ESR 11: Transcriptomic and metabolomic approaches to investigate molecular responses of human exposure to flame retardants

Training and progress to date

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Stockholm, Sweden 25-04-2012

INFLAME-WP3

- **Research Objectives of WP3**: to advance our knowledge of the effects of human exposure to FRs.

- ESR10: Mechanistic profiling of flame FRs in general systemic stress and endocrine disruption
- **ESR11: A transcriptomic and metabolomic approach to biomarkers of exposure and effect.**
- ESR12: The role of FRs in indoor dust in potentiating or facilitating allergic responses to inhaled allergens

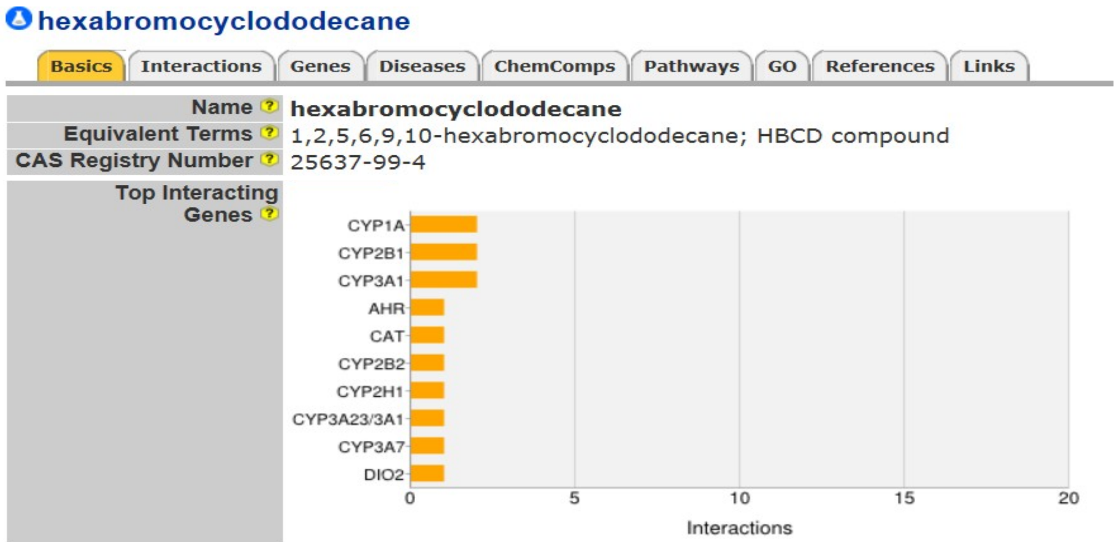
- **Project objectives of ESR11:** focus on the **molecular mechanisms** via which **FRs exert toxicity**, using **metabolomic** (FT-ICR mass spectrometry and ^{1}H NMR spectroscopy) and **transcriptomic** (Agilent microarrays) approaches in ***in vitro (mainly) and in vivo*** models.

Outline of project

Flame retardants	HBCD (Hexabromocyclododecane), (probably including α-, β-, γ- HBCD) TBBPA (Tetrabromobisphenol A); BDE-47(brominated diphenyl ethers); TBB (2-ethylhexyl-2,3,4,5-tetrabromobenzoate); TBPH (bis(2-ethylhexyl)-tetrabromophthalate)…			
Models	*In vitro* models		*In vivo* models	
	A549 cells; (adenocarcinomic human alveolar basal epithelial cells) BEAS-2B cells; HepG2 cells; …		asthma mice: lung, liver, blood, urine ?… in collaboration with UvA (ESR12)	
Cytotoxicity screening	MTT Assay; LDH (lactate dehydrogenase) release assay; *ROS; cell apoptosis assay*			
Approaches	**Transcriptomics**		**Metabolomics**	
Methodologies	Agilent gene expression Microarray	Real-time PCR	FT ICR-MS (Neg. vs Pos.)	NMR
Bioinformatics	Gene Ontology; Pathway analysis and mechanism interpretation			
Further analyses	Lipidomics; Targeted analyses using LC-MS in collaboration with UA (ESR10);			

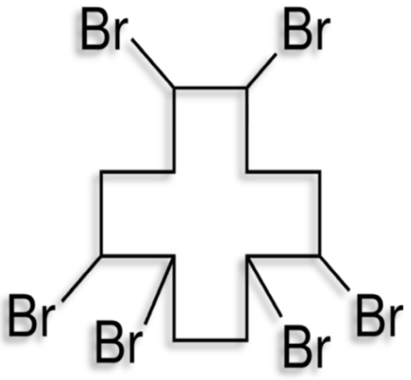
Sub-project 1 of ESR11 project

Transcriptomic and metabolomic approaches to investigate molecular responses of A549 cells to HBCD



hexabromocyclododecane

Name	hexabromocyclododecane
Equivalent Terms	1,2,5,6,9,10-hexabromocyclododecane; HBCD compound
CAS Registry Number	25637-99-4
Top Interacting Genes	



(From Comparative Toxicogenomics Database: http://ctdbase.org/detail.go?type=chem&acc=C089796)

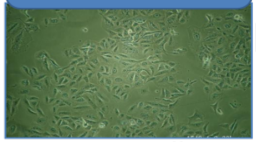
Aims:

- To optimise FT-ICR MS and NMR based metabolomic approaches using A549 cells exposed to HBCD
- To study transcriptomic and metabolic alterations in A549 cells upon HBCD treatment.

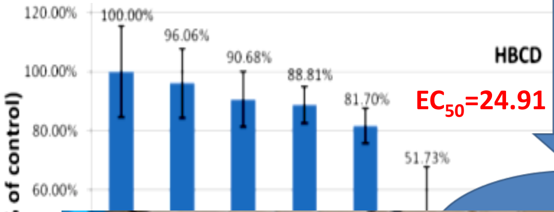
Experimental design

Media: DMEM supplemented with 2 mM glutamine, 10% fetal bovine serum (FBS), and 100 U/mL penicillin/streptomycin

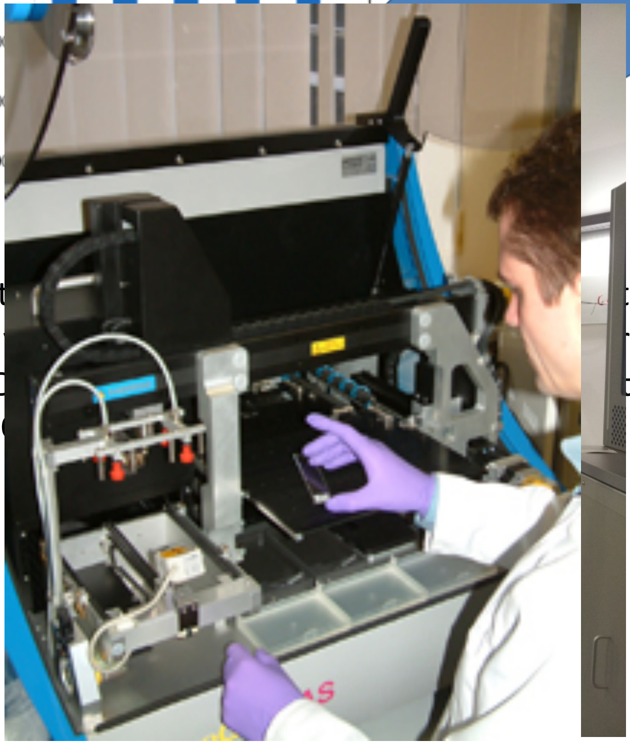
Cell culture



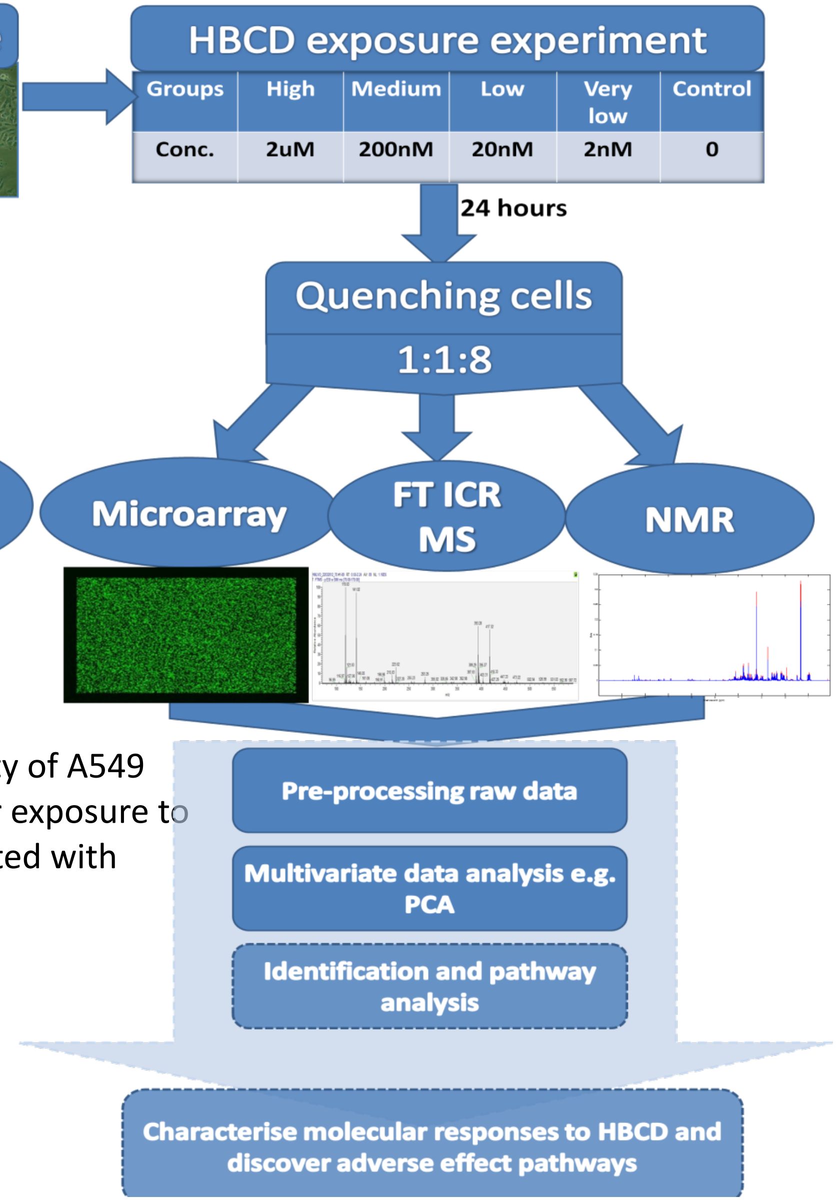
A549 cells



EC_{50}=24.91



Cytot … y of A549
cells … exposure to
HBCD … ted with
DMS…



HBCD exposure experiment

Groups	High	Medium	Low	Very low	Control
Conc.	2uM	200nM	20nM	2nM	0

24 hours

Quenching cells

1:1:8

Microarray

FT ICR MS

NMR

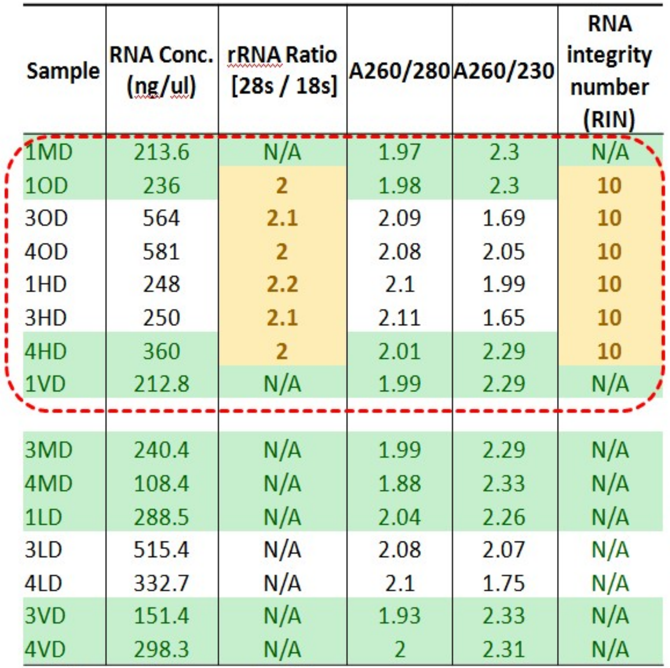
Pre-processing raw data

Multivariate data analysis e.g. PCA

Identification and pathway analysis

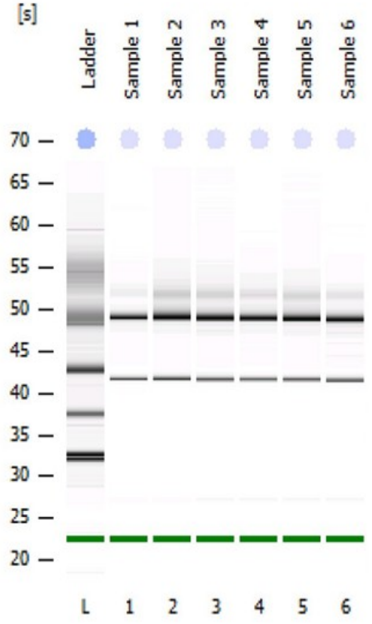
Characterise molecular responses to HBCD and discover adverse effect pathways

Pre-test of microarray in A549 cells study



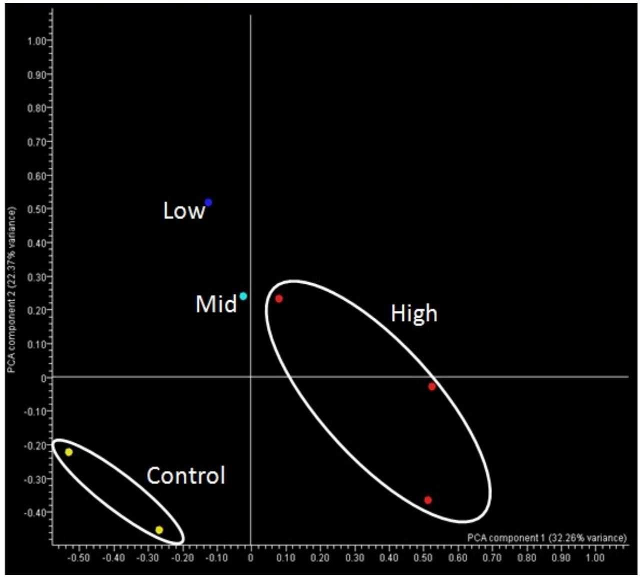
Sample	RNA Conc. (ng/ul)	rRNA Ratio [28s / 18s]	A260/280	A260/230	RNA integrity number (RIN)
1MD	213.6	N/A	1.97	2.3	N/A
1OD	236	2	1.98	2.3	10
3OD	564	2.1	2.09	1.69	10
4OD	581	2	2.08	2.05	10
1HD	248	2.2	2.1	1.99	10
3HD	250	2.1	2.11	1.65	10
4HD	360	2	2.01	2.29	10
1VD	212.8	N/A	1.99	2.29	N/A
3MD	240.4	N/A	1.99	2.29	N/A
4MD	108.4	N/A	1.88	2.33	N/A
1LD	288.5	N/A	2.04	2.26	N/A
3LD	515.4	N/A	2.08	2.07	N/A
4LD	332.7	N/A	2.1	1.75	N/A
3VD	151.4	N/A	1.93	2.33	N/A
4VD	298.3	N/A	2	2.31	N/A

Qiagen Rneasy mini kit
+ Ethanol/Acetate precipitate



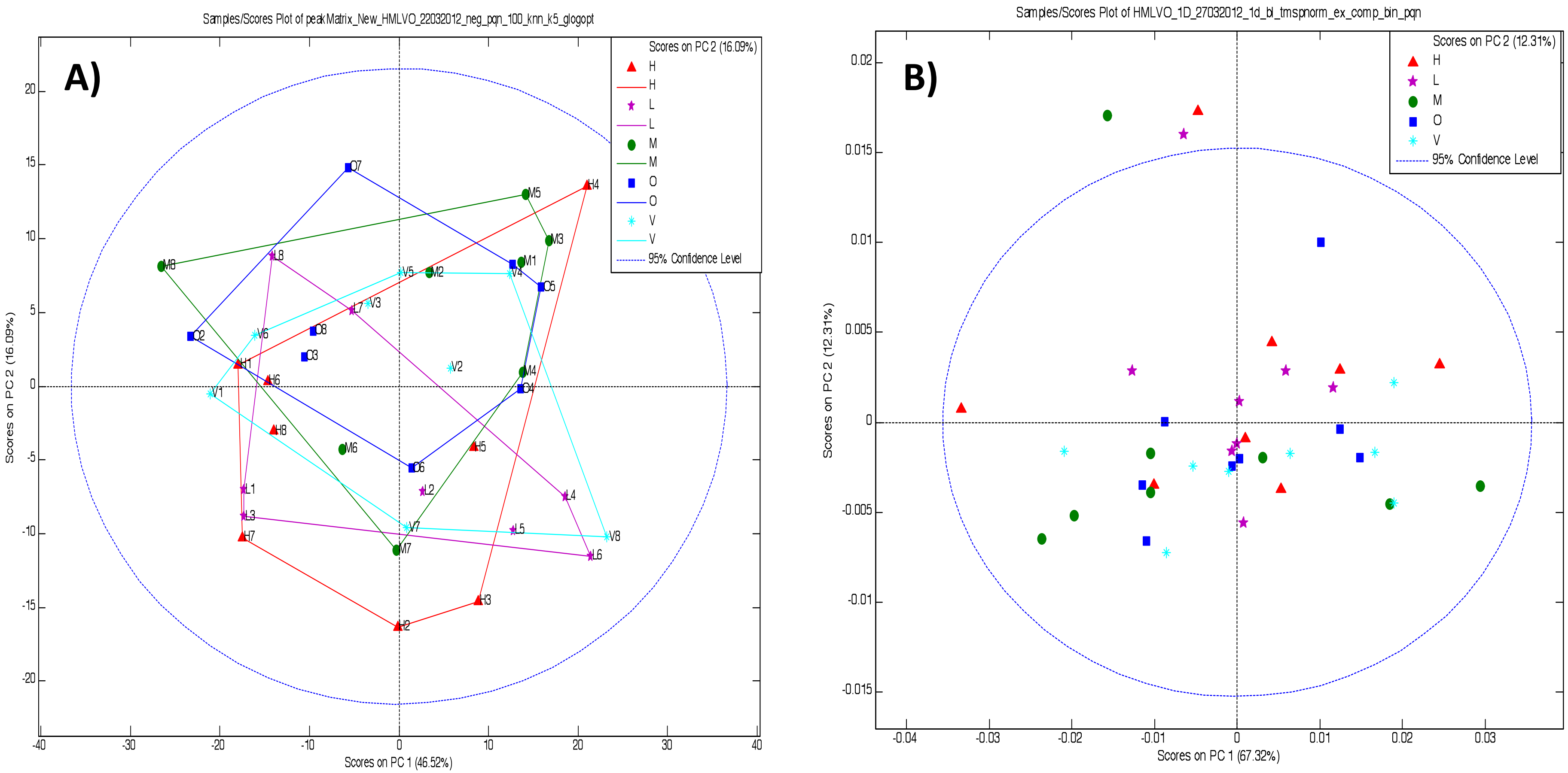
Electrophoresis File Run Summary by Agilent 2100 Bioanalyzer

RNA quality control



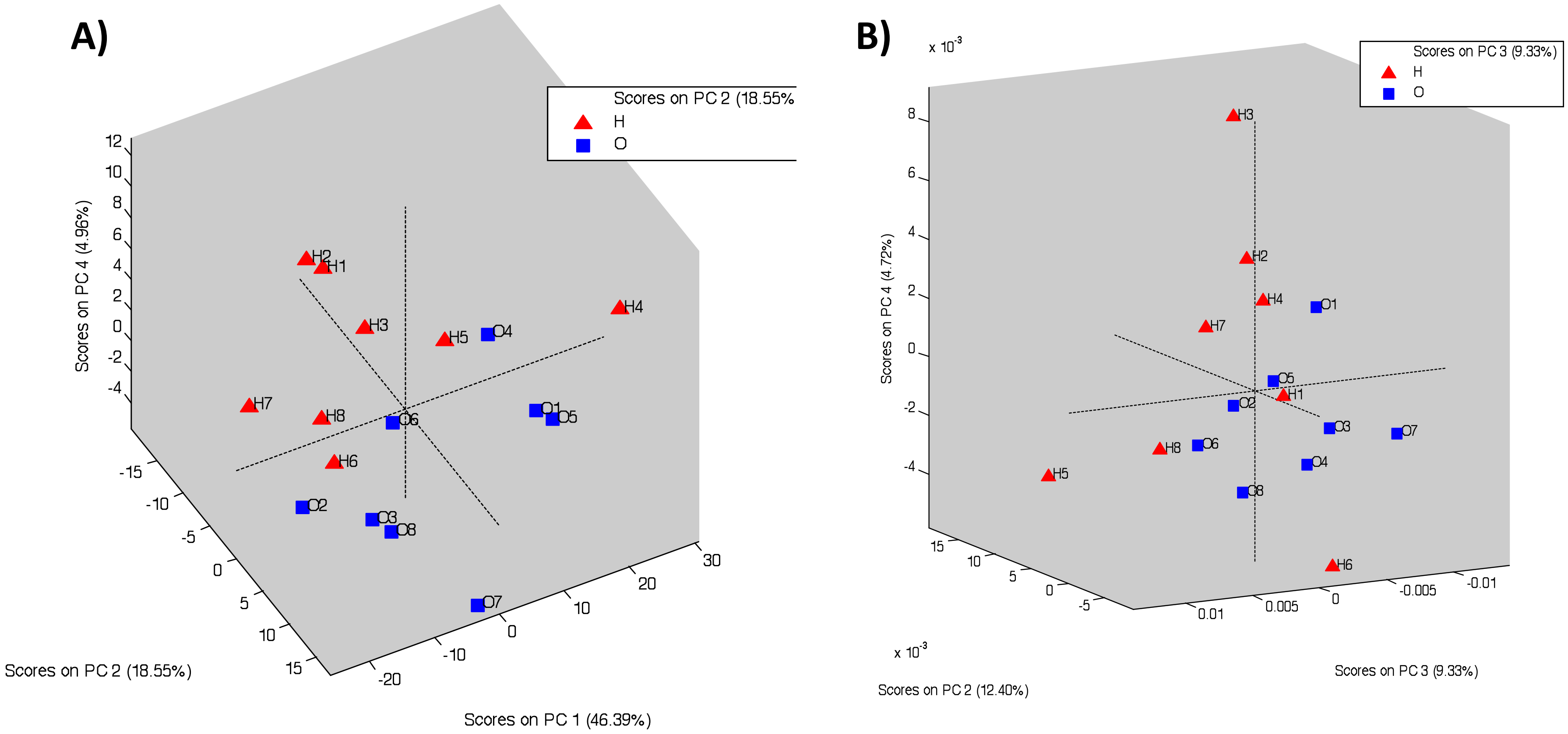
PCA scores plot of **microarray data** (passing quality cutoffs) in the pre-test of A549 cells exposed to HBCD (High dose n=3, control group n=2)

PCA analysis of MS and NMR data (1)



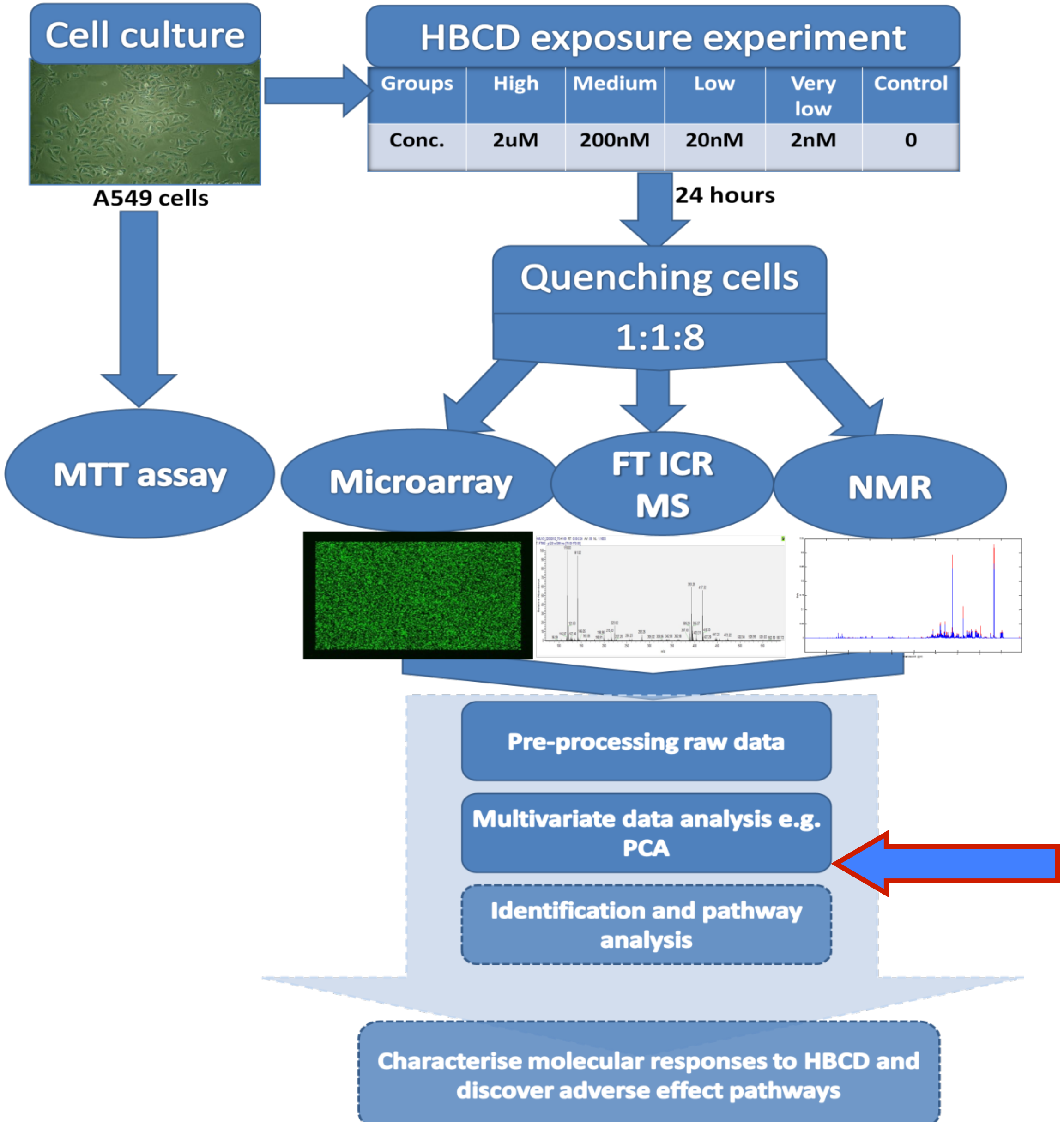
PCA scores plots of polar extracts of A549 cells following HBCD exposure (n=8), from negative ion analysis of FT-ICR mass spectra (A) and 1D NMR mass spectra (B).

PCA analysis of MS and NMR data (2)



PCA scores plots of A549 cells exposed to HBCD (High dose vs. Control) from analysis of MS spectra (A) and 1D NMR spectra (B).

Transcriptomic and metabolomic approaches to investigate molecular responses of A549 cells to HBCD



Cell culture

A549 cells

HBCD exposure experiment

Groups	High	Medium	Low	Very low	Control
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1:1:8

MTT assay

Microarray

FT ICR MS

NMR

Pre-processing raw data

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Main progress to date

- ✓ Screening toxicity of 3FRs in A549 cells (MTT assay)
- ✓ Optimise standard protocols for quenching cells and metabolites extraction in A549 cells study
- ✓ Optimise standard protocols for RNA and pre-test in oligo-microarray using A549 cells exposed to HBCD
- ✓ Practical skills training in operation of FT-ICR MS, NMR, Agilent microarray experiments
- ✓ Practical skills training in data collection and analysis using Matlab, Topspin, etc.

……

- ✓ 3 months report and 9 months report submitted to Biosciences Graduate Research School in Birmingham
- ✓ a poster presentation on the 6th POPs Network Conference, 17th-18th April, Birmingham

Next 6 months plan

(Apr.2011- Oct.2012)

- Continue training in transcriptomics and metabolomics (focusing on data pre-processing , statistical analysis, pathway analysis……)
- Completion of omics study of A549 cells exposure to HBCD mixture
- Omics studies of A549 cells exposed to alpha-, beta- and gamma -HBCD

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Acknowledgment

MARIE CURIE ACTIONS INFLAME

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THANK YOU!

TACK!