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

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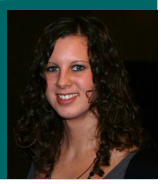


## INTRODUCTION

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. In parallel, the concentrations of MeHg, total mercury (T-Hg) and Se are determined in free-ranging harbour seal blood in order to follow their contamination levels.

**Figure 1. Juvenile harbor seal (*Phoca vitulina*)**

## 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.

Those blood samples were also used to determine the environmental concentrations of MeHg and T-Hg by atomic absorption spectrometry, and of Se by mass spectrometry.

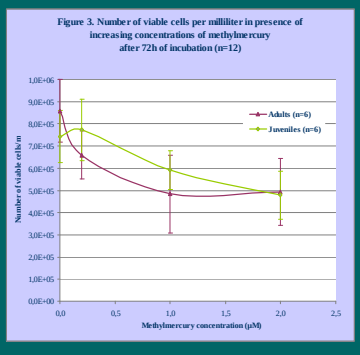
**Figure 2. Geographical location of sampling sites in the North Sea (pink circle) (Google 2010, Tele Atlas 2010)**

## RESULTS AND DISCUSSION

➢ THg 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 preys. 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).

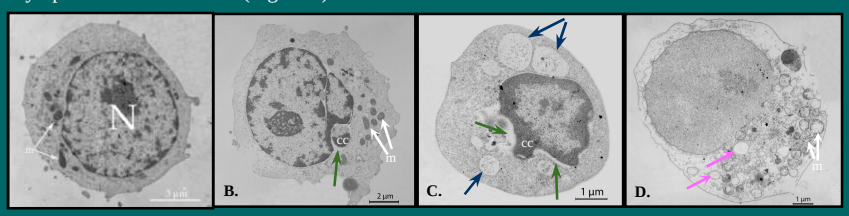



**Figure 4. A. Healthy activated lymphocyte with a big central nucleus (N) and mitochondrias (m). B-C-D. Suffering lymphocytes in presence of MeHg (1 µM) presenting a distorted plasmic membrane, a fragmented nucleus (B), perinuclear spaces (green arrows), vacuoles (blue and pink arrows), compacted chromatin (cc), swelling mitochondrias (D: m).**

## 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.**

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