



Accord Pelagos relatif à la création en Méditerranée
d'un Sanctuaire pour les mammifères marins

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di un Santuario per i mammiferi marini

2023 CALL FOR TECHNICAL AND SCIENTIFIC CONSULTANCY OF THE PELAGOS AGREEMENT

Final Administrative Report

June 2025



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General info:

Project title	Call 4 - PROTOCOLS AND ASSESSMENT OF PATHOLOGICAL EFFECTS, INCLUDING MORTALITY RESULTING FROM CHEMICAL AND BIOLOGICAL CONTAMINATION IN CETACEANS IN THE PELAGOS SANCTUARY
Consultant(s)	Dr. Letizia Marsili, University of Siena Dr. Cinzia Centelleghé, University of Padua Dr. H��l��ne Labach, Miraceti
Duration of the consultancy (beginning – end)	27/12/2023 – 30/06/2025
List of the deliverables submitted (number of the deliverable, title and date of submission)	<p>Deliverable 1. February 29th, 2024.</p> <p>Deliverable 2. Preliminary Technical Report. Bibliographic review on existing protocols to assess potential pathological effects on marine mammal species and relevant legislation at UNEP MAP, EU, RAMOGE, ACCOBAMS and national level. March 22nd, 2024.</p> <p>Deliverable 3. Joint protocol (FR, IT, MC) for long-term monitoring of chemical and biological contamination of marine mammals in the Pelagos Sanctuary and to assess links between pollutants and occurrence of pathologies. August 13th, 2024.</p> <p>Deliverable 4. Overall assessment of the relationship between the incidence of pollutants and any actual or potential impact of pollutants on individual species' health, including the link with any pathologies. April 22nd, 2025.</p> <p>Deliverable 5. Advisory fact sheet on the actual and potential impact of macro- and microplastics ingestion including relevant toxicological effects. April 22nd, 2025.</p> <p>Deliverable 6. Protocols and assessment of pathological effects, including mortality resulting from chemical and biological contamination in cetaceans in the Pelagos Sanctuary. Pelagos Agreement-funded project. Final Report. April 22nd, 2024.</p>

Abstract:

The Mediterranean Sea, and particularly the Pelagos Sanctuary, is subject to intense anthropogenic pressures, including maritime traffic, coastal urbanization, industrial discharges, and tourism, that contribute to elevated levels of chemical and biological pollution. As long-lived, high-trophic-level predators, cetaceans are particularly vulnerable to contaminant exposure and serve as effective sentinels of both ecosystem and human health. Increasing evidence links chronic pollutant exposure to adverse health effects such as immunosuppression, endocrine disruption, reproductive impairment, and increased disease susceptibility. Despite mounting scientific evidence, substantial knowledge gaps remain, particularly concerning the absence of harmonized, long-term monitoring frameworks on the regional scale. Research efforts have largely focused on legacy contaminants (e.g., PCBs, mercury), while emerging pollutants and biological stressors remain underexplored. Establishing causal links between contaminant burden and disease remains methodologically challenging, given the complexity of multiple stressor interactions, species-specific sensitivity, and the inherent difficulty of studying wide-ranging, long-lived, and legally protected species such as cetaceans.

In this context, the consultancy “Call 4 – Protocols and assessment of pathological effects, including mortality resulting from chemical and biological contamination in cetaceans in the Pelagos Sanctuary”, funded by the Permanent Secretariat of the Pelagos Agreement under the 2023 Call for Technical and Scientific Consultancy, aimed to address four key objectives: (i) A bibliographic review of existing protocols and relevant legislation concerning potential contaminant-related pathological effects in marine mammals; (ii) Development of a joint, cross-border protocol (France, Italy and Monaco) for long-term monitoring of chemical and biological pollution in cetaceans; (iii) An Assessment of the relationships between pollutant exposure and health outcomes in cetacean species; (iv) Production of an advisory fact sheet on the actual and potential impacts of macro- and microplastic ingestion.

Between January 2024 and April 2025, the consultants conducted an in-depth review of peer-reviewed scientific literature, validated monitoring protocols, and established best practices, alongside a thorough analysis of environmental regulatory frameworks at international, EU, regional, and national scales. The objective was to assess the risks associated with exposure to biological and chemical stressors and to evaluate the adequacy and effectiveness of current regulatory instruments for assessing, managing, and monitoring the long-term impacts of contaminants on cetacean populations within the Pelagos Sanctuary. The bibliographic and regulatory review revealed that, although major international agreements acknowledge pollution as a significant threat to cetacean populations, they lack standardized monitoring tools and clearly defined indicators for pollution and health. Despite their recognized role as sentinel species, cetaceans are rarely considered in pollution-related descriptors of key environmental policies, such as the Marine Strategy Framework Directive (MSFD) 2008/56/EC. This gap, combined with the lack of harmonized methodologies, underscores the urgent need for robust and integrated monitoring frameworks at both national and regional levels.

Based on the outcomes of the bibliographic and regulatory review, the consultants developed a harmonized cross-border protocol for the long-term monitoring of chemical and biological pollution in both stranded and free-ranging cetaceans within the Pelagos Sanctuary. The protocol draws on internationally recognized best practices, particularly the joint ACCOBAMS-ASCOBANS document “Best practice on cetacean post mortem investigation and tissue sampling” (IJsseldijk et al., 2019), and integrates procedures established by national stranding networks and relevant regional frameworks. The protocol standardizes methodologies for chemical, biological, and pathological analyses, incorporating advanced approaches such as biomarker-based diagnostics, omics technologies, and *in vitro* assays. It proposes a suite of candidate indicators for long-term monitoring, detailing target tissues, standardized reporting units, reference toxicity thresholds available in the scientific literature, and validated analytical

methods applied in the Mediterranean region. Chemical indicators include legacy pollutants, such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes, polybrominated diphenyl ethers (PBDEs), trace elements, selected for their toxicological relevance and consistency with international regulatory instruments (e.g., Stockholm Convention) and EU legislation (e.g., MSFD, WFD, REACH Regulation). Additionally, the protocol includes emerging contaminants of concern, such as plastic additives, per- and polyfluoroalkyl substances (PFAS) and halogenated natural products (HNPs), based on their bioaccumulative properties and potential adverse effects. For biological contamination, proposed indicators include emerging terrestrial pathogens identified in cetaceans within the Sanctuary (e.g., *T. gondii*, *Listeria* spp. and *E. rhusiopathiae*), highly pathogenic non-terrestrial agents with known cross-species transmission potential such as Cetacean Morbillivirus (CeMV), and understudied biological contaminants, such as harmful algal toxins, which may become increasingly relevant under future climate change scenarios. Finally, the protocol underscores the strategic role of tissue banks and stranding databases to enable retrospective analyses and support long-term epidemiological surveillance of pollution-related health risks in cetaceans.

Building on this framework, the third component of the project consisted in a comprehensive review of 60 peer-reviewed studies spanning nearly five decades of research (1973-2022) documenting contaminant levels in Mediterranean cetaceans. The literature review revealed substantial heterogeneity in the chemical compounds analyzed, biological matrices sampled, and analytical methodologies applied across studies, with limited integration between toxicological and pathological data. Despite these inconsistencies, widespread contamination was consistently reported in cetaceans, particularly odontocetes such as striped and bottlenose dolphins, with high levels of PCBs, mercury, and PBDEs. Elevated organochlorine concentrations were associated with CeMV infections and immune suppression, while mercury levels, especially in odontocetes, often exceeded thresholds for adverse effects, likely reflecting the so-called Mediterranean mercury anomaly. Although PBDEs were less frequently assessed, reported concentrations approached levels known to interfere with endocrine function. Emerging contaminants, including PFAS, plastic additives, and HNPs, were also detected, raising concern due to their persistence, bioaccumulative potential, and capacity to cross the blood-brain barrier, although their toxicological impact in cetaceans remains poorly characterized. In contrast, highly toxic substances such as polycyclic aromatic hydrocarbons and dioxins remain under-investigated, despite their known risks. While direct causal relationships between pollution and disease remain difficult to establish, the overall evidence supports a precautionary interpretation of chemical contamination as a significant risk factor for cetacean health. This highlights the importance of integrating toxicological and pathological data within coordinated, long-term monitoring frameworks, with cetaceans serving as key sentinels of ecosystem and human health.

Finally, the project produced a six-page outreach fact sheet summarizing current scientific knowledge on macro- and microplastic ingestion in cetaceans of the Pelagos Sanctuary. It describes exposure pathways, sublethal and lethal health effects, and interactions with other pollutants and pathogens, and provides recommendations for monitoring and mitigation in line with the “One Health” and “One Ocean” approaches.

This project identified key gaps in assessing pollution-related health risks in cetaceans within the Pelagos Sanctuary. These include the historical focus on a limited range of contaminants, methodological heterogeneity, limited integration between toxicological and pathological data, and the lack of standardized regional indicators for long-term monitoring. By addressing these challenges, the project laid the groundwork for a coordinated, cross-border strategy. Future priorities include implementing the joint long-term monitoring protocol, adopting harmonized indicators to ensure spatial and temporal comparability, integrating multidisciplinary data in post-mortem investigations, and enhancing inter-institutional collaboration and data sharing.