ACCOBAMS GUIDELINES

GUIDELINES CONCERNING BEST PRACTICE AND PROCEDURE FOR ADDRESSING CETACEAN MORTALITY EVENTS RELATED TO CHEMICAL, ACOUSTIC AND BIOLOGICAL POLLUTION

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1. GUIDELINES CONCERNING BEST PRACTICES AND PROCEDURES FOR ADDRESSING CETACEAN MORTALITY EVENTS RELATED TO CHEMICAL, ACOUSTIC AND BIOLOGICAL POLLUTION

[1] Document prepared by: Dr Marie-Françoise Van Bressem, Cetacean Conservation Medicine Group, CMED/CEPEC,Cra 74, 139-33, Bogota, Colombia

E-mail: mfb.cmed@gmail.com

1. GUIDELINES CONCERNING BEST PRACTICES AND PROCEDURES FOR ADDRESSING CETACEAN MORTALITY EVENTS RELATED TO CHEMICAL, ACOUSTIC AND BIOLOGICAL POLLUTION

1.1Role of chemical, biological and acoustic pollution in cetacean mortalities and diseases

1.1.1 Introduction

Since the detection of massive mortalities in seals (Osterhaus and Vedder, 1988) and dolphins (Domingo *et al*., 1990) in the last twenty years, diseases of marine mammals have gained growing attention. Several micro- and macro-parasites that may negatively influence population growth have been identified (Van Bressem *et al*., 2009) and the role of chemical pollutants in facilitating the emergence of morbillivirus epidemics has been thoroughly investigated (Aguilar and Borrel, 1994; Ross, 2002). Evidence suggests that polychlorinated biphenyls (PCBs) and related compounds might have contributed to the severity of morbillivirus outbreaks in seals and dolphins through toxicity at the level of the immune system (Aguilar and Borrel, 1994; Ross, 2002). More recently mid-frequency sonar operations induced cetacean mass-strandings in Europe, the US and Asia following decompression and gas and fat embolic syndrome (Jepson *et al*., 2003; Fernandez *et al*., 2005; Yang *et al*., 2008). Biological pollution is also of increased concern because of the findings of terrestrial pathogens in marine mammals, of a significant increased fecal coliform count in harbour seals (*Phoca vitulina*) living near urban developments and of cutaneous disorders of miscellaneous aetiology in coastal odontocetes (Mos *et al*., 2006; Van Bressem *et al*., 2007; Miller *et al*., 2008). Chemical and biological pollution will likely increase as a result of climate change (Boxall *et al*., 2009).

Below are summarized information on chemical, biological and acoustic pollution in cetaceans and their role in cetacean diseases and mortalities. A special insight is given into the effects of pollution in marine mammals from European waters, especially the Mediterranean Sea that receives persistent, organic contaminants from the most contaminated regions of the world (Lelieveld *et al*., 2002).

1.1.2. Chemical pollution

During the 20th century, the global environment became contaminated with several persistent, organic contaminants, commonly referred to as 'POPs'. Contamination has resulted from deliberate discharges and applications, as well as from the inadvertent formation of byproducts of incomplete combustion or industrial processes. Classes of these POPs include the organochlorine pesticides (*e.g.,* DDT, chlordane, toxaphene), the polyhalogenated-biphenyls (PHBs; including polychlorinated biphenyls PCBs), -dibenzo-*p*-dioxins (PHDDs; including polychlorinated dibenzo-*p*-dioxins PCDDs), -dibenzofurans (PHDFs; including polychlorinated dibenzofurans PCDFs), the polychlorinated naphthalenes (PCNs), carcinogenic polycyclic aromatic hydrocarbons (PAHs) and certain brominated flame-retardants. Several POPS have 'dioxin-like' properties, i.e. they bind to the Aryl hydrocarbon receptor (*Ah*R) and initiate toxic responses. POPs are fat-soluble chemicals and are resistant to metabolic breakdown, factors that result in their bioaccumulation in aquatic food chains and persistence in the environment (see Ross, 2002; Tabuchi *et al*., 2006).

Prey items from the freshwater and marine environment, and the terrestrial food chain are the main sources of these contaminants for marine mammals. POPs may accumulate in high concentrations, affect the reproductive, immune and endocrine systems and cause cancers (Reijnders, 1986; De Swart *et al.,* 1994; Ross *et al.,* 1996). High trophic level organisms are vulnerable to accumulating high concentrations of POPs, but considerable variation exists among species. For example, cetaceans appear to be able to metabolically eliminate many dioxin-like PCBs, PCDDs and PCDFs, but are prone to accumulating the nondioxin-like (or "globular") PCBs (Tanabe *et al.*, 1988; Kannan *et al.*, 1989).

Other problematic persistent chemical contaminants not included in the POP group include the organo-metallic compounds (chemical compounds that are used in anti-foulant paints) and methyl mercury (an organic form of mercury that is highly toxic) (reviewed in Ross and Birnbaum, 2003). Mediterranean cetaceans are exposed to a cocktail of toxic compounds, some time at very high concentrations, as indicated by the data compiled here below.

1.1.2.1 Polychlorinated biphenyls

PCBs are widespread in the environment. They bio-accumulate in wildlife occupying high trophic levels as a consequence of their chemical characteristics and persistence. Pinnipeds and cetaceans accumulate high levels of PCBs in their blubber because they are at the top of the food chain, have large lipid stores, have a long life span and a limited capacity for metabolism and excretion of compounds such as *p,p* –DDT and PCBs (Aguilar *et al*., 1999,2002; Ross *et al*., 2000). PCBs are immunotoxic causing thymus atrophy and reduced T-cell function through a common mechanism of action mediated by the cytoplasmic *Ah*R (Silkworth and Antrim, 1985; Kerkvliet *et al.*, 1990) that has been found in all mammals studied, including several marine mammal species (Hahn, 1998).

Studies carried out in seals that died during the 1988 seal epidemic and in the laboratory showed that: (1) ambient levels of environmental contaminants in the Baltic Sea herring were immunotoxic to harbor seals; (2) the pattern of effects implicated “dioxin-like” contaminants; (3) PCBs represented the major “dioxin-like” contaminant class; (4) many populations of free-ranging pinnipeds had PCB levels which exceeded those found to be immunotoxic in the captive study; and (5) environmental contaminants likely contributed to the severity of the 1988 PDV-associated mass mortality of harbor seals in northern Europe (Ross, 2002). Similarly, the striped dolphins (*Stenella coeruleoalba*) that died during the 1990-1992 epidemic had significantly higher loads of PCBs than the individuals that survived it. Given their well-known immunosuppressive effects, it was suggested that PCBs may have compromised the dolphin’s immune response and increased the severity of the outbreak (Aguilar and Borrell, 1994). Though the role of environmental contaminants in the 2007 morbillivirus epidemic in the Mediterranean remains inconclusive, recent pollutant data obtained through analyses of biopsies from apparently healthy striped dolphins in 1987-2002 suggested that PCB and DDT concentrations have gradually decreased (Aguilar and Borrell, 2005). Recent studies have demonstrated a significant association between chronic PCB exposure and infectious diseases in harbour porpoises (*Phocoena phocoena*) from the British Isles. Individuals that died in poor health had a significantly higher sum of the concentrations of 25 individual chlorobiphenyl congeners ($\sum$25CBs) than those that perished by traumatic death (Jepson *et al*., 2005a, Hall *et al*., 2006).

Altogether these data suggest that contaminant-related immunosuppression likely contributed to the severity of the 1988 phocine distemper virus outbreak in harbour seals and of the 1990-1992 dolphin morbillivirus epidemic and that they may increase susceptibility of porpoises to infectious diseases.

1.1.2.2. Brominated flame retardants

Brominated flame retardants (BFRs) are a diverse group of compounds that have been extensively applied to combustible materials, such as plastics, wood, paper, and textiles to meet fire safety regulations (Alaee *et al*., 2003; de Wit, 2002). Additive flame retardants, such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), are blended with the polymers and may leach out of the products (Alaee *et al*., 2003). Being environmentally persistent compounds resistant to physical and biochemical degradation and with high production volumes, PBDEs and HBCD are among the most abundant BFRs detected in the environment (Alaee *et al*., 2003). Initially the major commercial products, the penta- and octabromodiphenylether formulations were prohibited in all applications for the European Union Market in August 2004 (European Union 2003). The deca-mix product was also banned in Europe following a ruling by the European Court of Justice in 2008. HBCD and tetrabromobisphenol-A (TBBP-A) are however still widely used. PBDEs are similar in structure to thyroxine (T4) and triiodothyronine (T3) (Hamers *et al*., 2006). Biologic effects of PBDEs in rodents are similar to those of PCBs, with increased risks for reproductive and endocrine disruption and neurodevelopmental problems (Zhou *et al*., 2002; Siddiqi *et al*., 2006; Stoker *et al*., 2004; Kuriyama *et al*., 2005; Ellis-Hutchings *et al*., 2006; Lilienthal *et al*., 2006; Talsness, 2008). BFRs negatively affect the reproductive health, immune system and development in exposed mammals including pinnipeds and cetaceans (Law et al., 2002, 2003, 2006a; Ross, 2005). They have been detected in cetaceans from Europe, the United States and Asia (Isobe *et al*., 2007; Law *et al*., 2008, Johnston-Restrepo *et al*., 2008). Rising trends in the concentrations of HBCD in the blubber have been observed in harbour porpoises stranded or dying due to physical trauma along the coasts of Bristish Isles in 1994–2003 (Law *et al*., 2008). PBDEs have also been detected in Mediterranean Sea striped dolphins, bottlenose dolphins, Risso's dolphins, a long-finned pilot whale and a fin whale (Pettersson *et al*., 2004). The impact of these contaminants on Mediterranean cetaceans is poorly known and should be further investigated (Fossi *et al*., 2006).

1.1.2.3. Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a large class of molecules with condensed benzene rings. They are genotoxic and may induce cancers in humans and animals (Mastrangelo *et al*., 1996; Hakami *et al*., 2008; Topinka *et al*., 2008). Their lipophilic nature allows them to cross biological membranes and accumulate in organisms (Marsili *et al*., 2001). They are released in the environment by natural and man-made processes including combustion of wood and fossil fuels, oil plants and refineries and oil spills (Marsili *et al*., 2001). It has been estimated that an input of 635.000 tonnes of petroleum derived-hydrocarbons contaminates the Mediterranean each year (UNEP, 1988). Low molecular weight PAHs tend to remain in solution and are available to marine organisms through ingestion and respiration. Their solubility augments as temperature increases. These fat-soluble contaminants build up in fat and are mobilized with fat reserves during illnesses, reproduction and lactation and food scarcity (Marsili *et al*., 2001).

The contamination of the Saguenay River and immediate St. Lawrence estuary area by highly toxic PAHs such as the potent carcinogen benzo(a)pyrene (BaP) released massively by the local aluminum smelters over half a century and the exposure of belugas (*Delphinapterus leucas*) to these compounds were suggested as the most likely aetiology for a high prevalence of malignant tumours in belugas from the estuary (Ray *et al*., 1991; Martineau *et al*., 2002b). Total and carcinogenic PAHs were also detected in the subcutaneous blubber of fin whales (*Balaenoptera physalus*) and striped dolphins collected along the Italian coast of the Mediterranean Sea in 1993 and 1996, with naphthalene being the most ubiquitous compound (Marsili *et al*., 2001).

1.1.2.4. Perfluorinated compounds

Perfluorinated compounds (PFCs) refers to a group of man-made chemicals and their precursors, manufactured for their properties of providing resistance to heat, oil, and stains to products. Belonging to this group are subgroups of PFCs - perfluorinated carboxylic acids (PFCA) that includes perfluorooctanoic acid (PFOA) used as a polymerization aid in the manufacture of fluorinated polymers and elastomers; and perfluorinated alkyl sulfonates that includes perfuorooctane sulfonate (PFOS). Fluorotelomer alcohols are precursors to PFCAs. They are transformed in biota or in the atmosphere to produce PFCAs such as the extremely stable PFOA. They are persistent organic pollutants and are not known to degrade by any natural processes. PFCs and fluorotelomer alcohols are widely used in consumer product applications including lubricants, stain repellents (clothing and carpeting), food preparation (greaseproof packaging and non-stick cookware- Teflon), pharmaceuticals, insecticides and fire-fighting foams. They are ubiquitous and several of them have

adverse effects on neuroendocrine and reproductive systems, reduce neonatal survival, are carcinogenic and immunotoxic (DeWitt *et al*., 2008, 2009a,b).
General exposure to PFOS may occur through ingestion of contaminated fish and water, or with dermal contact with PFOS containing products and direct occupational exposure at workplaces where it is manufactured. PFOA is found in the blood of the general human population (Hansen *et al*., 2001; Nakayama *et al*., 2005). Concentrations of PFOS in animals from relatively more populated and industrialized regions, such as the North American Great Lakes, Baltic Sea, and Mediterranean Sea, were greater than those in animals from remote marine locations (Giesy and Kannan, 2001). PPFOS and PFOSA were found in cetaceans from around the globe including Japan, China, Brazil, the US and the Mediterranean (Kannan *et al*., 2001, 2002; Hart *et al*., 2008; Yeung *et al*., 2009). Transplacental transfer occurred at very high levels in at least two species (Dorneles *et al*., 2008; Hart *et al*., 2008). PFOS was the most predominant fluorochemical detected in the tissues of free-ranging Mediterranean odontocetes (short-beaked common dolphins *Delphinus delphis*, common bottlenose dolphins *Tursiops truncatus*, striped dolphins and long-finned pilot whales *Globicephala melas*) analyzed and in the blood of captive bottlenose dolphins fed mackerel and herrings caught in the Mediterranean and capelin from the North Sea. The greatest PFOS concentration was observed in the liver of a common dolphin (940 ng/g, wet wt) similar to those reported for dolphins from the Florida coast (Kannan *et al*., 2002).

A recent study in bottlenose dolphin epidermal cell cultures suggests that exposure to PFOS significantly alters normal gene expression patterns and causes a cellular stress response, a decreased cell cycle progression and cellular proliferation and reduced protein translation (Mollenhauer *et al*., 2009). Though no direct mortalities due to these compounds were reported their ubiquitous presence, high concentration in several species, maternal transfer and toxicity are cause for concern.

1.1.2.5. Heavy metals

Marine mammals accumulate high levels of mercury (Hg) and cadmium (Cd) (Wagemann and Muir, 1984; Aguilar *et al*., 1999). The natural occurrence of these elements in seawater has involved detoxification capacities to support elevated exposure to toxic metals in their environment (reviewed in Das *et al*., 2000). Cd can be stored over long periods in the kidneys of marine mammals (Lahaye *et al*., 2006). In odontocetes the demethylation of organic Hg occurs in the liver and leads to the production of non-toxic granules of tiemannite that are not excreted (Martoja and Berry, 1980). Since these granules are not excreted, inorganic Hg would be stored in the liver for the whole life resulting in elevated concentrations of Hg in this organ (Nigro and Leonzio, 1996; Lahaye *et al*., 2006). The immune system is susceptible to long-term mercury exposure. A reduced viability, metabolic activity as well as DNA and RNA synthesis were observed *in vitro* in stimulated lymphocytes from harbour seals following exposition to more than 1µM concentration of methylmercury (Das *et al*., 2008). In addition to immunosuppression, metal pollutants may induce immunoenhancement leading to hypersensitivity and autoimmunity **(**Kakuschke and Prange, 2007**).**

High Hg concentrations in harbour porpoises from the German Waters of the North and Baltic Seas were significantly associated with prevalence of parasitic infections and pneumonia (Siebert *et al*., 1999). The mean liver concentrations of Hg, Se, the Hg:Se molar ratio and Zn in harbor porpoises found dead along the coasts of the British Isles were significantly higher in those that died of infectious diseases than in those that died of a physical traumas (Bennett *et al*., 2001). Hg and Cd were also detected in the liver and kidneys of Mediterranean bottlenose dolphins and striped dolphins, respectively, at high concentrations in some individuals (Lahaye *et al*., 2006).

1.1.3. *Biological pollution*

Coastal ecosystems are continuously invaded by microorganisms from ballast waters, aquaculture waste and untreated run-off waters (Weber *et al*., 1994; Rhodes *et al*., 2000; Cabello, 2004, 2006; Drake *et al*., 2007). The discharge of water, sediments and biofilms from ships' ballast water tanks is a prominent vector of aquatic invasive species (Ruiz *et al*., 2000; Drake *et al*., 2007). The use in aquaculture of a wide variety of antibiotics in large amounts, including non-biodegradable antibiotics useful in human medicine, ensures that these remain in the aquatic environment, exerting their selective pressure for long periods of time. This has resulted in the emergence of antibiotic-resistant bacteria in aquaculture environments (including the Mediterranean Sea), in the increase of antibiotic resistance in fish pathogens and in alterations of the bacterial flora both in sediments and in the water column (Rigos *et al*., 2004; Cabello, 2006). Increasing water temperatures, a consequence of global warming, likely enhance the survival of some marine bacterial pathogens such as *Vibrio* spp. and increase exposure (Pascual *et al*., 2002). An increased pathogen exposure due to biological pollution has been detected in harbour seals inhabiting urban sites along the coast of Washington State and British Columbia (Mos *et al*., 2006). Biological contamination is also thought to have played a role in the emergence of miscellaneous skin diseases observed in cetaceans from the Americas and the Indian Ocean (Van Bressem *et al*., 2007; Flach *et al*., 2008; Kiszka *et al*., 2009).

1.1.4. *Acoustic pollution*

Cetaceans depend on sound to find food, communicate, detect predators and navigate. Escalating mechanized use of the sea, such as for shipping, military activities, oil and gas exploration and recreation, is increasing the amount of noise that humans introduce into the oceans, sometimes over very large distances. Anthropogenic underwater noise is a relatively novel environmental element for cetaceans and they may not be able to cope with it (Simmonds *et al*., 2004; Wright *et al*., 2007).

Powerful underwater sounds cause damage to the hearing systems, which can result in: (1) disorientation, (2) disconnection from school, pod or community, (3) internal bleeding; ruptured tissues, deafness and strandings as well as physiological harm. For example, exposure to an unexpected and unnatural loud noise could startle a deep-diving whale, causing it to bolt for the surface in a panic – such a rapid ascent could lead to bubbles forming in the tissues (a condition known in human divers as "the bends") and then to a stranding (Weilgart, 2007).

Anthropogenic sound sources vary in space and time but may be grouped into general categories: (1) explosions, (2) large commercial ships, (3) airguns and other seismic exploration devices, (4) military sonars, (5) navigation and depth-finding sonars, (6) research sound sources, (7) acoustic harassment devices (AHDs) and pingers, (8) polar icebreakers, (9) offshore drilling and other industrial activity, and (10) small ships, boats, and personal watercraft (Hildbrand, 2005). The following paragraphs summarize data on military sonars and seismic explorations.

1.1.4.1. Anthropogenic sonar signals

Sonar is an acronym for Sound Navigation and Ranging. A wide range of sonar systems is in use for both civilian and military applications. They intentionally create acoustic energy to probe the ocean. They can be categorized as low-frequency (<1 kHz), mid-frequency (1–20 kHz), and high-frequency (> 20 kHz). Low-frequency active (LFA) sonars are used for broadscale surveillance. Mid-frequency tactical antisubmarine warfare (ASW) sonars are designed to detect submarines over several tens of kilometers. They are incorporated into the hulls of submarine-hunting surface vessels (Hildbrand, 2005). All active sonars emit a noise pulse or "ping". These sound pulses bounce off a target (such as a submarine) and return as echoes that are detected by hydrophones.

Multiple mass strandings of beaked whales have been documented over the last decade following acoustic exposure to anthropogenic sounds, especially mid-frequency sonars, in Europe, the US and Asia (see Cox *et al*., 2006 for a review). These strandings affected Cuvier's beaked whale (*Ziphius cavirostris*), Blainville's beaked whale (*Mesoplodon densirostris*), northern bottlenose whale *Hyperoodon ampullatus* and Gervais' beaked whale *Mesoplodon europaeus* (see Cox *et al*., 2006 and Simmonds *et al*., 2004 for reviews). Affected whales had a condition called gas and fat embolic syndrome (GFES) characterized by extensive fat and gas bubble emboli, an ensemble of lesions most similar to decompression sickness (DCS) in human divers (Jepson *et al*., 2003, 2005b; Fernandez *et al*., 2005). The prevalent hypothesis is that GFES is induced through a precondition of tissue N2 supersaturation coupled with a behavioural response (increased or decreased surface interval, ascent rate, or dive duration, leading to increased supersaturation, thereby increasing DCS risk) to acoustic exposure (Jepson *et al*., 2003; Cox *et al*., 2006). Other suggestions include an acoustic signal that could (1) activate existing stabilized bubble nuclei allowing them to grow by passive diffusion, and/or (2) drive activated bubbles to expand through rectified diffusion (Cox *et al*., 2006). Each of these hypotheses assumed that these beaked whales live with significantly elevated blood and tissue tension N2 levels, a fact supported by a recent mathematical model (Hooker *et al*., 2009). In the Mediterranean strandings related to acoustic testing occurred in Greece in May 1996 (Frantzis, 1998).

1.1.4.2. Seismic surveys

Seismic airguns, used by the petroleum industry to detect pockets of oil or natural gas within the ocean floor and by researchers to locate sub-surface geological features, sound like underwater gun blasts and at times can be heard throughout entire ocean basins. Such impulsive sounds can be acutely harmful to nearby animals, but may also disturb (repeatedly startle) marine mammals to the point where they abandon important habitat (Nieukirk *et al*., 2004; Simmonds *et al*., 2004). The possibility that seismic noise can lead to strandings and/or death in marine mammals exists. Indeed, two Cuvier's beaked whales stranded in the Gulf of California in September 2002 coincidently with seismic reflections (Hildebrand, 2005). During the 2002 breeding season, three seismic surveys conducted in the Southern portion of Abrolhos Bank, Bahia and Espírito Santo States, Brazil may have been responsible for an increase in the strandings rate of adult humpback whales (*Megaptera novaeangliae*) (Engel *et al*., 2004). Hearing damage may also have indirectly killed humpback whales by compromising their navigation or sensory system (Todd *et al*., 1996).

1.2 Things to do in preparation for non-infectious unusual mortality events

Marine mammal strandings attract a lot of public attention. Several dolphins may beach over weeks along thousands of kilometres. The degree of response of each country will depend on the existence of active stranding networks and marine mammal research groups as well as on its economic and logistic possibilities. Some countries may be able to provide most of the scientific, technical and administrative infrastructure needed to face a massive stranding while others may only offer a more reduced support or none at all. Collaboration between Member States will be a plus to effectively attend these events. The foundation of an expert Sub-Committee on Cetacean Unusual Mortalities (CEUM) within the ACCOBAMS Scientific Committee would optimise the answer to die-offs in the Agreement Zone. The CEUM Sub-Committee should ideally have the equipment described in 1.2.2.1-1.2.2.3. Nevertheless, much can be done with a more reduced infrastructure and equipment (1.2.2.4).

1.2.1. Technical and administrative infrastructure needed in each Member State to best address emergencies caused by cetacean die-offs

All Member States should at least have an on-scene coordinator body (OSCB) that would contact the CEUM Sub-Committee and any other relevant institution in the case of a suspected mass-mortality, send data to the Mediterranean Database of Cetacean Strandings (MEDACES- http://medaces.uv.es/home_eng.htm), deal with the public and media, ensure that the proper samples are taken, be responsible to obtain all necessary permits and deal with the carcasses. The OSCB should ideally depend on an existing stranding network, a natural science museum, a university or a ministry (Agriculture, Environment, Fisheries). It should collaborate with existing national entities related to marine mammal stranding such as active stranding networks and marine mammal research groups, wildlife conservation and rescue centres, aquaria and oceanaria, coastguards, park officials and local authorities. It should also establish Memoranda of Agreement (MOA) with the Navy that could be directly involved in sonar activities as well as with Oil and Gas Companies involved in seismic surveys. Ideally, the Navy MOA should permit collaboration between the Naval Forces and the OSCB during stranding events possibly related to sonar activities by allowing the use of their planes, helicopters, boats and/or, trucks for transport of stranding responders or animals or assistance with aerial surveys to discern the extent of such an event. The MOA with the Oil and Gas Companies should facilitate access to OSCB marine mammal observers to their boats. The OSCB should also launch an agreement with universities or medical institutions willing to offer free tomographic examination of the cetacean's head stranded during acoustic operations and with universities or research institutes interested to collaborate on chemical and biological contamination. The OSCB should have all necessary addresses and phone numbers in the case of an emergency as well as a precise protocol to collect samples for research.

The OSCB basic technical and administrative infrastructure should include:

- A stranding hotline telephone, dedicated to record any stranding occurring along the coast and operating 24 hours, seven days a week;
- A computer with permanent internet access;
- A printer;
- Portable telephones;
- A GPS to register stranding locations;
- Digital cameras;
- DVD reader;
- Educative material;
- A specialized marine mammal library;
- A website describing the activities of the OSCB as well as the names of the persons in charge and to be contacted in the event of a die-off;
- A database on cetacean mortality events
- A centrifuge to spin blood samples;
- A large fridge to keep samples at 4°C;
- A –80°C freezer to store samples for longer periods of time.

1.2.2. Equipment list

The optimal and complete equipment list to face stranding of live and dead animals has been presented in another ACCOBAMS document (Van Bressem, 2009). A checklist for recording material, necropsy and sampling for chemical, acoustic and biological pollution is provided here below.

1.2.2.1. Recording material

- Waterproof pencils;
- Metal clipboards, waterproof labels;
- Data forms, necropsy and collection protocol forms;
- Camera and film, extra batteries, video camera with additional memory cards;
- Tape measure (metric), at least 20 meters long (plastic and metallic);
- Hoist/crane, scales to record organ weights (0,1-10kg);

1.2.2.2. Necropsy

- Rope, at least 20 meters, blankets, stretchers to move carcasses, if necessary;
- Gloves (non-powdered, vinyl)
- Necropsy instruments: multiple stainless steel scalpel handles, stainless steel scalpel blades, stainless steel scissors, stainless steel forceps forceps and knives;
- Stainless steel surgical scissors;
- Knife sharpener, if possible in secure pack;
- Stainless steel flensing knives and hooks with appropriate sharpening tools, chain saw, axe, or reciprocating saw to cut through the cranium, chest or vertebrae;
- Hammers, chisels and handsaws;
- Retractors of various sizes and shapes. Self-retaining retractors with one or two movable arms mounted on a slide bar are most useful;
- Sterile instruments for culture collection;
- Whirlpacks;
- Jars, vials;
- Buckets;
- Flashlights with extra batteries and light bulbs;
- Containers (from vials to garbage cans) for sample collection, including ice chest, dry ice and, if possible, liquid nitrogen;
- Gas generator and flood lights with extra bulbs and gasoline;
- Lights;
- Portable or electric circular saw;
- Accessible water supply with hose;
- Buckets;
- Garbage bags, dish soap, paper towels for clean-up.

1.2.2.3. Specific sampling (chemical, biologic and acoustic pollution)

- 10% neutral buffered formalin;
- 2.5% buffered glutaraldehyde and/or 4% paraformaldehyde (for transmission and scanning electron microscopy);
- Dimethyl sulfoxide (DMSO)
- methylene chloride or methanol
- Isopropanol alcohol for contaminant sampling;
- clean and sealed glass containers for contaminant sampling
- Teflon bags for contaminant sampling (precleaned)
- Needles and syringes;
- Heparinized syringes;
- ethylenediaminetetraacetic acid- and heparin-containing tubes
- Culture vials fro microbiology;
- Transport medium for microbiology and cell culture;
- Sterile swabs;
- Sterile urine cups;
- Glass slides;
- Serum tubes for blood and urine collection and gas burner to sear organ surfaces and sterilize scalpel blades;
- Coolers for samples refrigeration;
- Liquid nitrogen (if possible)

1.2.2.4. Minimal equipment

The following minimal equipment also permits to document the event and take valuable samples from freshly dead dolphins. In this case, all samples for toxicology should be large to allow further processing with stainless steel instruments.

- Recording material (waterproof pencils, metal clipboards, waterproof labels, data forms, necropsy and collection protocol forms);
- Camera;
- Mobile phone;
- Buckets;
- Water sprayer;
- Gloves, plastic boots and masks;
- Wide plastic sheets;
- Butcher knives;
- Butcher saws;
- Scalpel and scalpel blades;
- Vials and jars;
- Plastic bags (whirlpacks);
- Aluminium foils;
- Ropes.

1.3 Actions to take during non-infectious unusual mortality events

Several situations may occur during non-infectious unusual mortality events:
- Single stranded dolphins found dead or agonizing on different beaches;
- Several dead dolphins stranded together on the shore;
- Dead and live cetaceans stranded simultaneously on a beach.

In all cases, excellent coordination between the OSCB staff, the proposed CEUM Sub-Committee, other organizations specialised in these events and military institutions will be the key for a successful answer. The protocols given below are broadly based on Geraci and Lounsbury (2005). The second edition of '*Marine Mammal Ashore: A Field Guide for Strandings*' provides extensive information on how to deal with stranded, live or dead dolphins and whales and one or more copies should be in the library of all bodies involved with cetacean strandings. It would be wise to carry one copy to the field. Several papers cited in the present document are available online or upon request to the authors and would be worth to have in the library for more in-depth information.

1.3.1. Protocols for collection, transportation and storage of specimens and samples

1.3.1.1. Protocols for sample collection

Prior to sample collection, basic data should be collected in order to get crucial biological parameters. Recording the whale/dolphin condition is important to determine which samples should be given priority. Only the animals considered fresh or slightly decomposed are worth sampling for microbiology, toxicology and histopathology. All samples collected for microbiology and toxicology should be taken as aseptically as possible. The necropsy should be carried out by an experienced scientist. Notes should be taken by an assistant.

After collection of the basic data, the body should be opened, preferably on a wide plastic sheet or on a necropsy table. All instruments necessary for collecting biological samples such as bags, jars and vials with or without liquids should be clean, sterile and at hand before making the first incision. An assistant should label the containers and take notes and pictures.

Glass containers and Teflon bags are recommended for both organic compound and heavy metal analysis. Although glass containers should have a teflon-lined cap, foil-lined caps are acceptable for organic compound analysis. Sample jars should be cleaned with detergent, rinsed with tap water, soaked in 1:1 acid, rinsed with metal-free water, and rinsed again with high purity methylene chloride or methanol (PSEP 1989a,b). Containers should be kept capped and sealed after cleaning and prior to sample collection. Handling of containers should be kept to a minimum and the inside of the container should not be touched by anything other than the sample. Cross-contamination between tissues should be avoided. The scalpel and forceps should be cleaned after taking each sample. All tissue surfaces that come into contact with implements that were not cleaned (e.g., blubber when the body was opened) should be cut away with clean implements. The sample should not come into contact with the outside of the sampling container or the ground. When conditions are not ideal and sterility is not guarantee, remove a large slice (300-400 grs of the required tissue as hygienically as possible. Record whether the knife is ferrous or stainless or metal steel. The large samples may be collected in aluminium foil, plastic bags or buckets. They should be sealed, labelled with a waterproof pen, placed in a cooler with ice and transported to the laboratory quickly.

Skin samples for cell culture should be collected in culture medium with antibiotic and anti-fungi and kept on ice. They should be processed within 24h. These skin samples should be collected only in the case of an existing agreement with a university or research institute.

Small (1 cm^3) and representative samples of all organs and tissues from fresh cetaceans should be promptly fixed in 10% neutral buffered formalin solution for histopathology. The pancreas should be fixed as soon as possible, given the enhanced susceptibility of this organ to *post mortem* autolysis. The fixative containing the above tissue samples should be replaced with fresh formalin solution after 24 hours.

If there is suspicion of sonar-related stranding, if there is possibility to carry out tomography and if the specimens are fresh enough, the whole head should be collected and kept at on ice or in a 4°C till examination is carried out.

Samples for microbiology (skin lesions, blood, etc...) should be only taken from freshly dead cetaceans, collected in a sealed container previously cleaned and sterilized containing transport medium, identified and kept on ice or at 4°C. If laboratory tests are not planned within the next days, then freeze at –80°C.

1.3.1.1.1. Basic Data Protocol

- Investigator
    - Name:
    - telephone:
    - e-mail:
- Date:
- Location of stranding:
- Presence of other dead aquatic animals:
    - Species:
    - Number (estimation):
- Field number:
- Species[2]:
- Sex[3]:
- Standard body length[4]:

[2] Species identification should be done by qualified persons. Ideally a picture of each specimen with its field number should be taken.
[3] A picture of the genital region with field number will help to confirm the sex.

- Condition:
  - alive
  - fresh
  - early decomposition
  - advanced decomposition
  - mummified
- Fatness stage: fat, normal, thin, emaciated
- Indications for acoustic testing manoeuvres[5]:
  - presence of naval exercises YES/NO
  - number of boats:
  - distance from coast:
  - extension of the area:
  - frequency used, date and time of the exercises:
  - characteristic of the vessel (vessel length, speed and heading):
  - identify key characteristics of sound (e.g. frequency, amplitude, energy, directional transmission pattern, use of arrays vs. single sources, etc.)
  - characteristics of environmental parameters that may influence sound propagation
  - behaviour of cetaceans before stranding:
    - *continually circling or moving haphazardly in a tightly packed group – with or without a member occasionally breaking away and swimming towards the beach: YES/NO.*
    - *abnormal respiration including increased or decreased rate or volume of breathing, abnormal content or odour: YES/NO*
    - *presence of an individual or group of a species that has not historically been seen in a particular habitat, for example a pelagic species in a shallow bay when historic records indicate that it is a rare event: YES/NO.*
    - *abnormal behaviour for that species, such as abnormal surfacing or swimming pattern, listing, and abnormal appearance: YES/NO*
  - presence of external abnormalities (especially bleeding from the eyes and ears): YES/NO
    - Description - pictures
- Indication for an algal bloom: YES/NO
- Evidence for human interactions: YES/NO
  - Net marks
  - Knife cuts
  - Wounds caused by vessel strikes
  - Description-pictures

- Presence of skin lesions and wounds: YES/NO.
  - Description – pictures

[4] Precise how it was taken (measurements should be parallel to the dolphin body, e.g. total length from snout to fluke notch).

[5] This checklist should be filled by an assistant or an experienced volunteer while the principal researcher carries on with the rest of the protocol.

- Collect samples in 10% neutral buffered formalin solution, DMSO and, if possible, keep some unfixed samples at –80°C

- Lactating: YES/NO

1.3.1.1.2 Specific sample collection[6]

1.3.1.1.2.1. Reproductive tract

Ovaries and testes should always be examined, weighed, photographed and collected in 10% formalin (4% end concentration) to assess sexual maturity. The presence/absence of corpora albicantia and a corpus luteum should be recorded. Uterus should be opened to check for a foetus. The latter should be measured, weighed and sexed and, if small, conserved in formalin. Presence of sperm in the epidydimis should be evaluated. A piece of at least 1 cm^3 of both testes should be collected in formalin. The following questions may be answered in the field if time permits otherwise in the lab after addressing the mortality event.

- Ovaries:
  - presence of corpus albicans: NO, YES
  - presence of corpus luteum: YES, NO

- Foetus in uterus: YES, NO
  - sex
  - length
  - weight

- Testes: YES/NO
  - Right:
    presence of seminal fluid
    length
    weight
  - Left:
    presence of seminal fluid
    length
    weight

1.3.1.1.2.2. Biological pollution

- Document, describe and take pictures of any change in organ gross morphology.

- Collect cutaneous lesions and subcutaneous abscesses in 10% formalin (histology) and in containers with cell culture medium (microbiology);
- Collect 5-10grs samples from the kidneys, testes, uterus, placenta and foetus (if available), mammary glands and spleen, keep on ice and refrigerate at 4°C or freeze at –80°C if long delays are unavoidable (> 24h) before further analysis. When no freezing facilities are

[6] Basic and advanced data protocols are also available at the Medaces website: http://medaces.uv.es/home_eng.htm

available, smaller samples should be kept in DMSO. Preserve 1 cm^3 samples of the same organs in formalin.
- Collect pleural and peritoneal fluids, urine and pus from abscesses and store half in aerobic containers and half in anaerobic containers. Keep on ice and then freeze at –80°C if a laboratory is not at hand.
- Extract 5-10 ml blood directly from the heart or major blood vessels after disinfecting the surface with alcohol and put on ice. You may attempt to centrifuge the blood and take the supernatant before freezing to avoid further hemolysis;
- Collect water around the site of stranding (preferably before massive arrival of people) in a sterile container, seal and put on ice before freezing;

1.3.1.1.2.3. Chemical pollution

The following organs are useful to evaluate the burden of contaminants in cetaceans.
- Blubber: take a large sample (300-400 grs minimum) of blubber about 10 cm caudal to the blowhole or directly below the dorsal fin on the mid-lateral line, place in an aluminium foil, then in an sealed plastic bag with field number and store on ice;
- Skin: take a 10 cm^2 sample of clean skin, preserve in a container with culture medium containing antibiotics and anti-fungi, seal, identify and keep on ice;
- Liver: slice 300-400 grs from the caudal end of the liver, place in an aluminium foil, then in an sealed plastic bag with field number and place on ice;
- Kidney: take 500 grs of from the caudal end of the left kidney, place in an aluminium foil, then in an sealed plastic bag with field number and place on ice;
- Blood: collect 50 ml blood in a tube, seal, identify and keep on ice;

1.3.1.1.2.4. Acoustic pollution

With suspect sonar-related strandings, arrangements should be made for computerized tomography (CT) of the entire head or ears and close evaluation of the larynx should be undertaken for evidence of submucosal hemorrhage. Samples of peribullar adipose tissue should also be collected for histopathology. Tissues from all organs should be collected, if feasible.

- Live animal
  - blood
  - diagnostics such as auditory evoked potential (AEP) computerized tomography (CT) or ultrasound
  - rehabilitation
- Dead animal
  - When possible collect head for diagnostic imaging including CT/MRI scans or ultrasound of entire head;
  - Collect tissues (1 cm^3) from all organs and preserve in formalin 10%, with emphasis on the brain, peribullar adipose tissue, hypophysis, choroid plexus, cervical spinal cord, liver, lung, kidney, heart, lymph nodes, digestive tracts, reproductive tracts, and perilaryngeal tissues, including the trachea and thyroid and eyes. All sampled should be collected in separate bags (whirlpacks) and clearly identified.

1.3.2 Protocols for transportation and storage

Contact the local CITES Management Authority (http://www.cites.org/common/directy/e_directy.html) to know the requirements to obtain permits to export cetacean samples. Contact the laboratories that will analyse the samples and coordinate for sample dispatch according to the airline procedures. Make sure that somebody will collect the samples at their arrival and that the person in charge is not on holidays at the time you send the samples. Keep telephone and e-mail contact until you are assured that the samples arrived and were properly stored.

Microbiology: All fresh samples should be kept on ice or cold packs, away from the sun while waiting for further processing. Upon arrival in the laboratory, they should be kept at 4°C and immediately dispatched to the laboratory, if possible. If long delays are expected they should be frozen at –20°C or –80° C. Storage should be organized in a way that samples are easily found when the freezer is full. Records should be kept of any sample location.

Toxicology

Chemical analysis: samples en route to the analytical laboratory should be packed in dry ice. However, if delivery time is short (less than 6 hours, depending on ambient temperatures), then samples could be delivered in coolers filled with ice. All samples for toxicology should be stored in a freezer at –20°C or below until analysis. Storage time and temperature records should be recorded. The maximum holding times for tissues recommended by PSEP guidelines are 1 year for organics (with the exception of volatile organic compounds, which have a maximum holding time of 14 days), 28 days for mercury, and 2 years for all other metals. Samples held for longer periods may be suitable for analysis of some contaminants, but suitability should be evaluated based on the contaminants being tested and then described in a report presenting results for these samples.

Skin culture: skin samples to be used for cell culture should be maintained on cool packs and send as soon as possible to the laboratory. They should never be frozen nor left without ice.

Acoustic pollution

With suspect sonar related strandings, arrangements should be made for CT of the entire head or ears and close evaluation of the larynx should be undertaken for evidence of submucosal haemorrhage. Samples of peribullar adipose tissue should be collected for histopathology.

1.4Activities to implement after stranding

1.4.1. Debriefing meeting

Organize a debriefing meeting with all the people involved in the stranding and ask them their opinion on the event, the number of cetaceans they counted and attended, the presence of other dead aquatic animals on the beach, if live dolphins and whales were observed in waters close to the beach where the event happened, if the response to the stranding was adequate in their opinion, what material was missing. Thank all volunteers for their help and distribute any new information material and stickers. Speak with fishermen, members of the military and local people and ask if they have observed the occurrence of unusual species during the days preceding the stranding, if free-ranging cetaceans known to occur in the region exhibited an unusual behaviour, if military operations had taken place during the last days, or if there were reports of seismic surveys in neighbour waters.

1.4.2. Communication

1.4.2.1. Local government, Armed Forces, Ministry of External Affairs, Ministry of Environment and Ministry of Health

Call or write the local government, the Ministries o Health and Environment as well as the Navy and the Oil and Gas Companies if there are strong indications for strandings related to acoustic pollution.

1.4.2.2. Scientists

E-mail or call scientists that have signed a MOA. Ask for their comments and help. Send data to the Mediterranean Database of Cetacean Strandings.
(MEDACES- http://medaces.uv.es/home_eng.htm).

1.4.2.3. Press

Write a brief note on the event for the media. Alert the media and public for the possibility of more cetacean strandings on every beach and encourage them to report.

1.4.3. Preliminary report

Write an initial report as soon as possible. Points to summarize in the report should include the following (Geraci and Lounsbury, 2005):

- Date and location of the stranding
- Type of beach;
- Nature, timing, effectiveness of the initial response;
- Account of the scene as described by the team:
    - species involved and number of specimens per species,
    - pattern of stranding,
    - presence of other dead or sick aquatic animals,
    - presence of live cetaceans exhibiting an unusual behaviour in adjacent waters,
    - evidence for the use of mid-frequency sonar,
    - cetacean condition,
    - indication for an epidemic,
    - environmental conditions.
- Necropsy findings;
- Specimens collected, place where they are stored, condition for storage;
- Actions taken and reason for decisions:
    - intended response plan,
    - impediments to implementation,
    - eventual action.
- Additional information:
    - photographs, maps, drawings,
    - reports from independent groups (police, coastguards, stranding networks, rehabilitation facility, Navy, fishermen),
    - Things to be improved.

1.4.4. Follow-up

Ask for a follow-up of the analysis and prepare a manuscript on the findings together with all involved institutions.

2. CONTINGENCY PLAN DRAFT

Cetaceans from the Mediterranean harbour a cocktail of chemical, toxic pollutants, some likely to have increased the severity of disease epidemics. Mid-frequency sonar operations have caused the stranding of beaked whales in Greece (Frantzis, 1998). Biological contamination is of concern because of the release of untreated freshwater run-off, aquaculture, maritime traffic and discharge of ballast waters in Mediterranean waters. Thus, Member States should be ready for the eventuality of cetacean strandings, diseases and mortalities related to these agents. The development and strengthening of existing national and regional stranding networks will be key to properly address these events. Importantly, data on strandings along the coasts of the Black and Mediterranean Sea as well as the contiguous Atlantic waters should be sent to MEDACES (http://medaces.uv.es/home_eng.htm) set-up in 2001 to co-ordinate all national and regional efforts for riparian countries. The establishment of a CEUM Sub-Committee within the ACCOBAMS Scientific Committee would further improve answer to strandings by facilitating coordination between Member States and helping with infrastructure and capacity building. The foundation of CEUM Working Group that would communicate by e-mail would facilitate information diffusion. Memoranda of Agreement with the Naval Forces as well as with Oil and Gas Companies would improve answer to cetacean die-offs related to acoustic pollution.

2.1. OSCB

An efficient contingency plan will be based on the foundation of a national OSCB that will be responsible for the activities and decisions related to unusual mortality events as well as on timely relaying information on their occurrence to the Member States and to the suggested CEUM Sub-Committee. The easy and open communication between OSCBs will help determine when a die-off is underway, ensure a timely and adequate intervention and, ultimately, uncover the cause of the die-off and explore environmental factors that may have enhanced its severity. Minimal personal of an OSCB should be one scientist, preferably a marine mammal research veterinarian with good knowledge in the biology of cetaceans and of the different factors involved in cetacean strandings.

2.1.1. Administrative support team

At least one person should be in charge of the administration of the OSCB. His/her responsibilities should include:

- Coordination with local authorities;
- Coordination with the Naval Forces and Oil and Gas Companies;
- Contact with the authorities that will deliver CITES permits;
- Contact with the airlines that will transport the samples: ask for their specific requirements for the packaging and dispatch of biological materials;
- Communication with media and public;
- Development of education activities and material;
- Management of volunteers;
- Building of a website;
- Finance management.

2.1.2. Scientists

A biologist and a veterinarian, both ideally with experience with cetaceans, should be appointed by the OSCB. Their responsibility should include the following items:

- Develop a stranding network that can react quickly to cetacean mortality events;
- Develop protocols for attending strandings and for the collection of tissues for chemical, acoustic and biological pollution;

- Prepare the material necessary for attending a die-off (everything should be ready and at hand for instant leave);
- Provide field staff and build capacity;
- Recruit and manage volunteers;
- Timely intervention and incident control coordination: an educated decision on response level (equipment and personnel);
- Coordination with other similar networks within and outside the Member States;
- Adequate decision regarding the fate of live-stranded cetaceans (release, rehabilitation, euthanasia);
- Collection of biological data and pictures;
- Necropsy of dead cetaceans;
- Collection of samples;
- Contact with laboratories that will process the samples;
- Contact with research centres that could provide free CT examination;
- Prepare a protocol for packing and dispatching biological material;
- Send the samples;
- Carcass disposal in agreement with national regulation.

2.1.3. Volunteers

Volunteers should be recruited to help with strandings. They may have distinct backgrounds and personalities and should be given tasks according to their respective skills.

2.2. Memoranda of understanding with collaborators

Memoranda of understanding should be established with the Naval Forces, Oil and Gas companies as well as with universities, research/medical institutes and laboratories willing to help at the occasion of an outbreak of mortality. Laboratories (toxicology, microbiology and acoustic research) should be asked to send specific protocols for sampling, preserving and sending the samples. Ideally they should provide the vials, fluids and other material required for sampling. Otherwise they should specify the material needed for sampling and the firm where to buy it.

3. OUTLINE OF A PROGRAMME TO BUILD CAPACITY

Capacity building is a prerequisite to explore factors involved in a die-off. It should concern the staff of the OSCB, volunteers, coastguards and navy officials, fishermen and the general public (please see § 1.2.3.). The following programme outlines the steps that may be taken to realize this target.

- Organization of annual, national workshops on cetacean outbreaks of mortality for the staff of the OSCBs. National and international experts in the fields of toxicology, acoustic contamination and microbiology should ideally be invited to participate;
- Organization of training courses on cetacean strandings, on acoustic, chemical and biological contamination and sample collection for the staff of the nascent OSCBs. These training courses may take place at the OSCB, CEUM facilities or at the laboratory of a national stranding network;
- Organization of national meetings with other relevant bodies related to strandings (universities, coastguards, oceanaria, naval forces, fishermen, etc) and presentation of documents on cetacean mortality events;
- Acquire capacity building material (books, papers, reports, CDs, DVDs, protocols) from other stranding networks, universities, research groups, NGOs and scientists;

- Development of a library dedicated to marine mammal strandings, acoustic, biological and chemical contamination and epidemics;
- Communication with other OSCBs;
- Preparation of leaflets on the biology of cetaceans and the reasons of cetacean mortality events targeting the general public;
- Preparation of children booklets and posters on whales and dolphins and stranding events.

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