

The background of the slide is a photograph of a beach. A bright sun is in the upper left, creating a lens flare that extends diagonally across the frame. A single white cloud is positioned in the center-left. The right side of the image shows a sandy beach meeting the sea, with the water's surface reflecting the light.

# INDAGINI ECOTOSSICOLOGICHE SUI CETACEI SPIAGGIATI

LETIZIA MARSILI  
UNIVERSITÀ DI SIENA

SORVEGLIANZA SANITARIA DEI CETACEI SPIAGGIATI LUNGO LE COSTE DELLA REGIONE TOSCANA  
Pisa 15 Novembre 2013



# DESCRITTORE 8: CONCENTRAZIONE DEI CONTAMINANTI

All'interno della EU Marine Strategy, più precisamente all'interno del Descrittore 8 (Concentrazione dei Contaminanti), sono richiesti indicatori per i differenti habitat oceanici.

L'intensità degli effetti biologici o ecologici dovuti ai contaminanti deve essere mantenuta entro i livelli di variabilità naturale dei processi biologici degli habitat e dei gruppi funzionali.







Si definisce BIOINDICATORE o  
"organismo sentinella"  
ogni organismo vivente,  
animale o vegetale,  
che, campionato in un determinato  
ambiente, ci fornisce indicazioni sul  
livello di contaminazione di quella  
determinata area.





MUSSEL WATCH  
1970

PROGRAMMA DI MONITORAGGIO  
DEGLI AMBIENTI MARINO COSTIERI  
UTILIZZANDO IL  
*Mytilus galloprovincialis*  
COME BIOINDICATORE





### **Caratteristiche necessarie per un buon bioindicatore**

- **optimum ecologico ed ampia distribuzione nell'area di studio**
- **facile identificazione sistematica**
- **buone conoscenze su anatomia, fisiologia ed ecologia della specie**
- **uniformità genetica e lungo ciclo vitale (ma in casi particolari si usano anche microrganismi)**
- **facile reperibilità in tutte le stagioni (ma in casi particolari si usano anche organismi a ciclo stagionale)**
- **scarsa mobilità (stanzialità)**




EU Marine Strategy

PER INDIVIDUARE NUOVI  
INDICATORI PER IL  
DESCRITTORE 8,  
QUESTO STUDIO PROPONE  
VARIE SPECIE DI CETACEI.

to protect Europe's oceans and seas





A photograph of a dolphin leaping from the surface of the ocean. The dolphin is captured mid-air, its body arched as it moves from the water. The water is a deep blue with visible ripples and a splash of white foam where the dolphin exited. The text is overlaid on the lower half of the image.

Come si possono  
definire  
bioindicatori?



Delfino di fiume (*Lipotes vexillifer*)  
Baiji (cinese) del Fiume Giallo o Yangtze



Gli studiosi affermano che nel fiume, che è  
lungo oltre seimila chilometri, ci potrebbero  
ancora essere una decina di esemplari di  
"baiji", che non sono sufficienti per salvare la  
specie dall'estinzione.





# MAR DI CORTEZ - MESSICO

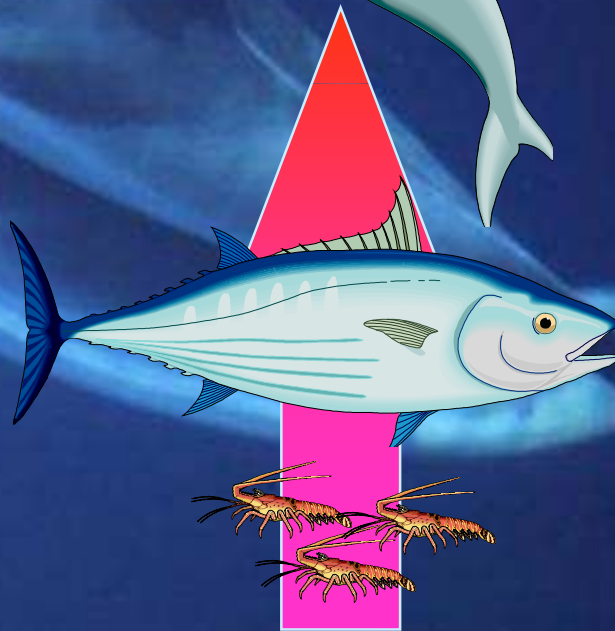
39 percent of the world's total number of species of marine mammals and a third of the world's marine cetacean species.



# BIOMAGNIFICAZIONE

*TOP PREDATORS*

*Alta "rischio" tossicologico*



*OCs*



Balenottera comune (*Balaenoptera physalus*),  
capodoglio (*Physeter macrocephalus*),  
tursiope (*Tursiops truncatus*),  
stenella striata (*Stenella coeruleoalba*),  
zifio (*Ziphius cavirostris*),  
delfino comune (*Delphinus delphis*) e  
grampo (*Grampus griseus*)  
sono nella "Red list" dell' "International Union for  
Conservation of Nature and Natural Resources" delle  
"specie a rischio".

**IUCN**  
The World Conservation Union

The IUCN Species Survival Commission

**2006 IUCN Red List of  
Threatened  
Species™**

[Introduction](#)  
[Partners & Credits](#)  
[Red List Programme](#)  
[Data Organization](#)  
[Summary Statistics](#)  
[Sources & Quality](#)  
[Categories & Criteria](#)  
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[Photo Gallery](#)  
[References](#)  
[Publications & Links](#)  
[FAQs](#)



**SEARCH** **EXPERT  
SEARCH**

What's new? Last updated on 09 March 2007.  
© International Union for Conservation of Nature and Natural Resources, [Contact](#)  
[Information](#)

 **SSC**  
Species Survival Commission

LA BALENOTTERA COMUNE, UNICO MISTICETE DEL  
MEDITERRANEO, IL CAPODOGLIO ED IL DELFINO  
COMUNE, SONO INDICATE ADDIRITTURA COME SPECIE  
"ENDANGERED", VALE A DIRE AD ALTO RISCHIO  
D'ESTINZIONE NEL PROSSIMO FUTURO.



# Multiple Stress Pressure in Mediterranean Cetaceans

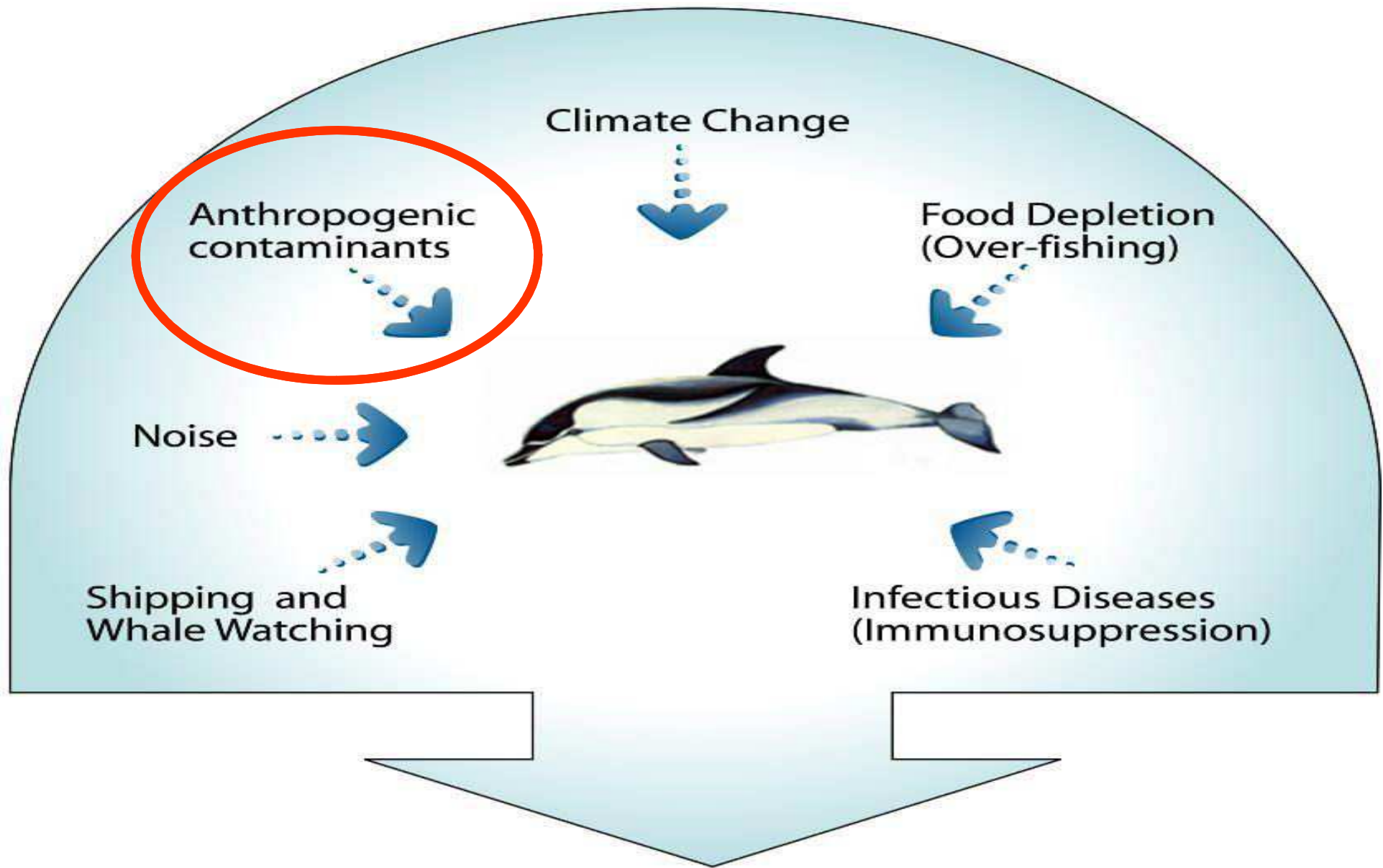
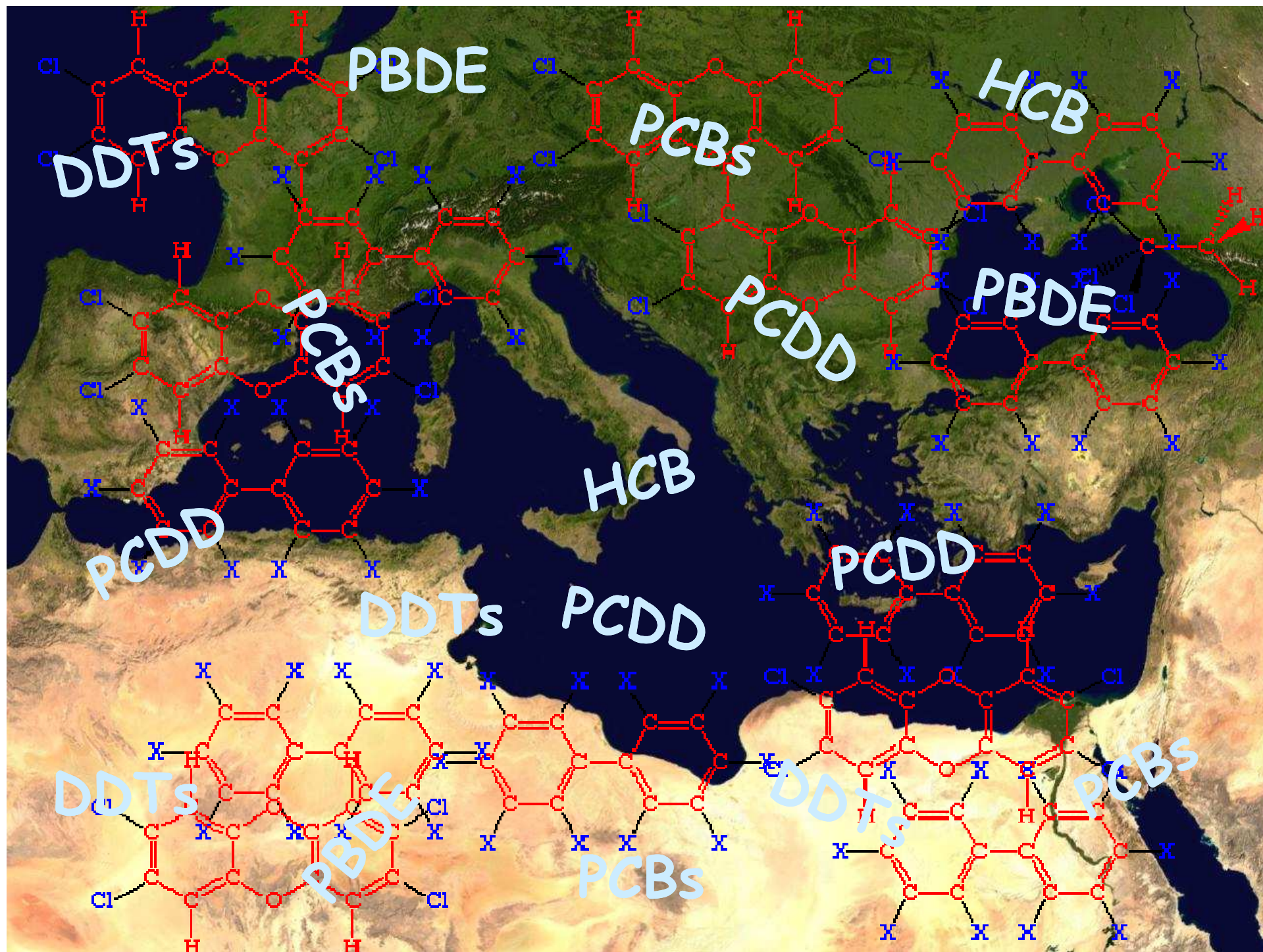
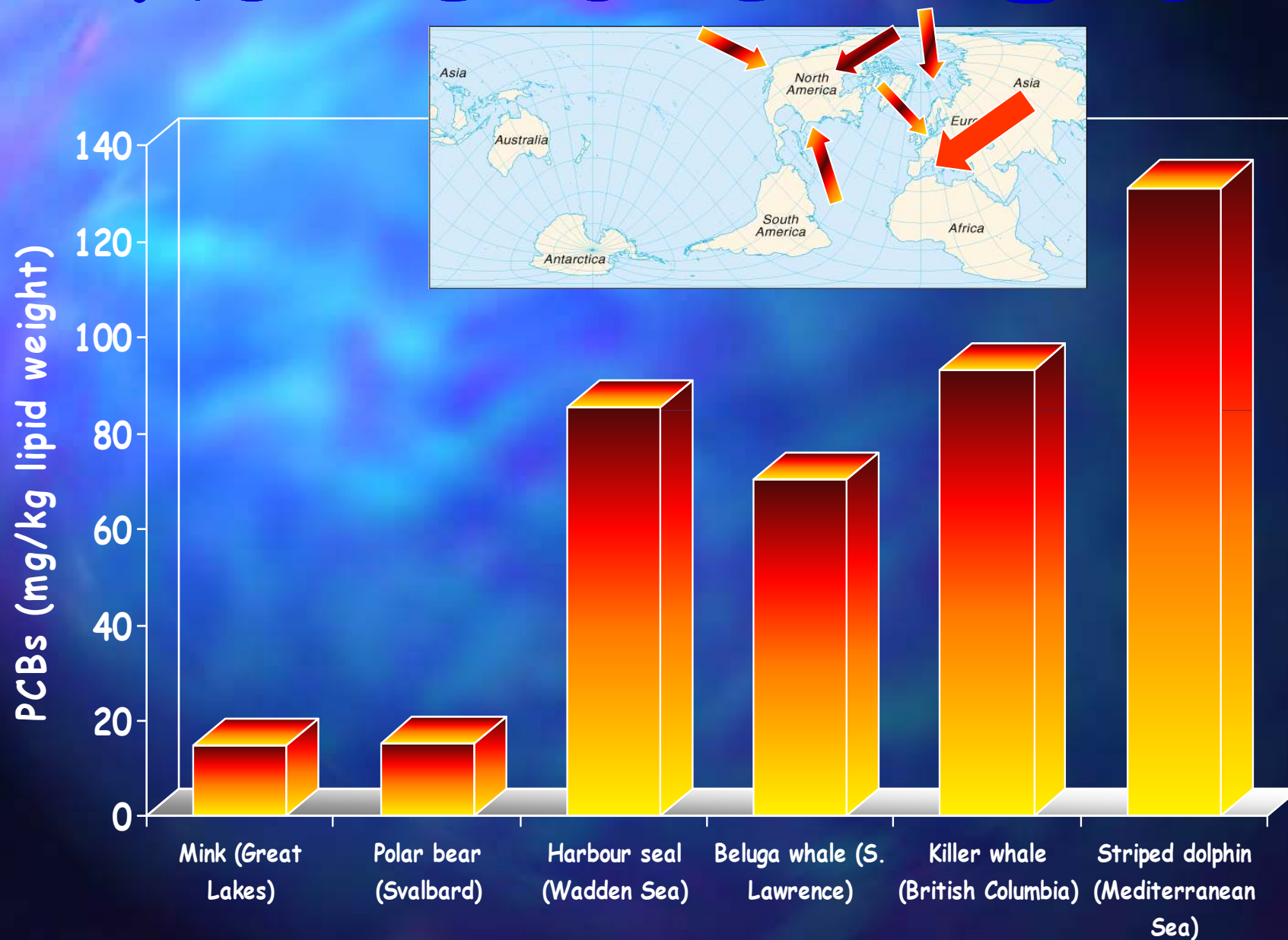


Fig1 .





# HOT SPOTS AREAS



# MEDITERRANEAN SEA



STRIPED DOLPHIN  
PCBs 6-60 ppm l.b.  
DDTs 21-43 ppm l.b.



STRIPED DOLPHIN  
PCBs 70-850 ppm l.b.  
DDTs 70-450 ppm l.b.

Marsili (2000) IJEP, 13, 416-52



A photograph of a dolphin leaping from the water, creating a large splash. The dolphin is captured mid-air, with its body arched and its tail visible. The water is a deep blue, and the dolphin's skin is a lighter, greyish-blue. The text is overlaid on the image in a bold, red, hand-drawn style font.

TECNICA DI  
CAMPIONAMENTO  
TOTALMENTE NON  
DISTRUTTIVA ED INVASIVA

# MATERIALE BIOLOGICO PER INDAGINI TOSSICOLOGICHE





Primo quesito  
ecotossicologico:

Quali sono i livelli  
dei diversi contaminanti  
ambientali negli organi e  
tessuti dei Cetacei del  
Mar Mediterraneo?

# Stenella striata



Marsili, 2000 – Int. J. Environm. Poll., 13(1-6): 416-452



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**Lipophilic contaminants in marine mammals: review of the results of ten years' work at the Department of Environmental Biology, Siena University (Italy)**

---

Letizia Marsili

Department of Environmental Sciences, University of Siena, Via Mattioli 4, 53100 Siena, Italy  
(e-mail: marsili@uni.si.it)

Int. J. of Environment and Pollution 2000 - Vol. 13, No.1/2/3/4/5/6 pp. 416-452

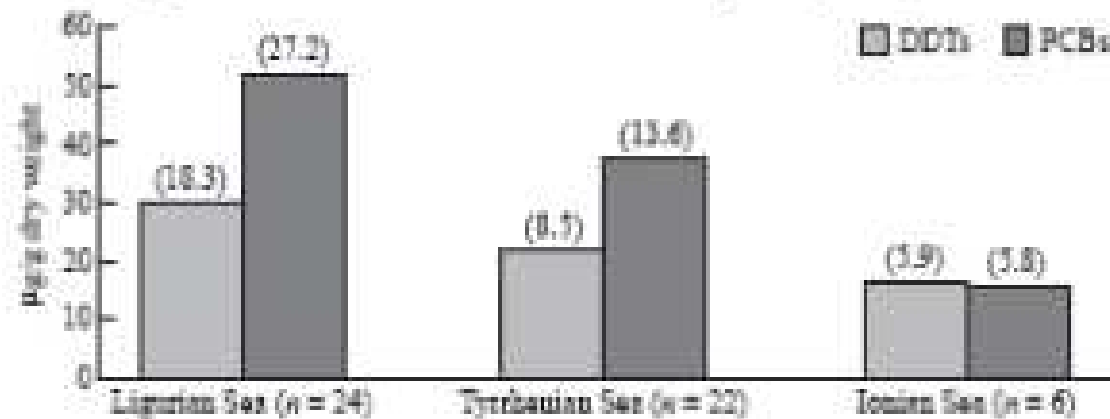
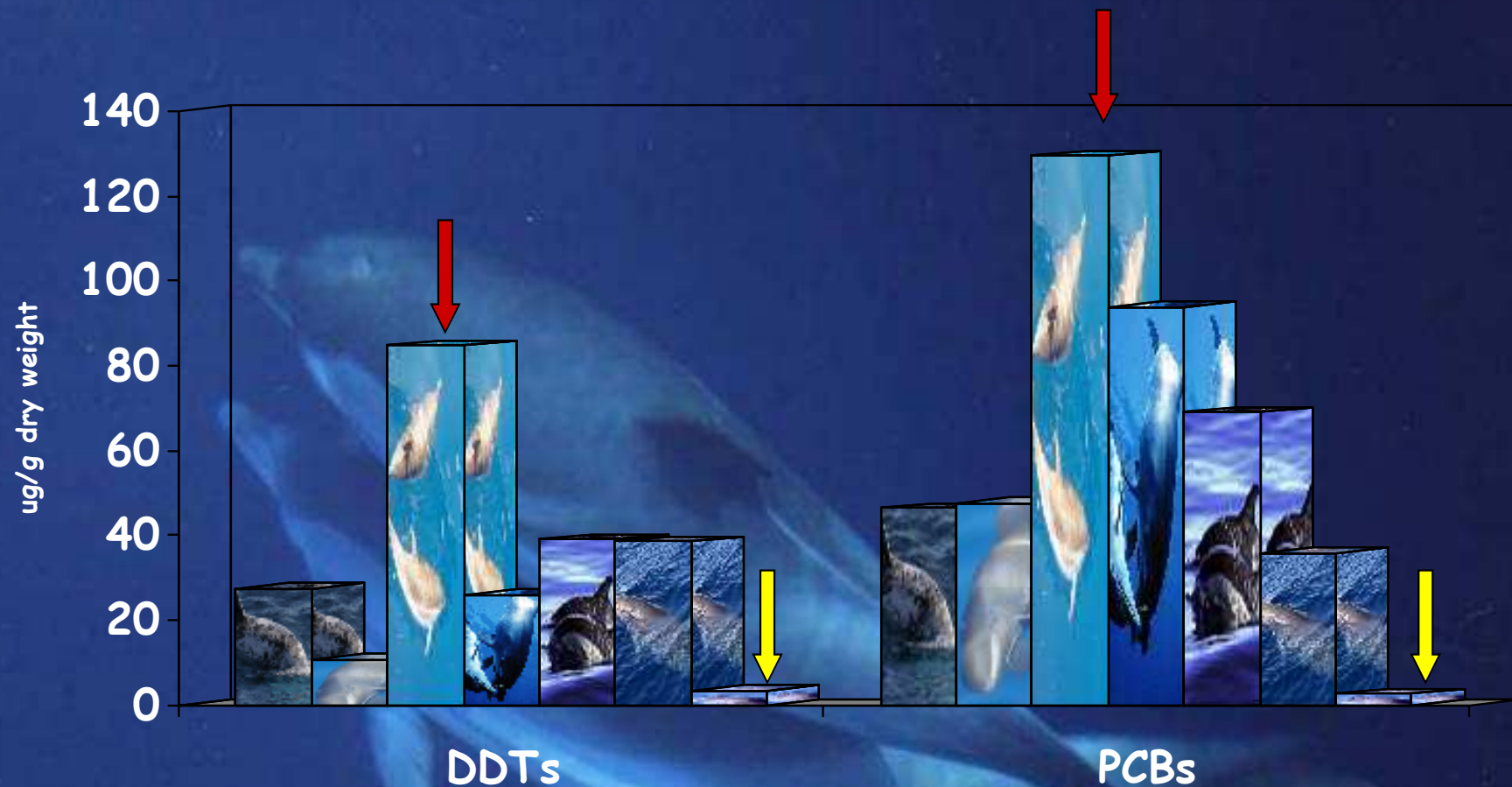


Figure 5. Average concentrations of DDTs and PCBs in striped dolphins from different seas in the summer of 1993.



- Risso's dolphin
- Long-finned pilot whale
- Striped dolphin
- Bottlenose dolphin
- Common dolphin
- Cuvier's beaked Whale
- Fin whale

Marsili e Focardi, 1997 - Environ. Mon. Ass., 45: 129-180  
 Marsili, 2000 - Int. J. Environm. Poll., 13(1-6): 416-452  
 Marsili 2007 - unpublished data



# Age, growth and organochlorines (HCB, DDTs and PCBs) in Mediterranean striped dolphins *Stenella coeruleoalba* stranded in 1988–1994 on the coasts of Italy

Letizia Marsili<sup>1,\*</sup>, Carolina Casini<sup>2</sup>, Luca Marini<sup>3</sup>, Andrea Regoli<sup>4</sup>, Silvano Focardi<sup>1</sup>

<sup>1</sup>Dipartimento di Biologia Ambientale, Università di Siena, Via delle Cerchia 3,

<sup>2</sup>Dipartimento di Biologia Animale e dell'Uomo, Università "La Sapienza", Viale dell'Univ

<sup>3</sup>Ufficio per i Diritti degli Animali, Comune di Roma, Viale del Giardino Zoologico 2

<sup>4</sup>Istituto Nazionale di Statistica, Via A. Ravà 150, I-00142 Roma, I

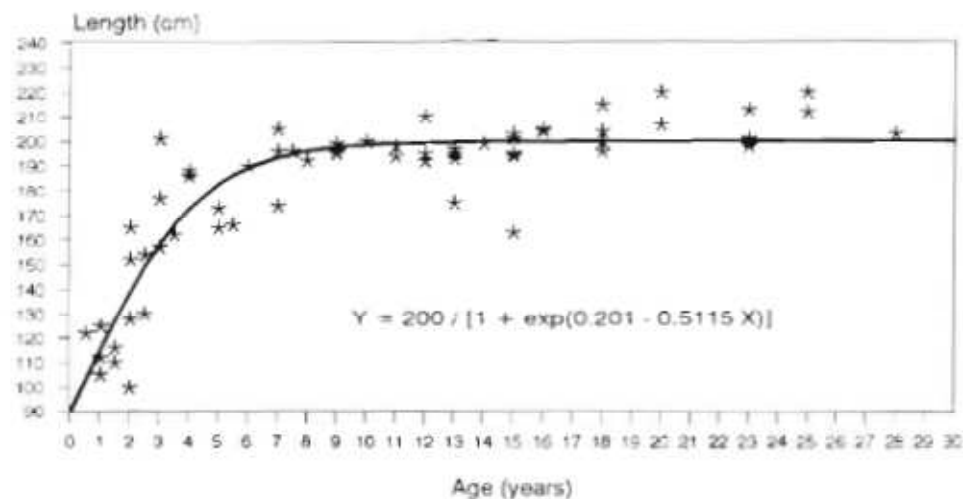


Fig. 1. *Stenella coeruleoalba*. Growth curve of dolphins in the Mediterranean Sea. Y: length; X: age

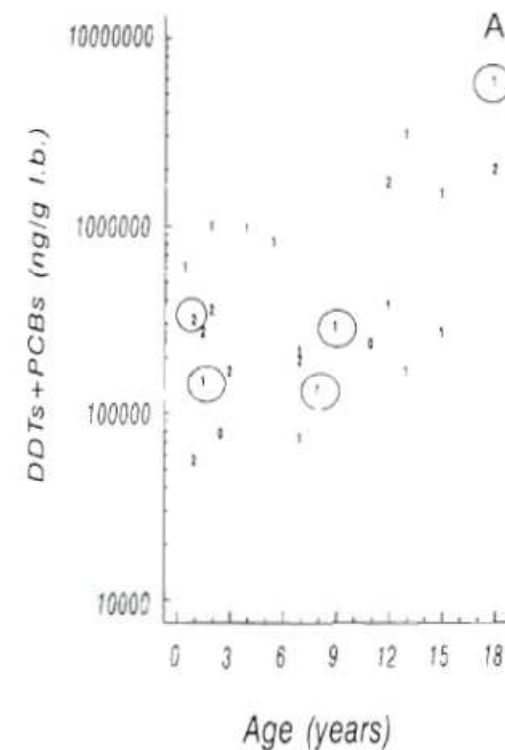
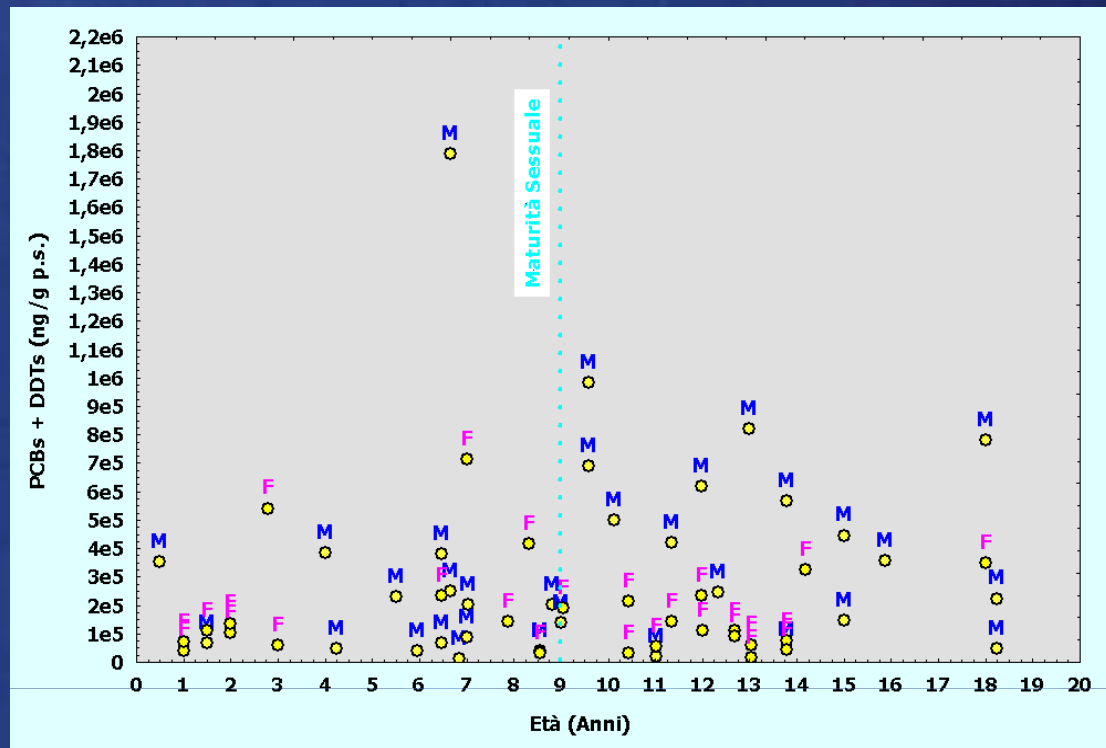
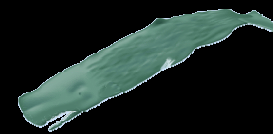


Fig. 2. *Stenella coeruleoalba*. Concentration of organochlorines



Fin whales



Sperm whale



Milk-cow

FOR 100 gr

Water → 53-55

→ 53-54

→ 90-91

Lipids → 31-33

→ 35-37

→ 3-4

\*Solid residues → 13-14

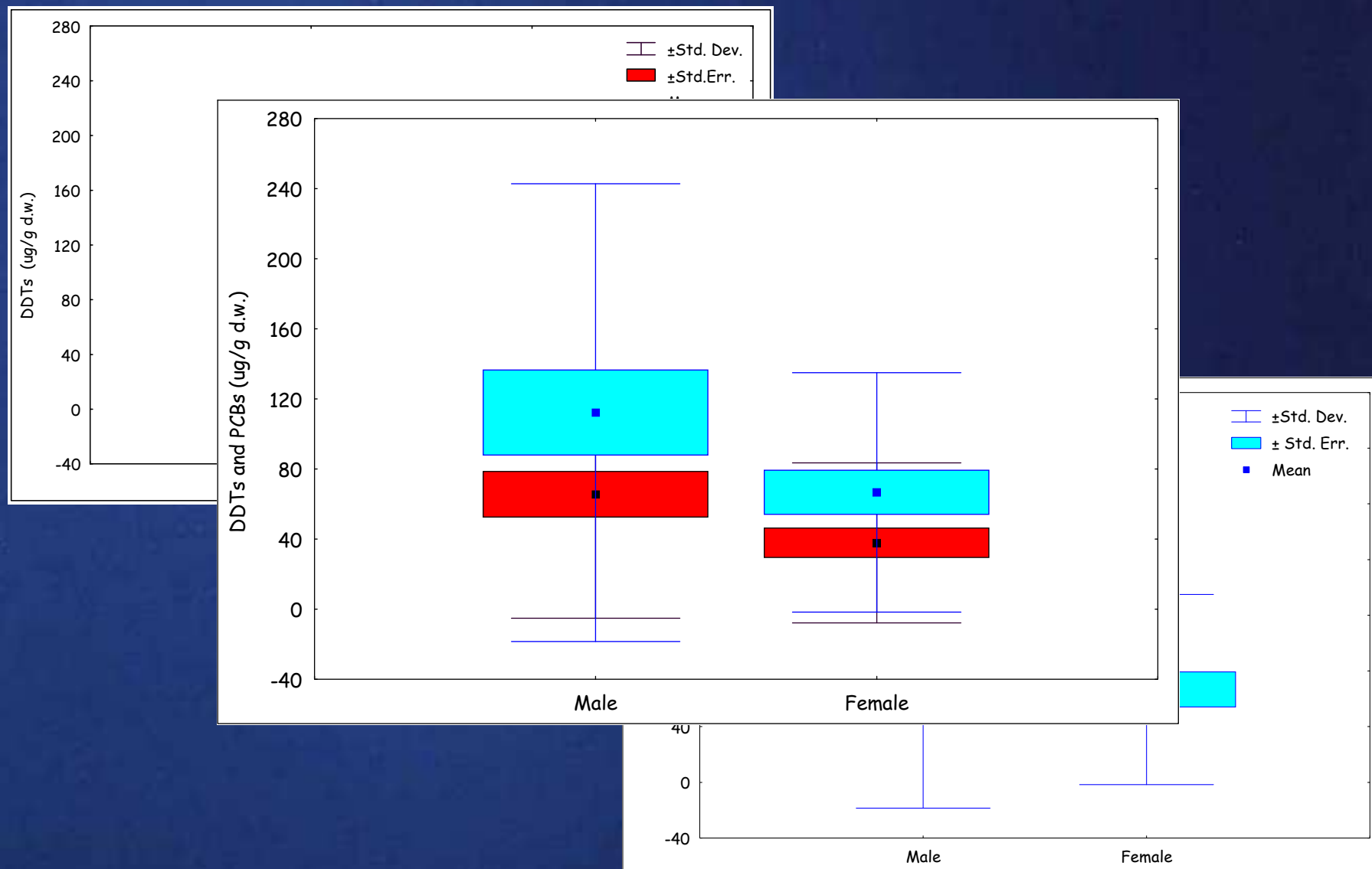
→ 7-8

→ 4-5

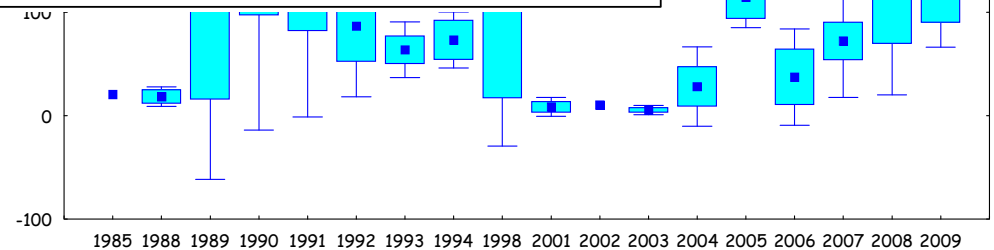
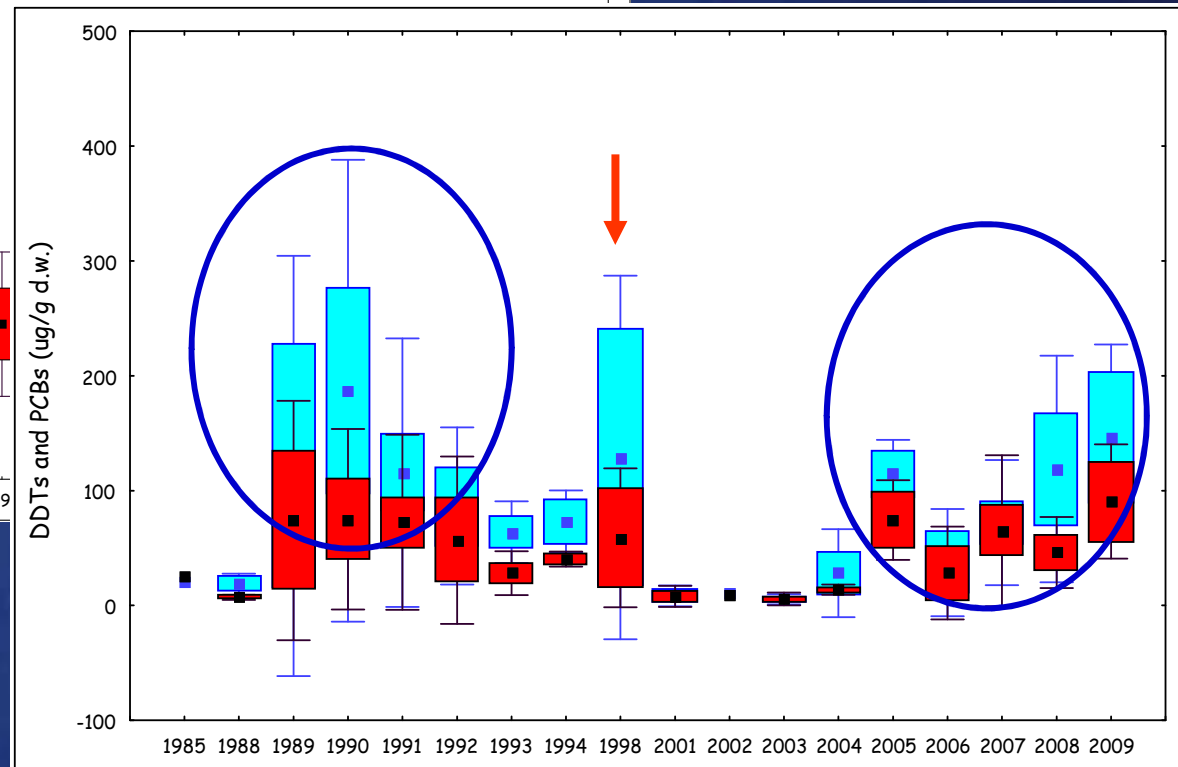
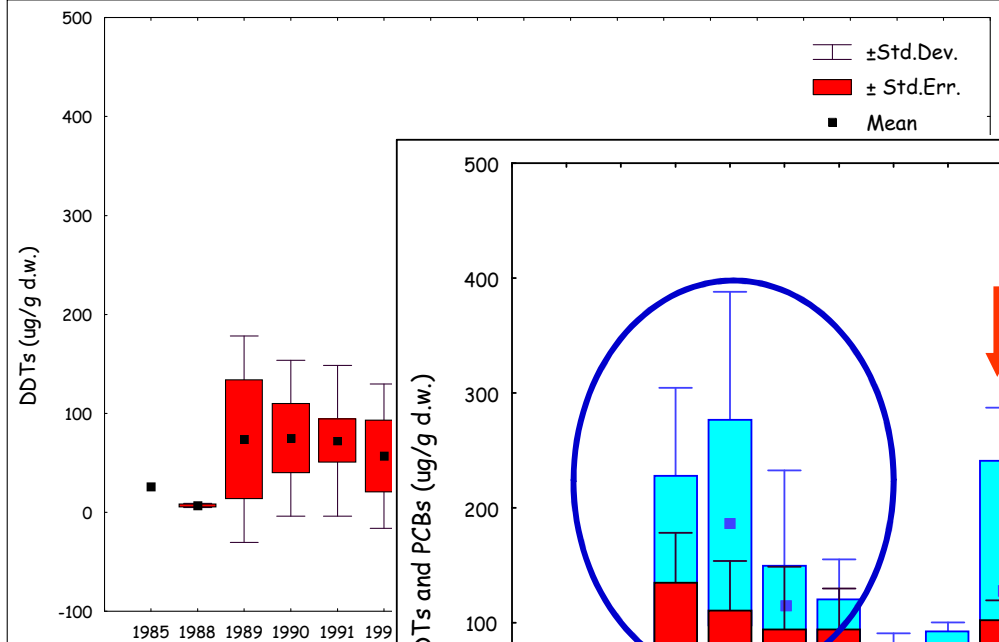
\* PROTEIN,S (CASEIN AND ALBUMIN), SUGARS (LACTOSE), MINERAL SALTS AND VITAMINS



# LIVELLI DI PCB<sub>1</sub> E DDT<sub>1</sub> IN MASCHI E FEMMINE DI ESEMPLARI SPIAGGIATI DI *Stenella coeruleoalba* 1985-2009



# TEMPORAL TREND OF $OC_2$ IN STRANDED SPECIMENS OF *Stenella coeruleoalba* 1985-2009





## Topic 4.4

### Effects of endocrine disruptors in aquatic mammals\*

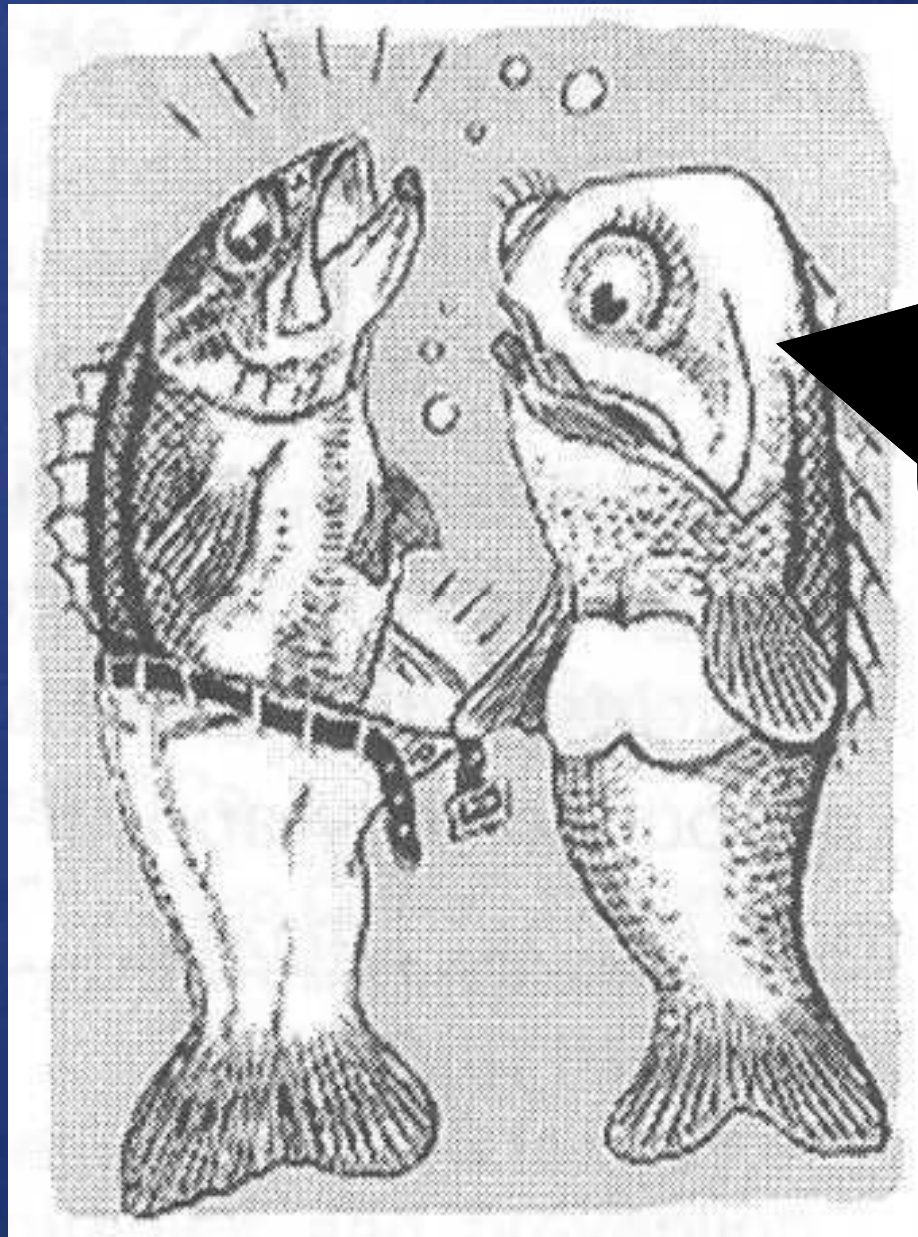
M. Cristina Fossi<sup>†</sup> and Letizia Marsili

Department of Environmental Sciences, Siena University, Via Mattioli 4, 53100  
Siena, Italy

**Abstract:** In the last few decades, various studies have shown that aquatic mammals are sensitive to the toxicological effects of certain xenobiotic compounds, including the large class of endocrine-disrupting chemicals (EDCs). Since some EDCs, particularly organochlorines, tend to bioaccumulate and biomagnify in the aquatic food chain, various aquatic mammals, particularly those high in the food chain, such as pinnipeds, odontocete cetaceans, and polar bears, are potentially “at risk”. The main aim of this chapter is to define the state of the art on effects of endocrine disruptors in aquatic mammals, both freshwater and marine. Another aim is to formulate recommendations for future research in this field and finally to define what can be done internationally for hazard/risk assessment and communication of the findings.

**Table 1** DDT metabolites and the PCB congeners with known estrogenic capacity commonly detected in aquatic mammals as measured in ER binding assays.

	Activity	Activity references	Potency <sup>1</sup>	aER binding IC <sub>50</sub> (nM) <sup>2</sup>	ER binding IC <sub>50</sub> (nM) <sup>4</sup>	ER binding RBA % <sup>5</sup>
DDTs	Estrogen	17				
<i>p,p'</i> -DDT	Estrogen	23		>50 <sup>3</sup>	>1000	
	Antiandrogen	17,20,24				
	ER agonist	23				
<i>o,p'</i> -DDT	Estrogen	17,20,23,25,26,27	++	9.1	5	0.1
	Antiandrogen	22	+	>50 <sup>3</sup>	>1000	
	Antiandrogen	22,23	+++			
	ER agonist	23				
<i>p,p'</i> -DDE	Estrogen	17,20,24,28	+			
	Antiandrogen	22,23	+			
	Androgen	22	+			
	Antiandrogen	22,23,29	++			
	ER agonist	23				
	AR agonist	23				
	AR antagonist	18,23				
<i>o,p'</i> -DDE	Estrogen	20,26		37.25		
	ER agonist	23				
<i>p,p'</i> -DDD	ER agonist	23			>1000	
<i>o,p'</i> -DDD	ER agonist	23		2.26		
PCBs		17,20,23,26,30,31				
Arochlor 1260	Estrogen	23,27,32				
	Effect on sexual differentiation	23,27,32				
	Gonadal abnormalities	23,27,32				
95	Estrogen	21,33	+			
99	Estrogen	21,33	++			
101	Estrogen	21,34				<0.001
118	Antiandrogen	21,34	++			
153	Estrogen	21,35	+++			0.004

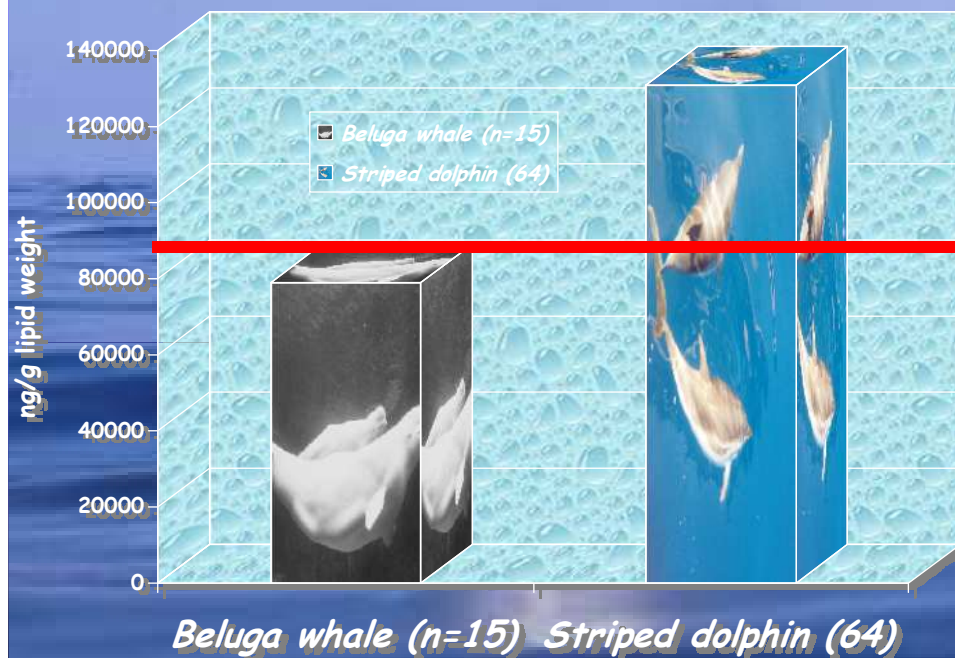


*Ma che cosa  
hai bevuto*

*PCBs o DDTs?*

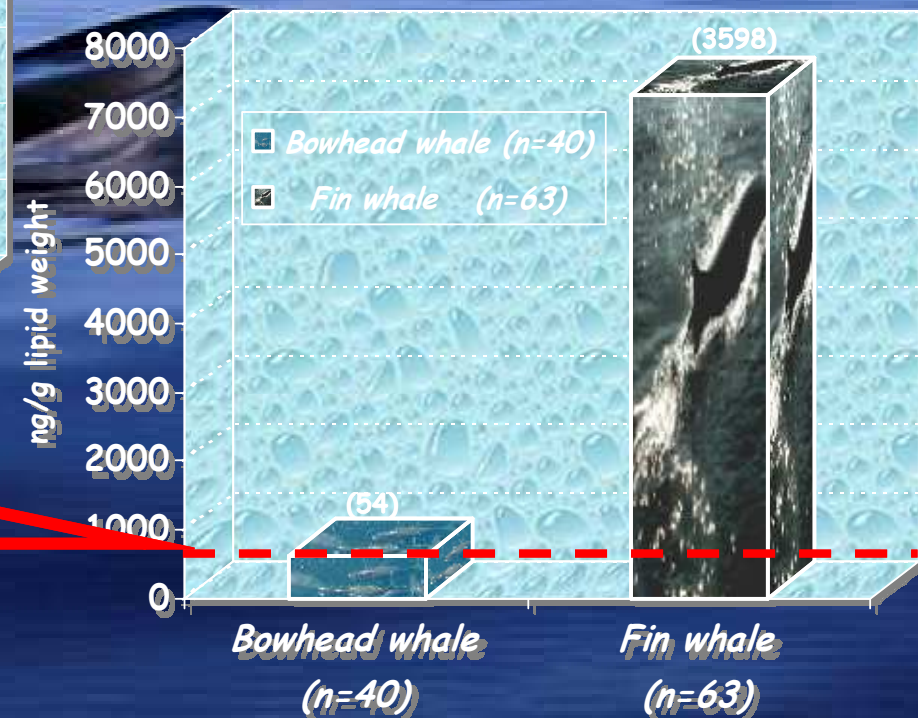


# "Potential Hazard" in Mediterranean Cetaceans



Soglia di ermafroditismo vero  
in beluga

Soglia di pseudoermafroditismo  
in balene franche della Groenlandia



## Abnormally high polychlorinated biphenyl levels in striped dolphins (*Stenella coeruleoalba*) affected by the 1990–1992 Mediterranean epizootic

Alex Aguilar<sup>a</sup> and Asunción Borrell<sup>a</sup>

<sup>a</sup>Department of Animal Biology (Vertebrates), Faculty of Biology, University of Barcelona 08071 Barcelona, Spain

Available online 1 July 2003.

### Abstract

PCB concentrations and total lipid content were determined in the blubber and liver of striped dolphins affected by the 1990 morbillivirus epizootic in the Mediterranean Sea, and in the blubber of striped dolphins from the same area sampled with a biopsy dart in 1987–1989 and 1991. PCB levels were found to be significantly higher in the individuals that succumbed to the epizootic than in the 'healthy' population sampled before or after the event. Although recent mobilization of lipid reserves was found to have occurred in some of the diseased dolphins, this had little effect on their PCB blubber concentrations and cannot explain the observed difference with the healthy individuals. Three hypotheses are put forward to explain the apparent link between high PCB levels and mortality caused by the epizootic: (i) depressed immunocompetence caused by PCBs leading to an increase in individual susceptibility to the morbillivirus infection, (ii) mobilization of fat reserves leading to increased PCB levels in blood which, in turn, may produce a liver lesion capable of increasing the individual's susceptibility to the morbillivirus infection, and (iii) previous existence of an unspecific hepatic lesion producing impairment of the liver function which, in turn, could lead to an increase both in tissue PCB concentrations and in individual susceptibility to the morbillivirus infection.

**Keywords:** Striped dolphin; *Stenella coeruleoalba*; Epizootic; Pollution; Polychlorinated biphenyls; Distemper; Mediterranean Sea; Mass mortality; Immunosuppression

*Science of The Total Environment, Volume 154, Issues 2-3, 16 September 1994, Pages 237-247*



## CAUSE:

HIGH LEVELS OF  
PCB<sub>1</sub>

REACTION OF  
IMMUNOSUPPRESSION

RELATIVE MORTALITY  
INCREASE IN  
SPECIMENS WITH HIGH  
PCB LEVELS

MOBILIZATION  
OF LIPID  
RESERVES

LIVER LESIONS DUE TO  
PCB<sub>1</sub>

## EFFECT:

CRONIC OR OLD  
LIVER LESIONS

HEPATIC FUNCTION  
DAMAGE

PCB LEVEL INCREASE  
IN AFFECTED  
DOLPHINS





Contents lists available at ScienceDirect

Chemosphere

journal homepage: [www.elsevier.com/locate/chemosphere](http://www.elsevier.com/locate/chemosphere)

## PCB and DDT levels do not appear to have enhanced the mortality of striped dolphins (*Stenella coeruleoalba*) in the 2007 Mediterranean epizootic

J. Castrillon<sup>a</sup>, E. Gomez-Campos<sup>a</sup>, A. Aguilar<sup>a</sup>, L. Berdié<sup>b</sup>, A. Borrell<sup>a,\*</sup>

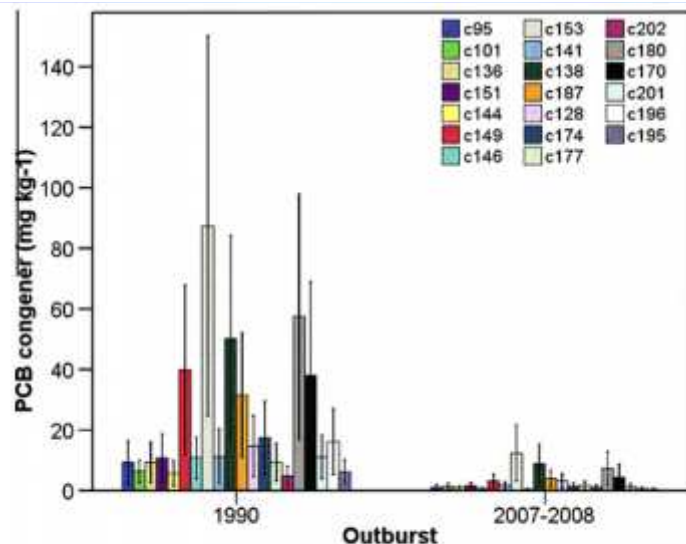


Fig. 2. Comparison of PCB-congener levels between the 1990 ( $n = 33$ ) and 2007–2008 ( $n = 20$ ) Mediterranean epizootics.

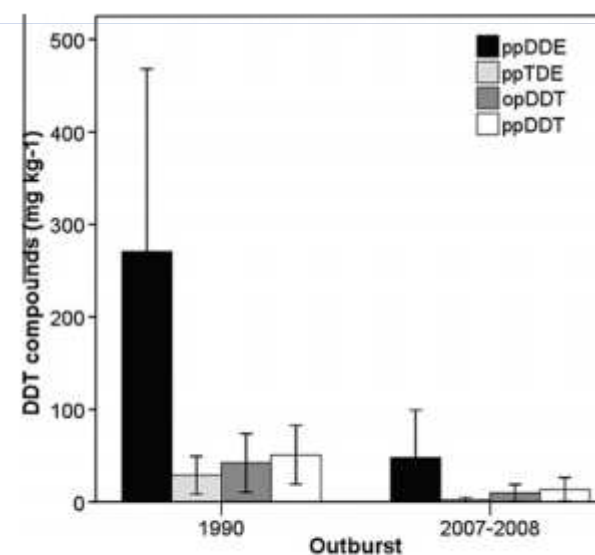


Fig. 3. Comparison of DDT-compound levels between the 1990 ( $n = 33$ ) and 2007–2008 ( $n = 20$ ) Mediterranean epizootics.

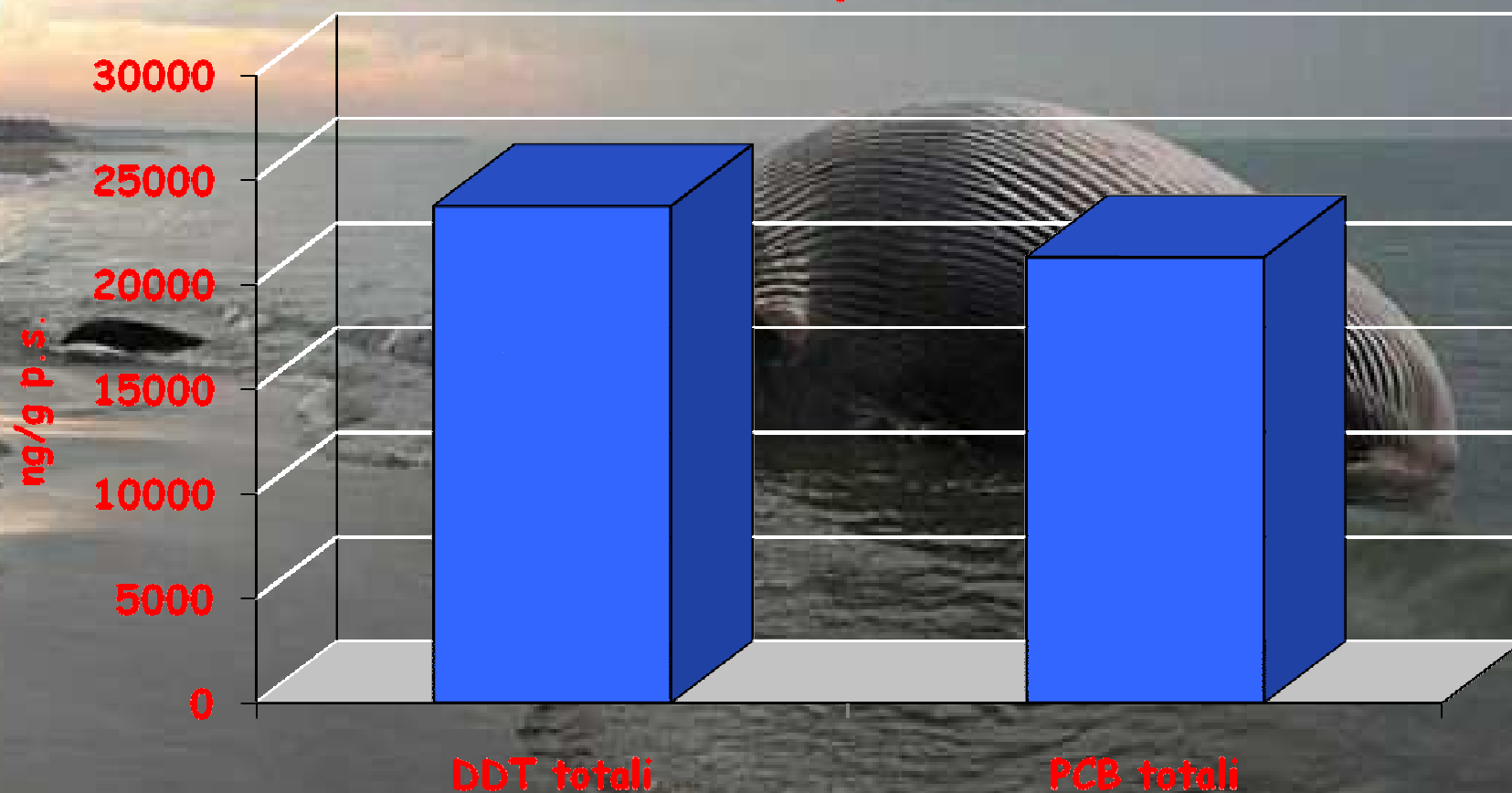


Di Guarda - LABORATORY DIAGNOSIS OF MORBILLIVIRUS AND TOXOPLASMA  
SPP. INFECTIONS IN STRANDED DOLPHINS *Medicine / Disease M13 ECS2009*  
Pretti - DETECTION OF TOXOPLASMA GONDII DNA AND SERUM ANTIBODIES  
IN STRANDED CETACEANS FROM THE COASTS OF  
TUSCANY (TYRRHENIAN SEA WESTERN MEDITERRANEAN)  
*Medicine / Disease M15 ECS2009*

NESSUNA EVIDENZA DI INFEZIONE DA  
MORBILLIVIRUS  
MA PRESENZA IN MOLTI ESEMPLARI DI  
TOXOPLASMA ED ALTRE PATOLOGIE

PISA. Era affetta da infezioni da morbillivirus e toxoplasma condii la balena spiaggiata lo scorso 27 gennaio sul litorale del Parco di San Rossore a Pisa.

## OCs Adipe RT25



DAL 2009 AD ORA, MOLTI ESEMPLARI DI STENELLA  
STRIATA E DI TURSIOPE HANNO MOSTRATO EVIDENZA  
DI INFEZIONE DA MORBILLIVIRUS.







**MAX ENTRO 12H;  
A 24H, CIRCA 50% DI  
PROBABILITÀ DI SUCCESSO**

**EPIDERMIDE:**

*Culture cellulari*  
*Analisi di Biomarkers*  
*Genetica*  
*Immunostochimica*

**GONADI:**

*Culture cellulari*  
*Analisi di Biomarkers*  
*Genetica*  
*Istologia*  
*Morfologia*  
*Immunostochimica*

**FEGATO:**

*Culture cellulari*  
*Analisi di Biomarkers*  
*Genetica*  
*Istologia*  
*Immunostochimica*



# Giannella Gennaio 2008





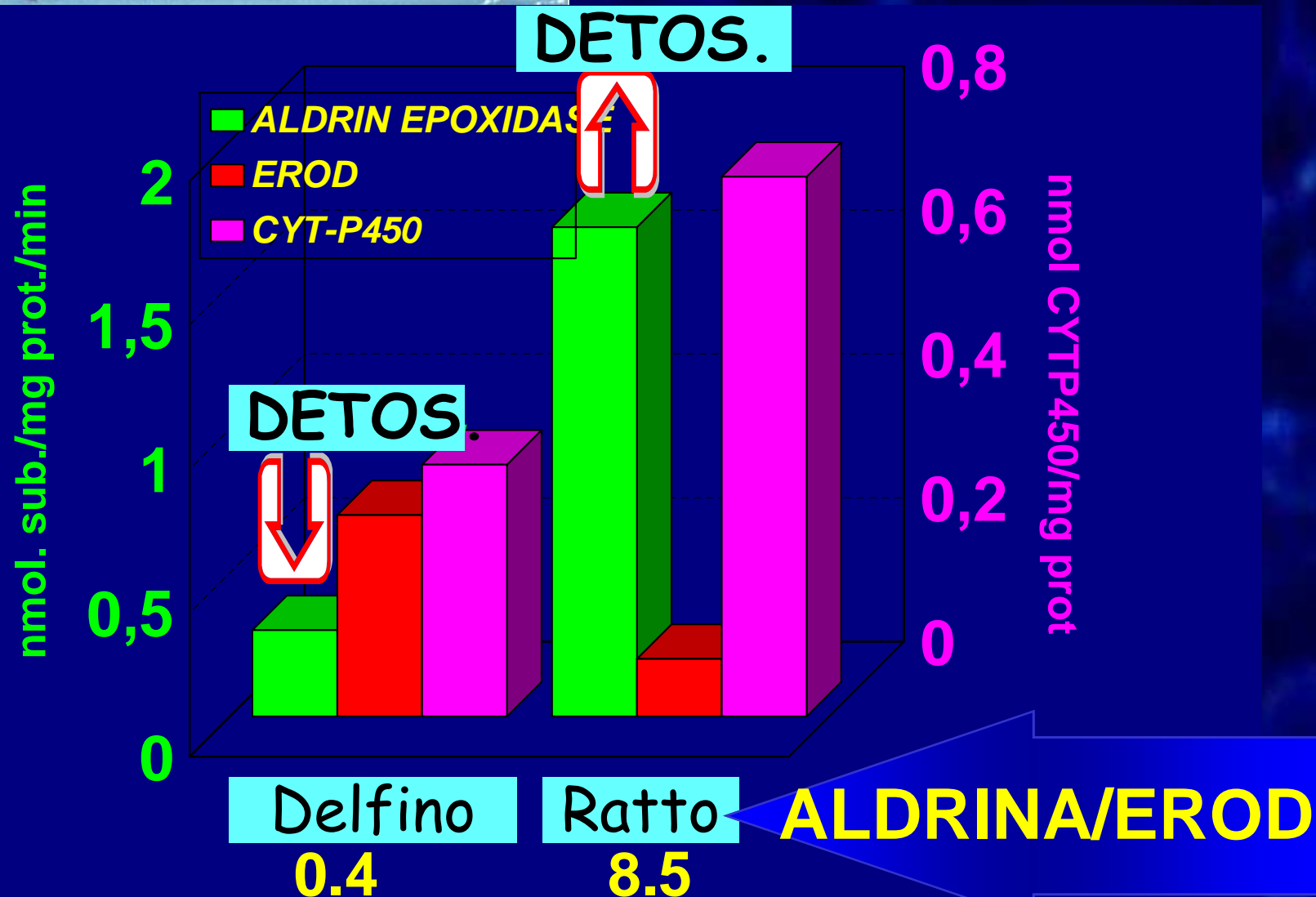
Azoto Liquido

RNA Later



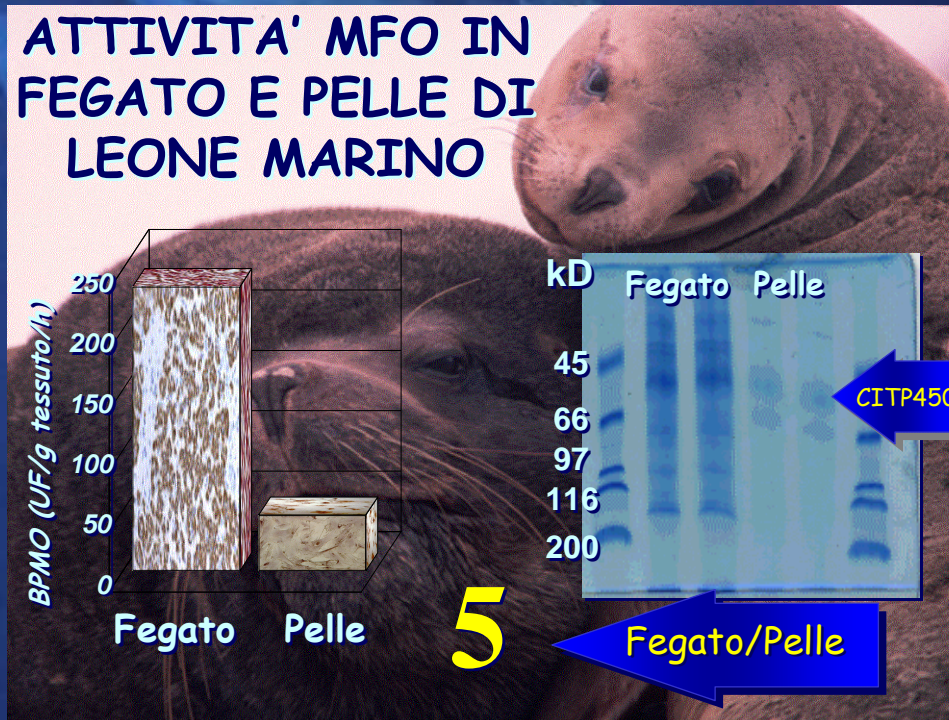
Tutti queste nuove  
indagini devono servire  
allo studio delle  
interazioni fra composti  
inquinanti ed attività  
metaboliche degli  
esemplari.

# ATTIVITA' MFO IN UNA STENELLA STRIATA





Inoltre questi campioni possono  
permettere di validare i test  
non distruttivi effettuati sulla  
biopsia cutanea



# INDAGINI DI BIOMARKERS (2005-2006)

## *Tursiops truncatus*



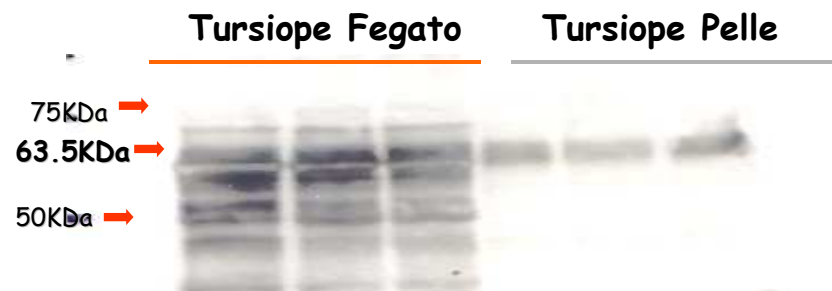
E' STATA VALUTATA, PER LA PRIMA VOLTA, LA PRESENZA DEL CYP1A1 E DEL CYP 2B4, ATTRAVERSO TECNICHE IMMUNISTOCHEMICHE, IN FRAZIONI CELLULARI EPATICHE E DI EPIDERMIDE DI UN CETACEO SPIAGGIATO



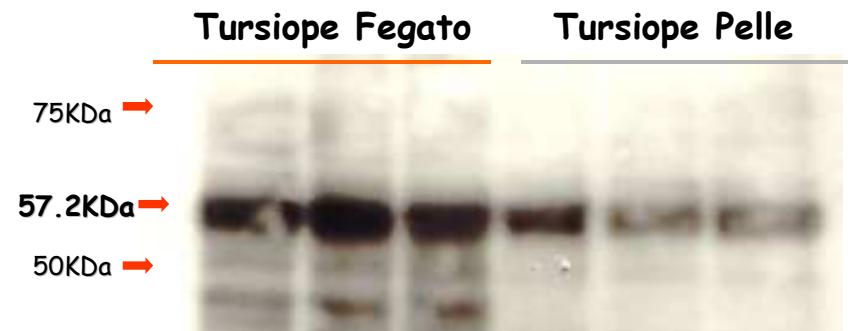
SONO STATE VALUTATE, IN MANIERA COMPARATIVA, SIA NELLA FRAZIONE EPATICA CHE IN QUELLA CUTANEA, LE ATTIVITÀ ENZIMATICHE DELLE MONOSSIGENASI A FUNZIONE MISTA (MFO) EROD, PROD, BPMD E NADPH-CYT P450 REDUTTASI, E LE ATTIVITÀ ANTIOSSIDANTI (CATALASI)

# Analisi Western Blot

## CYP 1A1



## CYP 2B





# ATTIVITÀ MFO ( EROD, PROD, BPMD, REDUTTASI) E CATALASI IN TESSUTO EPATICO ED EPIDERMIDE DI UN ESEMPLARE SPIAGGIATO DI TURSIOPS TRUNCATUS

	Fegato	Epidermide
EROD	752	16,25
pmol/mg prot./min		
PROD	12,50	8,75
pmol/mg prot./min		
BPMD	33,28	2,51
UAF/mg prot./h		
NADPH CitC Red.	2,50	n.d.
nmol/mg prot./min		
Catalasi	2,52	1,08
nmol/min		
Proteine Totali	48	4,8
mg/ml		

*Annotations:*


- Red arrow from 752 to 16,25:  $\times 46$  volte
- Red arrow from 33,28 to 2,51:  $\times 13$  volte



VIENE RICONFERMATO IL GRANDE  
DISEQUILIBRIO METABOLICO DEI SISTEMI  
DETOSSIFICANTI-BIOATTIVANTI TIPICO  
DEI MAMMIFERI MARINI (FOSSI E  
MARSILI, 2002).



QUESTO DATO CONFERMA ANCORA UNA  
VOLTA L'ELEVATA VULNERABILITÀ  
BIOCHIMICA DEI CETACEI NEI CONFRONTI  
DEI CONTAMINANTI XENOBIOTICI

A photograph of a dolphin leaping from the water at night. The dolphin is captured mid-air, its body arched as it moves from right to left. The water surface is dark, with some light reflecting off the dolphin's wet skin and the splash of water behind it. The background is a deep, dark blue/black, suggesting a nighttime setting.

Realizzare esperimenti su colture cellulari  
di fibroblasti di varie specie di cetacei al  
fine di stabilire la diversa sensibilità  
interspecifica a composti inquinanti.

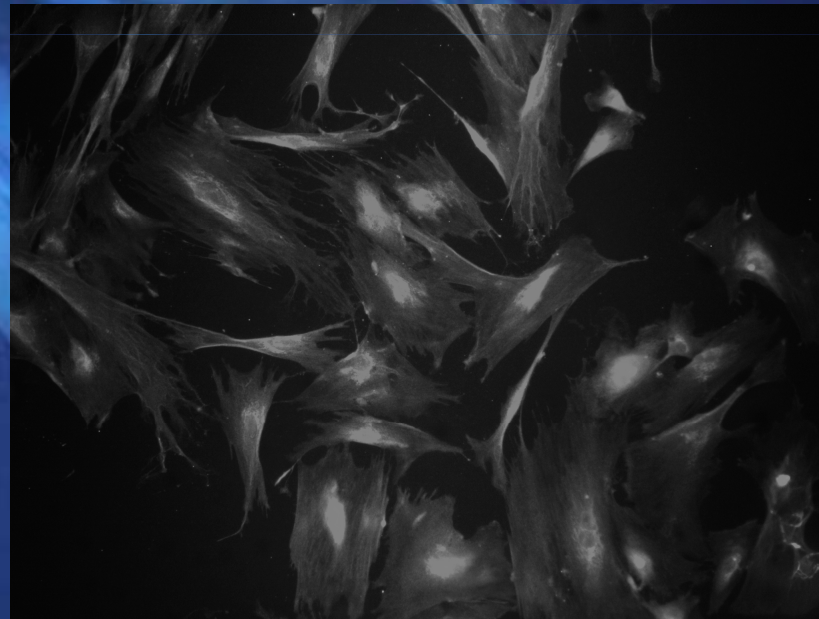


# Colture cellulari di Fibroblasti



“delfino in provetta”

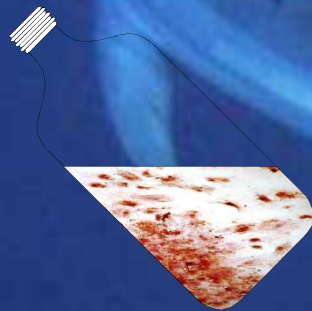
# ALLESTIMENTO COLTURE CELLULARI ED ESPERIMENTI DI IMMUNOFLUORESCENZA



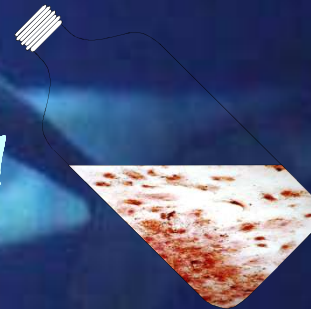
# PREPARAZIONE DELLE COLTURE CELLULARI



EPIDERMIDE



COLTURE CELLULARI  
DI  
FIBROBLASTI

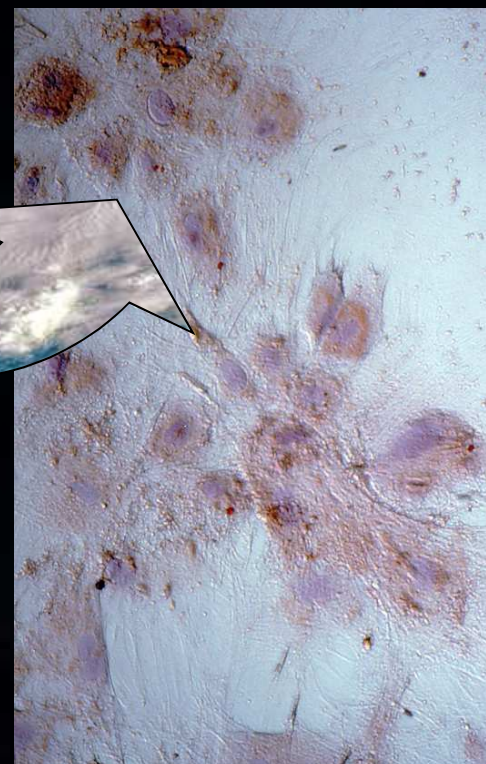
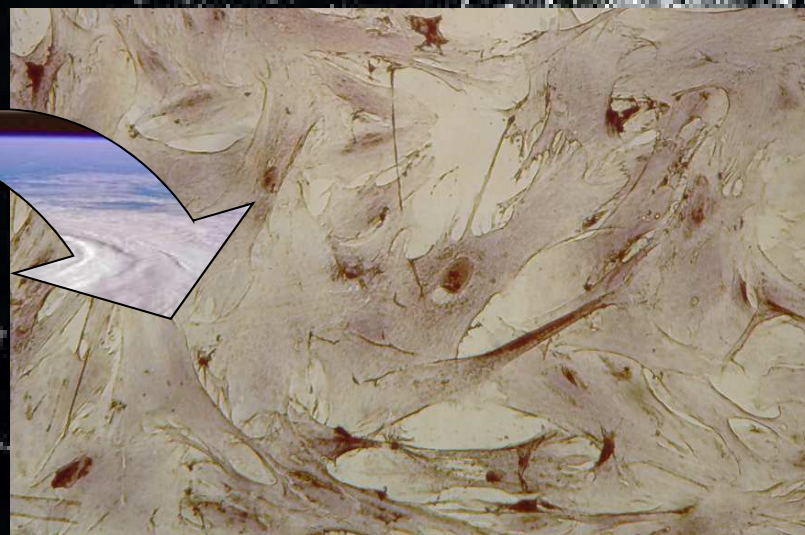






## COLTURA CELLULARI

Marsili *et al.*, 2000 – *Mar. Envir. Res.*, 50: 523-526





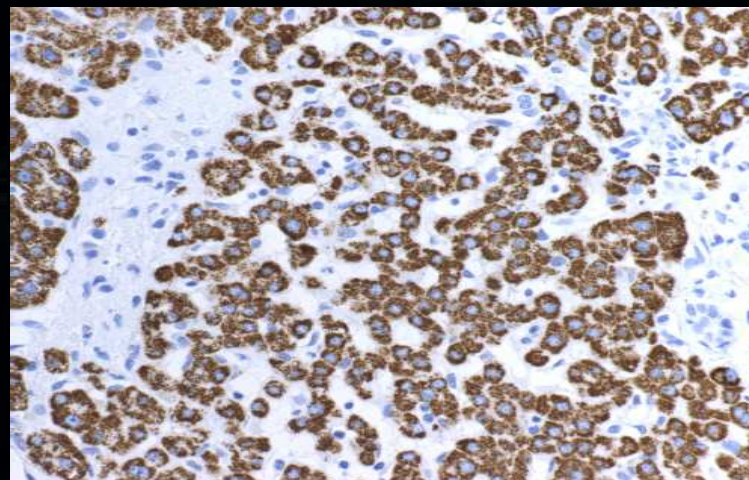
Fegato



*COLTURA CELLULARI*



Epatociti







Colture di  
fibroblasti

Colture di epatociti

Turles	1	2	3	4	
A					A
B					B



Trattamenti per 48 h con contaminanti ambientali  
(PCBs, DDTs, IPA, Ritardanti di Fiamma, ecc..)



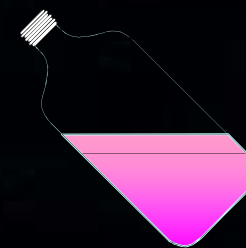
*Bianco*



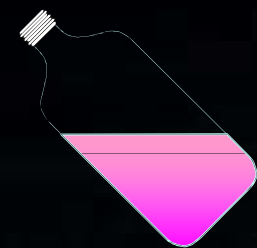
*Bianco Chimico*



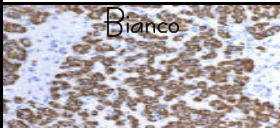

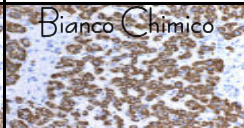
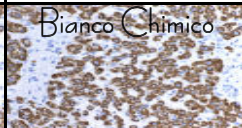
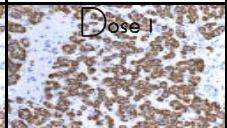
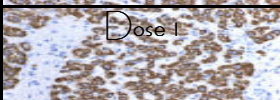
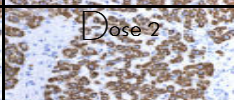
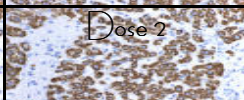

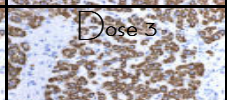
*Dose 1*



*Dose 2*



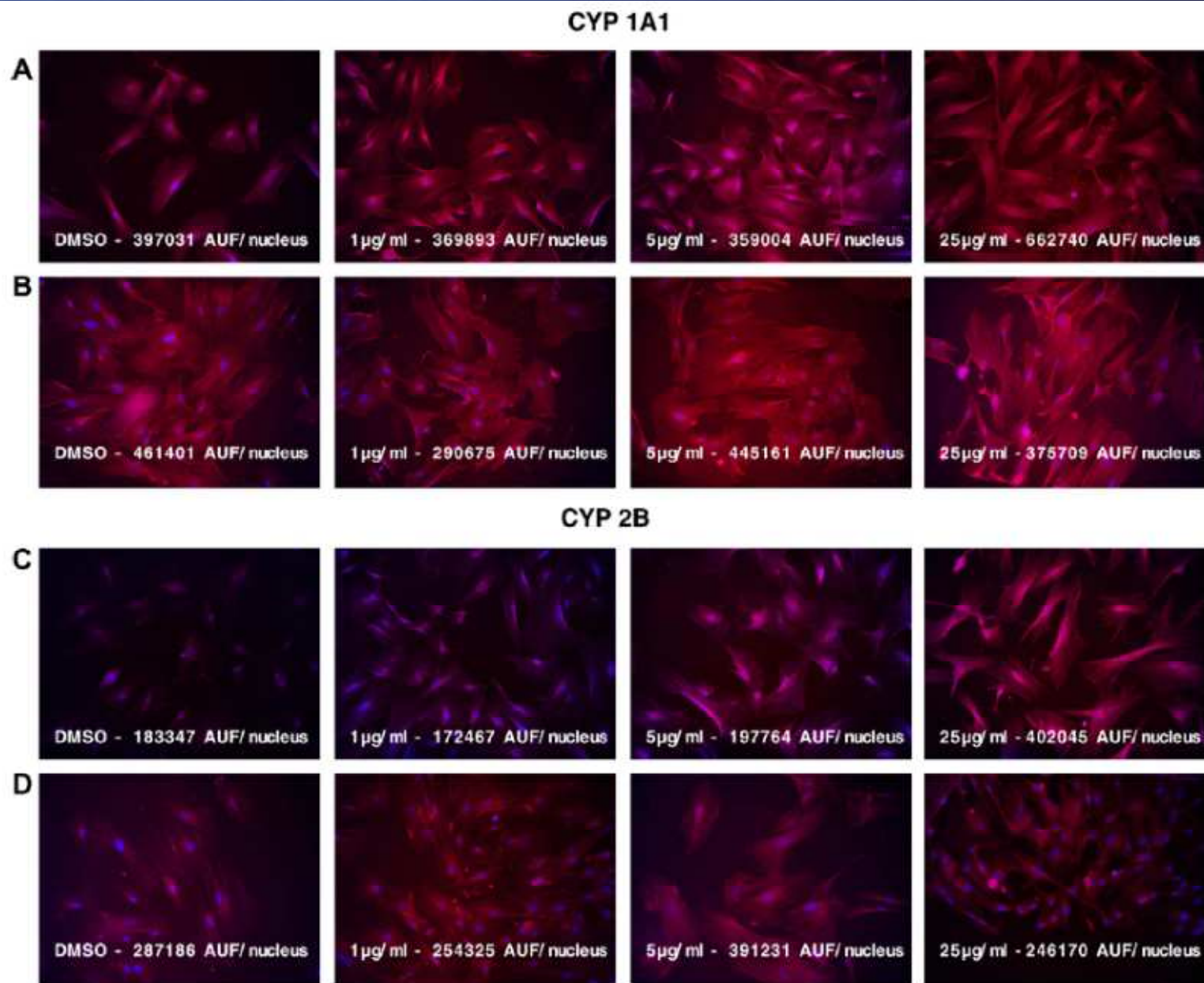
*Dose 3*

Turles	1	2	3	4		
A						A
B						B

**CYP450 1A1 - CYP450 2B4**

**Tecniche Innovative: Immunofluorescenza, WB, PCR**

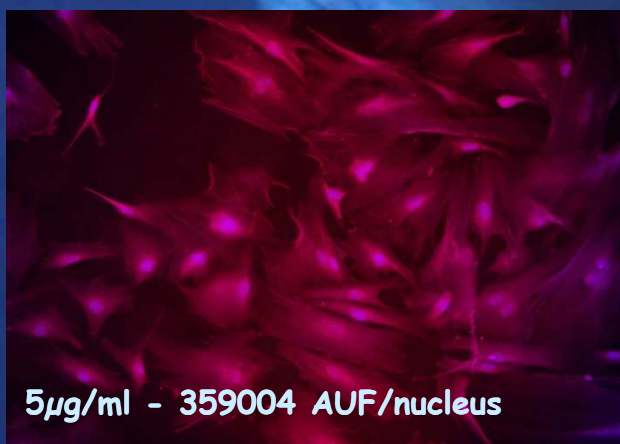
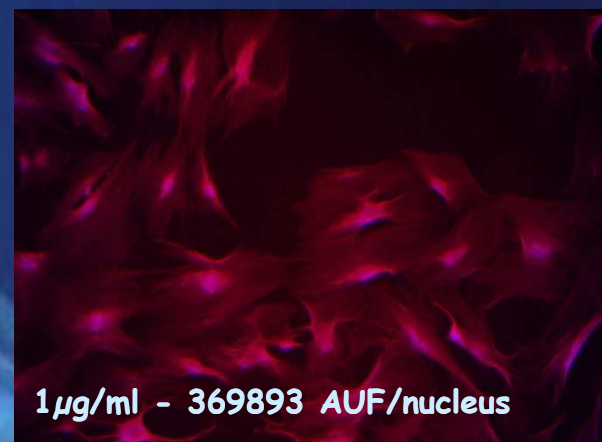
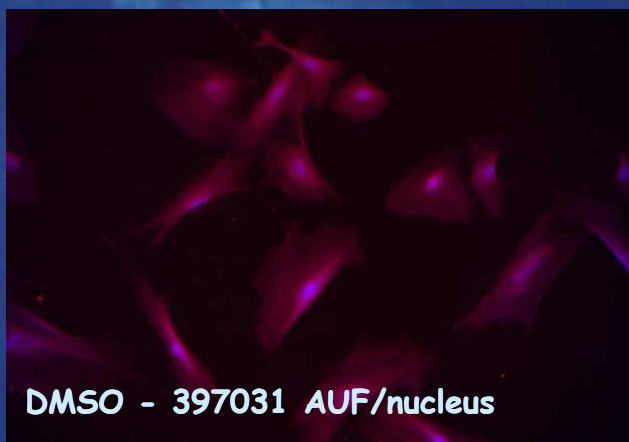




**Fig. 1.** Immunofluorescence of CYP1A1 and CYP2B in cultured fibroblasts of striped dolphin and bottlenose dolphin treated with the OC mixture. (A, C) Striped dolphin fibroblasts: DAPI and Alexa Fluor 594 (Intensity = 133 ms) images of DMSO and the three treatments. (B, D) Bottlenose dolphin fibroblasts: DAPI and Alexa Fluor 594 (Intensity = 50 ms) images of DMSO and the three treatments. The immunofluorescence is expressed in arbitrary unity of fluorescence (AUF) per cell.

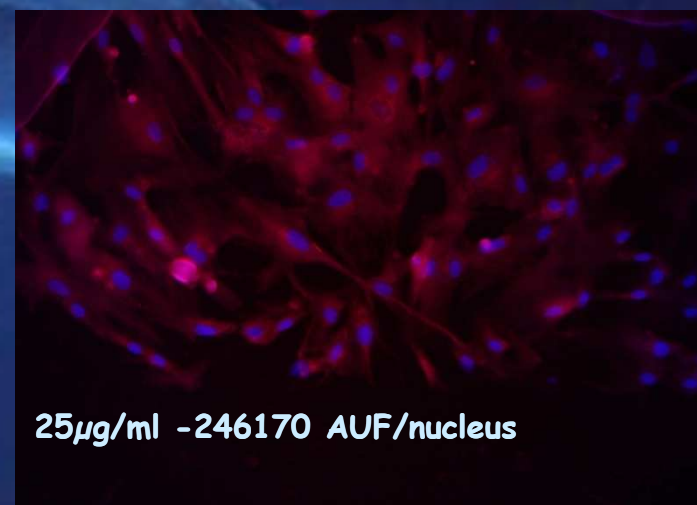
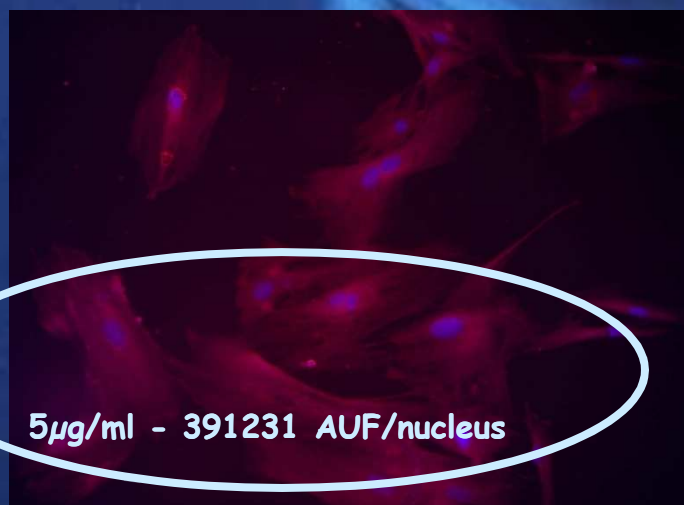
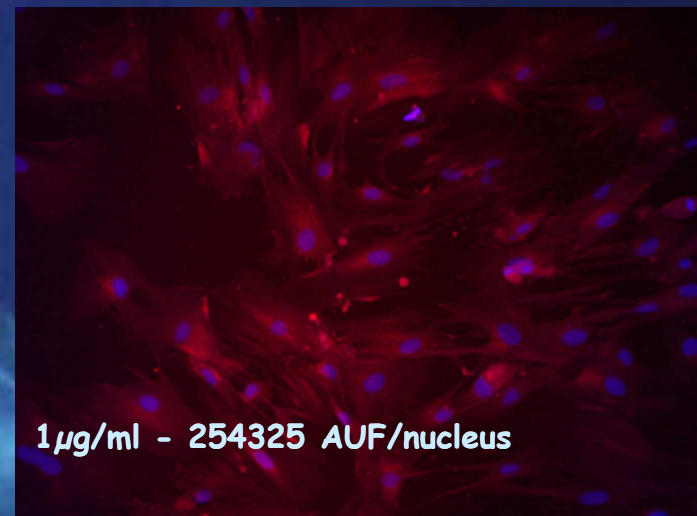
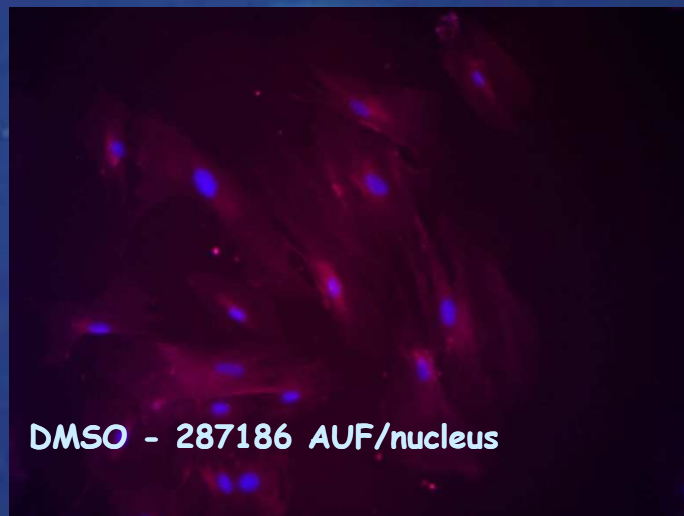
# *Stenella coeruleoalba*

## CYP 1A1



# *Turriopsis truncatus*

CYP 2B

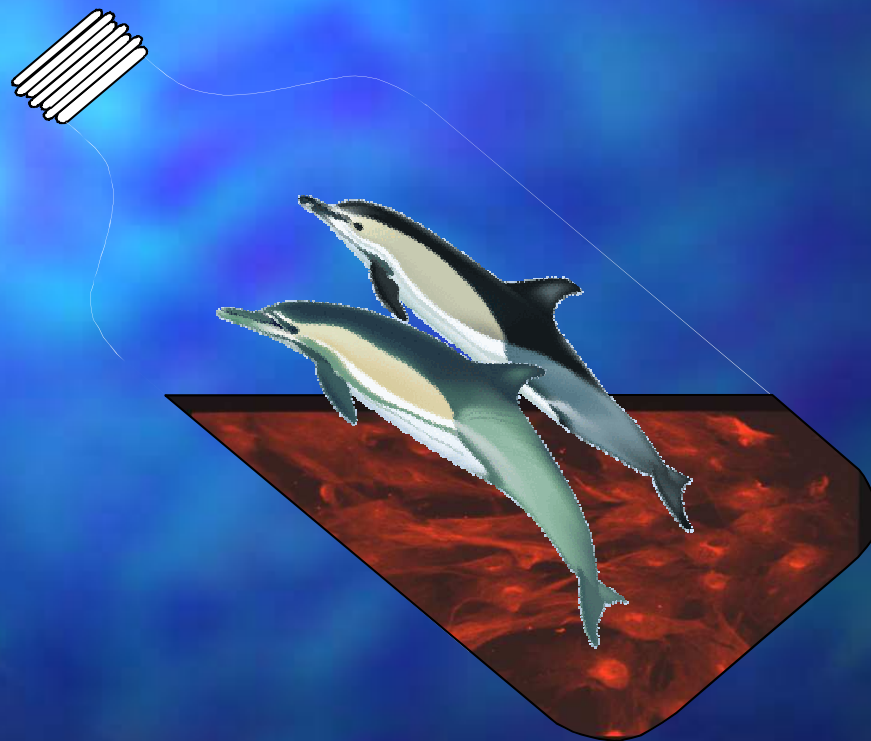




A photograph of a dolphin leaping from the water, creating a large splash. The dolphin is captured mid-air, with its body arched and its tail visible. The water is a deep blue, and the dolphin's skin is a lighter, greyish-blue. The text is overlaid on the image in a bold, red, hand-drawn style font.

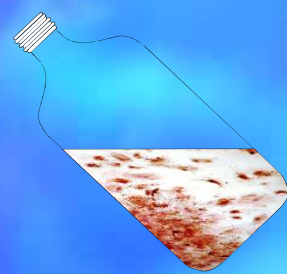
COME SI PUO' VALUTARE  
QUANTO E' IMPORTANTE  
IL RUOLO DI  
IMMUNOSOPPRESSORI DI  
QUESTI CONTAMINANTI?

# "CETACEO IN PROVETTA"

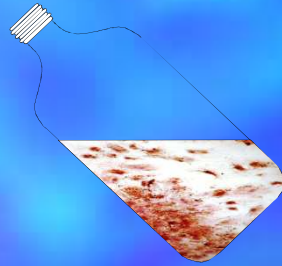


# OC EXPERIMENTAL TREATMENT

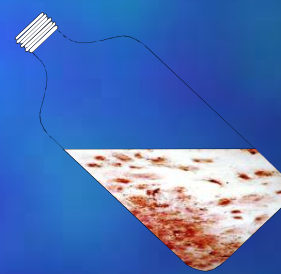
Mixture 48h: Arochlor 1260, pp'DDT, pp'DDE in DMSO



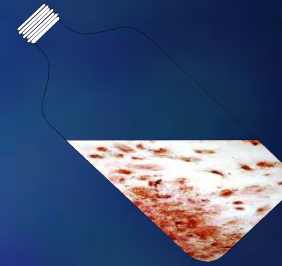
*DMSO (0.05%)*



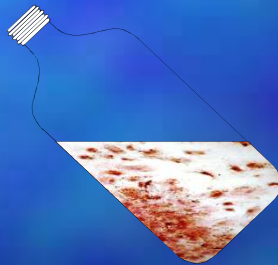
*1 ug/ml*



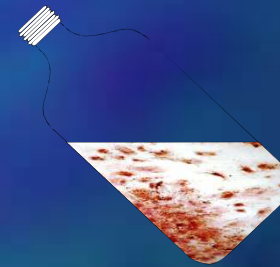
*5 ug/ml*



*25 ug/ml*



*Blank*



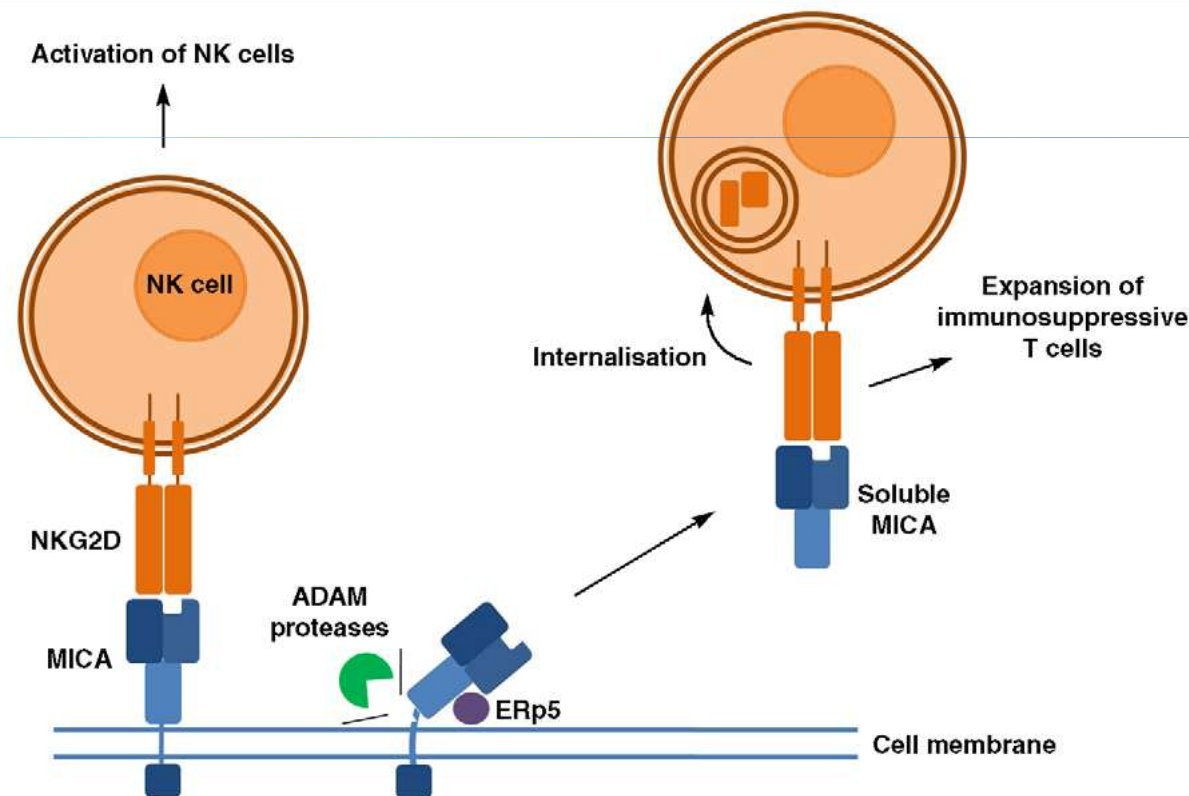
*Blank*

**MIC A**

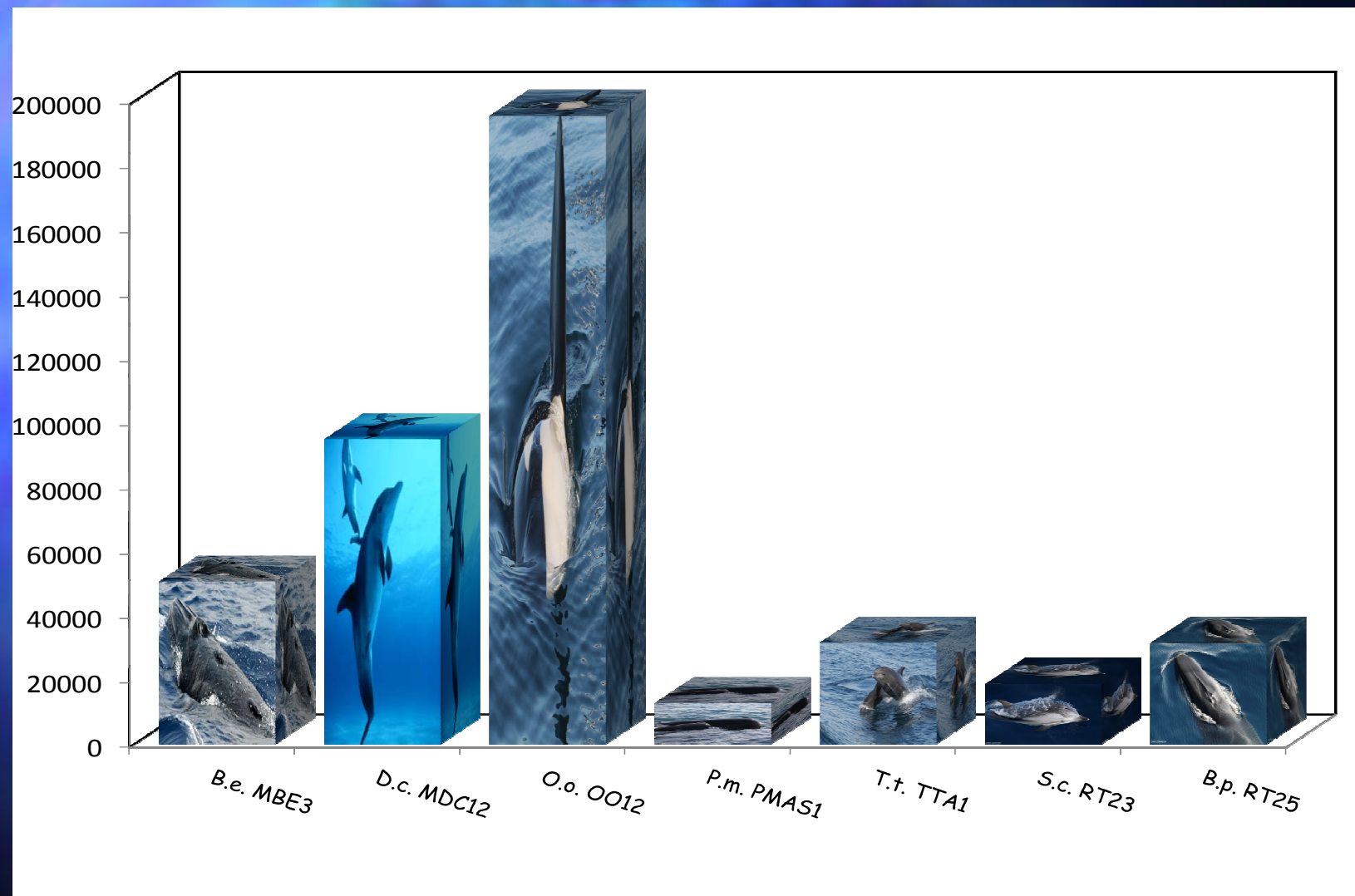


# MIC A:

## Major Histocompatibility Complex (MHC) Expressed in fibroblasts and endothelium when subjected to stress. Activate NK cells and gamma-delta



# RISULTATI




Attività basale della proteina MIC-A nelle varie specie di cetacei

- in tutti gli animali vi è almeno una dose di trattamento con OC che induce l'attività del MIC-A rispetto all'attività basale. In particolare nel capodoglio con le dosi più alte abbiamo un'induzione fino a circa 50 volte rispetto all'attività basale.

Sigla	Specie	BA	0,01 µg/ml (n.i.)	0,1 µg/ml (n.i.)	1 µg/ml (n.i.)	5 µg/ml (n.i.)	25 µg/ml (n.i.)
MBE3	<i>B. edeni</i>	100	242	419	149	/	/
MDC12	<i>D. capensis</i>	100	131	124	37	/	/
OO12	<i>O. orca</i>	100	99	80	81	148	69
PMAS1	<i>P. macrocephalus</i>	100	59	40	125	1090	4836
RT23	<i>S. coeruleoalba</i>	100	121	133	121	/	/
RT25	<i>B. physalus</i>	100	161	130	135	163	94





A photograph taken from the perspective of someone on a boat, looking out at a vast, deep blue ocean under a clear sky. In the bottom foreground, the back of a person's head with long, wavy blonde hair is visible. A large, white, hand-drawn thought bubble is superimposed over the center of the image, containing pink text. The thought bubble has a main large cloud-like shape and several smaller circles leading to it from the bottom left.

**Come rivisitare tutti  
i dati ottenuti per  
poter effettuare una  
stima di pericolo  
tossicologico da OCs  
per questi Mammiferi  
marini?**





*Creare dei modelli teorici  
statistici per valutare il  
potenziale "rischio"  
tossicologico da*

*OCs in Stenella coeruleoalba  
del Mediterraneo*



# Perchè la stenella?



Maggior numero di esemplari sia spiaggiati  
sia campionati liberi tramite biopsia



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CHEMOSPHERE

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Chemosphere 56 (2004) 791–801

[www.elsevier.com/locate/chemosphere](http://www.elsevier.com/locate/chemosphere)

Theoretical models to evaluate hazard due to  
organochlorine compounds (OCs) in Mediterranean  
striped dolphin (*Stenella coeruleoalba*)

L. Marsili <sup>a,\*</sup>, A. D'Agostino <sup>b</sup>, D. Bucalossi <sup>a</sup>, T. Malatesta <sup>a</sup>, M.C. Fossi <sup>a</sup>

<sup>a</sup> Dipartimento di Scienze Ambientali, Università di Siena, G. Sarfatti, Via Mattioli 4, 53100 Siena, Italy

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Received 3 December 2002; received in revised form 18 August 2003; accepted 28 March 2004

Canonical discriminant analysis (CDA) using normal Kernel density for DDTs + PCBs

Group	Free-ranging	Stranded
<i>Resubstitution summary</i>		
Free-ranging	97%	3%
Stranded	46%	54%
<i>Cross-validation summary</i>		
Free-ranging	89%	11%
Stranded	53%	47%
Correct classification rate	0.74	

*Classification Summary for striped dolphins sampled in 1990–1991*

	Free-ranging 1991	Stranded 1990–91
Free-ranging 1991	100%	0
Stranded 1990–91	14%	86%
Correct classification rate	0.93	

The results are in percentage of observations classified in the group (free-ranging and stranded populations).

Canonical discriminant analysis (CDA) using normal Kernel density for OCs with known mutagenic, carcinogenic and teratogenic activity

Group	Free-ranging	Stranded
<i>Resubstitution summary</i>		
Free-ranging	100%	0
Stranded	2%	98%
<i>Cross-validation summary</i>		
Free-ranging	98%	2%
Stranded	37%	63%
Correct classification rate	0.80	

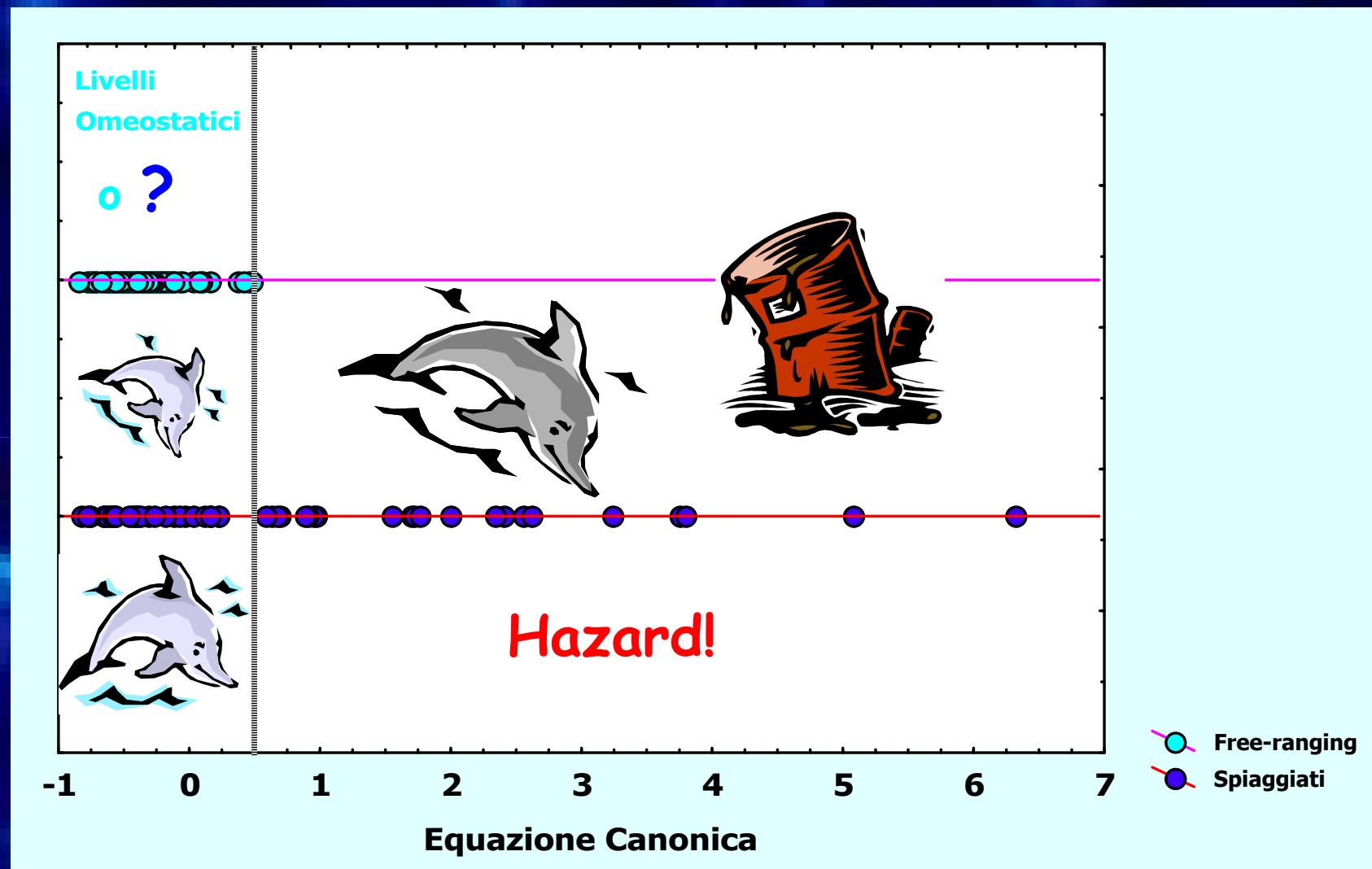
*Classification summary for striped dolphins sampled in 1990–1991*

	Ionian Sea free-ranging	Ionian Sea stranded
Ionian Sea free-ranging	100%	0
Ionian Sea stranded	50%	50%
Correct classification rate	0.75	

The results are in percentage of observations classified in the group (free-ranging and stranded populations).



# PCBs + DDTs



$$\text{Equazione Canonica} = (9.5124866 \times 10^{-6} \times \text{PCBs} + 4.3989707 \times 10^{-6} \times \text{DDTs}) - 0.9219903$$



ID	HCB	DDTs	PCBs	CAN value	Pathological findings
22	301.4	63493.0	85625.0	0.17	Parasites
23	179.5	25512.4	59594.4	-0.24	Parasites
31	<b>847.1</b>	<b>445582.0</b>	<b>471013.0</b>	<b>5.52</b>	<b>DMV (CNS, lungs); parasites</b>
57	50.7	3162.8	10096.2	-0.81	<i>Photobacterium damsela</i> (multiple organ)
58	234.2	225558.6	215694.5	2.12	DMV (lungs)
59	417.1	133704.0	159855.7	1.19	DMV (heart) <i>Photobacterium damsela</i> (lungs)
60	1287.9	97955.9	92773.9	0.39	Parasites
62	232.2	233717.7	318458.6	3.14	DMV (CNS, heart, lungs)
63	478.2	163043.3	178431.9	1.49	<i>Photobacterium damsela</i> (multiple organ)
64	200.3	83689.0	110450.6	0.50	DMV (CNS)
65	349.7	96539.1	140581.9	0.84	<i>Photobacterium damsela</i> (multiple organ)
66	473.7	11718.1	35953.3	-0.53	<i>Photobacterium damsela</i> (multiple organ)

Table 2 - Levels of HCB, DDTs and PCBs (ng/g d.w.) in blubber of 12 cetaceans with the evaluation of toxicological stress in function of the theoretical model of Marsili et al., 2004. In bold the animals with the hazard from organochlorines (CAN value >0.47).

Casalone *et al.*, Diseases of Aquatic Organisms, revisionato

# Stato ecotossicologico dei Cetacei.....







# COLD CASE

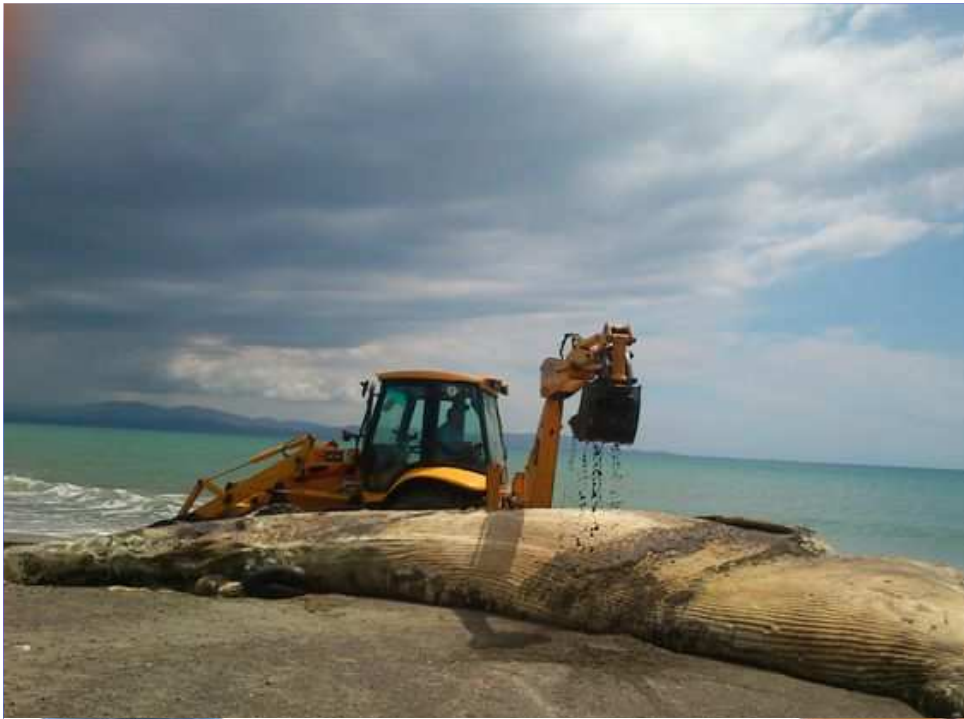
# Piombino Maggio 2012















A photograph of a dolphin leaping from the water, creating a large splash. The dolphin is captured mid-air, with its body arched and its tail visible. The water is a deep blue, and the dolphin's skin is a lighter, greyish-blue. The text is overlaid on the image in a bold, red, hand-drawn font.

INFORMAZIONI RELATIVE  
AD ESEMPLARI NON  
CONSIDERABILI  
"NO-STRESSED"



A photograph of a dolphin leaping from the water, creating a large splash. The dolphin is captured mid-air, with its body arched and its tail visible. The water is a deep blue, and the dolphin's skin is a lighter, greyish-blue. The text is overlaid on the image in a bold, red, hand-drawn style font.

L'ALTERNATIVA E' LA  
POPOLAZIONE CONSIDERATA  
"NO-STRESSED"  
CIDE'  
FREE-RANGING?



QUALI TECNICHE SUI  
FREE-RANGING  
NON INVASIVE E  
NON DISTRUTTIVE?

















# BIOLOGICAL MATERIALS IN FREE-RANGING CETACEANS

*CYP1A1-CYP2B WB*

*Cell Culture*

*RT-PCR*

*CYP1A1-BPMO*

*Heavy Metals*

*HCB*

*DDT<sub>s</sub>*

*PCB<sub>s</sub>*

*PAH<sub>s</sub> Dioxins*

*PBDE<sub>s</sub>*

SUBCUTANEUS BLUBBER

SKIN

Epidermis  
Dermis



Stato ecotossicologico dei  
Top-predators.....



ED ALTRI CONTAMINANTI



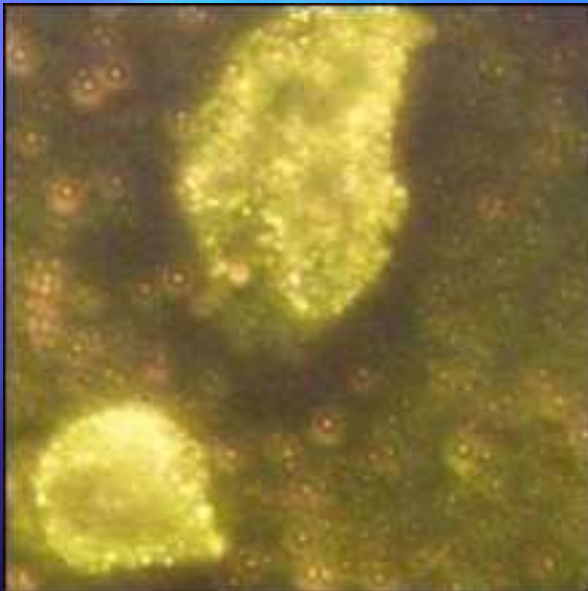


MA CI SONO E, QUALI SONO,  
GLI EFFETTI DELLE  
NANOPARTICELLE



# NANOPARTICELLE DI ORO ( $AuNP_1$ ) < 100 nm

OGGETTO DI INTENSA RICERCA PER LE PROPRIETÀ OTTICHE, ELETTRONICHE E DI RICONOSCIMENTO MOLECOLARE.



LE POSSIBILI APPLICAZIONI SPAZIANO TRA:

- ➔ L'ELETTRONICA,
- ➔ LA NANOTECNOLOGIA,
- ➔ LA SINTESI DI NUOVI MATERIALI CON PROPRIETÀ UNICHE,
- ➔ LA MEDICINA (AD ESEMPIO NELLA TERAPIA DELL'ARTRITE REUMATOIDE; COME TRASPORTATORI DI FARMACI IDROFOBICI; NELL'INGEGNERIA GENETICA E TERAPIA GENICA; NELLA RICERCA SUL CANCRO CON LA CAPACITÀ DI ARRIVARE SELETTIVAMENTE AI TUMORI).



A serene sunset scene over a calm ocean. The sun is low on the horizon, creating a bright, golden glow that reflects on the water's surface. The sky is filled with soft, wispy clouds. In the lower-left foreground, a dolphin is captured mid-leap, its dark silhouette contrasting with the shimmering water.

NANOPARTICELLE DI ORO ( $\text{AuNPs}$ )  $< 100 \text{ nm}$

"ciò che è affascinante è la possibilità di utilizzare oggetti che non si possono neanche vedere al microscopio ottico per produrre nei tessuti un effetto macroscopico"

Hugh Richardson



E DELLE MACRO- E MICRO-PLASTICHE





**Plastic**Oceans





## **RINGRAZIAMENTI**

Prof M. Cristina Fossi, Dr. Silvia Casini, Dr. Silvia Maltese,  
Dr. Daniele Coppola, Dr. Matteo Giannetti, Dr. Matteo  
Baini, Sig. Laura Carletti





# Ringraziamenti

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- L'Università di Padova (Sandro Mazzariol), di Teramo (Giovanni di Guardo) e lo Zooprofilattico di Pisa (Giuliana Terracciano)
- Forze dell'Ordine (Capitaneria, Forestale, ecc.)
- l'ISPRA ed i Ministeri dell'Ambiente e degli Esteri

# Grazie

