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**First detection of CYP1A1 and CYP2B induction in
Mediterranean cetacean skin biopsies and cultured fibroblasts by
western blot analysis**

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Abstract

The need to develop powerful tools to detect exposure and effects of POPs and emerging contaminants in Mediterranean cetaceans led us to develop a suite of sensitive non-lethal biomarkers in integument biopsies of free-ranging animals. In order to propose induction of CYP1A1 and CYP2B, detected by western blot analysis, as biomarkers of exposure to OCs, PAHs and PBDEs, a three-phase experimental protocol (in vitro experiments, calibration experiments and field applications) was followed using fibroblast cell cultures and biopsies of Mediterranean Stenella coeruleoalba and Tursiops truncatus. This methodology was confirmed to be sensitive and stable in comparison to previous methods used to detect CYP1A1 in biopsies, enabling analysis of several inducible proteins in non-lethal samples and analysis of material from stranded animals.

Keywords: Mediterranean cetaceans; Integument biopsy; Fibroblast cell cultures; CYP1A1; CYP2B; Western blot; OCs; PBDEs

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In the last 20 years, there has been growing concern about the potential risk to Mediterranean cetaceans of high bioaccumulation of organochlorines (OCs) (Fossi et al., 1992; Fossi et al., 2003a) and emerging contaminants, such as polybrominated diphenyl ethers (PBDEs) (Alaee et al., 2003; Fossi et al., 2006). While levels of organochlorine compounds are decreasing, concentrations of PBDEs seem to be increasing in the Mediterranean environment.

Several international institutions (IWC, ACCOBAMS) have encouraged research and development of sensitive non-lethal biomarkers in biopsies of free-ranging animals, to define the toxicological status of cetaceans. The objective of our lab was to develop a “multi-trial biomarker tool” applied to cetacean integument biopsies to detect “toxicological stress syndromes”. We used a multidisciplinary approach, merging genomic and proteomic studies (Western blot, RT-PCR, cultured fibroblasts) with analysis of POPs, PAHs and PBDEs, and genetic population studies. Here, we summarize the main results of part of this project, focusing on detection of CYP1A1 and CYP2B induction in cetacean biopsies and cultured fibroblasts by Western blot (WB) analysis, as biomarkers of exposure to OCs, PAHs and PBDEs.

In order to validate this methodology, a three phase experimental protocol was followed. In the first phase (*in vitro* experiments), we used WB to detect CYP1A1 and CYP2B induction caused by exposure of cultured fibroblasts of *Stenella coeruleoalba* and *Tursiops truncatus* to PBDEs and OCs. In the second phase (calibration experiments), we validated WB analysis of CYP1A1 and CYP2B as a semi-quantitative detection tool using calibration curves of CYP450 obtained from liver and skin of stranded specimens of *Tursiops truncatus*. Finally in the third phase (field applications), we applied WB analysis of CYP1A1-

CYP2B as a semi-quantitative tool to explore differences in exposure to OCs, PAHs and PBDEs of three sub-populations of *Stenella coeruleoalba*.

Sub-samples of skin and liver of stranded *Tursiops truncatus* (n=2) were obtained 4-8 hours after death. Integument biopsies (Fossi et al, 2003a) were obtained from free-ranging *Stenella coeruleoalba* (n=60) from the Mediterranean Whale Sanctuary, Ionian Sea and Strait of Gibraltar.

Gender was determined genetically according to Berube and Palsboll (1996). Fibroblasts were cultured from biopsies according to Marsili et al. (2000) and were subject to two different experimental protocols for 48 h, using two classes of CYP-inducers (Fossi et al., 2006). Firstly, a mixture of Arochlor 1260, pp'DDT and pp'DDE in DMSO were used at three concentrations: 1, 5 and 25 µg/ml, plus DMSO (0.05%) as control. Secondly, a mixture containing 27 PBDEs in nonane were used at three concentrations: 0.1, 0.05 and 0.01 µg/ml, plus nonane (0.01 µg/ml) as control.

Homogeneous sub-samples of biopsies (including epidermis, dermis and blubber layers; Angell et al., 2004) were homogenized in aryl-hydrocarbon-receptor (AhR) buffer (Wilson et al., 2007) in a Tissue Lyser (Qiagen). The homogenate was centrifuged twice and the supernatant (S9) was analyzed for total proteins and then by WB.

For WB analysis, S9 tissue homogenates (in duplicate) and fibroblast extracts were separated by SDS-PAGE (10% polyacrylamide gels) and blotted onto nitrocellulose; the membranes were saturated with blocking solution for 1h. Primary polyclonal antibodies were from Oxford Biochemical Research. Goat anti rabbit CYP1A1 and anti CYP2B4, diluted 1:5000 and 1:1000, respectively, in TTBS-1% gelatin, were incubated with cetacean proteins overnight. Incubation with anti-goat HRP-labelled secondary antibody (1:3000) was performed (1h) and detected according to the BioRad Immun-Star-HRP-Chemiluminescent-Kit booklet.

In order to validate WB analysis of CYP1A1 and CYP2B as a semi-quantitative detection tool in biopsies, a series of calibration curves of CYP450 from liver (ranging from 1.2 to 4.8 pmol of CYP450) and skin (from 0.095 to 2.400 pmol) of stranded *Tursiops truncatus* were analysed in triplicate. A triplicate skin standard (SS) was analyzed within the curves and maintained as internal standard in subsequent WBs. Semi-quantitative analysis was performed for each WB with Quantity-One software (BioRad) using Adjusted Volume (Intensity *mm²) as quantitative parameter. The data was correlated with increasing pmol of CYP450 and two Excel Macros (one for CYP1A1 and one for CYP2B) were produced incorporating the normalizing factor of SS for quantification of unknown biopsy samples. The calibration curves were validated for semi-quantitative analysis of CYP1A1 and CYP2B in three different populations of striped dolphin.

In the first phase (*in vitro* experiments), we applied the WB technique to detect CYP1A1 and CYP2B induction produced by PBDEs and OCs in fibroblasts of the two species of dolphins (Fossi et al., 2006). Increasing induction was detected in both species with increasing concentrations of contaminants. In female striped dolphin cells, CYP2B increased 3-, 15- and 16-fold with respect to control, with increasing concentrations of OCs. The highest trend of induction (CYP2B) was found in *Tursiops truncatus* cells treated with PBDEs. In the second phase (calibration experiments), we validated WB analysis of CYP1A1 (Fig. 1A) and CYP2B (Fig. 1B) as a semi-quantitative detection tool with the calibration curves of CYP450 obtained from liver and skin of stranded specimens of *Tursiops truncatus*. A linear correlation was found between relative pmol CYP1A1 and Adjusted Volume (Int. *mm²) (R=0.99) and between relative pmol CYP2B and Adjusted Volume (R=0.99). The two Excel Macros (CYP1A1 and CYP2B), incorporating the normalizing factor of SS (Skin Standard) (Fig 1A and 1B) will be used for quantification of unknown biopsy samples (third phase). In the third phase (field applications), we applied the WB analysis of CYP1A1 and

CYP2B as semi-quantitative detection tools, in biopsy samples, to explore different exposure to planar (polycyclic aromatic hydrocarbons and planar halogenated aromatic hydrocarbons) (Angell et al., 2004; Godard et al., 2004; Wilson et al., 2007) and globular (globular halogenated aromatic hydrocarbons) lipophilic contaminants, respectively, in three sub-populations of Mediterranean *Stenella coeruleoalba*. Here the calibration curves were validated (as pilot experiment) for semi-quantitative analyses of CYP1A1 (Fig. 2) and CYP2B in male sub-samples collected in the three areas. Future studies will concern correlations between CYP1A1/CYP2B and blubber levels of contaminants.

In conclusion this methodology applied to skin biopsies and stranded cetaceans was confirmed to be sensitive and stable in comparison to previous methods used to detect CYP1A1 in biopsies (enzymatic (Fossi et al., 1992; Fossi et al., 2003a) and immunohistochemistry techniques), enabling analysis of several inducible proteins in non-lethal samples and material from stranded animals. Future development of more sensitive techniques (RT-PCR and proteomics) with chemical data (OCs, PBDEs, PAHs) will provide more complete information about cetacean toxicological stress syndromes.

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References

Angell, C.M., Wilson, J.Y., Moore, M.J., and Stegeman, J.J. (2004). Marine Mammalian Sciences, 20, 554-566.

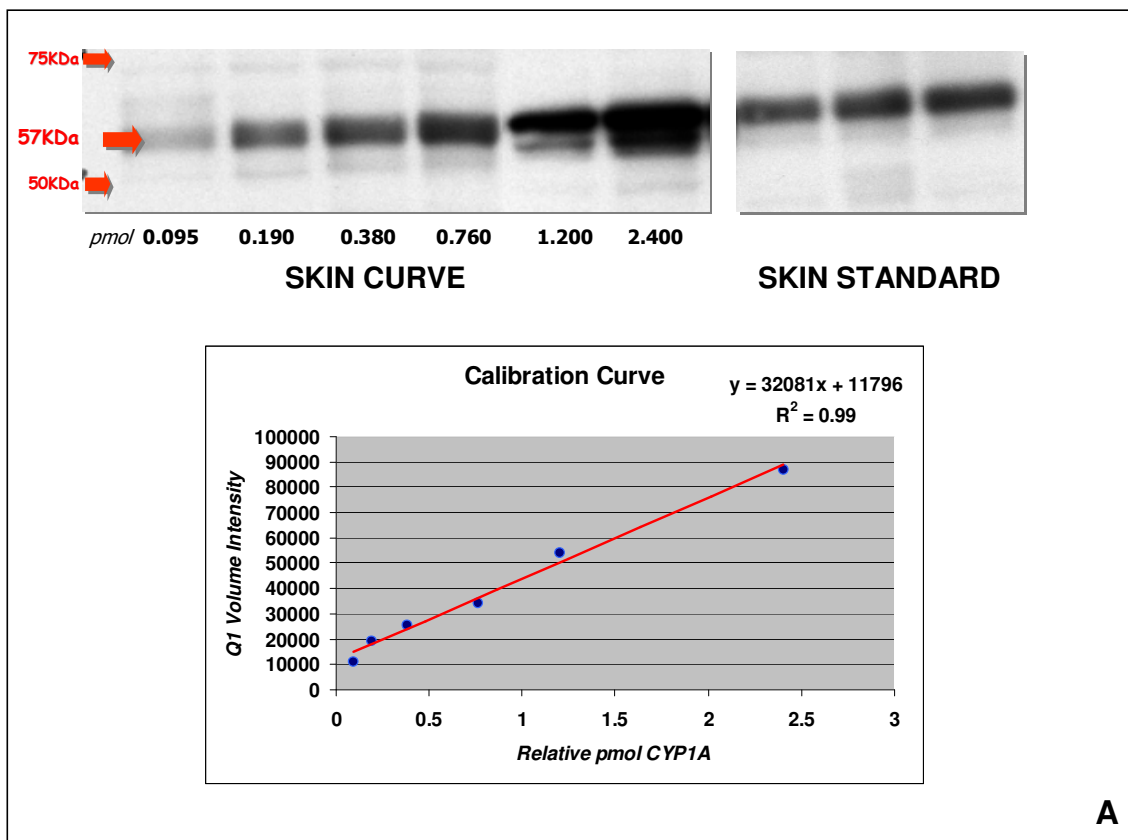
- Alaee, M., Arias, P., Sjödin, A., and Bergman, A. (2003). *Environmental International*, 29, 683-689.
- Berube, M., and Palsboll, P. (1996). *Molecular Ecology*, 5, 283-287.
- Fossi, M.C., Marsili, L., Leonzio, C., Notabartolo di Sciara, G., Zanardelli, M., and Focardi S. (1992). *Marine Pollution Bulletin*, 24, 459-461.
- Fossi, M.C., Marsili, L., Neri, G., Natoli, A., Politi, E., and Panigada, S. (2003a). *Marine Pollution Bulletin*, 46, 972-982.
- Fossi, M.C., and Marsili, L. (2003b). *Pure Applied Chemistry*, 75, 2235-2247.
- Fossi, M.C., Marsili, Casini, S., and Bucalossi D. (2006). *Marine Environmental Research*, 62, 200-2004.
- Godard, C.A.J., Smolowitz, R.M., Wilson, J.Y., Payne, R.S., and Stegeman, J.J. (2004). *Toxicological Sciences*, 80, 268-275.
- Marsili, L., Fossi, M.C., Neri, G., Casini, S., Gardi, C., Palmeri, S., et al. (2000). *Marine Environmental Research*, 50, 649-652.
- Wilson J.Y., Wells, R., Aguilar, A., Borrel, A., Tornero, V., Reijnerds, P., et al (2007). *Toxicological Sciences* (on- line).

Figure Captions

Fig. 1. Western blot analysis (WB) of CYP1A1 (A) and CYP2B (B) calibration curves of CYP450 from skin (ranging from 0.095 to 2.400 pmol of CYP450) of stranded specimens of *Tursiops truncatus*. A triplicate skin standard (SS) was analyzed within the calibration curve, and maintained as internal standard in subsequent WBs. Semi-quantitative analysis was performed for each WB (in triplicate) with Quantity One software (BioRad, 1-D Analysis Software) using Adjusted Volume (Intensity *mm²) as quantitative parameter.

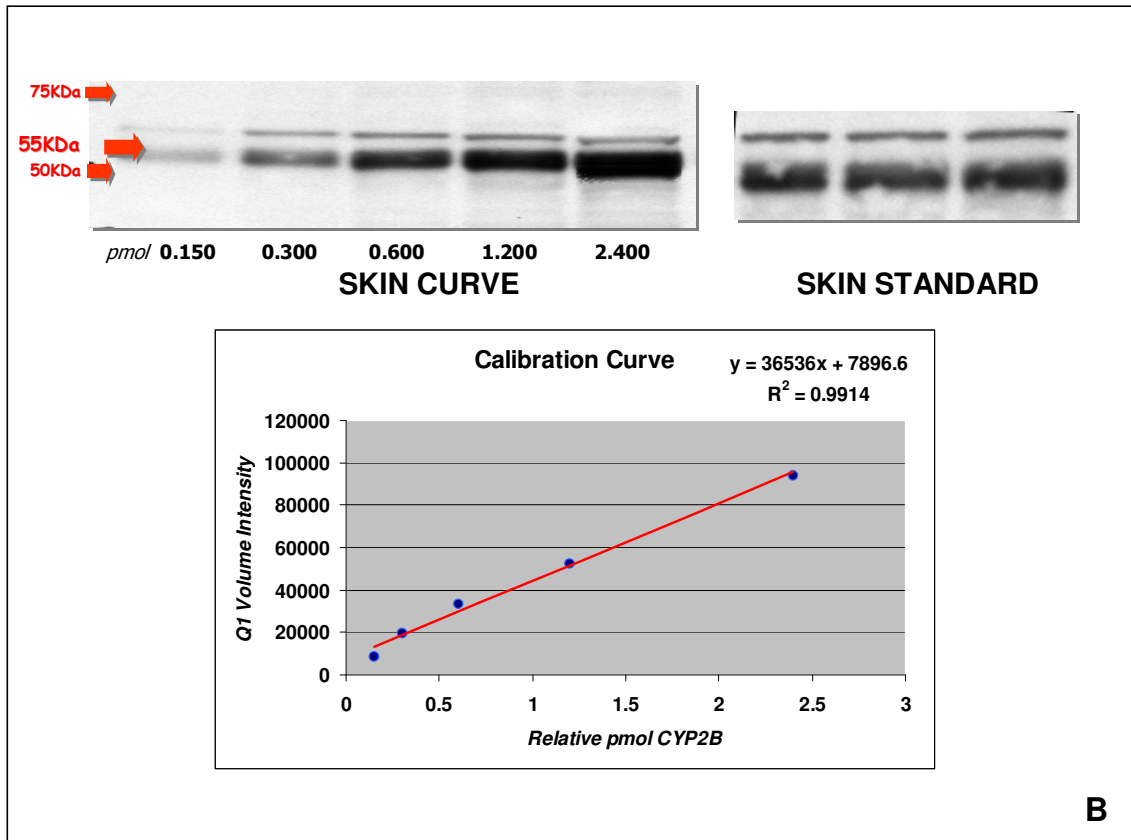
Fig. 2. Western blot (WB) analysis of CYP1A1 in male specimens of three sub-populations of Mediterranean *Stenella coeruleoalba* (biopsy samples). Different steps of the field validation of the methodology (WB, Quantity one analyses, Excel Macros and Relative pmol CYP1A1) are reported. The results are express as Relative pmol CYP/mg prot.

Figure 1a



A

Figure 1b



B

Figure 2

