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Effect-based approach for screening of chemical mixtures in whole blood of green turtles from the Great Barrier Reef

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WWF

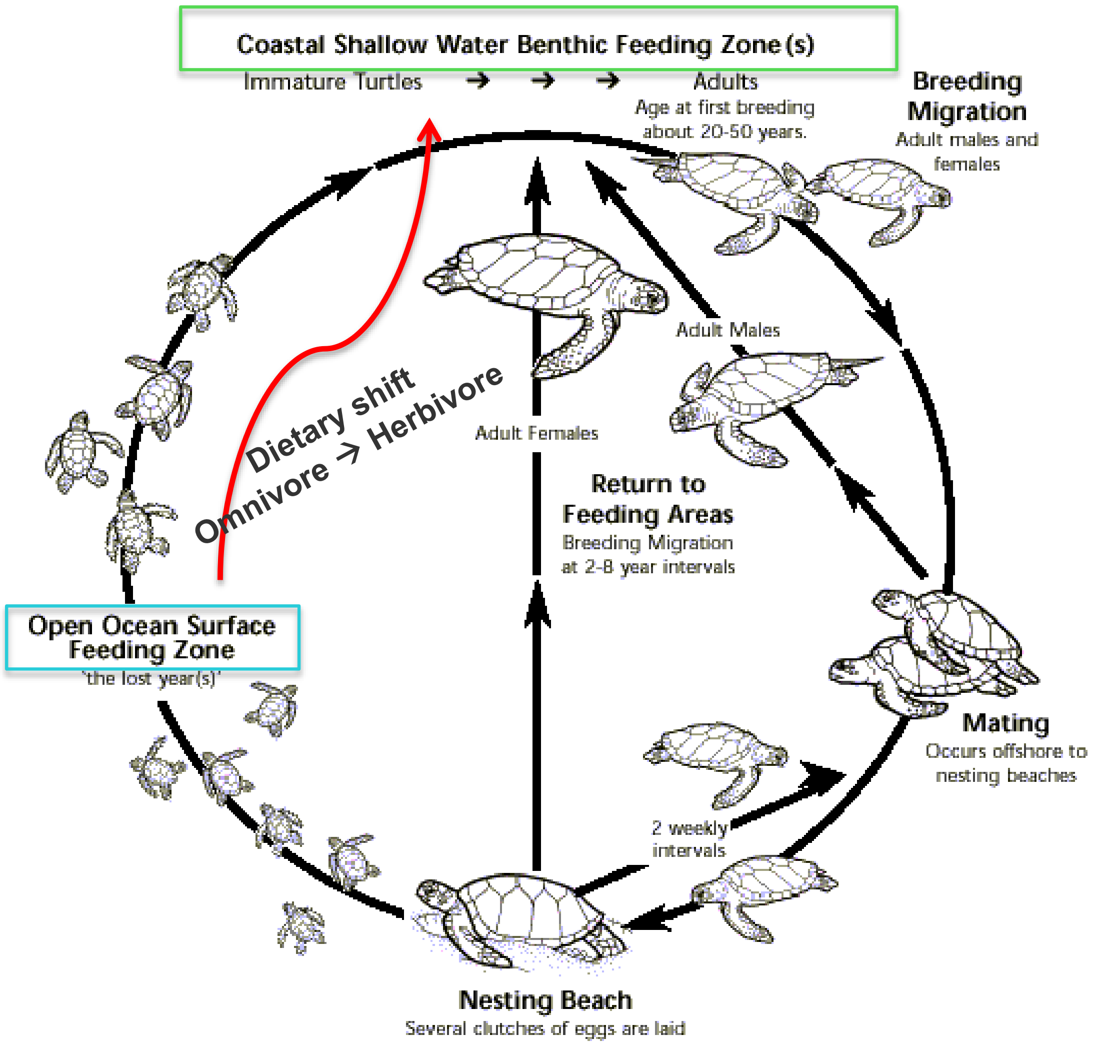
Rivers to Reef to Turtles Project

‘For more than 100 million years marine turtles have covered vast distances across the world’s oceans, performing a vital and integral role in marine and coastal ecosystems’

-WWF

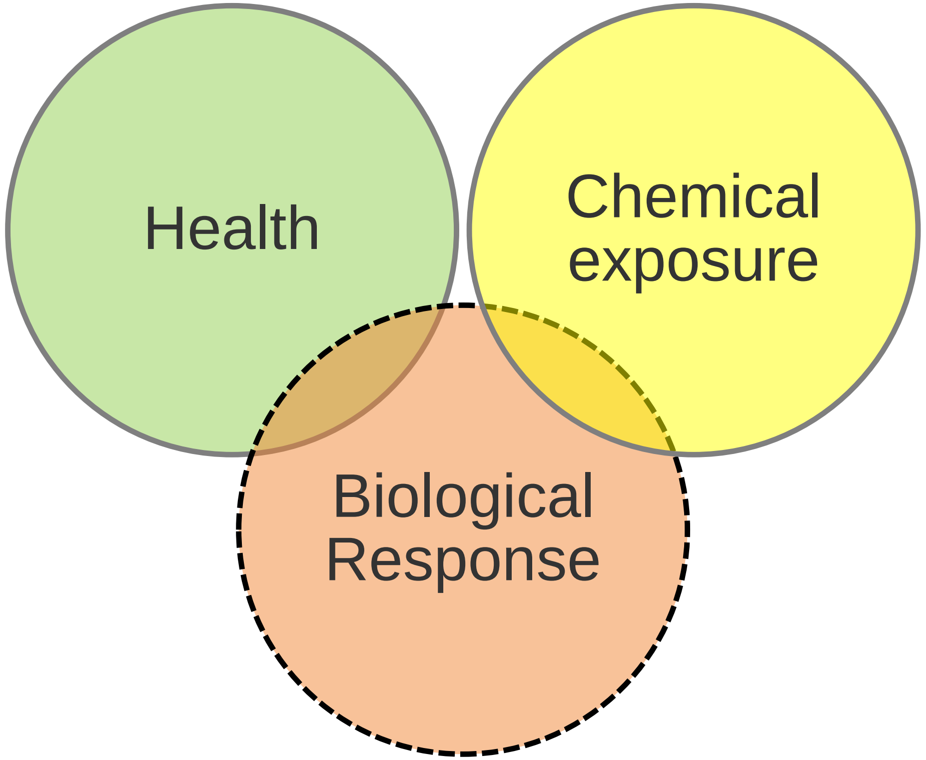

Oil spills
Marine debris
Chemical pollution
TOXIC
Multiple stressors
Boat strike
By-catch

Why green turtles?



Why *in vitro* bioassays?

- Mixture effects (known and unknown)
- Investigate actual molecular targets (MOA)
- Wide spectrum of MOA
- Time and cost-effective
- High throughput screening tool
- Ethical alternative to whole animal testing (3Rs (Replacement, Reduction, and Refinement)
- Very sensitive



Why whole blood?

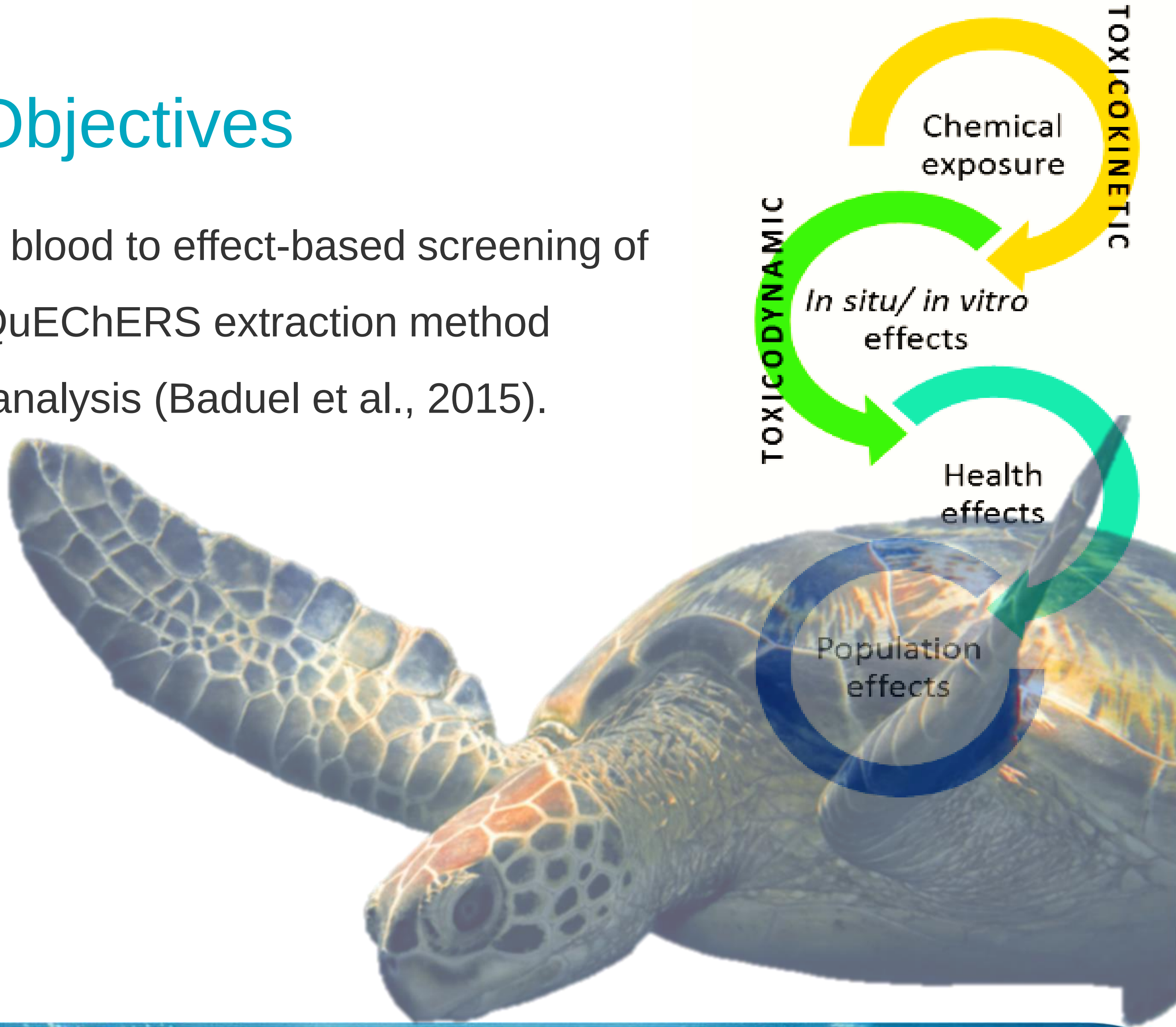
- Characterization of internal exposure to chemical mixtures
- Blood represents the transport medium for all (endogenous and exogenous) chemicals in a body
- Toxicokinetic processes/ADME

→ Matrix effects (e.g lipid, proteins) in cell-based tools?

*** Escher et al. 2017 From the exposome to mechanistic understanding of chemical-induced adverse effects**
**** Rappaport et al. 2014 The Blood Exposome and Its Role in Discovering Causes of Disease**

Objectives

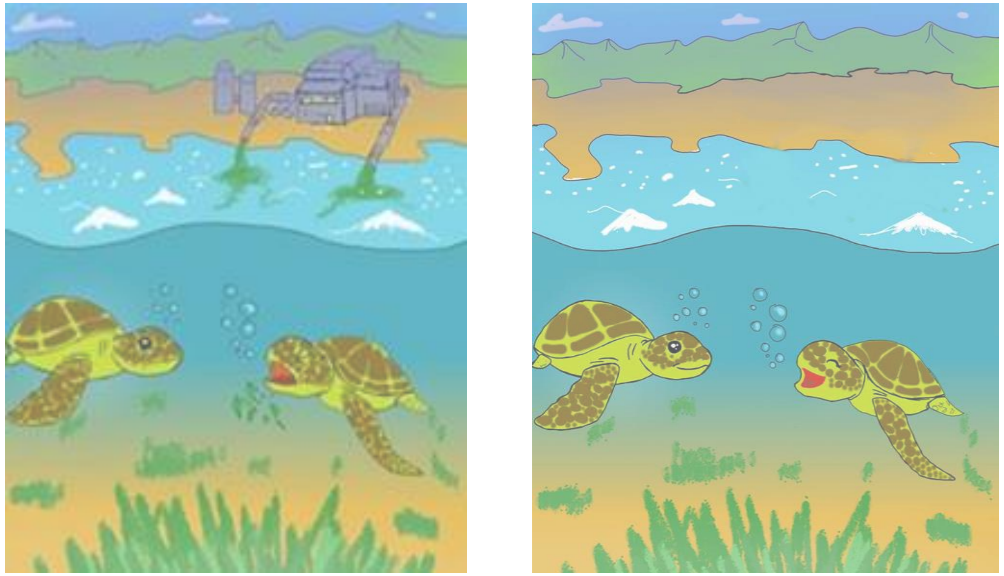
Investigating the application of whole blood to effect-based screening of complex chemical mixtures using a QuEChERS extraction method developed for non-target instrument analysis (Baduel et al., 2015).



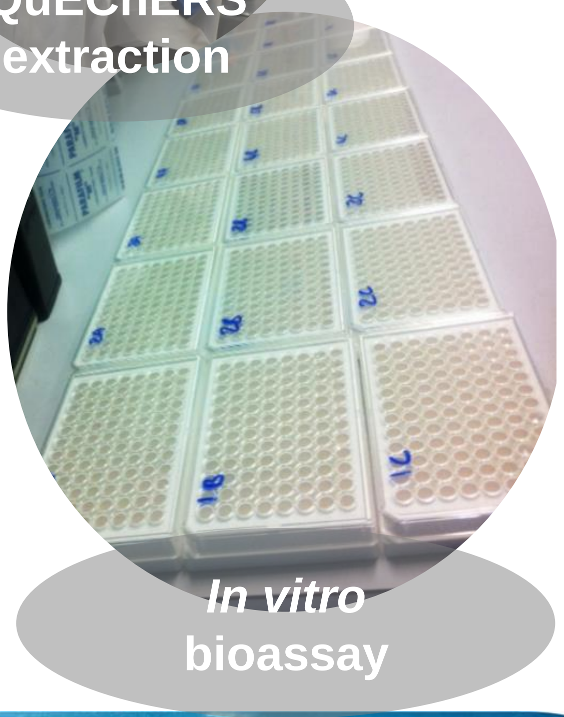
Objectives

1) Method validation - investigating the potential for interferences

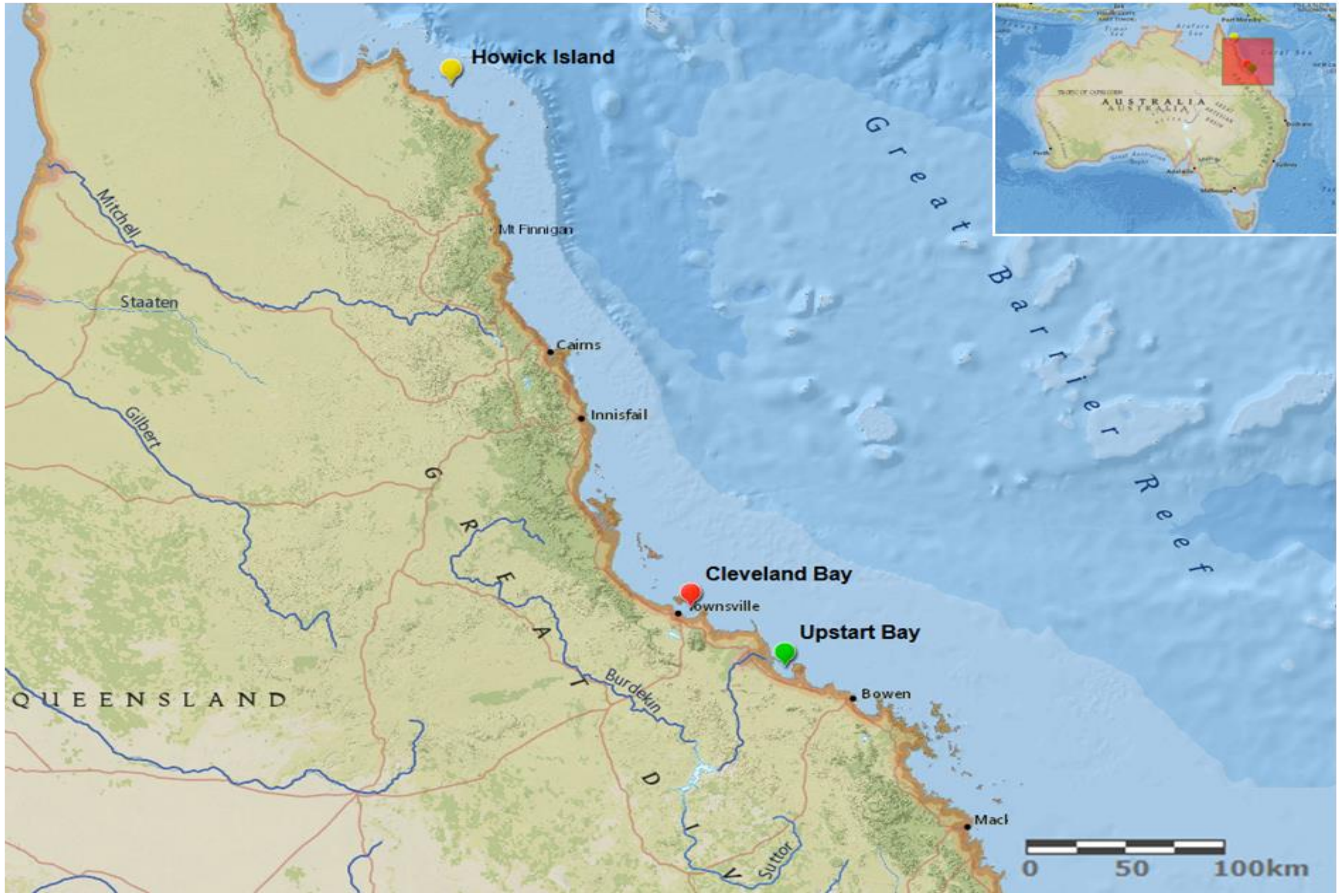
2) Method application – green turtles from 3 different foraging grounds



Habitat differences ?



Study area



Howick Island – Reference

Cleveland Bay – Industrial/ Urban

Upstart Bay- Agricultural

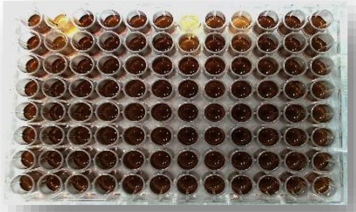
Method - Sampling

Method - Sampling

Method – Sampling/ Extraction

Modified QuEChRS

Blood extracts



Method - Toxicity pathway-based test battery of *in vitro* bioassays

Table 1. Overview of the different bioassays used in this study. .

Assay	Reference compound		Mode of action
Ahr-CAFLUX	2,3,7,8-tetrachloro-dibenzo-dioxin (TCDD)	Specific	Aryl hydrocarbon receptor (AhR) mediated receptor for dioxins and dioxin-like compounds
VM7Luc4E2	17β- estradiol		Receptor-mediated estrogen activity response
NFκB-bla	Tumor necrosis factor (TNF)-α	reactive	Nuclear Factor Kappa B mediated innate inflammatory response
AREc32	t-butylhydroquinone (tBHQ)		Nrf2-mediated oxidative stress response
Microtox	Phenol/virtual baseline toxicant	Non-specific	Baseline Toxicity- Cytotoxicity indicator by bioluminescence inhibition in *Vibrio fischeri*

Results (1)

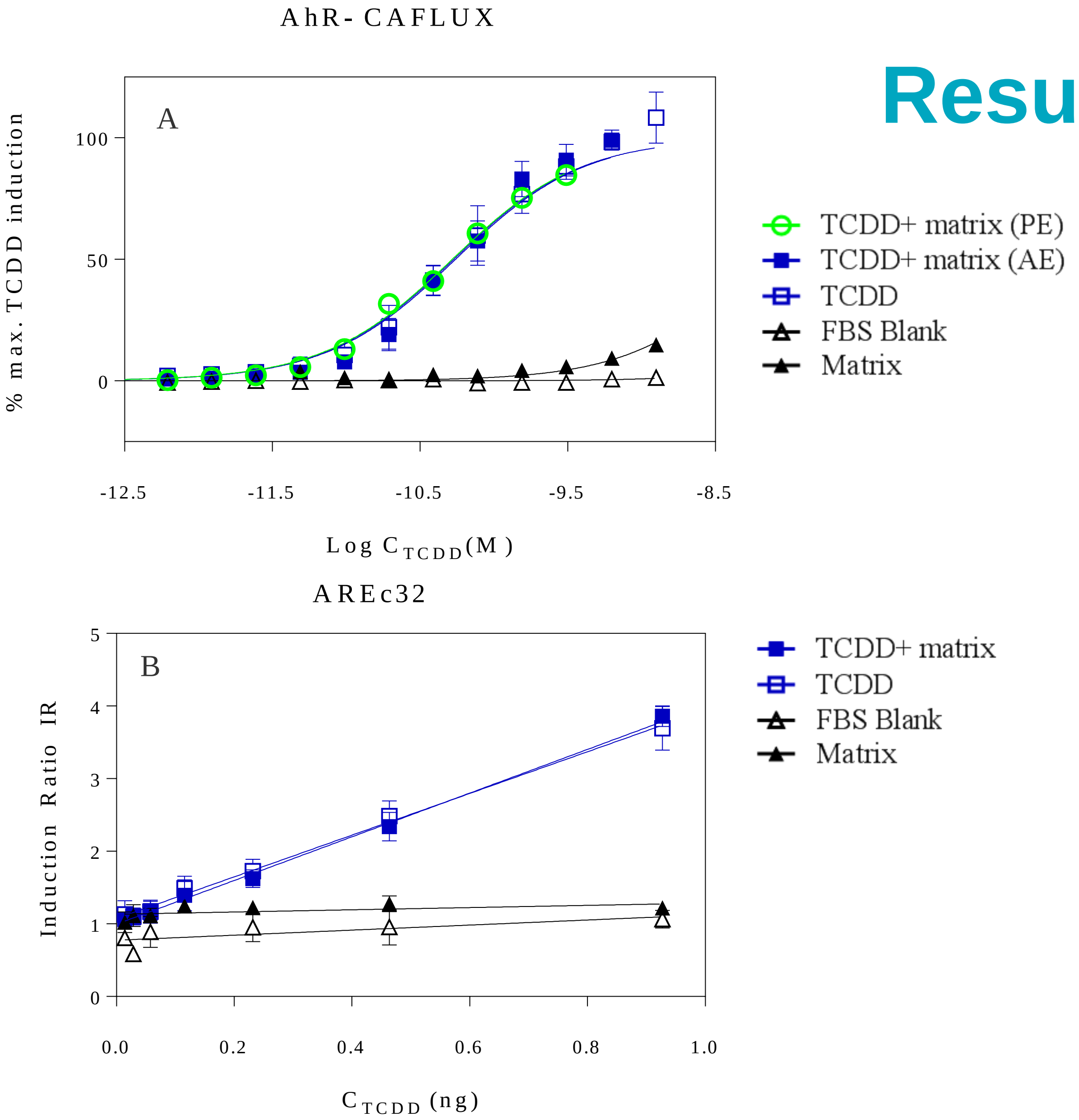


Figure 1. Comparison of responses of reference compounds and spiked or unspiked blood extracts for A: the dose-response from AhR-CAFLUX; B: the dose-response from AREc32.

Table 2. Comparison of effect concentrations (EC) of reference compound (x), which were spiked onto bioassays with (‘EC_x + matrix’) and without blood extract matrix (‘EC_x’).

Bioassay	Unit	Reference compound (x)	ECx	n	ECx + matrix	n
AREc32	mg/L	tBHQ	0.524	3	0.513	1
AREc32	mg/L	DDT	12.14	1	12.43	2
AREc32	pg/L	2,3,7,8-TCDD	290.3	4	296.9	4
AhR-CAFLUX	pg/L	2,3,7,8-TCDD	10.08	6	10.43	4
NF-kb bla	ng/L	TNF-alpha	0.039	1	0.039	1
VM7Luc4E2	ng/L	17β-estradiol	46.84	2	42.76	2

Howick Island – Reference

Cleveland Bay – Industrial/ Urban

Upstart Bay- Agricultural

Study outline

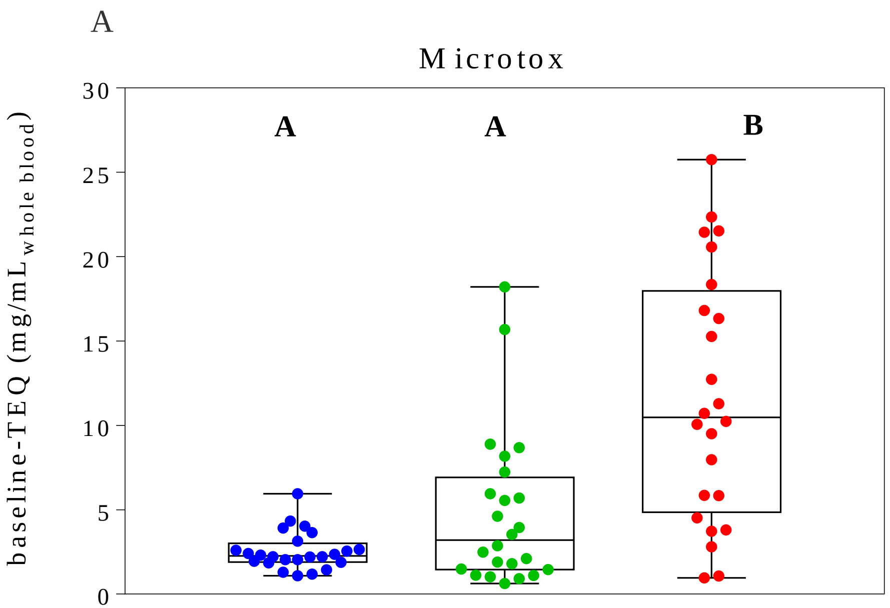
Pre-screening
n=5

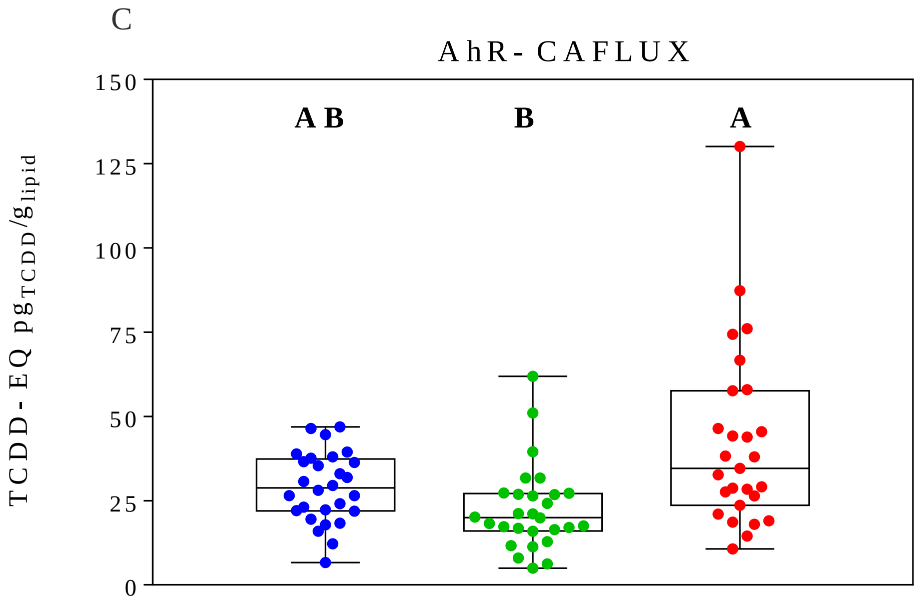
Selection of Bioassays

Screening population
(n=20-25)

Assay	Reference compound		Mode of action
Ahr-CAFLUX	2,3,7,8-tetrachloro-dibenzo-dioxin (TCDD)	Specific	Aryl hydrocarbon receptor (AhR) mediated receptor for dioxins and dioxin-like compounds
VM7Luc4E2	17β- estradiol	Specific	~~Receptor-mediated estrogen activity~~ response
~~**NFκB-bla**~~	Tumor necrosis factor (TNF)-α	reactive	Nuclear Factor Kappa B mediated innate inflammatory response
AREc32	t-butylhydroquinone (tBHQ)	reactive	Nrf2-mediated oxidative stress response
Microtox	Phenol/virtual baseline toxicant	Non-specific	Baseline Toxicity- Cytotoxicity indicator by bioluminescence inhibition in *Vibrio fischeri*

Results (2)





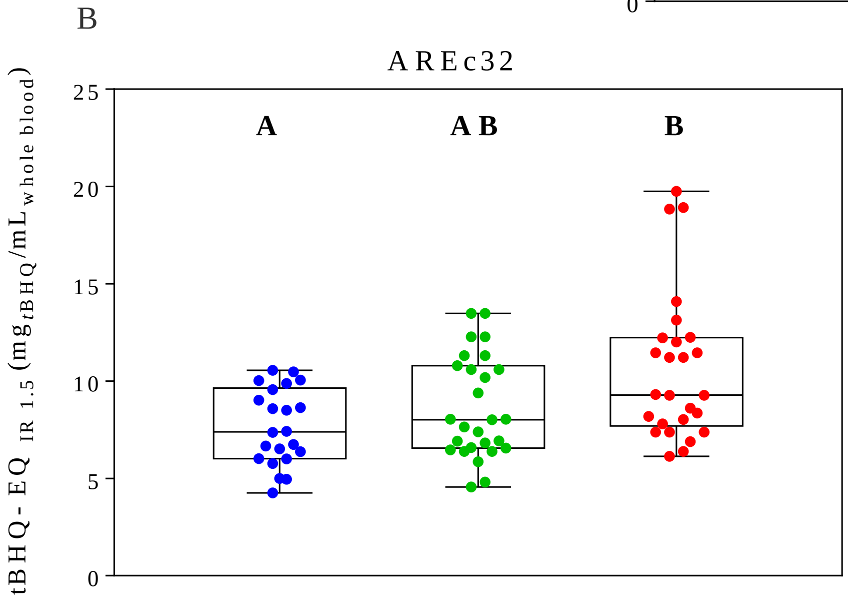


Figure 2. Comparison of the bioanalytical equivalent concentrations (BEQs) in QuEChERS extracted green turtle blood from Howick Island (HI; blue), Cleveland Bay (CB; green) and Upstart Bay (UP; red) for

A) Microtox (each point = 2 replicates)
B) AREc32 (3 replicates), and
C) AhR-CAFLUX (3 replicates) assays.

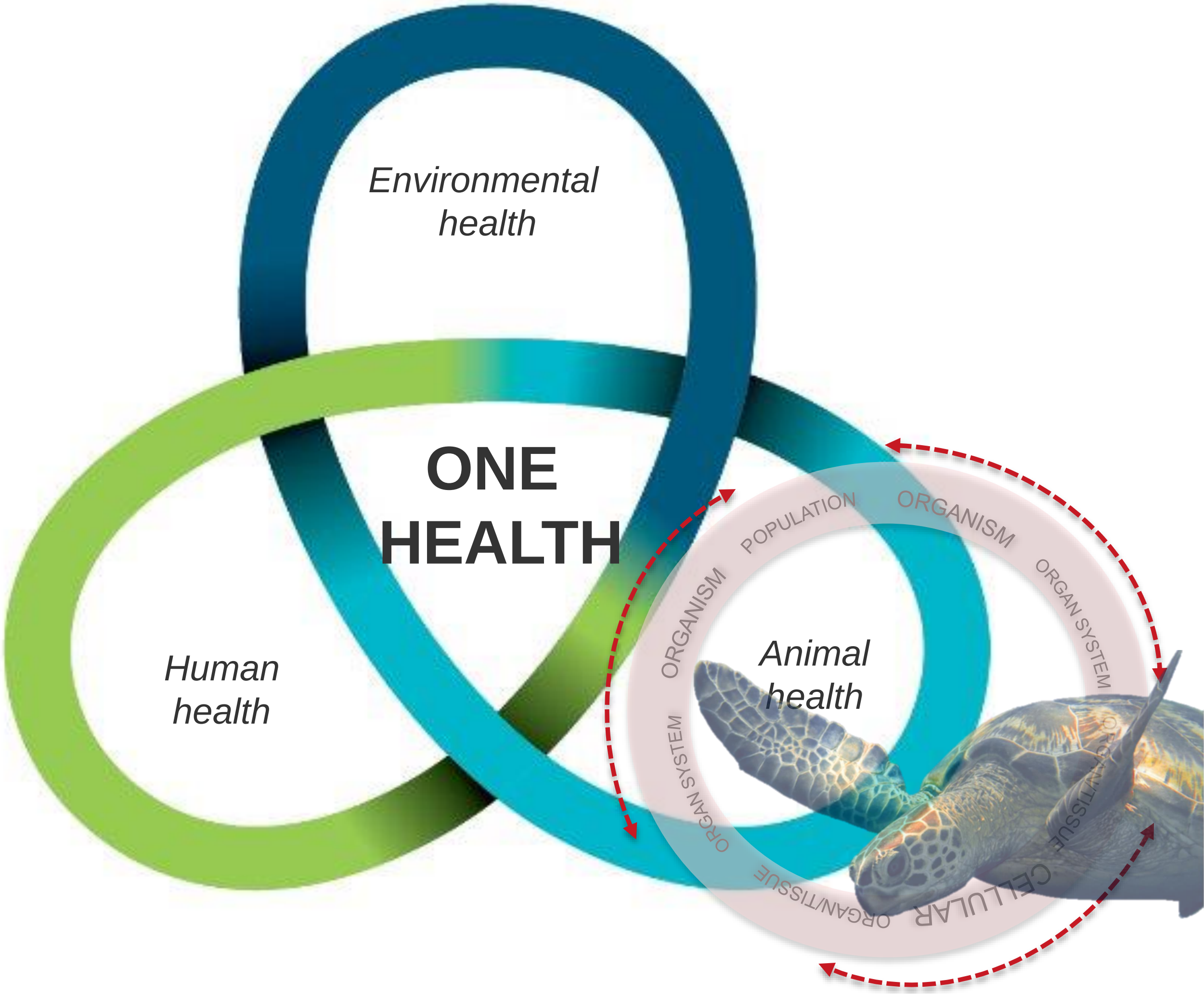
Whiskers = range, boxes = 1st and 3rd quartiles, line = median; statistically significant differences are indicated as letters (Microtox: $p < 0.0001$; AREc32: $p = 0.00328$; AhR-CAFLUX: $p = 0.0007$; Kruskal-Wallis test).

Conclusions

- Proof of concept for combining a non-selective extraction (QuEChERs, Baduel et al. 2015) of whole blood with effect-based screening in *in vitro* bioassays

- Blood as sample matrix to measure the internal exposure to complex mixtures of chemicals

- The agricultural habitat (Upstart Bay) showing higher responses compared to the other two sites → a reflection of the different land-uses



Science of The Total Environment
Volumes 599–600, 1 December 2017, Pages 1251-1262

Non-targeted, high resolution mass spectrometry strategy for simultaneous monitoring of xenobiotics and endogenous compounds in green sea turtles on the Great Barrier Reef

Amy L. Heffernan[a, b, 1], Maria M. Gómez-Ramos[c, 1], Caroline Gaus[a], Soumini Vijayasarathy[a], Ian Bell[d], Christine Hof[a, e], Jochen F. Mueller[a], Maria J. Gómez-Ramos[a, c]

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Environmental Pollution
Volume 220, Part B, January 2017, Pages 1465-1476

Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations ☆

C.A. Villa[a], M. Flint[b, c], I. Bell[d], C. Hof[e], C.J. Limpus[f], C. Gaus[a]

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Rivers to Reef to Turtles

KEYWORDS

great barrier reef marine turtles rangers

Acknowledgment

Ian Bell
Department of Environment and Heritage Protection, Threatened Species Unit, Townsville, Australia;

Christine Hof
Species Conservation, World Wildlife Fund for Nature, Australia

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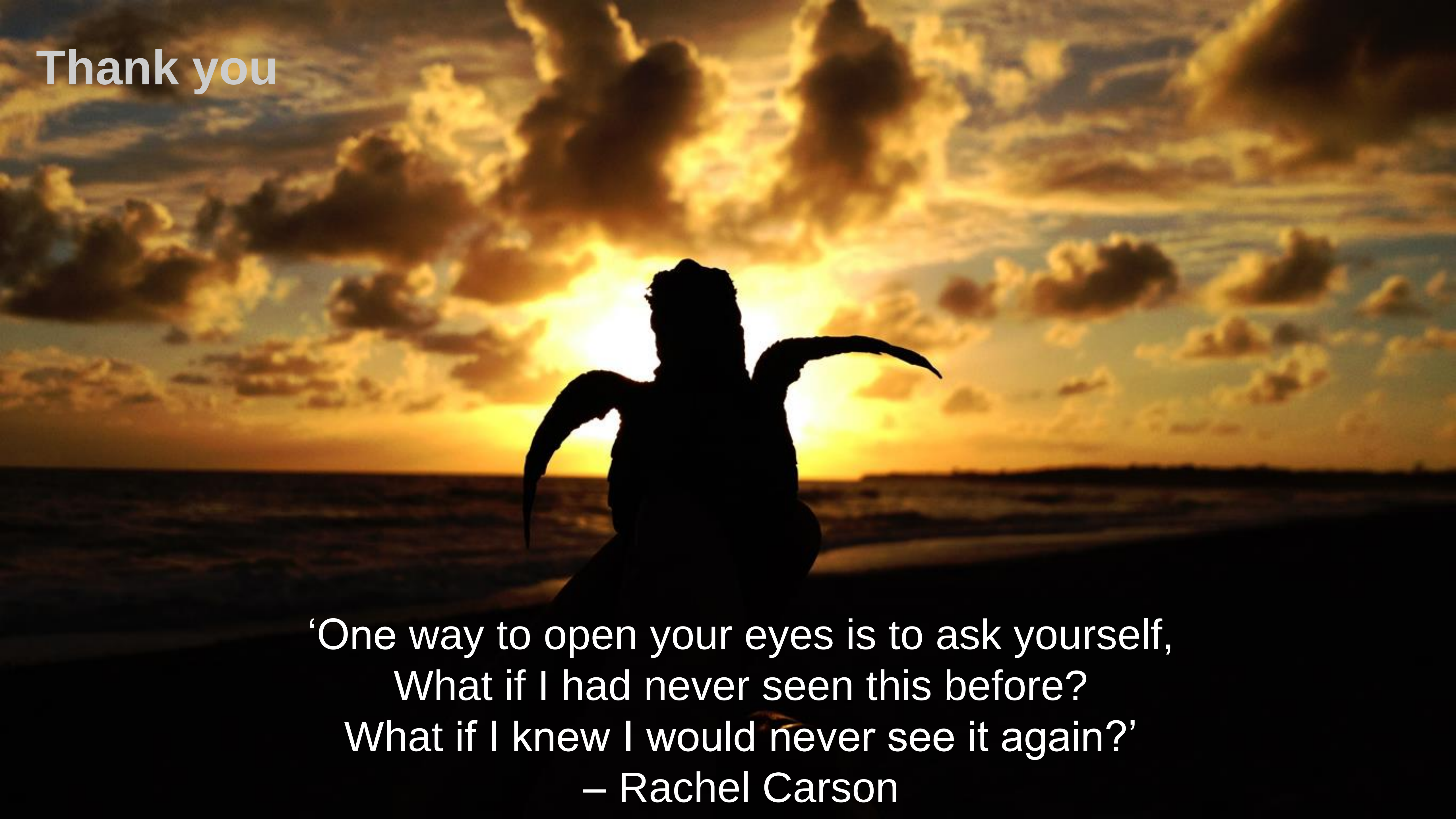

Thank you
‘One way to open your eyes is to ask yourself,
What if I had never seen this before?
What if I knew I would never see it again?’
– Rachel Carson

BACK UP

I) Validation of the QuEChERs method for *in vitro* bioanalytical screening

Green turtle blood

Chemical spiked → **ES**

Modified **QuEChERS method**

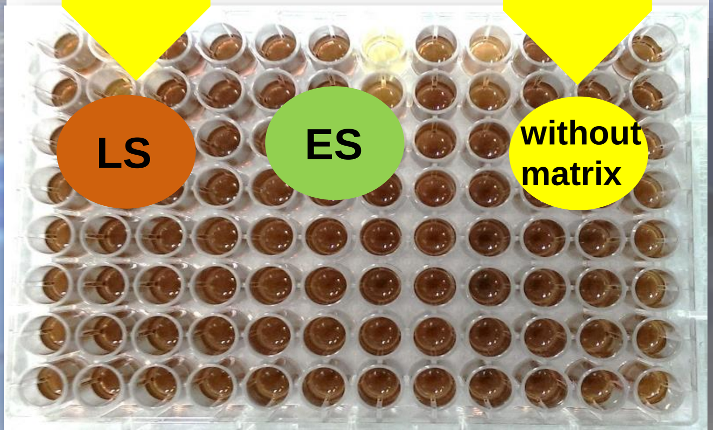
Sodium sulphate column

Recovery ?

Chemical spiked

***In vitro* bioassay**

Chemical spiked



Matrix Interference ?

LS= Late spiked

ES= Early spiked