NEW ANALYTICAL APPROACHES TO STUDY BIOACCUMULATION IN WHALES

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There is a great deal of measurements necessary to study environmental processes, but sometimes common routine measurements do not give adequate answers to characterize a fate of POPs or toxic elements in the environment. The development of novel approaches to determine new bio- or environmental markers are necessary. This and its application to environmental samples is the core business of environmental analytical chemistry. In this lecture the bioaccumulation of PFAS (per and polyfluorinated alkylated substances) as well as mercury (Hg) and selenium (Se) in pilot and sperm whales will be described.

The accumulation and biomagnification of trace elements such as mercury in the marine environment is well described. From sub ppt level of Hg in the seawater, it can increase in the food chain from one trophic level to the top predatorial species. Liver, the target organ of Hg in the top predators can have 1 million times higher Hg concentrations. The reason given is that the molecular form of methylmercury (MeHg) increases in proportion of total Hg from traces to almost 100% in fish. MeHg is known to penetrate the blood brain barrier and is a neurotoxin. Its lipophilicity is giving as a reason. However, MeHg needs to bind to something which has not been elucidated and the lipophilicity is not known. Furthermore, it is not clearly described why MeHg biomagnifies through the food chain, rather than being excreted. Furthermore, reports are available which indicate that cetaceans can accumulate extreme levels of Hg, but not in the form of MeHg as seen in fish. What is the reason? Is there a demethylation processes at work in a mammalian organism?

On the other hand, it is well known that persistent organic pollutants (POPs) are known to bioaccumulate in the food chain and that high levels of PCBs in whale livers have been discussed to be a reason why some whales such as Orcas can become infertile. Do we see biomagnification through life for all POPs such as PFAS? Do we see a change in the PFAS speciation over time since some PFAS have been banned by the Stockholm convention of POPs (C8 chemistry in the form of the octanoic acids and sulfonates, PFOA and PFOS).

To understand these processes and addressing open scientific questions such as; is the exposome responsible for the stranding of whales, is tackled by the analyses of all organs from a pod of stranded whales (long finned pilot whales). The animals are exposed to similar food and are from all ages so that the biomagnification during their lifespan can be studied. Our hypothesis is that Hg and PFAS bioaccumulate with age and that Hg biotransforms from MeHg to another Hg compound which bioaccumulates and cannot be excreted, while we may see a shift in the speciation of PFAS with age of the whales due to the introduction of the ban of PFOA and PFOS.

Here in this lecture we demonstrate that multi-method solutions are necessary to identify novel biomarkers. The following analytical methods have been used for determination of the speciation of Hg, which has been described thoroughly elsewhere[1,2].

1) Total element determination: acid digestion followed by ICPMS analysis reveal the linear increase of Hg and Se with age; hence biomagnification.
2) SS-ID-GC-ICPMS (species-specific isotope dilution gas chromatography linked to ICPMS) for quantification of MeHg and inorganic Hg (iHg) which can be derivatized by $NaBEt_4$. Results show that only a minor proportion, <10% of Hg is in the form of MeHg and reactive iHg regardless of age and tissue. Majority of Hg was in an inert form.
3) LA-ICPMS (laser ablation ICPMS of tissue thin sections for bioimaging) exhibit heterogeneous distribution of Hg with hot spots and a co-localization with Se. The size of the hot spots is often below the resolution of a few micrometers.
4) spICPMS (single particle ICPMS) revealed that for both elements Hg and

Se form particulate matter of the size from limit of size detection (100 nm) to larger micrometer size particles. More Hg and Se particles and larger particles were found in the enzymatic digest of liver tissues of adult whales compared to juveniles. Which means the NPs are growing with age and more new nuclei are formed.

5) Synchrotron-XRF bioimaging (pixel 800 nm) reveals nanoparticulate HgSe clusters. Larger clusters have an atomic ratio (Hg:Se) of 1.0, while the majority of particles are smaller and have a ratio of 0.7.[3]
6) XANES (x-ray near edge spectrometry) reveals that most of the Hg is tiemannite (HgSe) with some contribution of MeHg supporting the XRF and spICPMS measurements.
7) spICP-TOFMS: Here the atomic ratio of individual particles can be measured. Here the majority of NPs have an atomic ratio of 0.7 (Hg:Se), and this results supports the XRF maps and it can be concluded that the enzymatic digestion does not alter the Hg-Se clusters significantly.
8) NanoSIMS: Bioimaging of Hg with a resolution of 50 nm from the fresh tissue of a young sperm whale was investigated. Again, Hg co-localized with Se and form unregular clusters in the nanoparticle range[4]
9) MC-ICPMS (multi-collector ICPMS for Hg isotope fractionation) of the total digested tissues and only the NP fraction revealed that the Hg isotope signature is different in every tissue and that the isotope signature changes with methylation degree and age. This means that the formation of the HgSe clusters are formed in whale and specific in the organ where there are found[5].
10) Fractionation of selenoproteins in the tissues reveal that Hg is found in the two selenoprotein fractions where SelP and GPx can also be found.

What it means is that Hg is detoxified by the formation of nanoparticulate tiemannite (HgSe) which might have a corona of selenoproteins as zonation of these nanoparticles. This process would bind significant amounts of Se in the tissues and that might have an impact on the biological function of Se, so that the Hg detoxification can result in a Se deficiency in the tissues. If that happens in the brain (where HgSe was also found) neurodegenerative diseases may result. The association of Se deficiency and whether the whales are actually Se deficient requires further study. Another aspect is if this process can be found in other animals, since we have found Hg-Se clusters recently in Scottish Golden Eagles[6]. When compared to 5 other species of Scottish birds of prey, the Golden Eagles were unique in having a significantly higher fraction of iHg present in their liver. It is possible that this occurs via a comparable mechanism witnessed in cetaceans.

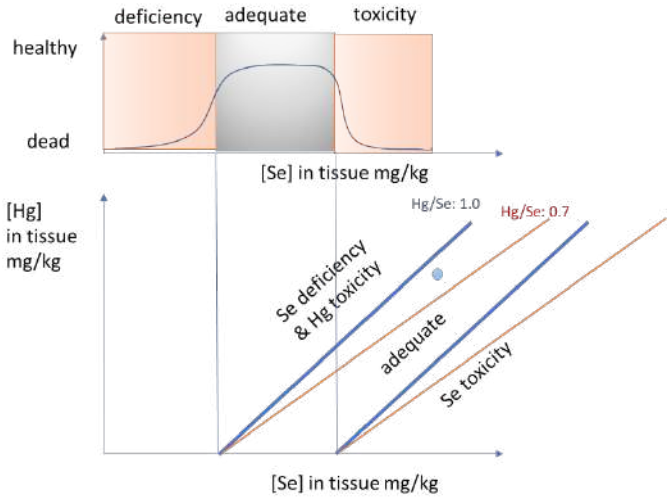

Figure 1: Selenium status measured by Se in tissue needs to consider the Hg concentration in the tissue.

The key to understanding the bio-accumulation of Hg in whales is the elucidation of which biological structures are responsible for the demethylation and transport of Hg in and out of cells. This can only be achieved by the application of several complimentary analytical techniques and strategies.

Shifting the attention to PFAS. It is expected that PFAS concentration would increase with age and that PFOA and PFOS are lower in the younger whales than in the older ones. Here, the following methodologies have been used:

Targeted HPLC-ESI-MS/MS: 35 of the most common PFAS have been determined in the different tissues of the stranded whales. Mainly PFOS and PFCA (perfluorinated carboxylic acids) have been identified and quantified in the liver, kidney and brain of the pilot whales. No PFOA has been found in any of the whales, but mainly longer chain PFCAs (C9-C11). However, no bioaccumulation with age has been identified.

The behaviour of PFAS appears to be different to that of PCBs and Hg which are known to bioaccumulate. This is still not understood yet and may need other analytical approaches. What the analytical state-of-the-art analytical methodology is lacking is that not all fluorinated compounds are determined in a sample. Clearly novel compounds can only be identified by a full non-targeted analytical methodology in which we employed HPLC simultaneously coupled to ESI-qTOFMS for molecular mass screening and ICPMS for fluorine-specific detection. This has been shown to generate promising results in water analysis[7,8]

What we miss when we use a targeted ESI-MS/MS are the following PFAS compounds:

1) Non-extractable PFAS (polymers, NPs)
2) Non-or less-ionizable PFAS (telomer alcoholic PFAS, fluorinated alkanes)
3) Non-target PFAS

All these PFAS can be determine if AF4-ICPMS is used for polymer and NPs, non- or less ionizable PFAS can be determine by HPLC-ICPMS even not fully identified by their molecular structure. Whereas novel PFAS can be determined by using accurate mass spectrometer like qTOFMs or Orbitrap and the use of an algorithm for poly or perfluorinated compounds. This needs to be demonstrated in the future and then the mystery why no bioaccumulation of PFAS in the whales can be observed by using targeted HPLC-MS/MS can be solved.

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