographic research on impact of contaminants in Cetaceans in the ACCOBAMS area with a particular focus on the most studied area: the Mediterranean Sea. Seventy-two papers were analyzed on contaminants load and interaction with cetacean species in the Mediterranean area and only 3 papers for the Black Sea. The analysis of the main results of the papers are reported below.

According to the distribution of the species and the research effort, we report below the area covered for the most investigated species in the Mediterranean Sea: striped dolphin, bottlenose dolphin, common dolphin, Risso's dolphin, pilot whale and fin whale (**Fig.1**).



Figure 1. Analysis of spatial distribution of the studies on contaminants for the most studied cetacean species in the Mediterranean Sea.

Regarding the following species, only few records are present in literature:

- Minke whale: a single study on contaminants on two organisms stranded along the French coast (Alzieu and Duguay, 1979)
- Cuvier's beaked whale: a single study on POPs on free-ranging organisms sampled in the Ligurian Sea (Baini et al., 2020) and heavy metals in stranded organisms (Shoham-Frider et al., 2014)
- Rough-toothed dolphin: a single study on a stranded organisms in the Adriatic Sea (Marsili and Focardi, 1997)

Regarding the Black Sea, the very few studies retrieved focus on western Black Sea coasts (Bulgaria; Evtimova et al., 2019), Crimean coastal waters (Malakhova et al., 2021) and widespread across the whole Black sea coast (Weijs et al., 2010).

As expected, striped dolphin and bottlenose dolphin are the most investigated species considering all the class of contaminants analysed in this document (Fig. 2).

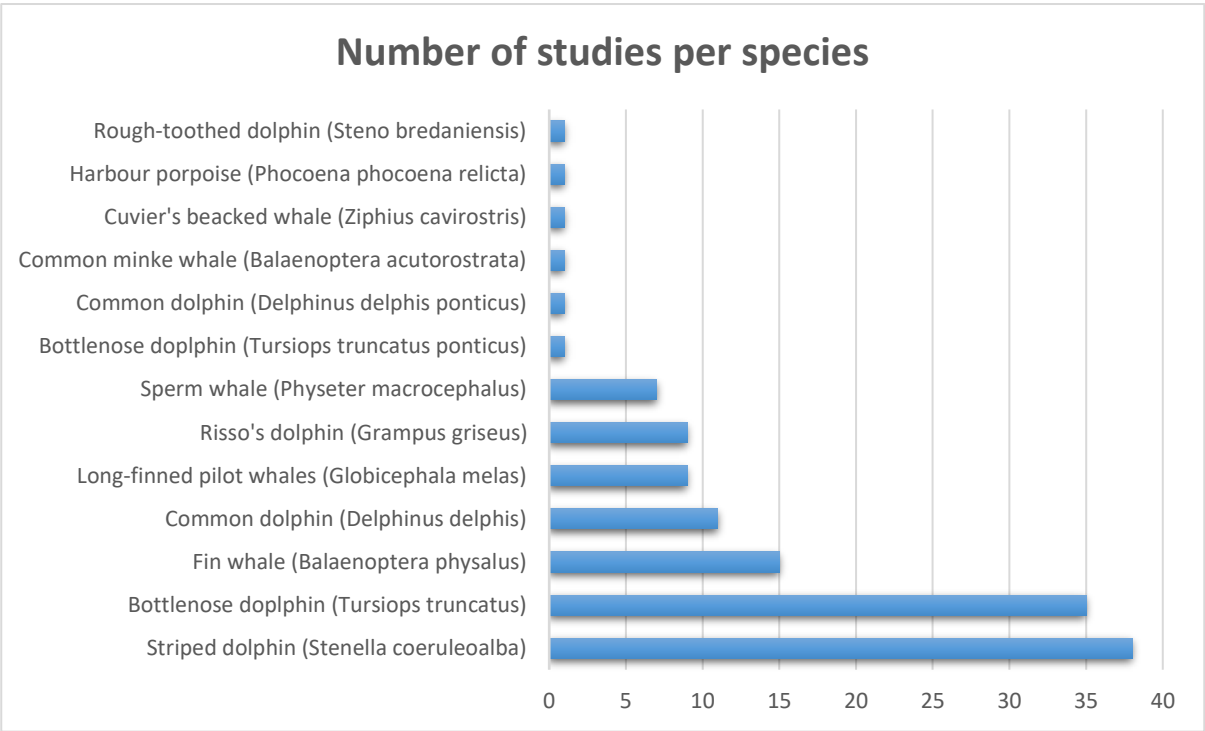


Figure 2. Analysis of the number of studies on contaminants per species in the ACCOBAMS area.

4. Main highlights on the impact of chemical pollution on cetaceans in the ACCOBAMS area

This section specifically describes the main conclusion derived from the analysis of the selected papers (Annex 1), subdivided in the main classes of contaminants that impact the cetaceans of the ACCOBAMS area.

4.1. Organochlorine Compounds (OCs)

This class of contaminants consider all those compounds characterized by the presence of chlorine, mostly of them are banned since several decades but still persist in the environment and biota.

Among the compounds analyzed in the cetaceans from the ACCOBAMS areas there are: several congeners of PCBs, DDT and its metabolites, hexachlorocyclohexane; exachlorobenze, polychlorinated diphenyl ether (PCDEs), polychlorinated terphenyls (PCTs), polychlorodibenzofurans (PCDFs), polychlorodibenzo-p-dioxins (PCDD). PCBs and DDTs are the most studied compound in cetaceans (both on stranded and free-ranging organisms) from the ACCOBAMS area, being amongst the oldest used xenobiotic compounds and due to their high persistence and affinity with lipophilic tissues. Striped dolphin, with more than 2000 individuals analysed, from early 70' to date is by far the most studied species.

Despite being studied for such long time still several issues regarding the comparability among results remains, in particular related to:

- number of congeners analyzed (from a few to more than 30, affecting the values of the Total Sum values reported), unit (e.g., ng/g d.w., ng/g w.w., ng/g l.b., m.g./kg l.w., etc.),
- tissue,
- condition (stranded/free-ranging),
- different analytical methodologies used from extraction, to instruments used with different sensitivity, different limit of quantification (LOQ) and limit of detection (LOD).

The main output and conclusion on this class of pollutants are reported below:

- **Most studied species:** *Stenella coeruleoalba*
- **Most affected species:** *Stenella coeruleoalba*, *Tursiops truncatus*, *Globicephala melas*
- **Levels (concentrations):** a wide range, difficult to quantify due to different units and congeners analyzed
- **Area of concern:** North-western Mediterranean Sea (however is the most studied area)
- **Effects (if studies available):** alteration of protein and gene expression, endpoints related to endocrine disruption, responses to oxidative stress, biotransformation systems, immunodepression
- **Knowledge gap:** very few studies in the Black Sea and in the less abundant species in the whole ACCOBAMS area

4.2. Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a class of semi-volatile organic compounds with at least two fused benzene rings, which may have branches of aliphatic chains (alkyl-PAHs). Petroleum is a rich source of PAHs, most of the PAHs in crude oil are low molecular weight (LMW) compounds, composed of two or three fused aromatic rings. Higher molecular weight (HMW) four- to six-ring PAHs, some of which are known or suspected mammalian carcinogens, are much less abundant in crude oils than they are in most pyrogenic PAHs assemblages. PAHs are widely distributed in the environment and their physical-chemical properties and their distribution and transformation processes, such as evaporation, dissolution, sedimentation, photo-oxidation and biodegradation, determine the fate of PAHs in the environment (Neff, 2002; Stout et al., 2002; Stout and Wang, 2007).

In the vertebrates most of the PAHs absorbed are efficiently biotransformed by enzymes that increase their solubility in water allowing their excretion; PAHs are not biomagnified in marine food chains (Broman et al., 1990; Hylland, 2006; Wan et al., 2007; Takeuchi et al., 2009).

Several PAHs, especially the HMW, are classified as toxic, some of which present mutagenic and carcinogenic properties: benz[a]anthracene, chrysene, dibenz[a,h]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-c,d]pyrene, benzo[ghi]perylene (Neff, 1979).

Despite their presence in the environment, studies on cetaceans inhabiting the ACCOBAMS area are scarce. The only species investigated are sperm whale (Marsili et al., 2014); striped dolphin (Marsili et al., 2001) and fin whale (Marsili et al., 2001; Fossi et al. 2010).

The main output and conclusion on this class of pollutants are reported below:

- **Most studied species:** *Stenella coeruleoalba*
- **Most affected species:** *Stenella coeruleoalba*
- **Levels:** 2000-36000 mg/kg lw
- **Area of concern:** too few data to identify an area of concern
- **Effects (if studies available):** *in vitro* studies available, altered protein expression of CYP1A and CYP2B
- **Knowledge gap:** very few data available for few species

4.3. Brominated flame retardants and brominated/halogenated natural compounds (BFR and B/HNC)

Brominated flame retardants are widespread in the environment due to their massive production and use, together with strong evidence of increasing contamination of the environment, wildlife, and people and limited knowledge of potential biological/physiological effects highlights the importance of identifying emerging issues associated with the use of BFRs. Within BFRs, PBDEs are a family comprising 209 congeners that are divided into 10 groups, from mono- to deca-BDE, according to their different degree of halogenation. PBDEs have been commercialized as three technical mixtures, i.e., penta-, octa-, and deca-brominated diphenyl ether, named so after the major congeners present in each mixture. They were primarily used as flame retardants in polyurethane (PUR) foams, computers, housing appliances, electrical and electronic products. Importantly, the ban on these two mixtures led to an increased production of deca-BDE formulation, made up of 96.8% BDE-209, until its more recent inclusion in the annex A of the Stockholm Convention in 2019 (UNEP, 2022). Toxicological relevance of higher brominated BDEs has not yet been fully investigated, but their debromination to lighter, more toxic and bioaccumulative congeners have been demonstrated (Eriksson et al., 2004; La Guardia et al., 2007; Rayne et al., 2003; Zhu et al., 2019). This fact is

worthy of attention due to the large historical production of deca-BDE estimated to be almost 10 times higher than those of penta and octa-BDE mixtures (Abbasi et al., 2019).

A growing alternative to halogenated flame retardants (HFRs) are organophosphorus flame retardants (OPFRs). They represented 20% of all FRs used in Europe in 2006; twice the quantity of brominated FRs (Van der Veen and de Boer, 2012). OPFRs are also released from materials and access environmental matrices through washout, infiltration, deposition, etc. (Schreder and La Guardia, 2014). Moreover, OPFRs are used as plasticisers, so they leak from the tons of plastic that reach the seas and oceans. Their presence has been reported in sediments, water, fish and marine mammals (Giulivo et al., 2016; Sala et al., 2019; Garcia-Garin et al., 2020) and their toxicity has been reported (Van der Veen and de Boer, 2012).

Methoxylated PBDEs (MeO-PBDEs) are natural analogues to PBDEs that are synthesized by some marine organisms (Malmvarn et al., 2008). They have been found in cetaceans and seafood (Alonso et al., 2014; Aznar-Alemany et al., 2017a, Aznar-Alemany et al., 2017b). Being natural compounds, their presence cannot be controlled. However, their structural similitude to PBDEs might confer them similar negative effects that would add to those of the anthropogenic FRs. As they can be analysed using the same method, it makes sense to collect the data.

Most of these compounds have been measured in cetaceans from the ACCOBAMS area, the main classes include: PBDEs, MeO-PBDEs, Polybrominated hexahydroxanthene derivatives (PBHDs), pentabromoethylbenzene (PBEB), Hexabromocyclododecane (HBCDs), decabromodiphenyl ethane (DBDPE), Hexabromocyclododecane (α -HBCD), Halogenated natural products

Data on brominated flame retardants and brominated natural compounds are available for: long-finned pilot whale, Risso's dolphin, bottlenose dolphin, Cuvier's beaked whale, striped dolphin, common dolphin, fin whale and harbor porpoise (Black Sea). The main output and conclusion on this class of pollutants are reported below:

- **Most studied species:** *Stenella coeruleaolba* and *Physeter macrocephalus*
- **Most affected species:** *Stenella coeruleaolba*
- **Levels:** PBDEs from about 20 ng/mg l.w. (sperm whale) to 5000 ng/mg l.w (Striped dolphin)
- **Area of concern:** most of the studies focus on the western Mediterranean Sea
- **Effects (if studies available):** in vitro studies available, altered protein expression of CYP1A and CYP2B, altered gene expression of endpoints related to endocrine disruption
- **Knowledge gap:** very few data in the Black Sea

4.4. Per- and polyfluoroalkyl substances (PFASs)

Per- and polyfluorinated alkyl substances (PFAS) are a large group of industrial chemicals (Jahnke and Berger, 2009) characterised by a linear or branched carbon chain (Ferrario et al., 2021) with an alkyl chain which is partly or fully fluorinated, typically containing between 4 and 18 carbon atoms (Jahnke and Berger, 2009). PFAS are employed in industrial and commercial applications, such as fire-fighting foams, non-stick coatings and cosmetics, due to their unique properties provided by the extreme strength of C–F bonds and their surfactant nature (Gredelj et al., 2020). Most of these compounds are under the attention of the Stockholm Convention. Many PFAS result to be bioaccumulative (Jahnke and Berger, 2009) with bioaccumulation increasing with the length of the carbon chain (Conder et al., 2008; Buck et al., 2011; Spaan et al., 2020). Although some PFAS, such as PFOS, are banned in some countries, they are still manufactured in other areas and might continue to be imported and used in consumer goods such as textiles, packaging, coatings and plastics (Fair and Houde, 2018). Due to their environmental behavior, PFAS may constitute a threat to wildlife

at high trophic levels (Galatius et al., 2013) and are expected to pose a serious threat to humans and the environment in which they live for many years to come (Bonato et al., 2020). However, knowledge about their toxic effects on wildlife is still scarce (Lam et al., 2016). Only few studies have investigated PFAS in cetaceans inhabiting the Mediterranean Sea. The studies in the ACCOBAMS area focus on the following species: bottlenose dolphin, common dolphin, striped dolphin, long-finned pilot whale, sperm whale and fin whales.

Most of these compounds have been measured in cetaceans from the ACCOBAMS area, the main classes include: several PFAS congeners, perfluorooctane sulfonic acid (PFOS), Perfluorooctanesulfonamide (FOSA), Perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS).

The main output and conclusion on this class of pollutants are reported below:

- **Most studied species:** *Tursiops truncatus*
- **Most affected species:** *Tursiops truncatus*
- **Levels (concentrations):** 6 to 790 ng/g ww (Σ PFAS)
- **Area of concern:** very few areas investigated to establish an area of concern
- **Effects (if studies available):** *in vitro* studies available on the effects on alterations of gene expression
- **Knowledge gap:** very scarce number of studies (some in the early 2000) both on species and areas

4.5. Heavy metals and trace elements

Heavy metals and trace elements are well known environmental pollutants that accumulate in the bodies of cetaceans and potentially constitute a toxicological risk for the species.

The main potential effects described for these pollutants are endocrine cycle disruption, failure of the reproductive process, immune system suppression and metabolic disorders that could generate cancer and genetic defects (Borrell et al., 2014). Numerous studies have been conducted over the last decades on trace metals in marine mammals (Riget and Dietz, 2000; Bennett et al., 2001; Caurant et al., 2006; Capelli et al., 2008; Law et al., 2012; Robin et al., 2012; Rojo-Nieto and Fernandez-Maldonado, 2017) since early '90 as well as on trace elements (Leonzio et al., 1992; Law, 1996; Cardellicchio et al., 2000; Das et al., 2003).

Among the trace elements and metals studied in cetaceans living in the ACCOBAMS area there are: chromium, copper, zinc, iron, manganese and vanadium, arsenic, mercury, selenium, cadmium, lead and nickel. Studies on the Mediterranean have been carried out on: bottlenose dolphin, common dolphin, Cuvier's beaked whale, Risso's dolphin, striped dolphin, rough-toothed dolphin, fin whale and minke whale. Regarding Black sea a single study on harbor porpoise was carried out. The main output and conclusion on this class of pollutants are reported below:

- **Most affected species:** depending on tissue analyzed and metal/trace element. *Tursiops truncatus* and *Stenella coeruleoalba*
- **Most studied species:** *Tursiops truncatus* and *Stenella coeruleoalba*
- **Levels (concentrations):** levels vary according to the tissue and metal/trace element analysed
- **Area of concern:** whole Mediterranean
- **Effects (if studies available):** neurotoxicity, hepatic damages
- **Knowledge gap:** many studies available, gaps on ecotoxicological effects

4.6. Phthalate esters (PAEs)

Phthalate esters (PAEs) presence has recently attracted the attention of the scientific community. This is a group of chemicals widely used as additives to make plastics more flexible and harder to break, and their content can be up to 10 – 60% by weight (Earls et al., 2003). Industrial formulations of phthalate esters include a large number of compounds, which vary in alkyl chain length, branching and molecular weight with a total European consumption accounting for approximately 1 million tons (Net et al., 2015; Mackintosh et al., 2004). Not chemically but only physically bound to the polymeric matrix, PAEs can easily be released into the environment directly and/or indirectly, during manufacture, use, and disposal (Net et al., 2015). PAEs can have various noxious toxic effects on organisms. In particular, they can act as endocrine disruptors (EDs) even at very low concentrations. EDs can interact with hormone synthesis and alter reproduction or other physiological and metabolic functions (e.g., causing oxidative stress, immunotoxicity) of organisms (Mathieu-Denoncourt et al., 2015; Talsness et al., 2009).

Eight phthalates have been measured in cetacean from the ACCOBAMS area: dibutyl phthalate (DBP), diisobutyl phthalate (DIBP), butyl benzyl phthalate (BBP), di-n-pentyl phthalate (DnPP), di(2-ethylhexyl) phthalate (DEHP), mono(ethylhexyl) phthalate (MEHP), di-n-octyl phthalate (DnOP), diisononyl phthalate (DINP), and diisodecyl phthalate (DIDP), including their metabolites (Baini et al 2017). Most of them are listed as priority pollutants by the Environmental Protection Agency and European Union taking into account their toxicity, prevalence in the environment and widespread use (Savoca et al., 2021).

The species studied are: bottlenose dolphin, Risso's dolphin, striped dolphin and fin whale.

The main output and conclusion on this class of pollutants are reported below:

- **Most affected species:** *Tursiops truncatus* and *Stenella coeruleoalba*
- **Most studied species:** *Balaenoptera physalus*
- **Levels (concentrations):** 3500-29000 ng/g d.w.
- **Area of concern:** n/a (few studies to assess an area of concern)
- **Effects (if studies available):** altered gene expression of endpoints related to endocrine disruption
- **Knowledge gap:** only few records in few studies

The results were presented considering the most relevant and studied classes of compounds in the ACCOBAMS area (and in cetaceans worldwide). Chlorinated compounds are by far the most investigated compounds, due to their use since several decades but also due to their persistence in the environment and in the organism tissues.

It should be noted that other contaminants of emerging concern (CECs) should be considered in the next future as, for instance, pharmaceutical and personal care products (PPCPs), new pesticides, microplastics and nanomaterials. Regarding these classes of contaminants, the attention of the scientific community is rapidly growing as well as the multiple and synergistic effects of the exposure of the organisms to a mixture of compounds in a rapid changing environment as the ACCOBAMS area.

5. Identification of ad hoc research projects aimed at assessing chemical pollution on cetaceans in the ACCOBAMS area

Apparently, very few “ad hoc” projects in the area have been funded in the last years (2010-today) or, if funded, results have not been made available yet nor through reports, papers or website.

An overview of the relevant projects and initiatives that aimed at assessing chemical pollution on cetaceans in the ACCOBAMS area are listed in **Table 1**. The list includes a core set of projects, implemented at Mediterranean and European level, that focused on different aspects of the Chemical pollution in Cetaceans.

Table 1. Overview of the relevant projects and initiatives that aimed at assessing chemical pollution on cetaceans in the ACCOBAMS area

ACRONYM	FULL TITLE	FUNDING SOURCE	THEMATIC FOCUS	WEBSITE
Pollution Project	Biological and toxicological contamination of cetaceans in the Pelagos Sanctuary: assessment, origin, monitoring and mitigation	Pelagos	Biological and toxicological contamination of cetaceans in the Pelagos Sanctuary	link
Plastic Busters MPAs	Plastic Busters MPAs: preserving biodiversity from plastics In Mediterranean Marine Protected Areas	Interreg Med	monitoring & assessment - entire management cycle	link
Plastic Pelagos	Il problema emergente delle microplastiche nel Mar Mediterraneo: il potenziale impatto sulla balenottera comune come modello di “descrittore ambientale”	IT-MATTM	Monitoring Plastic additives and Microplastic impact in Mediterranean Fin Whale	na

6. Conclusions and Recommendation for Future Work

In this document we summarize the main results of the bibliographic review on the impact of chemical pollution on cetaceans in the last 30 years, including the identification of *ad hoc* research projects aimed at assessing chemical pollution on cetaceans in the ACCOBAMS area. The analysis of the existing literature (more than 70 papers) supports the identification of priorities and knowledge gaps within the ACCOBAMS area related to pollution and chemical stress to cetacean populations.

The main conclusions obtained from the elaboration of the previous data allowed to draw some synthetic outputs on the impact of contaminants on cetaceans in ACCOBAMS area that can be summarize as following:

- OCs are the most investigated class of contaminants in the cetacean species of the ACCOBAMS area both in terms of detection and impacts.
- The striped dolphin is the most studied species both in term of concentrations of several classes of contaminants that in terms of potential toxicological effects.
- Only few studies reported the potential impact of contaminants and the related consequences at individual or population levels.
- Few studies investigated the connection between contaminants levels and emerging diseases.
- Several species are poorly investigated (e.g. Cuvier's beaked whale or Risso's dolphin) and very scattered and scarce data are available for the Black Sea
- Only few studies investigated the exposure multiple stressors (pollution, marine litter, climate change, emerging disease, etc.) in ACCOBAMS cetacean species.

Potential future research, mitigation, and governance actions, to be implemented in the future in the ACCOBAMS area, are suggested:

- a) to develop a transboundary health monitoring network on cetaceans stranded in the ACCOBAMS area;
- b) to harmonize the monitoring strategies and the analytical methodologies to detect the presence and impact of chemicals, both in stranded and free-ranging cetaceans in the ACCOBAMS area;
- c) to evaluate the origin and the impact of chemical pollutants of terrestrial origin on cetacean health in the ACCOBAMS area;
- d) to promote the mitigation of adequate biological and toxicological contamination in the ACCOBAMS area;
- e) the analysis of the presence and effects of contaminants of emerging concern (CECs) should be considered in the next future as, for instance, pharmaceutical and personal care products (PPCPs), new pesticides, microplastics and nanomaterials.

Annex 1 – Bibliographic research on the impact of chemical pollution in cetaceans in the ACCOBAMS area

Authors	Title	Doi	Abstract
Organochlorine Compounds, polybrominated diphenyl ethers, brominated/halogenated natural compounds			
Alzieu and Duguy 1979	Organochlorine compounds levels in Cetaceans and Pinnipedia living along the french coasts	na	The accumulation levels of PCB, DDT, DDE, and DDD were determined in samples from the blubber, muscles, liver, kidney and stomach of 87 marine mammals, most of which had been found stranded on the Atlantic and Mediterranean French coasts. Sampling covered 80 Cetacea Odontoceti (75 Delphinidae, 2 Physteridae, 3 Hyperoodontidae), 3 Cetacea Mysticeti (Balaenopteridae), and 4 Pinnipedia (Phocidae). Very high levels of PCB were found in the blubber from an immature pilot whale, <i>Globicephala melaena</i> (840 mg/kg of lipids) and from striped dolphin, <i>Stenella coeruleoalba</i> (833 mg/kg of lipids). In general, the results indicated considerable variations in concentrations of PCB and DDE according to the diet, age and location (Atlantic or Mediterranean) of the specimens examined. Tychophagous Odontoceti, and <i>Stenella coeruleoalba</i> and <i>Delphinus delphis</i> in particular, showed a higher degree of contamination than teuthophagous Odontoceti species. Lower levels of contamination were found in the Mysticeti; this is most probably due to the low contamination of zooplankton, their basic food. As a general rule, immature specimens appeared to be more contaminated than adults. Organochlorine content in the foetus was found to be important and was frequently at the same level as that detected in maternal organs. There is no close relation between PCB level and pathological features of the organs, but certain observations suggest that the health of the dolphin may be endangered if PCB level in the liver exceeds 20 mg/kg of lyophilised tissue.
Kannan et al., 1993	Isomer-specific analysis and toxic evaluation of polychlorinated biphenyls in striped dolphins affected by an epizootic in the western Mediterranean sea	doi.org/10.1007/bf00212134	Isomer-specific concentrations of polychlorinated biphenyls (PCBs) including planar, mono- and di-ortho congeners and concentrations of DDT were determined in striped dolphins affected by a morbillivirus epizootic in the western Mediterranean in 1990. Extremely high concentrations of PCBs ranging from 94 to 670 micrograms/g (wet wt) were detected in the blubber. Similarly, DDT concentrations were high, between 22 and 230 micrograms/g (wet wt). The concentrations of three non-ortho coplanar PCBs were 43 (3,3',4,4'-T4CB), 6.8 (3,3',4,4',5-P5CB), and 7.8 (3,3',4,4',5,5'-H6CB) ng/g (wet wt), respectively, the highest residue levels reported to date. The estimated 2,3,7,8-TCDD toxic equivalents of non-, mono- and di-ortho PCB congeners in striped dolphins were several times higher than those observed for other marine mammals and humans. Mono-ortho congeners contributed greater 2,3,7,8-TCDD toxic equivalents than non-ortho members. The higher ratio of 3,3',4,4',5,5'-H6CB/3,3',4,4',5-P5CB (IUPAC 169/126) suggested a strong induction of mixed function oxidase enzymes and highlighted the possibility of using this ratio as an index for risk assessment of PCB contamination in marine mammals. Elevated concentrations of PCBs may have played a role

			in the immune depression in striped dolphins, ultimately leading to the development of morbillivirus disease.
Aguilar and Borrell. 1994	Abnormally high polychlorinated biphenyl levels in striped dolphins (<i>Stenella coeruleoalba</i>) affected by the 1990–1992 Mediterranean epizootic	doi.org/10.1016/0048-9697(94)90091-4	PCB concentrations and total lipid content were determined in the blubber and liver of striped dolphins affected by the 1990 morbillivirus epizootic in the Mediterranean Sea, and in the blubber of striped dolphins from the same area sampled with a biopsy dart in 1987–1989 and 1991. PCB levels were found to be significantly higher in the individuals that succumbed to the epizootic than in the 'healthy' population sampled before or after the event. Although recent mobilization of lipid reserves was found to have occurred in some of the diseased dolphins, this had little effect on their PCB blubber concentrations and cannot explain the observed difference with the healthy individuals. Three hypotheses are put forward to explain the apparent link between high PCB levels and mortality caused by the epizootic: (i) depressed immunocompetence caused by PCBs leading to an increase in individual susceptibility to the morbillivirus infection, (ii) mobilization of fat reserves leading to increased PCB levels in blood which, in turn, may produce a liver lesion capable of increasing the individual's susceptibility to the morbillivirus infection, and (iii) previous existence of an unspecific hepatic lesion producing impairment of the liver function which, in turn, could lead to an increase both in tissue PCB concentrations and in individual susceptibility to the morbillivirus infection.
Guitart et al., 1996	Organochlorine residues in tissues of striped dolphins affected by the 1990 mediterranean epizootic: Relationships with the fatty acid composition	na	A simple and rapid method was developed for the simultaneous determination of fatty acids, organochlorine pesticides, and polychlorinated biphenyl (PCB) congeners in the same sample in order to explore possible connections between levels of contaminants and fatty acid composition. The method was applied to samples of melon, cerebrum, cerebellum, lung, liver, kidneys, and skeletal muscle obtained from 5 male and 5 female striped dolphins (<i>Stenella coeruleoalba</i>) found stranded in 1990 in the northeastern Spanish coasts during the morbillivirus epizootic that affected this cetacean in the Mediterranean Sea. The results indicate that PCBs were dominant in all tissues, with the highest geometric mean concentration being found in melon (903 µg g ⁻¹ wet wt); ΣDDTs were also found at high concentrations (111 µg g ⁻¹ wet wt, in melon). Statistical analysis indicate that organochlorine concentration was correlated with the fatty acid composition of tissues, although some of these variations can be interpreted as a consequence of a shift in the diet produced in the striped dolphin population. However, other changes such as the negative correlation with arachidonic acid may suggest that the eicosanoid production could have been affected by the extremely high concentrations of PCBs and ΣDDTs.
Marsili and Focardi, 1996	Organochlorine levels in subcutaneous blubber biopsies of fin whales (<i>Balaenoptera physalus</i>) and striped dolphins (<i>Stenella coeruleoalba</i>) from the Mediterranean Sea	doi.org/10.1016/0269-7491(95)00037-r	Polychlorinated biphenyls (PCBs) and DDT compounds were determined in subcutaneous blubber of fin whales (<i>Balaenoptera physalus</i>) and striped dolphins (<i>Stenella coeruleoalba</i>) from the Mediterranean Sea. From 1990 to 1993, 68 fin whale and 89 dolphin blubber biopsies were analysed. The whales were sampled while passing through the Ligurian Sea, whereas the dolphins were collected in different areas of the Mediterranean: the Ligurian, Tyrrhenian and Ionian Seas. Total PCBs and DDTs were 5.5-7.1 ppm and 4.2-9.5 ppm, respectively, in the whales and 15.5-86.0 ppm and 15.6-63.5 ppm, respectively, in the dolphins. Thirty PCB congeners were identified for each sample, IUPAC numbers 153, 138, 187, 180 and 170 being the most

			abundant, totalling an average of 55% of total PCBs in the whale and 60% in the dolphin. The large differences in accumulation are related to position in the food chain. In striped dolphins significant differences were found in relation to sampling site, and in fin whales in relation to sex.
Marsili and Focardi, 1997	Chlorinated hydrocarbon (HCB, DDTs AND PCBs) levels in cetaceans stranded along the Italian coasts: an overview	na	Concentrations of HCB, DDTs and PCBs in the tissues and organs of cetaceans (<i>Stenella coeruleoalba</i> , <i>Tursiops truncatus</i> , <i>Balaenoptera physalus</i> , <i>Steno bredanensis</i>), <i>Grampus griseus</i>) and <i>Globicephala melaena</i>) stranded along the Italian coasts in the period 1987–1993 are reported. The values are compared between species and between specimens of the same species. Chlorinated hydrocarbon (CH) levels were found to increase in relation to the quantity and type of lipids in each tissue and organ. Differences in accumulation encountered in the different species are principally due to different feeding habitats. Remarkable differences found between males and females of each species confirm that during gestation and lactation, females undergo disintoxication by passing much of their total burden of CHs to their young.
Marsili et al. 1998	Relationship between organochlorine contaminants and mixed function oxidase activity in skin biopsy specimens of Mediterranean fin whales (<i>Balaenoptera physalus</i>)	https://doi.org/10.1016/s0045-6535(98)00145-3	The relationship between organochlorine contaminants (PCBs and DDTs) and mixed function oxidase, benzo(a)pyrene monooxygenase activity (BPMO), was investigated in skin biopsy specimens from fin whales (<i>Balaenoptera physalus</i>) of the Mediterranean Sea. Skin biopsy material, sampled by a non invasive technique, is suitable for a wide range of chemical and biomarker analysis. In this study PCBs and DDTs were evaluated in subcutaneous blubber and MFO activity in epidermis. An interesting correlation was found in male specimens between the two variables.
Reich et al., 1999	Congener Specific Determination and Enantiomeric Ratios of Chiral Polychlorinated Biphenyls in Striped Dolphins (<i>Stenella coeruleoalba</i>) from the Mediterranean Sea	https://doi.org/10.1021/es9807385	Blubber and liver samples from six striped dolphins (<i>Stenella coeruleoalba</i>) found dead in the Mediterranean sea in 1989–1990 were tested for 37 coplanar and chiral polychlorinated biphenyls (PCBs), including the enantiomeric ratios of 9 chiral PCBs. The method includes a fractionation step using HPLC (PYE column) for separating the PCBs according to the number of chlorine atoms in the ortho positions. HRGC/ECD and HRGC/LRMS with an achiral column (DB-5) were used to determine the PCB congeners. The enantiomeric ratios of nine chiral PCBs were determined by HRGC/LRMS (SIM) with a chiral column (Chirasil-Dex) and by MDGC as the confirmatory technique. The total PCB concentration (sum of 37 congeners) ranged from 7.2 to 89.6 µg/g (wet weight) and from 0.52 to 29.2 µg/g (wet weight) for blubber and liver samples, respectively. PCB profiles were dominated by congeners 138, 153, 170, and 180. The toxic equivalent values (TEQ) ranged from 0.17 to 3.93 ng/g (wet weight) and from 0.02 to 0.73 ng/g (wet weight) for blubber and liver samples, respectively. PCBs 95, 132, 135, 149, and 176 revealed an enantiomeric excess of the second eluted enantiomer in almost all of the samples, whereas PCBs 136 and 174 were racemic or almost racemic. PCBs 88 and 91 were under the detection limits of the methodology used.
Fossi et al., 2000	Skin biopsy of Mediterranean cetaceans for the investigation	doi.org/10.1016/S0141-1136(00)00127-6	Various studies on Mediterranean cetaceans have revealed bioaccumulation of contaminants such as organochlorines (OCs) and heavy metals. The susceptibility of these animals to organic pollutants and the relationship between bioaccumulation and population decline (as in the case of

	of interspecies susceptibility to xenobiotic contaminants		Delphinus delphis) are unexplored fields. In this study, we used a non-destructive approach (skin biopsy) to explore OC bioaccumulation processes and mixed-function oxidase activity (BPMO) in four species of cetaceans: striped dolphin (<i>Stenella coeruleoalba</i>), bottlenose dolphin (<i>Tursiops truncatus</i>), common dolphin (<i>D. delphis</i>) and fin whale (<i>Balaenoptera physalus</i>). Significant differences in BPMO induction and OC levels were found between odontocetes and mysticetes, the former having mixed-function oxidase activities four times higher than the latter, binding with levels of OCs one order of magnitude higher in odontocetes. A significant correlation ($P<0.05$) between BPMO activities and OC levels was found in <i>B. physalus</i> . In an ongoing project, fibroblast cultures have been used as an alternative in vitro method of evaluating interspecies susceptibility to contaminants such as OCs and polycyclic aromatic hydrocarbons (PAHs). These results suggest that cetacean skin biopsies are a powerful non-invasive tool for assessing ecotoxicological risk to Mediterranean marine mammals species.
Hernandez et al., 2000	Persistent Organochlorines and Organophosphorus Compounds and Heavy Elements in Common Whale (<i>Balaenoptera physalus</i>) from the Western Mediterranean Sea	doi.org/10.1016/S0025-326X(99)00238-6	On March 1998, a female specimen of common whale (<i>Balaenoptera physalus</i>) appeared dead in the western Mediterranean coast of Valencia (Spain). Analyses of whale tissues (blubber, liver and kidney) were carried out in order to elucidate the contamination pattern present in the whale, as indicator of the contaminants present in its habitat from the Mauretanian coast to Ligurian Sea, sited in the western Mediterranean area. A multiresidue method based on normal-phase LC prior to GC-MS determination of residues of polychlorinated biphenyls (PCBs), organochlorine pesticides and derivatives and lipophilic organophosphorus (OP) pesticides was applied. Moreover, determination of heavy metals by ICP-MS was carried out, selecting for quantitative analysis Cr, As, Cd, Hg and Pb. Results obtained show high concentrations of PCBs and pp'DDE, at $\mu\text{g g}^{-1}$ level, as well as significant concentrations of As and Hg, which could affected the condition of the animal, and as a consequence, its survivor possibilities.
Jimenez et al., 2000	Evaluation of 2,3,7,8 Specific Congener and Toxic Potency of Persistent Polychlorinated Dibenzo-p-Dioxins and Polychlorinated Dibenzofurans in Cetaceans from the Mediterranean Sea, Italy	doi.org/10.1021/es990959q	The present study investigates individual 2,3,7,8-substituted polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) concentrations in cetaceans and assesses the PCDD and PCDF contributions to the total TCDD equivalent concentration. Liver samples of a variety of cetaceans species found stranded along the Italian coasts during the period of 1987–1992 were analyzed for PCDDs and PCDFs. The main aims were to scrutinize levels and patterns of PCDDs and PCDFs for the first time in cetaceans from the Mediterranean Sea and to estimate whether chlorinated compounds mentioned are implicated in the exceptionally high mortality of dolphins that occurred in the Mediterranean Sea during 1990–1992. PCDD and PCDF profiles were dominated by congeners OCDD and OCDF. The new toxic equivalency factors recommended by WHO in 1997 were used for calculation of dioxin-like toxicity. On the basis of our previous study of PCBs, the overall TEQ calculated from the 2,3,7,8-substituted PCDDs and PCDFs do not contribute as much dioxin-like toxicity as PCBs.
Troisi et al., 2001	Bioaccumulation of polychlorinated biphenyls	doi.org/10.1080/00984100050201622	Polychlorinated biphenyl (PCB) and dichlorodiphenylethane (DDE) methyl sulfone (MSF) metabolites possess high affinities for binding two homologous 16,000 Da homodimeric receptor proteins in the lung (Clara cell

	(PCBs) and dichlorodiphenylethane (DDE) methyl sulfones in tissues of seal and dolphin morbillivirus epizootic victims		secretory protein, CCSP) and the uterus (uteroglobin, UG), leading to selective bioaccumulation of MSFs in these tissues. As marine mammals are highly exposed to organochlorines, concentrations of PCBs, PCB MSFs, DDT, and DDE MSF were analyzed in blubber, lung, and uterus samples from harbor seal (<i>Phoca vitulina</i>) and striped dolphin (<i>Stenella coeruleoalba</i>) morbillivirus epizootic victims to investigate uterine and lung MSF accumulation. Mean uterus concentrations of PCB MSFs and DDE MSF in harbor seals were 0.61 and 0.04 microg/g lipid weight and in striped dolphins 0.05 and 0.01 microg/g lipid weight. Mean lung concentrations of PCB MSFs and DDE MSF in harbor seals were 0.96 and 0.02 microg/g lipid weight and in striped dolphins 0.16 and 0.01 microg/g lipid weight. To ascertain whether uterine and lung bioaccumulation of MSFs is possible due to the presence of CCSP and UG in seals, CCSP and UG proteins in uterine flushings and in uterine and lung and epithelial tissue from Baltic gray and ringed seals were characterized using gel electrophoresis and Western blotting techniques. UG- and CCSP-like proteins with molecular weights of 16,000 Da were resolved in all samples. This is the first demonstration of this protein in any marine mammalian species. The toxicological implications of MSF binding with UG and CCSP in marine mammals are discussed.
Borrell et al. 2001	Organochlorine compounds in common dolphins (<i>Delphinus delphis</i>) from the Atlantic and Mediterranean waters of Spain	doi.org/10.1016/S0269-7491(00)00213-X	Blubber of free-ranging common dolphins (<i>Delphinus delphis</i>) from the northwestern coast of Spain (Atlantic), sampled in 1984 and 1996, and of common dolphins entangled in fishing nets in the southwestern Mediterranean, sampled during 1992–1994, was analysed for organochlorine pollutants. In the Atlantic, concentration of all pollutants was significantly higher in males than in females. The overall tPCB/tDDT ratio in this area was 3.35, which indicates a predominance of industrial inputs over those associated with agriculture. Individuals sampled in 1996 showed significantly lower DDT concentrations but a higher ppDDE/tDDT ratio than those sampled in 1984, which reflects the aging of the environmental load. In the same period, tPCB concentration remained constant and, as a consequence, the tPCB/tDDT ratio more than doubled. In the Mediterranean, the reduced sample size of adult individuals precluded proper statistical investigation of sex-related variation. The overall tPCB/tDDT ratio was 1.12, suggesting a higher contribution of pollutants of agricultural origin. Individuals had significantly higher levels of all DDT forms and a higher ppDDE/tDDT ratio than their counterparts from the Atlantic, but similar PCB concentrations. However, the relative frequency of the different congeners in relation to the total PCB load was different in the two areas. This indicates that the two populations do not mix, at least in the short- or medium-term. Organochlorine levels in both areas are at the mid to low end of the range of concentrations detected in other common dolphin populations and in that of other Delphinidae species from the same region. Although the impact of the organochlorine concentrations on the common dolphin populations surveyed cannot be assessed, it is considered unlikely that they have played a significant role in the decline that the species has suffered in recent decades in the western Mediterranean.

Fossi et al., 2003	The use of a non-lethal tool for evaluating toxicological hazard of organochlorine contaminants in Mediterranean cetaceans: new data 10 years after the first paper published in MPB	https://doi.org/10.1016/s0025-326x(03)00113-9	In the Mediterranean Sea, top predators, and particularly cetacean odontocetes, accumulate high concentrations of organochlorine contaminants and toxic metals, incurring high toxicological risk. In this paper we investigate the use of the skin biopsies as a non-lethal tool for evaluating toxicological hazard of organochlorines in Mediterranean cetaceans, presenting new data 10 years after the paper published by Fossi and co-workers [Mar. Poll. Bull. 24 (9) (1992) 459] in which this new methodology was first presented. Some organochlorine compounds, now with worldwide distribution, are known as endocrine disrupting chemicals (EDCs). Here the unexplored hypothesis that Mediterranean cetaceans are potentially at risk due to organochlorines with endocrine disrupting capacity is investigated. High concentrations of DDT metabolites and PCB congeners (known as EDCs) were found in the different Mediterranean species (<i>Stenella coeruleoalba</i> , <i>Delphinus delphis</i> , <i>Tursiops truncatus</i> and <i>Balaenoptera physalus</i>). In this paper we also propose benzo(a)pyrene monooxygenase (BPMO) activity in marine mammal skin biopsies (non-lethal biomarker) as a potential indicator of exposure to organochlorines, with special reference to the compounds with endocrine disrupting capacity. A statistically significant correlation was found between BPMO activity and organochlorine levels (DDTs, pp(')DDT, op(')DDT, PCBs and PCB99) in skin biopsies of males of <i>B. physalus</i> . Moreover a statistical correlation was also found between BPMO activity and DDT levels in skin biopsies of the endangered Mediterranean population of <i>D. delphis</i> . These results suggest that BPMO induction may be an early sign of exposure to organochlorine EDCs and can be used for periodic monitoring of Mediterranean marine mammal toxicological status.
Storelli and Marcotrigiano, 2003	Levels and congener pattern of polychlorinated biphenyls in the blubber of the Mediterranean bottlenose dolphins <i>Tursiops truncatus</i>	doi.org/10.1016/s0160-4120(02)00081-8	Isomer specific concentrations of individual polychlorinated biphenyls (PCBs) including toxic non-ortho (IUPAC 77, 126, 169) and mono-ortho (105, 118, 156) coplanar congeners were determined in the blubber of nine bottlenose dolphins (<i>Tursiops truncatus</i>) stranded along the Eastern Italian coast. The total PCB concentrations ranged from 3534 to 24375 ng/g wet wt. The PCB profile was dominated by congeners 138 and 153 collectively accounting for 55% of the total PCB concentrations. Among the most toxic congeners the order of abundance was 126>169>77. The mean total 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalent of six coplanar PCBs in the blubber of bottlenose dolphins was 45596 pg/g. Non-ortho congeners contributed greater to the 2,3,7,8-TCDD toxic equivalents than mono-ortho members. Particularly, PCB 126 was the major contributor to the estimated toxic potency of PCBs in dolphins.
Fossi et al., 2004	Assessment of toxicological status of a SW Mediterranean segment population of striped dolphin (<i>Stenella coeruleoalba</i>) using skin biopsy	doi.org/10.1016/j.marenvres.2004.03.070	Various studies have revealed high concentrations of contaminants such as organochlorines (OCs) and heavy metals in Mediterranean cetaceans. A geographical trend of contamination (PCBs and DDTs) has been found for striped dolphin (<i>Stenella coeruleoalba</i>). In this study we used a non-lethal approach (skin biopsy) to investigate bioaccumulation of OCs, including polychlorobiphenyls (PCBs), DDTs, polychlorodibenzo-p-dioxins (PCDDs), polychlorodibenzofurans (PCDFs), trace elements (Hg, Cd, Pb) and CYP1A activity (BPMO) in nine striped dolphins sampled in the Aeolian area (Sicily – Italy) in summer 2002. The arithmetic mean value of BPMO activity in this group was 43.46 AUF/g tissue/h. This value is approximately 3 times and 5 times lower, respectively, than the value found in the Ionian and in the

			Ligurian groups. Skin biopsies of striped dolphins emerged as a suitable material for assessing the toxicological status of the various Mediterranean groups.
Petterson et al., 2004	Polybrominated Diphenylethers and Methoxylated Tetrabromodiphenylethers in Cetaceans from the Mediterranean Sea	doi.org/10.1007/s00244-004-3200-4	Eight tetrabrominated to hexabrominated diphenylethers were present at ppb levels in liver from cetaceans found stranded on the beaches of the Mediterranean Sea, Italy. The highest concentration was found in striped dolphin (sum polybrominated diphenyl ethers [PBDE] 8133 ng/g l.w.) and the lowest concentration in bottlenose dolphin (sum PBDE 66 ng/g lipid weight [l.w.]). The predominant congener in all samples was 2,2,4,4-tetraBDE (PBDE # 47) followed by, in decreasing order, the pentaPBDE # 99 and 100 and the hexaPBDE # 154 and 153. In 12 of the 14 analyzed samples, 3 different methoxylated PBDEs (MeO-PBDE # 1, 2, and 3) were detected at semiquantitatively calculated concentration ranges of 2 to 14 ng/g l.w.; 5 to 167 ng/g l.w.; and 7 to 628 ng/g l.w., respectively. In addition, several unidentified bromine compounds were seen when screening the samples in negativechemical ionization (NCI) mode monitoring m/z 79 and 81, which illustrates the importance of running both electronimpact ionization and NCI when analyzing environmental samples. Electron-impact ionization is more specific for monitoring the molecular ion compared with NCI, which might overestimate the concentration of certain PBDE congeners.
Aguilar and Borrell. 2005	DDT and PCB reduction in the western Mediterranean from 1987 to 2002, as shown by levels in striped dolphins (<i>Stenella coeruleoalba</i>)	https://doi.org/10.1016/j.marenvres.2004.06.004	Temporal trends in DDT and PCB contamination were recorded in the offshore waters of the western Mediterranean Sea during 1987-2002 using striped dolphins (<i>Stenella coeruleoalba</i>) as indicators. Despite the fact that the use of DDT and PCB was banned at the end of the 1970s-early 1980s, dolphins were still found to carry moderate to high levels of these chemicals in their tissues, reflecting their ubiquity and environmental persistence. Concentrations of both groups of compounds have slowly decreased, although the decline in PCB has been steeper than that of DDT. Consequently, the tDDT/PCB ratio increased significantly. Indices of metabolism of both DDT and PCB substantiated progressive aging of pollutant loads and degradation, suggesting that the offshore marine environment has not been exposed to significant releases of these contaminants in recent years. This all indicates a decline in organochlorine pollution in oceanic waters which is consistent, albeit not always, with trends observed in coastal surveys. Dolphins and other top predators are thus confirmed as useful indicators to assess long-term trends of pollutants in oceanic ecosystems and large water masses.
Borrell and Aguilar, 2005	Differences in DDT and PCB residues between common and striped dolphins from the southwestern Mediterranean	https://doi.org/10.1007/s00244-004-0039-7	rganochlorine concentrations (OCs) and stable isotopes were investigated in the blubber of common dolphins (<i>Delphinus delphis</i>) and striped dolphins (<i>Stenella coeruleoalba</i>) from the southwestern Mediterranean. Samples were obtained from dolphins entangled in fishing nets during the 1992-1994 fishing season and from biopsies taken in 1992. Intrapopulation variations were studied, but because most of the dolphins were juveniles or calves (90%), no significant differences were found on the basis of reproductive condition or sex. Only mature male common dolphins showed significantly higher levels of most of the compounds studied than immature individuals did. There were quantitative and qualitative interspecific differences in organochlorine compounds profile. As compared to common dolphins, striped dolphins carried higher concentrations of organochlorine

			<p>concentrations (OCs), their %DDE/tDDT and PCB/tDDT ratios were significantly higher, and recalcitrant PCB congeners were more abundant. Distribution and information on composition of stomach contents would in principle support a higher exposure to OCs in common dolphins as compared to striped dolphins, thus apparently contradicting the observed results. However, stable isotopes showed that striped dolphins exploit a higher trophic level, thus explaining observed differences. Interspecific dissimilarities in metabolic capacity to handle OCs may be an added factor. Although in the two species OC concentrations exceeded levels considered to be ineffective in marine mammals, pollution-induced effects on populations could not be properly assessed.</p>
Wafo et al., 2005	Accumulation and distribution of organochlorines (PCBs and DDTs) in various organs of <i>Stenella coeruleoalba</i> and a <i>Tursiops truncatus</i> from Mediterranean littoral environment (France)	doi.org/10.1016/j.scitotenv.2004.12.078	<p>The objective of the present study is to determine the levels of contamination by PCBs, DDT and its metabolites in dolphins failed on the coasts of the Mediterranean sea. Samples are represented by six <i>Stenella coeruleoalba</i> and a <i>Tursiops truncatus</i> collected in 2000 and 2003. The studies are achieved on the blubber, the heart, the liver, the kidney, the muscle and the lung. The concentrations of PCBs and DDT are very high in all tissues and organs analyzed. For the PCBs, the concentrations vary between 43,838 and 110,343 microg/kg lipid basis in the blubber, 601 and 39444 microg/kg dried weight in the liver, 1375 and 34512 microg/kg dried weight in the muscle, 3151 and 17082 microg/kg dried weight in the heart, 674 and 12365 microg/kg dried weight in the kidney and finally between 648 and 4118 microg/kg dried weight in the lung. These values are comparable to those previously obtained in our laboratory and by other authors during the years 1990 on the Mediterranean environment. Significant differences in concentrations are noted in tissues and organs, neither according to the age, nor according to the gender. In all the analyzed samples, the contents in PCBs are higher than those of DDT. The average ratios of pp'-DDE/SigmaDDT are close to 0.6 which shows the metabolization of these compounds along the years. The examination of the profiles of congeners shows that the hexachlorinated molecules are dominating in all tissues and organs which supposes the different animals were especially exposed to Pyralen-type compounds of transformer (Dp6).</p>
Tornero et al., 2006	Organochlorine contaminant and retinoid levels in blubber of common dolphins (<i>Delphinus delphis</i>) off northwestern Spain	doi.org/10.1016/j.envpol.2005.07.006	<p>The effect of age, sex, nutritive condition and organochlorine concentration on blubber retinoid concentrations was examined in 74 common dolphins incidentally caught off northwestern Spain. Age and blubber lipid content were strong determinants of the retinoid concentrations in males, while these variables did not account for the variation found in females. Retinoids were positively correlated with organochlorines in males and negatively in females. However, pollution levels were moderate and likely to be below threshold levels above that a toxicological response is to be expected. Thus, a cause-effect relationship between organochlorine and retinoid concentrations could not be properly established, and the observed correlation may be the result of an independent association of the two variables with age. Further research on the influence of the best predictor variables on retinoid dynamics is required to implement the use of retinoids as biomarkers of pollutant exposure in cetaceans.</p>

Borrel et al., 2006	Organochlorine compounds and stable isotopes indicate bottlenose dolphin subpopulation structure around the Iberian Peninsula	https://doi.org/10.1016/j.envint.2005.12.001	Isotopic signatures and organochlorine pollutant loads of organisms reflect the characteristics of the waters in which they live and feed. To investigate population structure of bottlenose dolphins around the Iberian Peninsula we determined $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the skin and organochlorine (OC) levels in the blubber of stranded bottlenose dolphins inhabiting the Mediterranean (Catalonia, Valencia and Balearic Islands) and adjacent Atlantic waters (Huelva and Portugal). OC levels were high in all regions, reflecting the predatory habits of the species, its coastal distribution and the existence of intense agricultural and industrial activity throughout the region. PCB congeners showed a gradient from the relatively more chlorinated forms to those that are less so, and followed a northeast to northwest direction across the Iberian Peninsula. This suggests that PCB inputs are more recent in the temperate latitudes of the eastern Atlantic Ocean than in the western Mediterranean Sea. Comparatively, OC ratios and isotopic signatures proved to be more efficient ways of discriminating groups than did raw OC concentrations. Significant differences in $\delta^{13}\text{C}$ and in PCB congener profiles indicate that dolphins from the Atlantic and the Mediterranean do not intermingle. In addition, the two Atlantic groups differed in $\delta^{15}\text{N}$ signature, tDDT concentration, DDT / PCB ratio and the PCB congener profile, which also suggests some degree of isolation between them. In the Mediterranean, dolphins from Catalonia and Valencia were indistinguishable, suggesting a common distribution area. However, dolphins from the Balearic Islands differed from those of the Peninsula in their DDT / PCB ratio and from all the other sample groups in their PCB congener profiles, which supports the hypothesis that the deep waters between the Islands and the Peninsula represent an effective barrier for the species.
Shoham-Frider et al., 2009	Persistent organochlorine pollutants and heavy metals in tissues of common bottlenose dolphin (<i>Tursiops truncatus</i>) from the Levantine Basin of the Eastern Mediterranean	doi.org/10.1016/j.chemosphere.2009.08.048	DDT's, PCBs and heavy metals (HM) were measured in tissues of common bottlenose dolphins, collected along the Israeli Mediterranean coast during 2004–2006. ΣDDT and PCBs concentrations were highest in the blubber, with a wide concentration range of 0.92–142 and 0.05–7.9 mg kg ⁻¹ wet weight, respectively. Blubber PCBs values were an order of magnitude lower than in tissues of this and other delphinid species in the Western Mediterranean. We found relatively high DDE/ ΣDDT percentage (85–96%); a common indicator of DDT degradation, which fitted the general trend of increase in the last 20 years in the Mediterranean Sea, indicating the progressive degradation of the remnant DDT and the absence of new inputs. Concentrations of HM ranged as follows: 0.01–123 mg kg ⁻¹ wet weight for Hg, <0.04–1.3 for Cd, 1–30 for Cu, 0.3–4 for Mn, 19–517 for Fe, 4.3–68 for Zn and 2.4–48 for Ni. These concentrations were similar to those found in specimens collected during previous years in the region, suggesting stability over time in the HM levels of the basin's food-web.
Castrillon et al., 2010	PCB and DDT levels do not appear to have enhanced the mortality of striped dolphins (<i>Stenella coeruleoalba</i>) in the 2007 Mediterranean epizootic	doi.org/10.1016/j.chemosphere.2010.08.008	In 2007, 17 years after the first reported Mediterranean epizootic of striped dolphins (<i>Stenella coeruleoalba</i>), a new strain of the morbillivirus caused the deaths of dozens of striped dolphins that appeared dead on Western Mediterranean beaches. DDT and PCB levels were determined in these dolphins, and in individuals from sporadic strandings in the surrounding years. Comparison between the two epidemic events showed that organochlorine (OC) levels in the dolphins from 1990 epizootic were more

			than 10-fold higher for tPCB and 6-fold higher for tDDT than levels in dolphins from the 2007 outbreak. In contrast to what occurred in 1990, OCs from individuals affected by the second outburst fit well with curves of OC trends in the Mediterranean. Because the virulence of the 2007 epizootic was much lower, and the deceased dolphins affected by it did not present OC concentrations that were more elevated than in presumably healthy individuals, this second outburst is not believed to have been enhanced by OC pollutants.
Fossi et al., 2010	A multi-trial diagnostic tool in fin whale (<i>Balaenoptera physalus</i>) skin biopsies of the Pelagos Sanctuary (Mediterranean Sea) and the Gulf of California (Mexico)	10.1016/j.marenvres.2009.10.006	The main objective of this study was to apply a set of sensitive non-lethal biomarkers in skin biopsies of fin whales (<i>Balaenoptera physalus</i>) to evaluate the toxicological status of this mysticete in the Pelagos Sanctuary (Mediterranean Sea) and in the Gulf of California (Sea of Cortez-Mexico). We developed a "multi-trial diagnostic tool" (based on field and in vitro studies), combining molecular biomarkers (western blot of CYP1A1, CYP2B) and gene expression (qRT-PCR of HSP70, ER α , AHR, E2F-1) with the analysis of OCs, PAHs and PBDEs. The study revealed a higher level of toxicological stress in the Mediterranean fin whales.
Weijs et al., 2010	Occurrence of anthropogenic and naturally-produced organohalogenated compounds in tissues of Black Sea harbour porpoises	10.1016/j.marpolbul.2009.11.022	Harbour porpoises are one of the three cetacean species inhabiting the Black Sea. This is the first study to report on polybrominated diphenyl ethers (PBDEs) and naturally-produced compounds, methoxylated PBDEs (MeO-PBDEs) and polybrominated hexahydroxanthene derivatives (PBHDs), in tissues (kidney, brain, blubber, liver, muscle) of male harbour porpoises (11 adults, 9 juveniles) from the Black Sea. Lipid-normalized concentrations decreased from muscle > blubber > liver > kidney > brain for the sum of polychlorinated biphenyls (PCBs) and for the sum of PBDEs. Among the naturally-produced compounds, levels of PBHDs were higher than of MeO-PBDEs, with tri-BHD and 6-MeO-BDE 47 being the dominant compounds for both groups, respectively. Concentrations of naturally-produced compounds decreased from blubber to brain, similarly to the sum of DDT and metabolites (DDXs). Concentrations of DDXs were highest, followed by PCBs, HCB, PBHDs, PBDEs and MeO-PBDEs. Levels of PCBs and PBDEs in blubber were lower than concentrations reported for harbour porpoises from the North Sea, while concentrations of DDXs were higher.
Praca et al., 2011	Toothed whales in the northwestern Mediterranean: Insight into their feeding ecology using chemical tracers	doi.org/10.1016/j.marpolbul.2011.02.024	Risso's dolphins, pilot whales and sperm whales rarely strand in the northwestern Mediterranean. Thus, their feeding ecology, through the analysis of stomach contents, is poorly known. The aim of this study was to gain further insight into the segregation/superposition of the diet and habitat of Risso's dolphins, pilot whales and sperm whales using chemical tracers, namely, stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and organochlorines. Significantly different $\delta^{15}\text{N}$ values were obtained in Risso's dolphins ($11.7 \pm 0.7\text{‰}$), sperm whales ($10.8 \pm 0.3\text{‰}$) and pilot whales ($9.8 \pm 0.3\text{‰}$), revealing different trophic levels. These differences are presumably due to various proportions of <i>Histioteuthidae</i> cephalopods in each toothed whale's diet. Similar $\delta^{13}\text{C}$ contents between species indicated long-term habitat superposition or corroborated important seasonal migrations. Lower congener 180 concentrations (8.20 vs. 21.73 $\mu\text{g/g}$ lw) and higher tDDT/tPCB ratios (0.93 vs. 0.42) were observed in sperm whales compared with Risso's dolphins and may indicate wider migrations for the former. Therefore,

			competition between these species seems to depend on different trophic levels and migration patterns.
Storelli et al., 2011	Contamination by polychlorinated biphenyls (PCBs) in striped dolphins (<i>Stenella coeruleoalba</i>) from the Southeastern Mediterranean Sea	doi.org/10.1007/s10661-011-2382-2	Concentrations of polychlorinated biphenyls (PCBs) including dioxin-like PCBs (non-ortho, PCB 77, PCB 126, and PCB 169 and mono-ortho, PCB 105, PCB 118, and PCB 156) were measured in different organs and tissues (melon, blubber, liver, kidney, lung, heart, and muscle tissue) of striped dolphins (<i>Stenella coeruleoalba</i>) from the Eastern Mediterranean Sea (Adriatic Sea). The mean highest levels were in blubber and melon, followed by liver, kidney, lung, heart, and muscle tissue. PCB profiles were similar in all tissues and organs being dominated by the higher chlorinated homologues (hexa-CBs, 55.8-62.1%; penta-CBs, 15.4-20.0%; and hepta-CB PCB 180, 12.7-16.5%). Major PCBs in all tissues were congeners 138 and 153 collectively accounting for 50.6-58.3% of the total PCB concentrations, followed by PCB 101, 105, 118, and 180 constituting from 27.0% to 31.0%. PCB levels were higher in adult males than in adult females. The estimated 2,3,7,8-TCDD toxic equivalents of non- and mono-ortho PCBs were much higher than the threshold level above which adverse effects have been observed in other marine mammals species, suggesting that striped dolphins in this region are at risk for toxic effects.
Lazar et. al, 2012	Organochlorine contaminant levels in tissues of a short-beaked common dolphin, <i>Delphinus delphis</i> , from northern Adriatic Sea	na	We analyzed 17 polychlorinated biphenyls (PCBs) and 7 organochlorine pesticides (OCPs) in blubber, liver, muscle, lung, heart and kidney of an adult male short-beaked common dolphin (<i>Delphinus delphis</i>) found dead stranded on the island of Cres (Croatia) in 2004. The PCB profile was dominated by hexachlorobiphenyls (39.4 – 63.2% of SPCB), with PCB-153 exhibiting the highest concentrations across all tissues. The pattern of PCB tissue distribution (SPCB) showed the highest burdens in blubber >> liver > kidney > heart > muscle > lung, which were positively correlated with tissue lipid content ($r_s = 0.986$, $p < 0.01$). Among OCPs, HCB and SDDT exhibited the same distribution between tissues, correlated with the tissue lipids ($r_s = 0.985$ and 0.986 , respectively, $p < 0.01$). Total HCHs showed highest levels in muscle > blubber > liver > kidney > lung > heart, with no correlation to tissue lipid content ($p > 0.05$). Total DDTs were lower than total PCB levels for all tissues, with SPCB/SDDTs ratios ranging from 1.3 in blubber to 5.9 in muscles. Blubber OC burdens recorded in our specimen were among the highest found in a dolphin in the Mediterranean after the year 2000. This result and the presence of mono-ortho substituted PCBs with dioxin-like toxicity in all our samples may present an additional factor of concern for the conservation of regional dolphin populations.
Storelli et al., 2012	Contamination by polychlorinated biphenyls (PCBs) in striped dolphins (<i>Stenella coeruleoalba</i>) from the Southeastern Mediterranean Sea	doi.org/10.1007/s10661-011-2382-2	Concentrations of polychlorinated biphenyls (PCBs) including dioxin-like PCBs (non-ortho, PCB 77, PCB 126, and PCB 169 and mono-ortho, PCB 105, PCB 118, and PCB 156) were measured in different organs and tissues (melon, blubber, liver, kidney, lung, heart, and muscle tissue) of striped dolphins (<i>Stenella coeruleoalba</i>) from the Eastern Mediterranean Sea (Adriatic Sea). The mean highest levels were in blubber and melon, followed by liver, kidney, lung, heart, and muscle tissue. PCB profiles were similar in all tissues and organs being dominated by the higher chlorinated homologues (hexa-CBs, 55.8-62.1%; penta-CBs, 15.4-20.0%; and hepta-CB PCB 180, 12.7-16.5%). Major PCBs in all tissues were congeners 138 and 153

			collectively accounting for 50.6-58.3% of the total PCB concentrations, followed by PCB 101, 105, 118, and 180 constituting from 27.0% to 31.0%. PCB levels were higher in adult males than in adult females. The estimated 2,3,7,8-TCDD toxic equivalents of non- and mono-ortho PCBs were much higher than the threshold level above which adverse effects have been observed in other marine mammals species, suggesting that striped dolphins in this region are at risk for toxic effects.
Wafo et al., 2012	PCBs and DDTs in <i>Stenella coeruleoalba</i> dolphins from the French Mediterranean coastal environment (2007–2009): Current state of contamination	https://doi.org/10.1016/j.marpolbul.2012.07.034	Organochlorinated compounds including PolyChloroBiphenyles, Dichloro-DiphenylTrichloroethan and metabolites are determined in <i>Stenella coeruleoalba</i> (n = 37) stranded on the french Mediterranean coasts from 2007 till 2009. Studies are carried out on lung, muscle, kidney, liver, and blubber. The sought-after compounds are all detected to variable levels in each tissue and organ. In general, total PCBs are the most abundant, followed by total DDTs. The concentration (in ng g ⁻¹ of lipid weight) in blubber of <i>S. coeruleoalba</i> , varied from 2,052 to 158,992 for PCBs and from 1,120 to 45,779 for DDTs. The ratios DDE/tDDTs are higher than 80% in almost all samples. The overall results of this work, compared to previous studies concerning the Mediterranean Sea, seems to confirm the tendency to a decrease of the contamination by organics compounds for the cetaceans in the Western Mediterranean Sea.
Wafo et al. 2012	Chlorinated pesticides in the bodies of dolphins of the French Mediterranean coastal environment	na	The concentrations of organochlorinated pesticides including lindane, heptachlor, aldrin, heptachlor-epoxide, endosulfan I, dieldrin, and endrin were determined in striped dolphins (<i>Stenella coeruleoalba</i>) (n = 26), and 2 bottlenose dolphins (<i>Tursiops truncatus</i>) (n = 2), stranded on the French Mediterranean coasts. Studies are carried out on the lung, the muscle, the kidney, the liver, and the blubber. The concentrations of all the analysed compounds were detected to variable levels in each tissue and organ. In blubber, dieldrin is generally the most abundant compound (215.3 ± 290.3 ng g ⁻¹ lw), followed by endrin (207.7 ± 217.5 ng g ⁻¹ lw), heptachlor-epoxid (106.6 ± 107.1 ng g ⁻¹ lw), endosulfan I (46.6 ± 32.8 ng g ⁻¹ lw), lindane (16.6 ± 12.1 ng g ⁻¹ lw), aldrin (11.9 ± 8.4 ng g ⁻¹ lw) and heptachlor (6.7 ± 4.2 ng g ⁻¹ lw). These values are comparable to those previously obtained by other authors during the years 1990.
Fossi et al., 2013	The Pelagos Sanctuary for Mediterranean marine mammals: Marine Protected Area (MPA) or marine polluted area? The case study of the striped dolphin (<i>Stenella coeruleoalba</i>)	doi.org/10.1016/j.marpolbul.2013.02.013	The concurrence of man-made pressures on cetaceans in the Mediterranean Sea is potentially affecting population stability and marine biodiversity. This needs to be proven for the only pelagic marine protected area in the Mediterranean Sea: the Pelagos Sanctuary for Mediterranean Marine Mammals. Here we applied a multidisciplinary tool, using diagnostic markers elaborated in a statistical model to rank toxicological stress in Mediterranean cetaceans. As a case study we analyzed persistent, bioaccumulative and toxic chemicals combined with a wide range of diagnostic markers of exposure to anthropogenic contaminants and genetic variation as marker of genetic erosion in striped dolphin (<i>Stenella coeruleoalba</i>) skin biopsies. Finally, a statistical model was applied to obtain a complete toxicological profile of the striped dolphin in the Pelagos Sanctuary and other Mediterranean areas (Ionian Sea and Strait of Gibraltar). Here we provide the first complete evidence of the toxicological stress in cetaceans living in Pelagos Sanctuary.

Lauriano et al., 2013	Biological threats and environmental pollutants, a lethal mixture for mediterranean cetaceans?	doi.org/10.1017/S0025315413000714	he possible existence of any cause–effect relationships between the concentrations of organochlorines (OCs) and the presence of Morbillivirus and Toxoplasma gondii infections was investigated in both free-living and stranded specimens of <i>Stenella coeruleoalba</i> , <i>Tursiops truncatus</i> , <i>Globicephala melas</i> , <i>Balaenoptera physalus</i> and <i>Physeter macrocephalus</i> from the Mediterranean Sea. High blubber concentrations of polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) were recorded in free-ranging <i>G. melas</i> . Tissue concentrations of PCBs and DDT in stranded <i>T. truncatus</i> (367 lipid weight (l.w.) and 143.7 mg/kg l.w., respectively) and <i>S. coeruleoalba</i> (139.9 l.w.; 92.9 mg/kg l.w.) were beyond the PCB threshold value for the appearance of adverse effects in marine mammals. Evidence of <i>T. gondii</i> infection was molecularly detected in three <i>S. coeruleoalba</i> and six <i>T. truncatus</i> .
Marsili et al., 2014	Ecotoxicological status of seven sperm whales (<i>Physeter macrocephalus</i>) stranded along the Adriatic coast of Southern Italy	doi.org/10.1002/aqc.2447	In December 2009 seven male sperm whales (<i>Physeter macrocephalus</i>) stranded along the Adriatic coast of Southern Italy. Genetic and photo-identification data indicated that these specimens belonged to the Mediterranean Sea population. 2. Complete necropsies were performed on the three best preserved animals. Blubber and muscle samples were collected from all seven specimens for contaminants analysis (DDTs, PCBs and PAHs) and biomarker responses (CYP1A1 and CYP2B) were evaluated from the three better preserved animals using western blot analysis. 3. Fibroblast cell cultures from two specimens were set up by culturing cell lines from integument tissue, to explore the susceptibility of these marine mammals to different xenobiotic compounds. 4. The levels of CYP1A1 and CYP2B, proteins that are induced by some POPs (DDTs and PCBs) and PAHs were detected using the indirect immunofluorescence assay. The results showed relatively high levels of contaminants, in particular from organochlorine xenobiotics, and strong responses of CYP1A1 and CYP2B reflecting toxicological stress of these sperm whales. 5. This is not considered to be their cause of death, but may have contributed to lowering the defences of their immune system.
Romanic et al., 2014	Organochlorine contaminants in tissues of common bottlenose dolphins <i>Tursiops truncatus</i> from the northeastern part of the Adriatic Sea	doi.org/10.1016/j.etap.2014.07.017	Levels of 24 organochlorine compounds, including toxic mono-ortho PCB congeners, were determined in the organs and tissues (blubber, kidney, lung, muscle, liver, heart) of 13 common bottlenose dolphins (<i>Tursiops truncatus</i>) stranded between 2000 and 2005 in the northern part of the Croatian territorial waters of the Adriatic Sea. Polychlorinated biphenyls (PCBs) were found at higher concentrations in comparison with organochlorine pesticides (OCPs) in all of the analyzed tissues. Sums of six indicator congeners ($\Sigma 6\text{PCB}$) constituted around 50% of the total PCB amount, while PCB-153 and PCB-138 were found to have the highest concentrations. Among the seven investigated OCPs, p,p'-DDE was found at the highest concentrations. In blubber, mean values of 22,048 and 11,310 ng g ⁻¹ wet weight were determined for ΣPCB and ΣDDT , respectively. Much lower concentrations were found in muscle samples, followed by similar concentrations in kidneys, liver and heart, while the lowest levels of organochlorine contaminants were found in lungs. The results indicate that p,p'-DDT is still being introduced in the Mediterranean region. PCB concentrations are

			among the highest found in this region and toxicological assessments indicate that the health of this specie is at high risk.
Baron et al., 2015	Halogenated Natural Products in Dolphins: Brain–Blubber Distribution and Comparison with Halogenated Flame Retardants	doi.org/10.1021/acs.est.5b02736	Halogenated natural products (MHC-1, TriBHD, TetraBHD, MeO-PBDEs, Q1, and related PMBPs) and halogenated flame retardants (PBDEs, HBB, Dec 602, Dec 603, and DP) in blubber and brain are reported from five Alboran Sea delphinids (Spain). Both HNPs and HFRs were detected in brain, implying that they are able to surpass the blood-brain barrier and reach the brain, which represents a new finding for some compounds, such as Q1 and PMBPs, MHC-1, TriBHD, TetraBHD, or Dec 603. Moreover, some compounds (TetraBHD, BDE-153, or HBB) presented higher levels in brain than in blubber. This study evidence the high concentrations of HNPs in the marine environment, especially in top predators. It shows the importance of further monitoring these natural compounds and evaluating their potential toxicity, when most studies focus on anthropogenic compounds only. While no bioaccumulation was found for Σ HNPs, Σ HFRs increased significantly with body size for both common and striped dolphins. Studies evaluating BBB permeation mechanisms of these compounds together with their potential neurotoxic effects in dolphins are recommended.
Pinzone et al., 2015	POPs in free-ranging pilot whales, sperm whales and fin whales from the Mediterranean Sea: Influence of biological and ecological factors	doi.org/10.1016/j.envres.2015.06.021	The pilot whale <i>Globicephala melas</i> , the sperm whale <i>Physeter macrocephalus</i> , and the fin whale <i>Balaenoptera physalus</i> are large cetaceans permanently inhabiting the Mediterranean Sea. These species are subjected to numerous anthropogenic threats such as exposure to high levels of contaminants. Therefore, selected persistent organic pollutants POPs (29 PCBs, 15 organochlorine compounds, 9 PBDEs and 17 PCDD/Fs) were analysed in blubber biopsies of 49 long-finned pilot whales, 61 sperm whales and 70 fin whales sampled in the North Western Mediterranean Sea (NWMS) from 2006 to 2013. Contamination profile and species feeding ecology were then combined through the use of stable isotopes. $\delta(13)C$, $\delta(15)N$ values and POPs levels were assessed through IR-MS and GC-MS respectively. To assess the toxic potency of the dioxin-like compounds, the TEQ approach was applied. $\delta(15)N$ values were $12.2 \pm 1.3\%$ for sperm whales, $10.5 \pm 0.7\%$ for pilot whales and $7.7 \pm 0.8\%$ in fin whales, positioning sperm whales at higher trophic levels. $\delta(13)C$ of the two odontocetes was similar and amounted to $-17.3 \pm 0.4\%$ for sperm whales and $-17.8 \pm 0.3\%$ for pilot whales; whilst fin whales were more depleted ($-18.7 \pm 0.4\%$). This indicates a partial overlap in toothed-whales feeding habitats, while confirms the differences in feeding behaviour of the mysticete. Pilot whales presented higher concentrations than sperm whales for Σ PCBs ($38,666 \pm 25,731$ ng g ⁻¹ lw and $22,849 \pm 15,566$ ng g ⁻¹ lw respectively), Σ PBDEs (712 ± 412 ng g ⁻¹ lw and 347 ± 173 ng g ⁻¹ lw respectively) and Σ DDTs ($46,081 \pm 37,506$ ng g ⁻¹ lw and $37,647 \pm 38,518$ ng g ⁻¹ lw respectively). Fin whales presented the lowest values, in accordance with its trophic position (Σ PCBs: 5721 ± 5180 ng g ⁻¹ lw, Σ PBDEs: 177 ± 208 ng g ⁻¹ lw and Σ DDTs: 6643 ± 5549 ng g ⁻¹ lw). Each species was characterized by large inter-individual variations that are more related to sex than trophic level, with males presenting higher contaminant burden than females. The discriminant analysis (DA) confirmed how DDTs and highly chlorinated PCBs were influential in differentiating the three species. Pollutant concentrations of our species were significantly higher than both their Southern

			<p>Hemisphere and North Atlantic counterparts, possibly due to the particular Mediterranean geomorphology, which influences pollutants distribution and recycle. Dioxin-like PCBs accounted for over 80% of the total TEQ. This study demonstrated (1) an important exposure to pollutants of Mediterranean cetaceans, often surpassing the estimated threshold toxicity value of 17,000 ng g(-1) lw for blubber in marine mammals; and (2) how the final pollutant burden in these animals is strongly influenced not only by the trophic position but also by numerous other factors such as sex, age, body size and geographical distribution.</p>
Jepson et al., 2016	PCB pollution continues to impact populations of orcas and other dolphins in European waters.	10.1038/srep18573	<p>Organochlorine (OC) pesticides and the more persistent polychlorinated biphenyls (PCBs) have well-established dose-dependent toxicities to birds, fish and mammals in experimental studies, but the actual impact of OC pollutants on European marine top predators remains unknown. Here we show that several cetacean species have very high mean blubber PCB concentrations likely to cause population declines and suppress population recovery. In a large pan-European meta-analysis of stranded (n = 929) or biopsied (n = 152) cetaceans, three out of four species:- striped dolphins (SDs), bottlenose dolphins (BNDs) and killer whales (KW)s had mean PCB levels that markedly exceeded all known marine mammal PCB toxicity thresholds. Some locations (e.g. western Mediterranean Sea, south-west Iberian Peninsula) are global PCB “hotspots” for marine mammals. Blubber PCB concentrations initially declined following a mid-1980s EU ban, but have since stabilised in UK harbour porpoises and SDs in the western Mediterranean Sea. Some small or declining populations of BNDs and KWs in the NE Atlantic were associated with low recruitment, consistent with PCB-induced reproductive toxicity. Despite regulations and mitigation measures to reduce PCB pollution, their biomagnification in marine food webs continues to cause severe impacts among cetacean top predators in European seas.</p>
Gonzalvo et al., 2016	The Gulf of Ambracia’s common bottlenose dolphins, <i>Tursiops truncatus</i> : a highly dense and yet threatened population.	doi.org/10.1016/bs.amb.2016.07.002	<p>The common bottlenose dolphin (<i>Tursiops truncatus</i>) is the only cetacean present in the semiclosed waters of the Gulf of Ambracia, Western Greece. This increasingly degraded coastal ecosystem hosts one of the highest observed densities in the Mediterranean Sea for this species. Photo-identification data and tissue samples collected through skin-swabbing and remote biopsy sampling techniques during boat-based surveys conducted between 2006 and 2015 in the Gulf, were used to examine bottlenose dolphin abundance, population trends, site fidelity, genetic differentiation and toxicological status. Bottlenose dolphins showed high levels of year-round site fidelity throughout the 10-year study period. Dolphin population estimates mostly fell between 130 and 170 with CVs averaging about 10%; a trend in population size over the 10 years was a decline of 1.6% per year (but this was not significant). Genetic differentiation between the bottlenose dolphins of the Gulf and their conspecifics from neighbouring populations was detected, and low genetic diversity was found among individuals sampled. In addition, pesticides were identified as factors posing a real toxicological problem for local bottlenose dolphins. Therefore, in the Gulf of Ambracia, high dolphin density does not seem to be indicative of favourable conservation status or pristine habitat.</p>

Cocumelli et al., 2018	Cetacean Poxvirus in Two Striped Dolphins (<i>Stenella coeruleoalba</i>) Stranded on the Tyrrhenian Coast of Italy: Histopathological, Ultrastructural, Biomolecular, and Ecotoxicological Findings	https://doi.org/10.3389/fvets.2018.00219	Tattoo skin disease (TSD) is a poxviral disease typical of cetaceans. Two juvenile and well-preserved male striped dolphins (<i>Stenella coeruleoalba</i>), found stranded along the Tuscany and Latium coasts of Italy in 2015 and 2016, respectively, showed typical skin lesions ascribable to TSD. Histological, ultrastructural and biomolecular investigations confirmed a poxviral aetiology for the aforementioned skin lesions. To our knowledge, this should be the first report of TSD in cetaceans stranded along the Italian coastline. As organochlorines like PCBs and DDTs are known to be highly immunotoxic, the tissue loads of these contaminants were evaluated, in order to increase our knowledge on their potential role as well as on the relationships between the level of exposure to these pollutants and poxviral infection's occurrence.
Zaccaroni et al. 2018	Halogenated flame retardants in stranded sperm whales (<i>Physeter macrocephalus</i>) from the Mediterranean Sea	doi.org/10.1016/j.scitotenv.2018.04.147	In recent years, decline of marine mammals' populations and increased frequency of strandings have arisen the interest on the role that pollution may have in these events. The present work aimed at quantifying levels of brominated flame retardants (BFRs) and dechloranes (DECs) in tissues of 3 adult females and one foetus of sperm whales stranded in the Southern Adriatic Sea coasts (Italy). Results proved the presence of different flame retardants (FRs) in tissues of sperm whales, including various polybrominated diphenyl ethers (PBDE) congeners (47, 99, 100, 154, entering the composition of PentaBDE mixture), hexabromocyclodecanes (HBCDs), Dec 602 and methoxylated polibrominated diphenyl ethers (MeO-BDEs). In blubber, a target tissue for contaminant accumulation, ΣPBDEs reached values of 160, 158 and 183 ng/g lw, α-HBCD of 5.75 ng/g lw, Dec 602 of 1632 ng/g lw and MeO-BDEs of 563 ng/g lw. The availability of foetal tissues allowed evaluating the potential maternal transfer on many of these compounds, and to discuss the potential adverse effects on foetal health. To the best of our knowledge, obtained data are the first reporting placental transfer of FRs in sperm whales. PBDE levels detected in foetus suggested a potentially long-term exposure to BFRs, which could cause severe damages to the developing organism, likely at the cerebral, endocrine and immunologic levels. Dec 602, which was detected at the highest concentrations among all FRs considered, could potentially cause dysfunctional effects on the immune system of adult females.
Bartalini et al., 2019	Evaluation of PCDD/Fs, dioxin-like PCBs and PBDEs in sperm whales from the Mediterranean Sea	10.1016/j.scitotenv.2018.10.436	Numerous studies to date have reported concentrations of Persistent Organic Pollutants (POPs) in different marine mammal species worldwide. Yet data on sperm whales are scarce from rich and unique biodiverse areas such as the Mediterranean Sea. This work aimed to assess levels of dioxin-like polychlorinated biphenyls (dl-PCBs), polybrominated diphenyl ethers (PBDEs), and polychlorodibenzop-dioxins and furans (PCDD/Fs) in blubber of sperm whales stranded along the Italian coast between 2008 and 2016. POP mean concentrations (dl-PCBs: 6410 ng/g l.w.; PBDEs: 612 ng/g l.w.; PCDD/Fs: 57.8 pg/g l.w.) were mostly in line with what has been previously reported on the same species in the Mediterranean environment and tended to be higher than those reported from other geographical regions. The relative abundance followed the order dl-PCBs > PBDEs >> PCDD/Fs. Interestingly, the non-ortho dl-PCB pattern (126 N 169 N 77) was similar to that described in other studies worldwide and different from what is described in its main prey. This could be linked to particular metabolic

			activities in sperm whales against these highly toxic contaminants. Total TEQs ranged from 275 to 987 pg/g l.w. and showed the pattern Σ non-ortho-dl-PCBs N Σ ortho-dl-PCBs N PCDDs N PCDFs, with PCBs' contribution about 96%. These findings highlight the high abundance of PCBs still found in the Mediterranean environment despite having been banned for decades. All sperm whales analyzed in this study surpassed the threshold of 210 pg WHO-TEQ/g l.w. proposed as starting point of immunosuppression in harbour seals; a level of contamination that may have contributed to an impairment of their immune system.
Genov et al., 2019	Linking organochlorine contaminants with demographic parameters in free-ranging common bottlenose dolphins from the northern Adriatic Sea	doi.org/10.1016/j.scitotenv.2018.12.025	Marine top predators, including marine mammals, are known to bioaccumulate persistent pollutants such as polychlorinated biphenyls (PCBs), a serious conservation concern for these species. Although PCBs declined in European seas since the 1970s–1980s ban, considerable levels still persist in European and Mediterranean waters. In cetaceans, stranded animals are a valuable source of samples for pollutant studies, but may introduce both known and unknown biases. Biopsy samples from live, free-ranging cetaceans offer a better alternative for evaluating toxicological burdens of populations, especially when linked to known histories of identified individuals. We evaluated PCB and other organochlorine contaminants in free-ranging common bottlenose dolphins (<i>Tursiops truncatus</i>) from the Gulf of Trieste (northern Adriatic Sea), one of the most human-impacted areas in the Mediterranean Sea. Biopsies were collected from 32 male and female dolphins during 2011–2017. All animals were photo-identified and are part of a well-known population of about 150 individuals monitored since 2002. We tested for the effects of sex, parity and social group membership on contaminant concentrations. Males had significantly higher organochlorine concentrations than females, suggesting offloading from reproducing females to their offspring via gestation and/or lactation. Furthermore, nulliparous females had substantially higher concentrations than parous ones, providing further support for maternal offloading of contaminants. Overall, 87.5% of dolphins had PCB concentrations above the toxicity threshold for physiological effects in experimental marine mammal studies (9 mg/kg lw), while 65.6% had concentrations above the highest threshold published for marine mammals based on reproductive impairment in ringed seals (41 mg/kg lw). The potential population-level effects of such high contaminant levels are of concern particularly in combination with other known or suspected threats to this population. We demonstrate the utility of combining contaminant data with demographic parameters such as sex, reproductive output, etc., resulting from long-term studies.
Aznar-Alemany et al., 2021	Temporal trends of halogenated and organophosphate contaminants in striped dolphins from the Mediterranean Sea	doi.org/10.1016/j.scitotenv.2020.142205	PBDEs, HBCD, novel DBDPE, PBEB and HBB, dechloranes, OPFRs and natural MeO-PBDEs were monitored in muscle of striped dolphins (<i>Stenella coeruleoalba</i>) from the Mediterranean Sea collected in three time periods (1990, 2004–2009 and 2014–2018). PBDEs levels decreased about 60% in under three decades, from 5067 ± 2210 to 2068 ± 2642 ng g ⁻¹ lw, evidencing the success of their ban. Most PBDEs were found in all the samples, with BDE-47, -99, -154, -100 and -153 as the main contributors. Found in 71.4% of the samples, α -HBCD was stable through time and usually <LOQ. DBDPE concentrations decreased by 89% from 1990 to

			<p>2004–2009 and have remained stable since. HBB occurred rarely and decreased by 94% to a current few ng g⁻¹ lw. Dec 602 was the main dechlorane with stable concentrations around 1200 ng g⁻¹ lw, but a declining trend might have started in the last years. OPFRs concentrations were stable and showed the highest concentrations of all FRs in 2014–2018: 6253 ± 11,293 ng g⁻¹ lw. TBOEP and TNBP contributed to most of the OPFR concentration, the former with decreasing levels by 96%. MeO-PBDEs showed mean concentrations between 600 and 700 ng g⁻¹ lw in all periods. Non-targeted analysis allowed the identification and semi-quantification of additional chlorinated pollutants, such as polychlorinated terphenyls (PCTs) (levels decreasing by 81% to a current 770 ng g⁻¹ lw mean) and polychlorinated diphenyl ethers (PCDEs) (decreasing by 83% to a current 3200 ng g⁻¹ lw) in Mediterranean marine mammals for the first time.</p>
Dron et al., 2022	Trends of banned pesticides and PCBs in different tissues of striped dolphins (<i>Stenella coeruleoalba</i>) stranded in the Northwestern Mediterranean reflect changing contamination patterns	doi.org/10.1016/j.marpolbul.2021.113198	<p>Although banned for years, organochlorine pesticides and PCBs continue to affect aquatic life, dolphins being particularly exposed. The concentrations of 31 PCB congeners, and 15 banned pesticides or metabolites were measured in 5 tissues of 68 striped dolphins stranded in the Northwestern Mediterranean coast in 2010–16. The results were compared to historical data (1988–2009) and, even though there is a slow decreasing trend, the levels in the 2010–2016 samples were still elevated based on common cetacean toxicological thresholds. A transition period in 2007–08, probably caused by a morbillivirus epizootic amplified the stranding, especially of highly contaminated specimens. From 2010, higher proportions in parent compounds towards metabolites were observed yet again. These changing patterns were likely reflect the exposure of dolphins to the remobilization of pollutants from contaminated soils and sediments, with a prominent role of rivers. This should lead to an even slower decline of these contaminants that could last for decades, requiring new efforts to reduce their dispersal to aquatic ecosystems.</p>
Pennino et al., 2022	Understanding the causes of mortality and contaminant loads of stranded cetacean species in Sardinian waters (Italy) using Bayesian Hierarchical Models	doi.org/10.1016/j.seares.2022.102170	<p>Cetacean strandings represent unique opportunities to collect biological material from these wild animals and obtain information on their population statuses. Here, we apply biological and pathological perspectives to analyze stranded cetaceans collected along the Sardinian coast (Italy) between 2006 and 2011. We quali-quantitatively explore the primary causes of deaths, and use Bayesian Hierarchical Models (BHM) to explore the potential effects of cetacean sex, age, body length, and month, year, and stranding location on Diclolo Difenil Tricloroetano (DDT) and Polychlorinated Biphenyl (PCB) contaminant loads. Although natural causes, such as bacterial and virus infections, were identified to be the main causes of death among the stranded cetaceans, fisheries also played an important role among the anthropogenic causes of death. The BHM revealed that both contaminants were positively related to the length, sex and age of the cetaceans, and that higher concentrations of these contaminants were mainly found in larger and older individuals. Despite the scattered nature of these data, the present study contributes valuable insights into the major causes of death of stranded cetaceans, and adds to growing worldwide efforts to biomonitor cetaceans.</p>

Bartalini et al., 2022	Global PBDE contamination in cetaceans. A critical review	doi.org/10.1016/j.envpol.2022.119670	<p>This review summarizes the most relevant information on PBDEs' occurrence and their impacts in cetaceans at global scale, with special attention on the species with the highest reported levels and therefore the most potentially impacted by the current and continuous release of these substances. This review also emphasizes the anthropogenic and environmental factors that could increase concentrations and associated risks for these species in the next future. High PBDE concentrations above the toxicity threshold and stationary trends have been related to continuous import of PBDE-containing products in cetaceans of Brazil and Australia, where PBDEs have never been produced. Non-decreasing levels documented in cetaceans from the Northwest Pacific Ocean might be linked to the increased e-waste import and ongoing production and use of deca-BDE that is still allowed in China. Moreover, high levels of PBDEs in some endangered species such as beluga whales (<i>Delphinapterus leucas</i>) in St. Lawrence Estuary and Southern Resident killer whales (<i>Orcinus Orca</i>) are influenced by the discharge of contaminated waters deriving from wastewater treatment plants. Climate change related processes such as enhanced long-range transport, re-emissions from secondary sources and shifts in migration habits could lead to greater exposure and accumulation of PBDEs in cetaceans, above all in those species living in the Arctic. In addition, increased rainfall could carry greater amount of contaminants to the marine environment, thereby, enhancing the exposure and accumulation especially for coastal species. Synergic effects of all these factors and ongoing emissions of PBDEs, expected to continue at least until 2050, could increase the degree of exposure and menace for cetacean populations. In this regard, it is necessary to improve current regulations on PBDEs and broaden the knowledge about their toxicological effects, in order to assess health risks and support regulatory protection for cetacean species.</p>
Malakhova et al., 2021	DDT in Crimea coastal waters and blubber of Black Sea cetaceans	10.1088/1755-1315/937/2/022019	<p>This research is based on the multi-year data on the distribution of organochlorine pesticides of the DDT group in the water of the coastal Black Sea areas of Crimea, obtained in expeditions of the "Professor Vodyanitsky" research vessel from 1999 to 2020. Study of content of DDT and its metabolites (DDTs) in the blubber of three species of Black Sea cetaceans that were stranded on the Black Sea coast from the 1980s to the 2020s was grounded on own and literature data. While there was an overall significant variability in ΣDDT content during the study period, there was a significant downward trend in their concentrations in the water of coastal areas of Crimea. Despite the significantly decreased DDTs pollution of the habitat of the Black Sea marine mammals over the last 10 years, high concentrations of DDTs were accumulated in their blubber, the maximum value of the sum of DDTs concentrations reached (163 µg/g of lipids) in the blubber of adult depleted bottlenose dolphin. The minimum concentrations were determined in the blubber of a newborn harbour porpoise female. In blubber samples up to 70% was of DDE, DDD - from 21 to 24%, and the concentration of the initial DDT varied from 6 to 14%. Based on the analysis of own and literature data, it is assumed that in the modern period, as compared to the 1990s, there is a downward trend in the content of the DDTs in the blubber of Black Sea cetaceans</p>

Per- and polyfluoroalkyl substances			
Kannan et al., 2002	Perfluorooctanesulfonate and Related Fluorinated Hydrocarbons in Marine Mammals, Fishes, and Birds from Coasts of the Baltic and the Mediterranean Sea	doi.org/10.1021/es020519q	<p>Perfluorooctanesulfonate (PFOS; C₈F₁₇SO₃-), Perfluorooctanesulfonamide (FOSA; C₈F₁₇SO₂NH₂), perfluorohexanesulfonate (PFHxS; C₆F₁₃SO₃-), and perfluorooctanoate (PFOA; C₇F₁₅CO₂-) were detected in 175 samples of liver and blood of bluefin tuna (<i>Thunnus thynnus</i>), swordfish (<i>Xiphias gladius</i>), common cormorants (<i>Phalacrocorax carbo</i>), bottlenose dolphins (<i>Tursiops truncatus</i>), striped dolphins (<i>Stenella coeruleoalba</i>), common dolphins (<i>Delphinus delphi</i>), fin whales (<i>Balenoptera physalus</i>), and long-finned pilot whales (<i>Globicephala melas</i>) from the Italian coast of the Mediterranean Sea and in livers of ringed seals (<i>Phoca hispida</i>), gray seals (<i>Halichoerus grypus</i>), white-tailed sea eagles (<i>Haliaeetus albicilla</i>), and Atlantic salmon (<i>Salmo salar</i>) from coastal areas of the Baltic Sea. PFOS was detected in all of the wildlife species analyzed. Concentrations of PFOS in blood decreased in order of bottlenose dolphins > bluefin tuna > swordfish. Mean PFOS concentrations (61 ng/g, wet wt) in cormorant livers collected from Sardinia Island in the Mediterranean Sea were less than the concentrations of PFOA (95 ng/g, wet wt). PFOS concentrations in cormorant livers were significantly correlated with those of PFOA. FOSA was found in 14 of 19 livers or blood samples of marine mammals from the Mediterranean Sea. The highest concentration of 878 ng FOSA/g, wet wt, was found in the liver of a common dolphin. Livers of ringed and gray seals from the Bothnian Bay in the Baltic Sea contained PFOS concentrations ranging from 130 to 1100 ng/g, wet wt. No relationships between PFOS concentrations and ages of ringed or gray seals were observed. Concentrations of PFOS in livers of seals were 2.7–5.5-fold greater than those in corresponding blood. A significant positive correlation existed between the PFOS concentrations in liver and blood, which indicates that blood can be used for nonlethal monitoring of PFOS. Trend analysis of PFOS concentrations in livers of white-tailed sea eagles collected from eastern Germany and Poland since 1979 indicated an increase in concentrations during the 1990s. Livers of Atlantic salmon did not contain quantifiable concentrations of any of the fluorochemicals monitored. PFOS is a widespread contaminant in wildlife from the Baltic and the Mediterranean Seas, while FOSA and PFOA were detected only in certain locations indicating their sporadic spatial distribution.</p>
Lopez-Berenguer et al., 2020	Stranded cetaceans warn of high perfluoroalkyl substance pollution in the western Mediterranean Sea	doi.org/10.1016/j.envpol.2020.115367	<p>Perfluoroalkyl substances (PFASs) are a class of organohalogenated compounds of environmental concern due to similar characteristics as the well-studied legacy persistent organic pollutants (POPs) that typically show environmental persistence, biomagnification and toxicity. Nevertheless, PFAS are still poorly regulated internationally and in many aspects poorly understood. Here, we studied liver and muscle concentrations in five cetacean species stranded at the southeastern coast of Spain during 2009–2018. Twelve of the fifteen targeted compounds were detected in >50% of the liver samples. Hepatic concentrations were significantly higher than those in muscle reflecting the particular toxicokinetics of these compounds. Bottlenose dolphins <i>Tursiops truncatus</i> showed the highest hepatic ΣPFAS (n = 5; 796.8 ± 709.0 ng g⁻¹ ww) concentrations, followed by striped dolphin <i>Stenella coeruleoalba</i> (n = 29; 259.5 ± 136.2 ng g⁻¹ ww), sperm whale</p>

			<p>Physeter macrocephalus (n = 1; 252.8 ng g⁻¹ ww), short-beaked common dolphin Delphinus delphis (n = 2; 240.3 ± 218.6 ng g⁻¹ ww) and Risso's dolphin Grampus griseus (n = 1; 78.7 ng g⁻¹ ww). These interspecies differences could be partially explained by habitat preferences, although they could generally not be related to trophic position or food chain proxied by stable N (δ¹⁵N) and C (δ¹³C) isotope values, respectively. PFAS profiles in all species showed a similar pattern of concentration prevalence in the order PFOS>PFOSA>PFNA≈PFUnA>PFDA. The higher number of samples available for striped dolphin allowed for evaluating their PFAS burden and profile in relation to the stranding year, stable isotope values, and biological variables including sex and length. However, we could only find links between δ¹⁵N and PFAS burdens in muscle tissue, and between stranding year and PFAS profile composition. Despite reductions in the manufacturing industry, these compounds still appear in high concentrations compared to more than two decades ago in the Mediterranean Sea and PFOS remains the dominating compound.</p>
Sciancalepore et al., 2021	Evaluation of per- and poly-fluorinated alkyl substances (PFAS) in livers of bottlenose dolphins (<i>Tursiops truncatus</i>) found stranded along the northern Adriatic Sea	doi.org/10.1016/j.envpol.2021.118186	<p>Per- and poly-fluorinated alkyl substances (PFAS) are a group of chemicals used in a wide variety of commercial products and industrial applications. These chemicals are persistent, can accumulate in humans' and animals' tissues and in the environment, representing an increasing concern due to their moderate to highly toxicity. Their global distribution, persistence and toxicity led to an urgent need to investigate bioaccumulation also in marine species. In 2013 PFAS contamination was detected in a vast area in Veneto region, mainly in Adige and Brenta rivers. In order to investigate any relevant presence of these substances in marine vertebrates constantly living in the area, PFAS were measured in hepatic tissue samples of 20 bottlenose dolphins (<i>Tursiops truncatus</i>) stranded along the northern Adriatic Sea coastline between 2008 and 2020. Using high performance liquid chromatography-mass spectrometry, 17 target PFAS (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTTrDA, PFTeDA, PFBS, PFHxS, PFOS, PFDS, PFHpS, PFPeS), were quantified in the samples. PFAS profiles were generally composed of the same five dominant PFAS (PFOS > PFUnA > PFDA ≈ PFDoA ≈ PFTTrDA). The greatest PFOS concentration found was 629,73 ng/g wet weight, and PFOS accounted until 71% in the PFAS profiles. No significant differences between sexes were found, while calves showing higher mean values than adults, possibly indicating an increasing ability in the elimination of PFAS with age. Finally, a temporal analysis was carried out considering three different periods of time, but no temporal differences in concentrations were found. The results suggest that long-chain PFAS are widespread in bottlenose dolphins along the North Adriatic Sea. Furthermore, they represent a baseline to investigate the impact of PFAS on marine mammals' conservation and health. Filling an important gap in the knowledge of PFAS accumulation in bottlenose dolphins, this study highlights the relevant role of Environmental and Tissue Banks for retrospective analyses on emergent contaminants.</p>
Heavy metals and trace elements			

Storelli and Marcotrigiano, 2000	Environmental contamination in bottlenose dolphin (<i>Tursiops truncatus</i>): relationship between levels of metals, methylmercury, and organochlorine compounds in an adult female, her neonate, and a calf	doi.org/10.1007/s001280000004	na
Shoham-Frider et al., 2002	Risso's dolphin (<i>Grampus griseus</i>) stranding on the coast of Israel (eastern Mediterranean).Autopsy results and trace metal concentrations	na	The stranding of Risso's dolphin (<i>Grampus griseus</i>) on the Mediterranean coast of Israel is reported in this study. High concentrations of trace metals (Hg, Cd, Zn, Fe and Se) were found in the various tissues analyzed, while Cu and Mn concentrations were naturally low.The specimen was found alive, but died a day later.The cause of death was attributed to bacterial bronchopneumonia in combination with endotoxemia, resulting in disseminated intravascular coagulation.Plastic bags found in its stomach contributed to the dolphin's poor physical condition. No connection was found between the high concentrations of trace metals in the internal organs and the cause of death.It is assumed that the high concentrations were a result of the high trophic level of this species, its diet and its advanced age. Anthropogenic influence could not be assessed due to the sparse database of trace metals for this species, in particular knowledge of the natural levels
Capelli et al., 2008	Distribution of trace elements in organs of six species of cetaceans from the Ligurian Sea (Mediterranean), and the relationship with stable carbon and nitrogen ratios	doi:10.1016/j.scitotenv.2007.10.036	Mercury (total and organic), cadmium, lead, copper, iron, manganese, selenium and zinc concentrations were measured in different organs of 6 different cetacean species stranded in an area of extraordinary ecological interest (Cetaceans' Sanctuary of the Mediterranean Sea) along the coast of the Ligurian Sea (North-West Mediterranean). Stable-isotopes ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) were also measured in the muscle. A significant relationship exists between $^{15}\text{N}/^{14}\text{N}$, mercury concentration and the trophic level. The distribution of essential and non-essential trace elements was studied on several organs, and a significant relationship between selenium and mercury, with a molar ratio close to 1, was found in the cetaceans' kidney, liver and spleen, regardless of their species. High selenium concentrations are generally associated with a low organic to total mercury ratio. While narrow ranges of concentrations were observed for essential elements in most organs, mercury and selenium concentrations are characterised by a wide range of variation. Bio-accumulation and bio-amplification processes in cetaceans can be better understood by comparing trace element concentrations with the stable-isotopes data.
Bellante et al., 2009	Trace elements and vanadium in tissues and organs of five species of cetaceans from Italian coasts	10.1080/02757540903193155	Trace element concentrations (chromium, copper, zinc, iron, manganese and vanadium) were determined in organs of five species of cetaceans (<i>Stenella coeruleoalba</i> , <i>Tursiops truncatus</i> , <i>Grampus griseus</i> , <i>Physeter macrocephalus</i> , <i>Ziphius cavirostris</i>) that were found stranded along Italian coasts in the period 2000– 2009. This dataset represents an important opportunity to verify and assess (particularly for V) patterns

			of incorporation of trace elements in different organs of cetaceans in a wide spectrum of species and related specimens distributed in all the age classes, and consequently determine the physiological and metabolic effects on the distribution modes of the same chemicals. In particular, Cu, Zn, and Fe accumulate preferentially in the liver of all studied specimens, while Mn and Cr values are found to be nearly constant in the analysed organs and tissues regardless of species. Comparable concentrations of trace elements, in different age classes, were measured for both specimens of <i>S. coeruleoalba</i> and <i>T. truncatus</i> (the most abundant dolphin species in the Mediterranean sea) in all analysed organs. On the other hand, unprecedented reported concentrations of V in tissues and organs of cetaceans from the Mediterranean show higher values when compared to levels measured in other marine mammals from the Atlantic Ocean.
Bellante et al., 2012	Stranded cetaceans as indicators of mercury pollution in the Mediterranean Sea	10.1080/11250003.2011.621072	Concentrations of Hg were determined in tissues (muscle, heart, kidney, lung and liver) of two cetacean species (<i>Stenella coeruleoalba</i> and <i>Tursiops truncatus</i>) stranded along the Italian coasts during the period 2000–2009 and compared with those previously reported by other authors from different areas of the Mediterranean basin. The highest concentrations of Hg were found in liver samples of both species (8.4–1752 mg · kg ⁻¹ dry weight for <i>S. coeruleoalba</i> and 9.6–1404 mg · kg ⁻¹ d.w. for <i>T. truncatus</i>). Statistically significant dissimilarities of Hg concentrations were recorded in muscle and liver tissues of <i>S. coeruleoalba</i> stranded in different geographical areas of the Mediterranean Sea as apparent direct response to uneven impacts of Hg pollution in the basin. A noteworthy outcome was that cetaceans stranded on the French coasts showed significantly higher levels of Hg contamination than those from other Mediterranean areas while the eastern part of the basin evidences the lowest Hg concentrations in the studied organisms. Moreover, the results of this study tentatively suggest the existence of different sub-populations of the two species in the basin.
Shoham-Frider et al., 2014	Trace elements in tissues of cetacean species rarely stranded along the Israeli Mediterranean coast	dx.doi.org/10.1016/j.marpolbul.2014.03.017	In this paper we present the concentrations of Hg, Cd, Se, Pb, Cu, Mn, Zn and Fe in organs of 6 non common specimens of cetaceans that were stranded along the Israeli Mediterranean coast (IMC), during 2002–2010: two fin whales, one minke whale, one Cuvier's beaked whale, one rough-toothed dolphin, and one Risso's dolphin. Most of the specimens were calves stranded by accident. Concentrations of Hg and Cd were low in tissues of the baleen whales and higher in the toothed whales, with maximum concentrations of 1067 mg kg ⁻¹ Hg in the liver of the Risso's dolphin and 29 mg kg ⁻¹ Cd in the kidney of the Cuvier's beaked whale. As far as we are aware, this is the first report of trace elements in baleen whales in the Eastern Mediterranean, and the first report of trace elements in minke whale and rough-toothed dolphin in the Mediterranean.
Martínez-Lopez et al., 2019	Trace metals in striped dolphins (<i>Stenella coeruleoalba</i>) stranded along the Murcia coastline,	doi.org/10.1016/j.chemosphere.2019.04.214	Mercury (Hg), cadmium (Cd), lead (Pb), selenium (Se) and arsenic (As) concentrations in internal tissues of 72 striped dolphins (<i>Stenella coeruleoalba</i>) from Murcia Region (Mediterranean coastline) have been investigated for the first time. Hg showed the highest concentration, followed by Se, Cd, As and Pb. In general, the levels of metal found in this study were similar to those described in similar studies in the Mediterranean

	Mediterranean Sea, during the period 2009-2015.		Sea. However, in some adult specimens, Hg liver concentrations were related with toxic effects in cetacean. A significant correlation was observed with age, likewise between Se and Hg and Cd in tissues, which agree with detoxify effect attributed to Se through inert complex formation. Molar ratio Hg:Se in liver was close to 1:1 in some specimens, which would indicate overload of the Hg-Se detoxify function and compromised health. These results could contribute to a better knowledge of the distribution of these persistent pollutants in the Mediterranean Sea.
Martinez-Lopez et al., 2019	Hg and Se in Organs of Three Cetacean Species from the Murcia Coastline (Mediterranean Sea)	doi.org/10.1007/s00128-019-02697-9	We determined Hg and Se concentrations in liver, kidney, brain, lung and muscle of five bottlenose dolphin (<i>Tursiops truncatus</i>), four common dolphins (<i>Delphinus delphis</i>) and four Risso's dolphin (<i>Grampus griseus</i>) stranded along the Murcia coast, Southeast Spain, in order to evaluate the risk of Hg toxicity. Hg concentrations showed similar concentrations to other individuals in the Mediterranean Sea with the same length in the same period. We observed a positive correlation of Hg and Se in liver ($r = 0.948$, $p < 0.001$) and kidney ($r = 0.939$; $p = 0.001$) and ratio the Se/Hg molar was higher than 1 in most cases. Our results suggest that the protective effects of Se against Hg toxicity occur in cetaceans. However, we detected levels of Hg described as responsible liver damage and neurotoxicological effects so other tools, as biochemical markers, should be included. Besides, more studies are needed to evaluate the risk of Hg exposure in dolphins from Murcia coastline.
Evtimova et al., 2019	Heavy metals in bones from Harbour Porpoises <i>Phocoena phocoena</i> from the Western Black Sea Coast	na	During the last few years, the Western Black Sea coast has documented increase in the number of stranded marine mammals, particularly the harbour porpoise (<i>Phocoena phocoena</i>). This species is a subject to threats such as exposure to contaminants, fishery by-catch and introduced new marine species. The aim of this study was to analyse spatial and age trends in bone metal concentration in harbour porpoises from the Western Black Sea Coast. Selected heavy metals (Cu, Pb, Zn, Cd and Ni) were measured in bones of 33 harbour porpoises stranded along the Bulgarian Black Sea Coast from 2017. Spatially, we found higher metal levels in the harbour porpoises stranded in the Northern region compared with those from Southern region. The effect of aging was evident only for Zn content – the levels were higher in juveniles than in adults. The obtained results suggested that heavy metal contamination represent an important threat encountered by harbour porpoises.
Polycyclic Aromatic Hydrocarbons			

Marsili et al., 2001	Polycyclic aromatic hydrocarbons (PAHs) in subcutaneous biopsies of Mediterranean cetaceans	doi.org/10.1016/s0045-6535(00)00206-x	The aim of the present study was to measure polycyclic aromatic hydrocarbon (PAH) levels in free-ranging Mediterranean cetaceans as they are likely to cause chemical stress in the organisms of this basin. Blubber samples were collected from live specimens of fin whales (<i>Balaenoptera physalus</i>) and striped dolphins (<i>Stenella coeruleoalba</i>) by means of biopsies, a non-destructive biological method. Fin whales were sampled in the Ligurian Sea, whereas striped dolphins were collected in the Ligurian and the Ionian Seas. A fingerprint of 14 PAHs was obtained for both species. In whales, the median value of total PAHs was 1970 ppb fresh weight (f.w.) while median carcinogenic PAH values were 89.80 ppb f.w.; in dolphins, the median values of total and carcinogenic PAHs were 29,500 and 676.00 ppb f.w., respectively. The different PAH values between the two species can be attributed to the different positions they take in the Mediterranean food web. The sampling period significantly influenced PAH concentrations of fin whales.
Fossi et al., 2010	A multi-trial diagnostic tool in fin whale (<i>Balaenoptera physalus</i>) skin biopsies of the Pelagos Sanctuary (Mediterranean Sea) and the Gulf of California (Mexico)	10.1016/j.marenvres.2009.10.006	The main objective of this study was to apply a set of sensitive non-lethal biomarkers in skin biopsies of fin whales (<i>Balaenoptera physalus</i>) to evaluate the toxicological status of this mysticete in the Pelagos Sanctuary (Mediterranean Sea) and in the Gulf of California (Sea of Cortez-Mexico). We developed a "multi-trial diagnostic tool" (based on field and in vitro studies), combining molecular biomarkers (western blot of CYP1A1, CYP2B) and gene expression (qRT-PCR of HSP70, ER α , AHR, E2F-1) with the analysis of OCs, PAHs and PBDEs. The study revealed a higher level of toxicological stress in the Mediterranean fin whales.
Marsili et al., 2014	Ecotoxicological status of seven sperm whales (<i>Physeter macrocephalus</i>) stranded along the Adriatic coast of Southern Italy	doi.org/10.1002/aqc.2447	In December 2009 seven male sperm whales (<i>Physeter macrocephalus</i>) stranded along the Adriatic coast of Southern Italy. Genetic and photo-identification data indicated that these specimens belonged to the Mediterranean Sea population. 2. Complete necropsies were performed on the three best preserved animals. Blubber and muscle samples were collected from all seven specimens for contaminants analysis (DDTs, PCBs and PAHs) and biomarker responses (CYP1A1 and CYP2B) were evaluated from the three better preserved animals using western blot analysis. 3. Fibroblast cell cultures from two specimens were set up by culturing cell lines from integument tissue, to explore the susceptibility of these marine mammals to different xenobiotic compounds. 4. The levels of CYP1A1 and CYP2B, proteins that are induced by some POPs (DDTs and PCBs) and PAHs were detected using the indirect immunofluorescence assay. The results showed relatively high levels of contaminants, in particular from organochlorine xenobiotics, and strong responses of CYP1A1 and CYP2B reflecting toxicological stress of these sperm whales. 5. This is not considered to be their cause of death, but may have contributed to lowering the defences of their immune system.
Phthalate esters			
Fossi et al., 2016	Fin whales and microplastics: The Mediterranean Sea and the Sea of Cortez scenarios	doi.org/10.1016/j.envpol.2015.11.022	The impact that microplastics have on baleen whales is a question that remains largely unexplored. This study examined the interaction between free-ranging fin whales (<i>Balaenoptera physalus</i>) and microplastics by comparing populations living in two semi-enclosed basins, the Mediterranean Sea and the Sea of Cortez (Gulf of California, Mexico). The

			<p>results indicate that a considerable abundance of microplastics and plastic additives exists in the neustonic samples from Pelagos Sanctuary of the Mediterranean Sea, and that pelagic areas containing high densities of microplastics overlap with whale feeding grounds, suggesting that whales are exposed to microplastics during foraging; this was confirmed by the observation of a temporal increase in toxicological stress in whales. Given the abundance of microplastics in the Mediterranean environment, along with the high concentrations of Persistent Bioaccumulative and Toxic (PBT) chemicals, plastic additives and biomarker responses detected in the biopsies of Mediterranean whales as compared to those in whales inhabiting the Sea of Cortez, we believe that exposure to microplastics because of direct ingestion and consumption of contaminated prey poses a major threat to the health of fin whales in the Mediterranean Sea.</p>
Baini et al., 2017	First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber	doi.org/10.1039/C6AY02674E	<p>Ingestion of plastics and microplastics by marine organisms may have physical and toxicological noxious effects creating a serious threat to marine species. There is a need to develop methods to evaluate the plastic exposure in free-ranging marine wildlife particularly related to microplastic ingestion. To this aim, seven phthalate esters (PAEs) were analysed, as plastic tracers, in neustonic/planktonic samples and skin biopsy samples of four cetacean species from the North-Western Mediterranean Sea. Among all phthalates, the highest frequency of detection was obtained from DEHP (78.9%), followed in descending order by MBzP (57.9%), MEHP and BBzP (both 52.6%), DIOIP (47.4%) and MBP and DNHP (both 42.1%). All 19 superficial tows analysed presented microplastic debris, confirming the widespread distribution of microplastics on the seawater surface in the Mediterranean Sea. The abundance of the smallest items (<0.5 mm) found in the samples was significantly correlated with MEHP, MBzP and BBzP, while the abundance of 0.5–1 mm and 2.5–5 mm items were related to BBzP and MBP, respectively. Regarding the PAEs, detected in the neustonic/planktonic samples correlated with microplastics (MEHP, MBzP, BBzP and MBP), appreciable levels of these compounds were found in all the four cetacean species. The present results represent a step forward in the application of a new non-invasive analytical method to evaluate PAEs as tracers of plastic exposure/ingestion in marine wildlife.</p>

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