University of Veterinary Medicine Budapest

# Tumours of Marine Mammals and their connection to Persistent Organic Pollutants: A Literature Review

# Tengeri emlősök daganatai és összefüggésük a perzisztens szerves szennyezőanyagokkal: Irodalmi áttekintés

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# Abstract

Persistent organic pollutants (POPs) make up a substantial portion of the ocean's contaminants. While their acute, high-concentration encounters are mostly lethal, their chronic consequences show more complex pathogenesis. These pathogenic pathways are suspected of having both direct and indirect POP aetiology.

Direct mutagenic effects through Aryl hydrocarbon Receptor (AhR) activation, DNA adduction or in utero foetal maldifferentiation have been associated with long-term POP accumulation in terrestrial mammals, marine mammals, and humans. Indirect effects through the CYP-enzyme biotransformation and MFO system are not fully understood yet.

Such effects lead to tumour formations in assorted tissues, including the liver, digestive tract, skin, lung, urogenital tract and lymphatic system of both cetaceans and pinnipeds.

Marine mammals serve as ocean health sentinels, which highlights the importance of further research, to fully understand POPs impact on environmental and human health.



# Absztrakt

A perzisztens szerves szennyezőanyagok (POP-ok) teszik ki az óceán szennyezőanyagainak jelentős részét. Míg heveny esetben, nagy koncentrációban többnyire halálosak, krónikus következményeik összetettebb patogenezist mutatnak. Feltételezhető, hogy a POP-ok kórtani hatásai részben közvetlenül, részben közvetett módon érvényesülnek.

Az Aryl hydrocarbon Receptor (AhR) aktiválásán, a DNS-addukción vagy a méhen belüli magzati maldifferenciálódáson keresztüli közvetlen mutagén hatások összefüggésbe hozhatók a szárazföldi emlősökben, tengeri emlősökben és emberekben a POP-ok hosszú távú felhalmozódásával. A CYP-enzim biotranszformációján és az MFO rendszeren keresztüli közvetett hatások még nem teljesen ismertek.

Az ilyen hatások különféle szövetekben daganatképződéshez vezetnek, beleértve a cetfélék és az úszólábúak máját, emésztőrendszerét, bőrét, tüdejét, urogenitális traktusát és nyirokrendszerét.

A tengeri emlősök az óceán egészségének az őrszemei, ami rávilágít a további kutatások fontosságára a POP-ok környezetre és emberi egészségre gyakorolt hatásának teljes megértése érdekében.

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# Abbreviations

ACA: Adenocarcinoma

AhR: Aryl hydrocarbon Receptor

CYP: Cytochrome P450

DDTs: Dichlorodiphenyltrichloroethane

ECM: Extracellular Matrix

FAO: Food and Agriculture Organization of the United Nations

HCH: Hexachlorocyclohexanes

IARC: International Agency for Research on Cancer

MFO: Mixed function oxidase

OCs: Organochlorines

PBDEs: Polybrominated diphenyl ethers

PCBs: Polychlorinated biphenyls

POPs: Persistent organic pollutants

SCC: Squamous cell carcinoma

TCC: Transitional cell carcinoma

TCDD: Tetrachlorodibenzo-p-dioxin

TEF: Toxic equivalency factor

TME: Tissue Microenvironment

TSG: Tumour Suppressor Genes

WHO: World Health Organization

# 1. Introduction

POPs: persistent organic pollutants are toxic organic chemical compounds used in industrial electric power transmissions, insulations, machinery, paints, and pesticides. They were predominantly produced from the 1930s until the 1980s. Human illnesses resulting from contaminated rice oil with PCBs in 1966 were the first time, these contaminants were recognized in environmental samples [1]. When their toxic traits became better known; the Stockholm Convention Treaty was initiated. The treaty is to aid the protection of human health and the environment from POPs. Nowadays 181 countries have signed the treaty vouching for phasing out the production and usage of machinery with POPs by 2025 and eventually eliminating their storage by 2028. At this present time, only 17% (equivalating 3 tons of waste material) have been eliminated [2]. Nonetheless, not all POP subgroups, such as the PBDEs, are regulated globally and course through the human and wildlife food chains unmonitored.

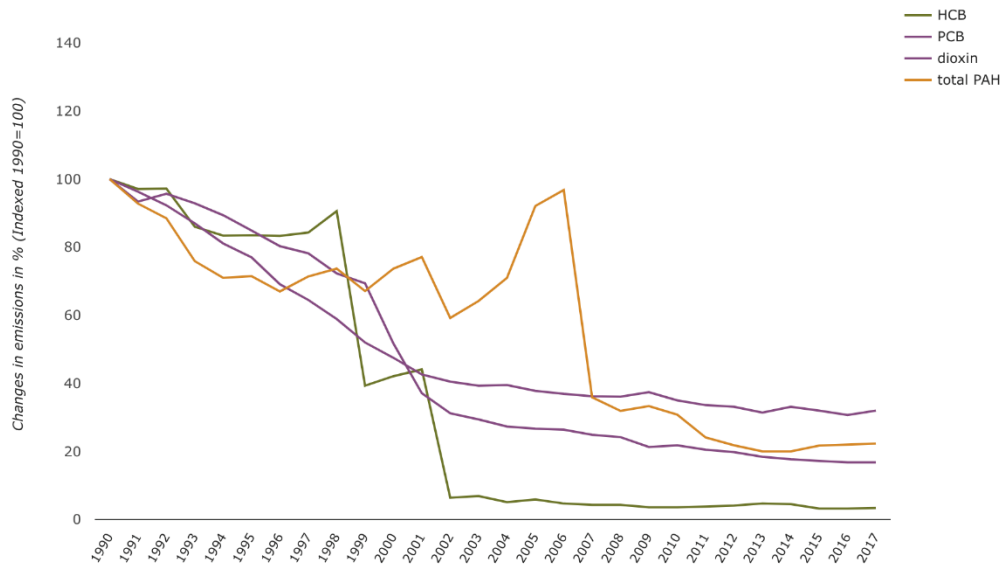


**Figure 1:** Emission trends of persistent organic pollutants [3]

POP production has substantially reduced since the 1990s, as illustrated in Figure 1. Comparing marine mammal and fish tissue samples monitored for POP concentrations; a decreasing trend can also be detected. It is impossible to pinpoint exact POP levels in the oceans as they fluctuate depending on weather, season, and coastal proximity. As wild marine mammals are not bound by jurisdictional borders, variability is extreme. Mass mortality events of marine mammals and

other ocean inhabitants have been caused by chemical spills before. In these cases, acute exposure led to lethal outcomes. Chronic exposure however brings a challenge to any scientist to put together accumulative factors to pathologic lesions showing several decades later or even jump to the next generation.

Marine mammals are among the largest long-living mammals alive. Less so pinnipeds, but cetaceans' life span can stretch from 17 years for Pygmy sperm whales to 211 years in the bowhead whale. Besides longevity, cetaceans' weight spectra also hold large ranges from 50-kg Maui dolphins to the 175-ton blue whale. Both their longevity and weight have puzzled researchers.

Peto's paradox, an observation made by Peto in 1977, discussed the risk of developing cancer should ultimately increase with size as there are a higher number of cell divisions and hence a higher risk of DNA damage and mutation. A paradox that remained unanswered until the depiction of the genome.

Genes have been linked to certain diseases for most mammals, many of such cancer-related. Through accelerated evolution, cetaceans developed a positive selection within the CXCR2 gene. This gene regulates DNA damage and the immune response to tumour propagation. Furthermore, cetaceans' tumour suppressor genes (TSGs) have a 2.4-times higher turnover rate than any other mammal. Whales belonging to the Mysticeti order, whose members are among the largest of cetaceans, showed an even faster TSG turnover rate. These genetic traits favoured marine mammals to longevity, gigantism and often thought cancer resistance [4].

The aim of this thesis is to compile research data relevant to tumours arising from long-term POP exposure in marine mammals. By comparing POP target cells and receptors, to these tissues' neoplastic changes reported by veterinary pathologists and marine biologists from all around the world. The focus will be put on the pathways of accumulation and possible causes for the tumours, by comparative analysis of published evidence regarding human and other mammal species. The topic of this thesis has been chosen to expand personal knowledge of marine mammals, with respect to future interests in environmental health and ocean wildlife preservation.

# 2. Literature review

## 2.1 Biological accumulation

Once in the environment, it as the name hints persists and accumulates. One of the most abundant and well-known POPs are PCBs, DDTs, PBDEs, TCDD, and OCs. Different isomers and forms of these groups vary in toxicity and bioaccumulation, although most studies do not differentiate. These chlorinated and lipophilic chemicals accumulate in adipose tissue and bio-magnify up the food chain [2]. Against the primary belief, that POPs dissolve in adipose tissue, it was later established that they merely deposit in it and bind to lipoproteins and albumin in blood circulation [5].

### 2.1.1 Pinnipeds

Trophic position and geographical range expose California sea lions (*Zalophus californianus*) to some of the highest levels of contaminants worldwide. Females are gregarious and do not migrate as far as the males outside the breeding season, making tracking and research convenient [6].

#### 2.1.1.1 Intrauterine exposure and Transplacental transfer

Exposure to POPs starts in-utero. During the 11-month gestation period, POPs transfer from the mother to the foetus via the placenta. As Albumin does not cross the placental barrier, the POPs attached to lipoproteins, such as cholesterol and triglycerides within the blood circulation; cross, distribute and deposit. The blubber concentration of pollutants is lower in the premature group than among the late-term group, most likely due to the higher adipose tissue ratio of foetuses closer to term and the longer accumulation time [7].

Theories mentioned whether the histologic differences in placental types would alter the POPs' ability to enter the foetus's bloodstream. However, no comparative studies have been published regarding marine mammals. Additionally, as all placenta types allow lipoproteins to pass as they are vital for mammalian foetal development, it may be insignificant. Although certain compounds are known to bypass tissue barriers more effectively due to their chemical composure and molecular weight.

**Table 1:** POP blubber concentration[1] and their transplacental transference[2] to the foetuses at different stages of pregnancy [7]

| | Mother | Premature | Late-term |
|---|---|---|---|
| DDTs' concentration | 18,567 | 14,560 | 22,738 |
| PCBs' concentration | 7,096 | 5,449 | 6,831 |
| DDTs' transfer | | 78.42 | 122.46 |
| PCBs' transfer | | 76.79 | 96.27 |

[1]All concentrations are reported as ng/g on a lipid weight basis, [2]Percentile transfer from the mother to the foetus

The sample concentrations in Table 1 show that 78.42% of the mother's DDT lipid concentration is passed on to the premature foetus. The late-term foetuses showing a 122.46% transfer concentration can be explained by the difference in body fat as the mother's mean lipid percentage is 25% more than the late-term foetuses [7].

At the time of birth, the pups are starting their lives carrying 122.46% of their mother's DDT levels and 96.27% of their mother's PCB levels. Meaning that throughout the decades of exposure the blubber POP levels have exponentially increased with every generation, even though emission rates are decreasing.

The concentration ratios in foetal and maternal blubbers were further examined to distinguish if any contaminant group was abundant. ΣDDTs showed a mean ratio concentration of 2.8 and ΣPCB of 3.2 [7].

PCBs being in the largest quantities can be explained by the longer half-life which ranges between 10-15 years while DDTs only last months in aquatic surroundings due to their photodegrading property and faster metabolism through the liver.

#### 2.1.1.2 Lactation and nursing

Further pollutant transference transpires during nursing. Mating, birthing, and nursing take place on land, consequently meaning a fasting period for the pinnipeds. The most common energy source for mammals is polymerized glycogen stores in the liver and muscle. Despite their importance, glycogen stores are small in marine mammals which makes lipids, stored as fat, even more essential [8]. The hypodermal blubber storages from winter and spring sustain the animals for these weeks however the lipid mobilization also causes shifts in the POPs deposits.

The ratios of adipose tissue to contaminant levels change unfavourably. In blubber samples comparing contaminant concentrations from California sea lions associated with weight loss, and gain during rehabilitation, the natural annual fasting period was simulated. The results showed the highest concentration of contaminants when the seals in question were at their lowest body mass and 89% showed an increased ratio during rehabilitation and weight gain. Meaning that only a low percentile of contaminants metabolized and left the body, while the others were yet again diluted through weight gain [9]. It is important to mention, that none of the dams included in this study were pregnant at that time, as this shows that not blubber loss alone can account for a decrease in POP tissue levels.

Despite that, it highly depends on the POPs in question. Several biochemical factors such as structure, lipophilicity, excretion, and redistribution kinetics, determine the changes. Chlorinated compounds of PCB, HCHs, and chlordane showed a larger decrease in concentration than any other compounds. As chlorination favours excretory metabolisms, the chlorinated compounds and their isomers may get excreted via urine and hence the trend was detected. Furthermore, the most abundant group found were DDTs, which compared to PCBs have a more active metabolism as mentioned earlier [9].

Pinnipeds' nursing duration varies greatly from only several days to up to 12 months depending on the scavenging possibilities and security of the birthing location. *Zalophus californianus* dams usually wean their pups at approximately 7 months of age.

Mothers spend the first week postpartum fully on land nurturing the newborn before taking turns of feeding trips in the water and suckling intervals on land. Even though females forage during lactation, their bodies still experience negative energy balance as shown by the presence of ketone bodies via triglyceride mobilization [10].

The energy shift and triglyceride storages being mobilized concurrently mobilize contaminants that deposit in the mammary tissue and milk.

Pinniped milk has an exceptionally high lipid percentage. In comparison: the average fat content of raw bovine milk is 4% whereas pinnipeds have an average of 45% [11].

As aforementioned, POPs bind and circulate while attached to lipoproteins. There are various studies on blood transport mechanisms and tissue transference. Tests showed such a high capacity for transport that the researchers concluded it's highly unlikely that pollutants transport is only mediated by specific binding sites and proteins.

While extraction proved difficult the transference from blood to tissue was rapid and near complete. Meaning that absorption from the pup's gastrointestinal tract, favoured by a simple concentration gradient, can account for the POPs absorption. From there the contaminants travel through the circulatory system and lastly deposit in the blubber. Inevitably, newborns most likely receive most of their hereditary bio-load of pollutants during nursing [5, 7].

#### 2.1.1.3 Gender differences

Researchers noticed the trend, that adult females had a lower PCB and DDT tissue concentration than adult males. Like in most mammalian species, marine mammal females tend to have a higher body fat percentage due to faster lipase production from sex hormones. Deducting from this, it was thought that adult females would have a higher blubber contaminant level coming with a higher body fat percentage.

However, transplacental transfer and nursing were not considered. As established above, POPs barely get metabolized, therefore the main way to lose contaminants is by passing them on to the offspring [7]. But the loss of POPs during the female annual reproductive cycle does not counterbalance the continuous uptake during feeding.

#### 2.1.1.4 Accumulation through feedstuff

Continuous uptake through feeding is inescapable. Marine mammals usually hold the top position in the ocean's complex food chain. Global studies demonstrated that xenobiotic substances are present even in the most remote areas of the world [1].

California Sea Lions mainly feed on market squid, pacific whiting, rockfish, jack, and pacific mackerel. A study on Harbour seal (*Phoca vitulina)* diets POP levels conducted in Washington USA, showed that the seals there had a seven times higher contaminants level than the Harbour seal colony in British Columbia, Canada. The study also showed that the estimated daily intake of PCBs exceeded the concentration guidelines of daily exposure, set by the WHO, as visible in Table 2 [12].

Harbour seals' and California sea lions' prey species are overlapping. Considering the flow direction of the north pacific gyre of the golf stream, both prey fish and contaminants travel south from Washington towards California USA. Therefore, POP levels in harbour seals' and the California sea lions' food baskets are comparable.

**Table 2:** Food basket contaminant concentrations [12] compared to U.S [13] and WHO/FAO guidelines values [14]

| Contaminants | Food basket contaminant concentration (µg/kg lipid ww) | WHO/FAO guideline values (µg/kg/day) |
|---|---|---|
| ΣPCBs | 2,904.93 | 6.0 |
| ΣDDTs | 253.20 | 1.0 |

ww: wet weight

As seen in Table 2, the mean PCB and DDT values highly outnumber the recommended health guidelines. Although these guideline standards are calculated for human toxicokinetics, there are barely any marine mammal standards set. Toxicokinetic values also differ regarding different organs. Harbour seals have an immunotoxic threshold of 17 µg/kg lipid while their endocrine altering threshold is as low as 1.3 µg/kg lipid [15].

As marine mammals outweigh most humans in body weight and body fat percentage, the toxicokinetics differs and the WHO guidelines should only be used as a rough comparison.

However, these values outnumber the standards by 484 times for PCBs and 253 times for DDTs and therefore the supposition of health risks for pinnipeds can be confidently deposed.

### 2.1.2 Cetaceans

Cetaceans' highly specialized physiology predisposes them to xenobiotic pollution. Cetaceans, even more so as pinnipeds, have adapted to their endothermic surrounding with their thick blubber layer. Giving lipophilic chemicals more space to accumulate.

The oxygen stores that enable cetaceans to dive for such a long duration is part of an exquisitely complicated cascade. Simply put, when they dive, oxygen supply is prioritized to the heart and brain. Not only are these vital organs therefore supplied with a higher concentration of in-blood lipoprotein-bound-POPs, but also a reduced biotransformation and excretion rate through the liver and kidneys occurs [8].

#### 2.1.2.1 Intrauterine exposure and Transplacental transfer

Same as in the seals, POP exposure starts in utero. Studies on placental toxin transfer are rare as samples from healthy foetal tissue and pregnant females are difficult to acquire. A unique study on free-ranging healthy arctic beluga whales resulted from collaboration with Inuvialuit community hunters aiding surveillance and sample retrieval, of these protected animals.

With the geographics of the animals' habitats being a secluded non-industrialized area, it is not surprising that blubber PBDE levels were far less than those measured in the St. Lawrence (525 ng/g) and Norwegian (72 ng/g) beluga population [16].

**Table 3**: Transplacental transfer of POPs from mother to foetus of Artic beluga whales (*Delphinapterus leucas*), taken in Northwest Territories of Canada [16]

| POPs | Mother concentrations (ng/g lw) | Foetus concentration (ng/g lw) | Transfer (%) |
|---|---|---|---|
| ΣPCB | 310 | 257 | 11.4 |
| ΣPBDE | 5.5 | 3.8 | 11.1 |

lw= lipid weight

Comparing the transfer percentages of Tables 1 and 3, cetacean foetuses are transferred far less POP during in utero development. Partition ratios suggest a similar transfer mechanism like in California sea lions, with a trend of congeners of fewer chlorine atoms and lower molecular weights having a higher transfer proportion. However, it is important to point out that results support theories of species-specific transfer characteristics.

Models on the St. Lawrence beluga whale used adapted figures from Dall´s porpoises, to estimate transplacental transfer ratios. The Artic beluga whale study showed a higher POP transfer than the St. Lawrence model, even though POP burdens are lower, further supporting species-specifics. This highlights the gravity of continuing research to understand pollution burdens on these ocean health sentinels [16].

#### 2.1.2.2 Lactation and nursing

Lactational transfer of POPs is estimated to make up 85% of reproductive transfer and POP inheritance between calves and mothers. As discussed above, transplacental transfer varies with species, making up 2.6-3.5% in melon-headed whales and 11.1-11.4% in belugas (Table 3) [16, 17]. Same as in Pinnipeds, the onset of lactation triggers POP mobilization, leading to high in-milk concentrations. Logically, the more lipid storages are exhausted during nursing, the more xenobiotics are mobilized from the blubber.

For hydrodynamic purposes, cetaceans only have two mammary teats located in slits on either side of the genital orifice. As whales lack lips, calves can´t form a closed space between the teat and their oral cavity. Myoepithelial cells in the mammary glands eject milk into the calf's mouth when stimulated by tongue-suction, requiring more energy.

Post-partum fasting also occurs in cetaceans. Through the vulnerability of the calve, for relying on the mother for protection, feeding, and aiding to breathe, parental care is extensive. Feeding and fasting strategies differ between Mysticeti and Odontocete cetaceans. Odontoceti females have been seen to nurse their offspring for several years by maintaining to forage during nursing ultimately teaching their offspring how to hunt, while Mysticeti females fast during nursing followed by an abrupt weaning [8].

Regarding these timed differences, it would be interesting to know whether longer nursing time equals a higher POP transfer, but comparative data are lacking.

Behavioural researchers described the trend of larger females, nursing longer which may be explained by bigger lipid storages and therefore longer fasting endurance [8]. According to a study on Harbour porpoises, smaller females had higher PCB milk levels than larger females [18]. Which may balance out duration versus concentration.

Nonetheless, the negative energy balance during nursing prevails. POP milk levels fluctuated throughout lactation and are highest during early lactation, measuring as high as 17.55 mg/kg lipid weight PCB and 0.29 mg/kg lipid weight of PBDEs [18].

#### 2.1.2.3 Accumulation through feedstuff

The order of cetaceans is divided into 2 major groups: the Mysticeti and the Odontoceti. Mysticeti are edentulous whales, while Odontoceti are the toothed whales and dolphins. Members of the Mysticeti clade feed using the lunge-and-gulp technique. They accelerate towards the prey and open their jaws up to a 90-degree angle. The pleats: creases located on the ventral side, extend from the mandibular symphysis to the navel and can expand up to four times their resting width.

After gulping, the prey-loaded water is filtered through the baleen plates and fringes, fibrous mats where the small animals get filtered out from the water. Mysticeti, also known as Baleen whales, evolved from toothed whales to adapt to small-sized prey found in aggregations, such as krill. Krills, being near the bottom of the food chain contain lower amounts of POPs than a larger prey fish with a longer life span and higher fat percentage, such as Salmon [19, 20]. This is evident by the assumption that POPs accumulate throughout life, which is directly correlated to a fish's size: an older fish is generally larger.

Odontoceti whales are active hunters, preying on large-bodied prey. Depending on the species in question, this varies from feeding on mackerel, squid, and salmon (like bottlenose dolphins, pinnipeds, and porpoises) to tuna (like killer whales). As already discussed, fish such as tuna, with a higher trophic level would have accumulated xenobiotics by feeding on lower trophic inhabitants. The pinnipeds and porpoises that are killer whale preys are the feed source with the highest POP levels.

Some fish such as the pacific herring overlap as a feed source for both smaller Odontoceti and Mysticeti order members, which is interesting as these fish are known to be considerably contaminated by POPs [15, 21].

### 2.1.3 Sirenians

The order Sirenia includes manatees and dugongs. The only herbivorous marine mammals. Being less susceptible to high contaminant concentrations, than their carnivorous peers, does not guard them against accumulation via ingestion of contaminated sediment. POP sedimentation is especially high in urban areas, close to river runoffs; a typical sirenian habitat [22].

The beluga whale, a carnivorous marine mammal, is also known to ingest sediment while feeding. Lethal health issues from chronic exposure such as malignant gastrointestinal carcinomas have been reported. The connection between sediment ingestion and gastrointestinal tumours will be discussed in chapter 2.2.2 digestive tract tumours. But data on sirenian blubber POP levels and long-term exposure consequences is scarce.

## 2.2 Health consequences and tumour prevalence

Tumours, in most cases, are considered a chronic development and consequence of many different factors, some of being a weakened immune system. Marine mammals are long-living marine inhabitants and have long-term exposure to precursors.

The cascade sequences of events leading to neoplastic growths are still not fully understood, but POPs are regarded as oncogene-promoters. Nonetheless the pervasiveness of neoplasia during marine mammal necropsies, it is difficult to determine whether the tumour is an ancillary finding or the cause of death.

First recordings of cancer in pinnipeds were made in California sea lions in 1979 when the population was suffering from transitional cell carcinomas primarily in the urogenital tract of females [23]. The first cancer among cetaceans was reported as early as 1962, in Fin whales [24]. Before that, tumours were rare pathological findings in stranded marine mammals and were thought to be an anomaly. After, 1983 the Canadian St. Lawrence beluga whale population showed such a high cancer mortality rate, that researchers were concerned that despite the hunting ban, the population was not recovering [25]. And thirdly, dolphins off the coast of Florida USA dying of immunoblastic malignant lymphomas was the first documented malignancy in dolphins [24].

Over the next decades, tumours were monitored throughout many long-living marine inhabitants, detecting an amplifying prevalence and omnipresent problem. The monitoring of such developments is important as most cetaceans and pinnipeds occupy high trophic levels and act as sentinels of ocean health.

In statistical comparison with pet animals, the numbers of cancer cases reported are low. The limitations of monitoring free-ranging animals, the rushed necropsies of stranded marine mammals, and the natural decay of the corpses before being spotted altering histopathologic results, are only some of the reasons why data is not as plentiful as with pet animals[8, 26].

Xenobiotics in acute high concentrations from oil or chemical spills have immediate effects, extending from toxaemia to suffocation. Lower concentrations facilitate chronic systemic changes ranging from cell-mediated immune system suppression, hormone insufficiencies, reproductive failures, and malformations as shown in Table 4 [1]. Regardless of the concentration or the type of xenobiotic present, the body attempts to metabolize these.

**Table 4:** Associations between organochlorines and marine mammal reproductive, hormonal, and morphological disorders [1]

| Phenomenon | Where in the process | Species | Mode of action | Contaminants |
|---|---|---|---|---|
| Implantation failure | Implantation | Harbour Seal, Beluga Whale | Enhanced hormone metabolism | PCBs and their metabolites |
| Stenosis and occlusions | Postpartum | Grey and Ringed Seals | Organochlorine-induced uterine lesions | PCBs, DDE, methylsulfones |
| Premature pupping | Late gestation | California Sea Lion | Microsomal enzyme induction; steroid mimicking | PCBs, DDTs |
| Low Vitamin A, Thyroid hormones | All ages | Harbour Seal | Binding competition | PCBs and metabolites |
| Reduced testosterone | Adults | Dall´s Porpoise | Unknown | PCBs and DDE |
| Lowered estradiol level | Implantation | Harbour Seal | Enhanced hormone metabolism/ enzyme induction | PCBs and metabolites |
| Skull lesions: osteoporosis, periodontitis | Predominantly adults | Harbour and Gray Seals | Infection/ hyperadrenocortical | PCBs, DDTs and their metabolites |
| Exostosis | All ages | Harbour Seal | Unknown | PCBs, DDTs and their metabolites |
| Testis abnormalities | Immatures and adults | Mink Whale | Unknown | Organochlorines |
| Adrenal hyperplasia | Unknown | Beluga Whale | Unknown | Organochlorines |
| Hermaphroditism | Foetal | Beluga Whale | Genetic/ environmental | PCBs and DDTs |

POPs are dioxin-laced chemical compounds. Each compound differs in toxicity and other biochemical properties. Among them, TCDD is known as the world's most toxic pollutant and has been listed as a Group I carcinogen by the IARC.

Dioxins bind to the aryl hydrocarbon receptors (AhR), which function as a ligand-activated transcription factor encoding xenobiotic metabolizing enzymes such as CYP1A1, CYP1A2, and glutathione S-transferase Ya. Upon binding AhR is transferred into the nucleus and forms the aryl hydrocarbon nuclear translocator (ARNT) protein which then binds to the xenobiotic responsive element (XRE) on the gene, ultimately leading to these genes' alteration. The adverse reaction of these oxidative metabolizing enzymes is the production of highly carcinogenic metabolites [27, 28]. Meaning the more dioxin-laced chemicals are present in the body, the more carcinogenic metabolites are produced, and the more gene alterations occur.

CYP enzymes bio-transform complex organic poisons, such as PCBs, via conjugation with oxygen and organochlorine substrates to form hydroxylated metabolites which make them more easily excretable [8, 29]. This metabolism is better known as the MFO system. The carcinogenic and procarcinogen metabolites that are formed, have five main modes of action: 1) DNA adduction, 2) epoxide intermediates with high reactivity making them mutagenic, 3) toxic O-esterification, 4) activation of nitrosamines to unstable metabolites, and 5) free radical formation/oxidative stress [30].

CYP enzymes are most abundantly found in the endoplasmic reticulum of the liver, but also occur in the small intestine, skin, lungs, placenta, and kidney [31, 32].

### 2.2.1 Liver tumours

The liver is considered the main organ involved in detoxification. Liver disorders are relatively common in wild and captive marine mammals. Hepatic lipidosis, hepatitis, and diffuse and focal cirrhosis are the most common of such.

In comparison with terrestrial mammals, especially cetaceans have large liver that tends to enlarge even further during pregnancy due to increased fat deposition [29]. The physiological fat deposition in the liver and muscles likely predisposes marine mammals to liver diseases.

**Table 5**: Reported neoplasms of the Liver in marine mammals [26]

| Species affected | Reported neoplasm |
|---|---|
| Bowhead Whale (*Balaena mysticetus*) | Lipoma |
| Blue Whale (*Balaenoptera musculus*) | Lipoma |
| Sperm Whale (*Physter macrocephalus*) | Haemangioma |
| Beluga Whale (*Delphinapterus leucas*) | Hepatocellular carcinoma |
| Bottlenose Dolphin (*Tursiops truncatus*) | Adenoma, Reticuloendotheliosis |
| California Sea Lions (*Zalophus californianus*) | Hepatic carcinoma, Bile-duct Adenocarcinoma |
| Fur Seal (*Callorhinis ursinis*) | Lipoma |
| Northern Elephant Seal (*Mirounga angustirostris*) | Gall bladder Adenocarcinoma |

As evident in Table 5, the most widespread liver neoplasm reported is the hepatic lipoma. Hepatic lipomas are benign, marginated masses composed of mature adipocytes without evidence of cellular atypia [33]. Research regarding the consequences of continued oxidative

stress caused by chronic toxin exposure in marine mammals is limited, but very well explored in humans, illustrated in Figure 2.

Cellular biochemistry of oxidative stress including the MFO system is mostly identical for all mammals, making human research applicable for discussion. Further factors as discussed in the above's biological accumulation chapter, of POP exposure levels being several hundred times larger than WHO guidelines, should be kept in mind. Evidence suggests that marine mammals must cope with far more oxidative stress than most humans, all while the MFO systems exhaust through chronic exposure leading to cell trauma.

Even though hepatic lipomas mainly remain asymptomatic, a statistically significant relationship to steatosis was determined in humans. 50% of patients with hepatic lipomas developed steatosis whereas only 9% of patients with non-lipomatous lesions also developed steatosis. Steatosis is histologically defined as an abnormal accumulation of cytoplasmic fat vacuoles resulting from an excessive number of triglycerides within hepatocytes [34]. Better known as fatty liver disease, if left untreated, progresses into liver cirrhosis and acts as a precursor to malignant neoplasms.

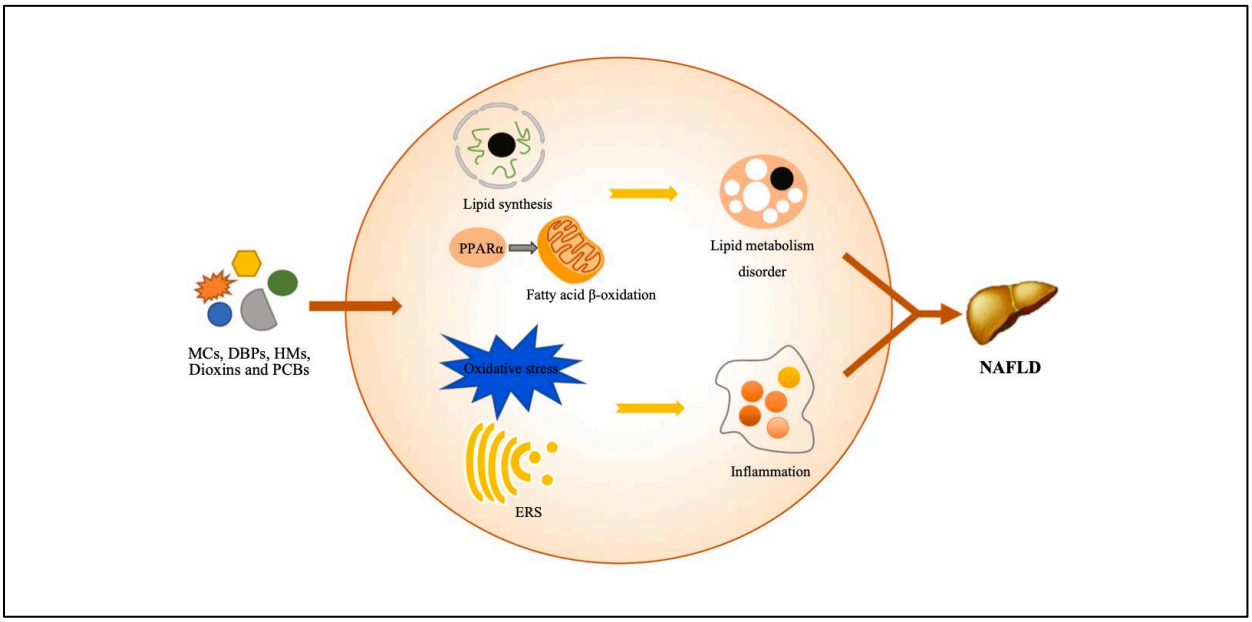


**Figure 2:** The common molecular mechanism of Fatty Liver Disease induced by environmental contaminants in water resources including dioxins and PCBs [28]

MC´s= microcystins, DBPs= chlorination disinfection by-products, HMs= Heavy Metals, PPAR= peroxisome proliferator-activated receptor, ERS= Endoplasmic Reticulum, NAFLD= Non-alcoholic fatty liver disease

Especially proteins and lipids are targets of oxidative attacks, during which the modification of these molecules increase the risk of mutagenesis [35]. TCDD, the chemical abbreviation for dioxin, being a class I carcinogen was found to be especially hepatotoxic and has been used to standardize biomarkers in MFO activity and many other bioassays [29].

Dioxins such as PCBs and DDTs are linked to fatty liver disease, hepatocyte hypertrophy, and centrilobular necrosis, therefrom resulting in cirrhosis, and from such chronic lesions developing hepatocellular carcinomas [28, 36]. These malignant neoplasms derive from hepatocytes and even though distant metastasis is uncommon, most likely will spread to lymph nodes within the cranial abdomen and lungs [37].

It is yet to be answered whether lipomas are direct precursors to malignant manifestations. Even so, the logical progression of events suggests it.

To identify where metastatic epithelial neoplasms originated, Immunohistochemical tests can be performed. A study on California Sea Lion tissues showed a positive cytokeratin 2 reaction meaning the carcinoma was of hepatic origin [38]. Cytokeratins (CK) are cytoskeletal proteins whose main function is to enable cells to withstand mechanical stress; therefore, their expression reflects tumour activity. The expression of CK is organ or tissue-specific; CK-2 reacts with hepatocytes and hepatocellular carcinomas [26, 37].

Despite the above's immunohistochemical findings, it is estimated that most tumours in California Sea Lions are of urogenital origins with liver metastasis, as seen in Figure 3.

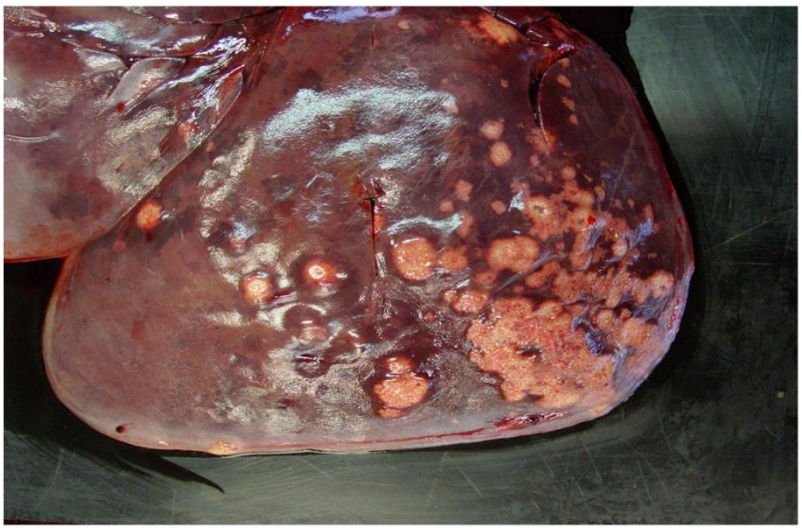

**Figure 3:** Foci of metastasis present within the hepatic parenchyma of a 25-year-old male California Sea lion [39]

Gall bladder and bile duct adenocarcinomas have only been reported in pinnipeds. Anatomical differences between marine mammals are responsible. While the pinniped gallbladder is large, cetaceans do not have gallbladders but instead, relatively wide distal bile ducts and other duodenal pouches. Cholangiocarcinomas has mostly been reported in California Sea Lions, which have an unusually high incidence of cancer compared to other pinnipeds. The aetiology of biliary adenocarcinomas remains unknown but has been associated with liver fluke infections in both humans and California Sea Lions but not in Northern Elephant Seals [40]. Liver fluke infections are known to exponentially increase with age, the same as tumour prevalence.

### 2.2.2 Digestive tract tumours

A large portion of POPs are accumulated in the food chain with a positive correlation among the trophic levels. Throughout the digestive processes, pollutants are set free via digestive enzymes and reabsorbed by the intestinal blood barrier. By the presence of xenobiotics in the gut lumen, Ah receptors activate gut immune cells. Through the constant inflammatory activity, immune cells deplete and tissue trauma via the MFO system leads to fibrosis. Such stiffening of mesenchymal and epithelial layers compromises anti-tumour immunity. ECM remodelling and fibrotic stiffening of the stroma have been linked to the degree of malignancy. Fibrosis decreases tissue mechanics and function and has been recognized as an important component of TME and a regulator of tumour aggression [41]. The digestive system tumours of cetaceans and pinnipeds vary greatly in their malignancy and location.

Through sudden creation of open space, the Mysticeti whales suck in the prey-loaded water. To make room for the huge volume, the tongue is only weakly muscularized and mainly composed of elastic and fatty tissue that can retract into the cavum ventrale of the buccal cavity [42]. With the tongue having a higher connective tissue percentile, it increases their chances of developing connective tissue tumours such as fibromas.

Being lipophilic and accumulating in fat tissue, a fish with a higher body fat ratio will carry a higher tissue contaminant level. Therefore, Baleen whales feeding on members of lower trophic levels are exposed to lower POP concentrations. That, and their higher TSG turnover rate, could explain the benign mesenchymal tumours reported in Fin, Humpback, and Sperm whales. As well as benign epithelial tumours like the tongue papilloma and upper lip benign melanoma in Blue and Sei Whales, as displayed in Table 6.

**Table 6**: Reported neoplasms of the Digestive tract in marine mammals [26]

| Species affected | Location and type of neoplasm | | | | | | |
|---|---|---|---|---|---|---|---|
| | Upper GI | | | | | Lower GI | |
| | Upper lip | Jaw | Tongue | Pharynx | Gingiva | Gastric | Intestinal |
| Fin Whale (*Balaenoptera physalus*) | | | Fibroma | | | | |
| Humpback Whale (*Megaptera novaeangliae*) | | | Fibroma | | | | |
| Blue Whale (*Balaenoptera musculus*) | | | Papilloma | | | Lipoma | Intestinal serosa Lipoma |
| Sei Whale (*Balaenoptera borealis*) | Benign melanoma | | | | | | |
| Sperm Whale (*Physter macrocephalus*) | | Fibroma | | | | | |
| Beluga Whale (*Delphinapterus leucas*) | | | SCC, ACA | | | ACA, Papilloma | ACA |
| Bottlenose Dolphin (*Tursiops truncatus*) | | | Sublingual SCC | | SCC | | |
| Pacific Whitesided Dolphin (*Lagenorhynchus abliquidens*) | | | | | Fibroma | | |
| Harbour Porpoise (*Phocoena phocoena*) | | | | | | ACA | |
| Atlantic Whitesided Dolphin (*Lagenorhyncus acutus*) | | | Fibropapilloma | Fibropapilloma | | | Leiomyoma |
| California Sea Lion (*Zalophus californianus*) | | | SCC | SCC, Lipoma | SCC | | |
| Ringed Seal (*Phoca hispida*) | | | | | | | ACA |

GI= gastrointestinal, SCC= Squamous cell carcinoma, ACA= Adencarcinoma

Another connection between Papilloma and benign Melanoma found in the oral cavity is the exhaustion of the immune system from chronic dioxin exposure. The Papillomaviruses causing dysfunctions in epithelial cells leading to stratum basale proliferations are usually self-limiting. In most animals', complete immunity follows the regression of lesions however lymphocyte ratios and abnormal cytokine expression suggest immunosuppression may be responsible for relapses [29]. Papillomata are benign, however, have been seen to overgrow and extensively proliferate in immune-suppressed individuals. Tongue papillomata are not invasive nor life-threatening but can become hindering during feed intake and in the worst cases can contribute to malnourishment. Papillomas, however, hold the risk of developing into SCC with contributing factors discussed later.

Benign Melanomas in cetaceans are a rarer finding and therefore not well-researched. A study on mice Ah dioxin receptors showed an inverse correlation of expression in melanoma cells. Meaning that a decrease in AhR due to overstimulation and immune exhaustion leads to an increase in Aldehyde dehydrogenase 1A1 (Aldh1a1) facilitating the growth of melanomas [43].

Viruses taking advantage of an immune deficiency can also lead to tumour growth. A study on the connection between Human Papillomavirus (HPV) infections and melanoma growths showed that 58% of people developing melanomas were also infected with HPV, and their prognosis was less favourable with the co-infection present [44].

It is important to point out that these studies were conducted on different mammal species with malignant melanomas. Cetaceans are one of the mammals least likely to develop malignant tumours due to their evolved TSGs, which may explain the mere benign neoplasms in these examples.

Standing out from Table 6 are the tumours found in the Beluga whales. They are the only Odontoceti whales mentioned to have malignant gastrointestinal tumours. Unique about the Belugas' feeding behaviour is that their diving pattern suggests that they ingest sediment through suction when feeding on benthic prey.

POPs are known to collect as a sediment layer over the ground due to their hydrophobic properties. That being the case, beluga whales ingest an unusually high concentration of carcinogenic chemicals. Belugas of the St. Lawrence area are one of the most consistently studied cetacean populations. In a case study, 50% of the examined carcasses showed tumours and half of these were of malignant nature [26].

Of all cetaceans, dolphins are the species where neoplasia is reported most frequently. GI tumours in dolphins are uncommon as the statistical comparison shows that they mostly develop lymphatic or dermal neoplasms.

The Fibropapillomas found in Atlantic whitesided dolphins most likely developed from Tursiops truncatus Papillomavirus Type 1 (TtPV-1) infections. TtPV-1 infections in free-ranging dolphins were discovered to be as high as 90% and 51% in captive dolphins [45]. The sublingual and gingival SCC of bottlenose dolphins may have developed from Papillomas in a pre-malignant status as the presence of carcinogens has been associated with SCC development in human studies [44].

Epithelial invasive tumours like the SCC, and ACA were also reported in Pinnipeds such as the California Sea lion and Ringed Seal.

Tissues which physiologically undergo fast regeneration like the gastrointestinal lining, dermis, and the uterus are more likely to develop neoplastic changes. If the tissue requires even faster regeneration because of constant mechanical erosion from sediment particles, desmoplasia formation, and functional tissue loss, the risk for neoplastic cell changes further increases [46].

### 2.2.3 Skin tumours

Cetacean skin has adapted to the marine environment. Neither hair nor glands are found in the dermis, to maintain osmosis. The skin, being the primary protective barrier against environmental stressors, is impermeable to water however highly susceptible to water quality changes such as salinity and the presence of xenobiotics [8]. Only a small portion of POPs circulate freely through the ocean water, most collecting as sediment or accumulating within individual organisms. Nonetheless, the skin has continual POP exposure and acts as the first line of defense against environmental chemical encounters.

Skin disorders such as ulcerative dermatitis in grey seals and northern elephant skin disease syndrome have been connected to POP exposure since the 1980s. In 1997, 90% of the skin biopsies taken from affected individuals showed hyperkeratosis of the surface and follicular epithelium, acanthosis, sebaceous gland squamous metaplasia, and atrophy [47]. Showing that high-concentration exposure to POPs results in skin lesions.

The emission of POPs, seen in Figure 1, has decreased by almost two-thirds, thanks to the Stockholm convention treaty. Skin disorders that were frequent in the 1990s now only occur after chemical spills as acute toxicosis symptoms.

First-order kinetic experiments on dermal absorption of TCDD and other dioxin-laced compounds showed a constant and yet slow absorption rate of 0.005 $h^{-1}$. Furthermore, these experiments suggest that the majority of compounds remain and accumulate within the epidermal layer and do not penetrate the deeper dermal layers.

The permeation coefficient of TCDD differs among mammals and has not yet been identified for marine mammals. Human skin TCDD permeability coefficient is one magnitude lower than that in mouse skin. The model comparing these in hairless mice and humans showed the shared trend of TCDD permeability increases with dosage. But, the percentile of dose permeating, decreases with increasing dose [48]. Consequently, one could argue; chronic exposure is worse than acute.

The thickness of the dermal layers varies between cetaceans and pinnipeds but are altogether thicker than terrestrial mammals. Meaning that TCDD permeation is likely to be lower than in humans.

TCDD permeation through the dermal layers is facilitated when the skin is damaged [48]. Infectious, parasitic, and mycotic skin diseases are frequent among wild cetaceans and pinnipeds. These diseases lead to skin trauma and aid dermal penetration. Even though cetaceans' epidermal turnover rate is 8.5 times higher than humans', healing time is longer. Bottlenose dolphins are the only cetaceans with a skin wound healing time similar to that of humans, while beluga whales take five times as long. Factors such as water temperature, salinity, and dermal thickness also play a role [8]. With the average skin wound healing time being longer, and the constant skin trauma, the dermal absorption rate of dioxin-laced chemicals increases.

Another factor that should be considered is the hormonal impact of chronic POP exposure, illustrated in Table 4. Hydroxylated POP metabolites compete with thyroxine by binding to the transport protein transthyretin leading to hypothyroidism. Characteristic clinical signs of thyroid hormone imbalance are skin disorders such as hyperkeratinization and thinning of the epidermal layer [49]. Not only would the skin thinning increase POP permeation but also increase the susceptibility to viral infections such as TtPV-1.

Skin tumours, displayed in Table 7, are common neoplasms found in cetaceans. Marine mammal bioassays are narrowed by ethical and variability limitations but have been tested for human health research using lab rodents. Even though dioxin permeability varies between mammals, the fact of uptake and its carcinogenicity is established.

**Table 7:** Reported neoplasms of the Skin in marine mammals [26]

| Species affected | Type of neoplasms |
|---|---|
| Fin Whale (*Balaenoptera physalus*) | Fibroma (also in subcutis) |
| Humpback Whale (*Megaptera novaeangliae*) | Fibroma |
| Killer Whale (*Orcinus orca*) | Squamous papilloma |
| Sperm Whale (*Physter macrocephalus*) | Fibroma |
| Beluga Whales (*Delphinapterus leucas*) | Subcutis penile haemangioma |
| Bottlenose Dolphin (*Tursiops truncatus*) | SCC |
| Striped Dolphin (*Stenella coeruleoalba*) | SCC |
| Pacific Whitesided Dolphin (*Lagenorhynchus obliquidens*) | SCC |
| Harbour Porpoise (*Phocoena phocoena*) | Squamous papilloma |
| California Sea Lions (*Zalophus californianus*) | SCC, ACA, Leiomyoma, Fibroma |

SCC= Squamous cell carcinoma, ACA= Adenocarcinoma

The U.S. Environmental Protection Agency (EPA) summarized the results of experimental models on rats, mice, and hamsters, regarding chronic dioxin exposure. Their results showed strong similarities to the tumours listed in Table 7.

21% of the Syrian golden hamsters, a species unresponsive to acute toxic effects, developed squamous cell carcinomas 8 months following TCDD subcutaneous injections. The total dose, of 600 µg/kg given, is below the maximum tolerated dose. In some individuals, metastases to the lungs were found [50]. As shown in Table 7, three dolphin species and the California sea lions developed SCCs. The reason why dolphins show a trend of developing SCCs is unknown but may be explained by their, in comparison to other cetaceans, thinner epidermal layer. Hence, a likely higher TCDD permeability coefficient.

Mice's skin showed induction of skin papillomas when dermally administered TCDD doses for 26 weeks consecutively. Interestingly the papillomata did not form at the site of administration and were observed in all experimental groups exposed to >7.3 ng/kg/day. The synergistic oral administration showed an increased papilloma formation in 25% of the test animals [50]. Likely due to the contributing immunosuppressive effect when taken per os.

The squamous papillomata found in Harbour porpoises and killer whales may be explained by their natural habitat. As their name suggests, Harbour porpoises stay within coastal regions and are often found in proximity to industrial areas. POP exposure is higher in human densely populated areas than in secluded spaces like the North and South Poles. Killer whales migrate with their pod and spend less time around industrialized coastal areas and therefore don´t fit the pattern of high exposure. Even so, killer whales are one of the main predators of the Harbour porpoise and must therefore hunt in regions of high contamination.

Another connection can be made when considering that the papilloma has a Papillomavirus infection background. Papillomaviruses require direct skin contact for transmission, which would be given when the porpoise is hunted down by the orca. The risk of infection increases when the skin is damaged, for example by ectoparasites.

While squamous cell carcinomas are also found in cetaceans, adenocarcinomas have, so far, mainly been reported among pinnipeds. As visible in Tables 5-9, all organs where CYP enzymes are abundantly found, developed adenocarcinomas. The California sea lion stands out with the most heavily affected and aggressive skin tumour types.

A long-term study on California Sea lions, published by the Marine Mammal Centre in California in 2020, stated that they have among the highest mammalian cancer rates. 18-23% of the adult animals examined throughout the 40 years had malignant tumours with widespread metastases. Researchers made a connection between POP exposure, Otariid Herpesvirus-1 infections, and inbreeding, being the multi-causative background to these malignancies [51]. The primary tumour sites remain undetermined, but cofactors suggest the urogenital tract.

### 2.2.4 Lung tumours

Near all wild marine mammals are infected with lung parasites which lead to secondary viral and bacterial infections, consequently leading to a high mortality rate. Captive marine mammals are treated with anthelmintics, and yet respiratory disease incidence is high. Even though lung tumours are not among the main mortality causes, it's a very frequent tumour type in dolphins [26].

Besides the increased risk of oncogenesis from constant tissue trauma, dioxins show a high pulmonary affinity.

There are several pathways through which TCDD and other POPs are lung tumour-promoting. The disruption of normal cell proliferation results in alveolar-bronchial metaplasia and hyperplasia in the epithelial layers. Additionally, intracellular tissue transduction pathways are interfered with by the transformation of related growth factors and interleukins.

The most direct carcinogenic effect is POPs ligation with the pulmonary AhR. However, complementary DNA micro-assay analysis has shown, not all genes altered by exposure to dioxin have xenobiotic response element (XRE) in their DNA. Suggesting a direct and indirect gene alteration [27].

Laboratory experiments on rats showed that the above-described pathways lead to adenomatous hyperplasia and keratinized squamous cell carcinomas in the lung [27]. These observed tumours are consistent with clinical reports of marine mammals.

As listed in Table 8, Adenocarcinomas and Squamous cell carcinomas are the only lung malignancies found among a wide field of marine mammals; ranging from the beluga, bottlenose, and Amazon River dolphins as well as the California and Stellar sea lions.

In humans, the majority of pulmonary adenocarcinomas are of broncho-genetic origin with a suspected xenobiotic background [27]. The pathology reports of the Table 8 specimens did not specify the origin of the tumours.

The EPA also stated that rats, animals with a very low background incidence of lung tumours, showed a 100% alveolar adenoma and carcinoma initiation when treated with both single and continuous doses of TCDD [50]. Making dioxins' affinity to pneumocytes ever more evident.

**Table 8:** Reported neoplasms of the Lung in marine mammals [26]

| Species affected | Type of neoplasm |
|---|---|
| Fin Whale (*Balaenoptera physalus*) | Pleural Fibroma |
| Blue whale (*Balaenoptera musculus*) | Fibroma |
| Beluga Whales (*Delphinapterus leucas*) | ACA, Chondroma, Lipoma |
| Bottlenose Dolphin (*Tursiops truncatus*) | SCC |
| Amazon River Dolphin (*Inia geoffrensis*) | SCC |
| California Sea Lions (*Zalophus californianus*) | SCC, ACA |
| Stellar Sea Lion (*Eumatopias jubatus*) | ACA |

SCC= Squamous cell carcinoma, ACA= Adenocarcinoma

Marine mammals, like all other terrestrial mammals, breathe air. Meaning their lungs don´t have direct contact with POPs like fish in their gill-respiratory cycle.

The Kociba study mentioned by the EPA health assessment summary is a lifetime study on Sprague-Dawley rats dosed with TCDD. It is the most long-term exposure bioassay done on lab rodents. In addition to neoplasms in other organs, lung squamous cell carcinomas developed even though the rats were given dioxin-laced feed and not exposed to an inhalant [50]. This shows that inhalation of POPs is not required, to cause lung tumours.

### 2.2.5 Urogenital tumours

The research institute and marine mammal centre in Sausalito California recently published an extensive study on the connections of persistent contaminants and the Otariid Herpesvirus-1 (OtHV-1), in association with cancer in California sea lions. The study includes necropsy findings from the past 40 years and is the most comprehensive research regarding this topic. Cancers primarily associated with OtHV-1 infections are urogenital tract carcinomas (UGC).

Like most Herpesviruses, the transmission between sea lions is mainly venereal. This exposes the urogenital tract of sexually active animals to several origins of vaginal, uterine, and penile tissue trauma. Tissue trauma and viral coinfections leading to mutagenesis are known and aforesaid.

According to the institute, the concourse of so-called cancer promoters, such as inbreeding, OtHV-1 infections, and POP presence, synergistically lead to neoplasms of varying malignancy. Blubber samples from sea lions with UGC showed significantly higher PCB and DDT levels than sea lions without carcinomas. The odds ratio of cancer, increased by 95% with increasing blubber contaminant concentrations, and a 28% higher odds ratio of each unit increase of POP concentration [51]. Such urogenital carcinomas cause frequent metastases inducing further systemic problems, such as discussed in chapter 2.2.3 skin tumours.

**Table 9:** Reported neoplasms of the Urogenital tract in marine mammals [26]

| Species affected | Types of neoplasm found | | | | |
|---|---|---|---|---|---|
| | Vaginal/ Penile | Uterus | Ovary/Testicle | Urinary Bladder | Kidney/Adrenal |
| Fin Whale (*Balaenoptera physalus*) | Fibroma | | Granulosa-cell tumour, Carcinoma | | |
| Blue Whale (*Balaenoptera musculus*) | | Fibromyoma | Mucinous cystadenoma, Granulosa-cell tumour | | |
| Killer Whale (*Orcinus orca*) | Papilloma | | | | |
| Blainsvilles Beaked Whale (*Mesoplodon densirostris*) | Fibroma | | | | |
| Pilot whale (*Globicephalamelaena*) | Fibroleiomyomas | Leiomyoma | | | |
| Sperm Whale (*Physter macrocephalus*) | Papilloma | Leiomyoma, Fibromyoma | | | |
| Beluga Whales (*Delphinapterus leucas*) | | Fibroleiomyomas, ACA, Leiomyomas | Granulosa-cell tumour | Haemangioma, TCC | Pheochromocytoma |
| Bottlenose Dolphin (*Tursiops truncatus*) | | ACA | | | Adenoma, ACA, Adrenal teratoma |
| Pacific Whitesided Dolphin (*Lagenorhynchus obliquidens*) | | | | | Teratoma |
| Harbour Porpoise (*Phocoena phocoena*) | Papilloma | | | | |
| Dusky Dolphin (*Lagenorhynchus phocaenoides*) | | Fibroleiomyomas | Dysgerminoma | | |
| Finless porpoise (*Neophocaena phocaenoides*) | Fibroma | | | | |
| Atlantic Whitesided Dolphin (*Lagenorhyncus acutus*) | Fibropapilloma | | | | Adenoma |
| Common Dolphin (*Delphinus delphis*) | | | Testicular Leydig cell tumour | | |
| Stellar Sea Lion (*Eumatopias jubatus*) | | Fibroleiomyoma | | | |
| California Sea Lions (*Zalophus californianus*) | SCC, Papilloma, Haemangioma | Carcinoma, SCC, Leiomyoma | ACA, Adenoma, Granulosa-cell tumour | Papilloma, TCC | Adenoma, Hypernephroma, ACA, Nephroblastoma |

| | | | | | |
|---|---|---|---|---|---|
| Fur Seal (*Callorhinis ursinis*) | SCC | | Granulosa-cell tumour | | Fibrosarcoma |
| Cape Fur Seal (*Arctocephalus pusillus*) | | | Granulosa-cell tumour | | |
| Southern Elephant Seal (*Mirounga leonina*) | | | Granulosa-cell tumour | | Adenoma, ACA |
| Gray Seal (*Halichoerus grypus*) | | Leiomyoma, SCC, Carcinoma | | | |

ACA= adenocarcinoma, TCC= transitional-cell carcinoma, SCC= squamous cell carcinoma

POP eliciting hormonal disorders, visible in Table 4, may have an influence on hormone-producing tumours such as testicular Leydig-cell tumours and ovarian granulosa-cell tumours. The background cause for the formation of these rare epithelial tumours is unknown. However, postmenopausal women have a higher risk of developing ovarian Leydig-cell tumours, supposedly caused by changes in sex-hormone levels [52]. Which may also be the cause in marine mammals.

The urinary tract is the main pathway for POP metabolite excretion. Direct carcinogenic effects of these metabolites could explain the urinary bladder carcinomas illustrated by the Joint Pathology Centre in the USA.

A single, yellow-white, 2x3cm nodule with 1cm thickness was found in a sea lion bladder (Figure 4, A). After a histopathologic review, the nodule was identified as a transitional cell carcinoma (Figure 4, B). With metastatic lesions in the liver parenchyma (Figure 3), multifocally in the lungs, and lymph nodes [39].

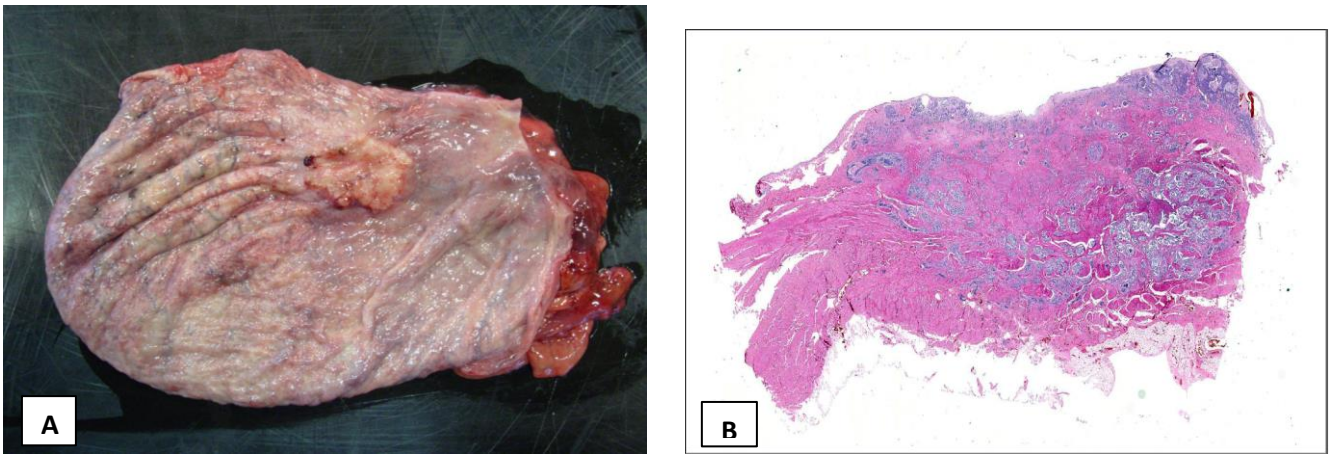


**Figure 4**: Urinary bladder transitional cell carcinoma in a male California sea lion **A**: Neoplasm in the bladder trigone, **B**: H.E. staining, 5x magnification with neoplastic cells extending from submucosa into deeper tissue with mild lymphocytic inflammation separating infiltrative nests [39]

Transitional cell carcinomas were also found in the urinary bladder of beluga whales (Table 9), which considering their high exposure habitat, fit the pattern.

The kidneys are the organ most sensitive to dioxin during foetal and neonatal development. In the mice model, TCDD exposure altered mesenchymal and epithelial differentiation mediated by the AhR [48]. Deriving from this, marine mammals are possibly born with insufficient kidney function.

Hydronephrosis, atrophic changes, and renal cysts are common pathological findings in all marine mammal species, especially sea lions, and have been identified as kidney neoplasm precursors in humans [53]. Additional precursors, such as immunosuppression and altered sex hormone excretion, fit the findings of Table 9, including adenocarcinomas, nephroblastomas, hypernephroma, fibromas, and adenomas.

### 2.2.6 Lymphatic tumours

Fin whales were the first marine mammals reported to develop lymphatic tumors, in 1962. Followed by killer whales in 1989 and bottlenose dolphins in 1997. Even though fin and killer whales were the first, bottlenose dolphins, beluga whales, and California sea lions have a much higher incidence rate.

A study on immunoblastic malignant lymphomas in dolphins conducted in 1997, suspected environmental factors as a partial etiology due to the close geographic proximity of the 5 dolphins affected but did not mention POPs specifically [24].

In the human study, of mother and infant immune response to PCBs, a significant increase in CD8+ T-cells was measured [1]. CD8+ T-cells are cytotoxic T-lymphocytes and play a central role in the body's neoplasm surveillance [54]. The increase in CD8+ T-cells indicates that the presence of PCBs results in neoplastic cellular changes, therefore activating the immune cascade responsible for tumour prohibition. The additional PCBs effect, of T-cell suppression, then limits the body to fight neoplasms and hence results in malignant growths. Contributing to this is the steadily increasing tissue POP levels, exhausting the body's immune system via MFO activation, throughout the years of exposure.

**Table 10:** Reported neoplasms of the Lymphatic System in marine mammals [26]

| Species affected | Type of neoplasm found |
|---|---|
| Fin Whale (*Balaenoptera physalus*) | Hodgkin´s lymphoma |
| Killer Whale (*Orcinus orca*) | Hodgkin´s lymphoma |
| Beluga Whales (*Delphinapterus leucas*) | Lymphoma |
| Bottlenose Dolphin (*Tursiops truncatus*) | Lymphoma |
| Striped Dolphin (*Stenella coeruleoalba*) | Myelogenous leukaemia |
| Pacific Whitesided Dolphin (*Lagenorhynchus obliquidens*) | Eosinophilic leukaemia, Lymphoma |
| California Sea Lions (*Zalophus californianus*) | Lymphoma |
| Fur Seal (*Callorhinis ursinis*) | Lymphoma |
| Harbour Seal (*Phoca vitulena geronimensis*) | Lymphoma |
| Harp Seal (*Pagophilus groenlandicus*) | Lymphoma |

As visible in Table 10, lymphoma makes up 82% of reported tumours in the lymphatic system. This includes normal lymphoma and Hodgkin´s lymphoma type. Hodgkin's lymphoma, the human medicine term for T-cell-rich large B-cell lymphoma, is characterized by the presence of binucleated prominent nucleoli [37].

Evidence from human studies explained in the above's paragraph shows that highly chlorinated PCBs and PBDEs contribute to the risk of lymphoma. Similar publications were made regarding beluga whales, California sea lions, and Harbour porpoises, connecting POPs to lymphatic oncogenesis. A role in lymphomagenesis is suggested, resulting from immunosuppression, leading to mutagenesis [15].

Despite the long-time, lymphoma is known to inflict marine mammals, research is minimal. Without continual samples and monitoring, we can only estimate the progression of the disease by comparing it to other mammal data; humans being the most extensively researched.

# 3. Methods

This thesis is a literature review regarding the POP tissue, milk, and feedstuff levels of marine mammals and their possible connection to tumour pathogenesis. To obtain information, and case studies scientific books on pathology, marine mammal physiology, biology, and medicine were used. Data from scientific journals were extracted by using scientific databases such as pubmed and google scholar.

# 4. Results

Comparing the data of infraorders in marine mammals shows that adult cetaceans have a higher POP blubber concentration than pinnipeds. While pinniped offspring receives the majority of its contaminant-heritage through the placenta (>122.46%), cetacean newborns receive most of their POPs during nursing via the mother-milk (80%). Reasons for these differences can only be assumed.

The variability of results is large, due to geographic differences and the inclusion of data from both wild and captive marine mammals. Captive marine mammals live in a more monitored and filtered environment, reducing tumour precursors. Filtered water, antibiotic/anthelmintic treatments, balanced nutrition, and constant care are the main of such. Their husbandry is challenging, especially for cetaceans. Animal welfare acts, ethical boundaries, and difficult logistics contribute to the rarity of reliable data and suppresses global significance.

Through the lack of detailed understanding of their physiology and therefore pathologic pathways arising from environmental contaminants, POPs have been thus far only connected to tumours in the urogenital tract. Exempting the metastases to other tissues. Tumour occurrence is recorded and has been categorically listed (chapter 2.2). POPs have been established to act on mammalian cells via the AhR, CYP-enzymes, and MFO metabolites. Even so, theories regarding exact aetiology could only be deducted from data on terrestrial mammals, including humans.

# 5. Discussion

Enviable genetic traits such as the TSGs and positive genetic selection within the CXCR2 gene give cetaceans the best prerequisites to withstand man-made pollutants, and yet chronic exposure did not leave them unimpaired.

As described, the variegated exposure and mode of action pathways overpowered evolution's failsafe. Saying POPs are solemnly responsible for the increased occurrence of tumours in marine mammals would be naive, but their substantial contribution is out of the question.

Their contribution to diminishing population numbers, may it be from reproductive impairments to preadolescence mortality, further threatens these animals. Some marine mammals are already on the IUCN red list. Authors of research papers from 1991, who were among the first to associate various health issues with POP exposure, said: “if the increase in ocean PCB concentration continues, it may ultimately result in the extinction of fish-eating marine mammals” [1]. The extinction of apex predators could finally tilt the oceans' health.

As top trophic predators, marine mammals' health is monitored as they share feed sources with humans. Humans are genetically far less equipped to handle the pollutants we produce. Terrestrial mammals and their food chains are also contaminated by POPs, which is not surprising considering the pollutant production occurs on land.

Pollution-free food does not exist. But monitoring every food for POPs would not be practical, therefore only regulations for high-risk sources, such as meat, fish, and eggs, are set by EC No 1881/2006. But again, not all members of POPs are monitored, which should be reconsidered.

# 6. Summary

To sum things up, POP accumulation starts as early as impregnation, when the foetus receives varying concentrations of contaminants through the placenta. The vulnerable foetus suffers developmental abnormalities, as even low concentrations show acute symptoms such as foetal death and abortion. If the pup or calf survives, environmental contaminants exposure leads to chronic changes, such as hormone imbalances, infertility, developmental abnormalities of gonads and skeletal system, and cellular mutagenesis, hence tumour formations. Lifelong accumulation occurs through various routes: via feed intake through the digestive tract and topical absorption through the skin. By systemic circulation, all other organs and systems are reached, while excess is stored among adipose tissue.

POPs are a large category of chemicals with hundreds of variants, all with different modes of action and toxicity. Therefore, only the response to the most researched xenobiotics is mentioned. The marine mammals' primary bodily response to POPs is the activation of the AhR. The organs in which AhRs are abundant show high tumour prevalence and have been discussed in individual chapters. The lymphatic system is the only tissue mentioned without AhRs but is connected within the cascade activation by AhR-Xenobiotic binding. Immunosuppression is one of the results of the cascade.

Secondary bodily responses occur through the MFO system and its carcinogenic metabolites. Evidence are suggesting the liver taking the most effect.

Further facilitating factors such as parasitic, bacterial, and viral infections contribute to tissue changes and immunosuppression all in all plausibly leading to tumour formations.

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**Dr. Dobra Péter Ferenc**

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