Linking the external and internal exposome for causal environment and health associations

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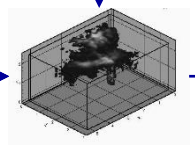
http://www.enve-lab.eu

The connectivity paradigm

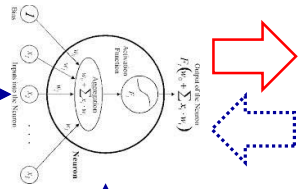
Environmental sensors

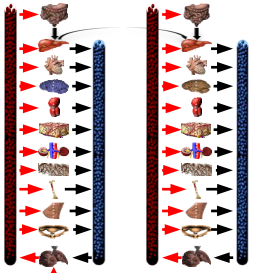
Remote sensing

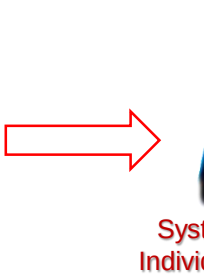


Environmental modeling / data management

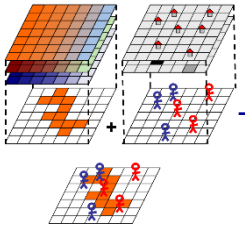
Exposure modelling







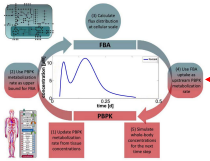
Environmental analysis



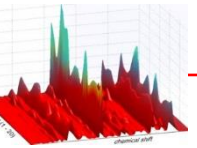
Agent based modelling

Human biosampling

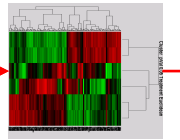
Ubiquitous personal sensors



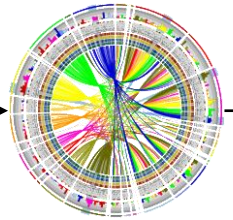
Fluxomics (dynamic flux balance analysis)



Metabolomics



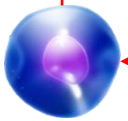
Transcriptomics



EWAS

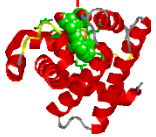
Systems Effects / Individual exposome

Community Effects / Cohorts



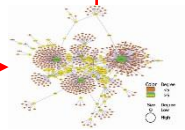
Cellular/tissue effects

Epigetics



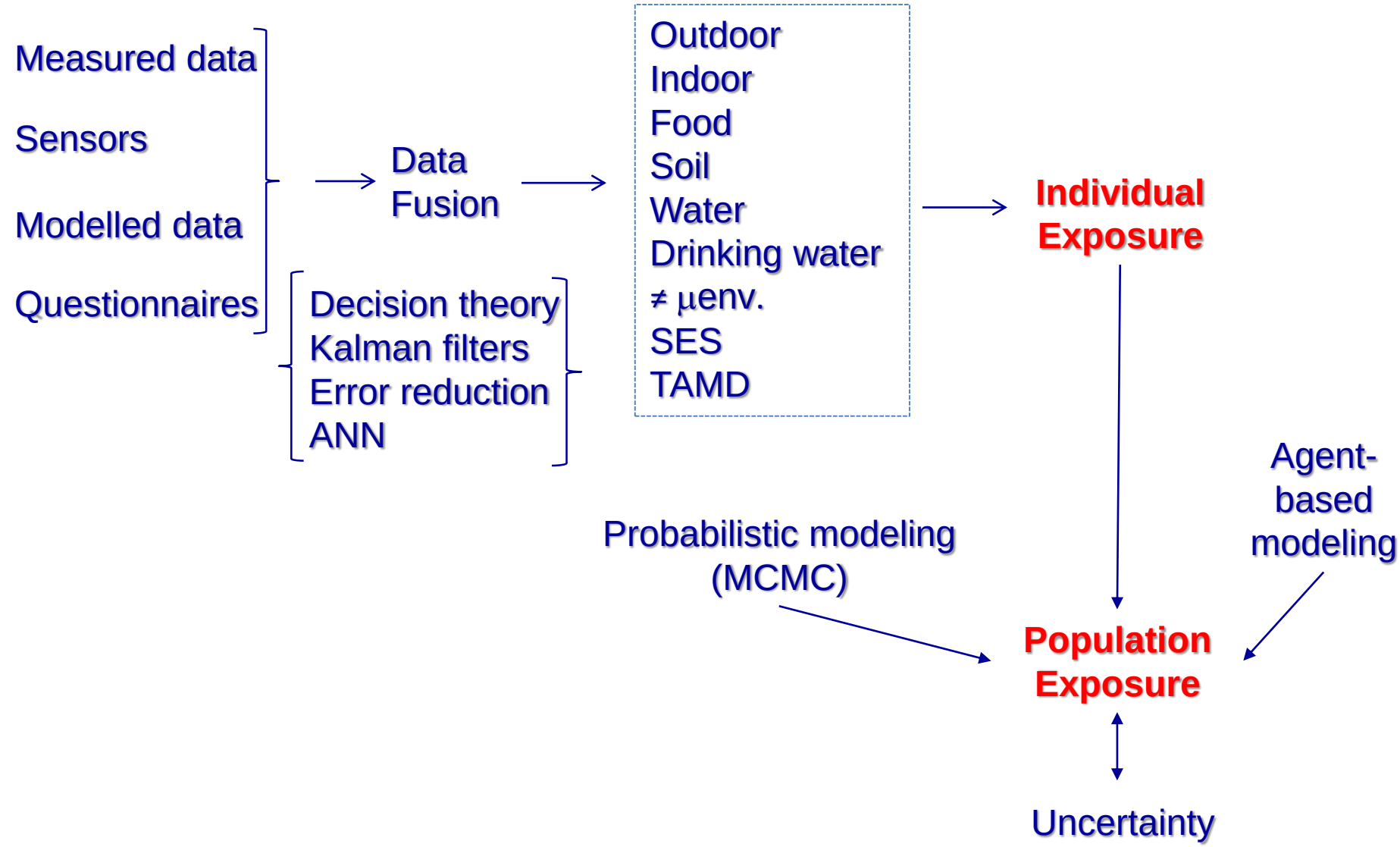
Molecular initiating events

Bioinformatics / systems biology



Pathway analysis

External Exposure workflow



An individual's space-time activity model

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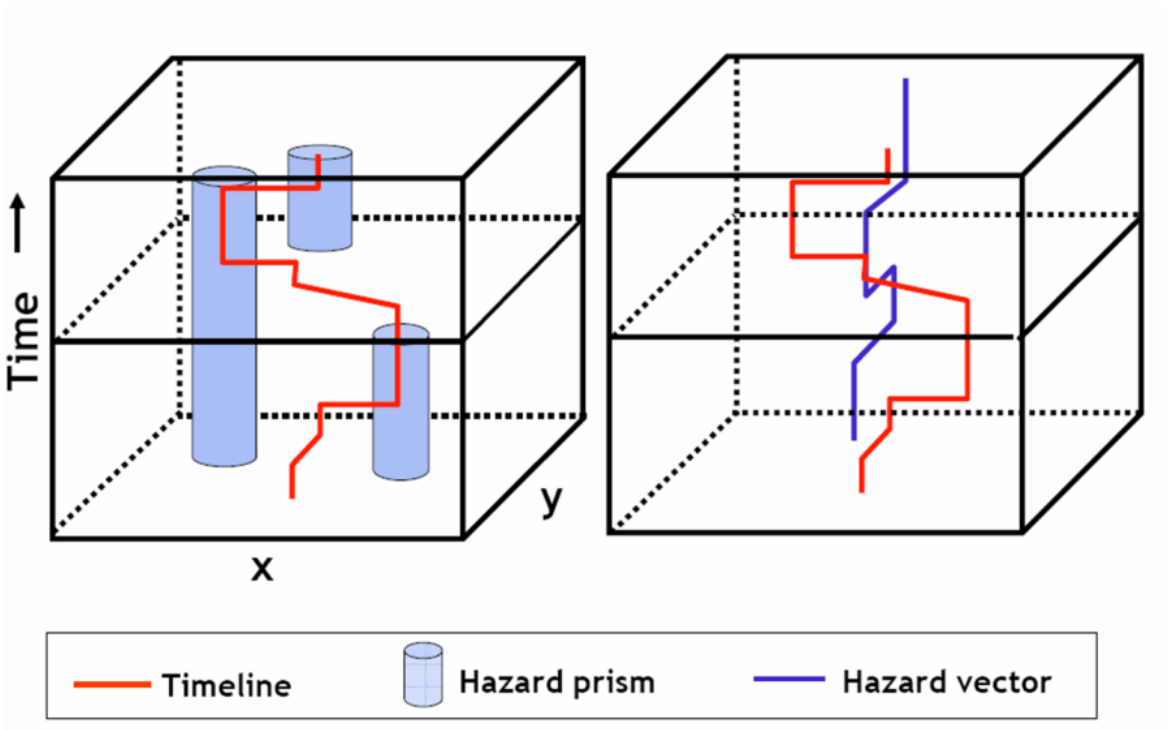
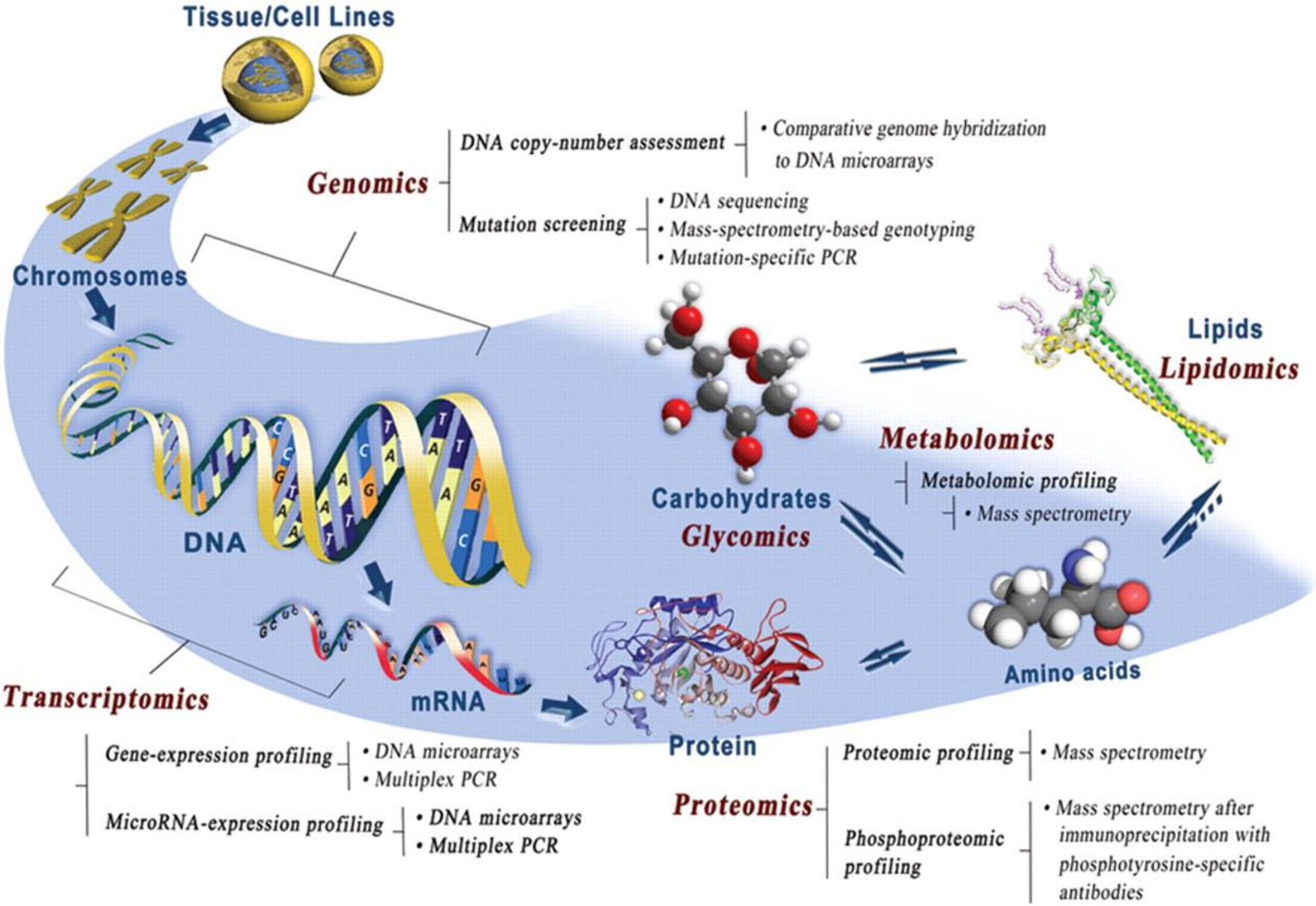
- Time-Geography provides a coherent ontological framework within which to explore spatio-temporal behaviour of individuals and their interaction with the environment

- By analysing and modelling these trajectories we can determine an individual's behaviour in terms of time geography, and thus begin to estimate individual level exposure



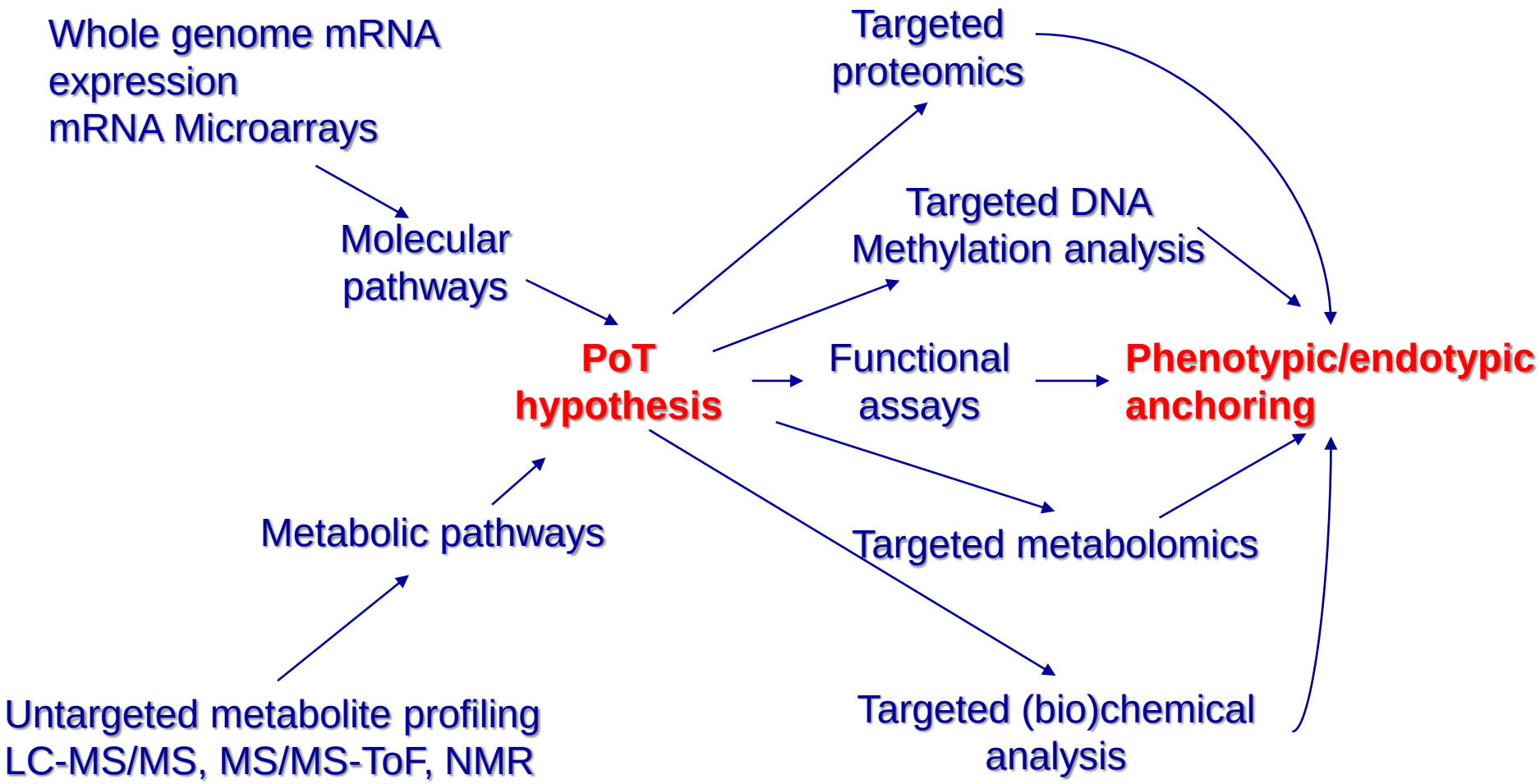
Adapted from Hägerstrand (1970)

High dimensional biology

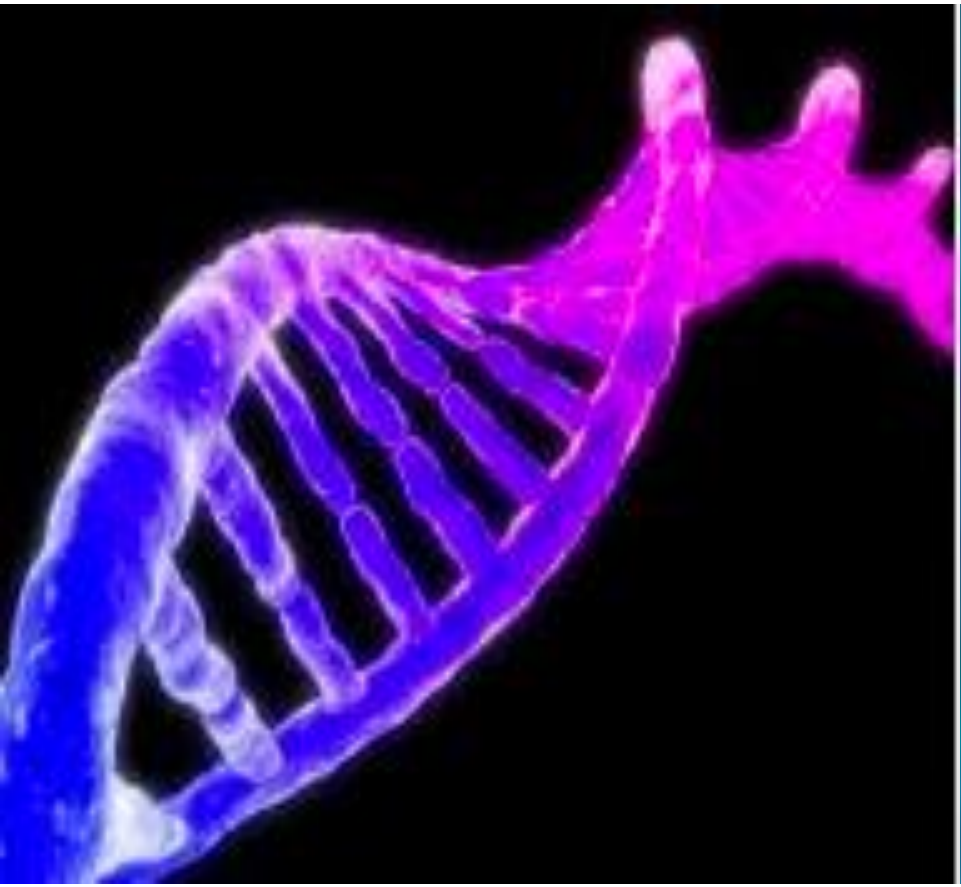


Exposure biology workflow

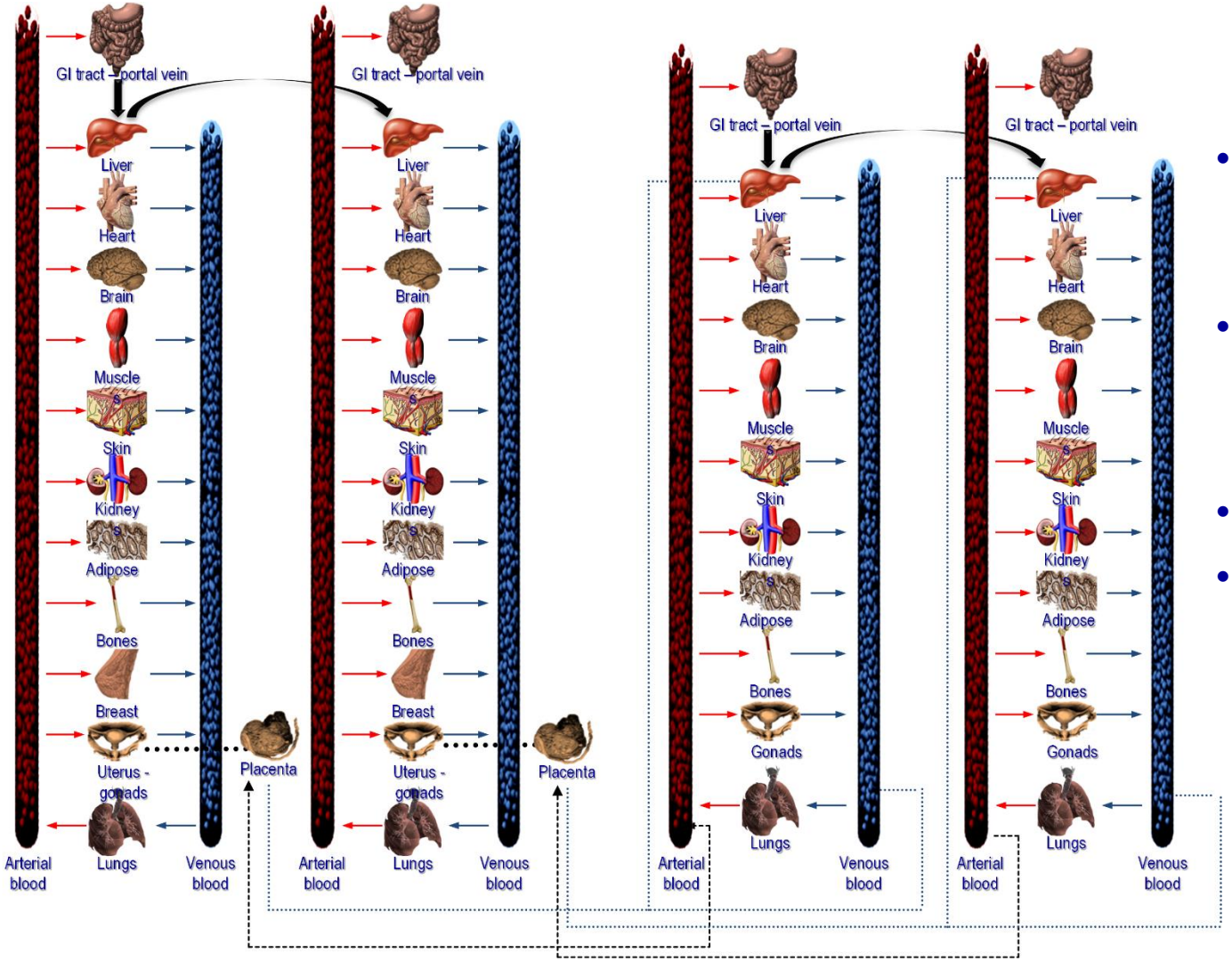
Rendering high dimension biology operational



The internal exposome



Concept of generic lifetime PBBK model



- Detailed description of compartments and tissue composition
- Lifetime evolving parameters
  - Organ volumes
  - Blood flows
  - Age-dependent clearance
- Mother – Fetus interaction
- Breast feeding

Expanding the chemical space – use of QSARs

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According to Abraham's solvation equation, a biological property *SP is described by the following equation*

$$\log SP = c + r \cdot R_2 + s \cdot \pi_2^{\mathrm{H}} + a \cdot \Sigma\alpha_2^{\mathrm{H}} + b \cdot \Sigma\beta_2^{\mathrm{H}} + v \cdot \log V_x$$

Where:

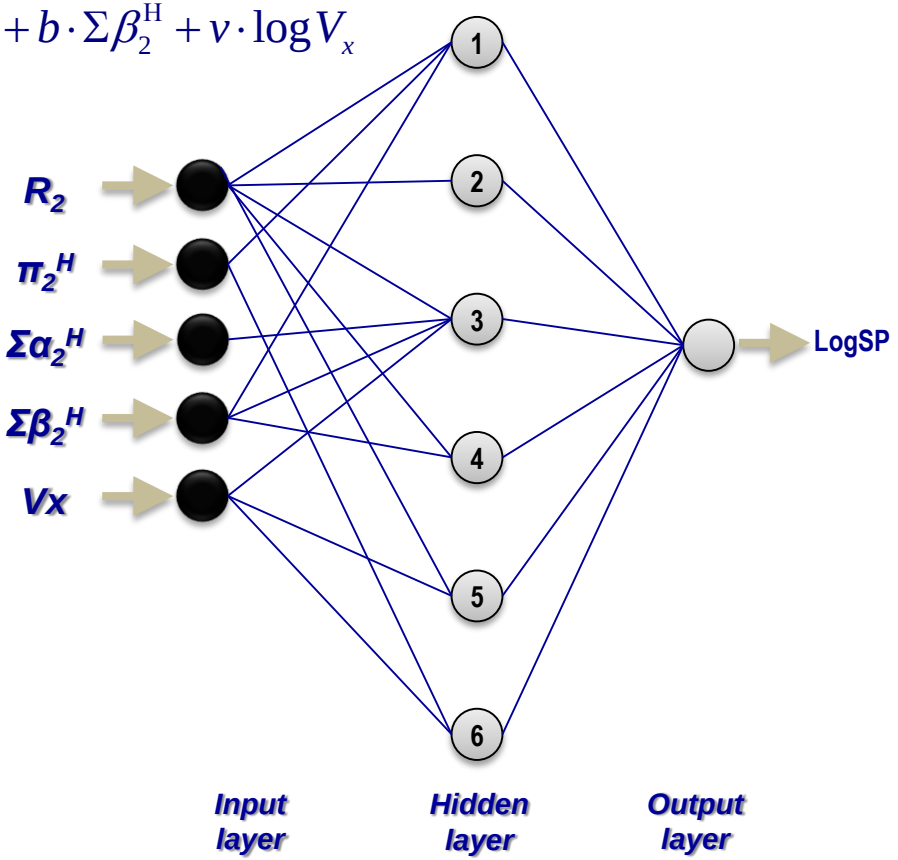
R_2 is an excess molar refraction that can be determined simply from a knowledge of the compound refractive index

π_2^H is the compound dipolarity/polarizability

$\Sigma\alpha_2^H$ is the solute effective or summation hydrogen-bond acidity

$\Sigma\beta_2^H$ is the solute effective or summation hydrogen-bond basicity

V_x is the McGowan characteristic volume



Coupling biokinetics and metabolic regulation

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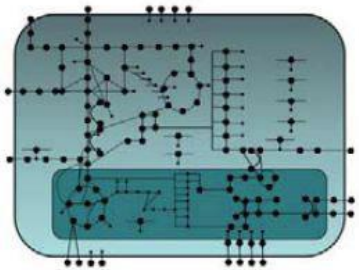
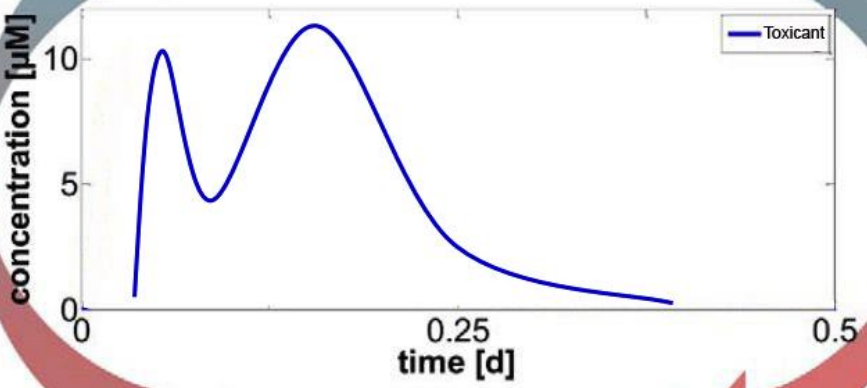


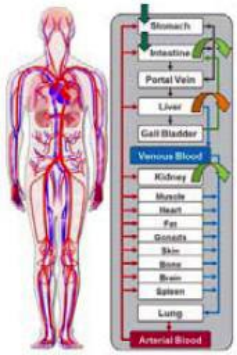
(3) Calculate flux distribution at cellular scale

FBA



(2) Use PBPK metabolization rate as upper bound for FBA

(4) Use FBA uptake as upstream PBPK metabolization rate



PBPK

(1) Update PBPK metabolization rate from tissue concentrations

(5) Simulate whole-body concentrations for the next time step

HBM data assimilation

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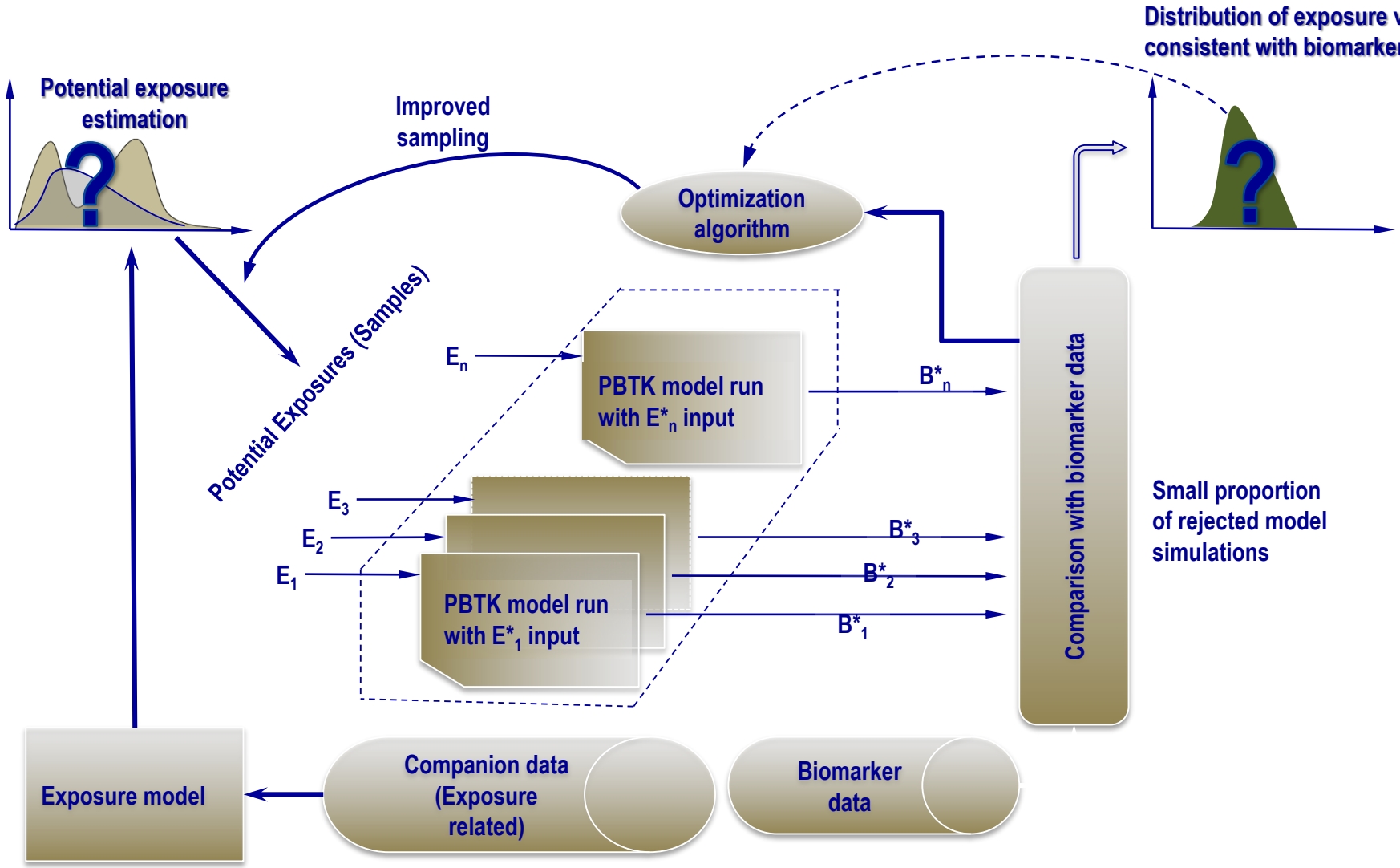
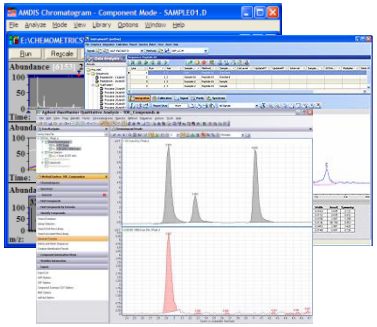
Optimal scheme for exposure reconstruction



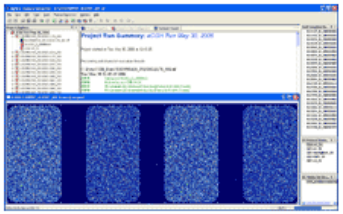
Exposure Biology Workflow

LC/MS
GC/MS



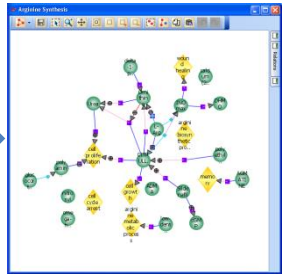
MassHunter Qual/Quant
ChemStation AMDIS

Microarrays



Feature Extraction

GeneSpring Platform



Biological
Pathways

NGS

Alignment to Reference Genome

BridgeDb for multi-omics pathway mapping

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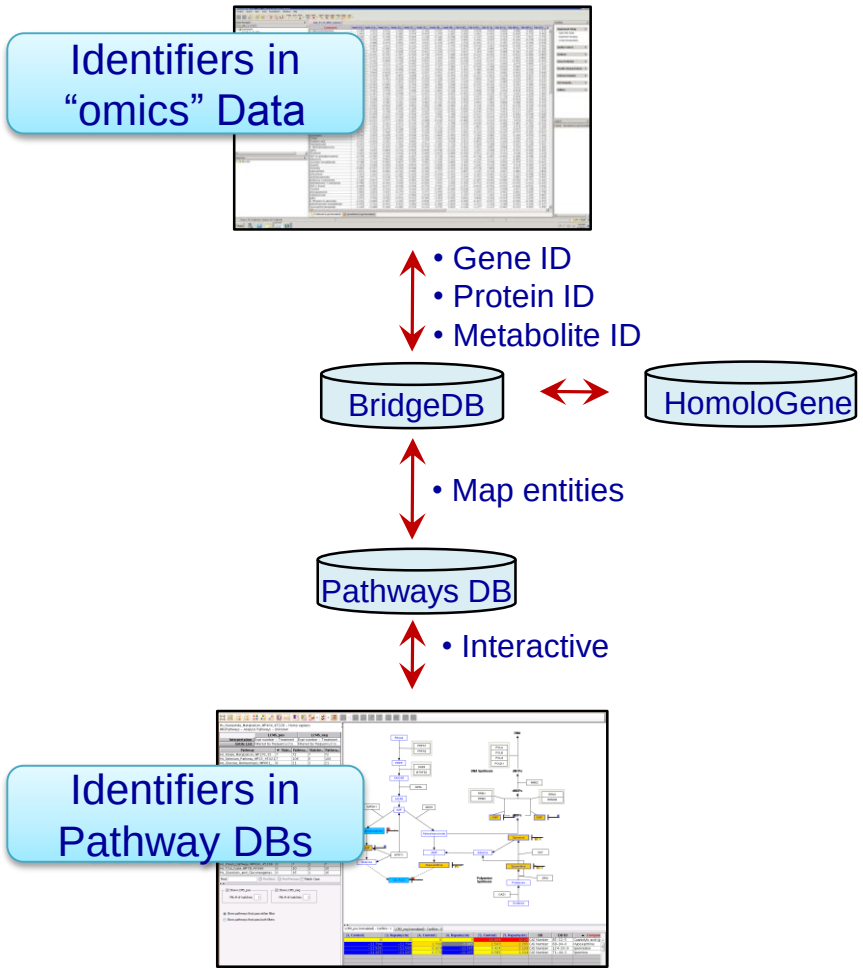

- **Metabolite Identifiers**
- KEGG
- HMDB
- ChEBI
- CAS
- **Protein Identifiers**
- Swiss-Prot
- UniProt
- UniProt/TrEMBL
- **Gene Identifiers**
- Entrez Gene, GenBank, Ensembl
- EC #, RefSeq, UniGene, HUGO
- HGNC, EMBL

Disease programming through life

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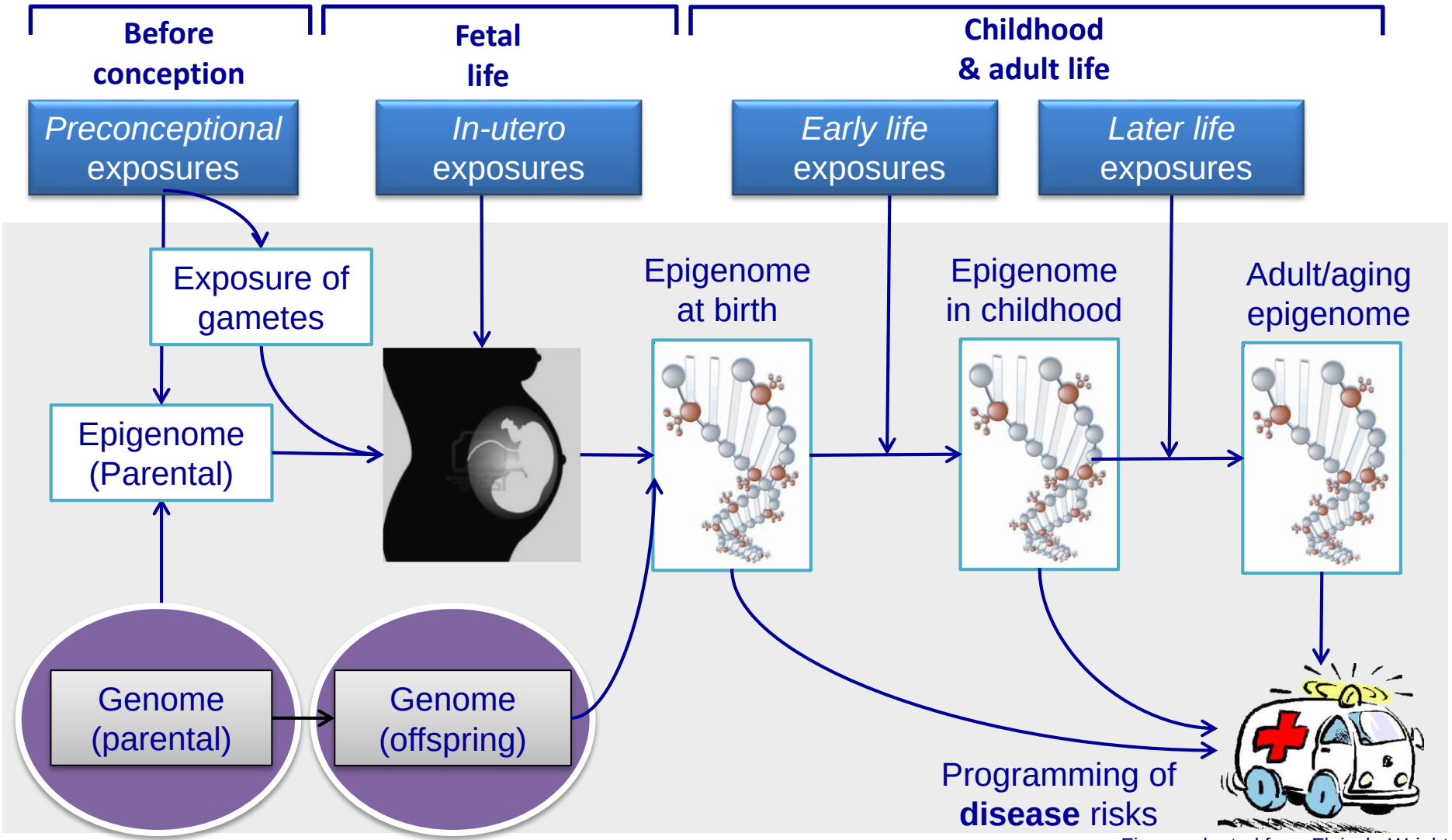
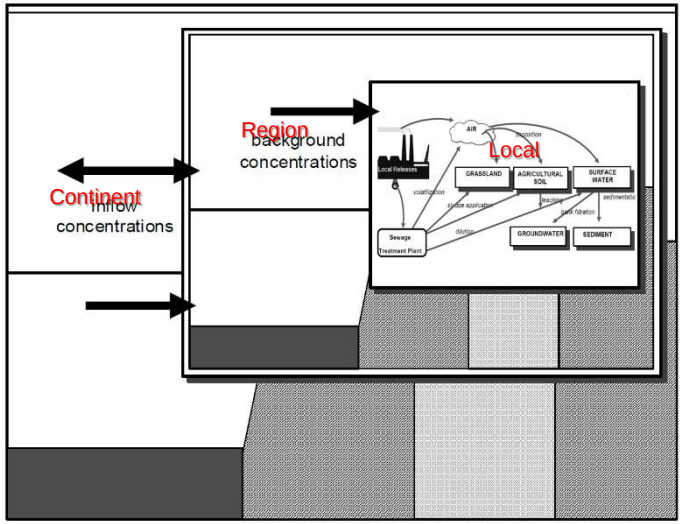


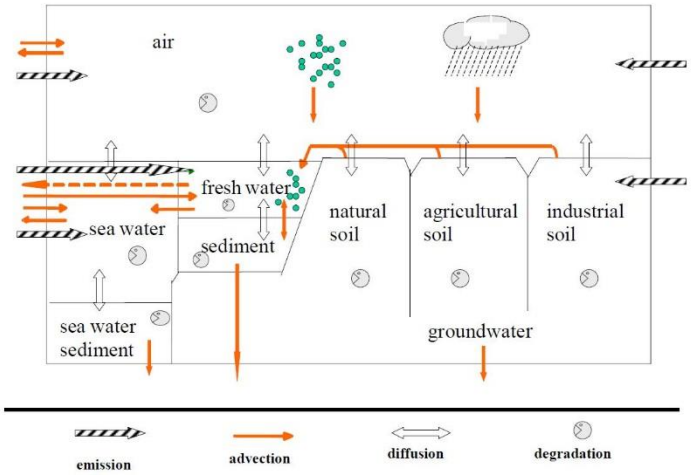
Figure adapted from Fleisch, Wright & Baccarelli, J Mol Endocrinol, 2012

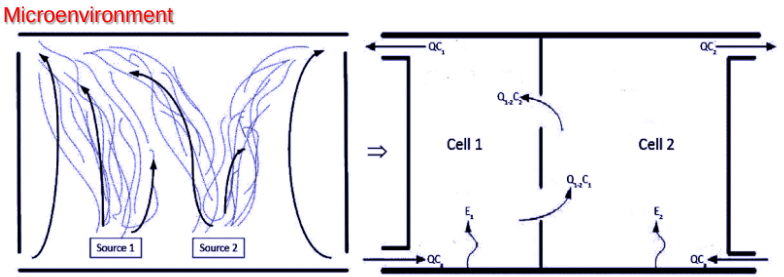
The external exposome

Multi-scale environmental contamination model

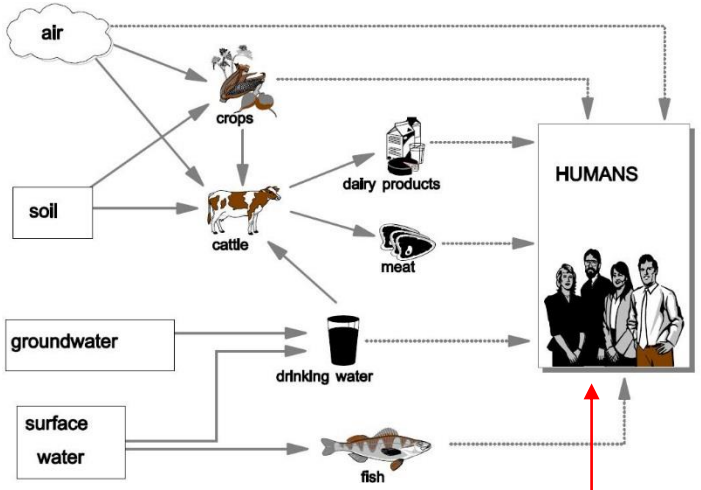


Multimedia environmental modelling, taking into account mass transfer and transformation across different scales and media, following ECHA recommendations





Detailed micro-environmental concentrations taking into account interactions among different media (gas, particles and dust)
Detailed exposure modelling taking into account multiple pathways and routes of exposure



Consumer products

External exposure advances

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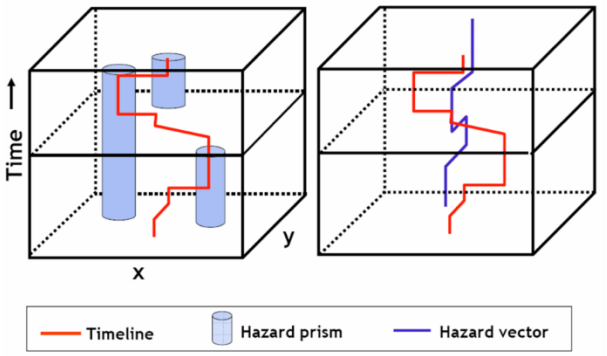
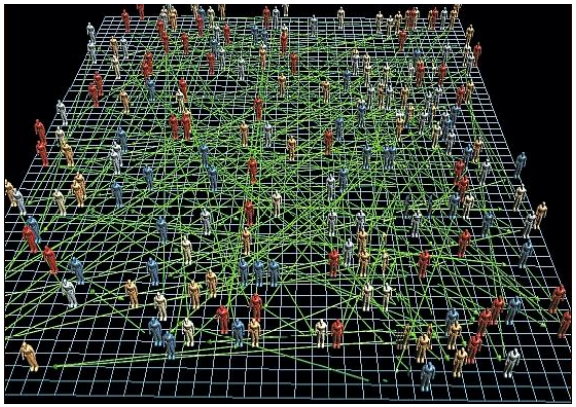
An individual's space-time activity model



Agent based modelling

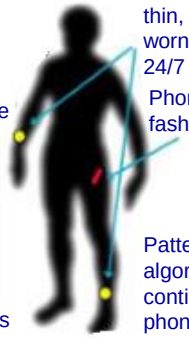


Sensors for exposure assessment

WOCKETS SYSTEM VISION

Multiple, low-cost 3-axis accelerometers stream data in real-time to mobile phone

Wearable sensors (test version 1)



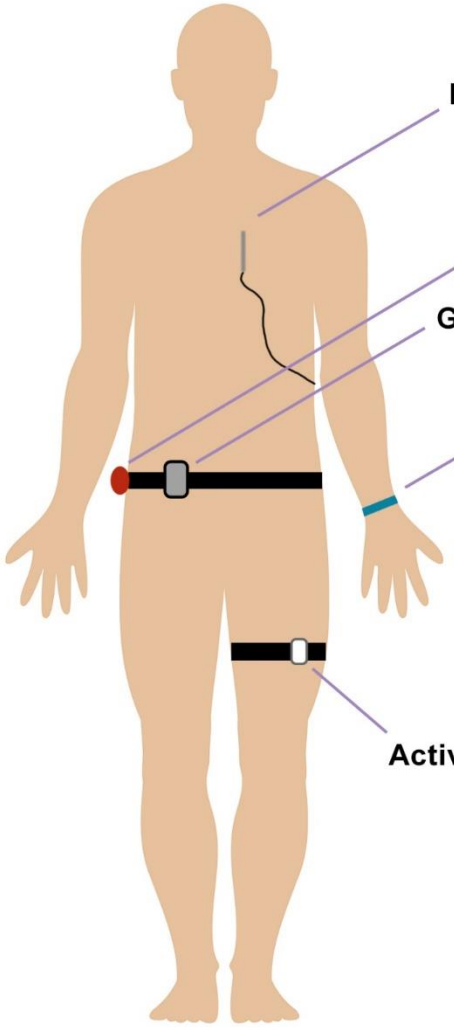
Sensors miniature, thin, and ergonomic; worn under clothing 24/7

Phone carried in typical fashion (e.g. in pocket)

Pattern recognition algorithms running continuously on phone detect physical activities in real-time

Innovative phone apps possible

Overall
wear - sync - export



Elitech Temperature Logger

- Worn on the upper torso
- Sensor should not touch skin or other object
- **Export data every day**

Actigraph — Just wear it all the time

GPS

- Keep GPS on at all times
- Logger should be on LOG
- **Charge it each night**

FitBit Flex

- Keep on your hand even in your sleep
- **Daily sync**
- **Charge after 3-4 days**

Activ8

- Worn around the middle of the thigh at all times
- **Sync and charge every 2-3 days**
- **Export data every day**

Moves (iOS / Android)

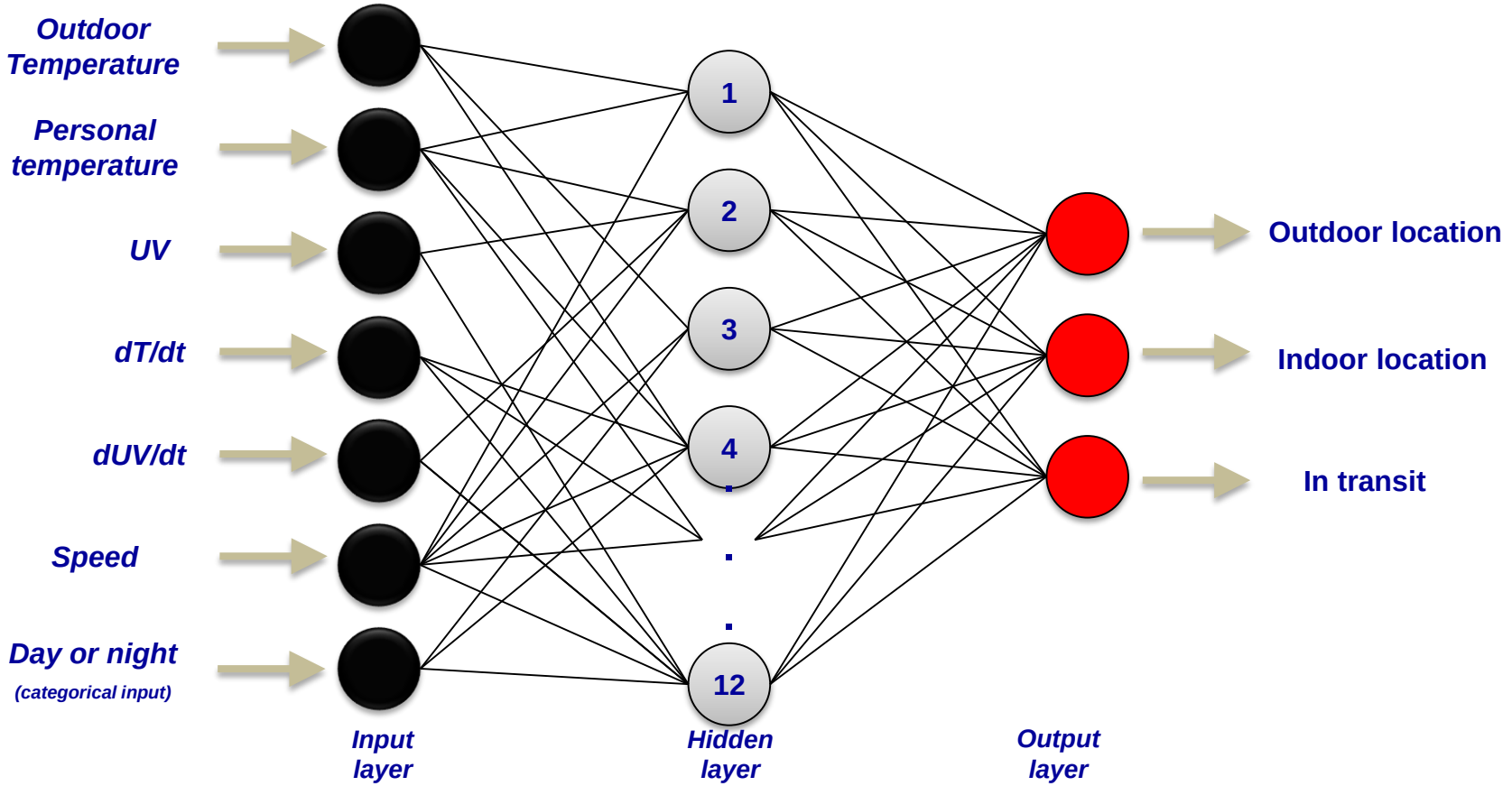
- Always carry your smartphone (pocket or bag)
- Charge your smartphone every night
- Code “home”, “work” etc.

Paper Log — Start time, End time, Location, Comments

"Intelligent" location tracking

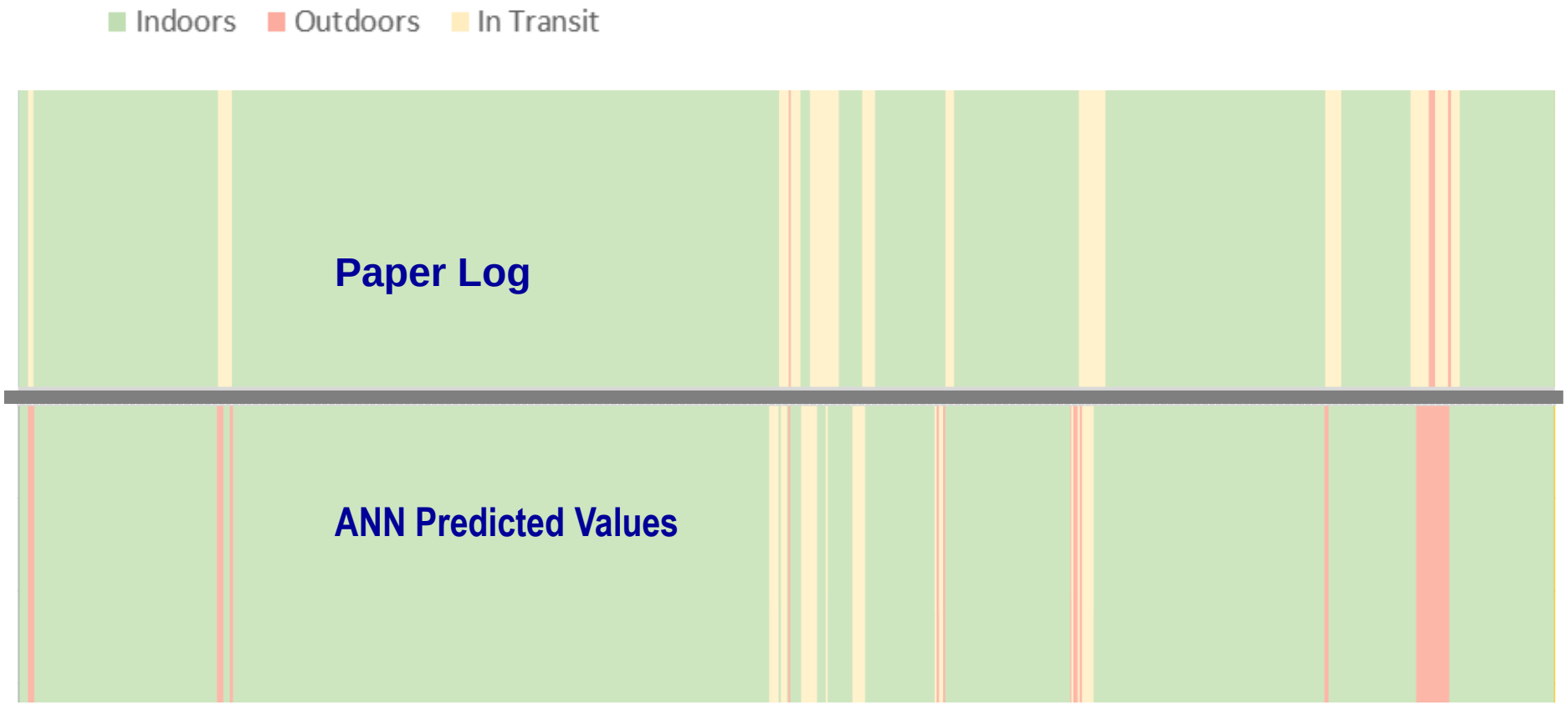
Statistical Method: Predicting location based on temp and UV logs through an Artificial Neural Network

7 input nodes (6 numerical and 1 categorical)
12 hidden nodes (found to yield the best results among several combinations)
3 output nodes (corresponding to the 3 different classes)
Data from 5 days were used for training the ANN model
Data from 2 days were used as an independent dataset to validate the ANN model



Predicting location

based on temp and UV logs through ANN



Indoor-to-outdoor transition captured satisfactorily

Identifying activity patterns Based on Agent Based Modelling

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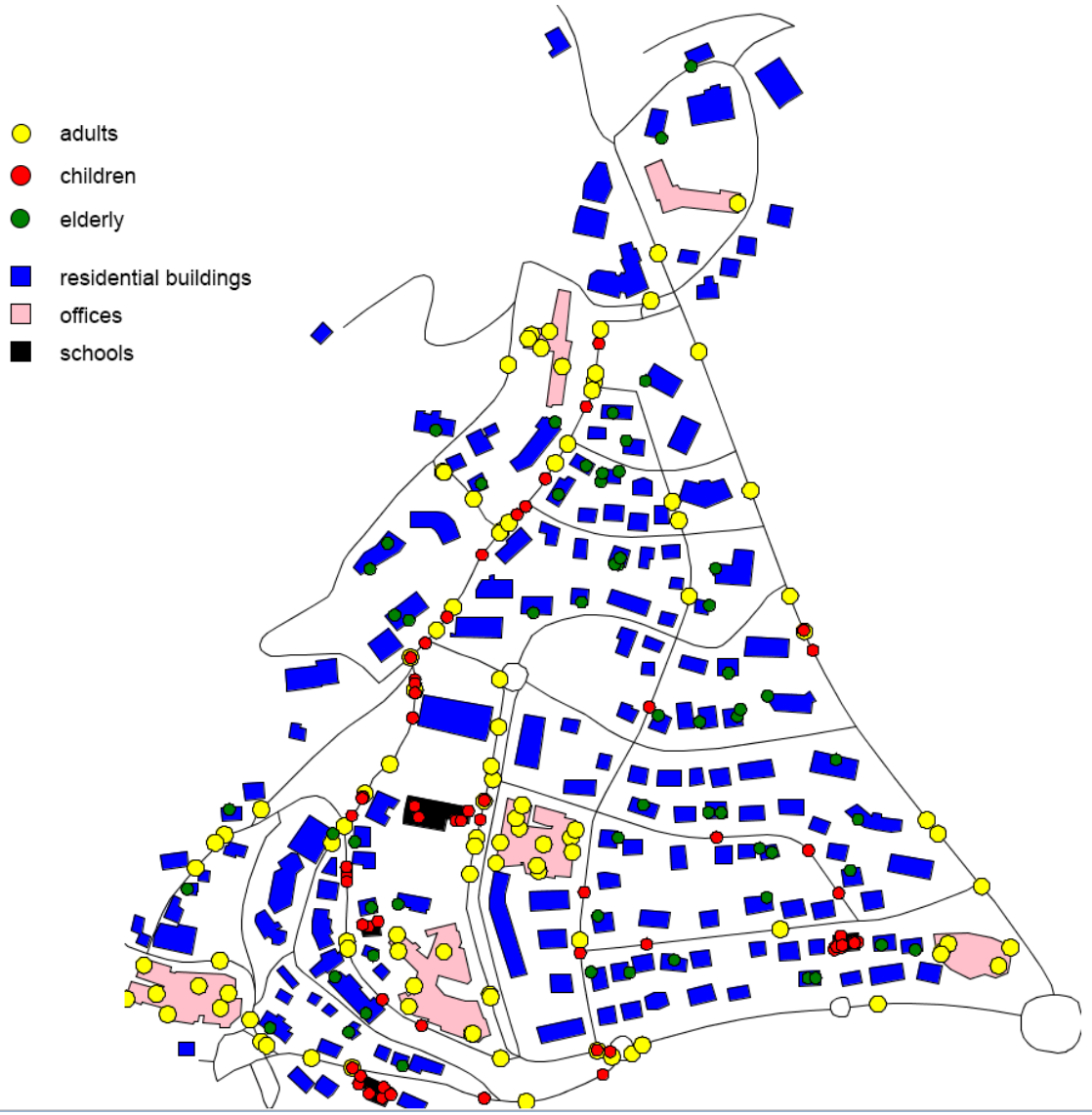
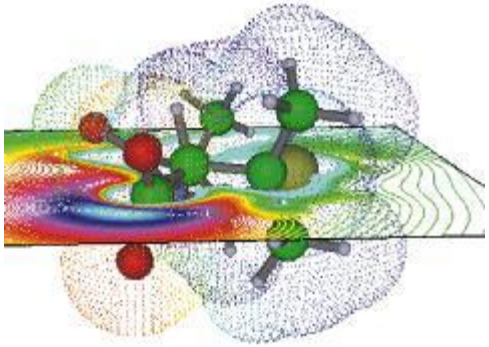
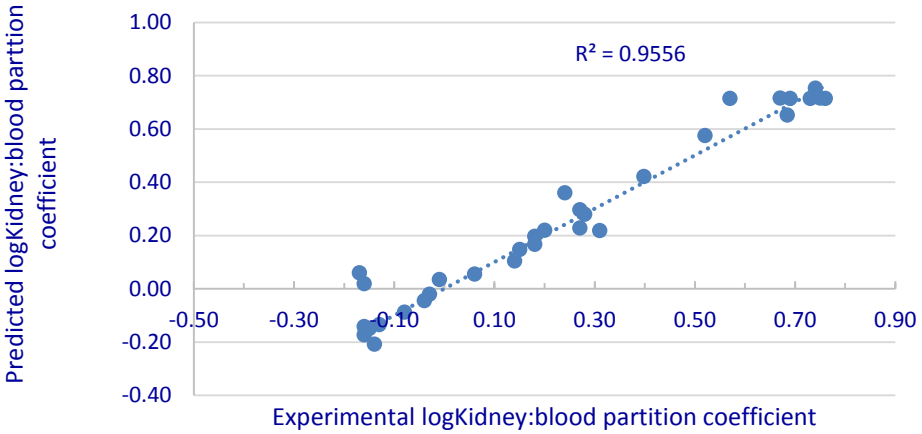


Enhancement of the chemical space through innovative use of QSARs

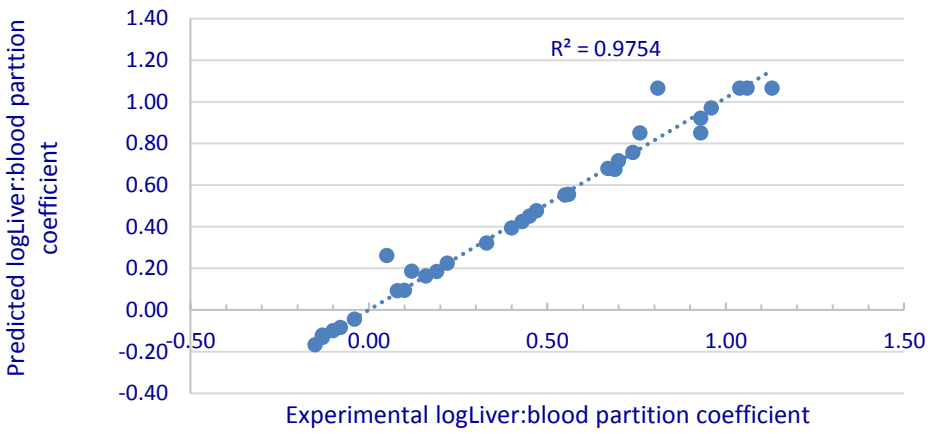


Expanding the chemical space: Coupled use of QSARs - Artificial Neural Networks

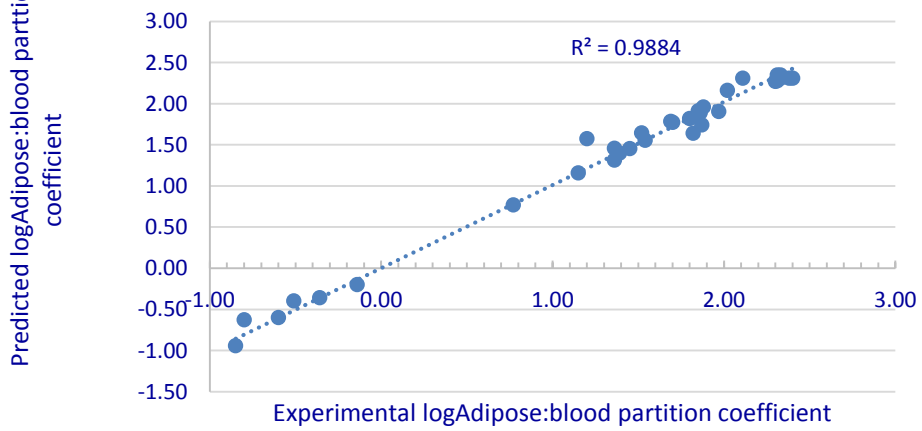
Logarithm of Kidney:blood partition coefficient - Abraham, ANN



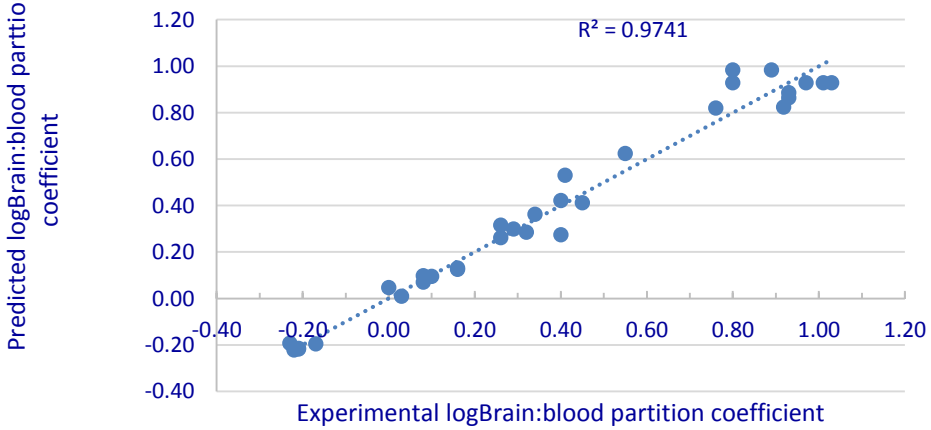
Logarithm of Liver:Blood Partition Coefficient - Abraham, ANN



Logarithm of Adipose:blood partition coefficient - Abraham, ANN

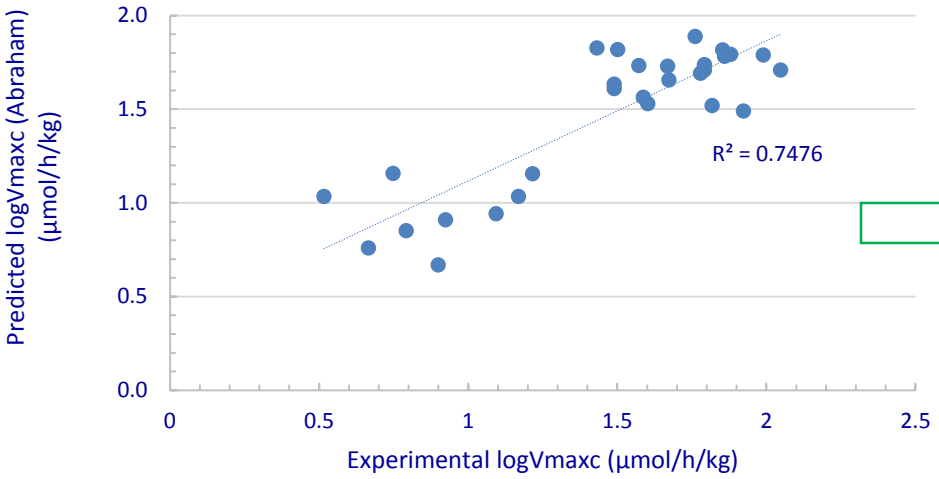


Logarithm of Brain:blood partition coefficient - Abraham, ANN

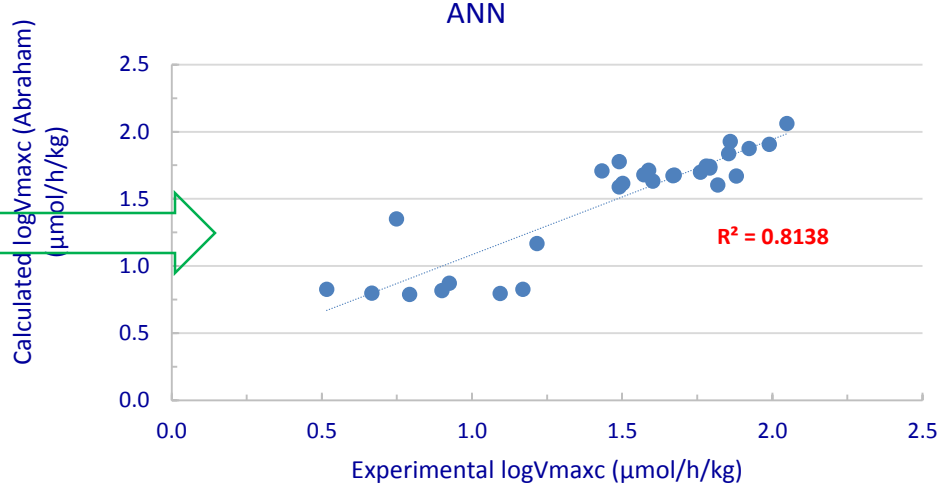


Expanding the chemical space: Use of QSARs - Artificial Neural Networks

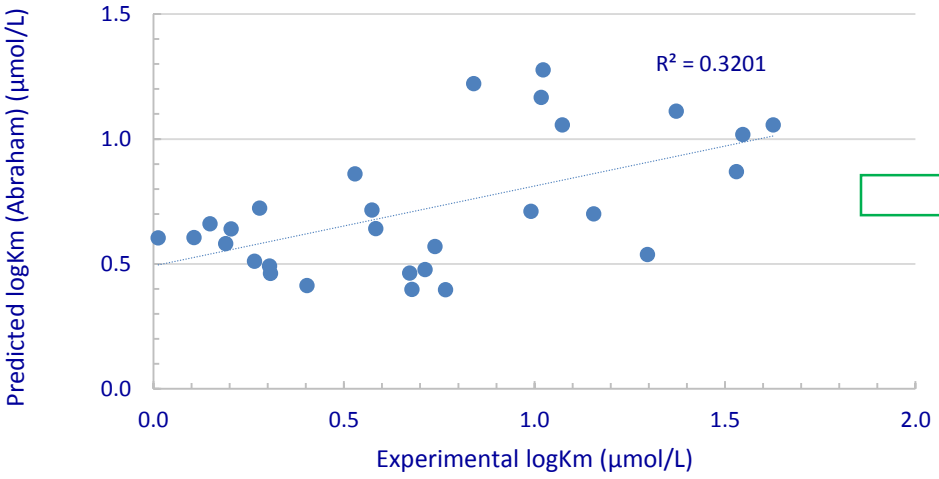
Logarithm of Normalized Maximal Velocity - Abraham, NLR



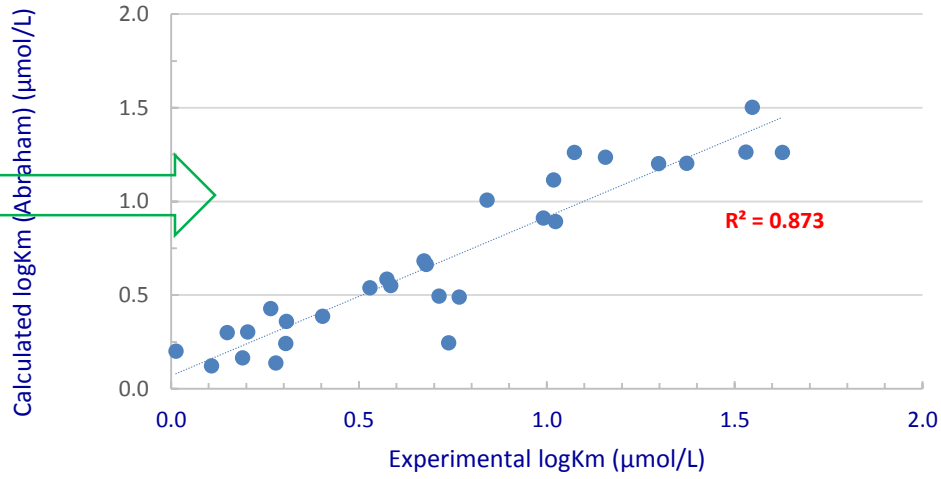
Logarithm of Normalized Maximal Velocity - Abraham, ANN



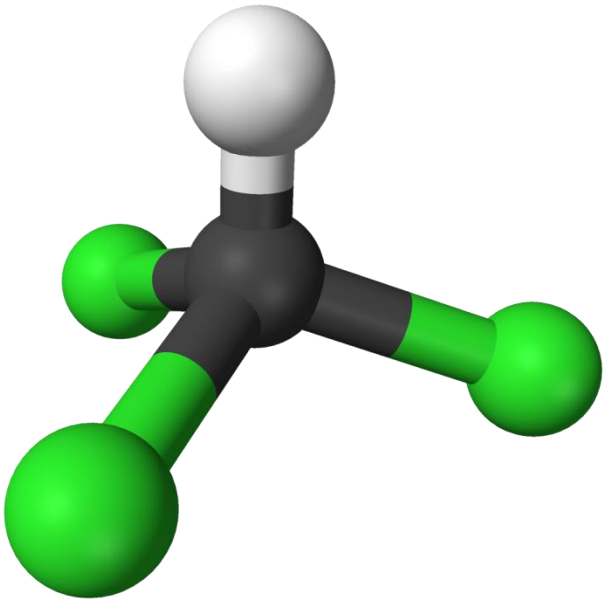
Logarithm of Michaelis - Menten Constant - Abraham, NLR



Logarithm of Michaelis-Menten constant- Abraham, ANN



TCM exposure reconstruction from domestic hygienic activities



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Reconstructing exposure from TCM spot samples

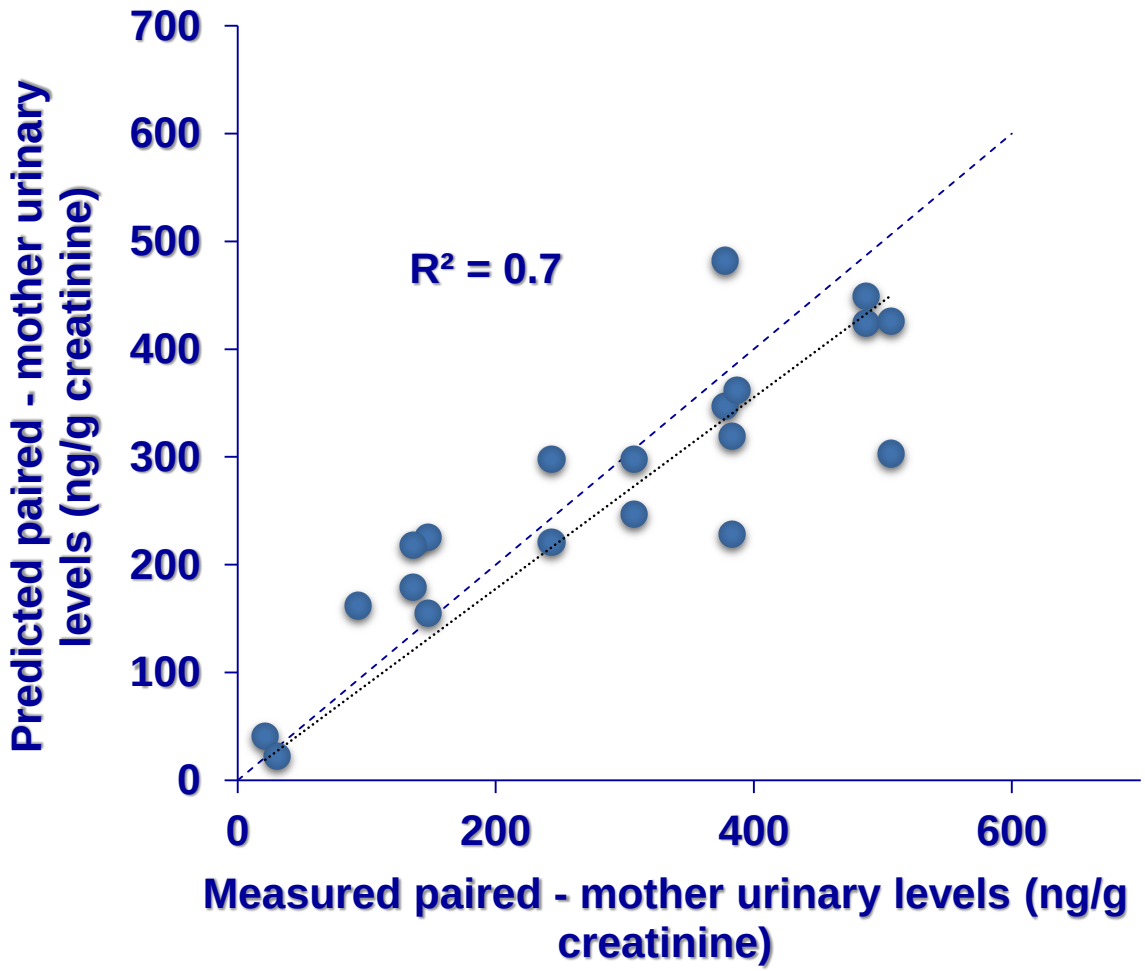
- Urinary TCM (morning voids) was measured in 20 matched mothers and children (paired)
- Using the children urinary TCM levels, indoor air background TCM concentrations were reconstructed
- These concentrations were used for estimating mother exposure

↓

Urinary TCM was predicted for the paired others (nested reconstruction)

↓

Re-running forward the model we estimated TCM blood levels (internal exposure)



Andra SS, Charisiadis P, Karakitsios S, Sarigiannis DA, Makris KC. Passive exposures of children to volatile trihalomethanes during domestic cleaning activities of their parents. Environmental Research 2015; 136: 187-195.

Reconstructing exposure from time-dynamic data

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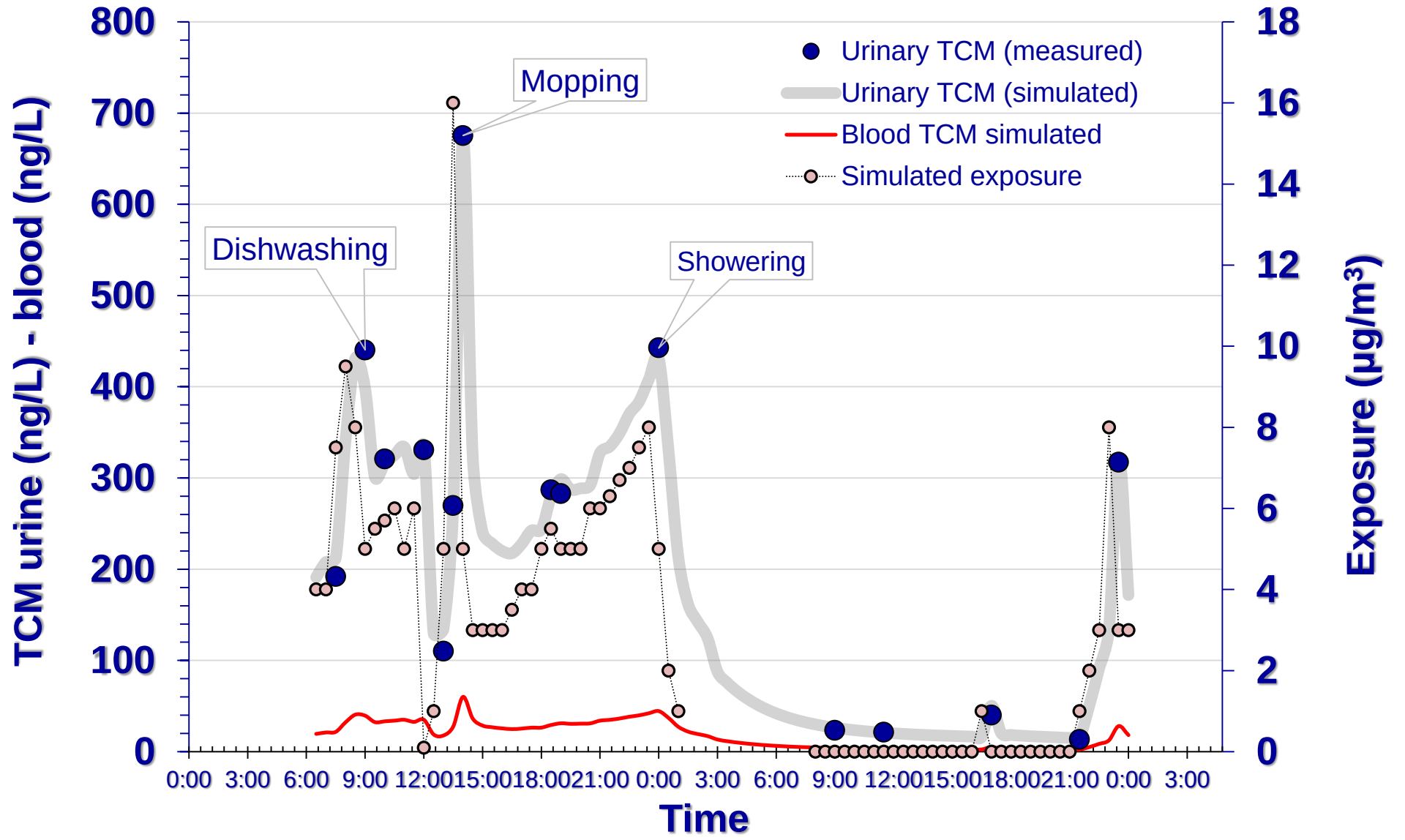
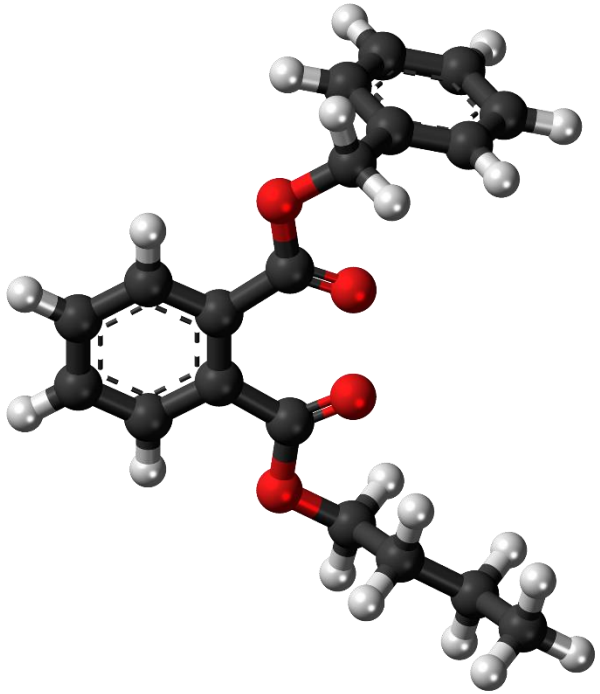


Bis(2-ethylhexyl) phthalate (DEHP)



DEHP - The vinyl floor case

- DEHP is emitted from electronic equipment and vinyl flooring mainly. Using the exposure scenario of vinyl flooring, environmental contamination at the regional and local scales is practically negligible. Exposure occurs at the micro-environmental levels of dwellings.
- Let's assume a typical common residential dwelling (size of 270 m^2 and air exchange rate equal to 0.5) characterized by total DEHP gaseous emissions of 200 μg/h (vinyl flooring and other plastic equipment).

Exposure pathways considered:

- Exposure through inhalation:
  - gas phase
  - particles phase
- Exposure through skin:
  - Rubbing of dust (0.001 g/day)
- Exposure through ingestion
  - Dust ingestion through hand to mouth behavior

	Infants	Toddlers	Children	Female Teens	Male Teens	Female Adults	Male Adults
House dust ingestion (g/day)	0.05	0.05	0.01	0.001	0.001	0.001	0.001

DEHP concentration in different media

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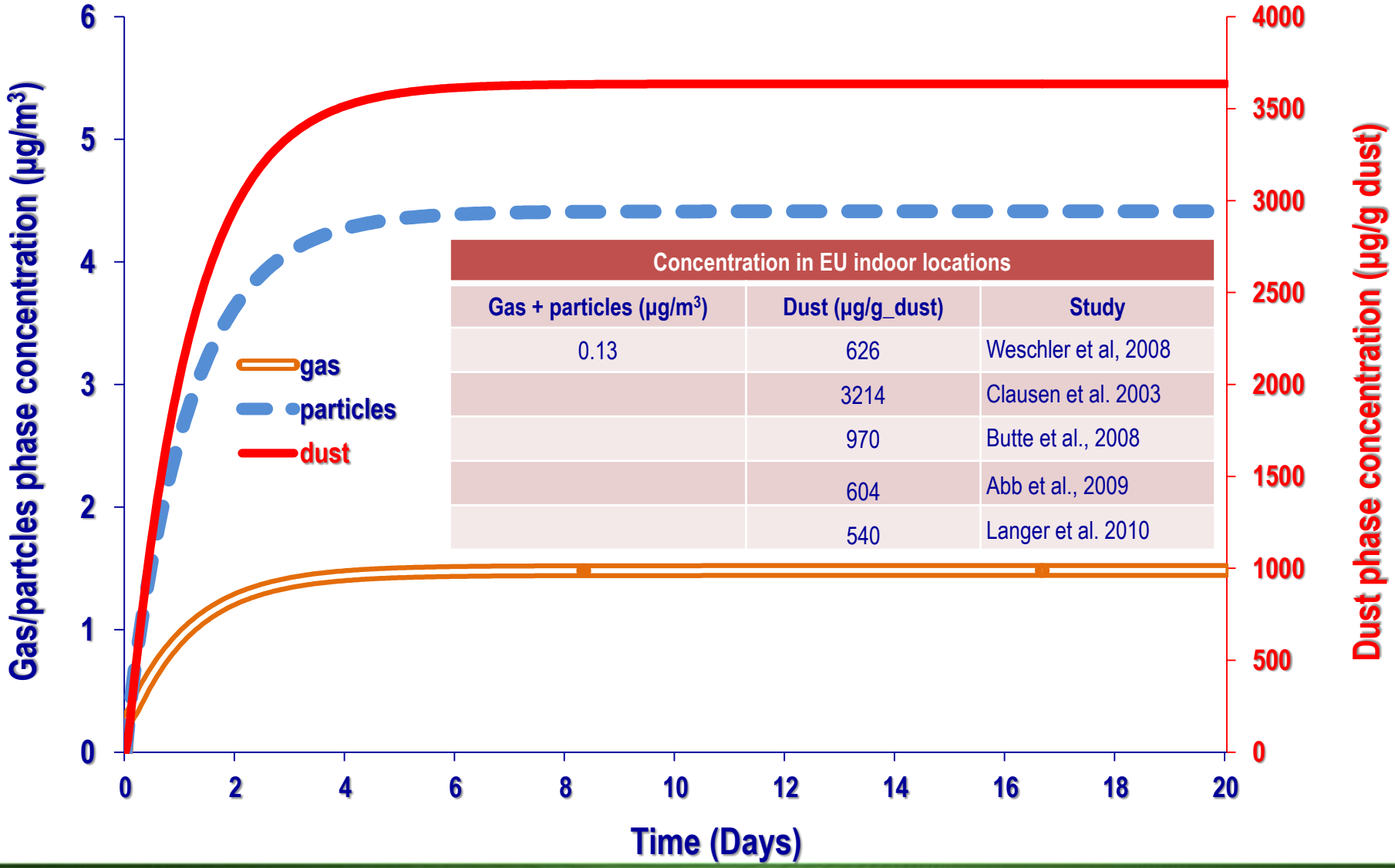

Concentration in EU indoor locations		
Gas + particles (μg/m³)	Dust (μg/g_dust)	Study
0.13	626	Weschler et al, 2008
	3214	Clausen et al. 2003
	970	Butte et al., 2008
	604	Abb et al., 2009
	540	Langer et al. 2010

DEHP daily uptake per route and age group

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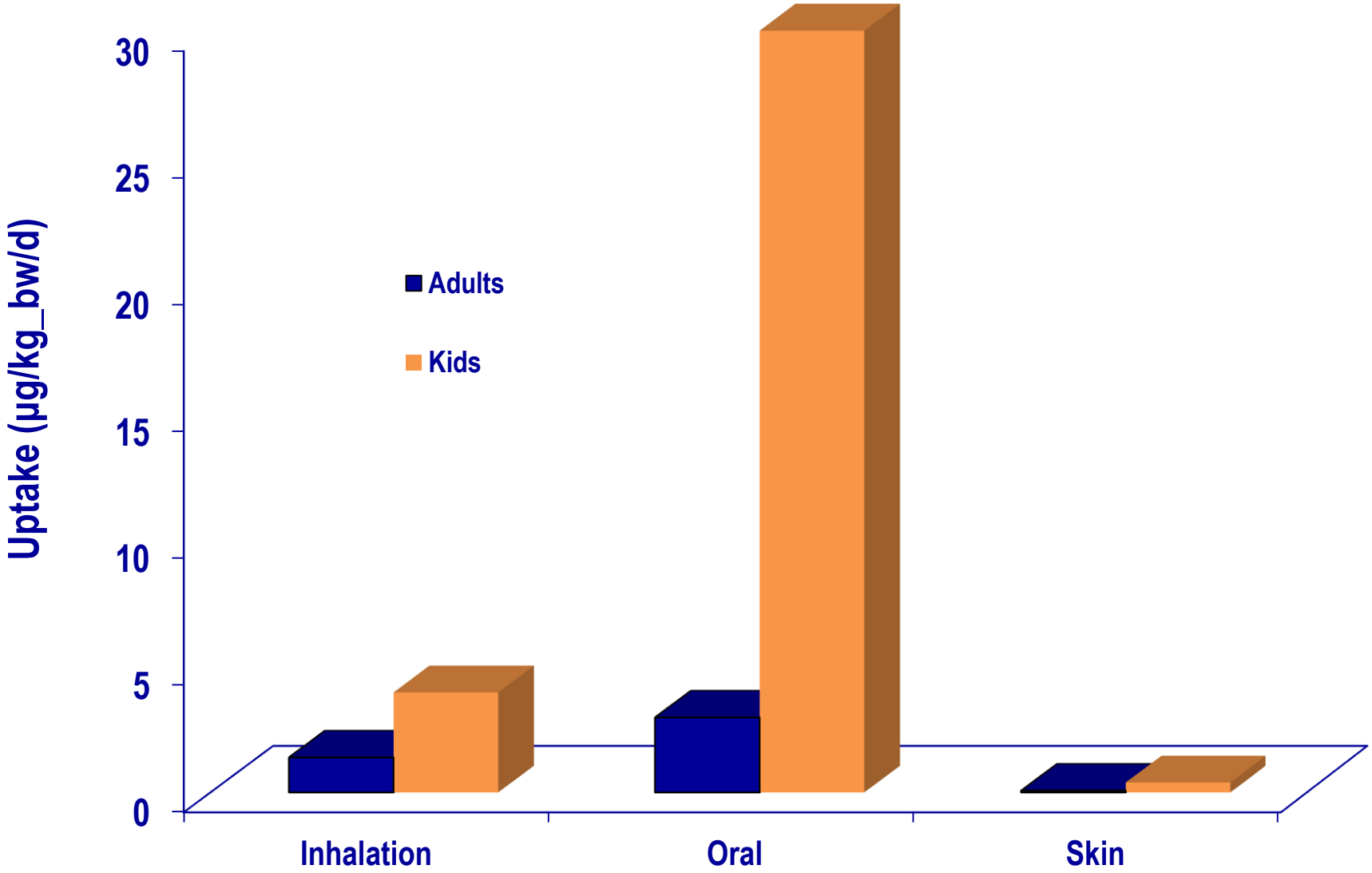

DEHP internal exposure

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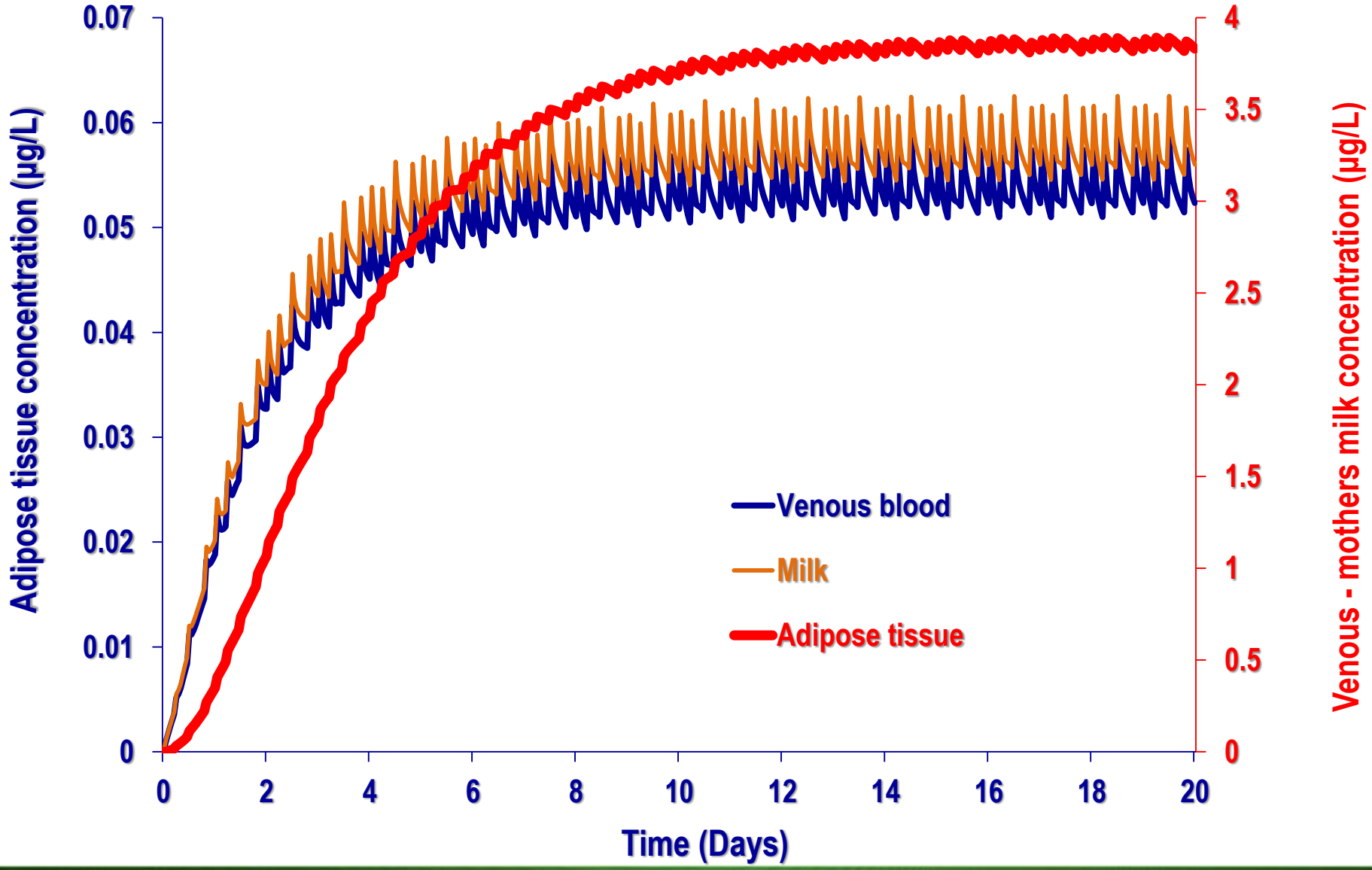
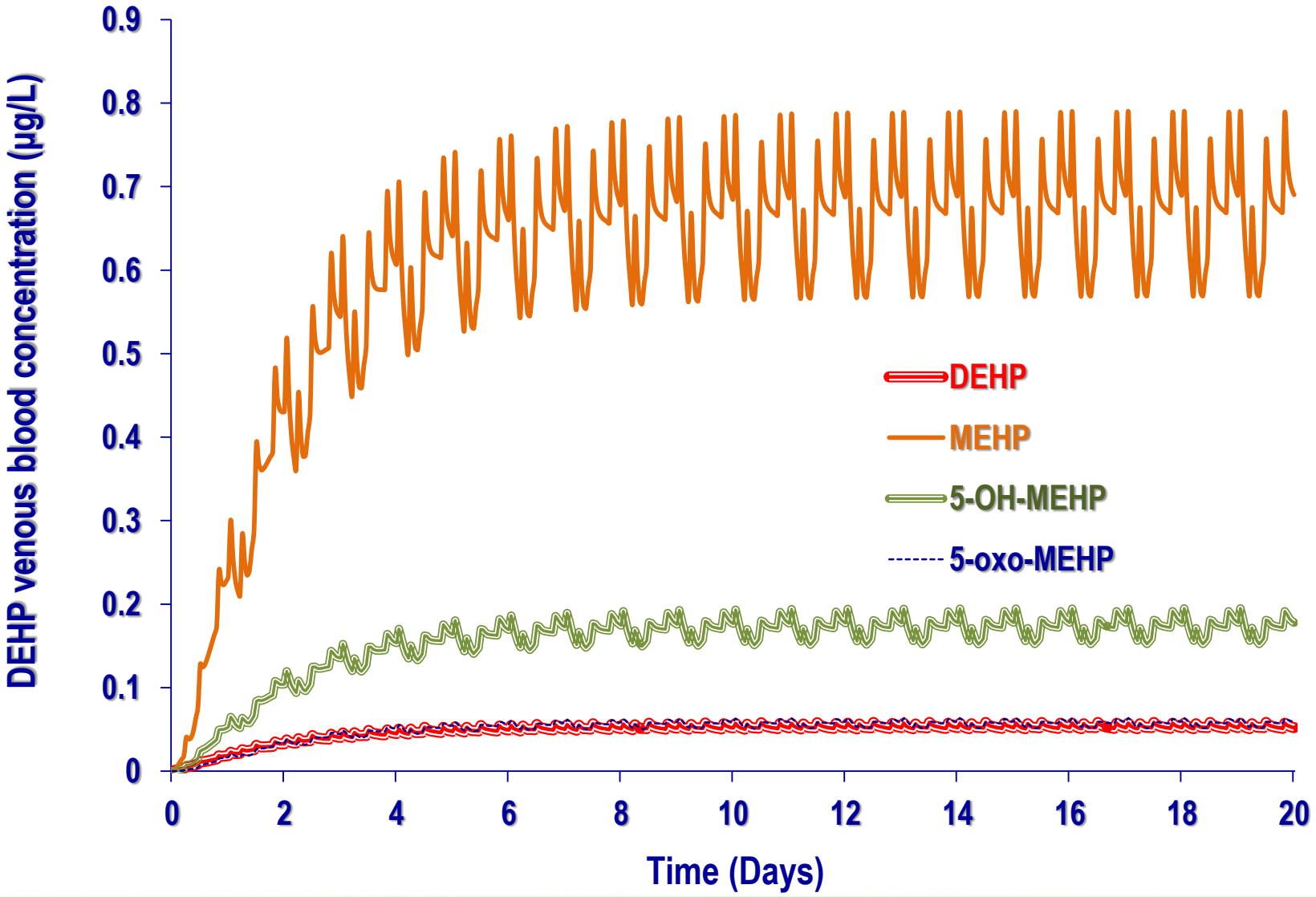
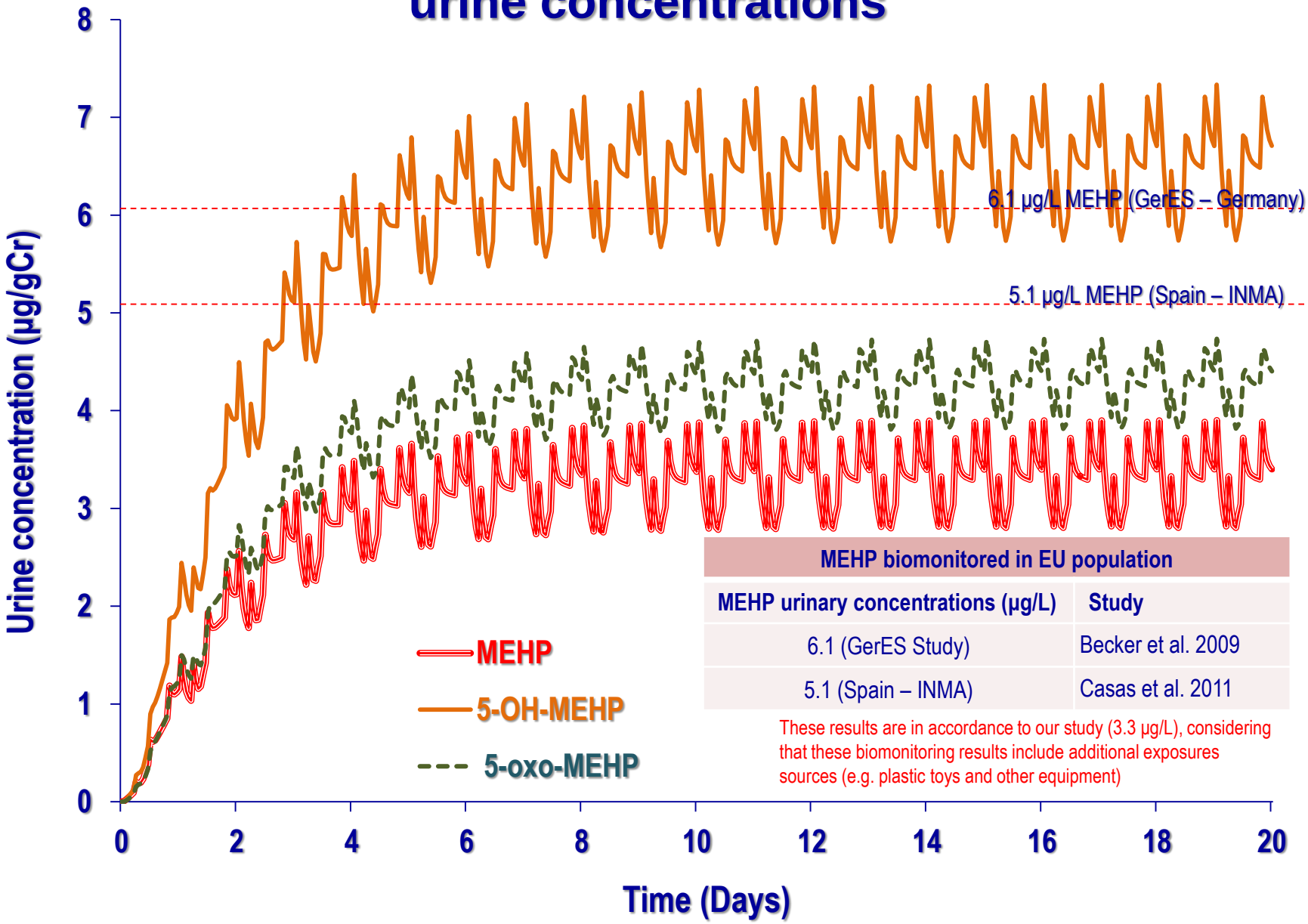


DEHP and major metabolites internal exposure



Major metabolites urine concentrations

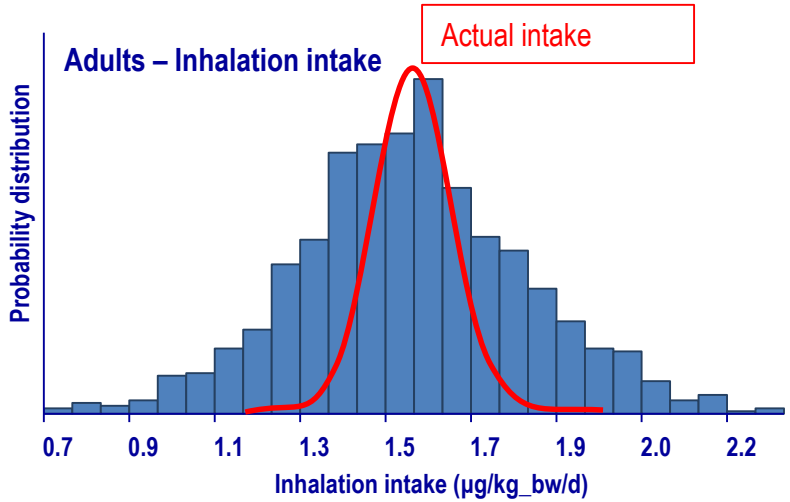


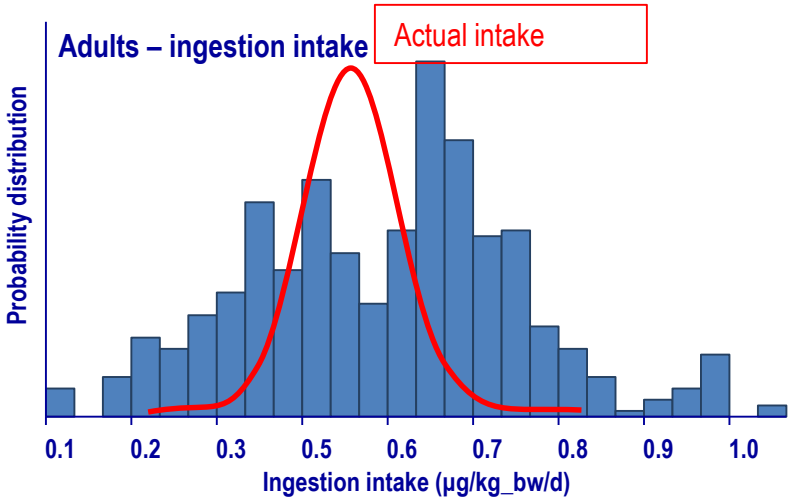
MEHP biomonitored in EU population	
MEHP urinary concentrations (µg/L)	**Study**
6.1 (GerES Study)	Becker et al. 2009
5.1 (Spain – INMA)	Casas et al. 2011

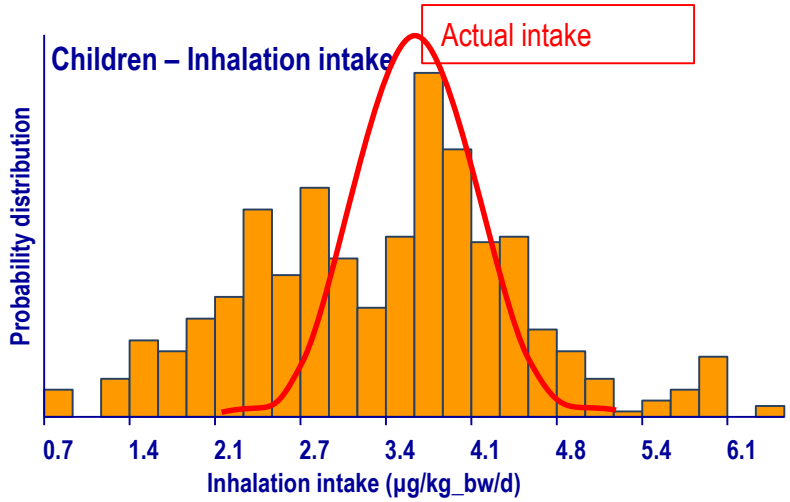
These results are in accordance to our study (3.3 µg/L), considering that these biomonitoring results include additional exposures sources (e.g. plastic toys and other equipment)

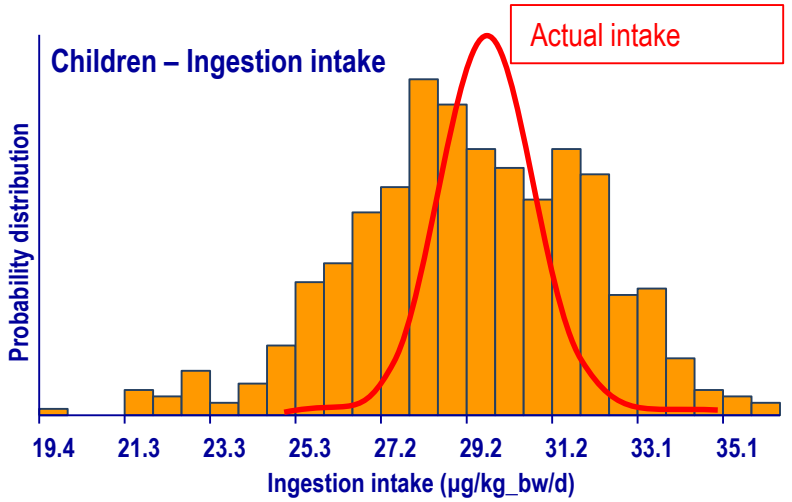
Exposure reconstruction based on urinary MEHP results

Using a distribution of spot samples of urinary MEHP at 8 o'clock in the morning, knowing environmental contamination (gaseous phase, particles and dust), what was the contribution from different routes?

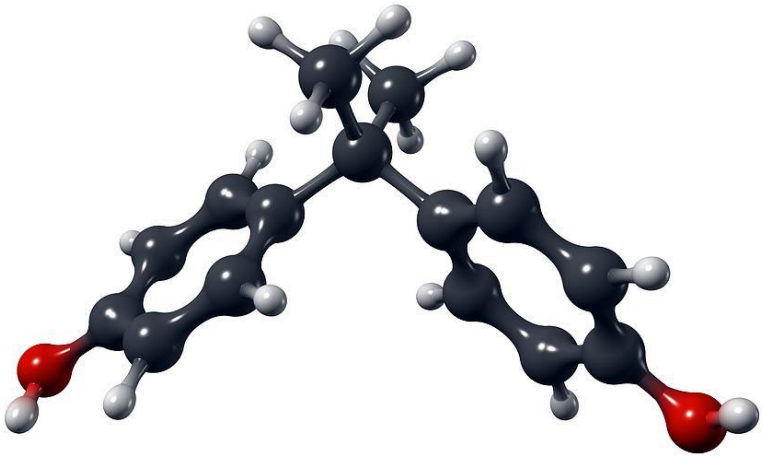








Bisphenol-A (BPA)



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BPA – daily intake under different exposure scenarios

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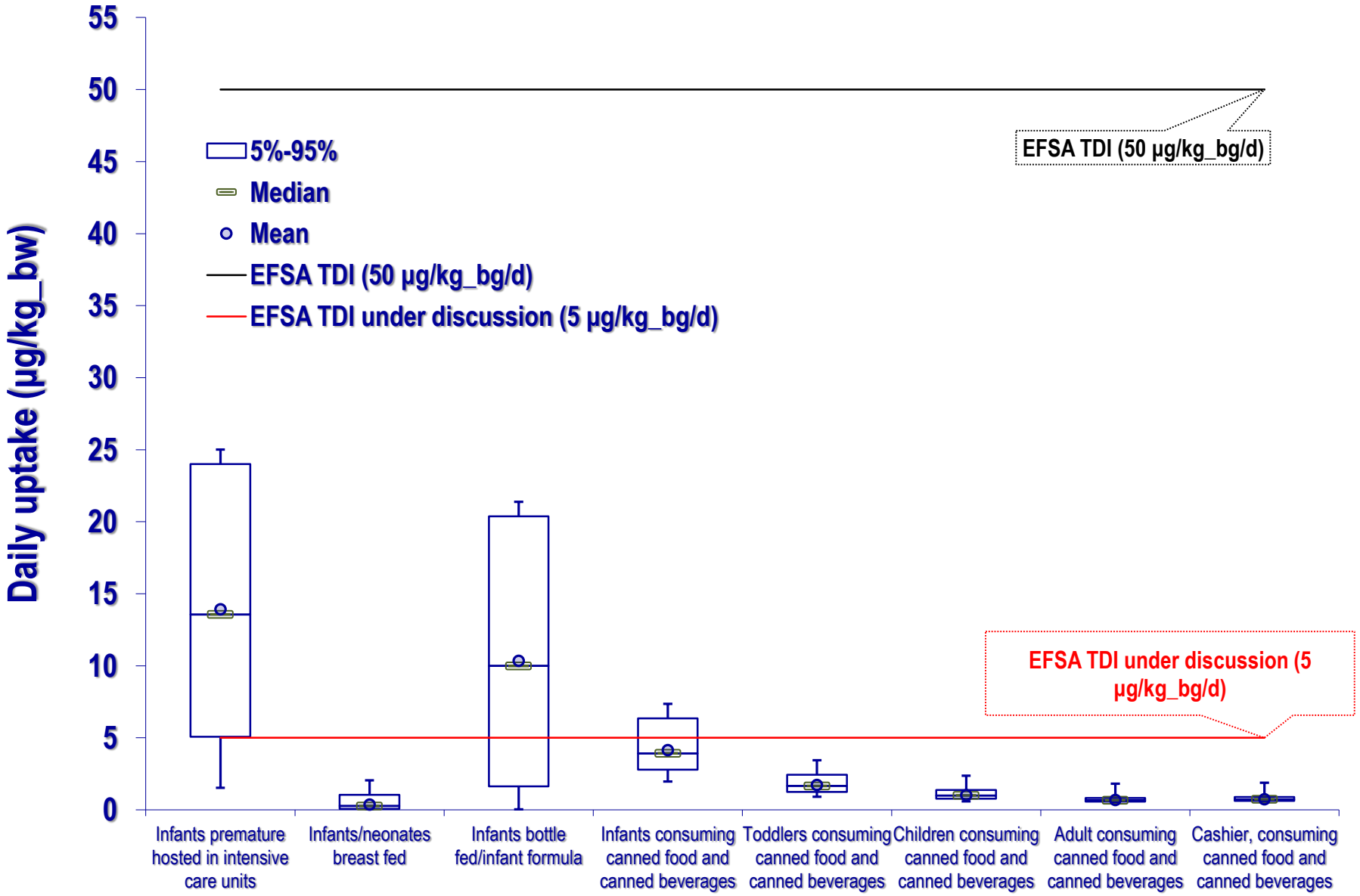
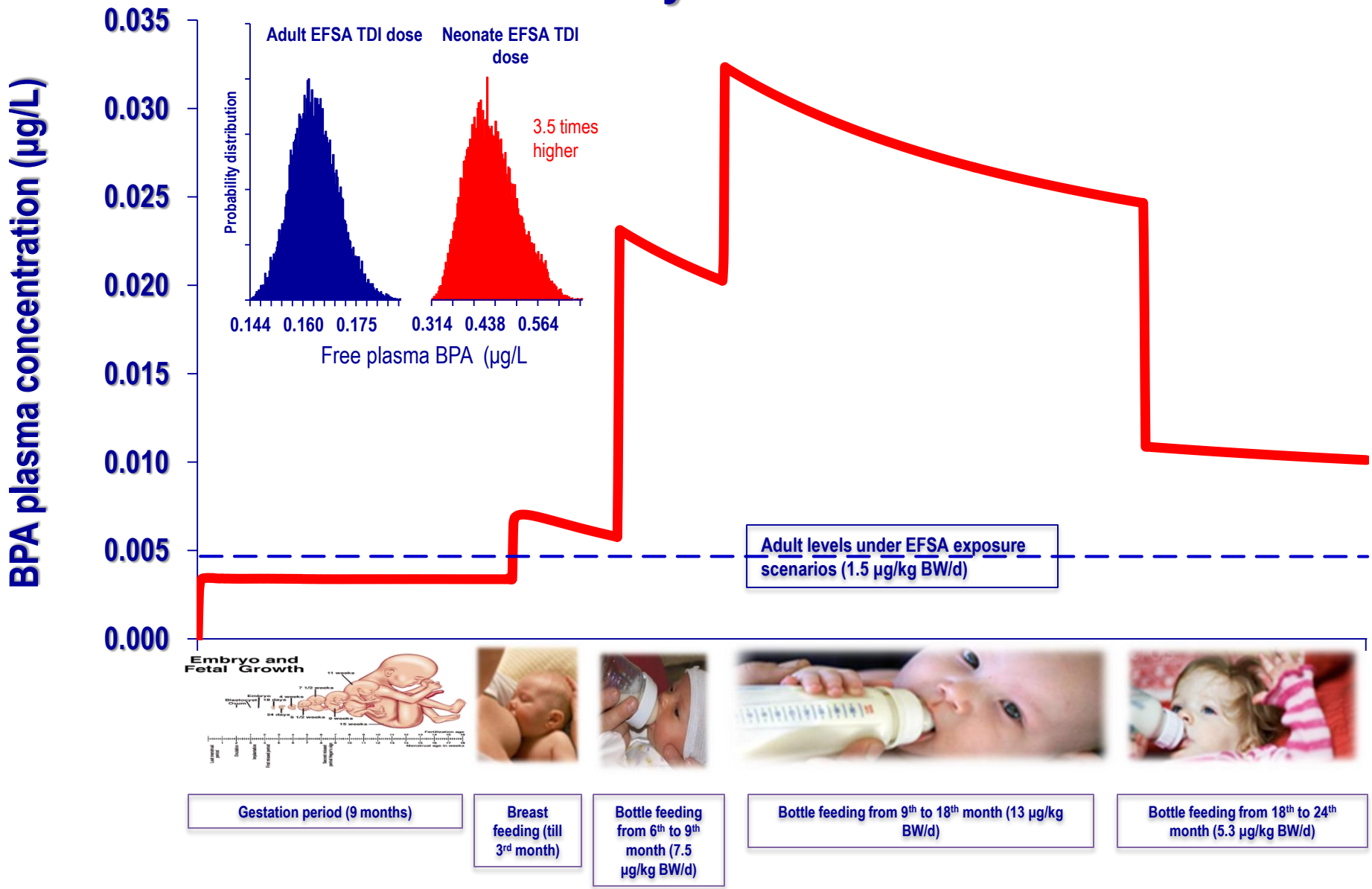


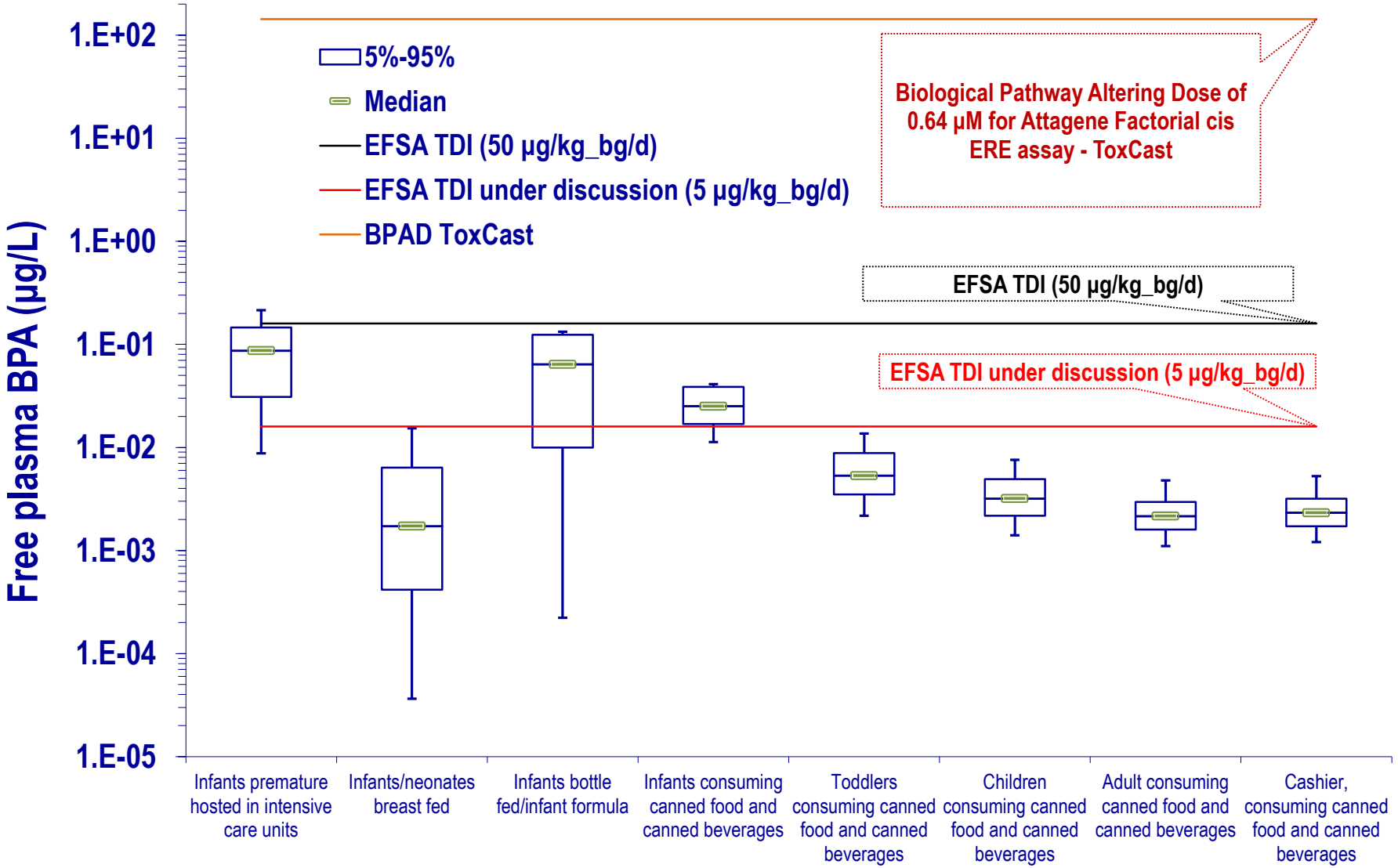
Internal dosimetry aspects of BPA toxicokinetics

- Wider inter-individual variability regarding glucuronidation capacity (significantly lower clearance for neonates/infants)
- Very strong plasma protein binding
- First-pass metabolism decisive for clearance – wide bioavailability differences are expected from routes beyond oral (up to six times higher internal dose concentrations for inhalation compared to oral)
- BPA-GLU de-conjugates to BPA in the stomach, increasing the actual dose during breast feeding, thus, the sum of BPA and BPA-GLU needs to be taken into account as BPA dose during breast feeding
- BPA-GLU de-conjugates to BPA in the placenta, increasing the actual dose during pregnancy

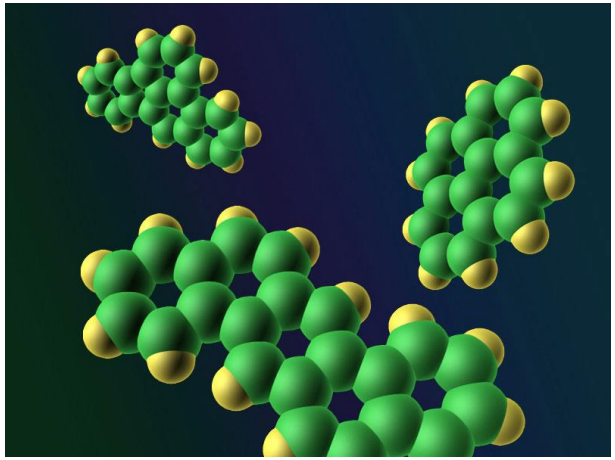
Importance of assessing internal dosimetry – focus on children



Importance of assessing internal dosimetry – focus on children



Exposure to PAHs from biomass burning emitted PM and associated lung cancer risk



Study design

– integrating detailed environmental information into enhanced exposure and health impact assessment

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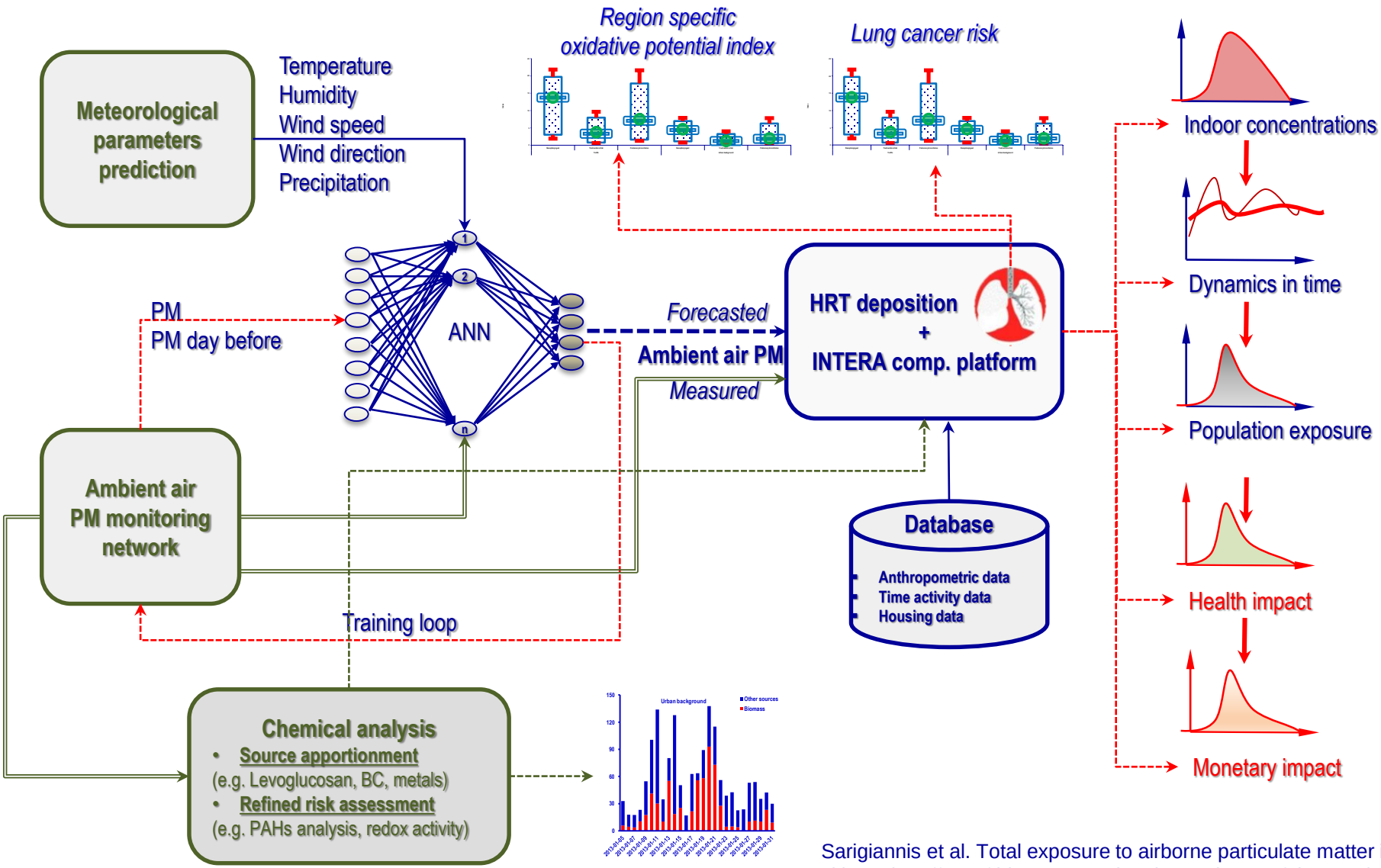

Sarigiannis et al. Total exposure to airborne particulate matter in cities: The effect of biomass combustion. Science of the Total Environment 2014; 493: 795-805.

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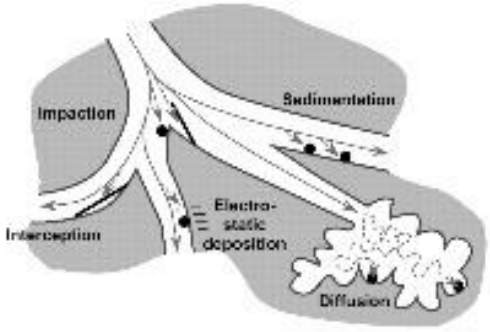
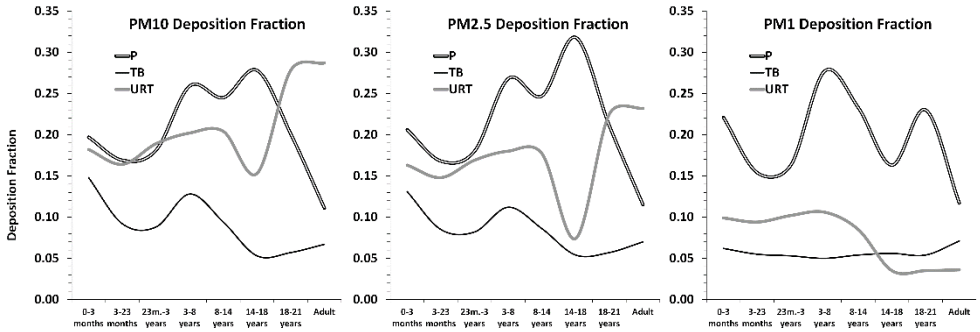
Translating environmental information into internal exposure metrics

How do they deposit

- *Size distribution*
- *Age (differences in physiology)*

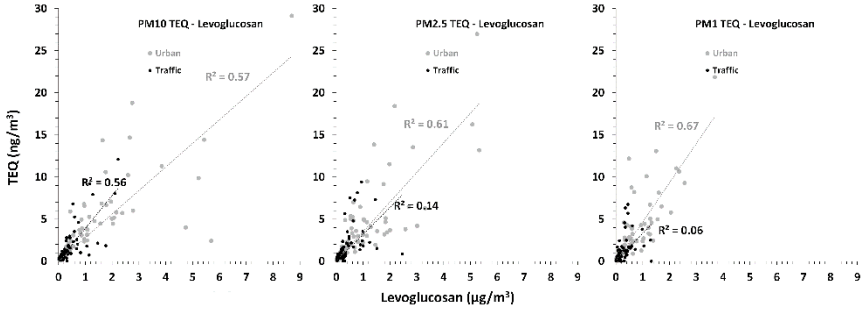


A higher fraction of smaller particles is deposited in the lower respiratory tract of children

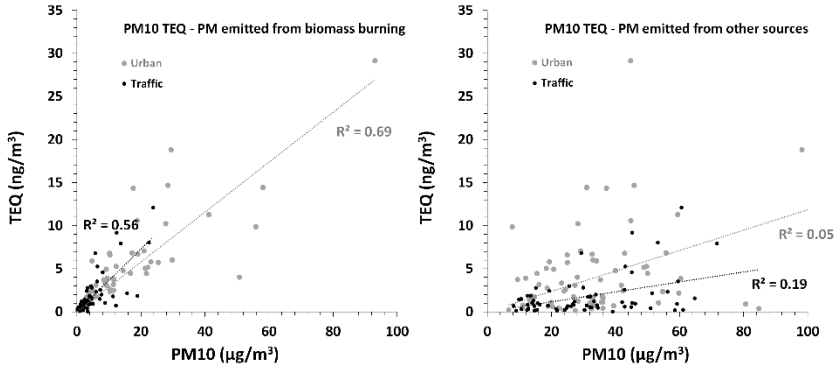


What about PM genotoxicity based on

- *Size distribution*
- *Origin (biomass or traffic)*

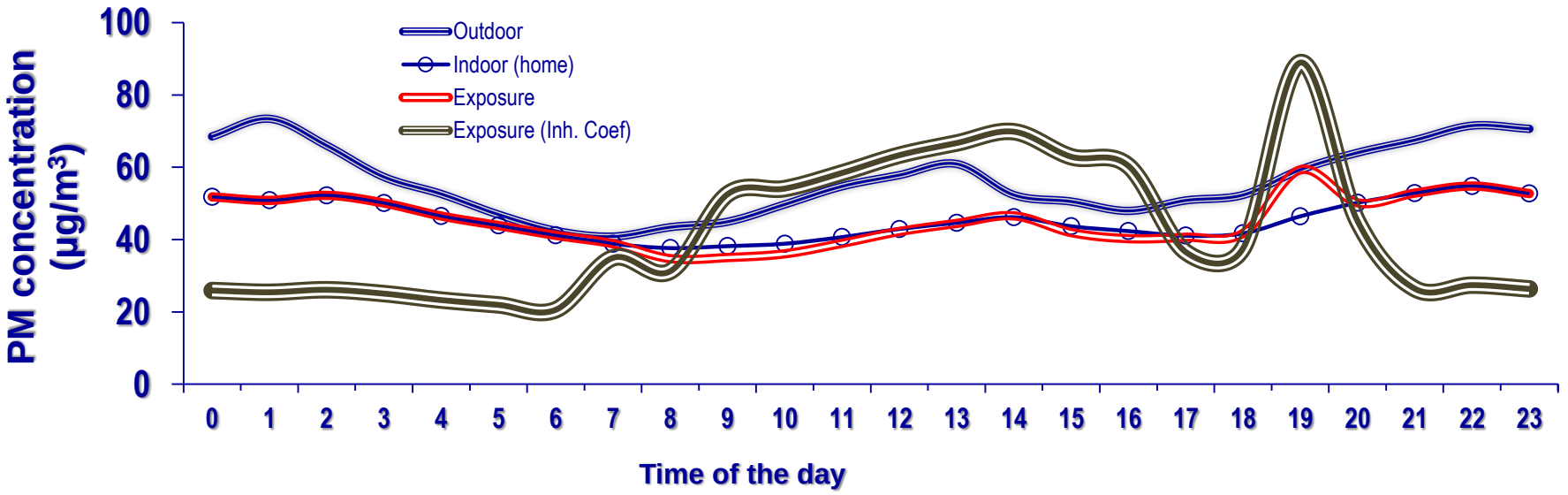


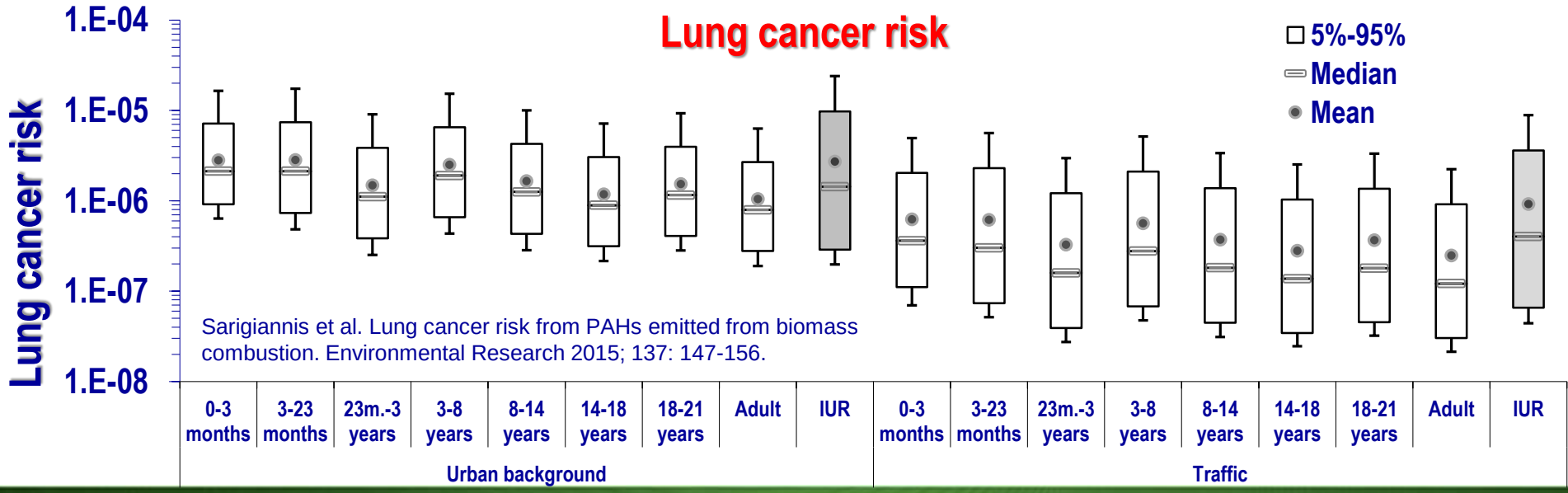
- Smaller particles have a higher PAHs content per mass of particle
- Biomass emitted particles are more genotoxic that the ones emitted from traffic



Sarigiannis et al. Lung cancer risk from PAHs emitted from biomass combustion. Environmental Research 2015; 137: 147-156.

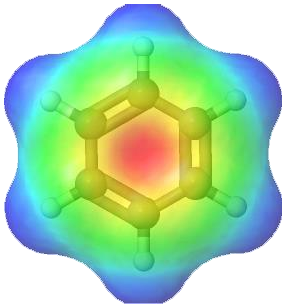
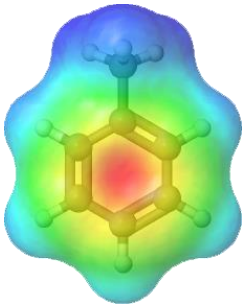
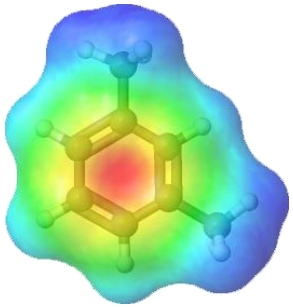
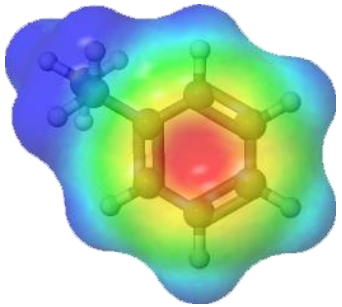
Biomass burning, PM exposure and lung cancer risk





Sarigiannis et al. Lung cancer risk from PAHs emitted from biomass combustion. Environmental Research 2015; 137: 147-156.

Co - exposure to BTEX and associated cancer risk

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A case on the exposome paradigm: Co-exposure to VOCs

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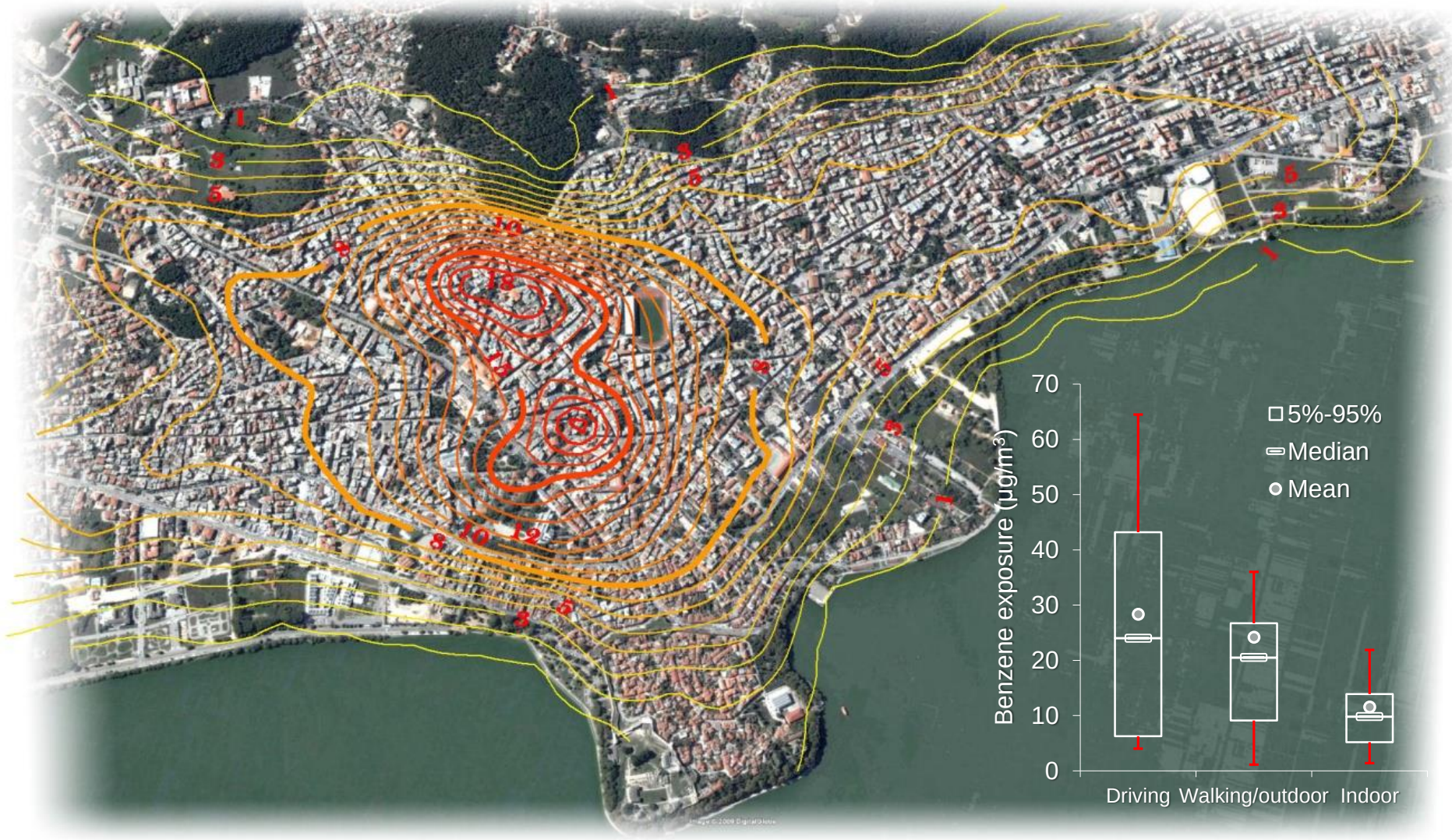
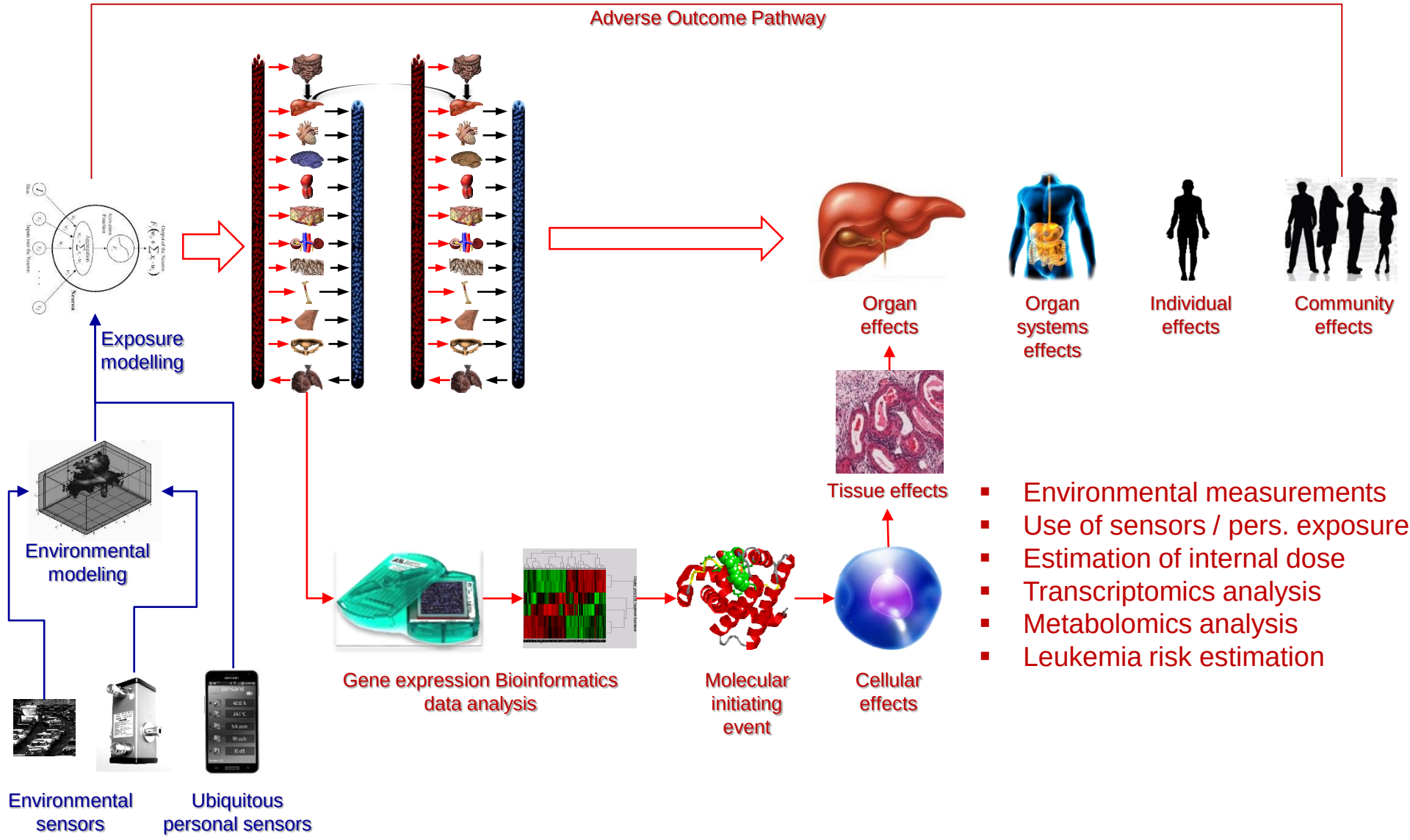
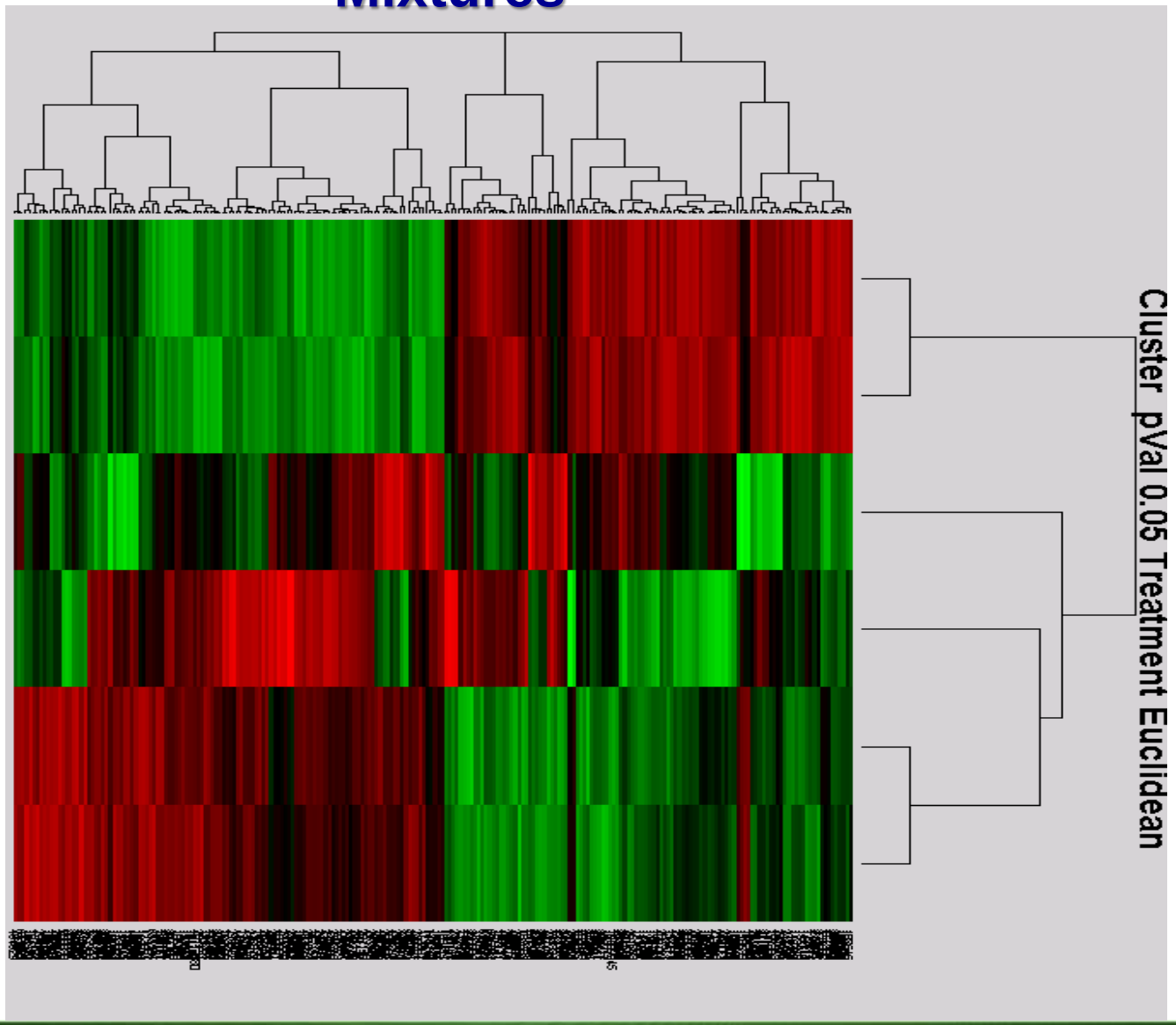


Co-exposure to VOCs



- Environmental measurements
- Use of sensors / pers. exposure
- Estimation of internal dose
- Transcriptomics analysis
- Metabolomics analysis
- Leukemia risk estimation

Comparative Cluster Analysis between Ha-CaT and A549 exposed to Indoor/Ambient Air Mixtures



p53 Pathway: differential modulation of gene expression in A549 cells by Indoor Air Mix and components

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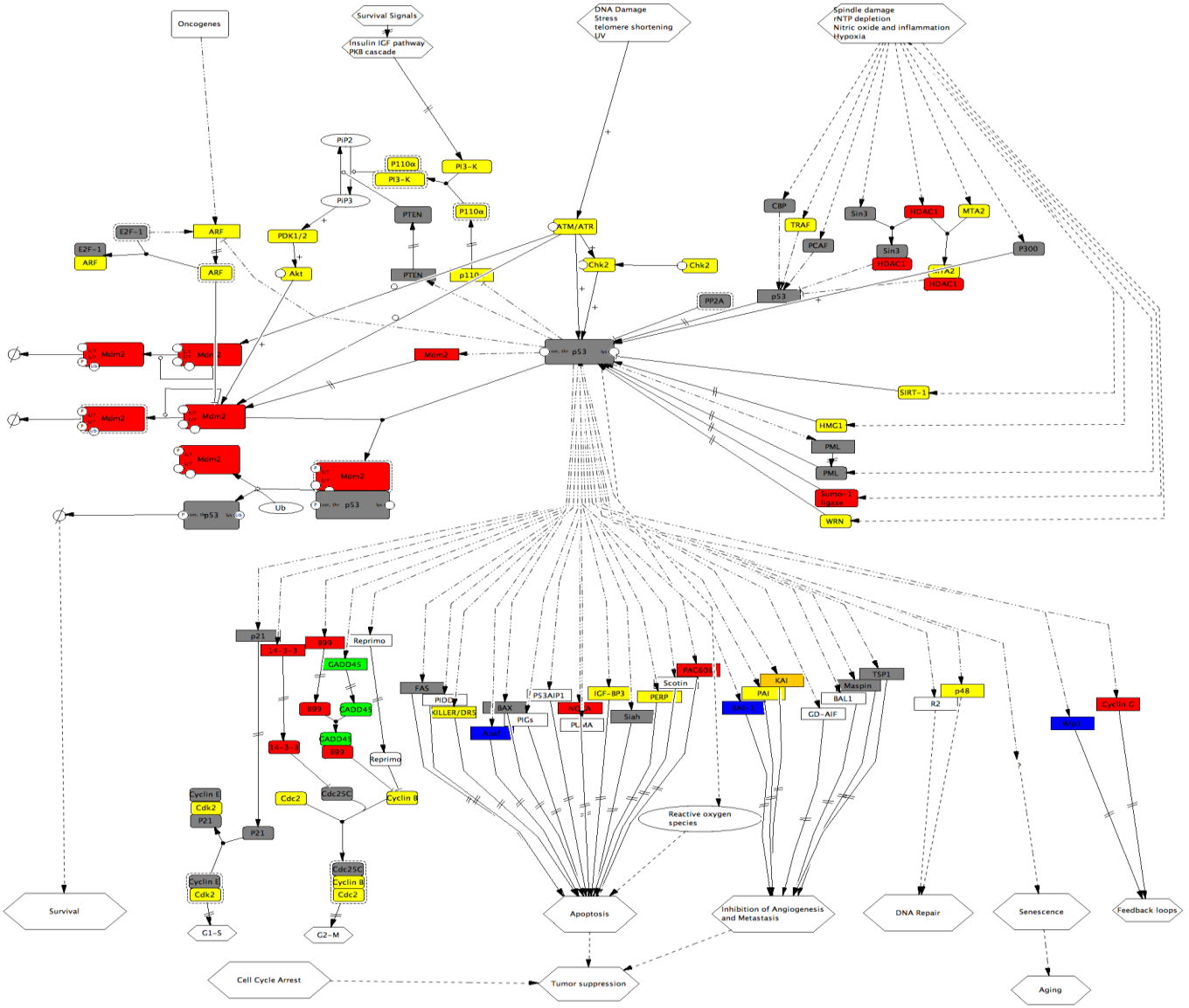
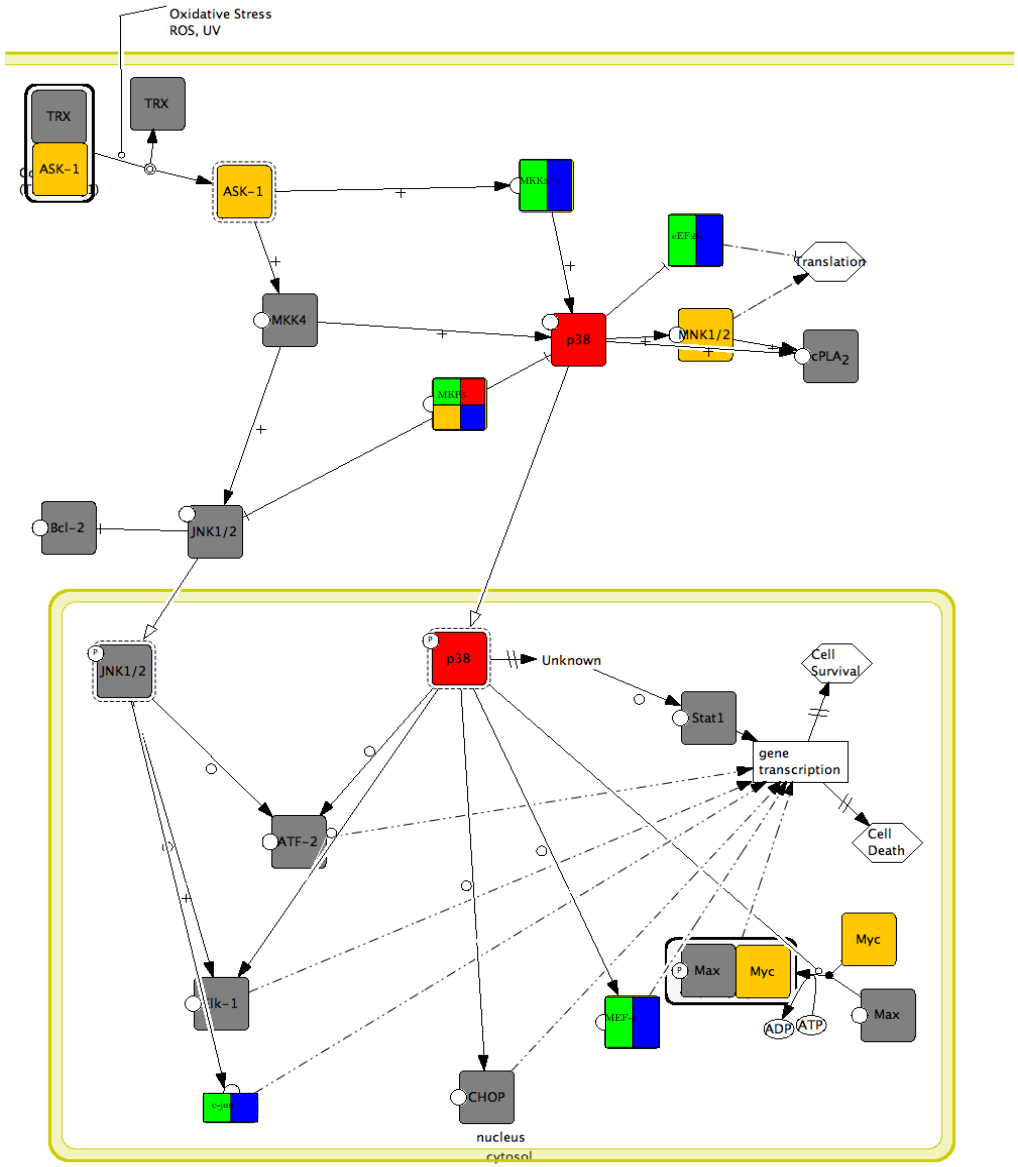
IAM: red
Aromatics: green
Aldehydes: orange
Terpens: blue

Yellow: components in more than one treatment



Oxidative stress pathway



= Indoor Air Mix

= Formaldheyde

= Terpenes

= Aromatics

Transcriptomics responses to chemical mixtures

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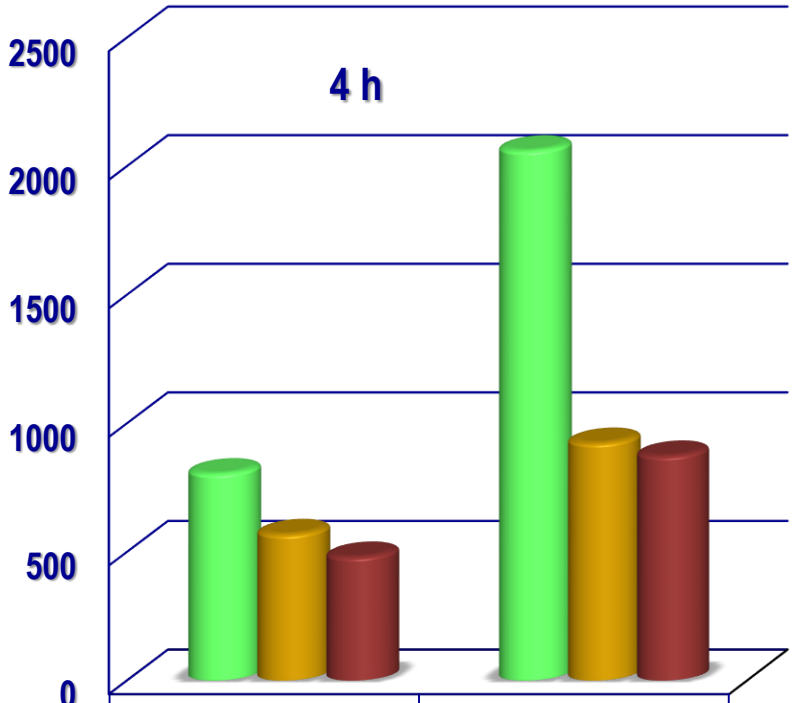
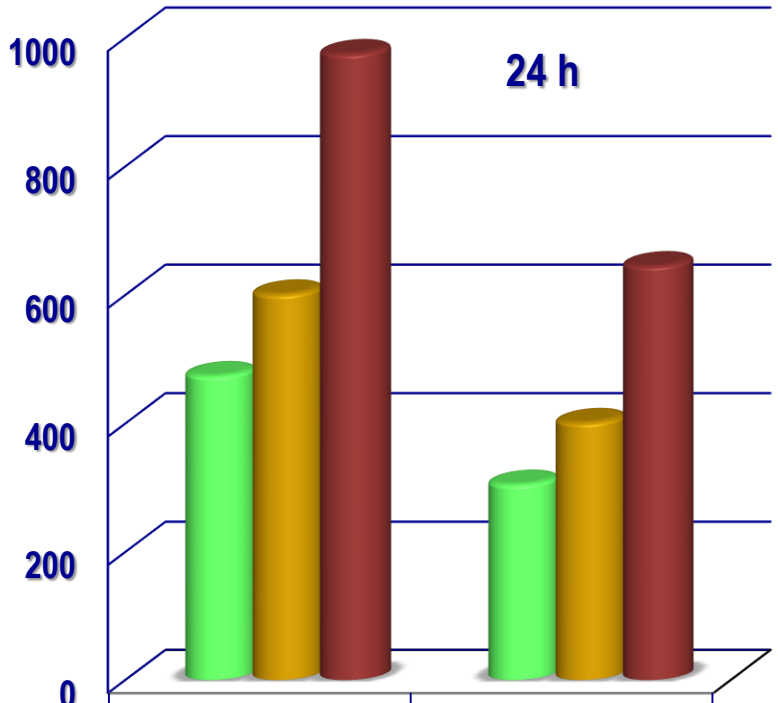


	Mix A	Mix B
10ng 4h	805	2061
100ng 4h	569	924
10ug 4h	481	874



	Mix A	Mix B
10ng 24h	474	305
100ng 24h	601	401
10ug 24h	975	645

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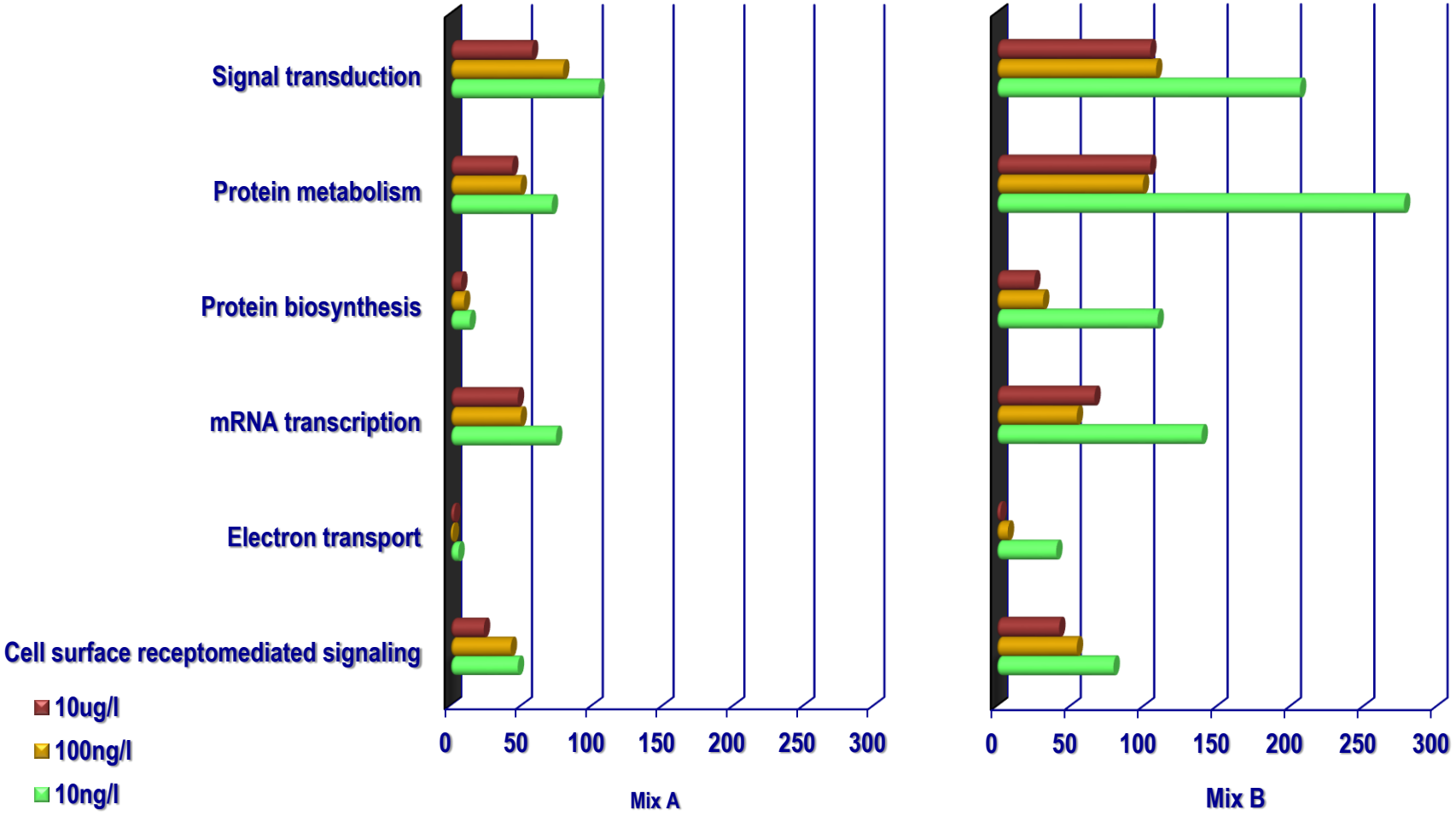
Transcriptomics responses to chemical mixtures



Transcriptomics responses to chemical mixtures

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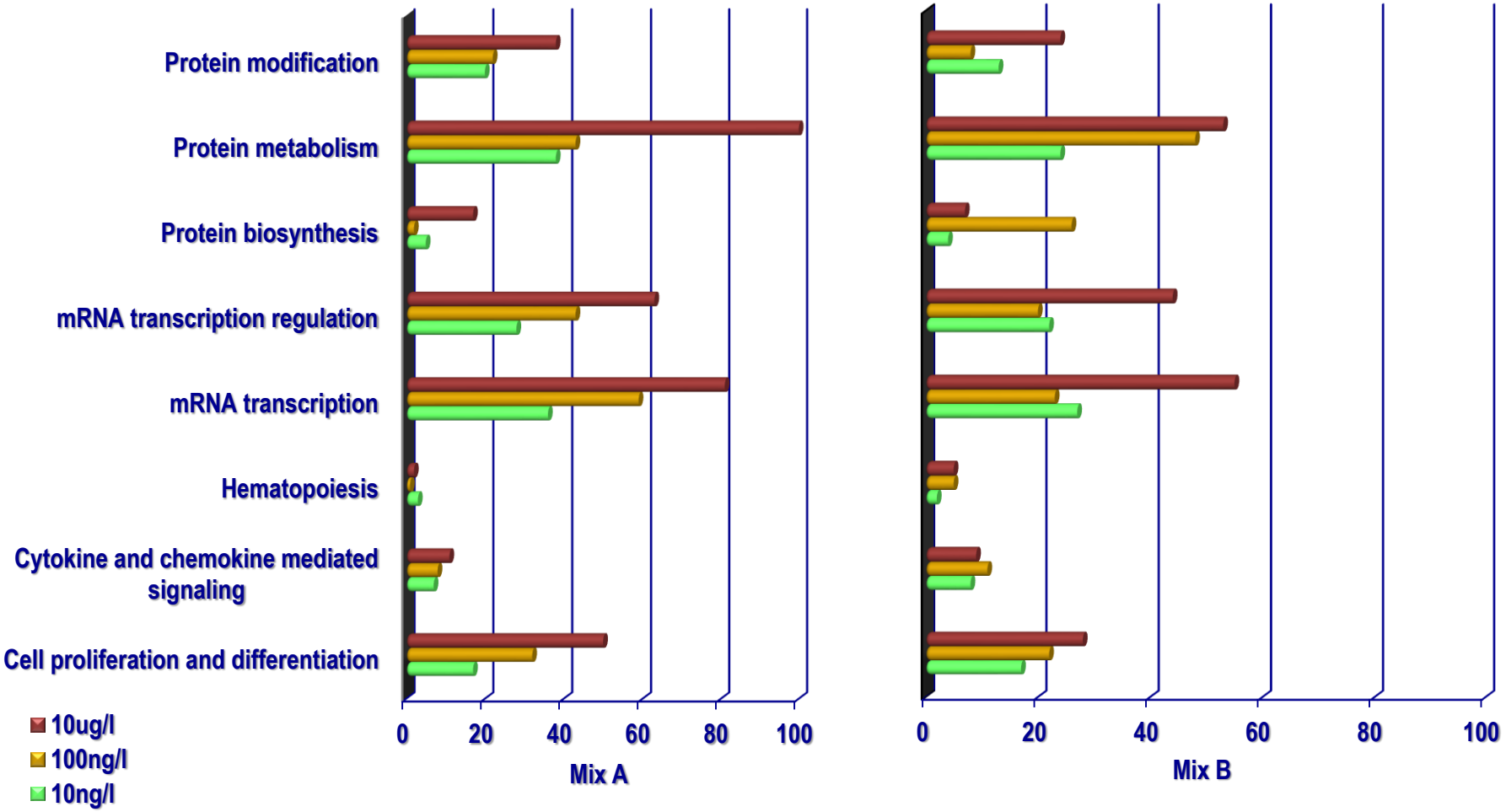

SNPs, micronucleus cells and comet assays

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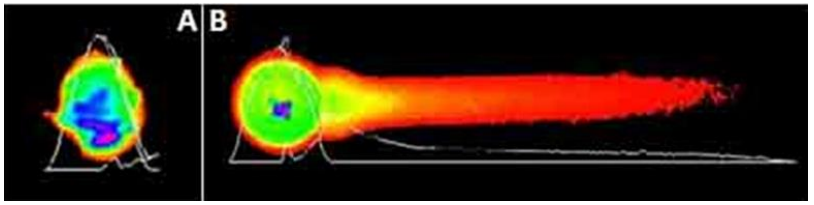
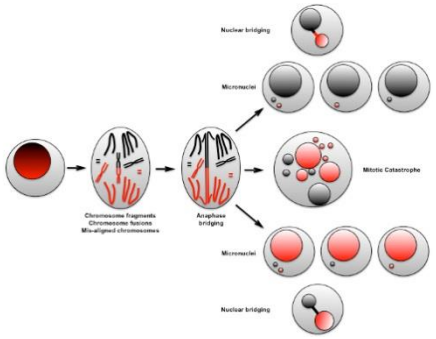
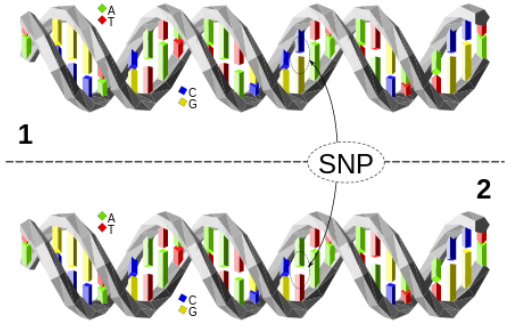
Comet assay to verify the genotoxic damage of the compounds of interest



Micronucleus assay (even from human saliva) quantifying the levels of genotoxic damage in exposed population



SNP profiling of genes affecting the metabolism (CYP1A1 gene, which is highly polymorfic) and genes affecting the response to xenobiotics (NR1I3)



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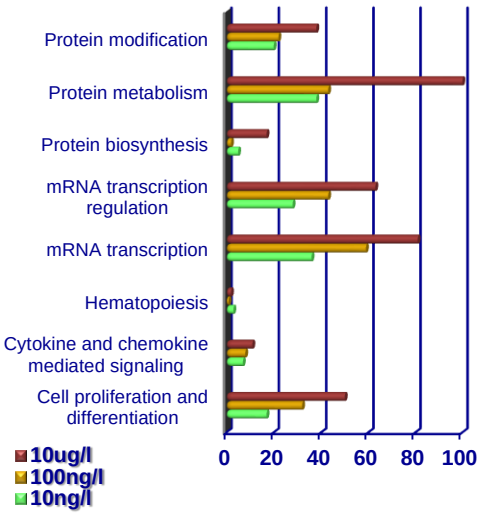
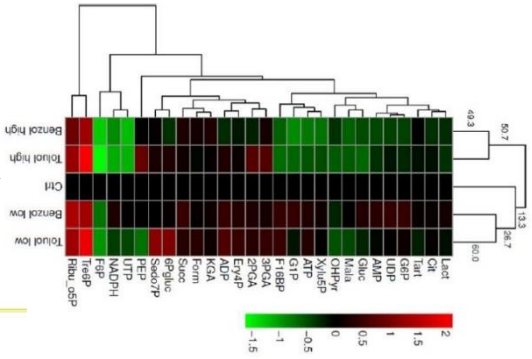
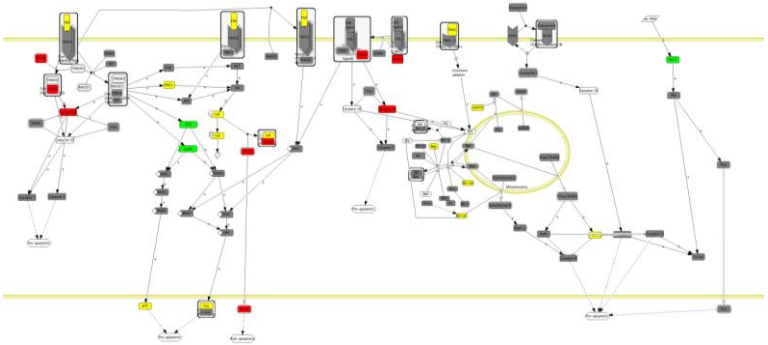
Multi-omics responses and associations

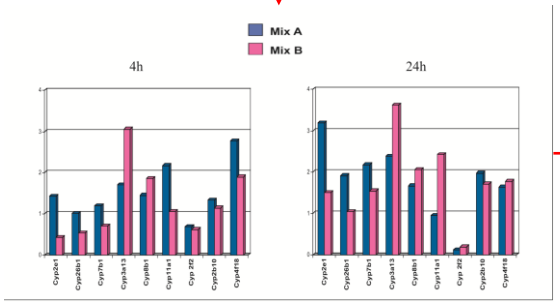


Identification of differentially expressed **proteins**

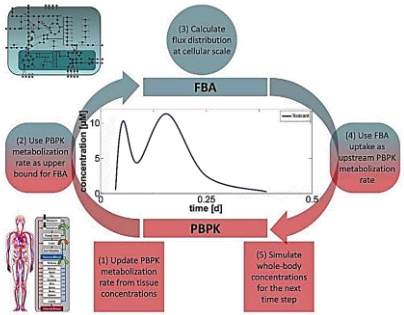


Apoptosis Signaling Pathway

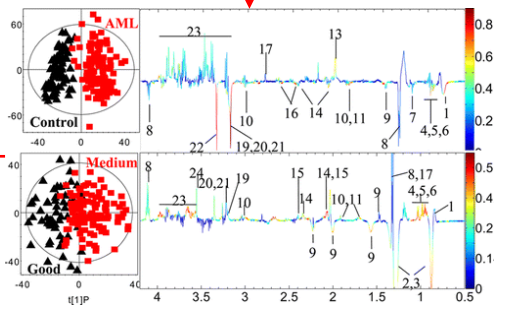




Transcriptomics responses to chemical BTEX mixtures



Extracellular perturbations on metabolic states



Identification of AML **metabolomic** fingerprint

Toxic metabolites in bone marrow concentration modulation

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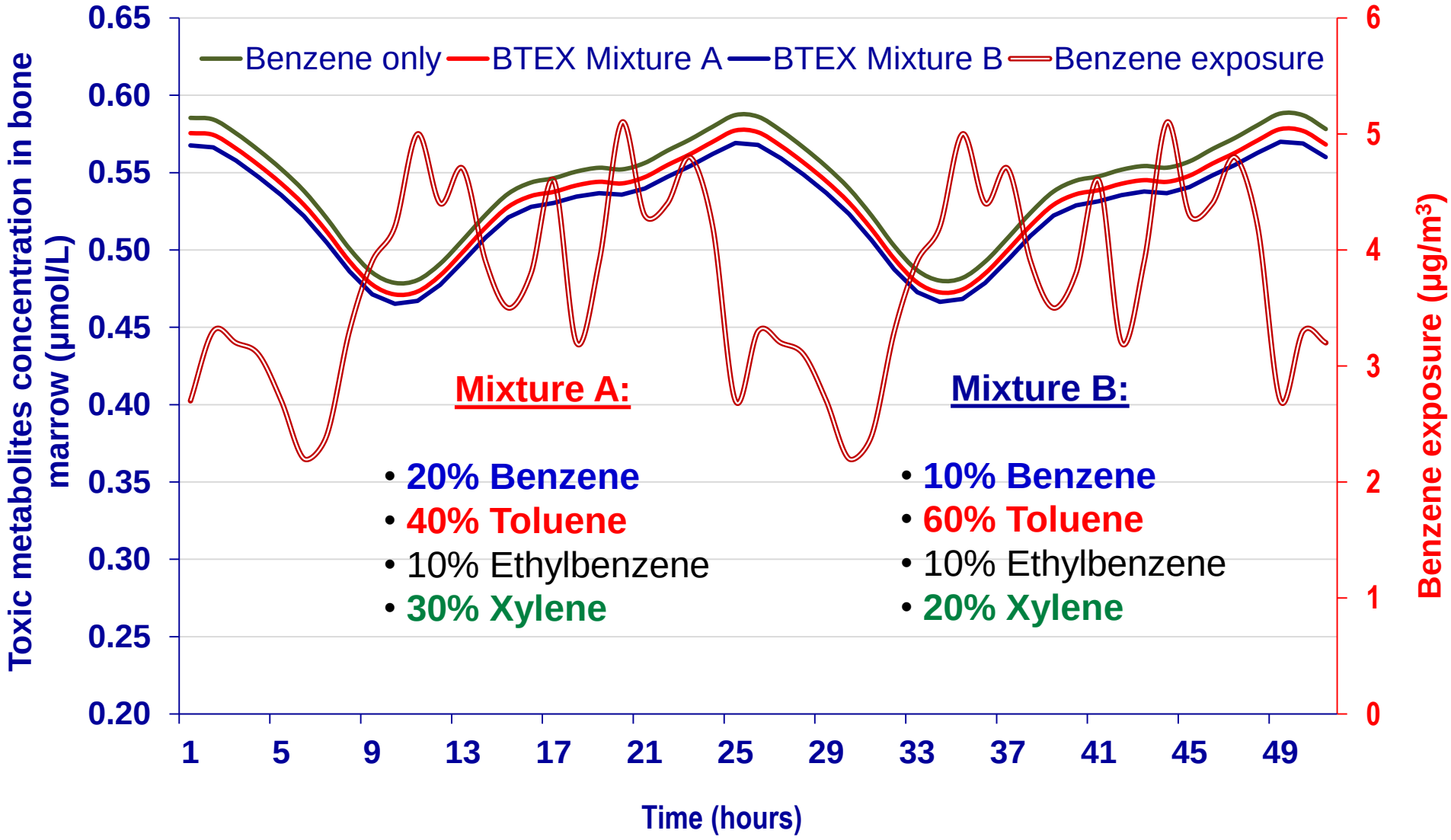
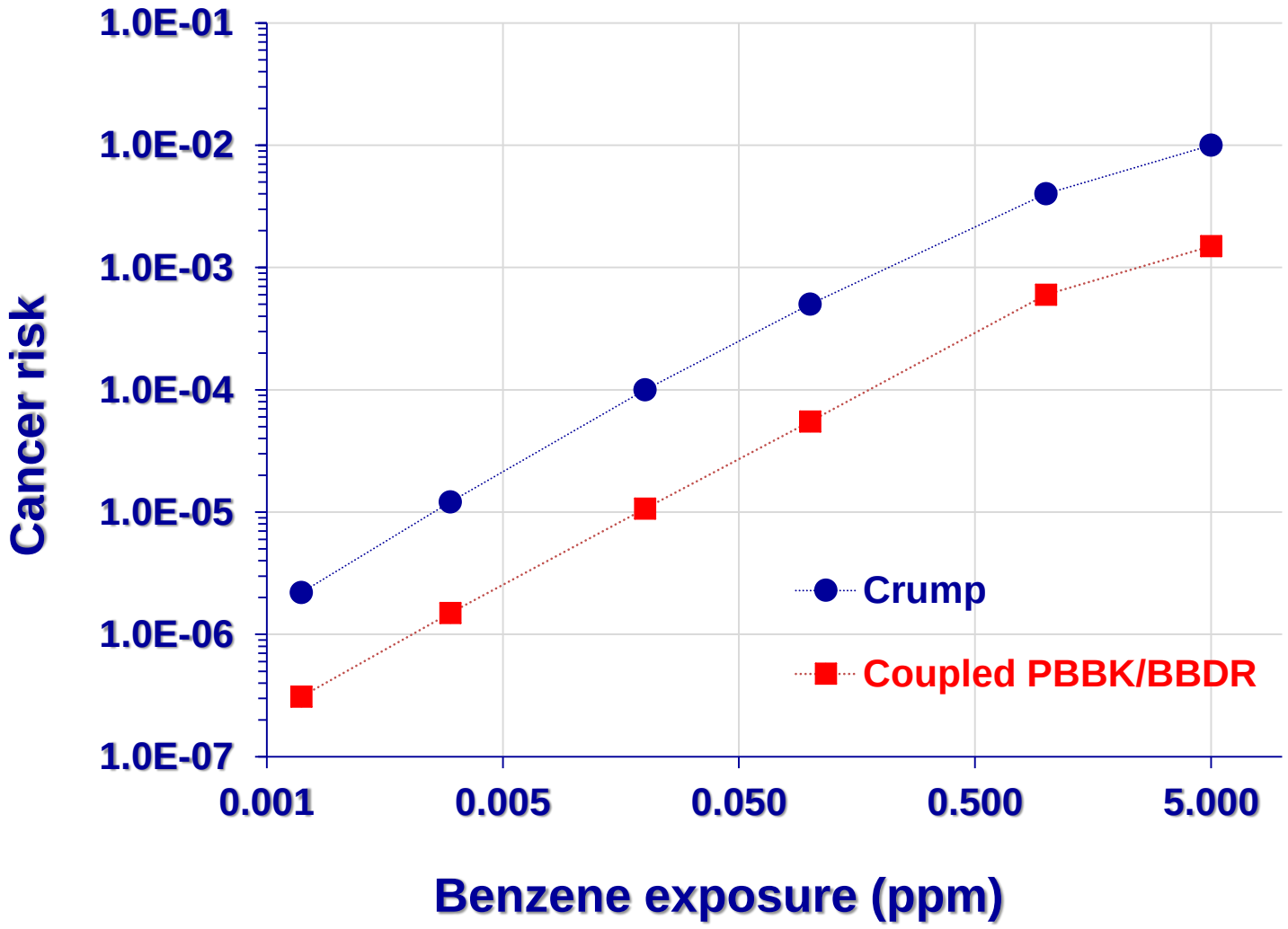
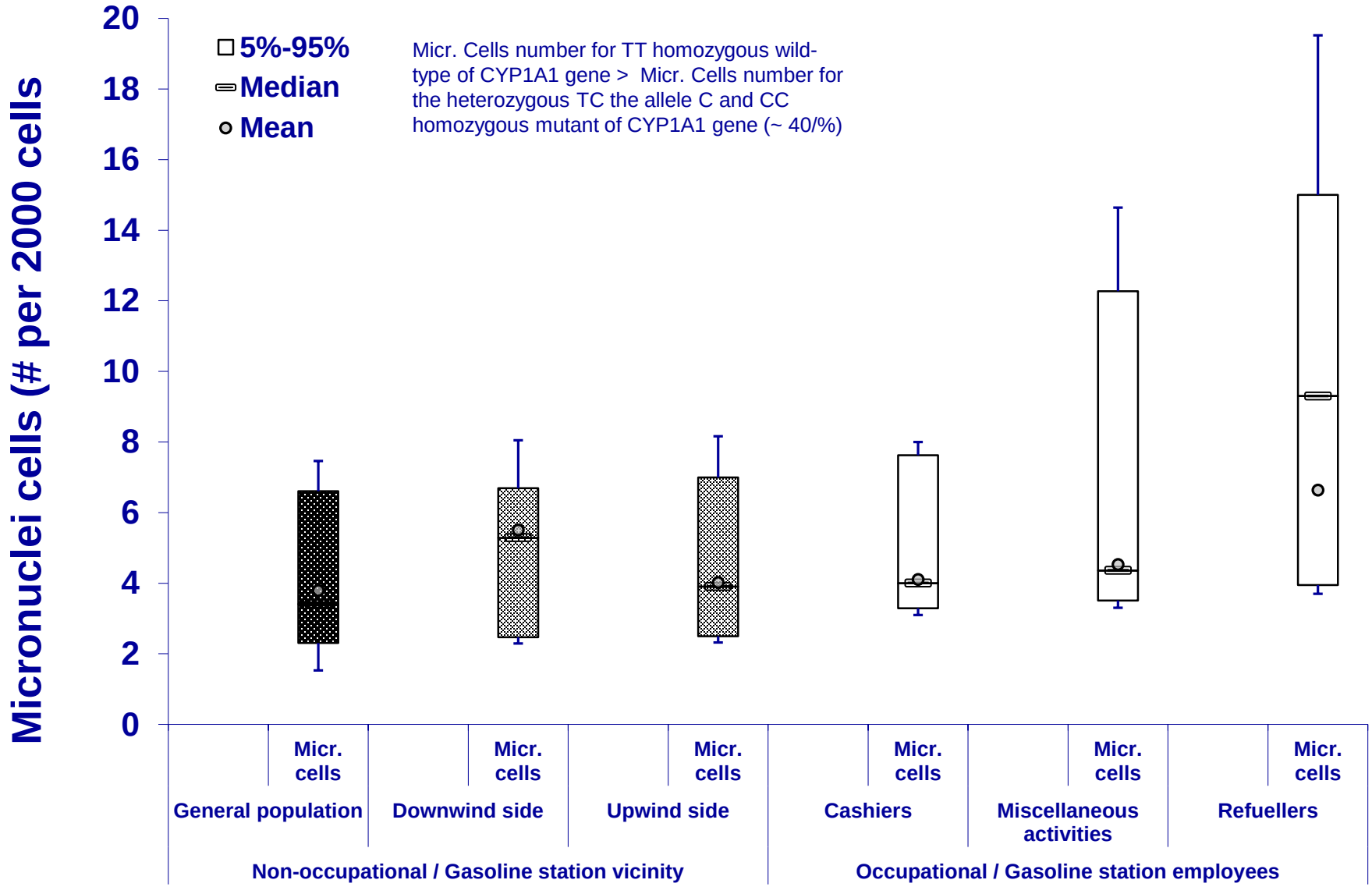


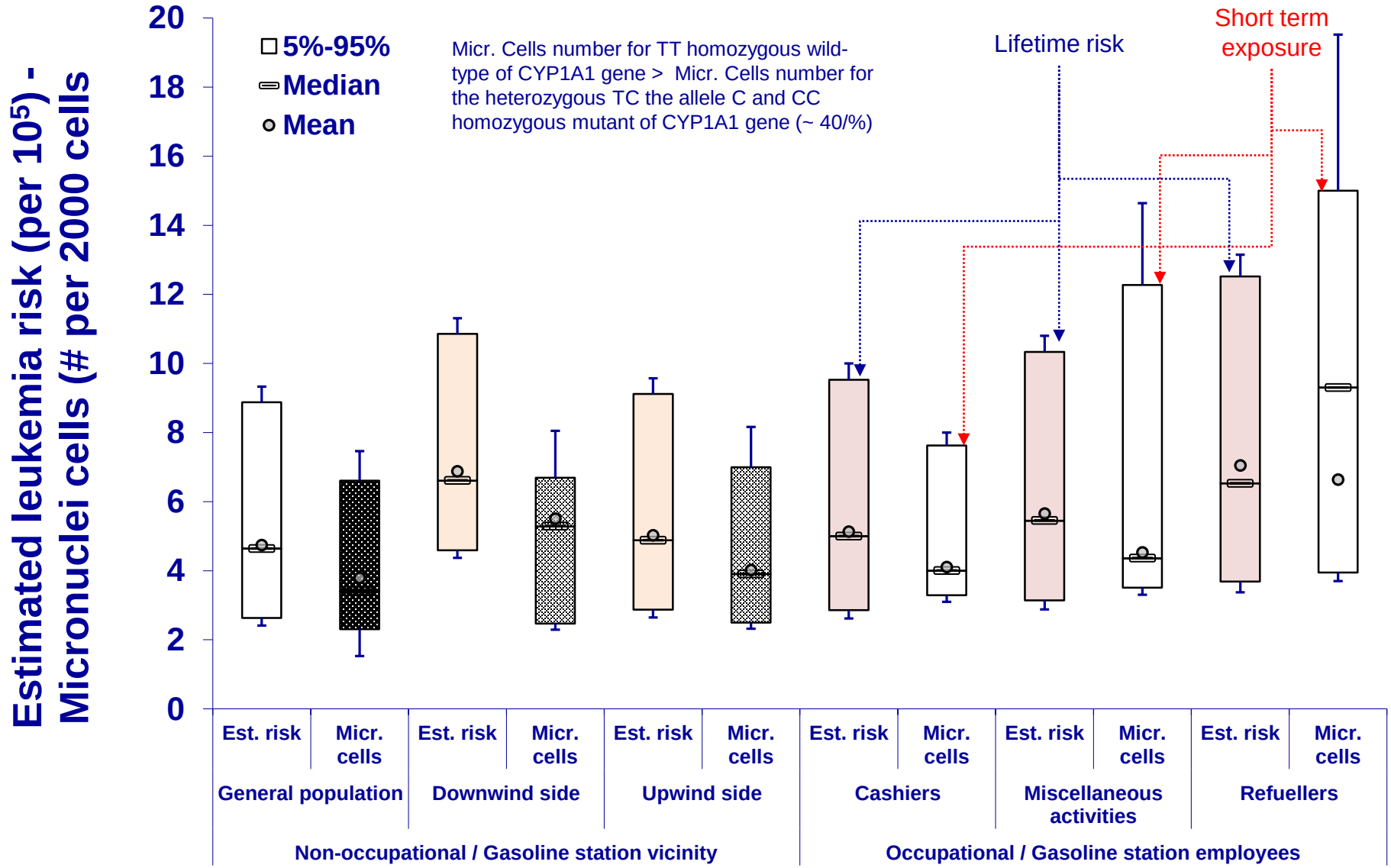
Biology based dose response for benzene



Estimated lifetime leukemia risk



Estimated lifetime leukemia risk



Conclusions

- As possible as accurate assessment of external exposure and translation into internal exposure provides the missing information that is “lumped” in environmental health associations and is a key element for completing genome and omics studies
- We have to better understand the level to which genomics affect health outcomes and incorporate this within a mechanistic framework – to differentiate polymorphisms related to internal dose and the ones related to the response/homeostasis maintenance
- “Functional integration” rather than “association” of all data is required.
- Dynamics in time of the different responses have to be taken into account
- Linking Emissions, Concentrations, Exposure and Internal dose within a “continuous” mathematical framework allows us to couple environmental and biological processes efficiently, validating each step of the way
- Integrated external and internal exposure assessment with a particular focus on tissue dosimetry, allows the use of toxicity testing recent developments of HTS – BPADs and incorporation of additional bio-chemical interactions

Bertold Brecht's *Life of Galileo*:
"*The main objective of science is not to open the door to infinite wisdom but to roll back the boundaries of infinite error*.

Thank you for your kind attention

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A connectivity perspective to environmental health