SESSION **PLENARY 2: THE CONTRIBUTION OF THE YOUNG GENERATIONS TO THE FUTURE OF THE EUROPEAN MARINE RESEARCH AREA: MARIE CURIE FELLOWSHIPS IN THE DOMAIN OF MARINE SCIENCES.**

TITLE **HEAVY METAL AND ENDOCRINE DISRUPTER IMPACT ON MARINE MAMMALS**

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SUMMARY

Due to their top position in the trophic network, their long life span and their low rate of pollutant elimination, marine mammals can accumulate high levels of chemicals, such as organic compounds and heavy metals. In the North Sea, high levels of mercury, PCBs, methyl sulfonyl PCBs, perfluorinated organochemicals, DDT and toxaphene have been found in the liver and blubber of the harbour porpoise *Phocoena phocoena* and the harbour seal *Phoca vitulina*. Such compounds may affect the immune and the endocrine systems of these species leading to e.g. an increase susceptibility to infectious diseases, cancers, reproductive and other endocrine dysfunctions.

INTRODUCTION

During these lasts four decades, there has been increasing awareness that chemicals in the environment can exert profound and deleterious effects on wildlife populations and that human health is inextricably linked to the health of the environment. Marine mammals are at the end of the food chain and therefore accumulate high levels of toxic compounds. Several toxicological investigations revealed a high body burden of Zn, Hg and organochlorine compounds in harbour porpoises (Siebert et al. 1999; Jepson et al. 2002, Bennet et al. 2001; Covaci et al. 2002, Van de Vijver et al. 2004; Das et al. submitted); some of these compounds have endocrine disrupting effects on other species (Brouwer et al. 1999).

This Marie-Curie project integrates in the research framework of transport pathway and impact of pollutant studies in the marine ecosystem and aims to understand the effects of some pollutants on the general health status, the endocrine and immune systems of the harbour porpoise (*Phocoena phocoena*). Pollutants such as trace metals, or organochlorines which have been recognised to have endocrine or immune disrupting effects (Zn, Hg, Cd, PCBs, toxaphene and polybrominated-ethers) are currently analysed. These investigations will examine if some endocrine and immune functions are impaired by those pollutants to the extend that harbour porpoises are susceptible to infectious agents which may decrease the population size.

Specific objectives include:

1. Investigation on the pollutant levels (trace metals and organochlorine compounds) in the tissues of stranded and by caught harbour porpoises from the European coasts in collaboration with several research teams (Liège University, Belgium, GKSS, Germany, Giessen University, Germany).
2. Study of the expression of pro- (IL-1β, IL-2, Il-6 and tumor necrosis factor TNF-α) and anti inflammatory (Il-4, IL-10, transforming growth factor TGF- β) cytokines in the full blood

porpoises freshly stranded porpoises from the Belgium coasts using reverse transcription polymerase chain reaction (RT-PCR).

3. Investigation on the harbour porpoise thyroid gland for evidence of lesions such as fibrosis and semi-quantitative evaluation of the relative thyroid tissues (solid tissue, follicles and connective tissues). 91 thyroid glands of fresh porpoises stranded along the French and Belgian and UK coasts were stained with haematoxylin and eosin (HE) and by elastica van Gieson for the detection of collagen. A commercial anti-thyreoglobulin antibody is also tested on harbour porpoises is also used to check for cross reactivity and thyroid activity investigations using the avidin-biotin-peroxidase technique. Data obtained on porpoises from UK, French and Belgian coasts will be compared with previously acquired results on porpoises from the German, Danish, Norwegian and Icelandic coasts.

RESULTS AND DISCUSSION

In some areas such as the Baltic sea, harbour porpoise population has decreased dramatically raising the need of a long term monitoring for this species. A more defined knowledge of the human pressure is primordial for protection and managements plans. The advisory committee and the parties of the Agreement on the Conservation of Small Cetaceans in the Baltic and North Seas (ASCOBANS 1998) as well as the International Whaling Commission (IWC 1997) and the International Council for the Exploration of the Sea (ICES 1998) have recommended strongly that systematic investigations should be conducted to provide information on the effects of pollutants on small cetaceans.

This project specifically addresses the study of effects of trace metals and organochlorines trophic pathways and potential impact on immune and endocrine systems. Only few European countries of the polluted North and Baltic Seas have performed systematic research to facilitate requirements for those investigations. The proposed project brings an European dimension to the study of the impact of contaminants on endocrine and immune systems of marine mammals as some of the samples will be provided by Belgium stranding network (Liège University), but also by others European countries such as Germany, Denmark, Norway and Iceland. (coordination by the host institution, *Forschungs-und Technologiezentrum Westküste*, FTZ).

The preliminary results are promising: the blood of by-caught and stranded harbour porpoises contained RNA of a quality high enough to amplify glyceraldeyde -3- phosphate dehydrogenase (GAPDH) mRNA, regardless it was collected from life or dead animals and independent of the post-mortem interval (by-caught or stranded). Cytokine detection by RT-PCR and Real-time RT-PCR are currently under progress but TGF-ß, IL-2 and IL-6 were often detected.

The rabbit polyclonal antibody designed to react against human thyreoglobulin cross-reacted positively in the thyroid of the harbour porpoises. Connective tissues were abundant in the thyroid of harbour porpoises stranded along the Belgian coasts reflecting interstitial fibroses.

The increase of connective tissues in the thyroid was previously correlated to the increase of PCBs concentrations in the blubber (Vossen et al. submitted). PCB-exposure was likely to be a causal agent for thyroid fibrosis in seals and porpoises from the German North Sea (Schumacher et al. 1993).

Porpoises from Iceland displayed striking differences in both their thyroid tissue proportion and their number of follicles < 62 µm. Icelandic porpoises displayed lower percentage (less than 3%) of connective tissues than porpoises from Norway, Germany and Belgium. These results clearly pointed out a relationship between toxaphene, PCBs, DDE and DDT compounds and histological characteristics of the harbour porpoises thyroids from Germany and Norway. The increase of connective tissues in this tissue was correlated to the increase of PCB concentrations in the blubber. Analyses of the thyroids of the Belgian porpoises and relationship with metals and organochlorines are still under investigation but first results indicated that the morphology of follicles of porpoises collected along the Belgian coasts are very similar to that of porpoises collected along the German coasts.

CONCLUSIONS

Preliminary results indicate some thyroid dysfunction in harbour porpoises from the southern North Sea (Northern France, Belgian and German coasts) as well as in porpoises from the Norwegian coasts. These thyroid lesions were linked to the high level of organochlorine compounds present in their tissues. Trace metals impact on thyroid are still under investigation but high Zn and Hg levels were previously related to the body condition of the harbour porpoises. The actual toxic effects and cellular mechanisms of trace metals and organochlorine on marine mammal immune and endocrine systems remain poorly known. Are they responsible - even in part - for the decline of the harbour porpoises in the North and Baltic seas? As quoted above, that decline is obviously multifactorial: past and present overfishing, increasing human activities and accumulation of pollutants cannot be neglected. Further understanding on human impact on the marine environment will be one of the greatest challenge of the 21st century and therefore should remain a research priority as human health (and life) has always been linked to the health of is marine environment.

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