Document: ACCOBAMS-SC14/2021/Doc32
Distribution: 09/11/2021

# DRAFT GUIDELINES ON THE BEST PRACTICES TO ASSESS THE IMPACT OF CHEMICAL POLLUTION ON CETACEANS / TO MEASURE THE CHEMICAL CONTAMINATION ON CETACEANS

# DRAFT GUIDELINES ON THE BEST PRACTICES TO ASSESS THE IMPACT OF CHEMICAL POLLUTION ON CETACEANS / TO MEASURE THE CHEMICAL CONTAMINATION ON CETACEANS

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

**Issue**: guidelines on the best practices to assess the impact of chemical pollution on cetaceans

## 1. Action requested

The Scientific Committee is invited to:

a) **Review** the draft Guidelines on the best practices to assess the impact of chemical pollution on cetaceans
b) Provide **advice** on their 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 hotspots 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 guidelines on the best practices to assess the impact of chemical pollution on cetaceans with a focus on emerging contaminants.

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.

UNIVERSITÀ
DI SIENA
1240

**(DRAFT)**

# GUIDELINES ON THE BEST PRACTICES TO ASSESS THE IMPACT OF CHEMICAL POLLUTION ON CETACEANS / TO MEASURE THE CHEMICAL CONTAMINATION ON CETACEANS

*By*

*M. Cristina Fossi and Cristina Panti*

*University of Siena*

fossi@unisi.it, panti4@unisi.it

# Executive Summary

## Objectives of the document

*The main objectives of this document is to define a guidelines on the best practices to assess the impact of chemical pollution on cetaceans / to measure the chemical contamination on cetaceans in ACCOBAMS are*

# 1. Standardization of protocols for samples collection and analysis on chemicals contaminants

A common protocol for samples collection and preparation for toxicological analyses need to be established. Chemical analysis and any biomarker investigations can be carried out on two types of samples: stranded organisms and skin biopsies obtained through remote sampling (Fossi et al 2018).

## 1.1 Toxicology investigation in Stranded Cetaceans

In theory, all stranded cetaceans in a good state of conservation can be used to some extent in ecotoxicologic investigation (Godard and Fossi 2018). Skin as well as blood and all internal organs and tissues can be suitable materials for a wide range of analyses. Different sample matrices may be more suited to particular biomarker or residue analyses than others, depending on the contaminant or class of chemicals of interest, the time between death and sampling, and the sample storage condition. Geraci and Lounsbury (1993) and Mazzariol et al. (2011) thoroughly covered specimen and data collection in the case of marine mammal strandings. Whenever possible and before postmortem examinations, the stranded cetaceans need to be measured for total length and weighed. When weight measurement is not possible, the total length may be used to estimate weight according to formulas specific to the species investigated. Ideally, sample collection for all organs and tissues needs to be performed by necropsy-authorized personnel for later microscopic examinations (i.e., histopathology, immunohistochemistry, and ultrastructural investigations), as well as virologic and microbiologic investigations. Teeth and baleens can also be sampled for age determination (two teeth for each animal in odontocetes), while gastric contents can be collected during opening of the stomach complexes for the examination of organic, inorganic (including marine litter), and parasitic loads. Skin samples can be collected for genetic, stable isotope, fatty acid, hormone, biomarker, and chemical analyses, as well as cell and organotypic cultures. Most analyses are suitable with frozen storage conditions, but some require storage at various temperature or in various media, buffers, or solutions.

Relevant information on this section derived from the Joint ACCOBAMS and ASCOBANS document “Best practice on cetacean post mortem investigation and tissue sampling” (Lonneke L. IJsseldijk, Andrew C. Brownlow, Sandro Mazzariol, 2019).


**Figure 1.** Joint ACCOBAMS and ASCOBANS document "Best practice on cetacean post mortem investigation and tissue sampling" (Lonneke L. IJsseldijk, Andrew C. Brownlow, Sandro Mazzariol, 2019).

Monitoring dead stranded cetaceans offers an often unique opportunity to gain insights into the health of, and threats and stressors affecting, marine ecosystems (Gulland & Hall 2009; Van Bressem et al. 2009). Information derived from the systematic examination of stranded carcases can provide insights into the at-sea population not easily acquired through other means, indeed strandings data is the major source of information available for some species (Reyes et al. 1991; Pyenson 2011).

Despite uncertainties inherent in determining the stage of decomposition, carcase quality is an important determinant in subsequent analyses. Carcasses are assigned to one of five decomposition condition categories (**DCC**), determined by specific characteristics, as specified below.

**CODE 1: Extremely fresh carcass, just dead**
Characteristics: Usually live stranded and died/ euthanized cases or those stranded right after death; exhibiting no post mortem changes (e.g. no bloating or sloughing of skin); fresh smell; clear, glassy eyes; blubber firm and white; muscles firm, dark red, well-defined; viscera intact and well-defined; GIT contains no to little gas (unless pathologic); brain firm with no discolouration, surface features distinct, easily removed intact.

**CODE 2: Fresh carcass**
Characteristics: Normal appearance, fresh smell, minimal drying and wrinkling of skin, eyes and mucous membranes; carcass not bloated, tongue and penis not protruded; blubber firm and white, occasionally tinged with blood.

**CODE 3: Moderate decomposition.**
Characteristics: Bloating evident, with tongue and penis often distended; skin cracked and started sloughing; characteristic (mild) odour can be expected; mucous membranes dry, eyes sunken. Blubber blood-tinged and oily; muscles are softer and poorly defined; gut segments contain gas; brain has soft consistency. Organs are largely intact, still distinguishable and can be easily removed and assessed, although colour is more uniform throughout thoracic and abdominal cavity and consistency, particularly kidneys and pancreas is soft and increasingly friable.

**CODE 4: Advanced decomposition**
Characteristics: Carcass may be intact, but collapsed; skin sloughing; epidermis may be largely missing, exposing underlying blubber. Strong odour; blubber soft, often with pockets of gas and pooled oil; muscles nearly liquefied and easily torn, effortless separation from the bones; blood thin and black; viscera often identifiable but friable, easily torn, and difficult to dissect; gut gas-filled; brain liquified, dark red, containing gas pockets, with decreased consistency.

**CODE 5: Mummified or skeletal remains**
Characteristics: Skin may be draped over skeletal remains; any remaining tissues are desiccated. Organs partially or totally disappeared, or if present not completely identifiable.

Below is reported the table where the conservation level of the specimens is related to the different types of applicable investigations, including contaminant investigations and identification of the Marine Litter ingested.

**Table 1.** Recommendation for tissue sampling considering carcass DCC. Shading: green ***V*** indicates the process is of potential use in carcasses of the indicated DCC; grey (*V*)indicates that there may be limitations and red ***V*** indicates the procedure is not recommended/very unreliable, due to post mortem autolysis (From Best practice on cetacean post mortem investigation and tissue sampling" (Lonneke L. IJsseldijk, Andrew C. Brownlow, Sandro Mazzariol, 2019).

| Analytical procedure | DCC 1 | DCC 2 | DCC 3 | DCC 4 | DCC 5 | Comments/recommendations |
|---|---|---|---|---|---|---|
| Genetics | ✓ | ✓ | ✓ | ✓ | ✓ | For DCC4 or 5: paleopathological procedures may be required on account of degraded DNA (eg extracting DNA from bone medulla) |
| Diet and marine debris | ✓ | ✓ | ✓ | ✓ | (✓) | If GIT is not intact, eg from post mortem scavenger damage, results are compromised |
| Age determination | ✓ | ✓ | ✓ | ✓ | (✓) | |
| Fatty acids and stable isotopes | ✓ | ✓ | ✓ | ✓ | (✓) | Depending on analysis planned |
| Parasitology | ✓ | ✓ | ✓ | ✓ | (✓) | Depending on analysis planned |
| Morphometrics | ✓ | ✓ | ✓ | (✓) | (✓) | Girth measurements can be disrupted by bloating due to autolysis in DCC4-5 |
| Gross pathology | ✓ | ✓ | ✓ | (✓) | (✓) | Recommended for DCC4-5 in cases of forensic investigation |
| Reproductive studies | ✓ | ✓ | ✓ | (✓) | ✗ | |
| Toxicology | ✓ | ✓ | ✓ | (✓) | ✗ | Depending on pollutants. DCC1-2 for biomarker investigation. |
| Ear investigation | ✓ | ✓ | ✓ | ✗ | ✗ | Inner ear analysis specifically: DCC1, histopathology of fixed ears possible up to DCC3 |
| Microbiology | ✓ | ✓ | (✓) | (✓) | ✗ | Depending on analysis planned. For DCC3-4 microbiology can still be worthwhile for detection of certain bacteria and fungi using specific culture methods. Should a septicaemia be suspected in DCC3-4 animals, then microbiological investigations should be undertaken on the kidney, as this is resilient to microbial post mortem invasion using specific culture methods. |
| Histopathology | ✓ | ✓ | (✓) | (✓) | ✗ | Recommended for DCC4-5 in cases of forensic investigation |
| Virology | ✓ | ✓ | (✓) | ✗ | ✗ | Depending on analyses planned. |

| | | | | | | |
|---|---|---|---|---|---|---|
| Biotoxins | ✓ | ✓ | (✓) | ✗ | ✗ | |
| Gas bubble analysis | ✓ | ✓ | ✗ | ✗ | ✗ | If this procedure is conducted: it should be done first, before undertaking further assessments and dissections, particularly prior opening any part of the vascular system or removing the head. |
| Serology | ✓ | (✓) | (✓) | ✗ | ✗ | Advisable both on blood serum and on cerebro-spinal fluid, the latter of which should be collected as soon as possible. In heavily autolyzed specimens, alternatives are "juice" obtained from skeletal muscle or lung, vitreous humour or pericardial fluid |
| Clinical chemistry | ✓ | ✗ | ✗ | ✗ | ✗ | Vitreous humour is a possible option in decomposed cases . Care is needed however to ensure sufficient baseline data are available for the analyte in the species under investigation. |

### 1.1.1. Samples Collection

The choice of sampling for toxicological assessment should be made from the Stranding Task Force, operating in the ACCOBAMS area, in collaboration with the laboratory undertaking the processing of the samples. It is recommended to archive duplicate samples of blubber, muscle, liver, kidneys and brain for subsequent persistent organic pollutants (POPs), plastic additives, trace elements and/or fatty acids analysis. Due to the potential for traces of contaminants in the sample to adsorb or absorb onto plastic, and vice versa, tissues destined for POP analysis should only come into contact with stainless steel, aluminium, glass or Teflon. Samples are most conveniently wrapped in standard catering-grade aluminium foil (shiny side out; do not use recycled foil as this might contain plastic particles) before being stored in standard plastic containers

Milk samples should be collected from any lactating females and stored in a glass container prior to POP analysis. If the container has a plastic cap, it is recommended to cover the opening with aluminium foil (shiny side out) to prevent the sample from contacting the (plastic) cap for the reasons outlined above.

Samples of brain, muscle, liver and kidney can be sampled for trace element analysis. These samples should not come into contact with any metals other than stainless steel and thus can be stored in plastic containers. Bone and/or blood samples are also suitable for heavy metal analysis.

If a foetus is present but too small for full post mortem examination, the whole foetus and (parts of) its placenta can be wrapped in aluminium foil (shiny side out) for POP analysis. For foetuses that can be dissected, if is recommended to conduct a full post mortem investigation with individual tissue sampling.

*Storage:* The minimal size of samples for trace elements and organochlorine analysis is 10g of solid tissue or 10 ml for milk. Samples should be stored frozen at -20°C until analysis. For DCC1-2, samples should be stored at -80°C in liquid nitrogen or RNA later for Real-Time (RT) PCR for Biomarkers investigations.

## 1.2. Toxicology investigation in Free-Ranging Cetaceans

Several international institutions, such as the International Whaling Commission (IWC) have encouraged research on panels of sensitive nonlethal biomarkers, combined with analyses of persistent, bioaccumulative, and toxic (PBT) residues in skin biopsies of free-ranging animals. This approach can help define the health status of cetacean species with respect to multiple threats and supports IWC projects such as Pollution 2000+ and Pollution 2020. From an ecotoxicologic perspective, it is preferable to obtain samples from live

freeranging animals with collection methodologies that pose no or minimum disturbance. The most useful samples for nondestructive studies in cetaceans are skin biopsy specimens, obtained remotely by dart. The biopsy dart method has been used successfully on a range of cetacean species worldwide and is considered relatively benign (Noren and Mocklin, 2012). The response of cetaceans to skin biopsy collection is considered low in odontocetes and low to moderate in mysticetes, while wound healing appears to be rapid, with no apparent adverse health effects (Noren and Mocklin, 2012). Cetacean skin biopsies are suitable for hazard assessment of free-ranging cetaceans (Fossi et al., 1992, 2013, 2014, 2016, 2018; Godard et al., 2004; Godard-Codding et al., 2011). Various dart methodologies have been used successfully, as reviewed in Noren and Mocklin (2012). Skin biopsies (epidermis and dermis/ blubber) from free-ranging dolphins (such as *Tursiops truncatus, Stenella coeruleoalba*) can be obtained using an aluminum pole armed with biopsy tips (e.g., 0.7 cm ø, 3.0 cm length) or with a crossbow and darts. Skin biopsies from large odontocete (*Physeter macrocephalus*) or mysticete (such as *Balaenoptera physalus* or other baleen whale) (Fig.XX) species can be obtained with a crossbow or air gun and darts armed with tips (e.g., 0.9 cm ø, 4.0 cm length). Several models of crossbows (such as a Barnett Wildcat II crossbow with a 150-pound test bow), air guns, and darts (preferably untethered and with or without prongs of different angles in the tip) are available. To avoid the possibility of infection, the bolt tip needs to be sterilized before deployment. Biopsy samples can be taken between the dorsal fin and the upper part of the caudal peduncle upon approaching the animal at a suitabledistance and speed as specifically permitted for the species and research project. The skin biopsy needs to be stored immediately in the proper conditions required for intended analyses. Common storage conditions include frozen, as is, in liquid nitrogen, dry ice, or −80 and −20 C freezers or stored either cold or at room temperature in cell medium, buffer, or specific reagents. Skin biopsy is a powerful tool for ecotoxicologic studies for the following reasons: (1) it allows collection of a large number of samples across a wide geographic range; (2) it allows collection of sequential samples from the same animal if identified by photo identification or genetics; (3) it is suitable for residue analysis of many contaminants including dioxin-group chemicals (suitable for calculation of TEQs), other halogenated aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), plastic additives (phthalates), and heavy metals; (4) it is suitable for several biomarker analyses (see the next section) and cell and organ culture. A number of successful studies show that cetacean skin biopsies are a powerful nonlethal tool for assessing ecotoxicologic risk in marine mammals and aspects of feeding ecology and food preferences (Fig. 2).

**Figure 2.** Skin biopsy sampling in fin whale (*Balaenoptera physalus*) in the Mediterranean Sea. Remote sampling of integument biopsies from mysticete can be obtained using a crossbow anddarts armed with tips (0.9 cm ø, 4.0 cm length) (Godard and Fossi 2018).

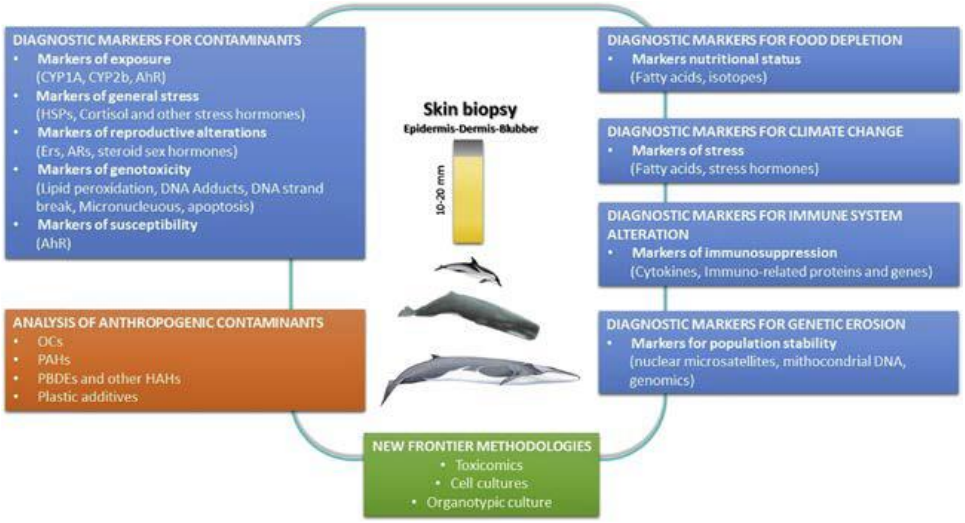


**Figure 3.** Biomarker and residue analyses relevant to the skin matrix.

## 1.3. A threefold monitoring approach to detect marine litter ingestion/related contaminants and toxicological impact in cetaceans

Given the multiple potential physical and ecotoxicological effects of marine litter ingestion, the impact of litter, and related contaminant, on marine organisms should be assessed using a threefold approach. The application of the threefold approach can elucidate not only the rate of ingestion in cetaceans, but also the multiple sublethal stresses that marine litter ingestion can cause in the short and long term. Each of the three investigation tools that make up the threefold approach can be applied independently or simultaneously using different methods according to the species and whether the animal is stranded or free ranging (Fig. 4).

The threefold approach comprises the following elements:
A) **Analysis of gastrointestinal content**: For stranded cetaceans, it is possible to detect the occurrence and rate of marine litter ingestion and any associated pathology through analysis of the gastrointestinal content, with a particular focus on plastics and microplastics.
B) **Analysis of the levels of plastic additives, as a proxy for ingestion**: An indirect approach can be used for free-ranging as well as stranded animals. The levels of plastic additives and associated PBT compounds can be measured to evaluate the exposure to marine plastic pollution.
C) **Analysis of biomarker responses**: Biomarker responses can be used to detect the potential toxicologic effect related to PBT and plastic additives related to plastic ingestion in free-ranging individuals or in stranded organisms up to a few hours after death.

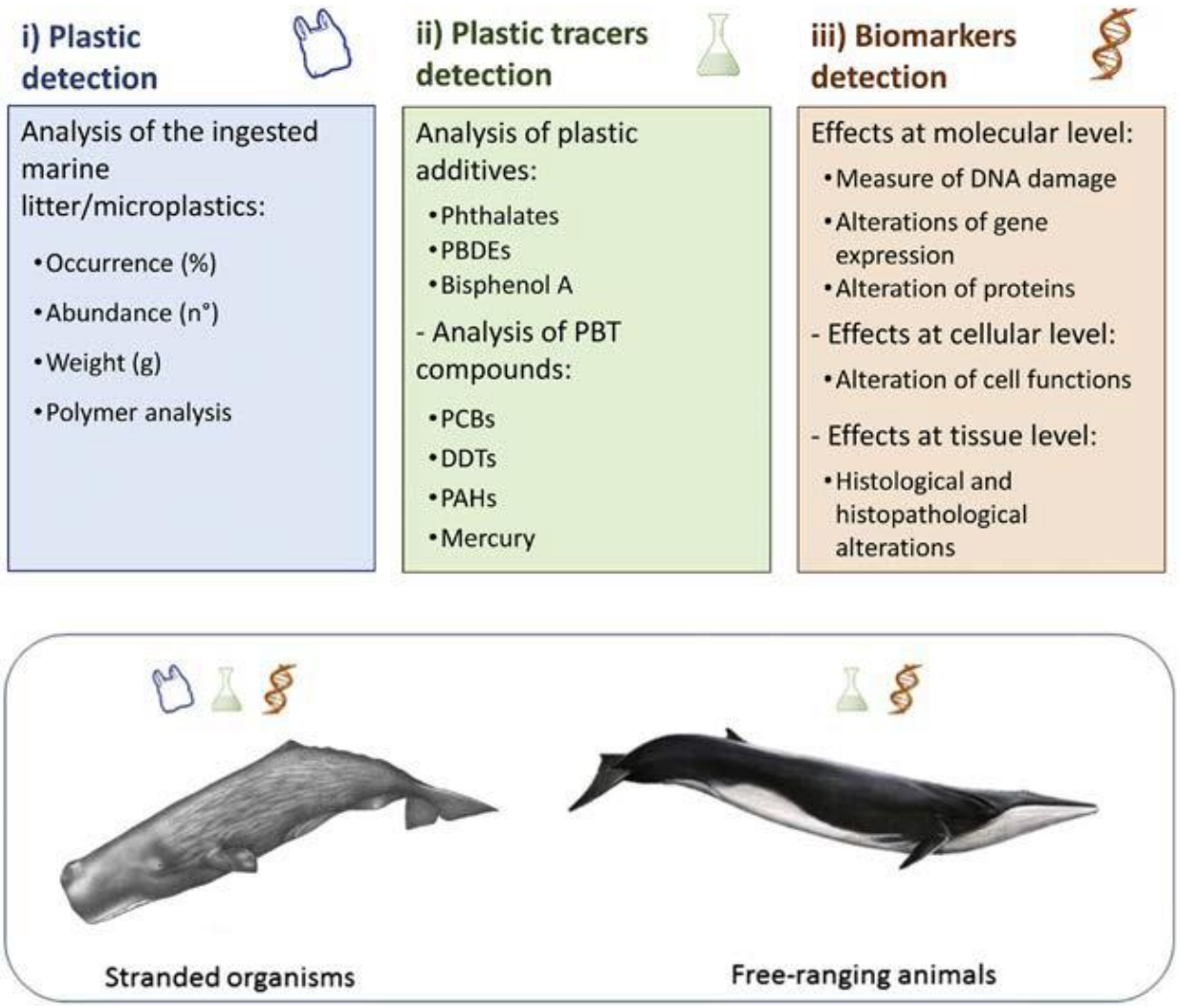


**Figure 4.** The threefold monitoring approach to detect marine litter presence and impact in cetacean species (stranded and free-ranging organisms). (Fossi et al 2018)

## 1.5. Synthetic workflows for:

- **Protocol for samples collection**
- **Analytical procedure of the samples for toxicological analyses**

*This section will be elaborated in the final report as figures and tables*