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Review

Impact of chemical pollution on threatened marine mammals: A systematic review

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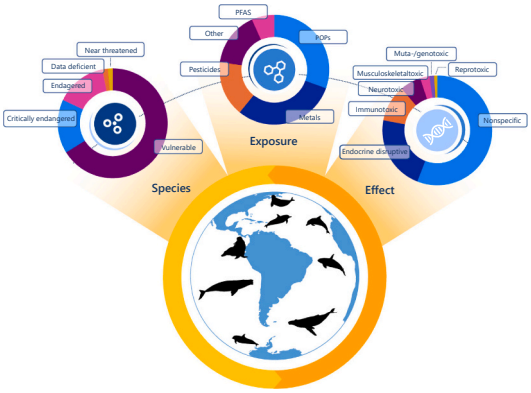
HIGHLIGHTS

- The higher the extinction risk, the fewer exposure-effect data are available.
- Lack of studies in the Southern Hemisphere shows a spatial bias in the literature.
- Commonly studied pollutants are persistent organic pollutants, metals, pesticides.
- Pollution-effect studies focus on molecular and cellular levels.
- *In silico* and in vitro approaches aid in assessing in vivo effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Marine mammals, due to their long life span, key position in the food web, and large lipid deposits, often face significant health risks from accumulating contaminants. This systematic review examines published literature on pollutant-induced adverse health effects in the International Union for Conservation of Nature (IUCN) red-listed marine mammal species. Thereby, identifying gaps in literature across different extinction risk categories, spatial distribution and climatic zones of studied habitats, commonly used methodologies, researched pollutants, and mechanisms from cellular to population levels. Our findings reveal a lower availability of exposure-effect data for higher extinction risk species (critically endangered 16%, endangered 15%, vulnerable 66%), highlighting the need for more research. For many threatened species in the Southern Hemisphere pollutant-effect relationships are not established. Non-destructively sampled tissues, like blood or skin, are commonly measured for exposure assessment. The most studied pollutants are POPs (31%), metals (30%), and pesticides (17%). Research on mixture toxicity is scarce while pollution-effect studies primarily focus on

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molecular and cellular levels. Bridging the gap between molecular data and higher-level effects is crucial, with computational approaches offering a high potential through *in vitro* to in vivo extrapolation using (toxico-)kinetic modelling. This could aid in population-level risk assessment for threatened marine mammals.

1. Introduction

Marine mammals are exposed to a wide variety of legacy (persistent) organic pollutants (e.g., PCBs and PBDEs), emerging contaminants (e.g., pharmaceuticals), heavy metals, and new types of engineered materials such as microplastics over their lifetime [1,16,35,50,89,91,106]. Due to their relatively long life span, key position in the food web (often apex consumers), and large lipid deposits, marine mammals are exposed to the highest concentration of pollutants of all marine wildlife. Among marine mammals, 60% of the species are threatened by pollution, which makes pollution the second most prevalent threat to marine biodiversity behind accidental mortality, such as by-catch [5,86]. Studies that have drawn empirical and quantitative links between pollutants and their adverse outcomes show, for example, that elevated PCB (polychlorinated biphenyl) concentration still is a significant cause of European cetacean populations' decline [104,61]. PCB levels in multiple dolphin species markedly exceed all known protective thresholds. Thus, population declines in these species were likely the result of a lack of recruitment arising from reproductive failure [33].

A recent publication by [17] predicted that many global killer whale populations are at a high risk of decline and might eventually collapse due to PCB toxicity. Particularly killer whale populations near industrialised regions and those feeding on high trophic levels regardless of location are at the highest risk. Furthermore, strong evidence shows a link between the susceptibility to diseases due to systematic suppression of immune function and the presence of accumulated contaminants (e.g. PCBs and Hg) [18,93]. Considering this and the differences in marine megafauna's sensitivity to pollutant exposure [103,23,46], mechanistic chemical risk assessment approaches, and more proactive conservation measures are urgently needed.

Environmental pollutants can become bioavailable to exposed marine mammals primarily via dietary intake or offloading to the next generations via gestation and lactation from the mother to the offspring [28,30,53]. Once the compounds are internally present, they can reach their toxicological target sites, such as specific organs or tissues, and initiate primary macromolecular interactions or Molecular Initiating Events (MIEs). This would then trigger key cellular and organ toxicity responses. Ultimately, this may result in adverse health effects on individuals or even negatively impact resilience at the population level [61,95]. This process represents a sequential relationship between toxicokinetics (fate and distribution of chemicals in the body) and toxicodynamics (effects of pollutants), the latter being initially called the 'adverse outcome pathway' (AOP) by [4].

Although the burden of pollutants can pose a severe health threat to these megafauna species and threaten the richness of marine mammal biodiversity, due to their large home ranges, migratory nature, and often protected status, research is limited. Species-specific factors such as lifecycle, physiology, and dietary preferences, influence chemical exposure, accumulation, and sensitivity [20,85]. Furthermore, many exposure-effect studies focusing on a single or mixture of chemical groups, insufficiently assess the hazardous potential of complex environmental exposure scenarios [44,48,85]. In reality, organisms are exposed to complex mixtures of known and unknown hazardous chemicals, which are diverse and variable, making them difficult and costly to characterise and monitor using traditional target analyses [21,44]. Therefore, understanding the impact of chemical pollutants on marine ecosystems has been a challenge for toxicologists and environmental scientists for decades.

The International Union for Conservation of Nature (IUCN) Red List supplies information about the range, population size, habitat and ecology, use and/or trade, threats, and conservation actions to guide conservation decisions for marine mammals. A quantitative cause-effect relationship, or species-specific sensitivity assessment to pollutants, is not yet included in the IUCN assessment criteria. Frameworks for marine mammal risk assessment have recently been suggested in the literature applying novel tools using *in vitro*, *in silico*, non-target screening, and effects-based analyses in an integrated approach [85]. Such information has great potential and may provide the means to include chemical exposure within future assessment criteria. However, whether adequate information is available to conduct such approaches for the most threatened species is unclear, and will be systematically explored within this review.

Marine mammals are valuable flagship species for marine conservation as they play an integral role in the marine ecosystem's function and resilience. Therefore, conserving marine mammal biodiversity can provide insurance for marine health that is experiencing stressors and can minimise the risk of drastic changes in marine ecosystems. The larger the number of functionally related species in an ecosystem, the greater the chance that some will be resilient to a particular stressor. Thus, research must focus on identifying the sensitivity of the most affected, and ecologically relevant species to chemical pollution in the hope of conserving existing biodiversity. This systematic review will examine the published literature (up to May 2022) on pollutant-induced adverse health effects in IUCN red-listed marine mammal species, focusing on gaps in the literature concerning (1) different extinction risk categories, (2) spatial distribution and climatic zones of studied habitats, (3) commonly used methodologies, (4) researched pollutants, (5) mechanisms from cellular to population level, and (6) discussion of future strategies in marine mammalian ecotoxicology.

2. Materials and methods

A systematic review helps to identify all the pollutant-effect relationships that have been studied to date and makes recommendations for future research. The advantages of this approach over conventional reviews are its structured and standardised approach, its objectivity, and its transparency, which minimises the risk of bias in the results [14]. The method for systematically surveying and selecting papers is reproducible and explicit, which enables researchers to repeat the procedure [14]. This review includes a quantitative systematic approach, according to Pickering and Byrne [76]. The method follows a review protocol that holds information on the research question(s), inclusion and exclusion criteria, search strategy, data extraction, and data analysis (Table 1).

2.1. Review questions and criteria

The research question, inclusion and exclusion criteria, can be found in the review protocol (Table 1). Since the research question is broadly defined, this review includes only literature from databases and publications describing a statistically significant pollutant-effect relationship. Publications using literature (e.g., thresholds or TEQ (toxic equivalency) values) that are not specific to individual species, and lack a mechanistic basis to describe a pollutant-effect response, were excluded since this review only focuses on mechanistic pollutant-effect responses. Species selection was based on the IUCN Red List of Threatened Species (Version 2021–3, https://www.iucnredlist.org accessed on 1 May 2022). Included are marine mammal species belonging to the Red List categories: CR - Critically Endangered (n = 5), EN - Endangered (n = 20), NT - Near Threatened (n = 11), VU - Vulnerable (n = 14) and DD – Data

Table 1
Review protocol applied in this systematic review.

Step	Method details
Research question	What is known about the pollutant-effect relationships in marine mammals with high-risk extinction status?
Inclusion criteria	**Marine mammals**: Species with conservation status CR - Critically Endangered, EN - Endangered, NT - Near Threatened, VU - Vulnerable and DD – Data Deficient. **Pollutants**: (heavy) metals, (persistent) organic pollutants (e.g., PCBs, PBDEs, PFAS (polyfluoroalkyl substances), etc.), pesticides (e.g., DDXs), and (micro and nano-) plastics. Single pollutants as well as mixtures (e.g., POP (Persistent Organic Pollutants) mixture) are included. **Effects**: Physiological- and biological responses to pollutant exposure. Only statistically significant pollutant-effect relationships are included. **Literature**: Primary English peer reviewed literature. Only full articles are included.
Exclusion criteria	**Marine mammals:** Marine mammal species of the category LC – Least concern **Pollutant:** A mixture of pollutants in which the composition is unknown (e.g., oil spill). **Effects:** Pollutant-effect relationships in which thresholds or other effect markers are used from literature. **Literature:** Presentations, (book) reviews, comments and all studies reported in a non-English language. Grey literature.
Literature search	**Databases searched**: Web of Sciences and Scopus Searches were optimised with Booleans (Table A.2) to account for title variations and included the following keywords. **Keywords**: *Phocoena sinus, Sousa teuszii, Balaenoptera ricei, Eubalaena glacialis, Lipotes vexillifer, Balaenoptera omurai, Mesoplodon bowdoini, Mesoplodon carlhubbsi, Mesoplodon ginkgodens, Mesoplodon hectori, Mesoplodon hotaula, Mesoplodon traversii, Orcinus orca, Tasmacetus shepherdi, Arctocephalus galapagoensis, Cephalorhynchus hectori, Enhydra lutris, Monachus monachus, Neomonachus schauinslandi, Neophoca cinerea, Neophocaena asiaeorientalis, Orcaella brevirostris, Phocarctos hookeri, Sotalia fluviatilis, Balaenoptera borealis, Balaenoptera musculus, Eubalaena japonica, Inia geoffrensis, Lontra feline, Mesoplodon perrini, Platanista gangetica, Platanista minor, Sousa plumbea, Zalophus wollebaeki, Cephalorhynchus eutropia, Cephalorhynchus heavisidii, Phocoena spinipinnis, Sotalia guianensis, Balaenoptera bonaerensis, Berardius minimus, Eumetopias jubatus, Hyperoodon ampullatus, Mesoplodon stejnegeri, Pseudorca crassidens, Tursiops aduncus, Cystophora cristata, Dugong dugon, Neophocaena phocaenoides, Odobenus rosmarus, Orcaella heinsohni, Sousa chinensis, Trichechus manatus, Balaenoptera physalus, Callorhinus ursinus, Physeter macrocephalus, Pontoporia blainvillei, Sousa sahulensis, Trichechus senegalensis, Ursus maritimus*, organics, persistent, organic pollutant, contaminant, pollutant, metal, heavy metal, plastic, microplastic, toxic, toxicity, response, relationship, effect, and disease.
Data extraction	**Extracted categories from selected publications**: Author(s), year of publication, title, journal, area of marine mammal origin within the related study, country of marine mammal origin within the related research, continent of marine mammal origin within the related study, climatic zone, type of method (*ex vivo,* in vivo*, in vitro*), method details, characterisation of biomarker, type of species, type of biological organisation level in which effect is seen, type of effect (e.g. neurotoxic), effect target (e.g. CYP1A1), type of correlation (positive or negative), tissue in which the pollutant is measured, dose dependency, and type of pollutant. **Software used for extracting data**: EndNote and Microsoft Excel.
Data synthesis	**Synthesis methods**: Narrative synthesis, developed categories from a detailed examination of all included studies. **Presentation methods:** Tables, and graphs. **Software used for synthesis**: Microsoft Excel, Microsoft Power BI, and Data wrapper (online).

Deficient (n = 9) (Table A.1). The climatic zones were based on the study areas and defined by a high-resolution (5 arc minutes) Google Earth plug-in described by Rubel et al., [83] and supplied online by the University of Veterinary Medicine Vienna, Climate change and infectious disease group (http://koeppen-geiger.vu-wien.ac.at/present.htm). The climatic zones are expressed according to Köppen-Geiger classification.

2.2. Publication selection process

The selection of publications followed the review protocol and the PRISMA 2020 flow diagram [69]. The publication selection process is presented in Fig. 1. The search output of two databases resulted in a total of 1055 records. All were collected and assessed according to a three-tier approach; identification (1), screening (2), and included (3). Tier 1 (n = 1055) consisted of removing duplicates (n = 161). Tier 2 (n = 894) included initial screening and examination of the titles and abstracts. In total, 799 records were excluded, for which one of the exclusion criteria was met. Tier 3 included thorough reading of selected Tier 2 reports (n = 95). To be included, studies needed to demonstrate a statistically significant relationship between a pollutant and a physiological or biological response associated with pollutant exposure. This evidence could be quantitatively presented in the form of a dose-response curve or be based on a single concentration. The full texts were assessed against the inclusion criteria. Reports were included when all the criteria were met. Seventy-one studies were included, and 24 reports were excluded due to insufficient data.

2.3. Data extraction

Data extraction followed the data extraction form as reported in the review protocol. It translated to the following categories: author(s), year of publication, title, journal, area of marine mammal origin within the related study, country of marine mammal origin within the related study, the continent of marine mammal origin within the related study, climatic zone, type of method (*ex vivo,* in vivo*, in vitro*), method details, characterisation of biomarker, type of species, type of biological organisation level in which effect is seen, type of effect (e.g., neurotoxic), effect target (e.g., CYP1A1), type of correlation (positive or negative), tissue in which the pollutant is measured, dose dependency, and type of pollutant. The review database was built in Microsoft Excel based on the selected categories.

2.4. Database synthesis and analysis

A narrative synthesis was applied to interpret the included studies on pollutant-effect relationships in IUCN red-listed species. The synthesis process consists of three steps: categorical organisation of the study's content, analysing findings based on categories and synthesising content across studies [75]. Category development is driven by the research question and detailed assessment of included studies. The following categories have been developed as the framework to assess the included studies:

1. **Species** – A type of species studied in in vivo (living animals), *in vitro* (cell lines) and *ex vivo* (biopsies and primary cells). E.g., dugong biopsies, North Atlantic right whale skin cell line, and free-ranging polar bears.
2. **Spatial distribution** – Area, country, continent, and climatic zone of study area/species origin.
3. **Approach** – The method used to study pollutant-effect relationships (*in vitro*, in vivo, *ex vivo*), approach details, and biomarker classification.
4. **Pollutant** – A type of pollutant defined as a single pollutant, a sum of pollutants from the same chemical class (e.g., PFAS), and POP mixtures. Furthermore, the tissue in which the pollutant is measured.
5. **Effect** – A type of effect, biological organisation level in which the effect is seen, target, type of correlation (positive or negative), dose-dependency.

To quantitatively analyse trends and relationships between multiple variables, cross-tabulation was performed. In this way, similarities,

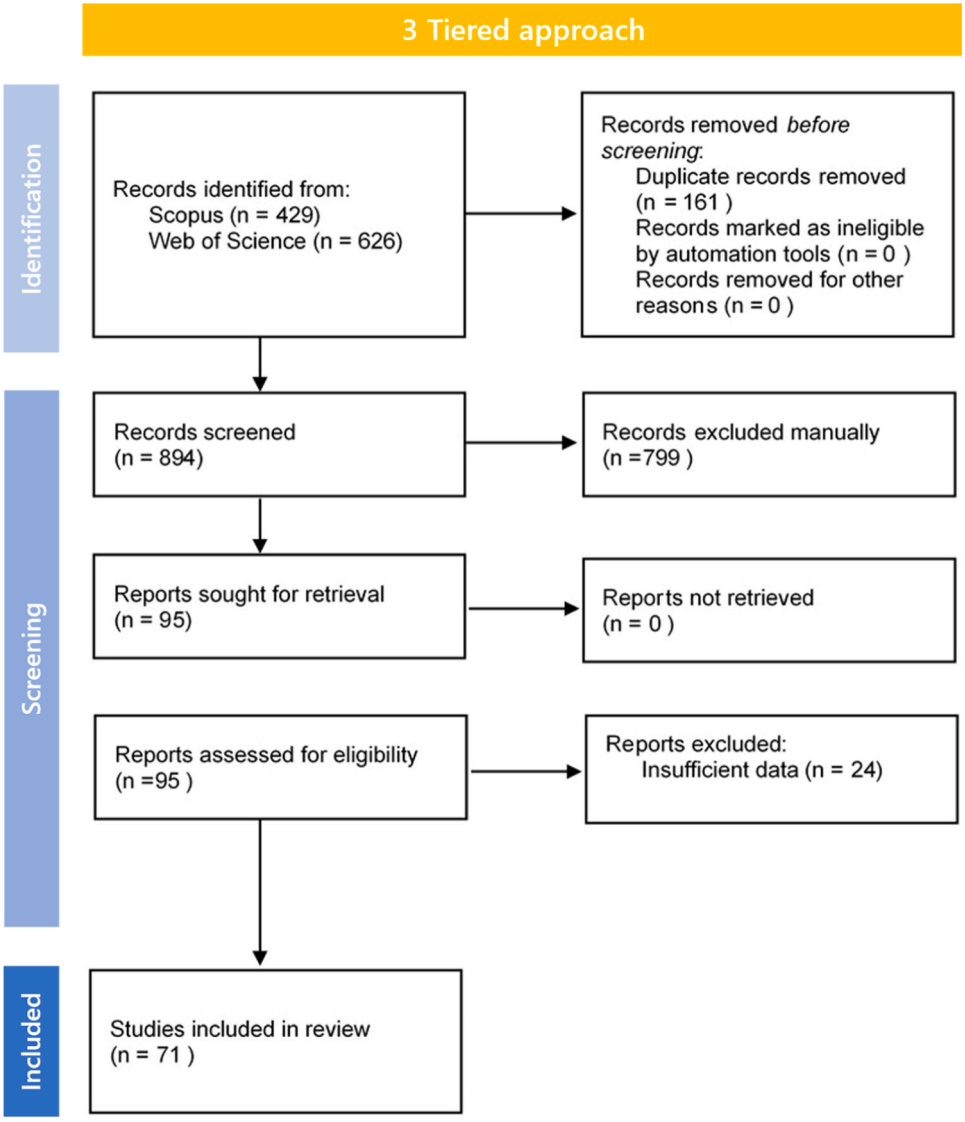

Fig. 1. Schematic overview of the tiered approach including the selection, screening, and inclusion of studies from the database query output.

differences, and analogies between pollutant-effect relationships can be explored between the studies. This analysis created clear and detailed tables and graphs aligning with the categories mentioned. The included studies were grouped in order to study common features.

3. Results and discussion

3.1. IUCN- species and categories

Marine mammals (n = 130) comprise species of cetaceans, pinnipeds, sirenians, polar bears, sea and marine otters. Many (n = 50) are classified as vulnerable or endangered and red-listed by the International Union for Conservation of Nature (IUCN). IUCN Red List classes are designed to reflect varying degrees of the threat of extinction, such as extremely high (critically endangered), very high (endangered), and high (vulnerable) risk of extinction. Taxa in these three categories are collectively referred to as 'threatened'. In this systematic review, we also included species that fall under the 'near threatened' and 'data deficient' categories. Species are classified as near threatened (NT) when they currently do not meet the criteria for critically endangered, endangered, or vulnerable, but are at risk of qualifying for a threatened category in the near future. The 'data deficient' category is based on inadequate information, which directly or indirectly assesses its risk of extinction based on distribution and population status.

The output of our literature search shows that most pollutant-effect interactions were studied in *Ursus maritimus*, the polar bear, with 380 reported pollutant-effect relationships, followed by *Eubalaena glacialis* (n = 138), *Physeter macrocephalus* (n = 67) and *Balaenoptera physalus* (n = 65) and *Neophocaena asiaorientalis* (n = 53) (Fig. 2). Although our literature review also focuses on marine mammals living in rivers and estuaries, no studies have been found researching the mechanistic understanding of pollutant-effect relationships in these species. Overall, the quantitative pollution-effect relationships in marine mammals showed that species in conservation groups critically endangered (16%) and endangered (15%) were less studied than species categorised as vulnerable (66%) (Fig. 2, Table A.1). Such a disproportate approach has been shown in other fields of study than toxicological research. Our findings align with the global marine mammal research which has been disproportionately directed towards less endangered species [39].

Scientific knowledge guides conservation and policy, which can lead to better funding allocation and efficient conservation strategy development. Research revealed a strong connection between existing conservation policies, scientific information, research priorities, and public concern [60]. Therefore, a bias towards specific species (e.g.,

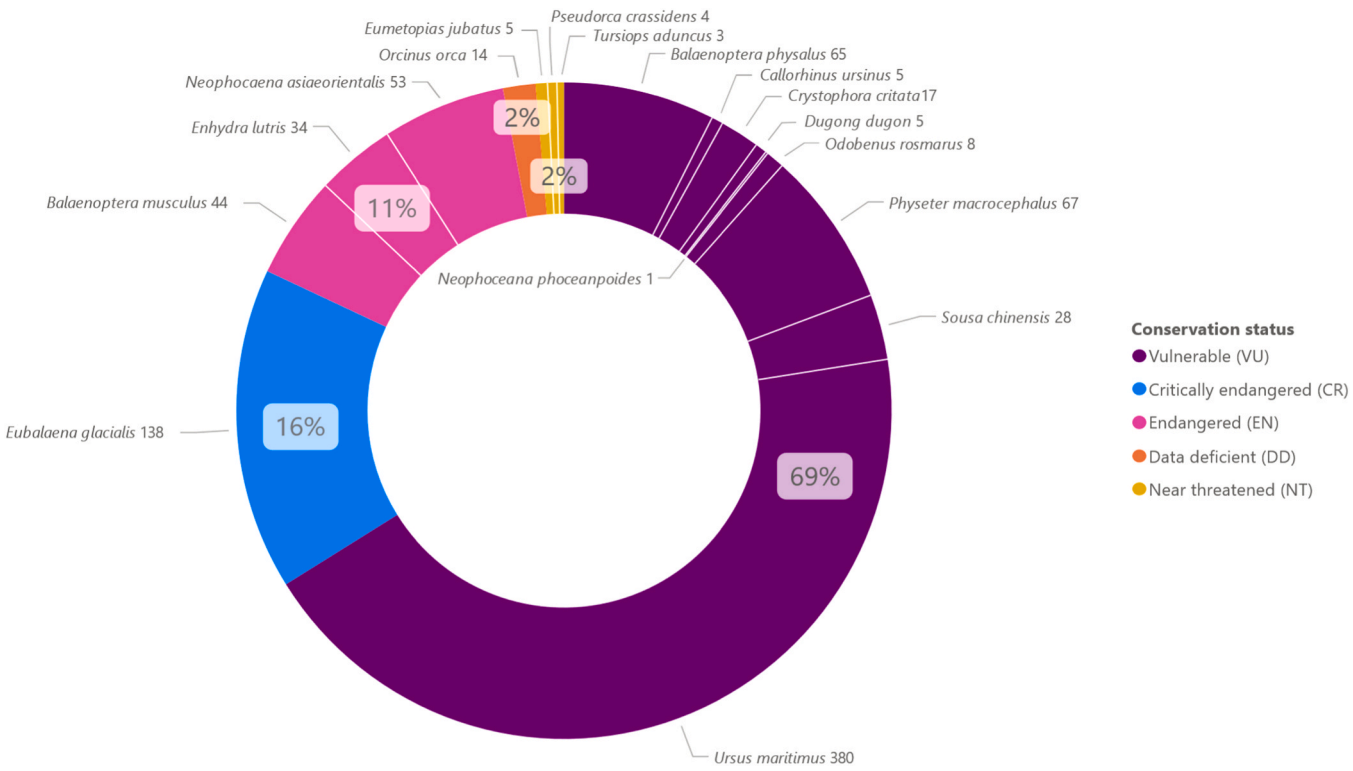

Fig. 2. Percentage of pollutant-effect relationships studied per IUCN category and number of pollutant-effect relationships per species. *Balaenoptera physalus* – Fin Whale, *Callorhinus ursinus* – Northern Fur Seal, *Cystophora cristata* – Hooded Seal, *Dugong dugon* – Dugong, *Neophocaena phocaenoides* – Indo-Pacific Finless Porpoise, *Odobenus rosmarus* – Walrus, *Physeter macrocephalus* – Sperm Whale, *Sousa chinensis* – Indo-Pacific Humpback Dolphin – *Ursus martimus* – Polar Bear, *Eubalaena glacialis* – Black Right Whale, *Balaenoptera musculus* – Blue Whale, *Enhydra lutris* – Sea Otter, *Neophocaena asiaorientalis* – Finless Porpoise, *Eumetopias jubatus* – Northern Sea Lion, *Pseudorca crassidens* – False Killer Whale, *Tursiops aduncus* – Indo-Pacific Bottlenose Dolphin, *Orcinus orca* – Orca.

charismatic species) is a well-known criticism in many conservation efforts. Furthermore, logistical and ethical challenges in researching endangered species, global resource distribution, and lack of access to rare wildlife species samples could lead to such uneven results. An uneven distribution of scientific focus exists among different cetacean and pinniped groups. Cetacean species with more extensive ranges and pinniped species with larger population abundances are studied the most. Challenges in correct taxonomic identification could also be one

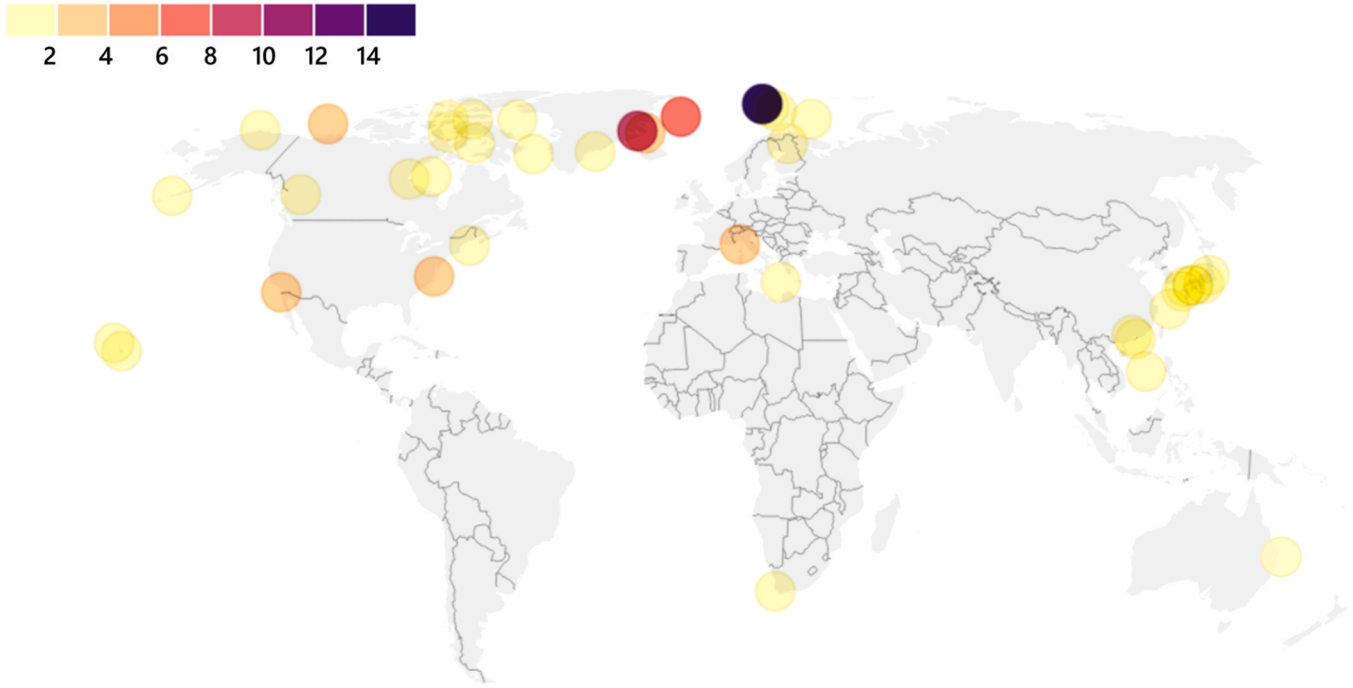

Fig. 3. The number of pollutant-effect relationships per region. (Map created with Datawrapper.).

reason why certain cetacean species are underrepresented [39]. Overall, our results highlight the need for more research on marine mammals with a higher risk of extinction, as the chemical burden these marine mammals experience is not considered in current conservation measures.

3.2. Spatial distribution and climate zones

The results of this review show that pollutant-induced health effects in marine mammals were almost exclusively performed in the Northern Hemisphere (Fig. 3). Only two studies were performed in the Southern Hemisphere (South Africa and Australia) that met the selection criteria of this systematic review. North America is leading this field of research with 32 studies performed throughout the continent. Overall, the location with most studies was Svalbard in Europe.

Since the data predominantly originates from the Northern Hemisphere, knowledge of pollutant-effect relationships in species living in southern climates is limited or lacking, such as the dugong (*Dugong dugon*), Indo-Pacific bottlenose dolphin (*Tursiops aduncus*), Hector's dolphin (*Cephalorhynchus hectori*), and Irrawaddy dolphin (*Orcaella brevirostris*). Economic considerations may lead to research bias in focusing on geographically favourable species [39]. The scarcity of studies that investigated data-deficient species and the prevalence of studies on threatened species in the Northern Hemisphere may likely result from extensive research efforts, capabilities, and a long history of research activities [86]. For example, 'data-deficient' is not a category of threat. Instead, this indicates that more information is needed. It has been argued that data-deficient species should have the same conservation status as threatened species until more knowledge becomes available [57]. It is unlikely that these species will get the protection they need soon, where some species may already experience a significant population decline [39]. Underlining this aspect, is a previous study which has shown that for amphibians, data-deficient species are, on average, more threatened than species listed as threatened by the IUCN [36].

Our results correspond to the relatively high biodiversity risk to marine mammals in the Northern Hemisphere by O'Hara et al. [66], whereby biodiversity risk is defined as the mean conservation status of marine biodiversity by combining threatened species' spatial range and extinction status. The systematic bias towards species in the Northern Hemisphere, however, may influence the outcome of biodiversity risk assessment. In contrast to O'Hara (2019), another study integrates data on species-specific vulnerabilities into threat types (e.g. by-catch, pollution, and direct harvesting) at specific locations and times and describes pollution threats to marine biodiversity at all coastal waters worldwide [5]. This study also identifies biodiversity risks to marine mammal communities in the Southern Hemisphere, with specific hotspot areas in Antarctica, Australia, South-East Asia, and South America [5].

The climate plays a crucial role in determining the environmental conditions that can impact the fate and distribution of pollutants in the marine environment and have significant implications for the uptake, bioavailability, and toxicity processes of pollutants in different species [31]. Therefore, the studied species were categorised into their climatic zones. The climatic zones in this study were based on the study area and defined by a high-resolution (5′) Google Earth plug-in described by Rubel et al., [83]. In the 71 studies included in this review, seventeen different mammal species and ten different climatic zones were covered (Fig. 4). *Balaenoptera physalus*, *Orcinus orca*, *Neophocaena asiaeorientalis*, *Physeter macrocephalus*, *Pseudorca crassidens*, and *Ursus maritimus* were studied in more than one climatic zone (migrating or far-ranging species).

The highest diversity of marine mammals was studied in the northern polar tundra (ET). The Arctic is strongly affected by climate change and has therefore received high research interest, and this could also explain the high diversity of marine mammals studied in an ET climate. Unfortunately, no studies investigated the pollution-effect relationship in species from the ET climatic zone in the Southern Hemisphere, even though it provides habitats for various marine mammals, many of which have a threatened or data-deficient IUCN status. For instance, beaked whales, of which 19 beaked whale species are data deficient, are particularly concentrated around Antarctica [86]. There is a lack of knowledge regarding marine mammals at high risk of extinction inhabiting tropical (A) climates. Additionally, data on marine mammals

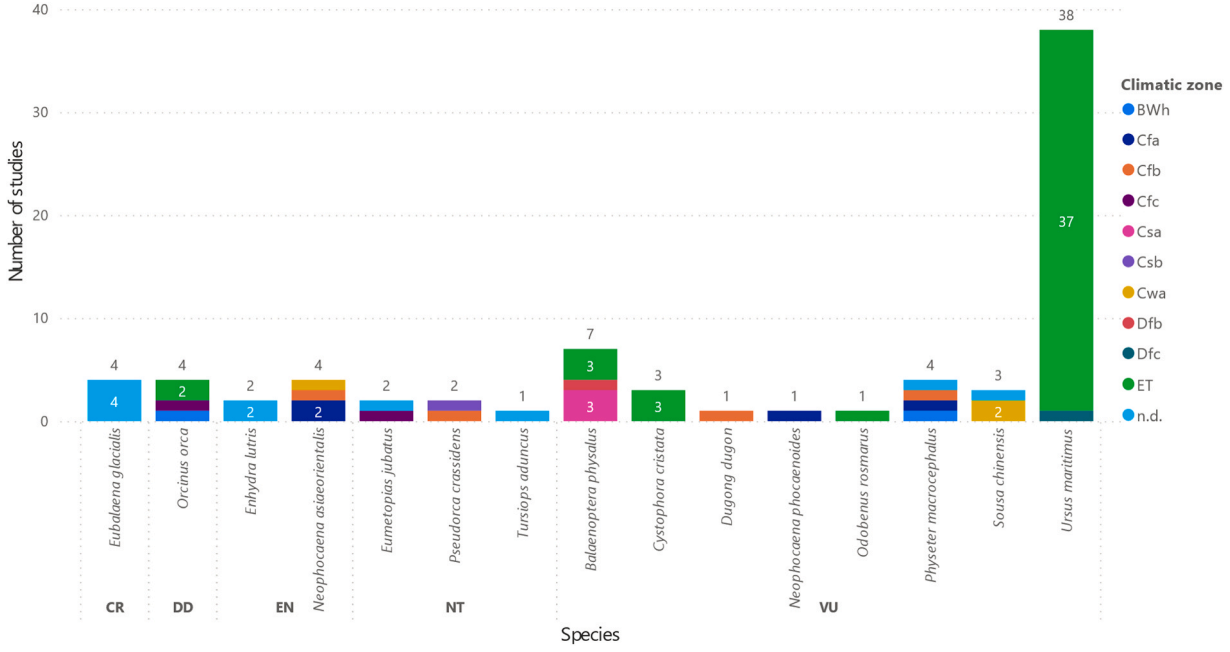

Fig. 4. The number of studies per species and climatic zone. IUCN red list categories: CR - critically endangered, DD – data deficient, EN – endangered, NT – near threatened, VU - vulnerable. BWh – hot desert climate, Cfa - humid temperate climate, Cfb – oceanic climate, Cfc - subpolar oceanic climate, Csa - hot-summer Mediterranean climate, Csb - warm-summer Mediterranean climate, Cwa - subhumid temperate climate, Dfb – warm summer hemiboreal climate, Dfc – regular subarctic climate, ET – polar tundra climate.

in arid (B = BW), continental (D = Df), and temperate (C) climates (e.g., Cf, Cs, Cw) is limited.

Marine mammals across the Northern Hemisphere, specifically the North Pacific Ocean, the Greenland Sea and the Barents Sea, are highly vulnerable to climate change. The most vulnerable species to global warming are the North Pacific right whale (*Eubalaena japonica* – IUCN conservation status: Endangered) and the Gray whale (*Eschrichtius robustus* – IUCN conservation status: Least concern) [2]. However, for both species, no pollutant-effect relationships are described in the literature. Also, some species listed within the data deficient IUCN-category (Baird's Beaked whale – *Berardius bairdi*) exhibit high vulnerability to climate change [2].

In Arctic ecosystems, higher temperatures will release stored POPs from glaciers, melting ice and thawing permafrost into marine environments [15,71,8]. Furthermore, changes in air mass movements, ocean currents, and, thus, pollutant distribution are aligned with changes in POP accumulation in some species in the (Arctic) environment [15,64]. Also, the declining state of sea ice affects the air-water exchange of pollutants, and prey accessibility [15,64]. It is evident that climate change will have far-reaching effects on a wide array of habitats, species, and environmental processes. These impacts, in turn, will influence the dynamics of pollutants in these environments. As a result, the species inhabiting these ecosystems may experience additive, or synergistic effects, as a consequence of both climate change and pollutant exposure.

3.3. Sampling matrices

The range of tissue biopsies used to investigate pollutant-effect relationships in IUCN red-listed species is relatively narrow, with the polar bear providing the most extensive data on various tissue analyses when assessing the impact of pollutants (as indicated in Fig. 5). This may partly be due to the increased research focus on the Arctic ecosystem and the greater accessibility of samples from species also inhabiting land. Therefore, the assessment of tissue-specific differences towards pollutant exposure stays challenging overall. One study applied *in tissue* passive equilibrium sampling in harbour porpoises (*Phocoena phocoena*), one harbour seal (*Phoca vitulina*) and one orca (*Orcinus orca*) from the North and Baltic Seas. This was achieved by using silicone polydimethylsiloxane (PDMS) to extract a broad range of pollutants from mammal tissue [81]. They applied these extracts onto bioassays indicative of different toxicological endpoints, showing that liver extracts elicit the highest activation of AhR-CALUX, PPARγ-bla and AREc32, followed by the kidney and brain extracts, while the blubber extracts of the animals were least active[81]. Interestingly, blubber samples are commonly taken from stranded and free-ranging animals.

Tissues obtained via non-destructive sampling from free-ranging species, such as blood (plasma), adipose, and skin tissue, make up the most measured tissues to assess pollutant-effect relationships (Fig. 5). For example, Pedro et al. [73] related POPs in blubber to vitamin A and E levels in killer whales (*Orcinus orca*) and Guo et al. [34] explored endocrine responses of the Indo-Pacific humpback dolphin (*Sousa chinensis*) to pollutant exposure in blubber samples. Grønnestad et al. [32] described the effects of a complex pollutant mixture in blood on thyroid hormones in breeding hooded seal (*Cystophora cristata*) mothers and their pups. However, potential risks due to pollutant-effect responses could be overlooked if studies focus mainly on adipose and blood/-plasma, since certain organ(s) (system(s)) are not covered. Other non-destructive sampling methods, such as hair, face, saliva, blow collection, skin, etc., should be explored more [12] and combined with a tissue residue approach in stranded animal responses [85] using modelling approaches (see Section 3.7.).

Five studies applied other non-destructive sampling methods to investigate pollutant-effect relationships in IUCN Red-Listed species. Three of the five studies used skin samples and the remaining two used hair samples. Mouton et al. [63] tried to link the occurrence of cutaneous opportunistic fungal invaders with elemental concentrations such as aluminium (Al), mercury (Hg) and nickel (Ni) in the false killer whale (*Pseudorca crassidens*) skin samples. In contrast, Fossi et al. [27] used skin samples to investigate the susceptibility of the Fin whale (*Balaenoptera physalus*) to xenobiotic pollutants. Skin biopsies of dugongs (*Dugong dugon*) were utilised to research the differences in marine megafauna cytotoxicity sensitivity towards heavy metals, dichlorodiphenyldichloroethylene (DDE) and perfluorononanoic acid (PFNA)

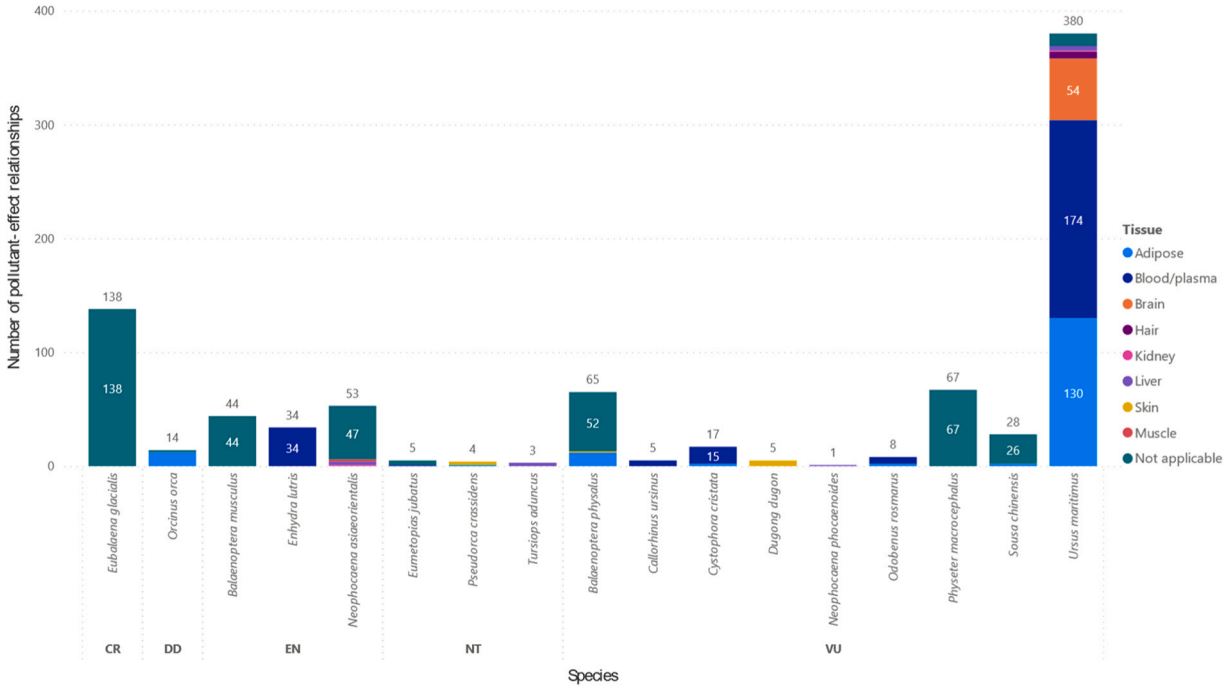

Fig. 5. Number of pollutant-effect relationships per exposure tissue in distinct species. IUCN red list categories: CR - critically endangered, DD – data deficient, EN – endangered, NT – near threatened, VU – vulnerable. Not applicable – non-tissue exposure (artificial dose/mixture).

by Finlayson and van de Merwe [23]. So far, Watson et al. [96] and McKinney et al. [62] provide the only studies in which hair samples are used to describe pollutant-effect relationships. Watson et al. [96] associated mercury levels in hair with the diversity and composition of the polar bear gut microbiota; and McKinney et al. [62] related the body mass index of Southern Beaufort Sea polar bears to hair mercury concentrations.

Generally, a tissue residue approach that relies on invasive biopsies to extract kidney, brain, liver, and muscle tissue from stranded animals is less used, probably due to the opportunistic nature of sampling and the constraints related to collecting tissue samples from deceased animals [38]. Invasive tissue sampling through necropsy can supply a wide variety of target tissues for toxicological analysis but these samples are also prone to bias regarding the state of the population they originate from [98]. Furthermore, many stranded animals are in morbid condition, meaning that they have starved before; thus, their bodies have probably endured a redistribution of pollutants in blubber to blood and other tissues/organs. Therefore, sampling should ideally be done on freshly stranded animals with good body conditions.

In additon, the sex of the animal should be considered during sampling and data interpretation. Kellar et al. [49] found that the long-beaked common dolphin (*Delphinus capensis)* showed different sex-linked behaviours depending on the biopsy platform due to disproportionate male sampling. Bow riding appeared to differ between males and females of the long-beaked common dolphin, for which males were disproportionately more likely to be biopsied than females [49]. The common dolphin (*Delphinus delphis*) did not observe this pattern, suggesting that bow-riding behaviours could differ between species [49]. Furthermore, the sex ratio of the common dolphin is strongly related to geographical location [49]. For example, a specific area may hold proportionally more males than another area.

Sex also influences the short-term response of humpback whales (*Megaptera novaeangliae*) to repeating boat approach and remote biopsy sampling [29]. Likewise, female reproductive status and social context affect the response of humpback whales to repeating boat approach and remote biopsy sampling in which mothers showed more frequent responses to approaching boats, but the weakest response to biopsy sampling [29]. In contrast, non-lactating females respond strongly to repeated biopsy sampling [29]. The group's competitiveness appears to impact the response of males towards repeated biopsy sampling. Males in a competitive group tend to respond less when sampled [29]. Many studies ignore these factors that introduce bias in sampling strategies. Therefore, a standardised reporting protocol, and sample strategy, that includes such side aspects is warranted and should be widely applied.

Generally, sampling non-destructive or invasive matrices for organic and inorganic analysis and health parameters requires an expert team in sampling and species biology. However, sampling of stranded animals is usually not common for many regions and is logistically costly. On top of this, the application of novel tissues needs to be validated and verified for use in instrumental or *in vitro* screening methods, which requires mostly an interdisciplinary team and is a time consuming and costly approach.

3.4. Experimental methodologies and approaches

Traditional toxicological laboratory studies (in vivo) are logistically and ethically impossible on large, free-ranging, protected marine species. Furthermore, in vivo experiments on laboratory organisms are often performed under strongly controlled exposure conditions and would be ill-suited to reflect a realistic exposure scenario of organisms within their natural environment. We found only three studies (4%) on pollution-effect relationships in polar bears (*Ursus maritimus*) that used in vivo techniques (Table 2), namely McKinney et al. [62], Knott et al. [52], and Bourque et al. [9]. Examples of effects at the tissue biological organisation level are receptor inhibition on a tissue scale, hormone levels (measured at a tissue scale), and tissue characteristics (i.e., testes weight). Nevertheless, toxicological effects on the organ system and organism level are endpoints that are easier to extrapolate at the population level. Pollutant-effect relationships at the organism and population level are absent, and only empirical studies are available without any mechanistic knowledge.

Table 2
Summary of experimental approaches used to investigate pollutant-effect relationships in the included literature on IUCN red-listed marine mammals.

Conservation status	Species	*ex vivo*	in vivo	*in vitro*	Total
Critically endangered	*Eubalaena glacialis*	1		3	4
Endangered	*Balaenoptera musculus*			1	1
	Enhydra lutris	2			2
	Neophocaena asiaeorientalis	2		1	3
Vulnerable	*Balaenoptera physalus*	3		4	7
	Callorhinus ursinus	1			1
	Cystophora cristata	3			3
	Dugong dugon	1			1
	Neophocaena phocaenoides	1			1
	Odobenus rosmarus	1			1
	Physeter macrocephalus	2		2	4
	Sousa chinensis	1		2	3
	Ursus maritimus	33	3	1	37
Near threatened	*Eumetopias jubatus*	1		1	2
	Pseudorca crassidens	2			2
	Tursiops aduncus	1			1
Data deficient	*Orcinus orca*	3		1	4
	Grand Total	**58**	**3**	**16**	**77**

In *ex vivo* studies, primary cells and/or tissues (blood, adipose, kidney, etc.) are extracted from living or dead organisms for culture or direct analysis. This approach has many advantages and is popular in ecotoxicology to assess toxicological effects in various species. Most pollutant-effect relationships in this review are described by *ex vivo* methods (58 studies - 76%) (Table 2). Since *ex vivo* techniques can be applied non-destructively for free-ranging animals (e.g. blood, skin, keratinised tissues) and invasively for freshly stranded animals (e.g. primary liver cells), *ex vivo* techniques are an ideal approach to assess pollutant-effect relationships in endangered, threatened, and vulnerable species. For example, blood or blubber can be collected non-destructively, and extracts can be used to screen for pollutant concentrations and their effects simultaneously. Villanger et al. [94] assessed pollutant concentrations and their effects on thyroid hormone levels in the blood of Hooded seals (*Cystophora cristata*). The impact of mercury concentrations on haptoglobin levels in the blood of Steller sea lions (*Eumetopias jubatus*) was also described by Kennedy et al. [51]. Aside from blood representing only a snapshot of exposure, the implication of alternative extraction methods for chemical analysis, such as passive sampling [45,47], may show that matrices other than blood and blubber are suitable for simultaneous exposure-effect assessment and provide the opportunity to understand exposure and mixture effects. Although these methods need to be validated, which demands substantial time and cost, they allow effect-based monitoring and toxicity analysis.

Cell-based approaches applying primary cells or cell lines gained momentum in ecotoxicology mainly due to the availability of innovative technologies, which is reflected in our database (Fig. A.1 and A.2). Cell-based technologies allow rapid screening of the potential toxicity of chemicals and environmental matrices, and are an ethical alternative for in vivo studies. Some primary cell cultures studies, and *in vitro*, techniques use cells or biological components (e.g., blood) isolated from animals. Here, we define *in vitro* studies as using immortalised cell lines for long-term culture application. Examples of *in vitro* techniques are bioassays (e.g. Blue whale peroxisome proliferator-activated receptor gamma (wPPARG) receptor gene assay). Examples of primary cells are Sperm whale primary skin fibroblasts and North Atlantic right whale

primary lung cells (Table 2).

Although *in vitro* techniques hold great promise, this approach exhibits limitations and presents multiple challenges that must be overcome for it to be useful in risk assessment [107,22]. Since *in vitro* studies are applied as an alternative to in vivo techniques, they require the right type of cells, or organoids which mimic the physiological conditions in vivo, such as the extracellular environment and cell-cell interactions at the tissue level [107]. Nevertheless, the current cell and tissue culturing technologies do not meet this level of detail. Most cells in toxicology are currently cultured using two-dimensional (2D) methods. However, new methods implementing three-dimensional (3D) cell culturing techniques suggest that more knowledge can be gained about cellular responses [40]. When performing 3D cell culture studies, the cell environment is manipulated to mimic an in vivo environment and provide more accurate data about cell-to-cell interactions [40]. With the use of organoids, 3D cell culture techniques have the potential, as an alternative to studying organ responses towards pollutants. They even could eventually link 2D cell culture with animal models [40]. This may one day overcome challenges in mimicking the real chemical kinetics cells experience in a tissue. However, to date, recreating realistic exposure scenarios remain a serious hurdle [107]. To the author's knowledge, 3D cell culture techniques are not applied in marine wildlife toxicology.

Challenges exist regarding describing the *in vitro* kinetics in culture media in which the stability and distribution of the test chemicals in the microplate environment (e.g., binding to assay medium and plastic and evaporation processes) could affect the toxicological response [107,77]. For instance, in dugong primary cells, it was observed that cadmium binding to media constituents resulted in a 50-fold decrease in the free accessible concentrations within the microplate environment, consequently, previous results were underestimating the sensitivity of dugongs to cadmium exposure [6].

Using species-specific primary skin cells showed differences in sensitivity to organic and exposed inorganic pollutants in marine megafauna species occupying lower trophic levels, such as green sea turtles and dugongs and higher trophic species, such as loggerhead turtles, hawksbill turtles, Burrunan dolphins, and common bottlenose dolphins [25]. The same study showed that marine mammals such as the dugong and the dolphin species were particularly vulnerable to the compounds studied. Interestingly, dose-response relationships for cadmium studied in the sea turtle skin fibroblasts were similar to those obtained from green turtle internal tissue cell cultures (e.g., liver, kidney). This indicates that the non-destructively extracted primary cells represent a good proxy for internal tissue effects [24]. However, this might largely depend on the studied toxicity endpoint and its mode of action, which can be categorised into non-specific and specific. The non-specific mode of toxic action, where chemicals can induce a generalised toxic effect by disrupting cell membranes and other cellular structures' normal functioning and leading to cell death. Furthermore, the specific mode of toxic action, where toxicants have a well-defined target site and produce a specific effect among the AOP, such as impairment of DNA synthesis or receptor binding.

Overall, *in vitro b*ioassays are based on activating cellular pathways at the initiating event of toxicity (e.g., cell viability), providing an early indication and mechanistic understanding of organ or system perturbations [58]. For *in vitro* assays to better mimic organ responses, experimental or technological improvement (e.g., organoid on a chip) and considering toxicokinetic processes within the microplate environment may provide an improvement in translating molecular effects to the organism level. To understand the species sensitivity of threatened species, non-destructively obtained primary skin cells show promising applications and avoid uncertainties that occupy extrapolations from model laboratory effect studies.

3.5. Exposure

Although several POPs have been banned since the introduction of the Stockholm Convention on Persistent Organic Pollutants (effective since May 2004), POPs are still found at significant levels in tissues of marine mammals and continue to affect marine mammal populations [41,42]. The persistent character of POPs in the environment and in organisms enables POPs to affect multiple generations through external and internal processes (e.g., maternal offloading) [61]. Therefore, the legacy of POPs is still a focal point in current marine mammal risk assessment and covers with a significant 31% of the total pollutant-effect relationships described in IUCN Red-Listed marine mammal species (Fig. 6). Amongst legacy POPs (total of 31%), polychlorinated biphenyls (PCBs) with 27% hold the greatest share of studied pollutant-effect relationships (non-coplanar PCBs 12.4%, sum PCBs 6.3%, coplanar PCBs 4.4%, and OH-PCBs 3.3%) and are followed up by polybrominated diphenyl ethers (PBDEs) with 4% (Fig. 6).

Pollutant-effect relationships of emerging POPs such as per- and polyfluoroalkyl substances (PFASs) are represented as well and cover 7% of all described pollutant-effect relationships in IUCN red-listed marine mammal species (Fig. 6). Tartu et al. [92] described the effect of PFAS on cholesterol levels in polar bears (*Ursus maritimus*), and Pedersen et al. [72] correlated perfluorooctane sulfonate (PFOS) levels with several neuronal biomarkers, including GABA-A receptor density and monoamine oxidases (MAO) activity, in polar bears. Except for PFAS, the focus on emerging pollutants in marine mammal toxicology is scarce and is underrepresented in current risk assessment due to the difficulties in evaluating their impact. The involvement of emerging pollutants in our water bodies continues to be a major concern for environmental health and consists of pharmaceuticals, cosmetic products, surfactants, and (micro)plastics [59,79]. Nevertheless, no pollutant-effect relationships are described for those emerging pollutants in IUCN red-listed marine mammal species. A recent review showed that four studies tried to research pollution-effect relationships of microplastics in marine mammals. However, as intensively discussed within the review, there are many concerns regarding the experimental design, and more standardised approaches are suggested [106].

Pesticides and metals make up 17% and 30% of the pollutant-effect relationships studied in marine mammals, respectively (Fig. 6). Both groups display a certain persistence in the environment and have been found in high concentrations in marine mammals and marine waters over decades [97,54,80]. The levels and impacts of these pollutants in marine mammals are generally well known. For example, Sonne et al. [88] described the impact of dieldrin on the size and density of East Greenland polar bears (*Ursus maritimus*) skulls. Yu et al. [105] defined induced cell cycle arrest and apoptosis of skin fibroblast in Indo-Pacific humpback dolphins (*Sousa chinensis*) by DDT exposure. Ciesielski et al. [13] also investigated the relationship between Mirex and testosterone levels in male polar bears from Svalbard. Metal-effect relationships are among others studied by Ierardi et al. [37] (transcriptome analysis of cadmium exposure in kidney fibroblast cells of the North Atlantic Right Whale (*Eubalaena glacialis*)) and Nakayama et al. [65] (correlation between organotin levels and lung nematode infection in Yangtze finless porpoise (*Neophocaena asiaeorientalis*)). However, the selection of specific pesticides and metals was quite narrow. It covered only eight metals (Al, Cd, Cr (VI), Cu, Hg, Mn, Se, Sn, and Zn) and 7 pesticides (DDX (DDT, DDE and DDD), sum CHL, sum OCPs, dieldrin, endosulfan, oxychlordane, and trans-nonachlor) in which pollutant-effect relationships for vulnerable marine mammals were described (Fig. 6). These metals and pesticides can be considered legacy pollutants and are still circulating within the marine system. Nevertheless, current-use pesticides (CUPs) and metal(loid)s, such as thallium (Tl) and arsenic (As) are rarely studied. Metribuzin, 2-methyl-4-chlorophenoxyacetic acid, phosalone, pendimethalin, tefluthrin, quizalofop-ethyl, and triallate are examples of CUPs and have even been newly discovered in remote regions, including the Arctic [7]. In turn, arsenic is a highly toxic element, and environmental arsenic exposure is strongly associated with lung, liver, kidney, skin, brain, cardiovascular diseases and several types of cancer in humans [78]. Interestingly, Tl is considered more toxic than

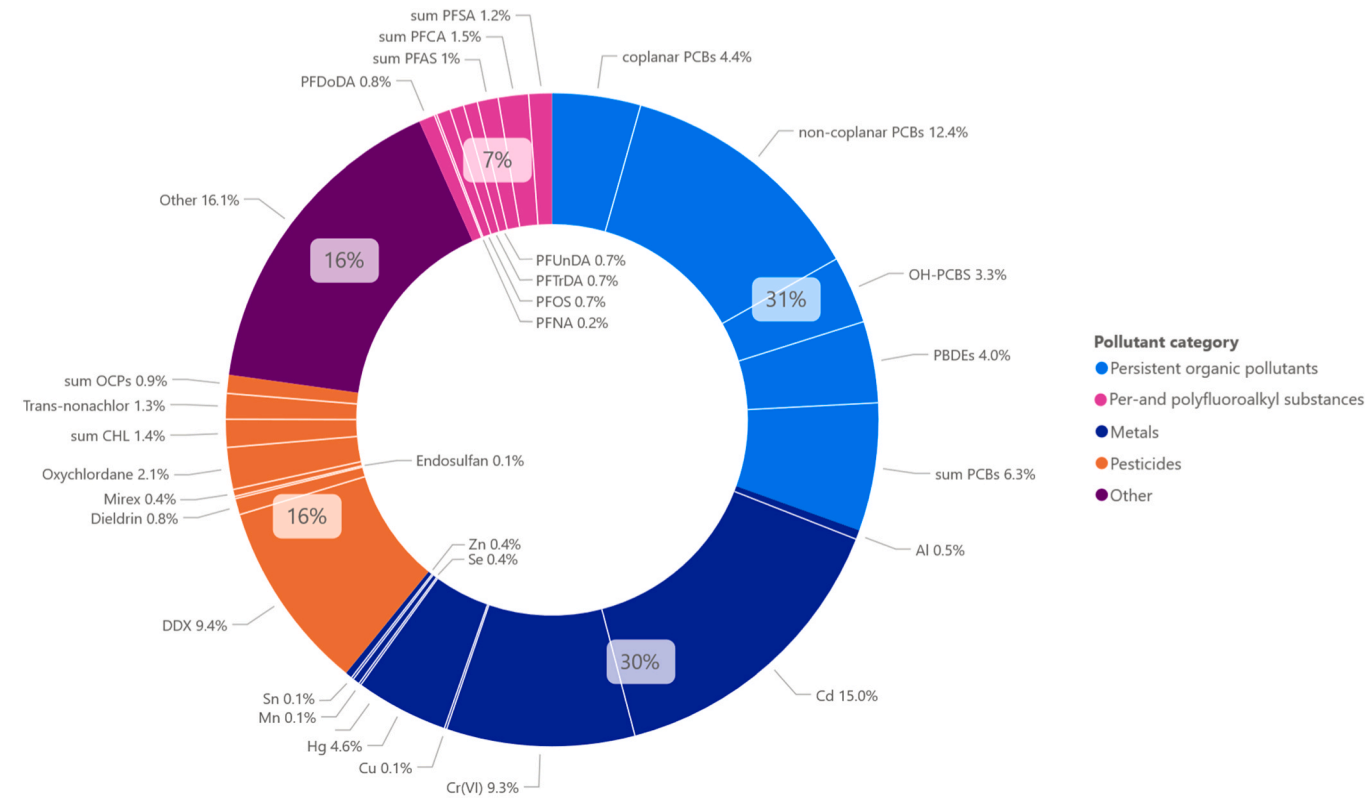

Fig. 6. Percentage of pollutant-effect relationships per pollutant category and percentage of pollutant-effect relationships per pollutant. Pollutant: non-coplanar PCBs - non-coplanar polychlorinated biphenyl, OH-PCBs - hydroxylated polychlorinated biphenyls, PBDEs - polybrominated diphenyl ethers, sum PCBs – mixture of polychlorinated biphenyls, Al – aluminium, Cd – cadmium, Cr(VI) – chromium, Cu – copper, Hg – mercury, Mn – manganese, Se – selenium, Sn – tin, Zn – zinc, coplanar PCBs – nonortho polychlorinated biphenyls: 2, 3, 11, 12, 13, 14, 15, 35, 36, 37, 38, 39, 78, 79, 80, 127 monoortho polychlorinated biphenyls: 1, 5, 6, 7, 8, 9, 20, 21, 22 23, 25, 26, 28, 29, 31, 33, 34, 55, 56, 57, 58, 60, 61, 63, 66, 67, 68, 70, 72, 74, 76, 106, 107, 108, 111, 120, 122, 124, 159, 162, dioxin-like polychlorinated biphenyl: 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189, DDX - DDT, DDE and DDD, sum CHL – mixture of chlordane, sum OCPs – mixture of organochlorine pesticides, PFDoDA - perfluoro dodecanoic acid, PFNA - perfluoro nonanoic acid, PFOS - perfluoro octane sulfonate, PFTrDA - perfluoro tridecanoic acid, PFUnDA - perfluoro undecanoic acid, sum PFAS – mixture of per- and polyfluoroalkyl substances, sum PFCA – mixture of perfluoroalkyl carboxylic acids, sum PFSA – mixture of perfluorinated sulfonates, other - hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), trichlorobenzene (TCB), pentachloro benzene (QCB), chlorobenzene, trans-nonachlor, bisphenol A, bisphenol A diglycidylether (BADGE), tetrabroombisfenol A (TBBPA), bis(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DiNP), benzo[*a*]pyrene, b-naphthoflavone, POP mixture (mixture of persistent organic pollutant, pesticides and per- and polyfluoroalkyl substances).

traditional metals, such as Hg, Zn, Cd, and Cr, as it targets the central and peripheral nervous system, including the lungs, heart, liver and kidney [74]. Hence, the detection of such CUPs and metal ions, and their elevated toxicity, highlights that future research needs to address new contaminants in presumed extensively studied groups of chemicals.

Marine mammals are exposed to complex mixtures of known and unknown or emerging pollutants from anthropogenic and natural origin [21]. Thus complicating the prediction of future risks by multiple stressors caused by chemicals and other stressors. It is important to recognise that pollutant-effect relationships of pollutant mixtures are predominantly restricted to pollutants corresponding to the same chemical group (e.g., sum PFAS, sum CHL, or POP mixture) (Fig. 6). Approaches to characterise and assess mixture exposure and emerging pollutants are rapidly developing, utilising multi-and non-targeted tools that are instrument based, effect-based (e.g., bioassays) or a combination of both [11,21]. Effect-based approaches consisting of *in vitro* techniques, such as bioassays based on cellular pathway activation at an early stage of toxicity, are particularly interesting as a complementing technique to traditional biomonitoring studies, as they can cover a wide spectrum of modes of action and thereby can provide information about the joint toxic potency of a mixture, as well as unknown emerging pollutants [10,21,3].

3.6. *Effects*

We have investigated the different toxicity endpoints across biological organisation levels to understand which perturbation of biological pathways in threatened marine mammals is studied. For eight species, pollutant-effect relationships on more than one biological organisation level are described, namely the Orca (*Orcinus orca*), Yangtze finless porpoise (*Neophocaena asiaeonientalis*), Steller sea lion (*Eumetopias jubatus*), False killer whale (*Pseudorca crassidens*), Walrus (*Odobenus rosmarus*), Indo-pacific humpback dolphin (*Sousa chinensis*), and polar bear (*Ursus martimus*) (Fig A.3). Remarkably, the polar bear is the only species in which pollutant-effect relationships are defined on all four levels of biological organisation, reporting 100 pollutant-effect relationships on a molecule/cellular level, 261 pollutant-effect relationships on a tissue level, 15 pollutant-effect relationships on an organism level, and four pollutant-effect relationships reported on a population level (Fig. 7).

Our findings unravel that most pollution-effect studies focus on early key events that occur on a molecular and cellular biological organisation level (Fig. 7) and thereby cover the early parts of adverse outcome pathways. Examples of molecular effects are up or down-regulation of a specific gene (e.g., reported by [68,55,37]) and inhibition of a certain receptor (e.g., reported by [82,56,43]) on a cellular scale. Cell death, phagocytosis, or inhibition of a cellular pathway are examples of effects on a cellular biological organisation level. On low biological levels of

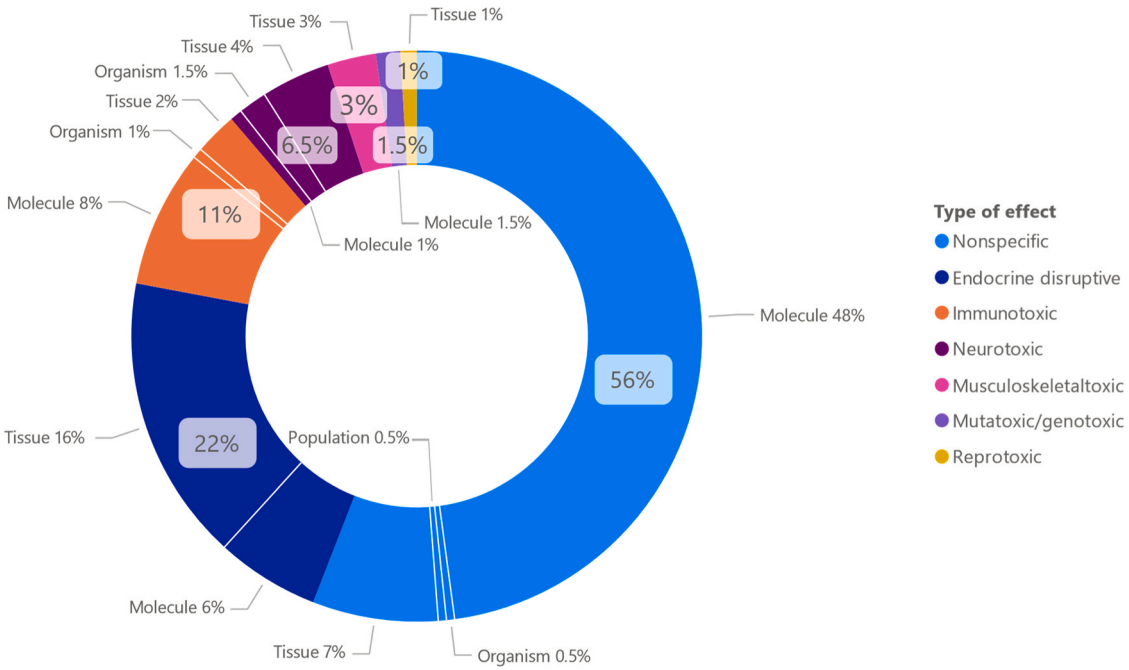

Fig. 7. Percentage of pollutant-effect relationships per toxicological endpoint including the biological organisation level. Non-specific – biological or physiological generic responses towards chemical pollution, endocrine disruptive - biological or physiological responses towards chemical pollution targeting the endocrine system, immunotoxic - biological or physiological responses towards chemical pollution targeting the immune system, neurotoxic - biological or physiological responses towards chemical pollution targeting the neurological system, musculoskeletal toxic - biological or physiological responses towards chemical pollution targeting the musculoskeletal system, mutatoxic/genotoxic - biological or physiological responses towards chemical pollution targeting the genetic system, the genetic system, reprotoxic - biological or physiological responses towards chemical pollution targeting the reproductive system. Molecule – effects on molecule and cellular biological organisation level, tissue – effects on tissue biological organisation level, organism – effects on organism biological organisation level, population – effects on population biological organisation level.

organisation effects were observed to be rather non-specific. Defined as non-specific effects from the adverse outcome pathway (AOP) perspective are responses such as CYP1A1 inhibition, PARGγ activation, cell death and phagocytosis made up 48% of a total of 56% of molecular effects (Fig. 7). Non-specific effects on a tissue level (e.g., haptoglobin levels in the blood) follow with 7% of the 56% of total non-specific pollutant-effect relationships, while effects on an organism (e.g., renal damage) and population level (e.g., body mass index) are limited and contribute with 0.5% respectively (Fig. 7). In contrast, effects are more specific with an increasing biological organisation level, which is reflected in the percentage of pollutant-effect relationships with endocrine disruptive, neurotoxic, musculoskeletal toxic, mutatoxic/genotoxic and reprotoxic properties.

Neurotoxic and immunotoxic pollutant-effect relationships are reported on organism or population level in IUCN red-listed species. Neurotoxic effects cover three biological organisation levels (all in polar bears) in which pollutant-effect relationships on the tissue level hold the largest share (4% of 6.5% total), followed up by organism (1.5%) and molecule (1%) respectively (Fig. 7). GABA (gamma aminobutyric acid) density across brain areas (organism level), MAO activity in the cerebellum (tissue level), and muscarinic acetylcholine receptor (mAChR) binding (molecule level) are clear examples of reported neurotoxic pollutant-effect relationships.

Immunotoxic effects are presented on three biological organisation levels (molecule, tissue and organism), in which effects on a molecule level are most predominantly reported with 8% of 11% total followed by tissue (2%) and organism level (1%) (Fig. 7). Typical immunotoxic effects presented in IUCN red-listed species are interleukin gene expression levels (molecule level), leukocyte viability in tissue (tissue level), and liver trematode (organism level). Unfortunately, certain types of effects are only studied at one biological organisation level, which includes musculoskeltaltoxic (tissue level; e.g., baculum bone length) and mutatoxic/genotoxic (molecule level; e.g., metaphase chromosome damage) effects (Fig. 7).

Pollutant-effect relationships affecting reproduction are hardly studied, with only 1% compared to pollutant-effect relationships affecting the endocrine system with 22% (Fig. 7). Scientists are increasingly looking into relating the disruptive endocrine properties of pollution to developmental and reproductive health effects seen in marine wildlife. Endocrine disruptive effects are predominantly presented at the tissue level (16% of 22% total), and less at the molecular level (6% of 22% total) (Fig. 7). Examples of endocrine disruptive effects on tissue level are testosterone levels in blood and estrogen receptor (ER) transcriptional activity on a molecular level.

Furthermore, chemical pollution can have direct and indirect effects at multiple levels of organisation (organism, population, species, and ecosystem) by influencing animal behaviour, such as increased or decreased predatory behaviour, or increased or decreased competition [84]. Altered behaviour reflects multiple physiological changes and links individuals to population-level processes [84]. Hence, this should be addressed in future research because behavioural studies were completely lacking in reported studies; however, they provide a valuable non-destructive method to have a first screening tool for any impact on populations. The second step would be a mechanistic approach to understand pollutant-effect relationships at the individual and population levels, which are essential to gain insight into these indirect processes [26].

Most pollutant-effect relationships researched in the literature for threatened species were non-specific, meaning that these effects are generic responses towards chemical pollution and can initiate multiple AOPs. Construction of AOPs is therefore not straightforward since the translation from molecule to organism may know an array of possible adverse outcomes. Nevertheless, the cytotoxicity endpoint has been used in modelling approaches to translate *in vitro* effects to in vivo levels [90].

Furthermore, non-specific endpoints can also be valuable in linking AOPs to generate an AOP network in which the potential impact of multiple pollutants could be assessed. Still, in light of this, our systematic and quantitative review revealed only one species with sufficient information available to conduct an AOP analysis, the polar bear. Ten mammalian AOPs were identified that contained similar specific and non-specific endpoints to polar bears, providing limited insight into the potential burden on the species represented in an AOP network.

3.7. Future directions

In human risk assessment, physiologically-based (toxico)kinetic modelling (PB(T)K) provides a sound scientific basis to interpret biomonitoring data. Such models translate specific biological (growth, diet), physiological (blood flow, heart rate), and biochemical processes (blood: tissue partitioning, assimilation efficiency) into a mathematical equation. PBK models usually consist of multiple tissue compartments connected by the circulating blood system of the body [99]. They integrate parameters dictating a compound's absorption, distribution, metabolism/detoxification, and excretion characteristics within these compartments. Such models already exist for harbour porpoises [100], pilot whales [102], and polar bears [19,87]. In marine mammals, such models can be evaluated with tissue concentrations detected in necropsied samples or non-destructively obtained metrics [20,70]. In addition, population characteristics can be studied by adding statistical supplementation (Monte Carlo simulations) [101]. These models can also be applied to estimate external exposure (intake) levels of chemicals (via reverse dosimetry) and further extrapolate routes of exposure, and exposure scenarios across species [70]. The OECD published a guidance document that provides a standardised approach to characterising and validating these models for regulatory purposes [67].

Most recently, PBK models have helped to facilitate quantitative *in vitro* to in vivo extrapolation (QIVIVE) approaches, enabling *in vitro* toxicity data to set safe intake levels in human risk assessment. They play a significant role in reducing animal testing and conducting risk assessments based on *in vitro* data [70]. However, QIVIVE modelling of pollutant-effect relationships in high-trophic marine species is still in its infancy. For example, one study has applied this approach to species-specific cell cultures in endangered marine wildlife. This study used the cell-based cytotoxicity results of primary cells extracted from green sea turtles' skin and correlated data with health parameters measured in the blood (*ex vivo*) quantitatively using a PBPK-based QIVIVE [20].

Our findings unraveled that most pollution-effect studies in threatened marine mammals focus on early key events that occur on a molecular and cellular biological organisation level and thereby cover the early parts of adverse outcome pathways. Furthermore, non-destructively obtained primary skin cells show promising applications for investigating species-specific effects of chemical exposure for highly threatened species. Therefore, we propose modelling approaches that can help (1) interpret biomonitoring data via PBK models and (2) ease the translation of molecular perturbations into organ, organism, or population effect data relevant for toxicological risk assessment for threatened marine mammal species (Fig. 8). Such applications can help to move away from arbitrary regulatory chemical threshold values towards science-based species-specific and chemical-specific safe limits

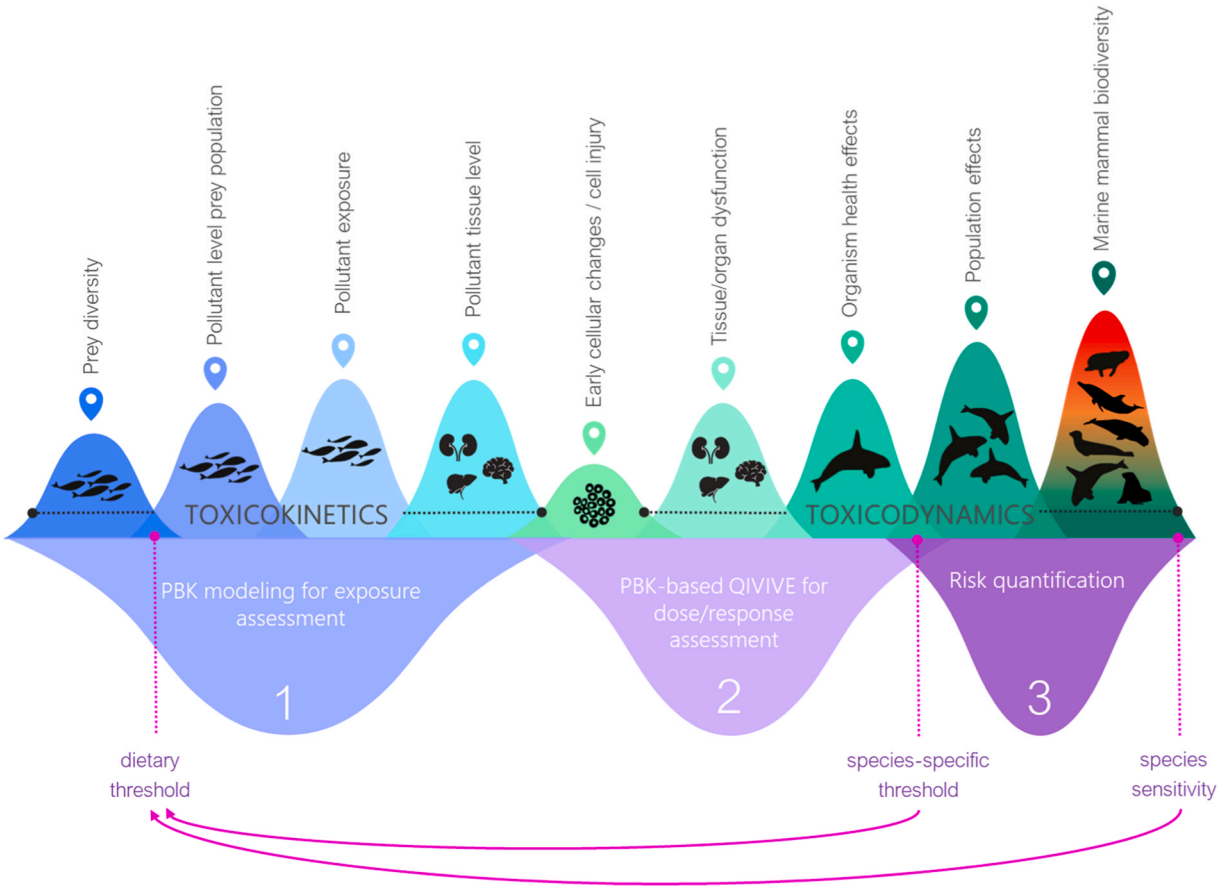

Fig. 8. Framework for conducting a mechanistic risk assessment in threatened wildlife species within a 3-step approach. 1. using PBK modelling to link external exposure via dietary input to internal concentrations and accumulation in different tissues of species; 2. linking tissue concentrations to effect concentration on a cellular level and extrapolating it to the higher biological organisation using PBK-base QIVIVE approach; 3. using the models in reverse mode (reverse dosimetry) reconstructing safe dietary threshold levels for risk assessment.

(Step 3 in Fig. 8). Hence, decision-makers can understand the safe boundaries marine wildlife populations can endure before their physiology is critically compromised.

4. Conclusions

The IUCN Red List plays a crucial role in guiding conservation decisions by providing valuable information about the range, population size, habitat and ecology, use and/or trade, threats and conservation actions of threatened marine mammals. However, current assessment criteria lack a quantitative cause-effect relationship to or species-specific sensitivity assessment of chemical pollutant exposure. The current systematic review identified critical gaps in our understanding of exposure-effect relationships in these species.

Our results showed a clear association between the higher extinction risk of marine mammal species and the scarcity of exposure-effect data available. Furthermore, we have identified a spatial bias in the literature, with a relative absence of studies in areas across climatic zones in the Southern Hemisphere. Even though some species may already be experiencing significant population decline (data deficient category), they are unlikely to be the focus of protection efforts in immediate future. This highlights the urgent need for increased research efforts on marine mammals facing a higher extinction risk and poorly researched regions, as well as data-deficient species. Doing so will ensure that comprehensive assessments and effective conservation strategies are put in place.

Our findings have discovered that most pollution-effect studies in threatened marine mammals focus on early key events that occur on a molecular and cellular biological organisation level, thereby covering only the early parts of adverse outcome pathways. As proposed, modelling approaches such as developing PBK-based QIVIVE would provide the means to ease the translation of molecular perturbations into organ, organism or population effect data relevant to toxicological risk assessment. However, physiological data for such models on threatened species may be limited. Thus, *in vitro*-derived parameters could serve as a surrogate and must be obtained. Overall, such applications can help to move away from arbitrary regulatory chemical threshold values, and decision-makers can understand the safe boundaries marine wildlife can endure before their physiology is compromised.

Environmental implication

Man-made release and enrichment of chemicals or engineered particles (e.g. plastics) in (marine) ecosystems are evolving rapidly and globally and reaching dangerous levels. However, governments or conservation measures fail to meet their responsibility to protect marine mammal species' biodiversity from chemical harm. This study systematically presents the available data (database in SI) on exposure and its effect on the most vulnerable marine mammal species (IUCN-listed) globally. By integrating novel computational and cell-based tools, this information can be applied within a new framework, as proposed in this article, to derive protective limits these species can endure before their physiology is compromised.

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CRediT authorship contribution statement

Iris Schaap: Conceptualisation, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Larissa Buedenbender**: Methodology, Formal analysis, Data curation, Writing – review & editing. **Sarah Johann**: Writing – review & editing. **Henner Hollert**: Writing – review & editing. **Gulsah Dogruer**: Conceptualization, Methodology, Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132203.

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