# BIOACCUMULATION OF PCB & DDE METHYL SULPHONES IN MARINE MAMMALS AND THEIR INTERACTIONS WITH RECEPTOR PROTEINS

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## Introduction

PCB and DDE-Methyl sulphone metabolites are the product of enzymatic and bile acid entero hepatic metabolism in the final phase (III) of PCB and DDE detoxification in mammals following hepatic microsomal cytochrome P450-dependent metabolism (phase I) and conjugation (phase II)[1]. There is good evidence that PCB and DDE methyl sulphone (MSF) metabolites interfere with steroid binding to a receptor protein in uterine epithelium (uteroglobin – UG)[2] and bronchial epithelium (clara cell secretory protein – CCSP)[3]. UG and CCSP are homologous 16,000 Da proteins with different tissue-specific functions[4].

UG binds progesterone in the pre-implantation uterus to signal localised endometrial thickening and capillary formation, vital for successful attachment of the fertilised embryo[4]. PCB-MSFs can displace progesterone in the mammalian uterus due to their higher affinity for UG, resulting in implantation failure or early fetal death[4]. CCSP however, functions to sequester phospholipase $A_2$ ($PLA_2$) released in response to stress (pathogenic infection / injury) to suppress inflammatory responses triggered by $PLA_2$ in bronchial epithelium[4]. CCSP is also known as retinol-binding protein (RBP) transporting retinol (vit A) to target epithelia for a functional immune response[4]. Studies with Harbour Seals demonstrated displacement of retinol from RBP by hydroxy-PCB metabolites resulting in immunosuppression[6]. PCB-MSFs have been shown to accumulate in clara cells and uterine epithelium in laboratory radioactive tracer studies and CCSP-knock out studies with mice[6-8].

PCB and DDE -MSFs burdens have been found in marine mammals[1, 9,10], suggesting they may be subject to reproductive and immuno-toxic effects of these metabolites. This study determines PCB and DDE-MSFs burdens in tissues (including lung & uterus) of Harbour Seal (*Phoca vitulina*) and Striped Dolphin (*Stenella coeruleoalba*) morbillivirus victims and characterises the marine mammalian UG/CCSP protein.

## Methods & Materials

For contaminant analysis, 2-5g of blubber, liver, lung and uterus were sampled from 10 Schleswig-Holstein (Germany) Harbour Seals that died in the phocine distemper virus (PDV) epizootic (1988) and from 12 west Mediterranean Striped Dolphins that died in the dolphin morbillivirus (DMV) epizootic (1990-91). Samples were stored in hexane-washed foil at -20°C until analysis. Methodology for preparation and analysis of ΣPCB (20 isomers), ΣDDT (DDD, DDT & DDE), ΣPCB-MSFs (13 isomers) and 3-DDE-$MeSO_2$ in samples is published elsewhere[1]. For characterisation of UG/CCSP, uterine flushings and 3-5g of uterine & bronchial epithelium were taken from an adult female Baltic Grey Seal (*Halichoerus grypus*). Samples were frozen at -80°C until use. Flushings were taken using phosphate buffer (pH 7.4). Tissues were homogenised in phosphate buffer (pH 7.4) and centrifuged to obtain soluble protein fraction (105, 000 x *g*).

UG/CCSP proteins were resolved using SDS-Page gel electrophoresis and western blotted with human anti rabbit UG/CCSP antibody (*Dako*, UK) to characterise UG/CCSP proteins using rat and human UG and CCSP as reference standards (*Dako*, Denmark).

**Results and Discussion**

As found in several other studies[1,9,10], concentrations of parent compounds were greater than MSF metabolites for all tissues in Harbour Seal and Striped Dolphin (Figs 1a & b). The ratio of parent compound / metabolite was significantly higher in Harbour Seal than Striped Dolphin (PCB:MSF; $p<0.001$ and DDT/3-DDE-MSF; $p<0.001$ for all tissues), suggestive of lower capacity for hepatic cytochrome P450 (CYP450) metabolism in Striped Dolphin. It has already been shown that cetaceans possess lower levels of hepatic CYP1A isozyme and lack CYP2B isozymes, as compared with pinnipeds[11]. These enzymes are responsible for phase I metabolism of PCBs and DDE by converting them into more polar intermediates necessary for MSF formation [1,9].

ΣPCB and ΣPCB-MSFs concentration in blubber of Schleswig-Holstein Habour Seals, were in the same range as those detected in other Harbour Seal PDV epizootic victims from the Swedish Coast[11]. ΣPCB blubber concentrations in Striped Dolphins, were in the same range as those reported in another study of epizootic victims from this population[12]. There are no previous reports of PCB-MSFs in west Mediterranean population, but PCB and PCB-MSF levels detected were greater than those found in epizootic victims from the east Mediterranean population[1]. This is likely due to the limited local sources of industrial pollution in the eastern Mediterranean basin.

PCB congeners 153, 138, 180, 187 and 170 were most strongly accumulated in both Harbour Seal and Striped Dolphin tissues. These PCBs typically constitute the bulk of total PCB burden as they are not metabolised by marine mammalian CYP450 detoxification systems due to their *ortho* chlorine substitution pattern[11]. PCB-MSF isomer pattern was similar for all tissues in both species, with 3-101 as the dominant isomer, and to a lesser or greater extent 4-101.

Gel electrophoresis highlighted a 16,000 Da between 14,200 and 20,100 Da molecular weight marker proteins for Grey Seal uterine flushing, uterine and lung epithelium samples. Alongside the human protein standard, western blots resolved an UG-like protein in uterine flushings and uterine epithelium samples and a CCSP-like protein in lung epithelium samples, by cross-reacting with human anti-rabbit UG/CCSP antibody (fig. 2). Further confirmation of the characterisation of UG/CCSP protein in marine mammals will be undertaken once specific anti-bodies have been raised to UG/CCSP proteins purified from seal and dolphin tissues.

It is not known whether uterine levels of PCB-MSFs in this study are sufficient to cause reproductive effects. Further research is needed to determine levels of exposure which can cause significant reduction in UG-progesterone binding in pre-implantation uterus and early pregnancy, when placental control of progesterone release is not yet established. In field studies, high organochlorine burdens have been correlated with reduced reproductive output in marine mammal populations. A feeding study with captive Harbour Seals found reproductive output was significantly reduced in females fed organochlorine-contaminated Wadden Sea fish compared with control females fed cleaner Atlantic Sea fish[13]. Uterine stenosis has also been correlated with high high organochlorine burdens in Baltic Seals [14]. Recent laboratory studies on mink dosed with PCBs showed uterine pathological changes and embryo toxicity, providing further support that organochlorines and their MSF metabolites are reproductive toxins[15]. It is thought that resorption of dead tissue accumulated from embryos which fail to implant and/or aborted early stage foetuses results in the blockage of uterine horns leading to stenosis and infertility. Preliminary results from an ongoing study in our laboratory, show progesterone binding with seal UG ($B_{max}$ 1.5-2.3 pmol/mg protein) and decreased progesterone binding in the presence of PCB-MSF 4-101 (10-20 pmol/mg protein) consistent with observations with rabbit UG[2]. This is preliminary evidence for the suggested anti-progestenic mechanism of PCB-MSFs-mediated reproductive toxicity.

It is not possible to predict whether lung PCB-MSFs levels observed are immunosuppressive since concentrations of PCB-MSFs necessary for toxic effect have not yet been established. $PLA_2$ catalyses hydrolysis of fatty acids into mediators of inflammation[4]. UG/CCSP functions to inhibit $PLA_2$ activity, as uncontrolled inflammation causes constriction of the airways, restricted blood flow and epithelial cell injury in conducting airways and alveolar regions[16]. Also, UG/CCSP

inhibits monocyte and neutrophil chemotaxis and phagocytosis to regulate immune response. Considering the demonstrated potential of PCB-MSF to bind UG/CCSP, it is feasible that PCB-MSF binding may inhibit $PLA_2$ binding thereby reducing anti-inflammatory response and affecting other UG/CCSP immune functions. Studies are underway to investigate seal CCSP-$PLA_2$ binding and $PLA_2$ displacement by PCB-MSFs. We are also investigating seal CCSP-retinol binding and retinol displacement by PCB-MSFs as it would reduce retinol delivery to target epithelia in the immune response. Such studies help to elucidate mechanisms of MSF-mediated immunotoxicity.

Due to the immunosuppressive nature of morbillivirus infection, the animals suffered from secondary infections, particularly in the lung. Pathological examination of epizootic victims showed a high incidence of pneumonia, alveolar atelectasis, alveolar collapse and emphysema[17,18]. Similar observations have been made in humans poisoned with PCB in the Yusho disaster[19]. PCB and PCB-MSFs levels in sputa, blood, lung and adipose of exposed humans correlated well with severity of pulmonary effects and reduced circulating immunoglobulin levels[19,20], suggesting immune function in morbillivirus victims was not only compromised by immuno-toxic PCBs, but also by MSF metabolites accumulated in lung tissue.

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**Fig 1. Mean (+/- s.e.) Sum Concentrations of PCB, MSF-PCB, DDT and 3-DDE-MSF in**

**a. Harbour Seal** **b. Striped Dolphin**

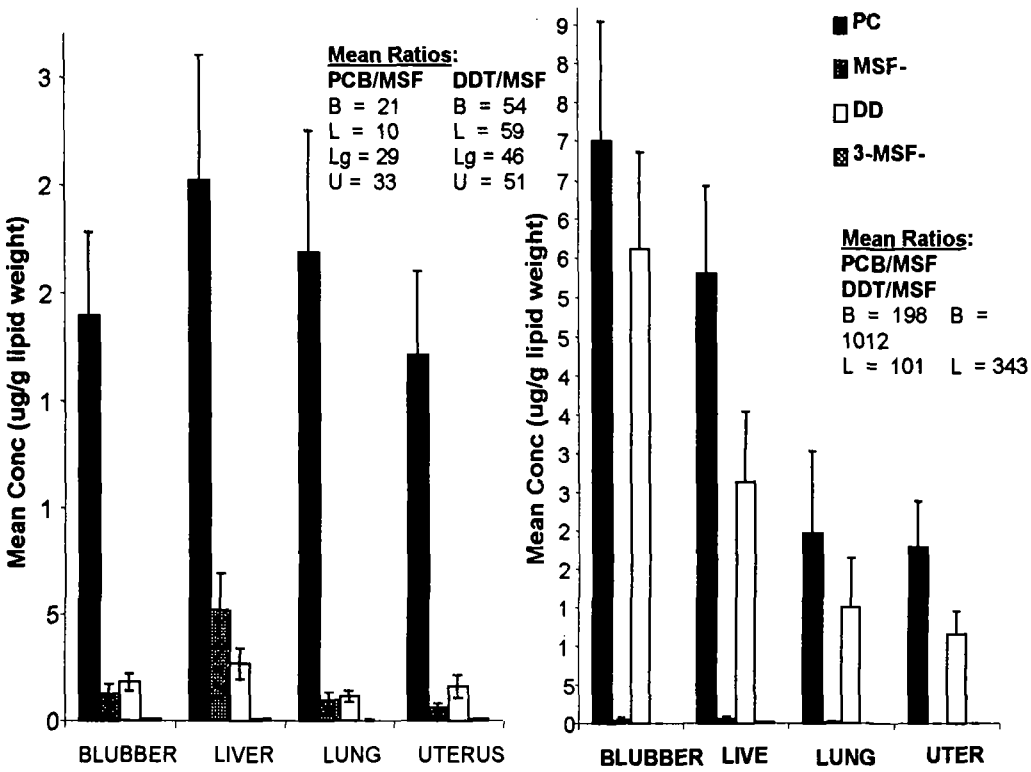
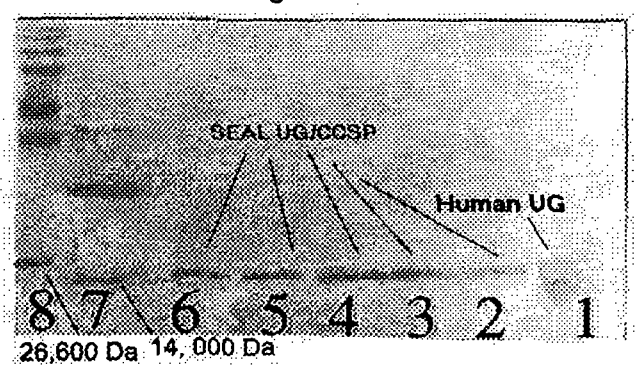



**Fig 2. Western blot characterisation of UG/CCSP in Grey Seal;**




**Lane 1** - Human UG/CCSP
**Lane 2** - Seal uterine flushing
**Lane 3** - Seal uterine flushing
**Lane 4** - Seal uterine uterus
**Lane 5** - Seal uterus
**Lane 6** - Seal lung
**Lane 7** - M.wt marker SDS 7 14,200 to 66,000
**Lane 8** - M.wt marker SFS 6H 26,600 to 205,000