

Introduction to marine ecotoxicology

1. Definitions and aim of ecotoxicology

Ecotoxicology: study of the contamination of ecosystems and of the mechanisms of contaminant accumulation and effects in living organisms at all biological levels (from molecules to ecosystem)

Contamination: occurrence of increased concentrations in comparison with background in a given site and a given organism

Pollution: anthropic contamination, directly or indirectly harmful to living beings
(for some authors pollution is by definition from human origin; for others it also includes natural contaminations)

Xenobiotic: element or compound which is not involved in the normal chemistry of an organism

An ecotoxicological study will have to address several aspects:

- (1) to identify the contamination: nature, concentration, distribution, sources
- (2) to determine the dynamics of the contaminants: fate of contaminants in abiotic and biotic compartments of the ecosystem.
- (3) to assess the effects of contaminants on individuals, populations, and communities
- (4) to evaluate the impact on human health and activities (food resources, commercial interests, nature preservation); conflicts may arise between different interest (economical, social, well-being); Examples include basic aspects as the cost of reducing contamination (see fig.: reduction of organic wastes from a sugar beet factory), conflicts between economical interests –e.g. tourism- and nature preservation (use of dispersants in shore cleaning after oil spills) or society choices (use of private cars vs. reduction of greenhouse gas production).

2. Categories of contaminants

2.1. Systematic classification

Physical: temperature, radioactivity

Chemical

Biological: excess of organic matter, pathogenic microorganisms

This classification is not very used because each category encompasses contaminants with very different effects.

2.2. Functional classification

Degradable contaminants

Degradable contaminants are organic material that is subject to bacterial attack, essentially by an oxidative process that will ultimately result in CO_2 , H_2O , and NH_3 .

These wastes include a large part of urban sewage, agricultural wastes (fertilizer leaching), food processing wastes (e.g. pulp from sugar beet factory), oil spillages etc.

The effects of degradable contaminants are principally through the increased bacterial activity they generate. This induces an increased oxygen demand that can eventually result in anoxic conditions that will kill most metazoans and metaphytes.

Dissipating contaminants

Dissipating contaminants rapidly lose their damaging properties after entering the water.

Heat (coming from cooling water from power stations) is usually of low concern in temperate waters but in tropical seas, where summer temperature are already near to the thermal death point of many organisms, the increase in temperature can cause substantial loss of life.

Acid and alkali are very quickly buffered by sea water and their discharges have only very localized effects.

Solid and particulate contaminants

These include drilling muds, clays, and degradable organic wastes whose first effect is usually due to their particulate nature. Particulate compounds reduce light availability for primary

producers, modify the granulometry of sediments and clog the feeding and respiratory structures of animals.

Conservative contaminants

Conservative contaminants are not subject to bacterial attack and are not dissipated but are reactive in various ways and possibly toxic for living organisms. They include heavy metals, halogenated hydrocarbons (pesticides, PCB, dioxins etc.), and radioactive elements.

3. Distinctive features of the marine environment in relation with ecotoxicology

The marine environment shows important differences from the freshwater and terrestrial environments from the ecotoxicological point of view.

It shows a high degree of dilution with the consequence that contaminant concentrations in sea water are usually very low, close to the detection limit of analytical methods. This means that reproducing field-level contamination in microcosms is particularly difficult except if radioactive tracers are used. This emphasizes the need for *in situ* observations and experiments.

Sea water itself influences the physico-chemistry of contaminants. Most contaminants are readily adsorbed on particulate matter. The complex ionic composition of sea water affects the speciation of contaminants, especially of metals (see table). This is the so-called matrix effect of sea water.

The marine environment is relatively homeostatic (in comparison with freshwater), pH and O₂ levels showing fewer variations.

4. Dynamics of contaminants in living organisms

4.1. Absorption

Absorption of contaminants may direct (through sea water) or indirect (through food). The relative importance of each will depend on the considered contaminant and organism. This will be modulated according to factors affecting these, namely speciation, physico-chemical conditions (pH, redox potential, temperature, salinity), physiology, reproductive state and so

on (see tables). A very typical example is the very different absorption rates of inorganic mercury vs. organic mercury.

4.2. Elimination and accumulation

4.2.1. Elimination

Elimination of a contaminant may occur through diffusion or excretion. Different organs can be involved: gut and liver will eliminate contaminants through the faeces, the kidney will ultrafiltrate some contaminants to urine, and in the gills contaminants will diffuse to the outer medium. Another strategy is to reduce absorption of the contaminant, e.g. by increasing the production of mucus (teleostean fishes).

4.2.2. Accumulation

This occurs with conservative contaminants and will be detailed in the chapters on halogenated hydrocarbons and metals.

Briefly, metals are complexed by specific or unspecific proteins and stored in lysosomes or granules of the hepatopancreas. Organic compounds are accumulated in lipidic structures due to their hydrophobicity.

The balance between accumulation and elimination processes will be expressed as the bioconcentration factor (BCF), which is the ratio between contaminant concentration in the organism and in sea water (direct BCF) or food (indirect BCF). If bioconcentration occurs at several successive levels of a food chain, the contaminant concentration in the organism will increase at each trophic level up to top predators. This process is called biomagnification. Biomagnification occurs with some metals (e.g. Hg) and with some halogenated compounds (DDT, dieldrin, etc.) (see fig.). However, it is absolutely not the rule and absence of biomagnification is much more frequent (see fig. the example of Cd).

5. Effects of contaminants

5.1. Molecular/biochemical effects

When a contaminant enters an organism, it may induce two types of reactions, protective or deleterious. Protective reactions may be preventive or curative. Preventive reactions are detoxification reactions whose goal is either to reduce the concentration of dissolved or reactive contaminant by complexation (e.g. metallothioneins, see chapter on metals) or which induce enzyme activities that can metabolize the contaminant (e.g. mixed function oxidases, see chapter on halogenated hydrocarbons). Curative reactions will repair the molecular damage caused by the contaminant. These repair mechanisms include DNA repair enzymes and stress proteins (e.g. heat shock proteins).

Deleterious effects can be very different depending on the contaminant or conditions. They include alterations of DNA and enzymatic activities, inhibition of receptors, etc. An example is the inhibition of acetylcholinesterase (AChE) by organophosphorous compounds. In the normal condition, the complex between acetylcholine (ACh) and AChE has a very short half-life (0.1msec). When organophosphorous compounds (pesticides and neurotoxic gas) bind to AChE, the complex is much more long-lived: half-life reaches 10^6 msec, blocking the enzyme activity and thus leaving the synapse in stimulated state. This may cause death or very serious irreversible paralysis.

In some instances, detoxification mechanisms may give rise to more toxic metabolites, as in the case of polycyclic aromatic hydrocarbons (PAH) (see fig.).

The question is if biochemical or molecular effects are transmitted to higher levels of biological integration, in particular to the individual, i.e. if there is a phenotypic effect. However, notice that there is always an energy cost for detoxification.

5.2. Cytological and organ effects

Effects on the cell physiology may range from different organelle alterations to induction of cell death (including apoptosis). Classical organelle alterations include vacuolization, reduction in microvilli, dilated RER cisternae, affected nucleus membrane or reduction of mitochondria cristae (see figs).

If a given organ is a favoured target of a contaminant, this organ may be particularly affected with resulting effects for the rest of the organism. An example of such targeted toxicity is ^{125}I from nuclear explosions that accumulates in the thyroid gland of vertebrates resulting in a cancer of this organ. Cadmium accumulates in the liver and kidney of mammals, inducing alterations of kidney cells and resulting decrease of the renal function. This emphasizes the need to analyse different organs separately.

5.3. Individual effects

These may include behavioural and reproductive effects. Some contaminants acting on the nervous system may reduce vigilance, which will increase vulnerability to predators and a resulting increase in mortality rate in the population (e.g. effect of Temephos, an organophosphorous compound, on escape response of crabs; see fig.). Behavioural alteration may also result in reduced foraging abilities and subsequent production.

The effects on the energy budget are measured by the scope for growth (SFG) (see fig), i.e. the difference between energy intake (food) and metabolic losses through respiration, excretion, faeces production and detoxification. SFG responses may show a threshold (see fig: effect of TBT on mussel SFG).

5.4. Effects at the population and community levels

Contaminants that affect production and/or survival of individuals, reproduction or embryo development may influence population dynamics. Effects of organochlorine pesticides that reduce the thickness of eggshells in top predators are particularly well-known. The thinned eggs were crushed by brooding adults, dramatically reducing the reproduction success (see fig). This dramatically reduced raptor populations in North America and Europe.

Disappearance of a species may have consequences on another (which may be unaffected by the contaminant itself!). This occurs, for instance, when the consumer is removed by the contaminant. A well-known example is the effect of the *Tampico Maru* oil spill that occurred on the Mexican coast of Baja California in March 1957. The dominant sublittoral grazers were the abalone *Haliotis* and two species of sea urchin, *Strongylocentrotus*. The three species were either killed or left the area. A dense growth of giant kelp followed and persisted in the bay before the grazers became re-established (see fig.).

6. Monitoring of a contamination

6.1. Nature and contamination levels

The complete characterization of the contamination of a marine ecosystem would require the analysis of contaminants in sea water, sediments and organisms. All these compartments present positive and negative aspects on the monitoring point of view. Analysis of sea water and sediments would provide the actual concentrations in the environment and information on sources and fluxes of contaminants. However, analysis of sea water is difficult due to the low concentrations and the biological signification of sea water and sediment concentrations is low due to unknown bioavailability. Sea water concentrations are also submitted to fast temporal fluctuations questioning the representative value of these analyses.

Living organisms present the advantages of easily analysed concentrations (due to bioconcentration), direct measures of bioavailability, and an integration of environmental concentrations over a longer period than sea water. However, it is impossible to measure contaminants in all organisms from an ecosystem although these may present very contrasted responses to the same contaminant. For this reason, monitoring programmes use bioindicators, i.e. organisms used to quantify the abundance and bioavailability of contaminants. To get a good monitoring of an ecosystem requests to use several bioindicators of contrasted phylogenetic positions, physiologies, and trophic levels. A good bioindicator should also be endowed with several characteristics:

- Sessile or sedentary
- Abundant, easy to identify
- Providing enough material for analysis
- Tolerant to high concentrations of contaminants
- Reflecting the contaminant concentration in the environment

It is also necessary to determine the time scale at which the bioindicator reflect the contamination (time to reach equilibrium concentration and time of elimination) (see figs for *Posidonia* example).

6.2. Effects of contaminants

6.2.1. Toxicity bioassays

The goal of toxicity bioassays is to establish the relationship between contaminant concentration and toxicity (establishment of dose-response relationships). This allows comparing the relative toxicity of very different compounds. It is important to realize that any substance may be toxic, it is only a matter of dose ("All substances are poisons; only the dose distinguish the poison from the cure" Paracelsus, 1493-1541). For instance, oxygen is essential to life for numerous metazoans, but pure oxygen atmosphere would be lethal.

The end points of bioassays are very frequently death, expressed as lethal concentration or dose at which 50% of individuals are killed in a given period of time (usually 48 to 96h) (LC50 or LD50). This is of limited value in the marine environment, lethal concentrations being very rarely encountered in the field. The main use of these lethal tests is to allow an easy comparison of the relative toxicity of very different compounds. Sublethal effects are also used (e.g. embryotoxicity tests), the results being expressed as the concentration at which 50% of the tested individuals are affected (effective concentration 50, EC50). In both lethal and sublethal tests, it is also possible to determine the lowest observed effect concentration or dose (LOEC or LOED) and the No observed effect concentration or dose (NOEC or NOED) (see fig.). Notice that the two latter are dependent of the range of concentrations tested: the narrower the gaps between tested concentrations, the more precise the NOEC and LOEC. It is also noteworthy that toxicity is strongly affected by numerous factors. Thus it is very important to standardize the bioassays. Presence of several contaminants may result in antagonist or synergic effects that should of course be taken into account.

6.2.2. Biomarkers

A biomarker is a biological response to a contaminant that gives a measure of the exposure to and possibly toxicity of this contaminant.

Exposure biomarkers indicate that the organism met the contaminant but do not give information on the resulting possible toxicity. Metallothionein levels and activity of cytochrome P-450 (see chapter on halogenated hydrocarbons) are frequently used exposure biomarkers.

Toxicity biomarkers do indicate an actual toxicity of the contaminant. A good example is the imposex induced by TBT in neogastropods (see chapter on metals).

Current biomarkers currently encompass molecular to individual biological levels. It should be noted that not all toxic effects are useful biomarkers, for instance because they are not specific enough or because confusing normal physiological processes affect them.

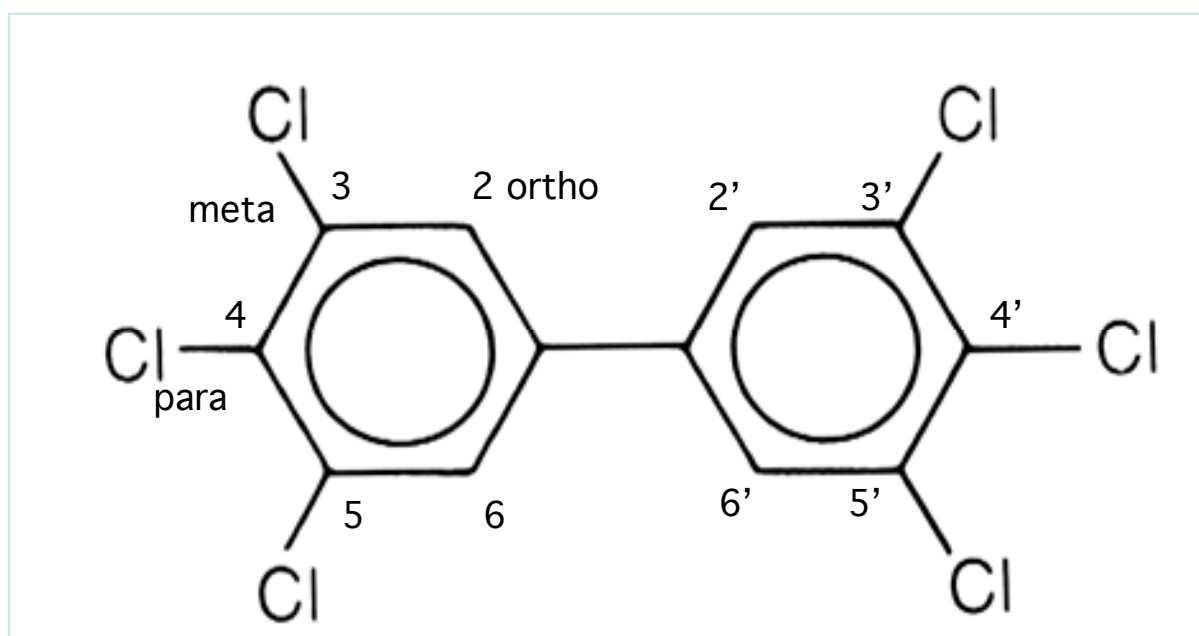
Specificity of biomarkers may range from absolute (imposex and TBT) to fairly broad (induction of mixed function oxidases can be due to several exogenous coplanar compounds but also to endogenous substrates; see chapter on halogenated hydrocarbons).

Polychlorinated biphenyls (PCB), Polychlorinated dibenzodioxins (PCDD), Polychlorinated dibenzofurans (PCDF)

1. Nature, sources

The three classes of contaminants are halogenated aromatic hydrocarbons (HAH).

1.1. PCB



PCB are made of 2 benzene rings linked by a C-C covalent bond. Hydrogen atoms may be substituted by 1 to 10 chlorine atoms. There are 209 different PCB compounds possible, called congeners (see table). Only 130 of them are found in the environment, always as mixtures. The stereochemistry of congeners depends on the positions of substitution of Cl atoms. In non-ortho-substituted and mono-ortho-substituted congeners, the two benzene rings remain in the same plane (coplanar PCB). Substitution of 2 or more ortho-positions by chlorine leads to the movement of the rings out of plane, due to the interaction of adjacent chlorines in different rings (non-coplanar PCB).

Solubility of PCB in water is low: 2-20 $\mu\text{g/l}$ and depends on the number of substituting chlorine atoms. Hydrophobicity is usually expressed as the partition coefficient between

octanol and water (K_{ow} = concentration in octanol/ concentration in water). PCB have an important chemical and thermal stability, a low vapour pressure and a very high dielectric constant. Stability increases with the number of chlorine atoms by reducing the possibilities for electrophilic substitution. Only particular processes allow the destruction of PCB, like temperature above 1300°C (found in cement works furnaces). In conventional incinerators, PCB are emitted as gas or transformed in PCDD. For these reasons PCB are very persistent contaminants in the environment.

PCB were produced industrially from 1929 till 1977. They were used for numerous industrial applications linked to their exceptional stability, namely as dielectric fluids, heat transformer fluids, lubricants, vacuum pump fluids, plasticizers (in paints for instance) and for making carbonless copy paper. Commercial PCB are always mixtures. Monsanto (USA) produced 9 different mixtures called Arochlor 12xx (e.g. Arochlor 1254, 1242, etc.). The first two digits refer to the 12 C in the benzene rings, the last two to the weight percentage chlorine in the mixture. In France, Clophen A50, A60 was produced, the number also referring to the percentage of chlorine. In the 30's, the annual production averages 1000 tons; in 1975, 2 million tons were produced. The production was stopped in 1977 due to the dispersion of PCB in the environment and their persistence and toxicity.

1.2. PCDD and PCDF

PCDD and PCDF are planar molecules formed by the linking of two benzene rings by respectively, two oxygen bridges and one oxygen bridge and one C-C covalent bond with varying substitutions of chlorine on the available ring positions (see fig). 75 different congeners are possible. Stereochemistry of these compounds is similar to that of coplanar PCB. They share with these their important stability. They are very poorly soluble in water (< 1µg/l at 20°C). They are also poorly soluble in organic solvents despite their hydrophobicity. PCDD and PCDF are not produced commercially but are unwanted by-products of the synthesis of chlorophenols, PCB, and phenoxy-herbicides (like 2,4D and 2,4,5T). They are also formed during the inappropriate combustion of PCB. 2,3,7,8-TCDD became sadly famous due to several releases in the environment with dramatic outcomes. This dioxin occurred as a contaminant in the "Agent Orange" (because it was stored in orange barrels), a formulation containing 2,4D and 2,4,5T, which was used by the US army as a defoliant during the Vietnam War. This resulted in affected people (both Vietnamese people and

American soldiers) getting cancer and other health problems and babies born from contaminated Vietnamese mothers presenting severe malformations. TCDD was also released into the air by the explosion of the Seveso Chemical Works in Northern Italy in 1976. Exposed people developed a severe skin affection called chloracne. Other effects of this accident are still discussed, including a possible increase in the occurrence of cancers.

2. Presence in the environment

Inputs of HAH in the environment are due to wastes of the manufactures contaminating rivers which will bring them to the sea, leaking from waste discharges, and inappropriate burning and atmospheric transport on particles. Due to their low solubility (see table below), they accumulate in the superficial film (a thin layer a few μm to maximum 1mm thick at the very surface of the ocean). This may result in severe contamination of animals feeding specifically on this film (like some petrels for instance; see table). Their adsorption on particles also favoured their subsequent accumulation in sediments at concentrations ranging between 0.1 and $10\mu\text{g/g}$. Sediments will then act as secondary source of contamination, especially for suspensivorous organisms (see example of *Mytilus edulis* in San Francisco Bay).

	PCB concentration (ng/l) (dissolved and particulate)
Offshore waters (0-200m)	0.1 - 150
Coastal waters	0.1 - 700
Estuaries	10 - 1000

In sediments mono- and dichlorosubstituted PCB are degraded by aerobic bacteria (see fig.). PCB substituted by more than 2 chlorines are only taken in charge by anaerobic bacteria (which use them as electron acceptors) and transform them in mono- and dichlorosubstituted PCB. However this process is slow.

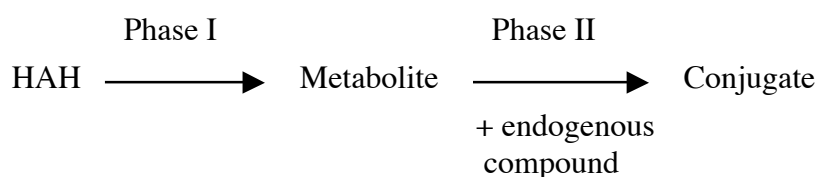
3. Fate in organisms

3.1. Uptake and accumulation

Invertebrates and fishes take up HAH from sea water, sediments or food depending on the hydrophobicity of the considered compounds (expressed as K_{ow} , see fig.) and the biology of the organism. Mammals and birds accumulate these compounds principally from food and biomagnification may occur. A well-known example is the accumulation of PCB in belugas from the St Lawrence River (Québec). Concentrations reach $575 \mu\text{g/g}$ in the blubber and $1750 \mu\text{g/g}$ in the milk. Accumulation in these lipid-rich tissues means that redistribution of the contaminant will occur when they are used during, respectively, starvation periods and lactation. These processes are well illustrated in killer whales (*Orcinus orca*) from waters of British Columbia (Canada) (see fig). Males show increasing PCB concentrations with age indicating bioaccumulation. Females show the same pattern when immature, then their blubber concentrations decrease during their reproduction period, and increase again after age 40 when they stop breeding. This decrease during reproduction years is due to the elimination of organochlorines (> 60% of the body burden) in milk (which will thus contaminate juveniles).

3.2. Detoxification

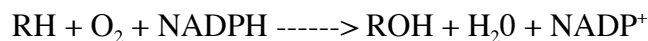
Detoxification of HAH occurs in two phases:



3.2.1. Phase I

Phase I enzyme activities are located in cisternae of the endoplasmic reticulum in the liver (vertebrates), hepatopancreas or gut (invertebrates). The enzymes catalyse oxidation, hydrolysis, hydration or reduction reactions that will produce metabolites with a hydroxyl group. This increases the molecule solubility and will be the basis for phase II reactions. Main

classes of Phase I enzymes are mixed function oxidases (MFO), esterases, and epoxide hydrolases (see table). MFO are present in all Metazoa. They show a broad specificity, most lipophilic xenobiotics being potential substrates, with the exception of highly chlorinated PCB. They catalyse the reaction:



The MFO fixes oxygen and transfer to this an electron, causing its dissociation in water and an oxidant radical which will oxidize the xenobiotic.

One of the best studied MFO is cytochrome P-450 (CYP). Several families and forms of this exist, some of them being inducible by xenobiotics. This is usually adaptive except for highly chlorinated compounds that effectively induce the CYP synthesis but are not metabolized by this. This means that electron transfer to oxygen does occur but the resulting oxidant radical does not oxidize the xenobiotic. Consequently, these very reactive but unspecific radicals will threaten cell structures. Anti-oxidants do protect the cells against them but may be overflowed.

3.2.2. Phase II

Phase II enzyme are principally located in the cytosol (thanks to their increased solubility, metabolites produce by Phase I enzymes pass in the cytosol). Phase II enzyme activities further increase the hydrosolubility by adding an anionic conjugate to the metabolite. This will help the organism to excrete the modified xenobiotic. The conjugation reaction depends on the involved enzyme activity (see fig.)

4. Biological effects

The toxicity of HAH depends on the considered congeners. Coplanar molecules are more toxic than non-coplanar ones. 2,3,7,8 TCDD is the most toxic HAH, followed by other dioxins, furans and coplanar PCB. As these compounds usually occur as mixtures, the toxicity of the mixture is tentatively expressed in terms of "dioxin equivalents". "Toxic equivalent factors" (TEF) are first determined. By convention TEF of 2,3,7,8 TCDD is 1. TEF for other HAH are then calculated according to their capacity to induce the aryl hydrocarbon

hydroxylase (a MFO activity) (see table). Then the TEF of each compound is multiplied by the concentration of this, which give the "dioxin toxic equivalent" (TEQ) for this compound. Summing all TEQ of the compounds present in the sample give the total dioxin equivalent toxicity of the sample (Σ TEQ). It is noteworthy that in the marine environment, despite the much higher TEF of dioxins, coplanar PCB account for most of the Σ TEQ due to their much higher concentrations (see fig.).

Accumulation in adipose tissues also influences toxicity as this generates delayed effects when lipids are used during starvation periods or for activities implying heavy energy requirements (like migration in birds). HAH often co-occur with organochlorine pesticides making the identification of the causal agent difficult.

Effects documented in animals include endocrine and immune effects. HAH are endocrine disruptors, meaning that they interfere with the normal hormonal physiology (they act as antagonists of some organs. In particular, they interfere with ovulation and embryo development in mammals and birds with consequences on the population dynamics of some species like the ringed seal (*Phoca hispida*) in the Baltic Sea or the common seal (*Phoca vitulina*) in Puget Sound (Washington state, USA). Fifty % of females in these populations were shown to contain $77\mu\text{g/g}$ PCB in their blubber and to have one or both uterine horns blocked by inclusions, rendering them sterile. Indeed, in the Baltic populations, only 28% of females were pregnant each year instead of the usual pregnancy rate of 80%. Furthermore, HAH are known to reduce the levels of androgenic hormones resulting in a reduced fertility in males. Endocrine effects may also alter neuronal branching resulting in reduced cognitive and motor functions. Effects on the immune system include increased production of reactive oxygen species by macrophage-like cells (see fig.). Mass mortalities of striped dolphins in the Mediterranean has been attributed to reduced immune reaction towards a viral disease, due to PCB contamination.

Effects of HAH in man have been reported due to several accidents. TCDD release during the Vietnam War and the Seveso accident were presented previously. In 1968, in Japan, 1291 persons were contaminated by PCB accidentally present in rice oil at $2\text{-}3000\mu\text{g/g}$. After 2 months 29 persons died and several suffered chloracne and hepatic troubles. Babies of some exposed women showed reduced size and weight. Until now, no indication of toxic effects due to the ingestion of contaminated sea products was reported in men but high TEQ recorded in some seafood raise growing concern.

Heavy metals and organometallic compounds

1. Classifications of metals

1.1. Functional classification

Major elements include Na, K, Ca, Mg, i.e. metals present in high concentration in organisms, usually transported as cations, and soluble at rather high concentration in biological fluids.

Minor elements are transition metals necessary for normal metabolism at low concentration; they include Cu, Zn, Fe, Mn, Co, and Cd (only in some microalgae). They are part of respiratory pigments (Cu, Fe), cofactors of enzymes (Zn in carbonic anhydrase, carboxypeptidase A and B, Mn in pyruvate carboxylase, Cd in carbonic anhydrase of some microalgae) or of vitamins (Co in cyanocobalamin = vitamin B12).

Trace elements are not necessary for metabolic activities and are generally toxic at rather low concentrations. They include Hg, Pb, Sn, Se, As, Cd, etc.

1.2. Practical classification

A more pragmatic classification only differentiates *light metals* (major elements) and *heavy metals* (metals whose atomic number is higher or equal to 21). The latter are distinguished as *essential* or *non-essential*.

2. Accumulation and elimination mechanisms

2.1. Uptake

Direct uptake is mainly a passive process depending on the concentration gradient between dissolved metals in the environment and inner fluids (thus metals fixed by proteins are not involved in this equilibrium). In some cases uptake is active like for Cd that is taken up by Ca transporters. Indirect uptake is linked to metal included in and adsorbed on food. The latter may be the main contamination route for filtering organisms.

2.2. Fate of metals in organisms

2.2.1. Transfer

Once absorbed, metals are usually linked to proteins either of high molecular weight (HMWP) in the plasma (like metalloenzymes) or by metallothioneins. They are then transferred to tissues together with these proteins.

2.2.2. Metallothioneins

Metallothioneins (MT) are:

- proteins of low molecular weight (6 – 10kD)
- linking metals (through thiol bonds on Cys), mainly Zn, Cu, Cd, Hg
- with a high % of cysteines (10-33 mole %), without aromatic amino acids nor histidine

MT are known from all reigns. Their 3D structure usually forms two domains (□ and □) able to link metals in a "pocket" (see fig.).

Their affinity for metals differ. Usually, the affinity order is as follows: Hg> Cu> Cd> Zn. This means that non-essential metals are able to displace essential ones. However, different isoforms may exist with specific affinities for one or another metal. In the snail *Helix pomatia*, two isoforms were recognized. One with a high affinity for Cd (Cd:Cu:Zn = 100:2:6.6) expressed in the mid-gut gland and the other with a high affinity for Cu (Cu:Cd:Zn= 100:1:6) expressed in the mantle.

MT are involved in the regulation of essential metal bioavailability for metalloproteins and in the detoxification of non essential metals. MT synthesis can be induced by increased metal concentrations. Regulation occurs at the transcription level. Metal-binding regulatory factors bind metals in the cytosol; this induces a conformation modification making their binding on metal-responsive elements upwards the coding region of the MT gene (see fig.). This binding initiates the transcription. It is noteworthy that other factors may control the transcription of MT genes, namely hormones or interferon. Main inducing metals are Zn, Cu, Cd, and Hg. When different isoforms co-exist, their induction depends on their affinity. In the case of *H.pomatia*, the synthesis of the isoform with a high affinity for Cd is induced by this metal

while the isoform with a high affinity for Cu is not affected by environmental Cd concentrations.

2.2.3. Elimination

Elimination of metals can be carried out by three different processes. Excretion is occurring in kidneys, liver or hepato-pancreas. In particular the complex MT-Cd is partly excreted in mammal kidneys although the process is slow. Metals trapped in residual bodies (see below) are eliminated in the gut lumen in some invertebrates (molluscs, echinoderms). Finally diffusion through the body wall and gills occur in numerous taxa.

2.2.4. Accumulation

Accumulation may occur in two structures, lysosomes and hepatopancreatic granules. The former happens in deuterio- and protostomians, the latter only in protostomians.

When MT-metal complexes fuse with a primary lysosome, the protein part is hydrolysed and metals are linked to a ligand, usually lipidic (see fig.). The latter get usually peroxidized forming highly insoluble lipofuscin in which metals are trapped, the lysosome becoming a residual body.

In hepatopancreatic granules, metals co-precipitate with anions (phosphate, carbonate or oxalate) on an organic matrix with affinity for divalent ions. This mechanism does not involve hydrolytic enzymes. It is responsible for the very high metal concentrations that are sometimes encountered in molluscs or crustaceans, for instance.

3. Toxicity of heavy metals and effects on organisms and ecosystems

Toxicity and concentration in organs may vary according to numerous factors like metal speciation, co-occurrence with other contaminants, physiological state, and genetic adaptations.

Of course, effects will differ according to the considered metal.

3.1. Mercury

Sources

- chlor-alkali industry (production uses electrolytic processes with mercury as a catalyst); half the world contamination by mercury is due to this industry
- pesticides
- anti-fouling paintings
- gold and silver extraction

Speciation

In the sea, mercury is principally adsorbed on particulate matter. Dissolved forms are HgCl_4^{2-} and HgCl_3^- . Mercury is also complexed with sulphur proteins and with humic acids. All three inorganic forms may be transformed in methyl mercury by prokaryotes.

Toxicity

The organic forms are more toxic than the inorganic ones. In molluscs, mercury is principally found in gut and associated organs. In fishes, muscle concentration (i.e. the part eaten by man) averages $0.15 \mu\text{g/g}$, $1 \mu\text{g/g}$ in top predators and may reach $4.9 \mu\text{g/g}$. Mercury is biomagnified, in particular methyl mercury which is hardly eliminated. Birds are able to demethylate mercury in the liver. They are also eliminating a part of their body burden when moulting as mercury is accumulated in feathers, associated to sulphur proteins (see fig.). For these reasons, birds may tolerate high mercury concentrations.

On the contrary, man suffers badly from mercury poisoning. This toxicity has been known for centuries. Mad hatters in Victorian England got their name from the convulsions and loss of neuro-muscular coordination due to chronic poisoning from the mercury used in the treatment of felt. Mad hatters were illustrated in "Alice in Wonderland".

Inorganic mercury can be readily excreted and, while it is dangerous to those exposed to it occupationally, it is not a hazard for the general public. On the contrary, organic mercury cannot be excreted easily and so may accumulate to toxic concentrations as a result of chronic contamination. It is a cumulative poison and since it can cross the blood-brain barrier, it causes progressive and irreversible brain damage. In particular it causes the destruction of the nerve myelin sheath, which leads to blindness and motor problems.

Human exposure to methyl mercury occurs through consumption of contaminated fish and seafood. A sadly famous methyl mercury poisoning occurred in the small Japanese coastal town of Minamata (and was called Minamata disease). Part of the population living around this bay was fishermen eating locally fished sea products. In 1952, a factory began producing vinyl chloride and acetaldehyde, both processes involving the use of mercury catalysts, large quantities of which were lost in washing the product and discharged into the bay. This inorganic mercury was transformed in methyl mercury by bacteria in the bay and contaminated the fishes and seafood to concentrations up to, respectively, 10-55 $\mu\text{g/g}$ and 10-39 $\mu\text{g/g}$. The lethal dose for methyl mercury in man is 10-60mg (i.e. the amount present in only 1kg of fishes from the Minamata bay!). More than 2000 people were poisoned; of these more than 100 died and over 700 of the survivors were left with severe permanent disabilities and babies born from contaminated mothers also suffered malformations and poor viability. The Minamata disease prompted the realization that environmental pollution is a case of concern for human health. This led the World Health Organization to recommend a maximum tolerable consumption of mercury in food of 0.2 mg of methyl mercury or 0.3 mg of total mercury per week. Most countries set limits between 0.5 and 1.0 $\mu\text{g/g}$ FW of total mercury as working concentration in fishes. The higher limit, in most countries, applies only to top predators (swordfish, shark, tuna) that are known to accumulate high levels of mercury and to avoid that a substantial proportion of the catch would be excluded from the market. In some countries this is coupled with advice to the public to limit its consumption of these species.

3.2. Cadmium

Sources

- stabilizers and pigments in plastics
- solders and alloys
- batteries
- phosphated rocks and consequently phosphate-enriched fertilizers
- zinc ore

Speciation

Cadmium is principally present in sea water as chlorides and is apparently not methylated in the environment. Cd is one of the most soluble heavy metal.

Toxicity

Cd is absorbed by the phytoplankton but is not biomagnified. Therefore, top predators do not show high concentrations. Cd is accumulated by molluscs in the hepatopancreas. Concentrations up to 2000 $\mu\text{g/g}$ were reported in a *Pecten*. In man, it was claimed to be responsible for an outbreak of itai-itai disease in a Japanese village on the Jintsu River. This painful disease affected the bones and joints of old women, and resulted in a number of deaths. At the time, it was attributed to contamination of rice by cadmium in the effluent from a zinc smelter. However, this is now questioned, and the disease could be associated with malnutrition and vitamin deficiency. High concentrations of Cd (173 $\mu\text{g/g}$) and Zn (57 600 $\mu\text{g/g}$) in oysters from the Derwent estuary in Tasmania caused nausea and vomiting in people that consumed them, but there were no further effects. Notice that Cd inhalation is known to cause lung diseases including cancer. This has been evidenced, among others, among workers from "Métallurgie Hoboken-Overpelt" in Antwerpen.

3.3. Copper

Sources

- erosion of rocks
- electrical equipment
- alloys
- anti-fouling paintings
- algicides
- wood preservative

Speciation

Cu in the marine environment is principally (83%) adsorbed on particulate matter. Dissolved forms include CuCO_3 , Cu^{2+} , and CuOH^+ . Speciation of copper in dissolved form is particularly complex.

Toxicity

Cu is an essential metal. It is present at high concentrations in crustaceans, gastropods, and cephalopods which use hemocyanin as respiratory pigment. Excess are usually stored in the hepatopancreas at concentrations up to 1000 $\mu\text{g/g}$. Cu is usually not biomagnified. Despite

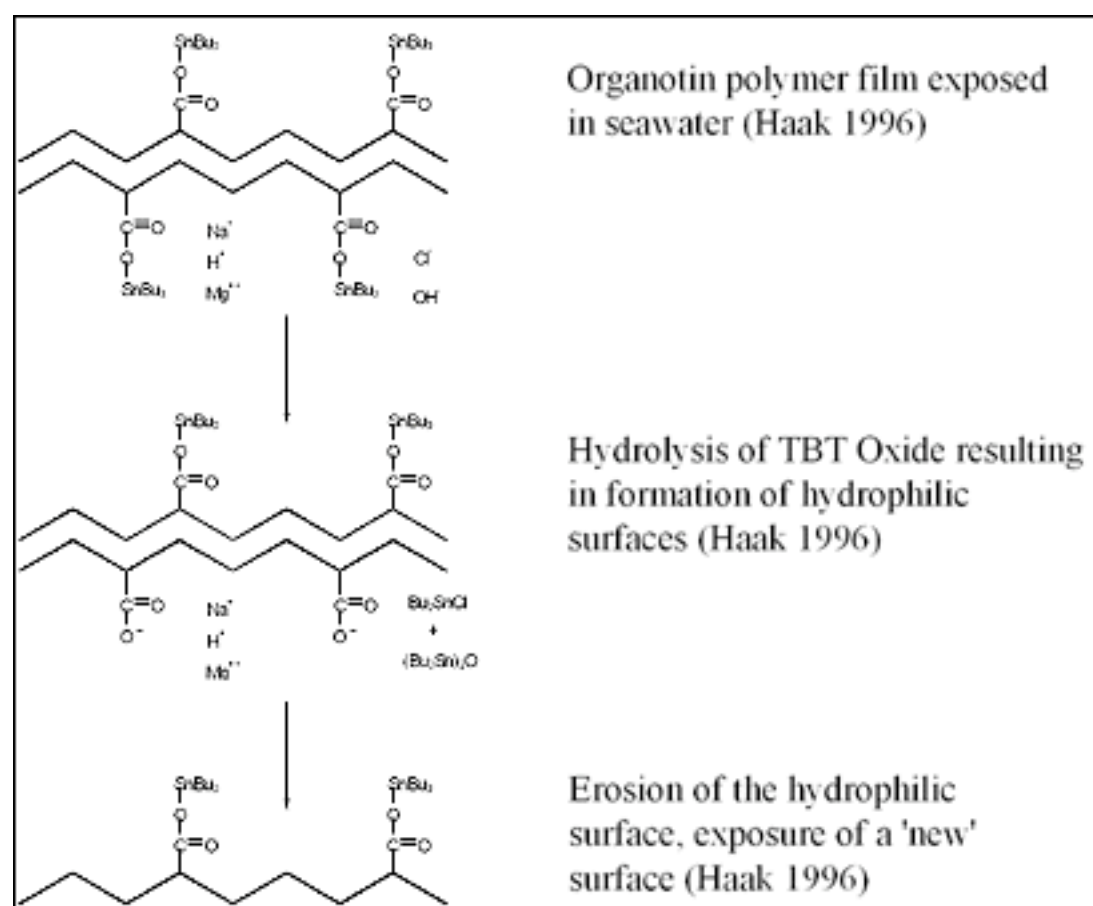
its essential character, Cu is the most toxic metal, after mercury and silver, to a wide spectrum of marine life, hence its use in anti-fouling paintings. Larvae are particularly sensitive to copper.

Humans are not at risk of copper poisoning from seafood: the lethal dose is about 100mg but the human taste threshold for copper is low (5.0 – 7.5 $\mu\text{g/g}$) and the taste is repulsive.

3.4. Tributyltin (TBT)

Sources

TBT is used in lixiviating anti-fouling paintings since the 60's (see fig.). It usually contains ca. 20% (w/w) TBT. Therefore it is principally found as contaminant in marinas, shipyards and dry docks, and maricultures. It was also used as wood preservative.



Speciation

Dissolved forms of TBT include chlorides, hydroxide, and carbonate. TBT can be degraded by bacterial activity and phytoplankton into dibutyltin (DBT) and monobutyl tin (MBT). Generally, in the marine environment, TBT concentrations are higher than DBT and MBT ones. Half-life of TBT in solution ranges from 3-13 days to 50 days. However, the main form of TBT in the sea is adsorbed on particles. The ratio between sediment and dissolved concentrations ranges from 2400 to 15000. Degradation of adsorbed TBT is much slower than that of dissolved TBT. Half-life in aerobic sediments is 162 days and up to 1 year in anaerobic sediments. This means that sediments are a very important secondary source of TBT (see the example of the Bay of Brest, fig.).

Toxicity

In Neogastropods, like *Nucella lapillus*, TBT induces the so-called imposex condition. This is the superimposition of male characters (penis, vas deferens) on female genitals (see fig.). First signs of imposex can already be detected at concentration below 1 ng/l. One third of females exposed to 4 ng/l are sterile and all females become sterile when exposed to 10 ng/l. Classical concentrations observed in estuaries range from 10 to 100 ng/l and may reach 1000 ng/l in harbours and near shipyards (see the example of Bay of Brest). The imposex is supposed to be due to a modification of the hormonal balance, TBT acting as an endocrine disruptor. Imposex caused by TBT resulted in an important decline of neogastropods populations, including complete disappearance in the more heavily contaminated zones (see fig.). This is further worsened by the fact that neogastropods are direct developers without planktonic stage, making recolonization a slow process.

In bivalves, impact of TBT is highly dependent on the considered species. In the oyster *Crassostrea gigas*, TBT causes an abnormal development of the shell, called "chambering". Additional shell layer separated by cavities filled with abnormal organic matrix grow, making the shell much thicker, greatly reducing the size of the animal inside and rendering the oysters unmarketable (see fig.).



Normal and chambered oysters *C.gigas*

Chambering is occurring from concentrations of 2ng/l. Furthermore, oyster larvae are affected by TBT at 10ng/l, with serious mortality occurring from 50ng/l and complete mortality from 200 ng/l.

In other organisms, effects are observed from 20 to 100 ng/l in copepods, echinoderms, polychaetes and tunicates. Fishes and decapod crustaceans appear more resistant (effects recorded from 500ng/l). This is apparently linked to a capacity to degrade TBT.

Effects on man are not documented.

Thus, TBT appears as one of the most toxic compound ever released in the marine environment by man. This is worsened by its relayed action due to its trapping in sediments. The damage caused by TBT to shellfisheries prompted countries from the European Union, North America, Australia and New Zealand to ban the use of TBT in mariculture installations and on boats less than 25m length. This ban was implemented in 1982 in France and in 1987 in the UK. This brought a serious improvement in oyster mariculture where chambering which affected 62 to 91% of oysters in Arcachon in 1980-1982 decreased to 0 –11% in 1983-1987. On October 5th, 2001, the International Maritime Organization (IMO) adopted a convention prohibiting, from January 1st, 2003, to apply or re-apply organotins compounds on all ships. According to this convention, from January 1st, 2008, all ships and platforms shall not bear any organotin compound on their hull or external surface of all ships or shall bear a coating that forms a barrier to such compounds leaching from the underlying non-compliant anti-fouling systems. The convention will enter in force 12 months after 25 states representing 25% of the world's merchant shipping tonnage have ratified it. By January 2004, there were 7 contracting states to the convention, including Belgium, in which the convention effectively entered in force in Belgium on January 1st, 2003.

