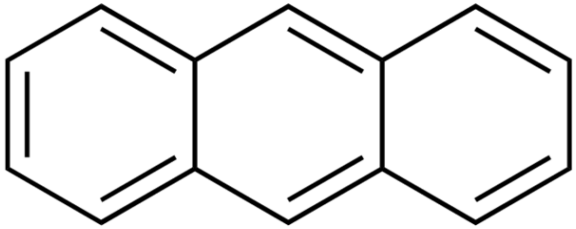
Chemitrace Limited

The development and characterisation of novel immunoassays for Polycyclic Aromatic Hydrocarbon (PAH) biomonitoring

Dr. Lathan Ball - CEO

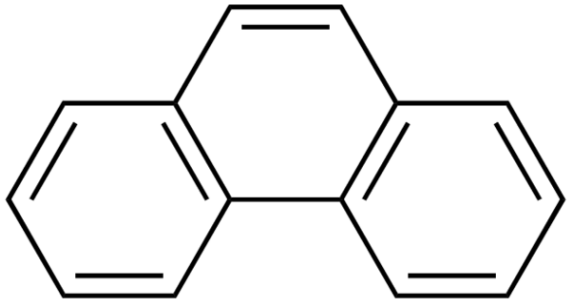
Polycyclic Aromatic Hydrocarbons - PAHs



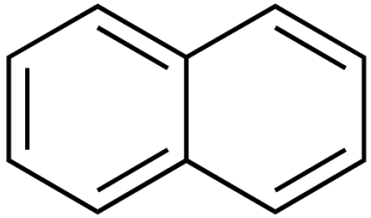
Anthracene

PAHs are a class of more than 100 chemicals produced during the incomplete burning of organic materials.

PAHs contain two or more single or fused aromatic rings



Phenanthrene



Naphthalene

Sources of PAHs

Sources of PAHs can be both natural and anthropogenic.

Natural sources

- forest fires
- oil seeps
- volcanoes
- green plants, fungi, and bacteria

Anthropogenic sources

- oil and petroleum refinery
- electricity generation
- waste incineration
- home heating
- production of coke, carbon black, coal tar, and asphalt
- vehicle exhaust

PAH Health Effects

PAHs have been classified as –

- Carcinogenic
- Mutagenic
- Immunosuppressent

PAH exposure occurs through -

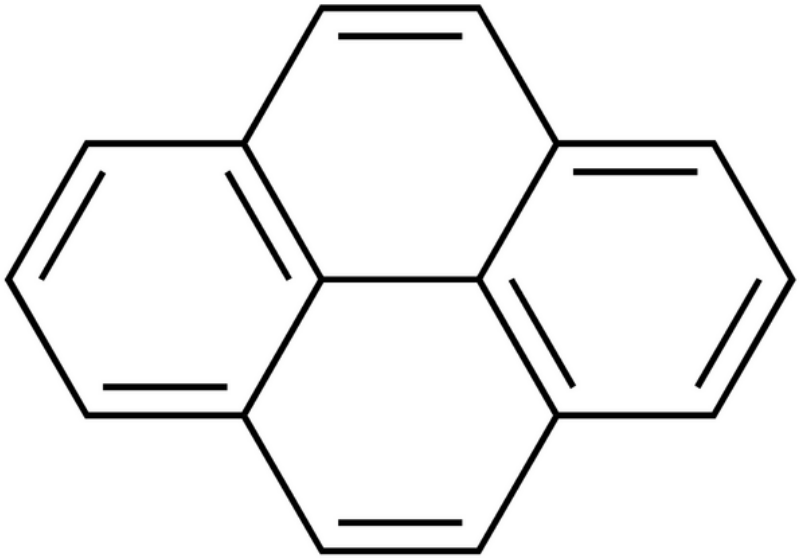
- Inhalation
- Absorption
- Ingestion

PAH exposure occurs on a regular basis

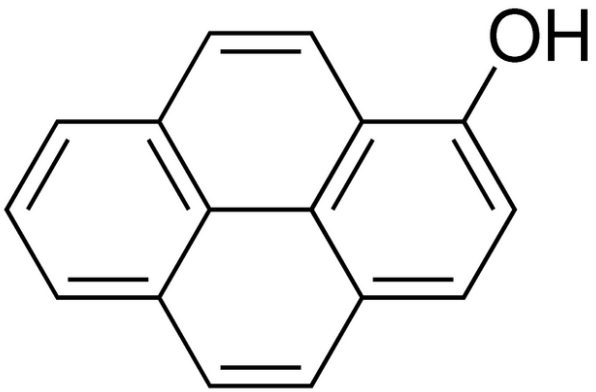
PAH BIOMONITORING

Pyrene is a common component of PAH mixtures.

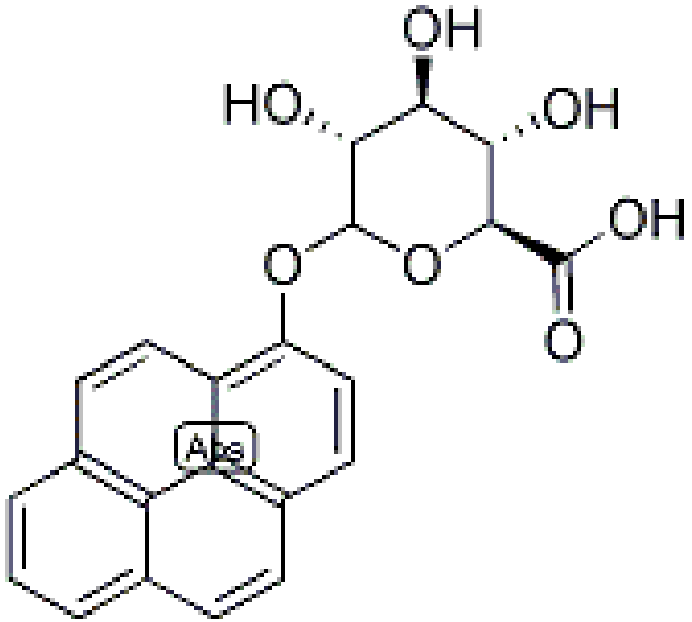
Pyrene



PAH BIOMARKERS



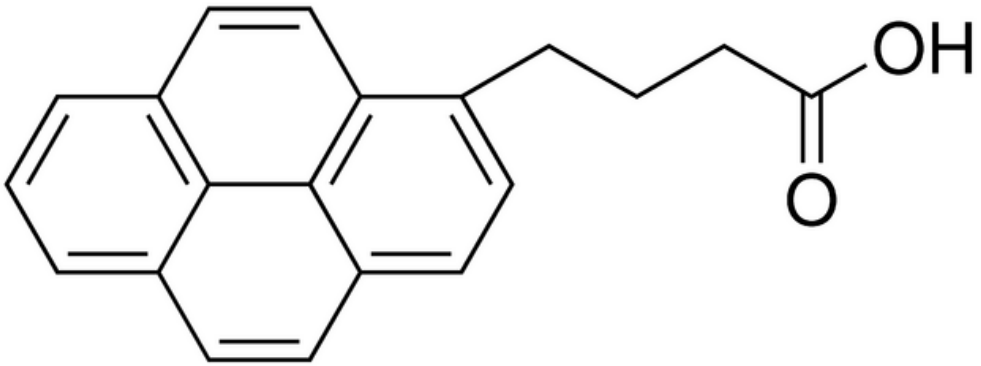
1-Hydroxypyrene



1-Hydroxypyrene Glucuronide

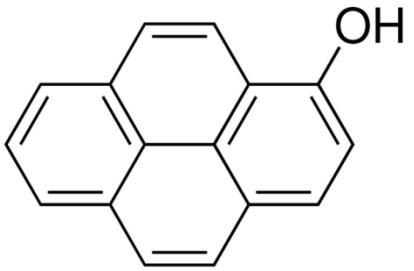
The metabolism of pyrene leads to the excretion of 1-hydroxypyrene and 1-hydroxypyrene glucuronide in urine

ANTIBODY PRODUCTION

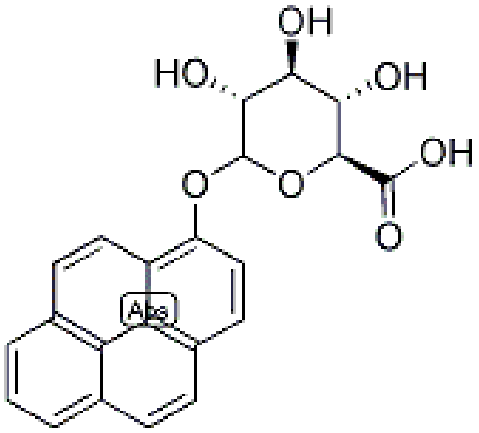


1-Pyrenebutyric

1-Pyrenebutyric acid was coupled to KLH with the cross-linker EDC (*N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide) and the conjugate used to induce antisera to OHPyr and OHPyr Gluc in sheep



1-Hydroxypyrene



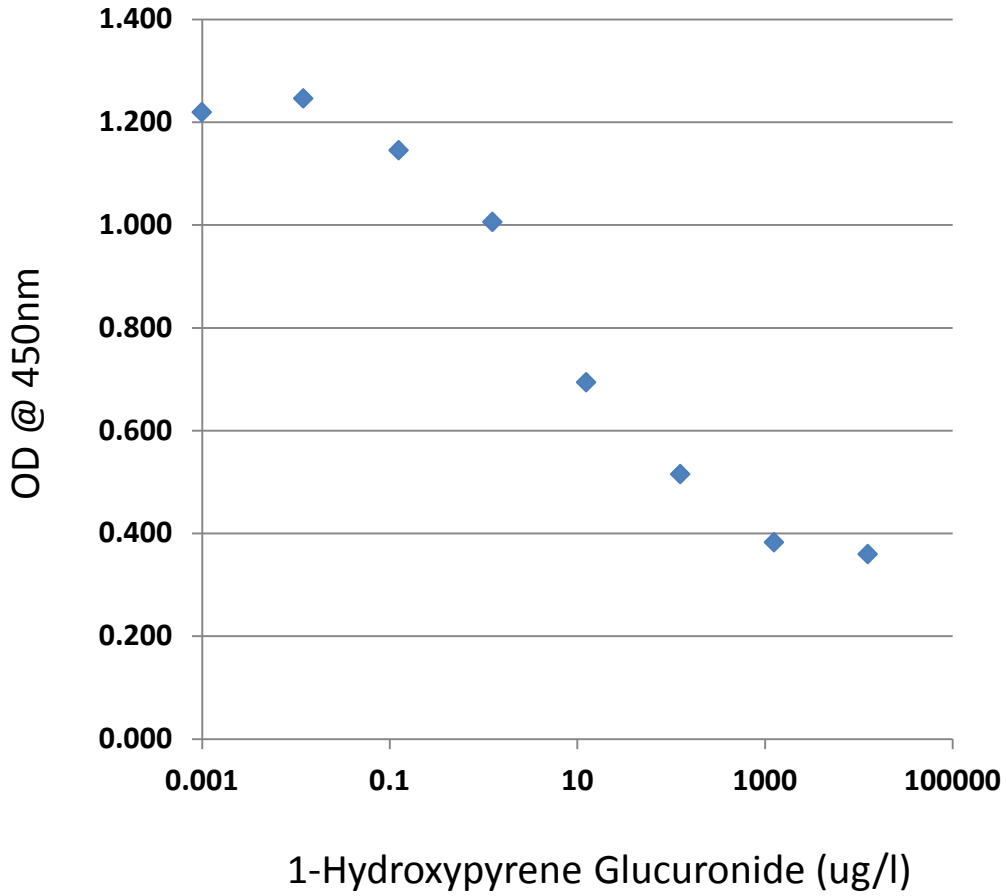
1-Hydroxypyrene Glucuronide

ANTIBODY PRODUCTION - TITRE

Bleed Summary			
Bleed	Sheep 1645 Titre	Sheep 1646 Titre	Sheep 1647 Titre
1	1 in 2000	1 in 4000	1 in 32000
2	1 in 2000	1 in 4000	1 in 35000
3	1 in 3500	1 in 4000	1 in 35000
4	1 in 3000	1 in 4000	1 in 16000
5	1 in 6000	1 in 3000	1 in 12000
6	1 in 3000	1 in 6000	1 in 16000

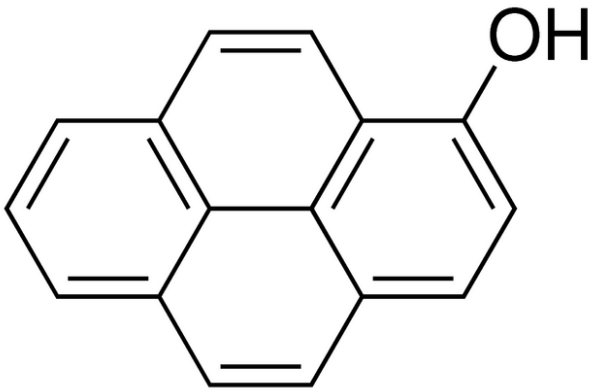
Comparison of Antisera 1645, 1646 and 1647

ANTIBODY PRODUCTION - AFFINITY

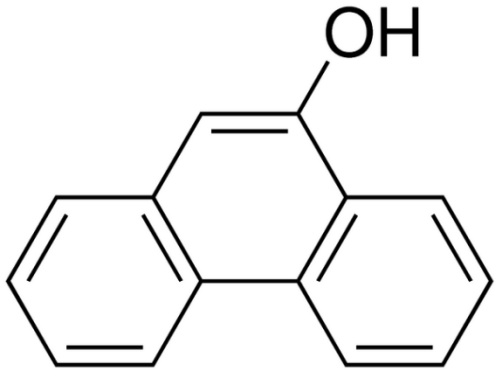


Binding Study with Sheep 1646

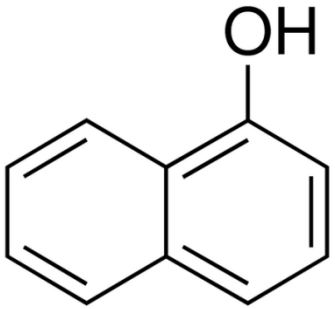
ANTIBODY PRODUCTION - SPECIFICITY



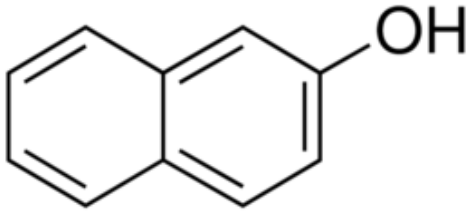
1-Hydroxypyrene



9-Hydroxyphenanthrene/
9-Phenanthrol



1-Naphthol



2-Naphthol

ANTIBODY PRODUCTION - SPECIFICITY

	Cross Reactivity	Urine Background (CDC)
OH-Pyrene	0.5 - 50 nM	90 ng/l (0.4nM)
1-Naphthol	<10 mM	2680 ng/l (18.6 nM)
2-Naphthol	<10 mM	2470 ng/l (17.1 nM)
9-Phenanthrol	0.05 mM	267 ng/l (0.37nm)

Cross-reactivity studies with 1647/6

ASSAY OPTIMISATION

1. **Assay Matrix**

   Variations in sample urine (salt, pH……)

2. **Plate Coating**

   Hapten-protein incorporation ratio

   Optimum incorporation ratio 8:1 biomarker:protein

3. **Affinity Purification**

   Improved assay sensitivity with 1646

   No change with 1647

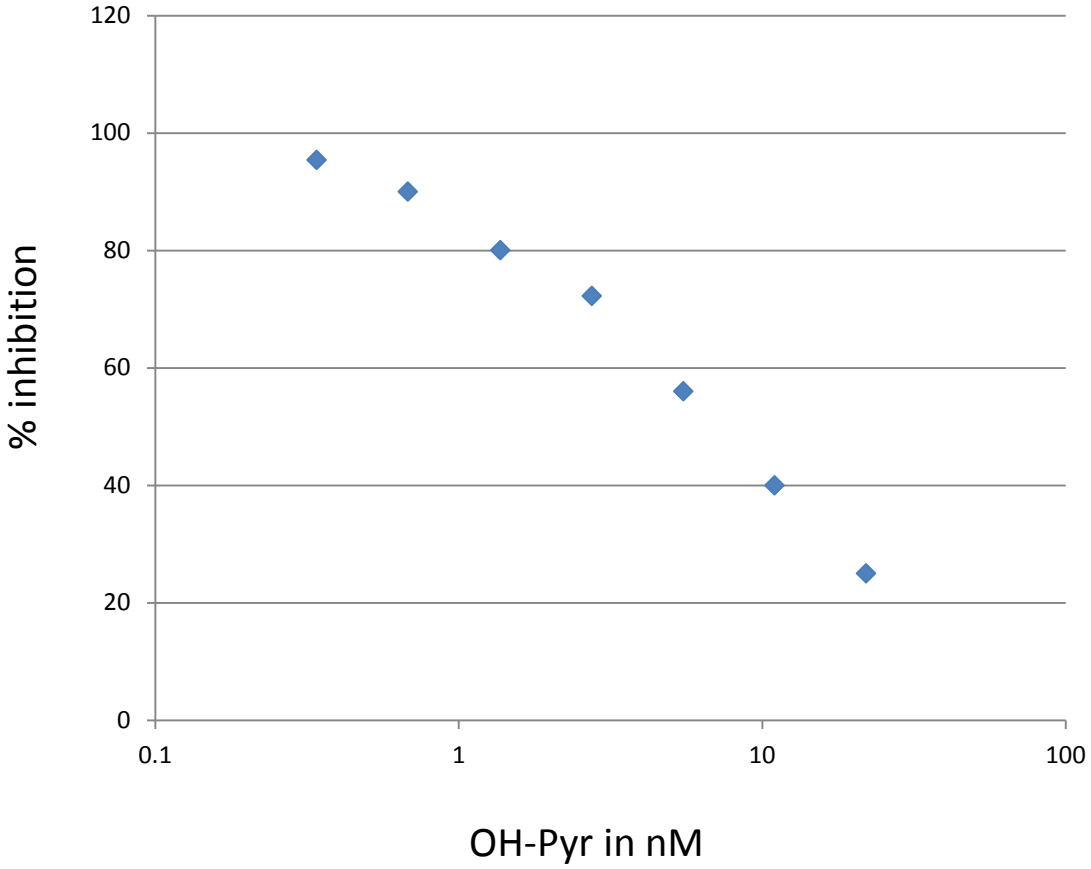
   Very low column binding

ELISA Protocol

Pipette	50ul Standards, Controls and Samples in to wells
Pipette	50ul Assay Diluent in to wells
Pipette	50µL of Anti-S-PMA 1° ab. into all wells.
Mix. Incubate	at room temperature for **120 minutes**.
Wash wells	three times with Wash Solution.
Pipette	100µL of Anti-Sheep-HRP into all wells.
Mix. Incubate	at room temperature for **30 minutes**.
Wash wells	four times with Wash Solution.
Pipette	100µL TMB Substrate reagent into all wells.
Mix. Incubate	at room temperature for **30 minutes**.
Pipette	100µL Acid Stop Solution 1 into all wells.
Mix. Read	wells at **450nm** wavelength.
Calculate	OH-Pyr results for all Controls/Samples.

ASSAY DEVELOPMENT

Calibration plot developed with 1647/6



Limit of Detection = 0.5nM

Assay completed in less than 4 hours and allowed 40 samples to be determined in duplicate

ASSAY REPRODUCABILITY

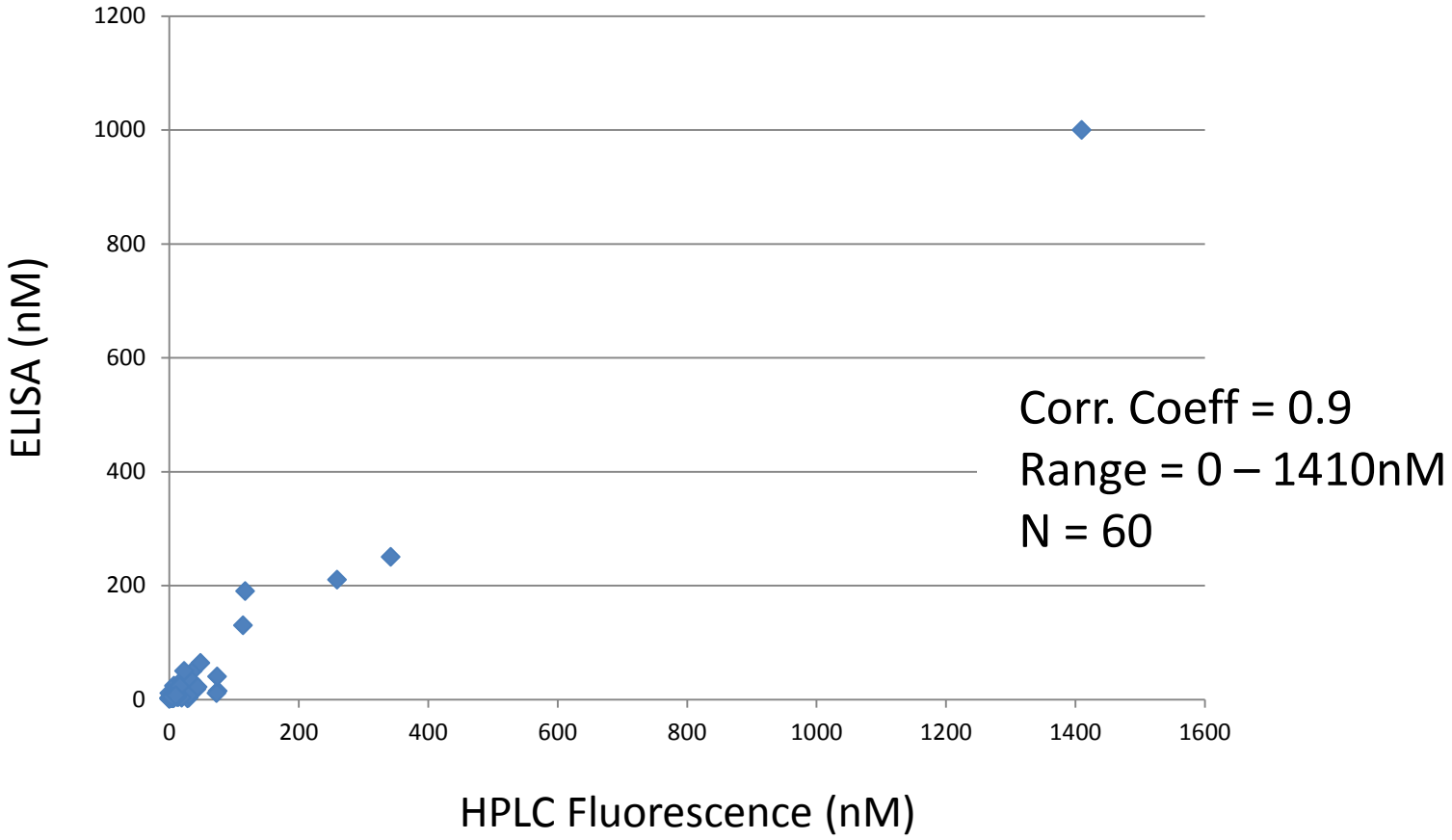
The assay robust and reproducible -

	With-in Assay Variation		Between Assay Variation		
Mean	17.8	4.9	**Mean**	28	4.5
SD	0.8	0.4	**SD**	2.6	1.0
CV	4.7%	8.5%	**CV**	9.6%	22.2%
N	4	4	**N**	4	4

ASSAY VALIDATION

- Urine samples collected from potentially exposed workers (5 week period)
- Samples split for ELISA and HPLC Fluorescence (UK-HSL) measurement
- HPLC samples determined immediately
- Samples stored frozen (-20^{0}C) until determination by ELISA.

ASSAY VALIDATION



Determination of OH-Pyr in urine samples from occupationally exposed workers – a comparison between ELISA and a HPLC Fluorescence method

Point of Care Testing

Allows “Real-time” Analysis

- Monitor immediate impact of Good Working Practice
- Allow immediate removal of potentially exposed workers
- Provides on the job reassurance to workers

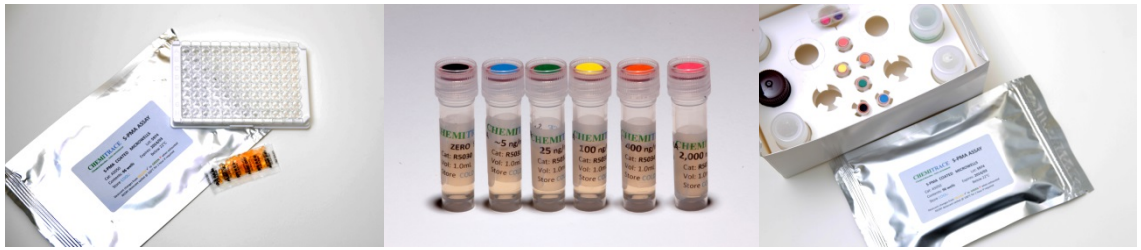
Low Cost

- Eliminate negative samples
- Selection of positive samples for further analysis
- Encourages increased testing

Chemitrace Limited

Simple and Cost-effective Polycyclic Aromatic Hydrocarbon Biomonitoring

THANK YOU



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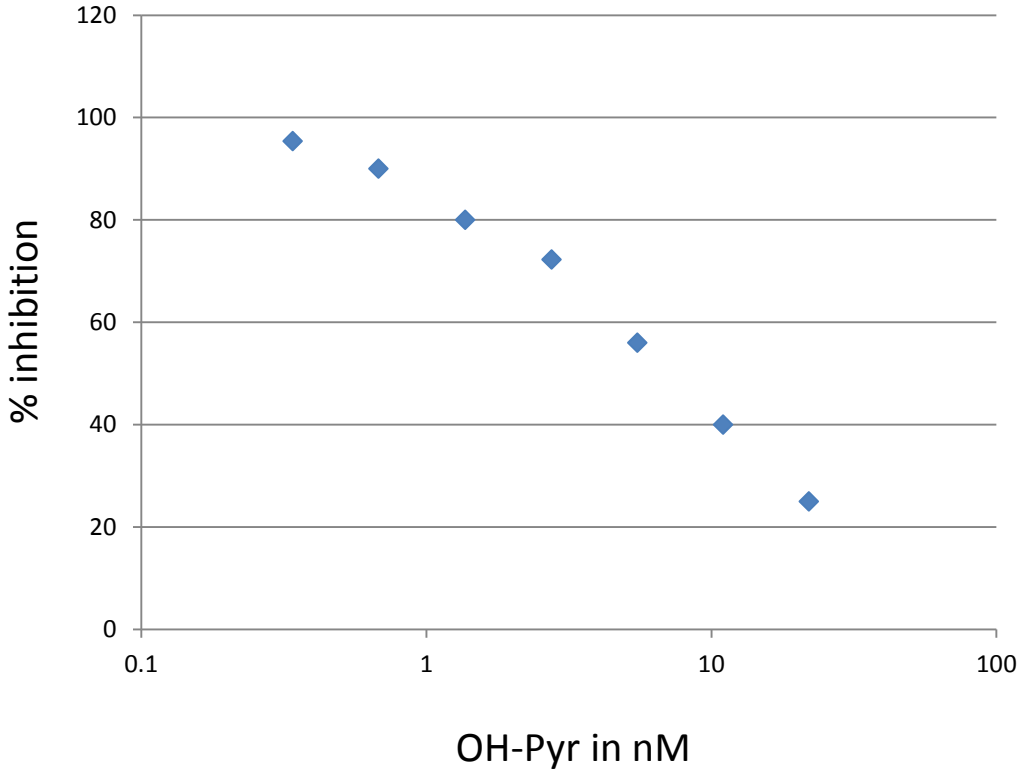
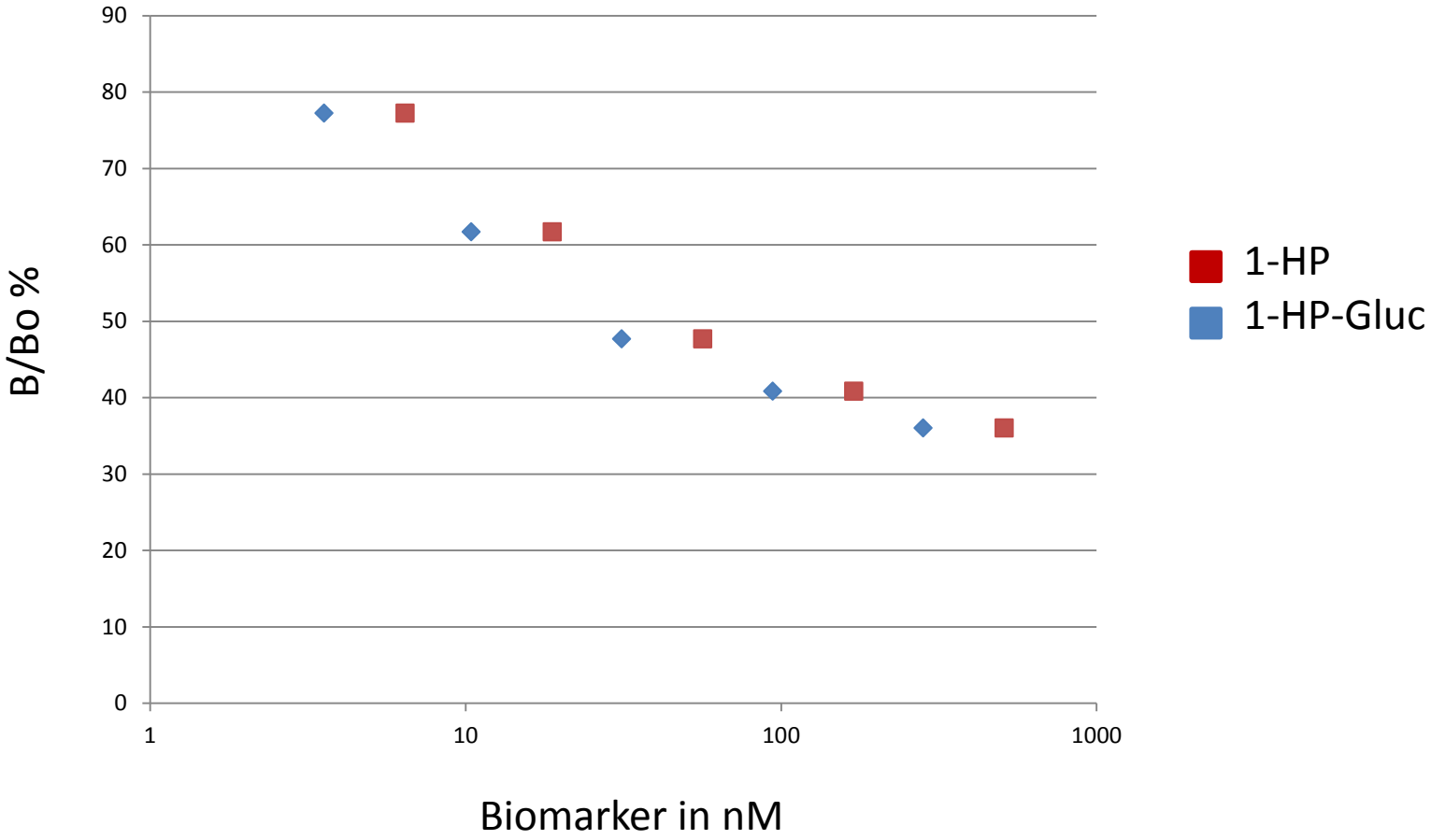


3800 samples	
HPLC - LOD 1nM	
29%	ND
58%	>3nM
37%	<10nM
Max	2800nM

ANTIBODY PRODUCTION - AFFINITY



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