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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 – DRAFT REPORT

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 - DRAFT REPORT

Presented by Cristina Fossi, Member of the Scientific Committee, Task Manager on Marine litter & chemical and biological pollution and Cristina Panti, Expert

Issue: bibliographic review on the impact of chemical pollution on cetaceans

1. Action requested

The Scientific Committee is invited to:

a) **Review** the draft report of the 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
b) Provide **advice** on its development.

2. Background

In 2019, the Italian Ministry of Environment provided a voluntary contribution to ACCCOBAMS in order to develop specific activities on marine litter and chemical pollution.

As part of the 2020-2022 ACCOBAMS Programme of Work, these activities aim:

- to identify potential hotspot areas of interactions between cetaceans and marine litter (ingested marine litter / microplastics / entanglements in ghost nets)
- to establish a state of the art on the impacts of chemical pollution on cetaceans and to develop guidelines on the best practices to assess the impact of chemical pollution on cetaceans with a focus on emerging contaminants.

The overall objective of these activities is to raise awareness of ACCOBAMS Parties on the impacts of marine litter and chemical pollution on cetaceans and to provide useful tools and guidelines to the scientists in the ACCOBAMS area to assess chemical contamination on cetaceans.

The following document is a first draft of the 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.

Once finalized the final draft will be circulated by email to the Scientific Committee Members before its presentation at the Eighth Meeting of the Parties to ACCOBAMS in November 2022.

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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

By

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Executive Summary

To be done once the document is completed

1. 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.

Objectives of the document

The main objectives of this document is to describe the results of a 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

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, PAHs, …). Coll et al. (2010) listed approximately 17,000 marine species; additionally, undescribed species will be added in the future. 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 coralliferous 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 basin, but only eight are consistently found in the basin when we exclude the Black Sea. The most common species are the striped dolphin (*Stenella coeruleoalba*), common bottlenose dolphin (*Tursiops truncatus*), short-beaked common dolphin (*Delphinus delphis*), sperm whale (*Physeter macrocephalus*), and fin whale (*Balaenoptera physalus*). Thus, these five species were used as case studies in this report (as previously reported by Marsili et al 2018).

Globally, as apex predators with long life spans, Cetaceans are particularly sensitive to contaminants, in particular to Heavy metals, PCBs, PBDEs, 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 Pelagos Sanctuary have shown that animals were highly contaminated by chemical pollutants (Marsili et al., 2018). Until recently, most research has been focused on PCBs, PBDEs, DDT and some trace elements (Hg). There is evidence indicating that some of these 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. Pesticides (including insecticides, herbicides and fungicides) based on current available compounds have never been investigated in these species, although marine contamination (Brumovsky et al. 2017, Salvat et al. 2016) and exposure of mammals (dugong/manatee) have already been described in several areas (Romero-Calderon et al. 2016, Hermanusen et al. 2008, Kunito et al. 2008, Harino et al. 2007). 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. 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.

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.

2. General methodology for bibliographic research

The first methodological step of this project consists in carrying out an accurate bibliographic research on the selected topic (hotspots of interactions between cetaceans and marine litter in the ACCOBAMS area) using the main research platform used for scientific publications (Scopus, PubMed, Google Scholar, etc.) and also grey literature (e.g. project's reports). This chapter will be supported by info-graphic material obtained from the data elaboration.

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 September 2021 are reported in Annex 1 with the indication of species investigated, the main classes of contaminants and the levels identified both in the tissues of stranded organisms and in skin biopsies.

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. ***61 papers*** *report studies on contaminants load and interaction with cetacean species in the Mediterranean area; the main results of the papers are reported below.*

Research to be implemented till June 2022 (also including the Black Sea)

Annex 1 – Excel file

Species	Ocean basin	Study Area	Year of Study	n°	sex	Conditions	Tissue	ΣPCBs (mg/kg lw)	ΣDDTs (mg/kg lw)	HCB (mg/kg lw)	H
Cuvier's beacked whale (*Ziphius cavirostris*)	Mediterranean Sea	Ligurian Sea	1999-2015	4	juvenile	free ranging	–	11.82 ± 4.68	–	–	
Cuvier's beacked whale (*Ziphius cavirostris*)	Mediterranean Sea	Ligurian Sea	1999-2015	8	sub adult	free ranging	–	25.11 ± 19.91	–	–	
Cuvier's beacked whale (*Ziphius cavirostris*)	Mediterranean Sea	Ligurian Sea	1999-2015	6	males	free ranging	–	28.17 ± 14.4	–	–	
Cuvier's beacked whale (*Ziphius cavirostris*)	Mediterranean Sea	Ligurian Sea	1999-2016	2	females	free ranging	–	0.49 ± 0.15	–	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Sapin	2009-2018	1	–	stranded	Liver	–	–	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Sapin	2009-2018	1	–	stranded	Muscle	–	–	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	North western sector	2003–2009	14	1 male and 13 U	12 free-ranging and 2 strandec	–	107.81 ± 108.72	115.98 ± 112.35	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Ligurian and Adriatic Sea	2008. 2009. 2016	9	All males	free ranging	–	6.420 ± 6.150	–	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	North western sector	2006-2013	61	47 males and 14 females	free ranging	–	22.849 ± 15.566	37.647 ± 38.518	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Adriatic Sea	2014	4	3 females and 1 foetus	–	Blubber	–	–	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Adriatic Sea	2014	4	3 females and 1 foetus	–	Muscle	–	–	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Adriatic Sea	2014	4	3 females and 1 foetus	–	Heart	–	–	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Adriatic Sea	2014	4	3 females and 1 foetus	–	Liver	–	–	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Adriatic Sea	2014	4	3 females and 1 foetus	–	Brain	–	–	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Adriatic Sea	2014	4	3 females and 1 foetus	–	Faeces	–	–	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Adriatic Sea	2014	4	3 females and 1 foetus	–	Umb. Cord	–	–	–	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Adriatic Sea	2009	7	males	stranded	Blubber	296,29841	1436,23468	4,62813	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Adriatic Sea	2009	7	males	stranded	Muscle	204,45835	220,13726	0,5562	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Adriatic Sea	2009	7	males	stranded	Liver	193,25467	196,398	0,54506	
Sperm whale (*Physeter macrocephalus*)	Mediterranean Sea	Ligurian Sea	2007-2009	2	–	free ranging	–	30.32 ug/g lw	21.7 ug/g lw	–	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	Ligurian Sea	2007-2009	12	–	free ranging	–	268.31 ug/g lw	224.22 ug/g lw	–	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	Ligurian Sea	1990	1	male	stranded	Melone	150765 ng/g dw	81354 ng/g dw	277 ng/g dw	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	Ligurian Sea	1990	1	male	stranded	Blubber	125025ng/g dw	58122 ng/g dw	143 ng/g dw	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	Ligurian Sea	1990	1	male	stranded	Muscle	4637 ng/g dw	1751 ng/g dw	11 ng/g dw	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	Ligurian Sea	1990	1	male	stranded	Liver	2967 ng/g dw	1594 ng/g dw	16 ng/g dw	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	Ligurian Sea	1990	1	male	stranded	Kidney	5932 ng/g dw	3243 ng/g dw	24 ng/g dw	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	Thyrrhenian Sea	1996	1	–	stranded	Muscle	–	–	–	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	Thyrrhenian Sea	1996	1	–	stranded	Liver	–	–	–	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	North western sector	2006-2013	49	26 males and 23 females	free ranging	–	38.666 ± 25.731	46.081 ± 37.506	–	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	All basin	1990-1992	1	male	stranded	Liver	–	–	–	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	–	2003–2009	5	? females, 1 male and 2 U	free-ranging	–	66.02 ± 57.91	–	–	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	France	1972-1977	14	–	stranded	Blubber	189 ± 298 mg/kg ww	94.2 mg/kg ww	–	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	France	1972-1977	11	–	stranded	Liver	18 ± 20.5 mg/kg ww	7.2 mg/kg ww	–	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	France	1972-1977	7	–	stranded	Kidney	31.1 ± 44.2 mg/kg ww	9 mg/kg ww	–	
Long-finned pilot whales (*Globicephala melas*)	Mediterranean Sea	France	1972-1977	16	–	stranded	Muscle	5.6 ± 7.5 mg/kg ww	2.8 mg/kg ww	–	

Evaluation of PCDD/Fs, dioxin-like PCBs and PBDEs in sperm whales from the Mediterranean Sea
A. Bartalini, J. Muñoz-Arnanz, L. Marsili, S. Mazzariol, M.C. Fossi, B. Jiménez
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 N 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 Σnon-ortho-dl-PCBs N Σortho-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.

Toothed whales in the northwestern Mediterranean: Insight into their feeding ecology using chemical tracers

Emilie Praca, Sophie Laran, Gilles Lepoint, Jean-Pierre Thomé, Antoni Quetglas, Paola Belcari, Paolo Sartor, Frank Dhermain, Denis Ody , Nathalie Tapie, Hélène Budzinski, Krishna Das

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 (d13C, d15N) and organochlorines. Significantly different d15N values were obtained in Risso's dolphins (11.7 ± 0.7‰), sperm whales (10.8 ± 0.3‰) and pilot whales (9.8 ± 0.3‰), revealing different trophic levels. These differences are presumably due to various proportions of Histioteuthidae cephalopods in each toothed whale's diet. Similar d13C contents between species indicated long-term habitat superposition or corroborated important seasonal migrations. Lower congener 180 concentrations (8.20 vs. 21.73 lg.g1 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.

Ecotoxicological status of seven sperm whales (Physeter macrocephalus) stranded along the Adriatic coast of Southern Italy

Marsili L., Maltese S., Coppola D., Carletti L., Mazzariol S., Fossi M.C.

In December 2009 seven male sperm whales (Physeter macrocephalus) 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.

POPs in free-ranging pilot whales, sperm whales and fin whales from the Mediterranean Sea: Influence of biological and ecological factors

Marianna Pinzone, Hélène Budzinski, Aurélie Tasciotti, Denis Ody, Gilles Lepoint, Joseph Schnitzler, George Scholl, Jean-Pierre Thomé, Nathalie Tapie, Gauthier Eppe, Krishna Das

The pilot whale Globicephala melas, the sperm whale Physeter macrocephalus, and the fin whale Balaenoptera physalus 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. δ13C, δ15N 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. δ15N values were 12.271.3‰ for sperm whales, 10.570.7‰ for pilot whales and 7.770.8‰ in fin whales, positioning sperm whales at higher trophic levels. δ13C of the two odontocetes was similar and amounted to 17.370.4‰ for sperm whales and 17.870.3‰ for pilot whales; whilst fin whales were more depleted (18.770.4‰). 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 ΣPCBs (38,666725,731 ng g1 lw and 22,849715,566 ng g1 lw respectively), ΣPBDEs (7127412 ng g1 lw and 3477173 ng g1 lw respectively) and ΣDDTs (46,081737,506 ng g1 lw and 37,647738,518 ng g1 lw respectively). Fin whales presented the lowest values, in accordance with its trophic position (ΣPCBs: 572175180 ng g1 lw, ΣPBDEs: 1777208 ng g1 lw and ΣDDTs: 664375549 ng g1 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 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 g1 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

Polybrominated Diphenylethers and Methoxylated Tetrabromodiphenylethers in Cetaceans from the Mediterranean Sea

A. Pettersson, B. van Bavel, M. Engwall, B. Jimenez

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.

Halogenated flame retardants in stranded sperm whales (Physeter macrocephalus) from the Mediterranean Sea

Zaccaroni, R. Andreini, S. Franzellitti, D. Barceló, E. Eljarrat

In recent years, decline of marine mammals' populations and increased frequency of strandings have arised 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

Organochlorine compounds levels in Cetaceans and Pinnipedia living along the french coasts

Cl. Alzieu, R. Duguy

The accumulation levels of PCB, DDT, DDE, and DDD were determinated 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 Physeteridae, 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, Globicephala melaena (840 mg/kg oflipids) and from striped dolphin, Stene/la coeruleoalba (833 mg/kg of lipids). ln general, the results indicated considerable vanat1ons in concentrations of PCB and DDE according to the diet, age and loca tion (Atlantic or Mediterranean) of the specimens examined. Tchtyophagous Odontoceti, and Stene/la coeruleoalba and Delphinu.1· delphis in particular, showed a higher degree of contamination than teuthophagous Odontoceti species. Lower Jevels of contamination were found in the Mysticeti ; this is most probably due to the low contamination of zooplancton, their basic food . As a general rule, immature specimens appeared to be more contaminated than ad ults. Organochlorine content in the fœtus was found to be important and was frequently at the same level as th<;t detected in maternai organs. There is no close relation between PCB leve! and pathological features of the organs, but certain observations suggest that the health of the dolphin may be endangered if PCB Jevel in the liver exceeds 20 mg/ kg of lyophilisated tissue.

Organochlorine levels in subcutaneous blubber biopsies of Fin whales (Balaenoptera physalus) and Striped dolphins (Stenella coeruleoalba) from the Mediterranean Sea

Letizia Marsili, Silvano Focardi

Polychlorinated biphenyls (PCBs) and DDT compounds were determined in subcutaneous blubber of fin whales (Balaenoptera physalus) and striped dolphins (Stenella coeruleoalba) from the Mediterranean Sea. From 1990 to 1993,

68fin 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.

Relationship between organchlorine contaminants and mixed function oxidase activity in skin biopsy specimens of Mediterranean fin whales (Baluenoplera physalus)

Marsili L., Fossi M.C., Notarbartolo di Sciara G., Zanardelli M., Nani B., Panigada S., Focardi S.

The relationship between organochlorine contaminants (PCBs and DDTs) and mixed function oxidase, benzo(a)pyrene monooxigenase activity (BPMO), was investigated in skin biopsy specimens born hn whales (Baluenoptera physalus) 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.

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

M. Cristina Fossi, Letizia Marsili, Giovanni Neri, Ada Natoli, Elena Politi, Simone Panigada

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 (Stenella coeruleoalba, Delphinus delphis, Tursiops truncatus and Balaenoptera physalus). 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, pp0 DDT, op0 DDT, PCBs and PCB99) in skin biopsies of males of B. physalus. Moreover a statistical correlation was also found between BPMO activity and DDT levels in skin biopsies of the endangered Mediterranean population of D. delphis. 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

Perfluorooctanesulfonate and Related Fluorinated Hydrocarbons in Marine Mammals, Fishes, and Birds from Coasts of the Baltic and the Mediterranean Seas

Kurunthachalam Kannan, Simonetta Corsolini, Jerzy Falandysz, Gunter Oehme, Silvano Focardi, And John P. Giesy

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 (Thunnus thynnus), swordfish (Xiphias gladius), common cormorants (Phalacrocorax carbo), bottlenose dolphins (Tursiops truncatus), striped dolphins (Stenella coeruleoalba), common dolphins (Delphinus delphi), fin whales (Balenoptera physalus), and long-finned pilot whales (Globicephala melas) from the Italian coast of the Mediterranean Sea and in livers of ringed seals (Phoca hispida), gray seals (Halichoerus grypus), white-tailed sea eagles (Haliaeetus albicilla), and Atlantic salmon (Salmo salar) 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.

Organochlorine compounds in common dolphins (Delphinus delphis)from the Atlantic and Mediterranean waters of Spain

Borrell, G. Cantos, T. Pastor, A. Aguilar

Blubber of free-ranging common dolphins (Delphinus delphis) from the northwestern coast of Spain (Atlantic), sampled in 1984and 1996, and of common dolphins entangled in fishing nets in the southwestern Mediterranean, sampled during 1992±1994, wasanalysed for organochlorine pollutants. In the Atlantic, concentration of all pollutants was significantly higher in males than infemales. The overall tPCB/tDDT ratio in this area was 3.35, which indicates a predominance of industrial inputs over those asso-ciated with agriculture. Individuals sampled in 1996 showed significantly lower DDT concentrations but a higher ppDDE/tDDTratio than those sampled in 1984, which re¯ects the aging of the environmental load. In the same period, tPCB concentrationremained constant and, as a consequence, the tPCB/tDDT ratio more than doubled. In the Mediterranean, the reduced sample sizeof 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 formsand a higher ppDDE/tDDT ratio than their counterparts from the Atlantic, but similar PCB concentrations. However, the relativefrequency of the di erent congeners in relation to the total PCB load was di erent in the two areas. This indicates that the twopopulations do not mix, at least in the short- or medium-term. Organochlorine levels in both areas are at the mid to low end of therange of concentrations detected in other common dolphin populations and in that of other Delphinidae species from the sameregion. Although the impact of the organochlorine concentrations on the common dolphin populations surveyed cannot be asses-sed, it is considered unlikely that they have played a signicant role in the decline that the species has suffered in recent decades in the western Mediterranean.

Differences in DDT and PCB Residues Between Common and Striped Dolphins from the Southwestern Mediterranean

A.Borrell, A. Aguilar

Organochlorine concentrations (OCs) and stable isotopes were investigated in the blubber of common dolphins (Delphinus delphis) and striped dolphins (Stenella coeruleoalba) 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 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.

Skin biopsy of Mediterranean cetaceans for the investigation of interspecies susceptibility to xenobiotic contaminants

M.C. Fossi, L. Marsili, G. Neri, S. Casini, G. Bearzi, E. Politi, M. Zanardelli, S. Panigada

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 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 (Stenella coeruleoalba), bottlenose dolphin (Tursiops truncatus), common dolphin (D. delphis) and fin whale (Balaenoptera physalus). 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 B. physalus. 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.

Organochlorine contaminant levels in tissues of a short-beaked common dolphin, Delphinus delphis, from northern Adriatic Sea

Lazar, B., Holcer, D., Mackelworth, P., Klini, D. & Herceg Romani, S.

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 (Delphinus delphis) 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.

Organochlorine contaminant and retinoid levels in blubber of common dolphins (Delphinus delphis) off northwestern Spain

Victoria Tornero, Assumpcio´ Borrell, Alex Aguilar, Jaume Forcada, Christina Lockyer

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 causeeeffect 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.

Cetacean Poxvirus in Two Striped Dolphins (Stenella coeruleoalba) Stranded on the Tyrrhenian Coast of Italy: Histopathological, Ultrastructural, Biomolecular, and Ecotoxicological Findings

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Tattoo skin disease (TSD) is a poxviral disease typical of cetaceans. Two juvenile and well-preserved male striped dolphins (Stenella coeruleoalba), 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.

PCB pollution continues to impact populations of orcas and other dolphins in European waters.

Jepson, P.D., Deaville, R., Barber, J.L., Aguilar, À., Borrell, A., Murphy, S., Barry, J., Brownlow, A., Barnett, J., Berrow, S., Cunningham, A.A., Davison, N.J., Ten Doeschate, M., Esteban, R., Ferreira, M., Foote, A.D., Genov, T., Giménez, J., Loveridge, J., Llavona, Á., Martin, V., Maxwell, D.L., Papachlimitzou, A., Penrose, R., Perkins, M.W., Smith, B., de Stephanis, R., Tregenza, N., Verborgh, P., Fernandez, A., Law, R.J.

Organochlorine (OC) pesticides and the more persistent polychlorinated biphenyls (PCBs) have wellestablished 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 (KWs) 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.

Halogenated Natural Products in Dolphins: Brain−Blubber Distribution and Comparison with Halogenated Flame Retardants

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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 ∑HNPs, ∑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.

The Pelagos Sanctuary for Mediterranean marine mammals: Marine Protected Area (MPA) or marine polluted area? The case study of the striped dolphin (Stenella coeruleoalba)

Maria Cristina Fossi, Cristina Panti, Letizia Marsili, Silvia Maltese, Giacomo Spinsanti, Silvia Casini, Ilaria Caliani, Stefania Gaspari, Juan Muñoz-Arnanz, Begoña Jimenez, Maria Grazia Finoia

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 (Stenella coeruleoalba) 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

Contamination by polychlorinated biphenyls (PCBs) in striped dolphins (Stenella coeruleoalba) from the Southeastern Mediterranean Sea

Maria Maddalena Storelli, Grazia Barone, Roberto Giacominelli-Stuffler, Giuseppe Onofrio Marcotrigiano

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 (Stenella coeruleoalba) 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 monoortho 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

PCBs and DDTs in Stenella coeruleoalba dolphins from the French Mediterranean coastal environment (2007–2009): Current state of contamination

Emmanuel Wafo, Véronique Risoul , Thérèse Schembri, Véronique Lagadec, Frank Dhermain, Chacha Mama, Henri Portugal

Organochlorinated compounds including PolyChloroBiphenyles, Dichloro-DiphenylTrichloroethan and metabolites are determinated in Stenella coeruleoalba (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 abondant, followed by total DDTs. The concentration (in ng g1 of lipid weight) in blubber of S. coeruleoalba, 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.

PCB and DDT levels do not appear to have enhanced the mortality of striped dolphins (Stenella coeruleoalba) in the 2007 Mediterranean epizootic

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In 2007, 17 years after the first reported Mediterranean epizootic of striped dolphins (Stenella coeruleoalba), 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

Chlorinated pesticides in the bodies of dolphins of the French Mediterranean coastal environment.

Wafo, E., Mama, C., Risoul, V., Schembri, T., Dhermain, F., Portugal, H., 2012a

PCBs and DDTs in *Stenella coeruleoalba* dolphins from the French Mediterranean coastal environment (2007–2009): current state of contamination

Wafo, E., Risoul, V., Schembri, T., Lagadec, V., Dhermain, F., Mama, C., Portugal, H., 2012b.

Accumulation and distribution of organochlorines (PCBs and DDTs) in various organs of Stenella coeruleoalba and a Tursiops truncatus from Mediterranean littoral environment (France)

Emmanuel Wafo, Luc Sarrazina, Catherine Dianaa, Frank Dhermainb, Therese Schembria, Veronique Lagadeca, Magali Pecchiaa, Pierre Rebouillon

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 Stenella coeruleoalba and a Tursiops truncatus collected in 2000 and 2003. The studies are achieved on the blubber, the heart, the liver, the kidney, the muscleand 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 Ag/kg lipid basis in the blubber, 601 and 39444 Ag/kg dried weight in the liver, 1375 and 34512 Ag/kg dried weight in the muscle, 3151 and 17082 Ag/kg dried weight in the heart, 674 and 12365 Ag/ kg dried weight in the kidney and finally between 648 and 4118 Ag/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 ppV-DDE/ADDT 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).

DDT and PCB reduction in the western Mediterranean from 1987 to 2002, as shown by levels in striped dolphins (Stenella coeruleoalba)

Aguilar, A. Borrell

Temporal trends in DDT and PCB contamination were recorded in the offshore waters of the western Mediterranean Sea during 1987–2002 using striped dolphins (Stenella coeruleoalba) 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 metabolisation 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.

Assessment of toxicological status of a SW Mediterranean segment population of striped dolphin (Stenella coeruleoalba) using skin biopsy

M. Cristina Fossi, Letizia Marsili, Giancarlo Lauriano, Caterina Fortuna, Simone Canese, Stefania Ancora, Claudio Leonzio, Teresa Romeo, Ruben Merino, Esteban Abad, Begona Jimenez

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 (Stenella coeruleoalba). 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.

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

Begona Jimenez And Maria Jose Gonzalez, Olga Jimenez, Sabine Reich, Ethel Eljarrat And Josep Rivera

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 OCCD 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

BIOACCUMULATION OF POLYCHLORINATED BIPHENYLS (PCBs) AND DICHLORODIPHENYLETHANE (DDE) METHYL SULFONES IN TISSUES OF SEAL AND DOLPHIN MORBILLIVIRUS EPIZOOTIC VICTIMS

G. M. Troisi, K. Haraguchi, D. S. Kaydo, M. Nyman, A. Aguilar, A. Borrell, U. Siebert & C. F. Mason

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 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 (Phoca vitulina) and striped dolphin (Stenella coeruleoalba) morbilli- virus 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 µg/ g lipid weight and in striped dolphins 0.05 and 0.01 µg/ g lipid weight. Mean lung con- centrations of PCB MSFs and DDE MSF in harbor seals were 0.96 and 0.02 µg/ g lipid weight and in striped dolphins 0.16 and 0.01 µg/ 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.

Congener Specific Determination and Enantiomeric Ratios of Chiral Polychlorinated Biphenyls in Striped Dolphins (Stenella coeruleoalba) from the Mediterranean Sea

Sabine Reich, Begona Jimenez, Letizia Marsili, Luis Manuel Hernandez, Volker Schurig, And Mariä Jose Gonzalez

Blubber and liver samples from six striped dolphins (Stenella coeruleoalba) 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.

Chlorinated hydrocarbon (HCB, DDTs AND PCBs) levels in cetaceans stranded along the Italian coasts: an overview
Marsili L. and Focardi S.
Concentrations of HCB, DDTs and PCBs in the tissues and organs of cetaceans (*Stenella coeruleoalba*, *Tursiops truncatus*, *Balaenoptera physalus*, *Steno bredanensis*, *Grampus griseus* and *Globicephala melaena*) 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.

Evaluation of toxicity and sex-related variation of PCB levels in Mediterranean striped dolphins affected by an epizootic.
Borrell, A., Aguilar, A., Corsolini, S., & Focardi, S
Individual PCB congener concentrations, including non-ortho chloro substituted, were determined in 30 striped dolphins (*Stenella coeruleoalba*) affected by the 1990–1992 Mediterranean epizootic to investigate their toxic potential. PCB congener concentrations in these dolphins were among the highest ever found in comparable studies on marine mammals. Concentrations in males and females were significantly different because of pollutant transfer to offspring by females. Thus, PCB concentrations and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxic equivalents (TEO) in males were approximately double those in females. Non-ortho, mono-ortho and di-ortho coplanar congeners accounted for approximately one third of the overall toxicity assessed through toxic equivalent factors (TEFs), as defined by Ahlborg *et al.* (1994). Di-ortho congener 170 and non- ortho congener 126 were the major contributors to TEQ (33% and 30% respectively).

Abnormally high polychlorinated biphenyl levels in striped dolphins (*SteneUa coeruleoalba)* affected by the 1990-1992 Mediterranean epizootic
Alex Aguilar, Asuncion Borrell
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.

Isomer-Specific Analysis and Toxic Evaluation of Polychlorinated Biphenyls in Striped Dolphins Affected by an Epizootic in the Western Mediterranean Sea
Kurunthachalam Kannan, Shinsuke Tanabe, Assumpcion Borrell, Alex Aguilar, Silvano Focardi, and Ryo Tatsukawa
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 morbiUivirus epizootic in the western Mediterranean in 1990. Extremely high concentrations of PCBs ranging from 94 to 670 Ixg/g (wet wt) were detected in the blubber. Similarly, DDT concentrations were high, between 22 and 230 ixg/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- PsCB), 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-PsCB (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.
Corsolini, S., Borrell, A., Focardi, S., 1995a. Toxicity assessment of polychlorinated biphenyl isomers including non-ortho coplanar PCBs in cetaceans from the Mediterranean Sea.

Congener profile and toxicity assessment of polychlorinated biphenyls in dolphins, sharks and tuna fish from Italian coastal waters.

Corsolini, S., Focardi, S., Kannan, K., Tanabe, S., Borrell, A., Tatsukawa, R., 1995b.

Bottlenose and Risso's dolphins found dead along the Italian coast in 1992 were analysed for the presence of PCB isomers and DDT. High concentrations of PCBs (90-1400 pg/g wet wt) were detected in the blubber of stranded carcasses. The concentrations were higher than those found in animals showing reproductive failure and physiological impairment following prolonged PCB exposure, suggesting that the contamination by PCBs as well as DDT may be a major causative factor for the large-scale deaths of dolphins in the Mediterranean Sea. The 2,3,7,8-TCDD toxic equivalents estimated for bottlenose and R&o's dolphins were 18.8 and 20.8 rig/g, respectively, with a major contribution fmrn mono-ortho PCBs. The monoortho congeners of IUPAC Nos 105, lI8 and 156 accounted for most of the toxicity exerted by PCBs in these dead dolphins. An increase in the proportion of non-ortho coplanar PCB congener of IUPAC No. 169 to No.126 with an increase in the total PCB concentration in the blubber suggested a strong induction of drug metabolizing enzymes. It is possible to use CB-169/CB-126 concentration ratio to indicate whether there has been a strong activation of cytochrome P450 enzyme system in severely exposed/contaminated dolphin populations. The total PCB concentrations in the adipose fat of sharks from Italian coasts ranged from 70 to 4000 rig/g wet wt and that of DDTfrom 14 to 300 nglg wet wt. In bluefin tuna, the total PCB and DDT concentrations were 170-2200 and 56-780 nglg wet wt, respectively. These values were comparable to those reportedfor the same species in the Mediterranean Sea during the 197Os, suggesting the existence of PCB sources near this marine ecosystem.

Differences in DDT and PCB residues between common and striped dolphins from the Southwestern Mediterranean

Borrell, A., Aguilar, A.

Organochlorine concentrations declined during 1987-2002 in western Mediterranean bottlenose dolphins, a coastal top predator

Borrell, A., Aguilar, A

We collected blubber samples from bottlenose dolphins stranded on Spain's Mediterranean coasts over a 25-year period (1978–2002).Samples were analyzed to determine time trends in levels of: HCB (hexachlorobenzene), PCB (polychlorinated biphenyls) and tDDT(dichlorodiphenyltrichloroethane and its metabolites). Overall, levels were high relative to other areas. This reflects both the ubiquityof organochlorine pollution in the western Mediterranean and the sampled species' coastal habit. There was a significant decline overthe study period in the concentrations of all the compounds analyzed. However, the DDE/tDDT, which is indicative of DDT ageing,significantly increased. This suggests there has been no significant use of HCB, DDT or PCB in the region for a long time. It also indi-cates that the pollutant loads in the environment are gradually being reduced; either by degradation or by migration of the compounds toother regions. A comparison with dolphin species that have an oceanic distribution suggests that PCB decline at a comparable pace incoastal and offshore water bodies. However, the decline of tDDT is faster near the coast.

Evaluation of toxicity and sex-related variation of PCB levels in Mediterranean striped dolphins affected by an epizootic.

Borrell, A., Aguilar, A., Corsolini, S., Focardi, S., 1996.

Organochlorine compounds in common dolphins (Delphinus delphis) from the Atlantic and Mediterranean waters of Spain.

Borrell, A., Cantos, G., Pastor, T., Aguilar, A

Accumulation and distribution of organochlorines (PCBs and DDTs) in various organs of *Stenella coeruleoalba* and a *Tursiops truncatus* from Mediterranean littoral environment (France).

Wafo, E., Sarrazin, L., Diana, C., Dhermain, F., Schembri, T., Lagadec, V., Rebouillon, P

The objective of the present study is to determine the levels of contamination by PCBs, DDT and its metabolites indolphins failed on the coasts of the Mediterranean sea. Samples are represented by sixStenella coeruleoalbaand aTursiopstruncatuscollected in 2000 and 2003. The studies are achieved on the blubber, the heart, the liver, the kidney, the muscleand the lung. The concentrations of PCBs and DDT are very high in all tissues and organs analyzed. For the PCBs, theconcentrations vary between 43,838 and 110,343Ag/kg lipid basis in the blubber, 601 and 39444Ag/kg dried weight in theliver, 1375 and 34512Ag/kg dried weight in the muscle, 3151 and 17082Ag/kg dried weight in the heart, 674 and 12365Ag/kg dried weight in the kidney and finally between 648 and 4118Ag/kg dried weight in the lung. These values are comparableto 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 thegender.In all the analyzed samples, the contents in PCBs are higher than those of DDT. The average ratios of ppV-DDE/ADDT areclose 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 andorgans which supposes the different animals were especially exposed to Pyralen-type compounds of transformer (Dp6).

Biological threats and environmental pollutants, a lethal mixture for mediterranean cetaceans?
Lauriano, G., Di Guardo, G., Marsili, L., Maltese, S., Fossi, M.C
The 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 Stenella coeruleoalba, Tursiops truncatus, Globicephala melas, Balaenoptera physalus and Physeter macrocephalus from the Mediterranean Sea. High blubber concentrations of polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) were recorded in free-ranging G. melas. Tissue concentrations of PCBs and DDT in stranded T. truncatus (367 lipid weight (l.w.) and 143.7 mg/kg l.w., respectively) and S. coeruleoalba (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 T. gondii infection was molecularly detected in three S. coeruleoalba and six T. truncatus.

Organochlorine contaminants in tissues of common bottlenose dolphins *Tursiops truncatus* from the northeastern part of the Adriatic Sea
Romanić, S.H., Holcer, D., Lazar, B., Klinčić, D., Mackelworth, P., Fortuna, C.M
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 (Tursiops truncatus) 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 (6PCB) 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 11,310ng g−1 wet weight were determined for PCB and DDT, 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

Environmental contamination in bottlenose dolphin (*Tursiops truncatus*): relationship between levels of metals, methylmercury, and organochlorine compounds in an adult female, her neonate, and a calf.
Storelli, M.M., Marcotrigiano, G.O
Bottlenose dolphin (Tursiops truncatus) occurs in all cold temperate to tropical seas worldwide. It appears to be common in the Mediterranean Sea. This species is primarily coastal in its distribution and it forms small groups of 10-100 individuals. These dolphins grow to a maximum length of ca 3 m, with a weight of around 300 kg and have a longevity of at least 30 years. They feed on a wide variety of fish available in their habitat, with a large fraction consisting of mullet. squid and octopus. Sexual maturity is attained between 5-12 years for females and from 10 years for males, According to estimates of the reproductive cycle, females give birth every two years. The pregnancy lasts 12 months while lactation lasts 12-18 months (Duguy & Robineau, 1982). These marine mammals, since they are inshore organisms. have been considered to be subject directly to pollution because coastal areas, though highly productive systems providing important nursing zones and habitats, are also the repository for toxic agents and hazardous materials from industrial. agricultural and urban sources such as heavy metals and organochlorine compounds. This, together with the consideration that dolphins are typical end-points in the biomagnification of persistent pollutants in the pelagic food chain led to many studies on the levels and effects of contaminants in marine mammals (Beck et al. 1997: Wood and Van Vleet 1996: Gauthier et al.. 1998). Since organochlorine compounds and methylmercury may be transferred from mother to offspring (Tanabe et al.. 1982: Ridgway and Reddy, 1995) it is-important to gather information for a particular species in different stages of life if possible. The present study reports the changes in concentrations of metals, methylmercury, polychlorinated biphenyls and organochlorinated pesticides in various tissues and organs of a female, her neonate and a calf of bottlenose dolphin (Tursiops truncatus) species.

Levels and congener pattern of polychlorinated biphenyls in the blubber of the Mediterranean bottlenose dolphins *Tursiops truncatus*
Storelli, M.M., Marcotrigiano, G.O
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 (Tursiops truncatus) 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.

Tissue-related polychlorinated biphenyls accumulation in Mediterranean cetaceans: assessment of toxicological status
Storelli, M.M., Barone, G., Piscitelli, G., Storelli, A., Marcotrigiano, G.O

Contamination by polychlorinated biphenyls (PCBs) in striped dolphins (*Stenella coeruleoalba*) from the Southeastern Mediterranean Sea.
Storelli, M.M., Barone, G., Giacominelli-Stuffler, R., Marcotrigiano, G.O

Persistent organochlorine pollutants and heavy metals in tissues of common bottlenose dolphin (*Tursiops truncatus*) from the Levantine
Shoham-Frider, E., Kress, N., Wynne, D., Scheinin, A., Roditi-Elsar, M., Kerem, D
DDT's, PCBs and heavy metals (HM) were measured in tissues of common bottlenose dolphins, collected along the Israeli Mediterranean coast during 2004–2006. RDDT 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 Mediterranea. We found relatively high DDE/RDDT 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.

The Gulf of Ambracia's common bottlenose dolphins, *Tursiops truncatus*: a highly dense and yet threatened population.
Gonzalvo, J., Lauriano, G., Hammond, P.S., Viaud-Martinez, K.A., Fossi, M.C., Natoli, A., Marsili, L.
The common bottlenose dolphin (Tursiops truncatus) 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 skinswabbing 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 where 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.

Linking organochlorine contaminants with demographic parameters in free-ranging common bottlenose dolphins from the northern Adriatic Sea
Genov, T., Jepson, P. D., Barber, J. L., Hace, A., Gaspari, S., Centrih, T., Leskak, J & Kotnjek, P
Marine top predators, including marine mammals, are known to bio-accumulate 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 (*Tursiops truncatus*) 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.

Organochlorine compounds and stable isotopes indicate bottlenose dolphin subpopulation structure around the Iberian Peninsula

Borrell, A. Aguilar, V. Tornero, M. Sequeira, G. Fernandez, S. Alıs

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 δ13C and δ15N 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 δ13C 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 δ 15N 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.

Fin whales and microplastics: The Mediterranean Sea and the Sea of Cortez scenarios

Maria Cristina Fossi, Letizia Marsili, Matteo Baini, Matteo Giannetti, Daniele Coppola, Cristiana Guerranti, Ilaria Caliani, Roberta Minutoli, Giancarlo Lauriano, Maria Grazia Finoia, Fabrizio Rubegni, Simone Panigada f , Martine Berube, Jorge Urban Ramírez, Cristina Panti

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 (Balaenoptera physalus) and microplastics by comparing populations living in two semi-enclosed basins, the Mediterranean Sea and the Sea of Cortez (Gulf of California, Mexico). The 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.

Temporal trends of halogenated and organophosphate contaminants in striped dolphins from the Mediterranean Sea

Òscar Aznar-Alemany, Berta Sala, Karl J. Jobst, Eric J. Reiner, Asunción Borrell, Àlex Aguilar, Ethel Eljarrat

PBDEs, HBCD, novel DBDPE, PBEB and HBB, dechloranes, OPFRs and natural MeO-PBDEs were monitored in muscle of striped dolphins (Stenella coeruleoalba) 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 ± 2642ng 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 < LOD. DBDPE concentrations decreased by 89% from 1990 to 2004–2009 and have remained stable since. HBB occurred rarely and decreased by 94% to a current few ngg−1 lw. Dec 602 was the main dechlorane with stable concentrations around 1200 ngg−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 ± 11,293ngg−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 700ngg−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 3200ngg−1 lw) in Mediterranean marine mammals for the first time.

Persistent contaminants in dolphin: an indication of chemical pollution in the Mediterranean Sea
N. Cardellicchio
Concentrations of metals (mercury and methylmercury, selenium, cadmium, lead), chlorinated pesticides and polychlorinated byphenyls(PCB)havebeen determined in tissuesand organs of Stenellacoeruleoalba dolphins beached along the Adriatic and Ionian coasts of Apulia (Southern Italy). The results obtained confirm that in Mediterranean dolphins the accumulation of contaminants is higher than in similarspecies living in the Atlantic. Therefore cetaceans can be considered "organisms indicating pollution". Accumulation is influenced by factors such as diet, age and sex, Reproduction plays a fundamental role in the elimination of toxic compounds in females. Correlation betweencontamination and death has not been found; however it is clear that pollutants at sub-lethal doses have neuro-toxic effects, increase stress and alter immunedefences, leavingthe organismat a greaterrisk fromdisease.

Insecticide pyrethroids in liver of striped dolphin from the Mediterranean Sea
Oscar Aznar-Alemanya , Joan Gimenez, Renaud de Stephanis, Ethel Eljarrat, Damia Barcelo
Pyrethroid pesticides were analysed in liver of striped dolphin (Stenella coeruleoalba) from the Alboran Sea (south of Spain, Mediterranean Sea). The occurrence and bioaccumulation of pyrethroid insecticides in marine mammal tissues from the northern hemisphere had never been determined before. Pyrethroids were detected in 87% of the specimens with a mean total concentration of 300 ng g-1 lw ± 932 (range 2.7e5200 ng g-1 lw). Permethrin and tetramethrin were the main contributors to the pyrethroid profiles, with enantiospecific accumulation for the first and isomer specific accumulation for the latter. Bioaccumulation of pyrethroids was unlike that of persistent organic pollutants (POPs), as pyrethroid concentrations were not correlated to the maturity stage of the specimens. Concentrations slightly increased from calves to juveniles, whereas juveniles presented similar concentrations to adults. Metabolization of pyrethroids after achieving sexual maturity might account for this pattern

Age, growth and organochlorines (HCB, DDTs and PCBs) in Mediterranean striped dolphins Stenella coeruleoalba stranded in 1988-1994 on the coasts of Italy
Letizia Marsili, Carolina Casini, Lucarini Andrea, Silvano Focardi
This study was based on data from 62 specimens of Stenella coeruleoalba found stranded along the ltallan coasts between 1988 and 1994 The age of each dolphln was determined by countlng dentine growth layer groups in the teeth and a growth curve was plotted Organochlorlne contanllnants (HCB DDTs and PCBs) were analysed In the blubber, hver, braln and muscle of 25 of the dolphins In all dolphlns and all tissues analysed, HCB concentrations (ng g ' dry weight) were the lowest and the PCBs were the highest, except in 1 blubber sample In which total DDTs welc higher than PCBs Correlations were sought between levels of these contaminants and age sex cause of death, locallty and year of stranding

Organochlorine Residues in Tissues of Striped Dolphins Affected by the 1990 Mediterranean Epizootic: Relationships with the Fatty Acid Composition
R. Guitart, X. Guerrero, A. M. Silvestre, J. M. Gutitrrez, R. Mateo
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 (Stenella coeruleoalba) 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 p,g g-1 wet wt); ~DDTs were also found at high concentrations (111 txg g-~ 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 EDDTs.

Stranded cetaceans warn of high perfluoroalkyl substance pollution in the western Mediterranean Sea
G. Lopez-Berenguer, R. Bossi, I. Eulaers, R. Dietz, J. Penalver, R. Schulz, J. Zubrod, C. Sonne, E. Martínez-Lopez
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 e2018. 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 Tursiops truncatus showed the highest hepatic SPFAS (n ¼ 5;

796.8 ± 709.0 ng g1 ww) concentrations, followed by striped dolphin Stenella coeruleoalba (n ¼ 29; 259.5 ± 136.2 ng g1 ww), sperm whale Physeter macrocephalus (n ¼ 1; 252.8 ng g1 ww), short-beaked common dolphin Delphinus delphis (n ¼ 2; 240.3 ± 218.6 ng g1 ww) and Risso's dolphin Grampus griseus (n ¼ 1; 78.7 ng g1 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 (d15N) and C (d13C) isotope values, respectively. PFAS profiles in all species showed a similar pattern of concentration prevalence in the order PFOS>PFOSA>PFNAzPFFUnA>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 d15N 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.

Polycyclic aromatic hydrocarbons (PAHs) in subcutaneousbiopsies of Mediterranean cetaceans

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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 werecollected from live specimens of fin whales Balaenoptera physalus) and striped dolphins Stenella coeruleoalba)bymeans of biopsies, a non-destructive biological method. Fin whales were sampled in the Ligurian Sea, whereas stripeddolphins were collected in the Ligurian and the Ionian Seas. A fingerprint of 14 PAHs was obtained for both species. Inwhales, 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., re-spectively. The different PAH values between the two species can be attributed to the different positions they take in theMediterranean food web. The sampling period significantly influenced PAH concentrations of finn whales.

Persistent Organochlorines and Organophosphorus Compounds and Heavy Elements in Common Whale (Balaenoptera physalus) from the Western Mediterranean Sea

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On March 1998, a female specimen of common whale (Balaenoptera physalus) 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 lipophylic 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 pp0 DDE, at lg gÿ1 level, as well as significant concentrations of As and Hg, which could a□ected the condition of the animal, and as a consequence, its survivor possibilities.

4. Main conclusion 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 previous paper, subdivided in the main classes of contaminants that impact the cetaceans of the Mediterranean and contiguous areas, considering in particular the differences between Odontocetes and Mysticetes.

Currently, one of the most comprehensive document on the topic is:

"Biological and toxicological contamination of cetaceans in the Pelagos Sanctuary: assessment, origin, monitoring and mitigation" by Jourdan J., Berny P., Grattarola C., Casalone C., Berio E., Keck N., Mignone W., Labach H. March 2020.

The outcomes of the aforementioned report on the Pelagos Sanctuary will be integrated in a comprehensive analysis of the literature presented in the previous chapters.

The final conclusions will be elaborated once the bibliographic research and analysis will be completed (June 2022).

Outline of the structure of the main conclusions.

4.1. Persistent organic pollutants (POP)

4.1.1. Chlorinated compounds

- **Most affected species**
- **Levels (concentrations)**
- **Area of concern**
- **Effects (if studies available)**
- **Knowledge gap**

4.1.2. Polycyclic aromatic hydrocarbons

- **Most affected species**
- **Levels (concentrations)**
- **Area of concern**
- **Effects (if studies available)**
- **Knowledge gap**

4.1.3. Organotin compounds

- **Most affected species**
- **Levels (concentrations)**
- **Area of concern**
- **Effects (if studies available)**
- **Knowledge gap**

4.1.4. Brominated compounds

- **Most affected species**
- **Levels (concentrations)**
- **Area of concern**
- **Effects (if studies available)**
- **Knowledge gap**

4.1.5. Perfluorinated compounds

- **Most affected species**
- **Levels (concentrations)**
- **Area of concern**
- **Effects (if studies available)**
- **Knowledge gap**

4.2. Heavy metals

- **Most affected species**
- **Levels (concentrations)**
- **Area of concern**
- **Effects (if studies available)**
- **Knowledge gap**

4.3. Plastic Additives

- **Most affected species**
- **Levels (concentrations)**
- **Area of concern**
- **Effects (if studies available)**
- **Knowledge gap**

4.4 Emerging contaminants

- **Most affected species**
- **Levels (concentrations)**
- **Area of concern**
- **Effects (if studies available)**
- **Knowledge gap**

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 has been funded in the last years (2010-today) or, if funded, results have not been made available yet nor though reports, papers or website. The research will continue till June 2022.

A preliminary overview of the relevant projects and initiatives that aimed at assessing chemical pollution on cetaceans in the ACCOBAMS area are listed in Table 2. 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 2. *Overview of the relevant projects and initiatives that aimed at assessing chemical pollution on cetaceans in the* ACCOBAMS *area (**to be finalized**)*

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	
PLASTIC Busters MPAs	Plastic Busters MPAs: preserving biodiversity from plastics In Mediterranean Marine Protected Areas	Interreg Med	monitoring & assessment - entire management cycle	
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	

6. Discussion and Conclusion

In this session we will briefly summarize the main conclusions obtained from the elaboration of the previous data in order to obtain a synthetic picture on the impact of contaminants on cetaceans in ACCOBAMS area.

This section will be elaborated in the final report

7. Recommendation for Future Work

In this last session, potential future research, mitigation action and governance interaction, to be implemented in the future in the ACCOBAMS area, will be suggested.

The main prelaminar recommendations are:

a) To develop a transboundary health monitoring network on cetaceans stranded in the ACCOBAMS area;

b) To evaluate the origin and the impact of chemical pollutants of terrestrial origin on cetacean health in the ACCOBAMS area;

c) To promote the mitigation of adequate biological and toxicological contamination in the ACCOBAMS area;

Recommendations will be fed from the ongoing IWC Workshop: “Pollution 2025 initiative: workshop on cumulative effects” (1-4 November 2021).

This section will be elaborated in the final report