Methylmercury and selenium in vitro effects on harbor seal (Phoca vitulina) lymphocytes: a multidisciplinary approach

A. Dupont1*, U. Siebert2, T. Rosenberger3, J.-M. Bouquegneau1, M.-C. De Pauw-Gillet4 and K. Das1

1 Laboratory of Oceanology, University of Liège, Belgium
2 Forschungs- und Technologie-Zentrum Westkante (FTZ), University of Kiel, Germany
3 Seehundstation Friedrichskoog, Friedrichskoog, Germany
4 Laboratory of Histology and Cytology, University of Liège, Belgium.

*Contact person: Aurelie.Dupont@ulg.ac.be

INTRODUCTION
Mercury (Hg) is a widespread pollutant which organic form, methylmercury (MeHg), gains particular attention because of its numerous toxic properties, notably towards the immune system of mammals. MeHg bioaccumulates along the food web, leading to very high concentrations in tissues of predatory species. Mainly absorbed by the digestive tract of marine mammals [1], it constitutes the predominant form of mercury present in their blood [2]. The blood cells, including the immune cells, are therefore exposed to the toxic properties of that chemical. Nevertheless, selenium (Se) is an essential element, absorbed concomitantly to MeHg, which could modulate this toxicity, but the interaction mechanisms between MeHg and Se at the marine mammal lymphocyte level are still unknown.

OBJECTIVES
The goal of this study is to evaluate the immunotoxicity of MeHg on the harbor seal (Phoca vitulina) T lymphocytes, highly important in the adaptive immune response, taking into account the potentially modulating effect of Se on that toxicity. It is also to highlight the mechanisms of interaction of MeHg and Se at that lymphocyte level.

MATERIAL AND METHODS
Blood samples were collected from 12 harbor seals (figure 1) inhabiting the North Sea (figure 2). The lymphocytes were isolated from the whole blood and exposed in vitro to increasing MeHg concentrations (0.2, 1 and 2 µM equivalent to 50, 250 and 500 µg/l) and to 5 µg/ml of mitogenic ConA, specifically stimulating T lymphocytes [3]. Their responses were estimated in the different culture conditions after 72 hours of incubation by functional tests including the evaluation of viability and proliferation by nucleocounting (propidium iodide staining), metabolic activity by MTS test, DNA and protein synthesis by dosages, and by morphological analysis by Transmission Electron Microscopy after osmium tetroxide staining.

RESULTS AND DISCUSSION
- T-Hg whole blood concentrations varied widely, from 43 to 611 µg/l (mean value: 172±143 µg/l), reflecting interindividual variations (n=22) [4].
- Results for the in vitro cultures showed a decreasing number of viable cells with increasing concentrations of MeHg (figure 3) after 72 hours of incubation.
- The numbers of viable cells per milliliter differed between juveniles and adults at lower MeHg concentrations, and remained similar at 2µM (figure 3).
- Immune cells from adults are generally exposed to higher environmental pollutant concentrations than those from juveniles, because adults ingest higher quantities of contaminated prey. That could maybe in part explain the significant decrease of viable lymphocytes observed for the adults at the lowest concentration (p=0.0009), which is not the case for the juveniles (p=0.67).
- After exposure to MeHg, the number of viable cells and their biological functions were reduced, suggesting deleterious effects in concentrations naturally encountered in free-ranging seals.
- Microscopic investigations evidenced a higher frequency of apoptotic cells in presence of 1µM of MeHg, notably displaying plasmic membrane distortions, nucleus fragmentations, swelling mitochondrias and cytoplasmic vacuolisation (Figure 4).

CONCLUSIONS
Those results highlighted various immunotoxic effects of MeHg, both at the functional and ultrastructural levels.

Preliminary results of the MeHg effects on T lymphocytes in presence of different selenium forms are currently under analysis.