



# Differential toxic sensitivity of crude oil in fish embryos depending on weathering status

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## Hebei Spirit oil spill?

- Hebei Spirit 좌현에 3개의 파공  
(#1 Tank : 30 x 3 cm, #3 Tank : 160 x 10 cm, #5 Tank : 200 x 160 cm)
- 3종의 중동산 원유 총 12,547 톤 유출

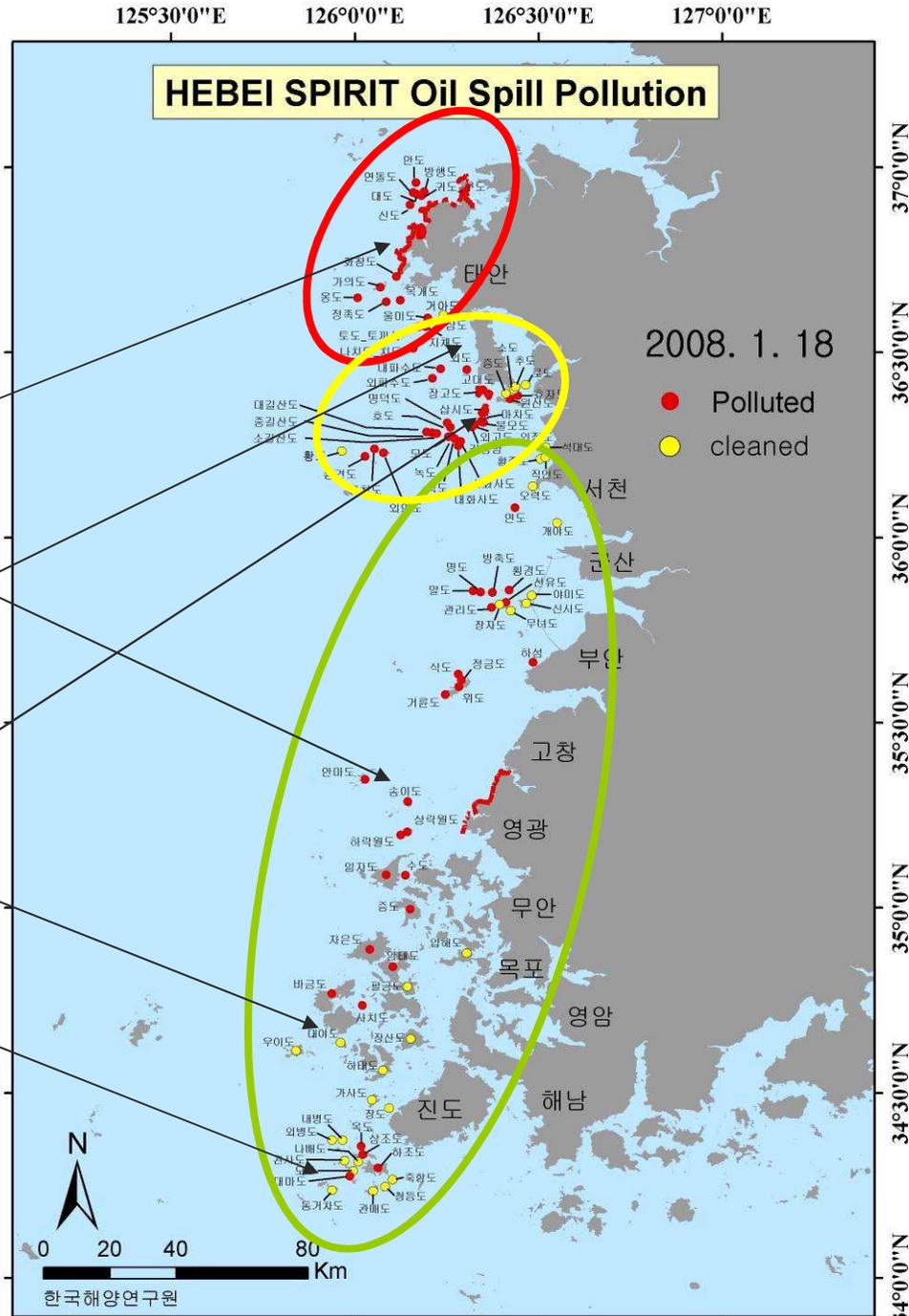
UAE Upper Zakum / #1

Kwait Export Crude / #3

Iranian Heavy Crude / #5



유출유 표착 해안  
유류오염 현황  
(2008.1.18 현재)



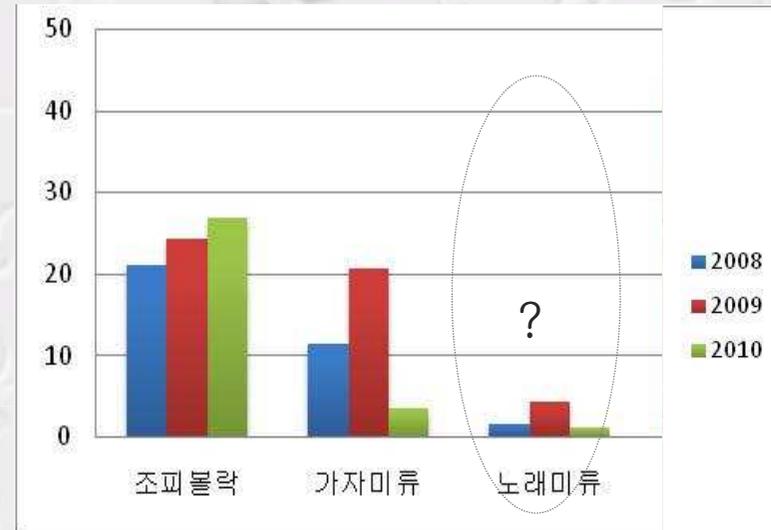
(해경 SCAT 자료 재구성)

# THE “HEBEI SPRIT” OIL SPILL



- ✓ Many marine species were found dead on the rocky shores and beaches
- ✓ More than 8,571 ha of land-based fish aquaculture facilities were directly affected by the crude oil
- ✓ There is still continuing controversy over the impact of residual oil on the ecosystem

# Toxic effects of crude oil on fish embryos



(Chungnam University)



Greenling



# Why fish embryos?



EVOS Trustee Council

**Exxon valdez oil spill**  
March 24, 1989



pink salmon habitat

NOAA Auke Bay Lab



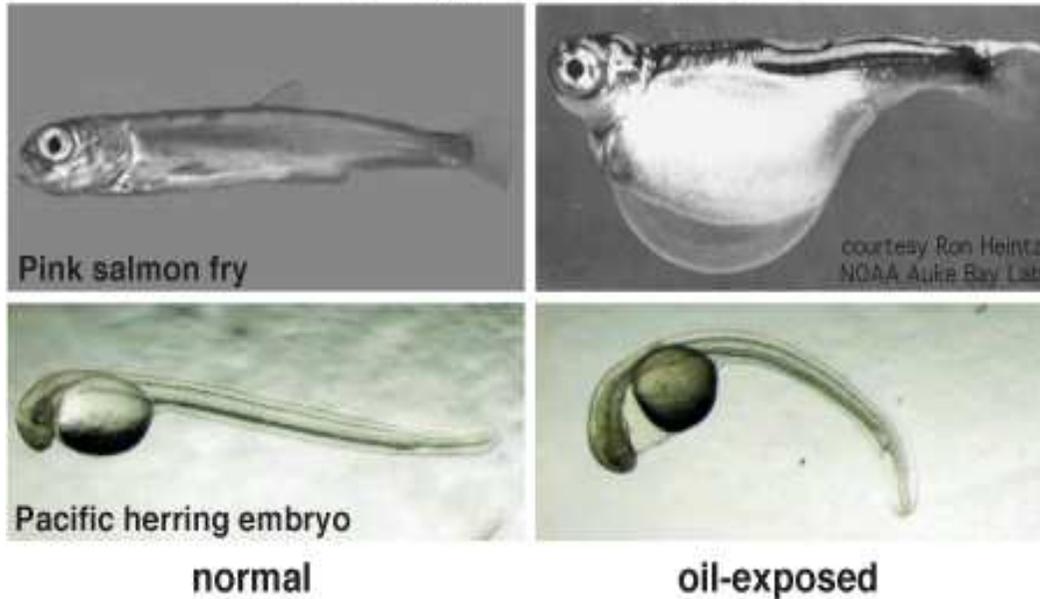
herring habitat

(Incardona, 2011)

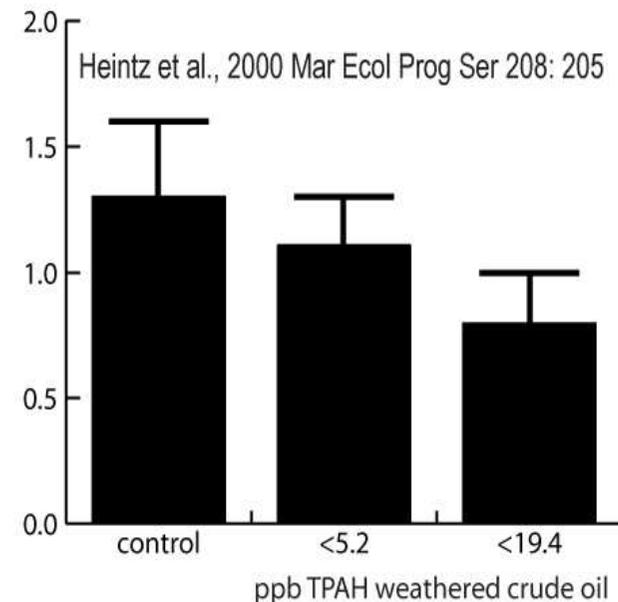
©FO Canada

# Acute and long-term impacts of oil spills on embryonic fish

Morphological abnormalities resulting from embryonic PAH exposure



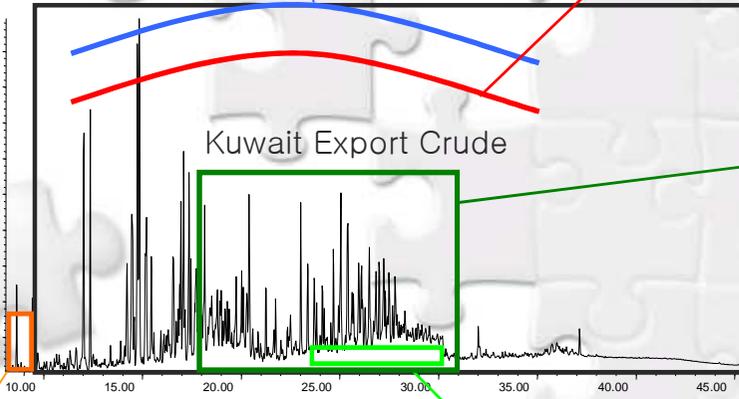
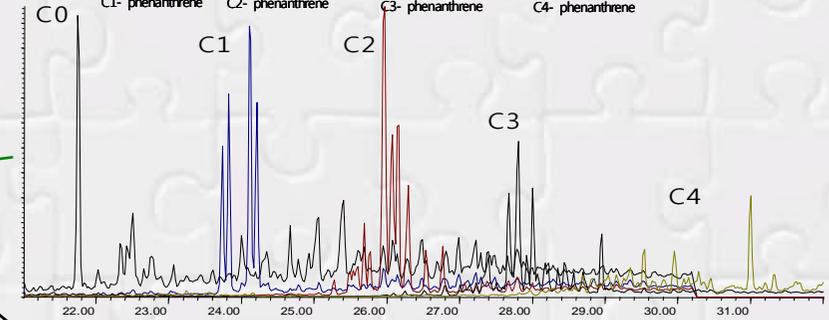
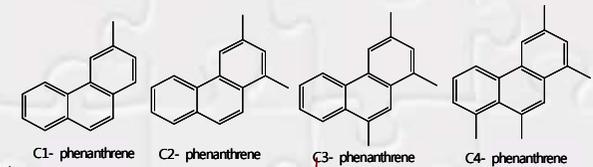
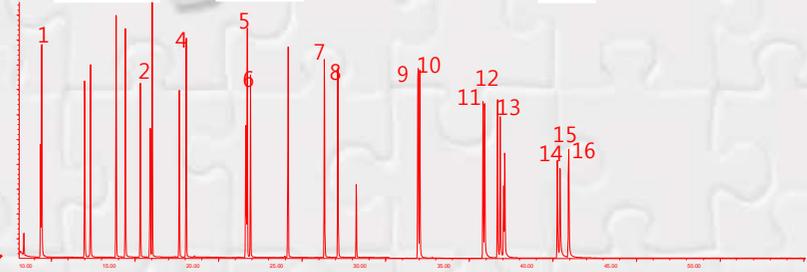
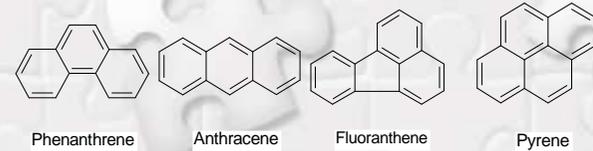
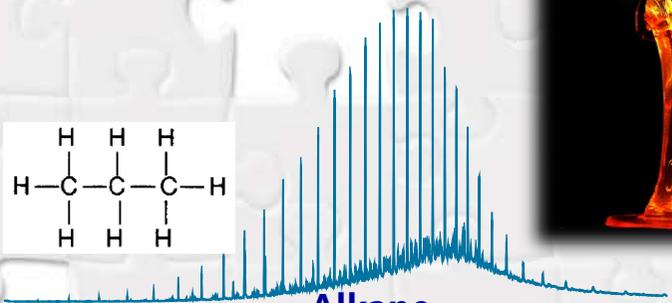
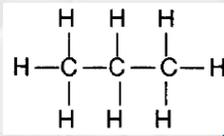
Reduced marine survival of morphologically normal pink salmon exposed to oil as embryos



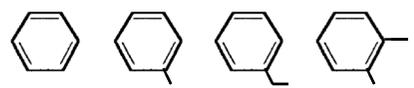
Oil compounds produce a chemical phenocopy of cardiac function mutants

(Incardona, 2011)

# Composition of crude oil



BTEX

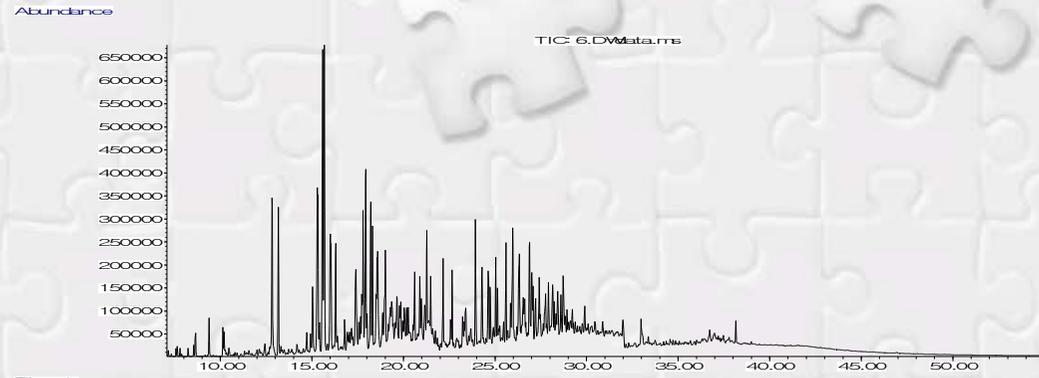


Total Petroleum Hydrocarbons (TPH)

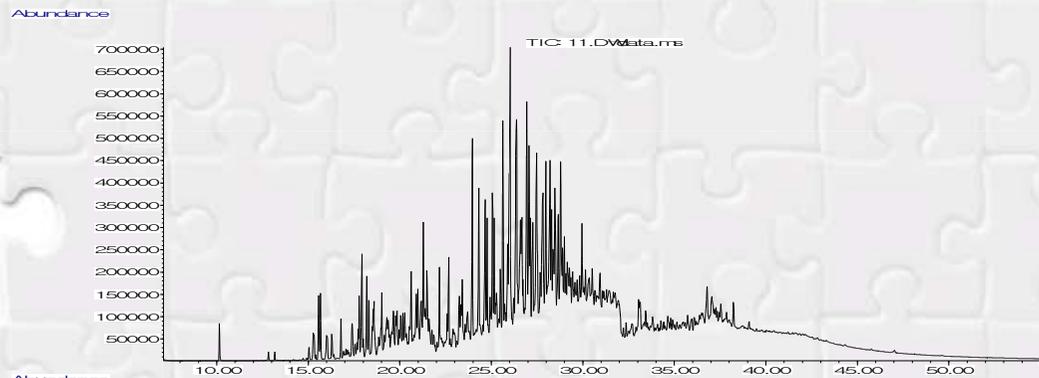
(Yim, 2008)

# Weathering status of crude oil

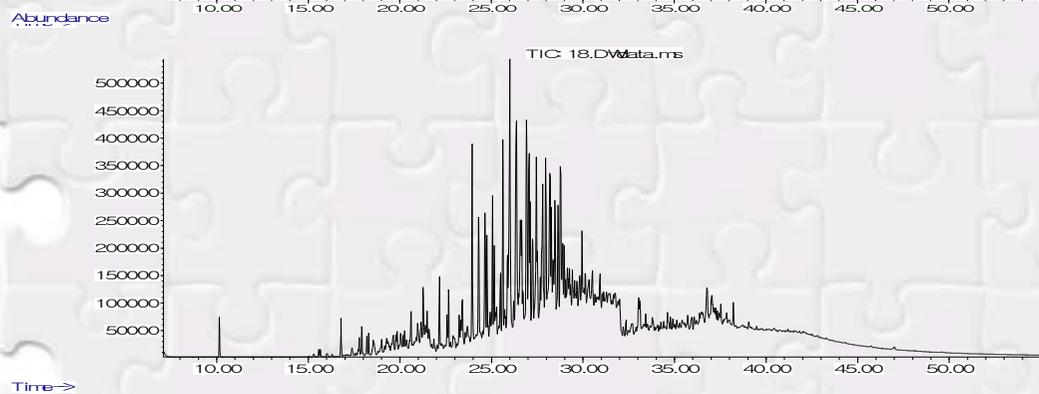
Fresh oil



After 14 days

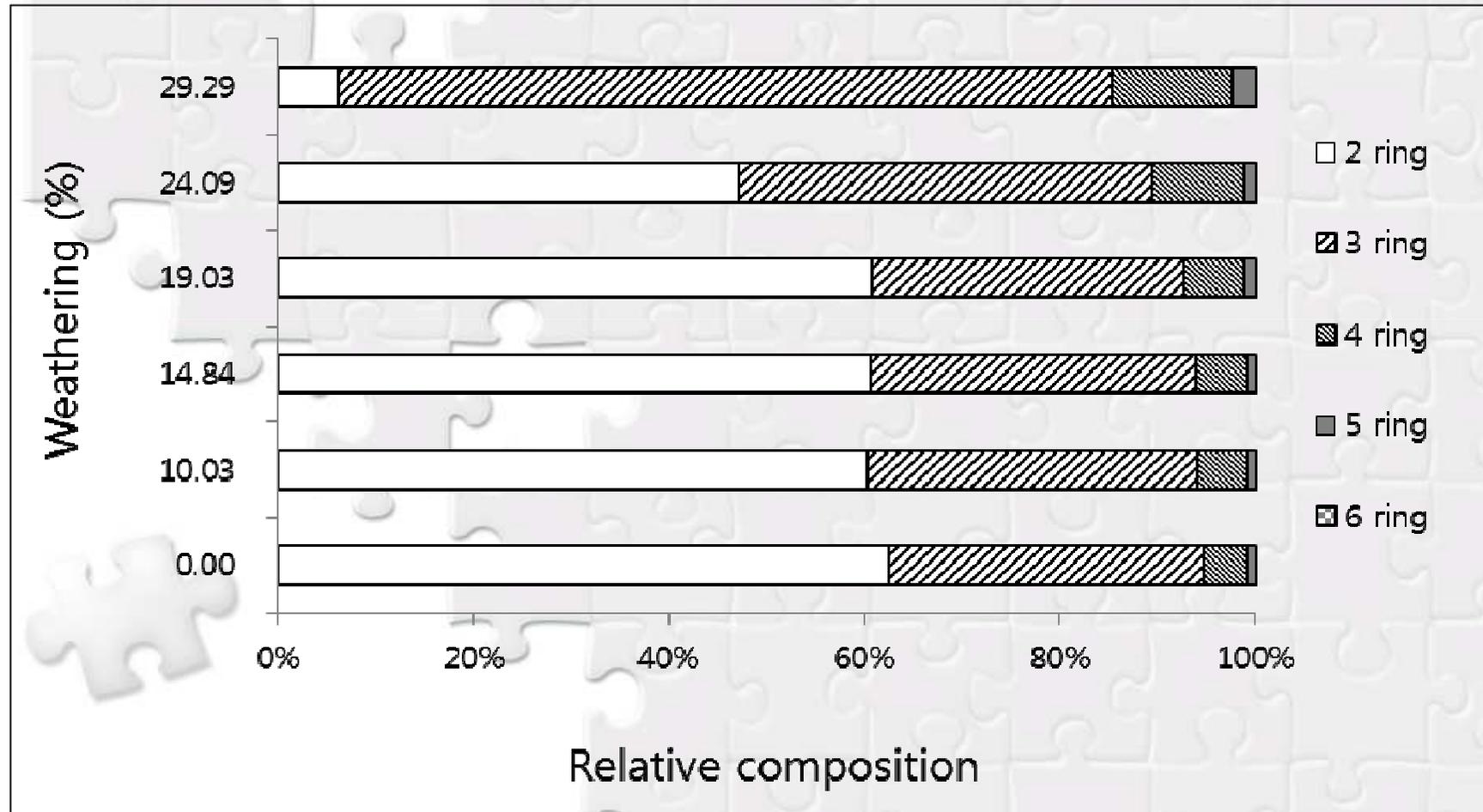


After 3 months



(Yim, 2008)

# Compositional changes of 16 PAHs in Iranian Heavy Crude oil according to the weathering rate



(Kim, 2012)

## A. Introduction

- ✓ Crude oil from different origins have distinct chemical composition, hence differing toxicity?
- ✓ There have been several studies comparing the general toxicity (e.g. LC50) of different type of crude oils
- ✓ There has been no systematic comparison of different oil types using cardiotoxic endpoint in fish embryos (most sensitive indicators of petroleum toxicity)

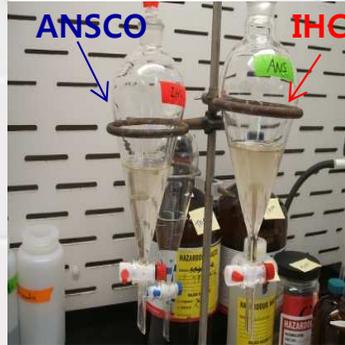
### **To clarify**

- ✓ **the potential for impact of the Hebei sprit spill**
- ✓ **expand our understanding of basic toxicity mechanisms of different crude oils**

# B. Materials & Methods

## Experiment 1

High-energy WAF  
1: 10,000 in system water



(50 embryos , 28.5°C)



Zebrafish embryos (24hpf)



PAHs analysis  
GC/FID & /MS



Live observation of embryos ,  
video-microscopy



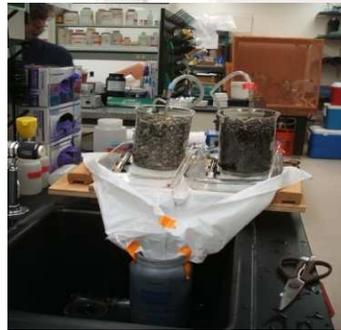
Immunofluorescence  
by confocal microscopy



Zeiss LSM 5 Pascal Confocal system  
with Ar and HeNe lasers

## Experiment 2

Oiled gravel column  
(OGE, 6g/kg)

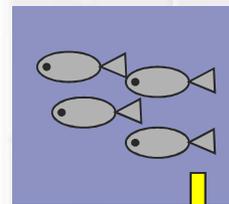


20% Weathered Alaska North Slope Crude oil(ANSCO)  
Fresh Iranian Heavy crude oil (IHC)



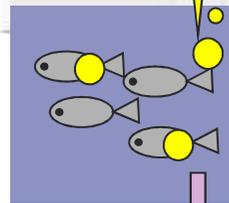
## *In situ* hybridization

### ◆ CYP1A & Myocin HC protein by Immunochemistry (whole organ)

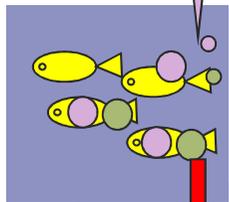


**FIX** at 4% paraformaldehyde

↓  
**Washing** at PBST

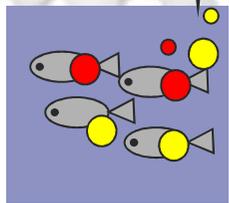


↓  
**Blocking** with goat IgG



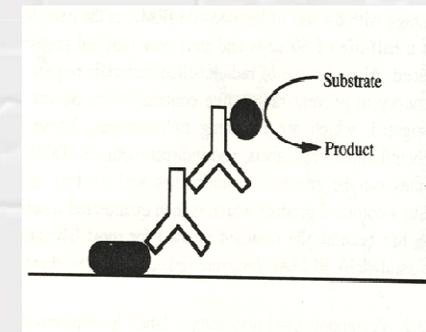
↓  
**1<sup>st</sup> ANTIBODY**(anti mouse CYP1A+ myocin HC)

↓  
**Washing** at PBST

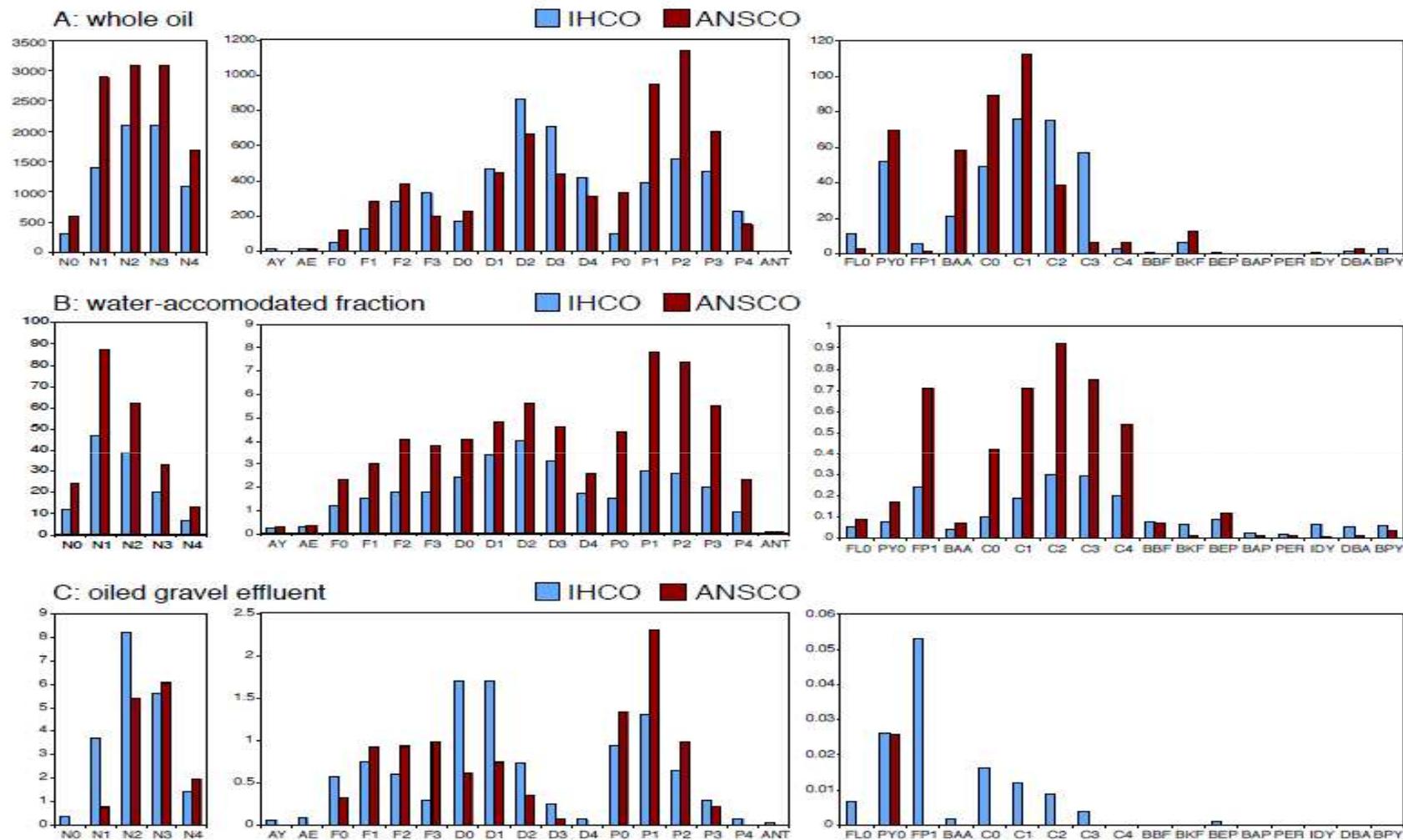


↓  
**2<sup>nd</sup> ANTIBODY**(goat anti mouse IgG<sub>3</sub>(g3)  
+ goat mouse myocin IgG<sub>2b</sub>(g2b))

↓  
**Washing** at PBST



## C. Results – PAHs composition



✓IHCO: NAP(37%), F(48%), DbT(21%)

✓IHCO OGE: parent and C1-alkyl PAHs

✓High weathered ANSCO OGE: Alkyl –phenanthrehes and alkyl-fluorences.

## C. Results – morphological defect

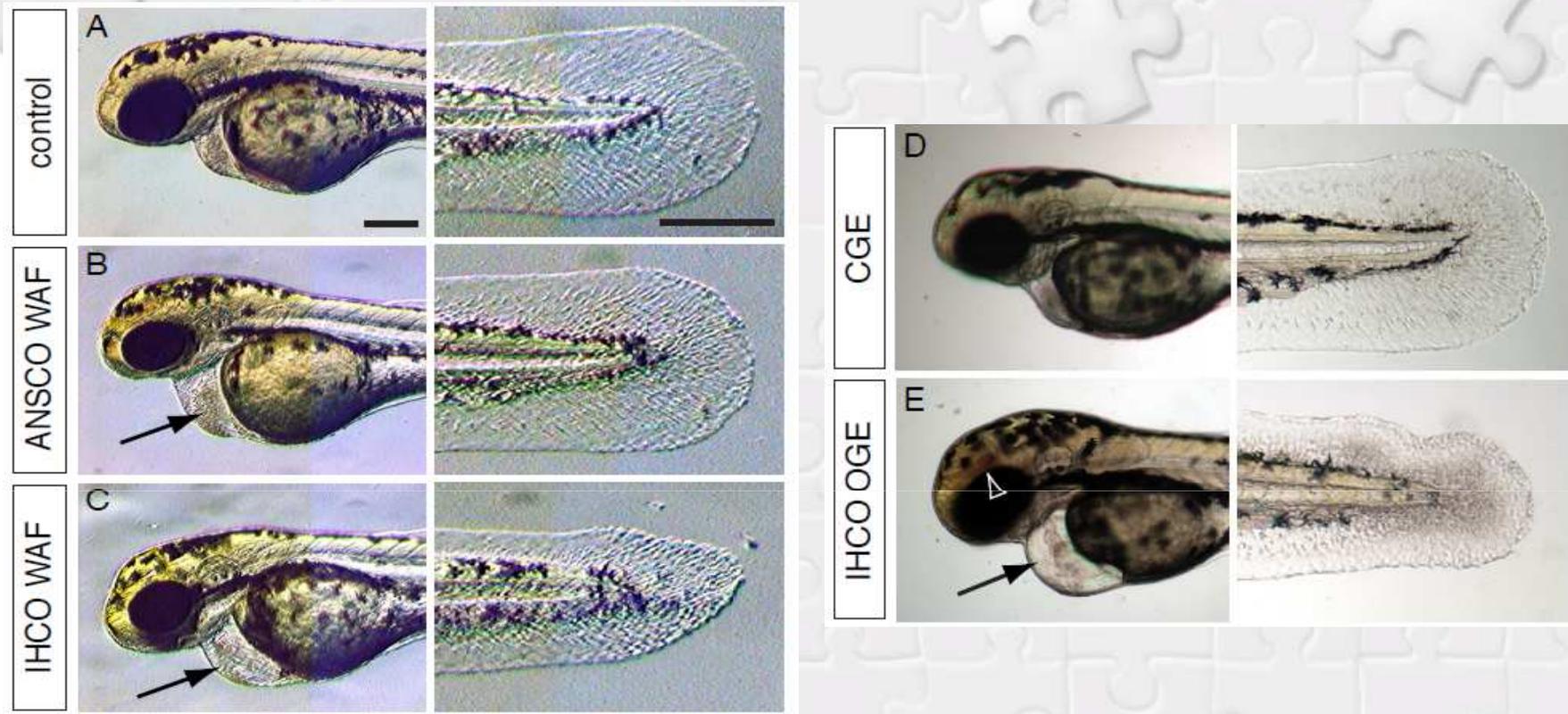
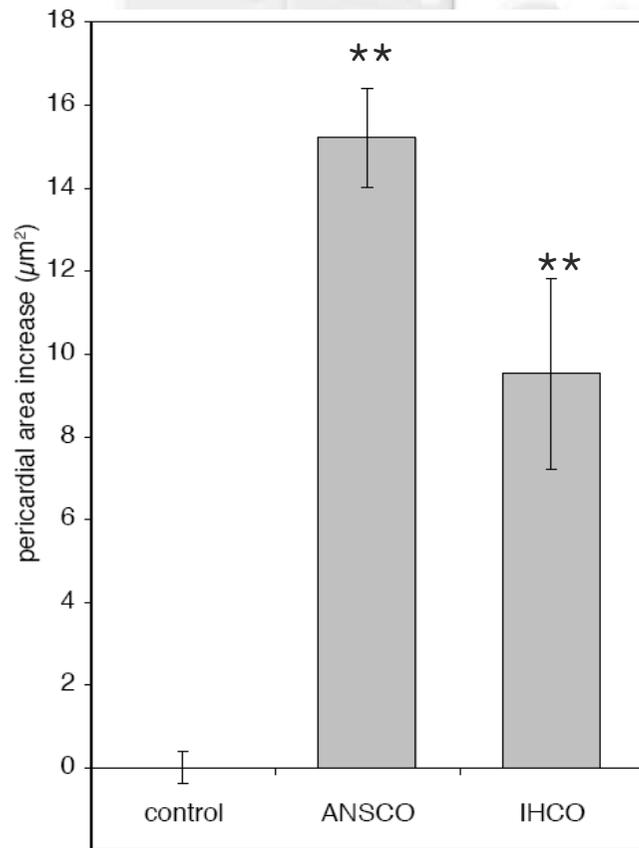


Table 1: Comparative toxicity of ANSCO and IHCO

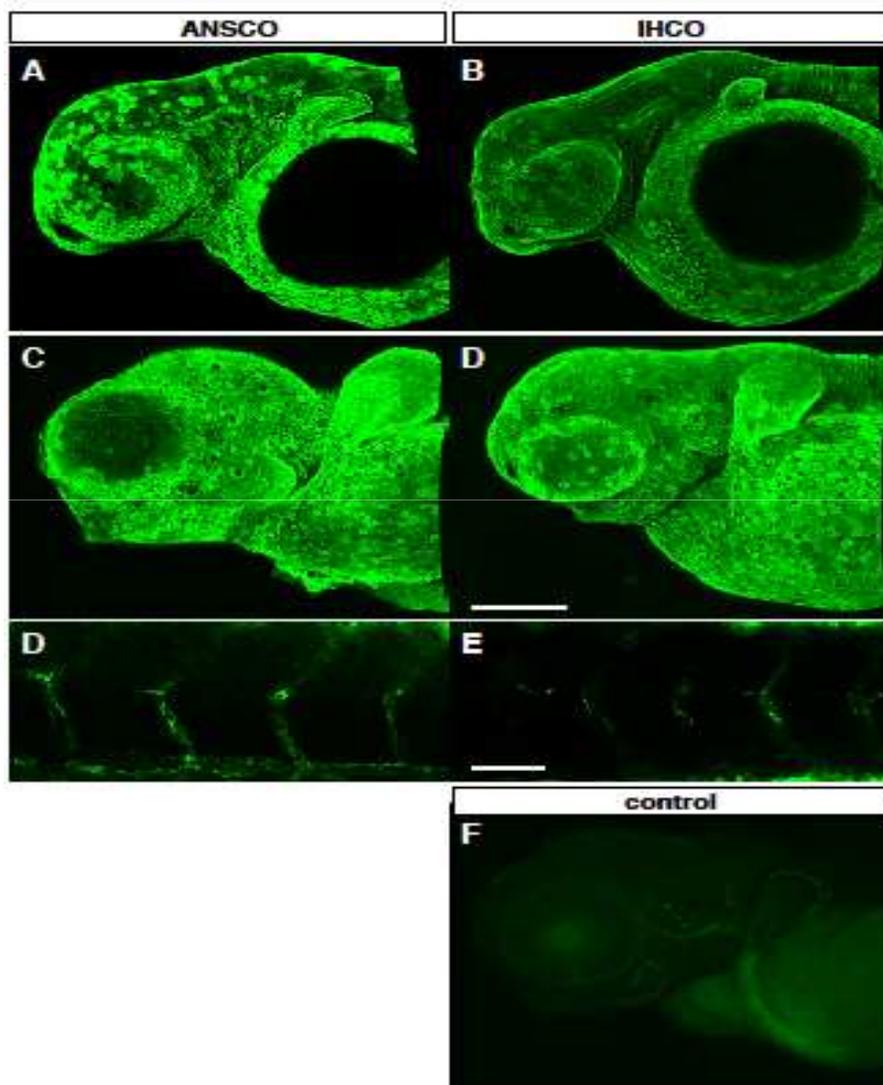
endpoint	control	ANSCO WAF	IHCO WAF(f)	ANSCO OGE	IHCO OGE(f)
pericardial edema	0	<b>86%</b>	63%	34%	63%
intracranial hemorrhage	0	63%	38%	19%	18%
tail malformation	0	0	<b>80%</b>	0	<b>80%</b>

## C. Results – IHCO & ANSCO



- Arrhythmia
- Bradycardia
- Tail (fin)defect

## C. Results – CYP1A & MHC protein expression

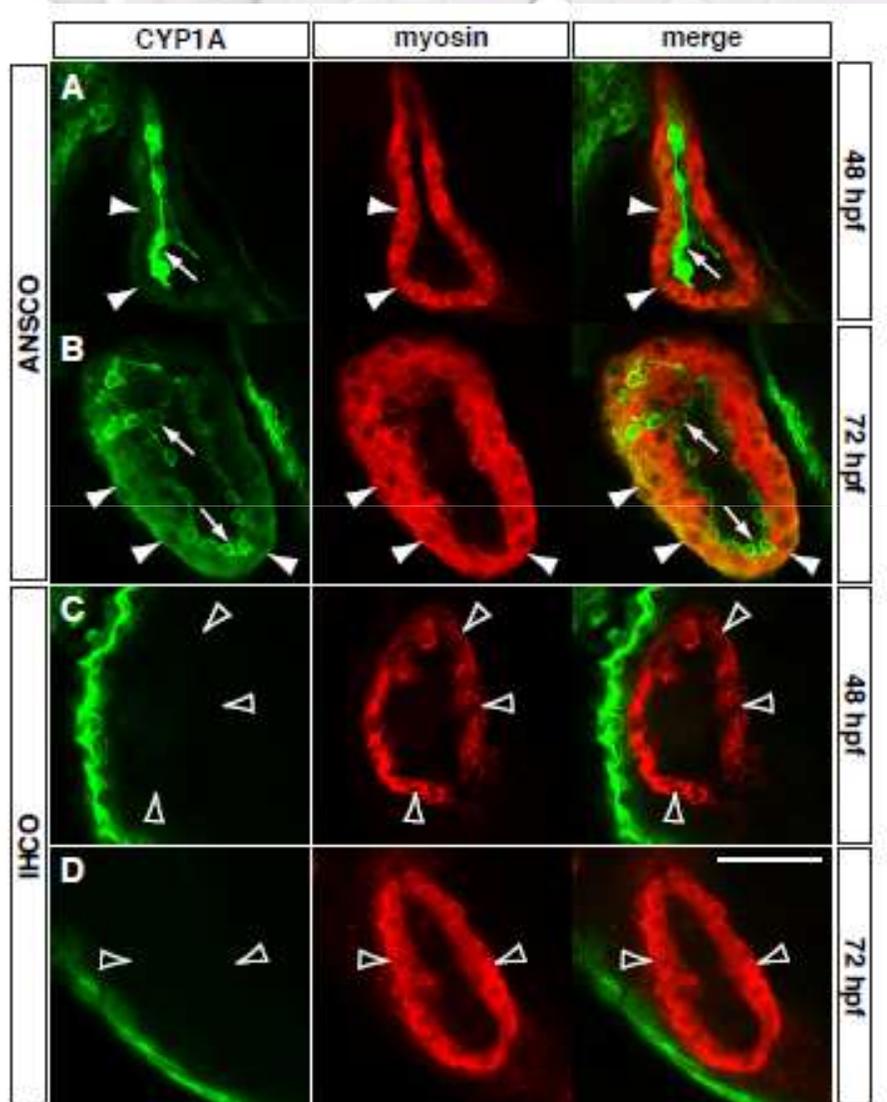


✓ Strong CYP1A immunofluorescence in epidermis after exposure to WAF and OGE

✓ CYP1A immunofluorescence activity was stronger in the epidermis of head and eyes exposed to weathered ANSCO than that fresh IHCO

✓ This pattern was similar at both 48 and 72 hpf.

## C. RESULTS – CYP1A & MHC protein expression

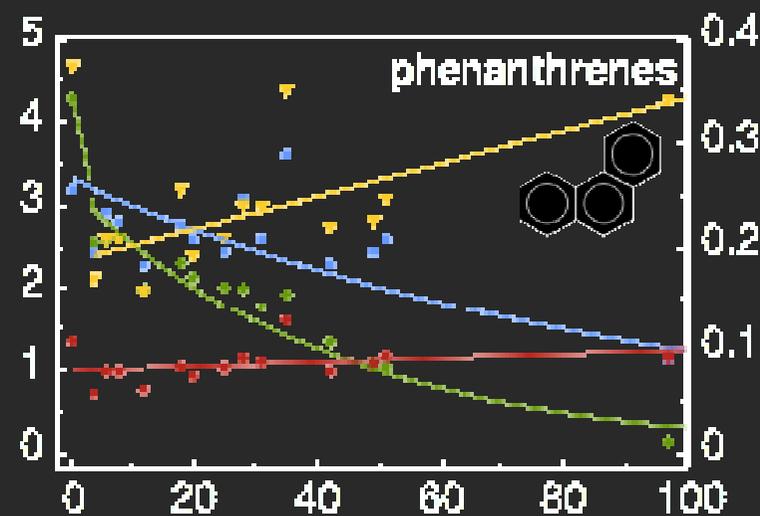
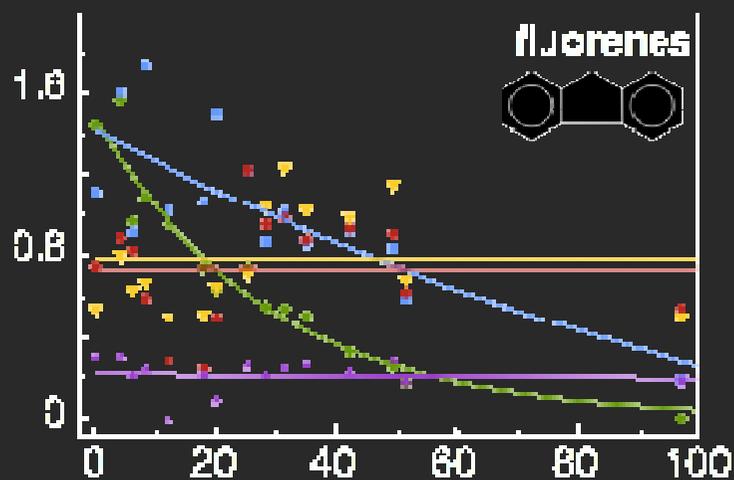
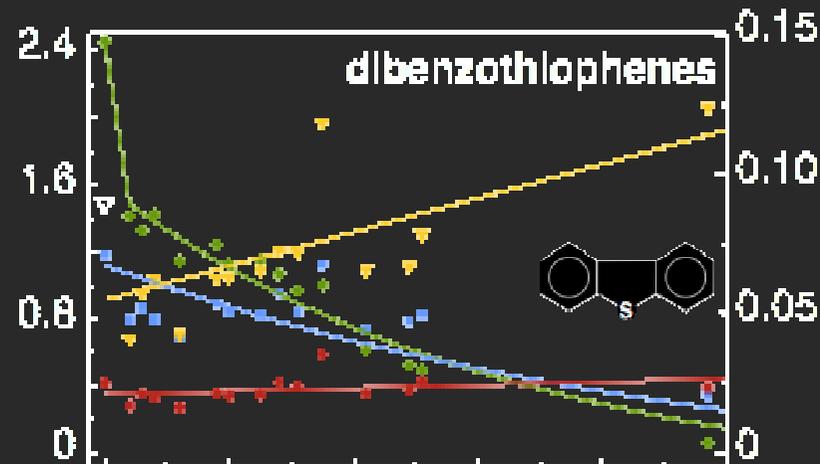
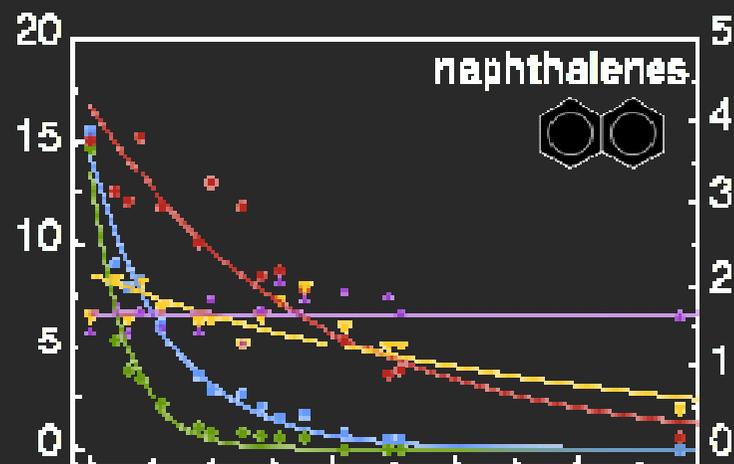


✓ Strong CYP1A immunofluorescence in endocardium at both 48 and 72 h hpf.

✓ ANSCO toxicity (oil cardiotoxicity) was independent of Ahr activation (persisted after & Ahr1a knockdown)  
 -> CYP1A induction in the endocardium but not myocardium.

✓ This pattern was similar at both 48 and 72 hpf.

# Changes in dissolved PAHs with weathering



- C0
- C1
- C2
- C3
- C4

## C. Results – Changes in dissolved PAHs with weathering

\* D0/D2 = Parent DbT/Dimethyl DbT -> Solubility

	Whole Oil	WAF (water accommodated fraction)			OGE (Oiled gravel effluent)		
	D0/D2	D0/D2	Edema (%)	CYP1A	D0/D2	Edema (%)	CYP1A
ANSCO	0.30	0.68	86%	Skin/eye Endocardium	1.6	34%	Skin/eye Endocardium
Fresh IHCO	0.30	0.60	63%	Skin/eye	2.3	64%	Skin/eye
Weathered IHCO	0.30	0.53	70%	Skin/eye Endocardium	1.9	58%	Skin/eye Endocardium

\* High weathered ANSCO(42days weathering)-> CYP1A expressed at Endocardium & Myocardium

-> cardiac defect from AhR-independent

## D. Summary

- ✓ Overall, the two types of crude oil studied here produced highly similar toxicity, primarily characterized by the well-known effects on cardiac function.
- ✓ On the other hand, a distinct chemical component of IHCO is evident from the high frequency of tail fin defects.
- ✓ These findings show that two crude oils from distinct sources have similar toxicity, and that the weathering state of dissolved PAHs is the more important determinant of toxicity.
- ✓ In some respect, the parent PAH compounds may be damaging. (edema....in equivalent  $\Sigma$ PAH)
- ✓ The toxicological difference between the HSCO and ANSCO may seem minor, compositional differences is much more strongly.

## D. Summary

- ✓ Although, developing heart defect may be shift from predominantly AHR independent mode during acute exposure
- ✓ AHR-mediated cardiotoxicity in function (by dioxin & PAHs)  
– circulatory function act (48h to 72h) still.
- ✓ AHR-independent actions of parent tricyclic PAHs  
- circulatory function loss at 36h to 48h.

# Acknowledgement



- **Co-authors**

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