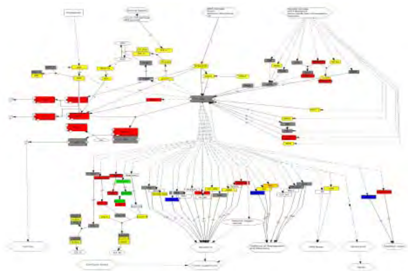
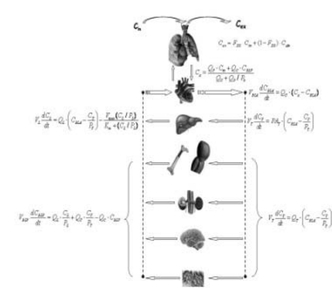
Exposure Science Research at the JRC Institute for Health and Consumer Protection



Dimosthenis A. SARIGIANNIS, PhD

Scientific Coordinator



European Commission - Joint Research Centre, Institute for Health and Consumer Protection, 21027 Ispra (VA)

Policy needs for health and safety data:

Consumer Policy and REACH: need for data on chemical safety of consumer products and on aggregate exposure

Env & Health Action Plan: Address mixture effects/Indoor air

Food safety: safety of chemicals in FCM/foodstuff

Methodological Problems linked to:

- Complexity of exposure pathways
- Cocktail (beyond additive) effect of mixtures
- Dose extrapolation
- Integrated use of exposure data (incl. human epidemiological and biomonitoring data)

JRC involvement

International collaborative research projects:

HEIMTSA (Integrated Health Impact Assessment Toolbox)

2-FUN (Health Risk Assessment for Future Scenarios)

HENVINET (Health and Environment Network)

CAIR4HEALTH (Air quality and Health)

HEREPLUS (Health Risk from Environmental Pollution Levels in Urban Settings)

GENESIS (Generic EU Sustainable Information Space for Environment)

In-house projects:

- Human Exposure Data Centre (with EEA)
- Biology based dose-response modeling
- Toxicogenomics for mixture toxicity assessment and exposure/effect biomarker identification

Challenges for Exposure Science

- Plethora of analytical/monitoring data
- 30-100,000 chemicals in the market

In the European Union:

- REACH introduces exposure-based waiving of toxicity testing
- REACH uses exposure surrogates: market volume p.a.
- Very ambitious time plan for evaluating risk of 30,000 chemicals
- E&H action plan: poses the problem of chemical mixtures and of susceptible population groups

Challenges for Exposure Science

- Adequate support of green chemistry towards the Sustainable Development goals
- Increased public awareness of risks of chemicals
- Need to set priorities for efficient risk assessment of chemicals
- The most plausible avenue/greatest challenge is to link all available data, incl.:
  - environmental
  - human biomonitoring
  - “sentinels”
  - surrogates

Exposure assessment

How we can optimize exposure assessment of chemicals?

A **Holistic Approach** is needed, regarding:

Full chain assessment

- Sources-emissions
- Media concentrations
- Personal exposure
- Internal dose
- Biology Based Dose Response

Methodological tools exploitation

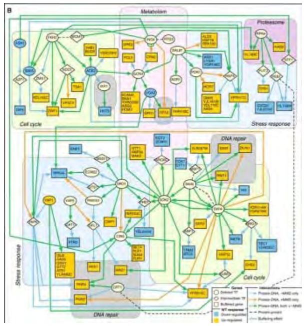
- Measurements data
  - environmental parameters
  - concentrations
  - personal exposure
  - biomonitoring
- Toxicity testing
  - animal data
  - gene expression and other omics
- Epidemiological data
- Clinical data

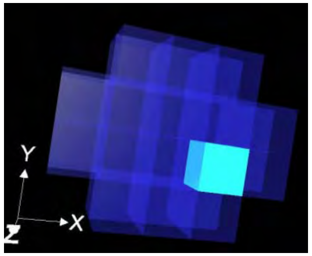
How can we connect all these elements?

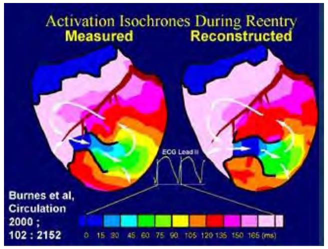
A single-word answer: the Exposome

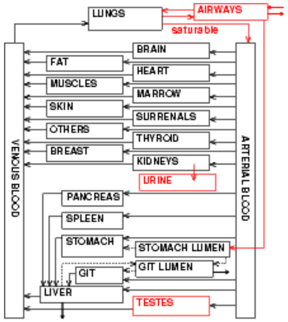
Toward Exposure Biology, through modelling and data assimilation

“Systems Biology” Approach







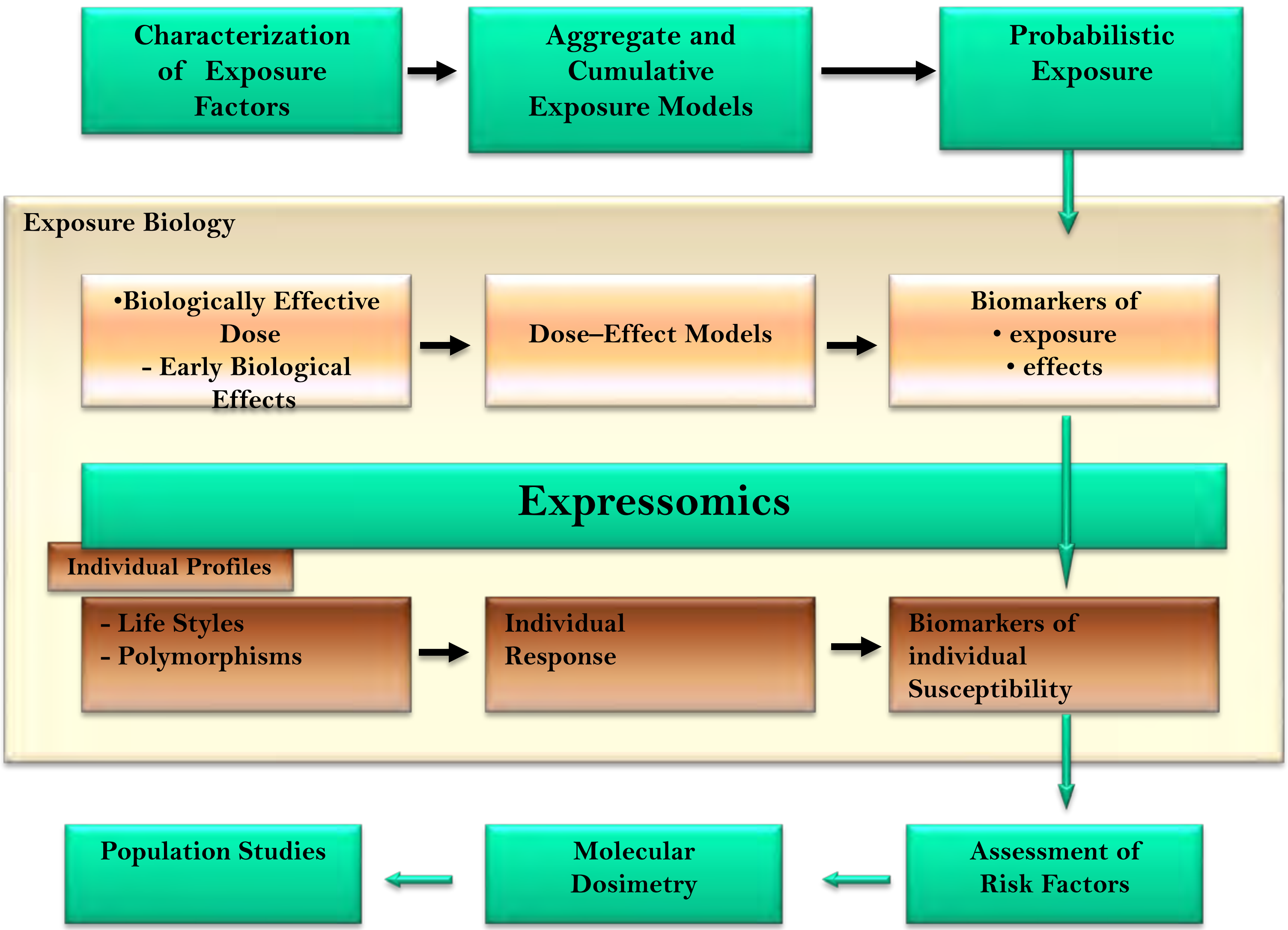


cell organ organism

“Physiome” Approach

Physiologically Based Pharmacokinetic (PBPK) Models

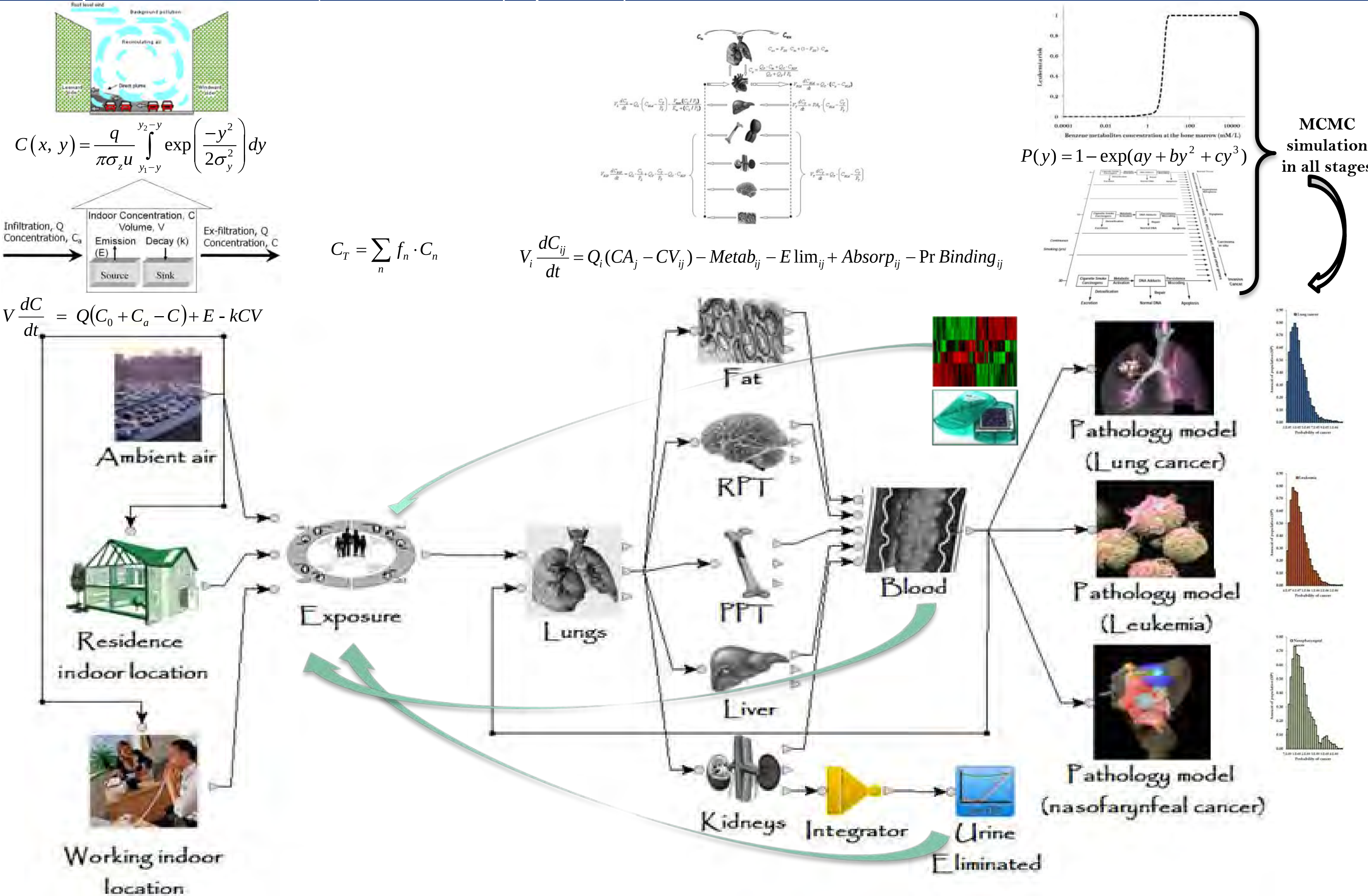
Integrated Multi-layer computational Approach



Tool Development

Full chain exposure assessment

Platform components



$$C(x,y)=\frac{q}{\pi\sigma_z u}\int_{y_1-y}^{y_2-y}\exp\left(\frac{-y^2}{2\sigma_y^2}\right)dy$$

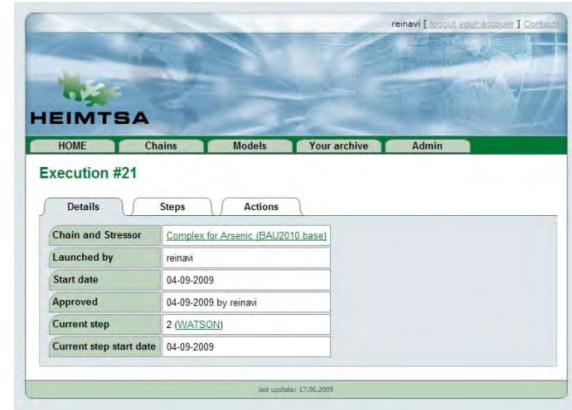
$$V\frac{dC}{dt}=Q(C_0+C_a-C)+E-kCV$$

$$C_T=\sum_n f_n\cdot C_n$$

$$V_i\frac{dC_{ij}}{dt}=Q_i(CA_j-CV_{ij})-Metab_{ij}-E\lim_{ij}+Absorp_{ij}-\Pr Binding_{ij}$$

$$P(y)=1-\exp(ay+by^2+cy^3)$$

Full chain approach-Platform User Interface



Generic PBTK model

General formula describing ADME:

Absorption

Distribution

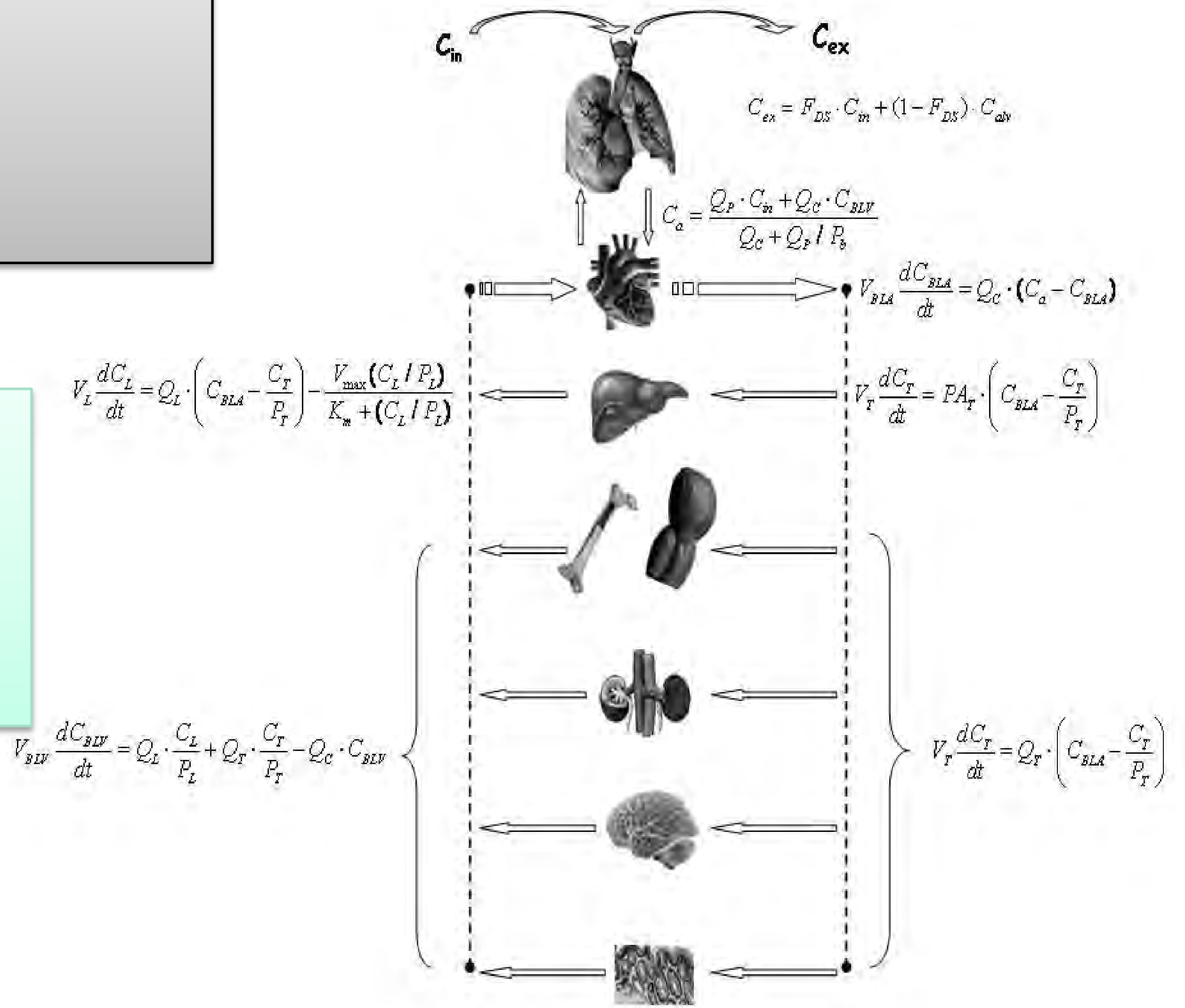
Metabolism

Elimination

$$V_i \frac{dC_{ij}}{dt} = Q_i(CA_j - CV_{ij}) - Metab_{ij} - E\lim_{ij} + Absorp_{ij} - \Pr Binding_{ij}$$

Tissue characteristics that affect the internal concentrations are:

- **Blood flow**
- **Perfusion**
- **Protein binding**
- **Metabolic and elimination activity**



Expressomics for the Exposome

Experimental Design

Tissues RNA
Mice, Rats, Humans

Whole Genome Discovery
Systems
(32.000 genes)

Gene Identification

Validation by Quantitative PCR

Statistical Evaluation

Genes Modulation
Genes Classification
Genes Pathway

Integrated approach
with
Proteomics/Metabonomics

Biomarkers and
Systems Toxicology
Models

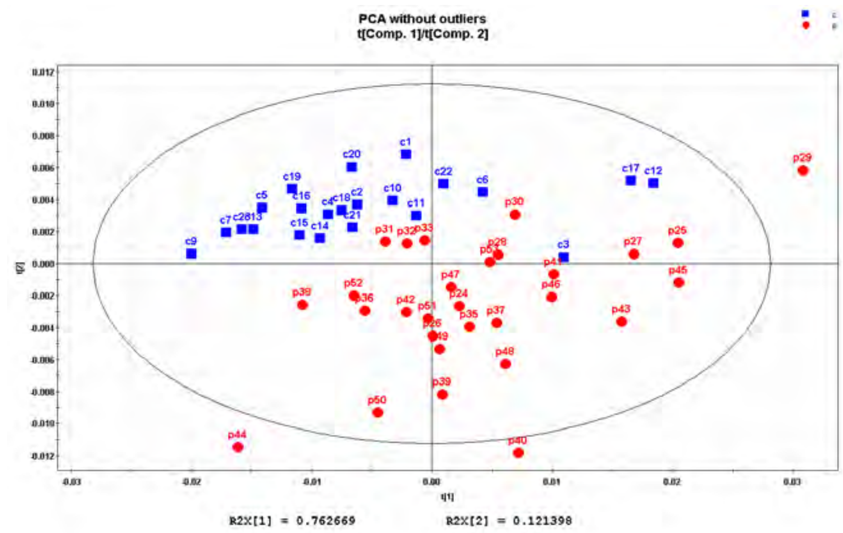
Environment and Health
Signature of chemicals in products
Implementation of Risk Assessment

BIOINFORMATICS

Addressing Variability and Uncertainty

Human Biomonitoring

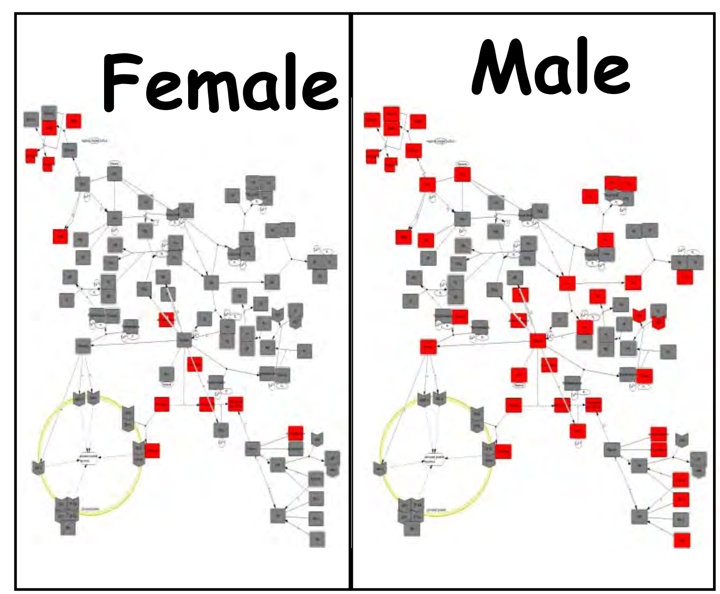
Early diagnosis of cardiovascular disease associated with exposure to chemicals through analysis of metabolites can be used for easy, non-invasive monitoring of health effect indicators



Biomarkers of exposure and effects

The difference in susceptibility to chemical exposure between males and females was demonstrated by analysis of the whole genome in cell lines and tissues exposed to mixtures of chemicals

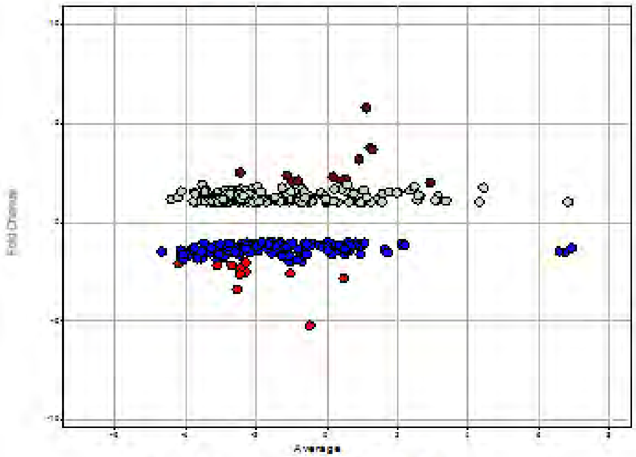
By identifying the difference in gene expression we can have early warning about anticipated health effects



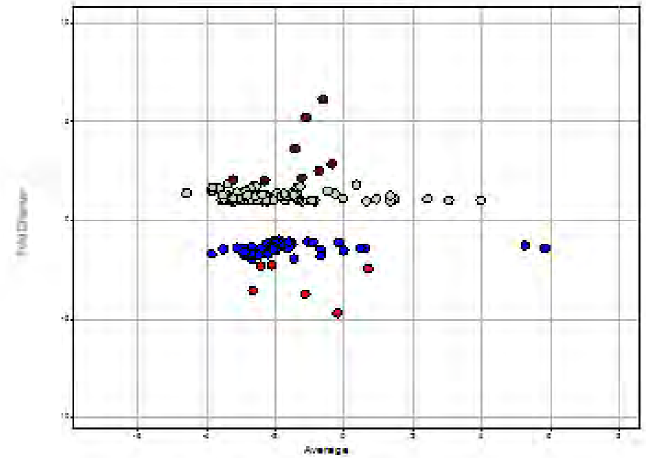
Chemical Mixtures – Cumulative Exposure

Chemical mixtures: molecular fingerprinting

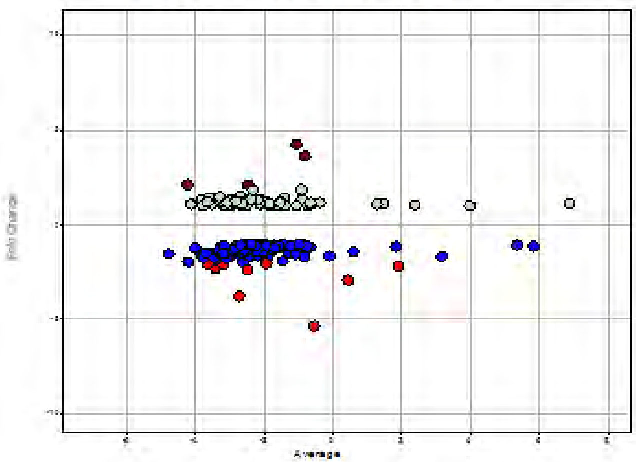
Milan Outdoor Air Mix



Fly Ash

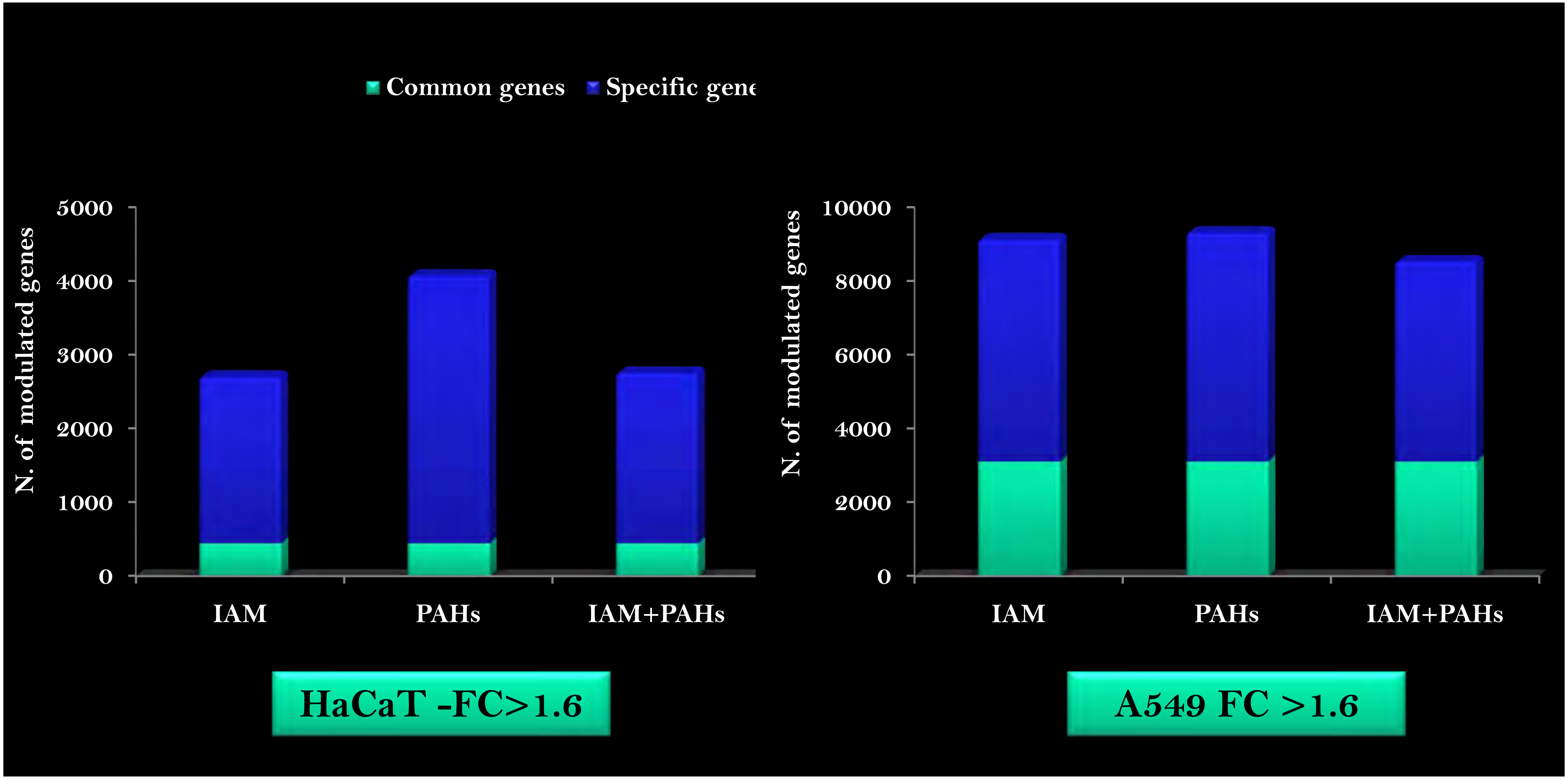


Indoor Air Mix

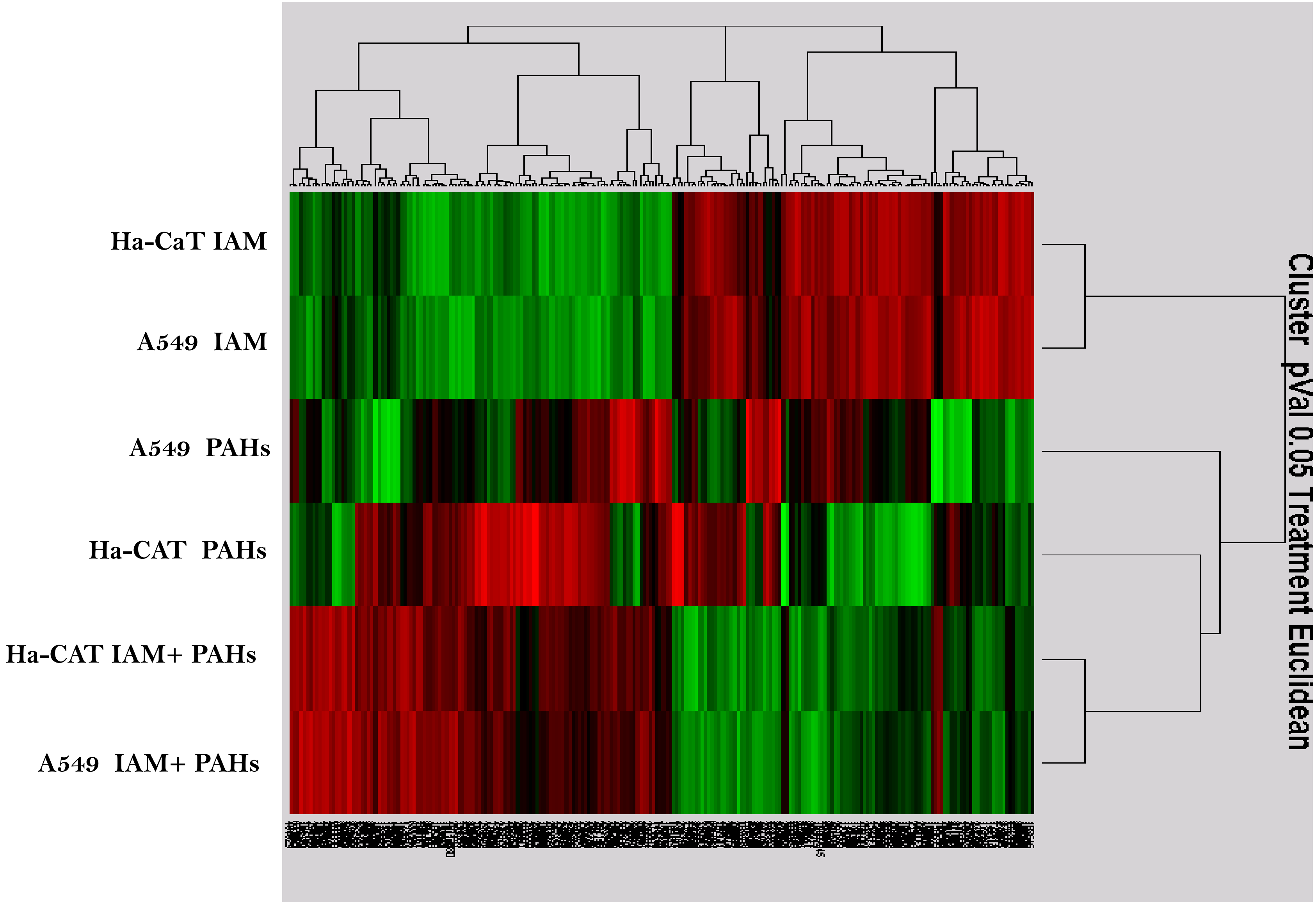


Regulated genes, FC: ± 2

	total genes	up	down
Milan Air Mix	376	209	167
Fly Ash	145	92	53
Indoor Air Mix	214	66	148


Common genes
Specific gene
N. of modulated genes
5000
4000
3000
2000
1000
0
IAM
PAHs
IAM+PAHs
HaCaT -FC>1.6
N. of modulated genes
10000
8000
6000
4000
2000
0
IAM
PAHs
IAM+PAHs
A549 FC >1.6

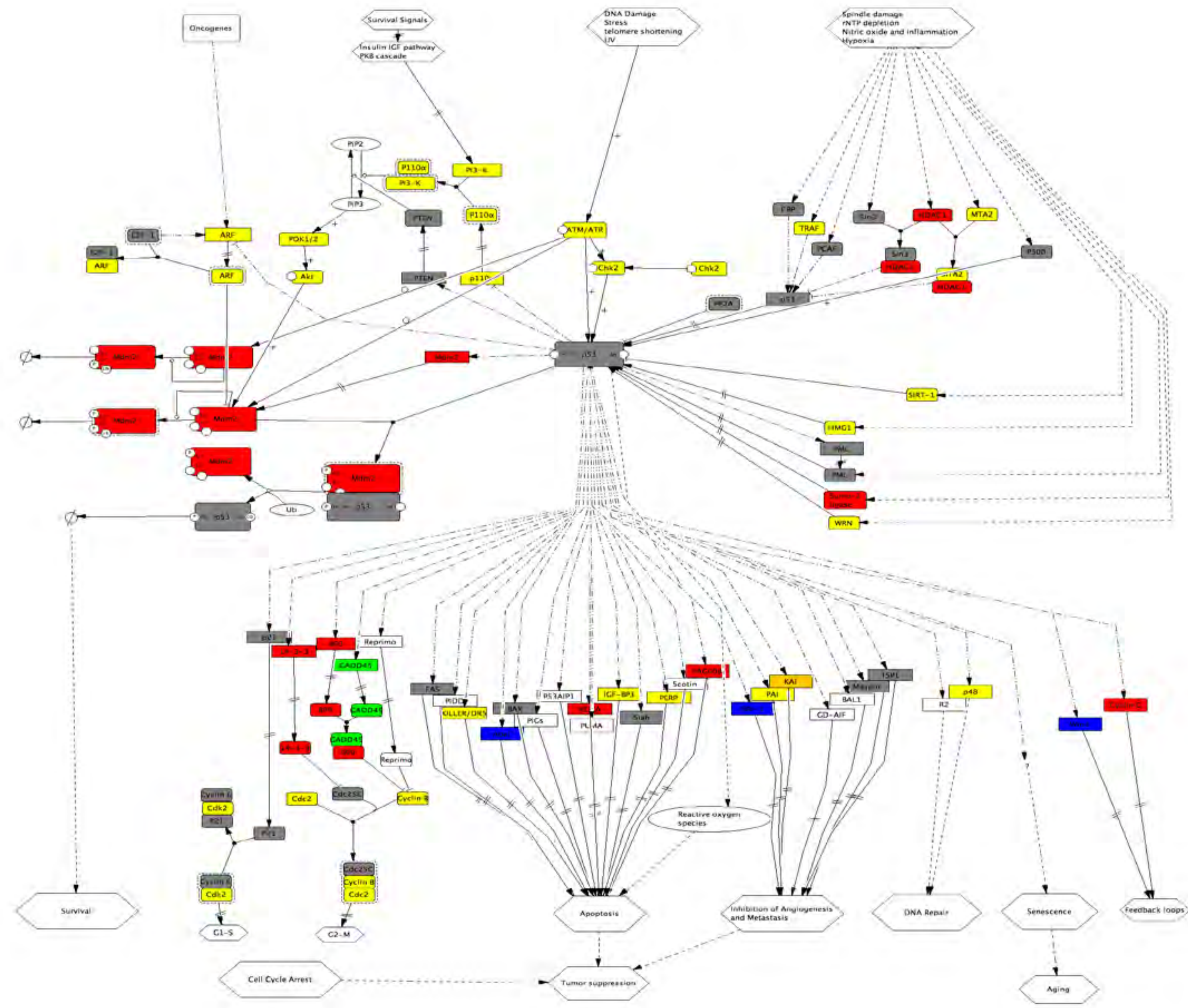
Comparative Cluster Analysis between Ha-CaTand A549 exposed to Air Mixtures



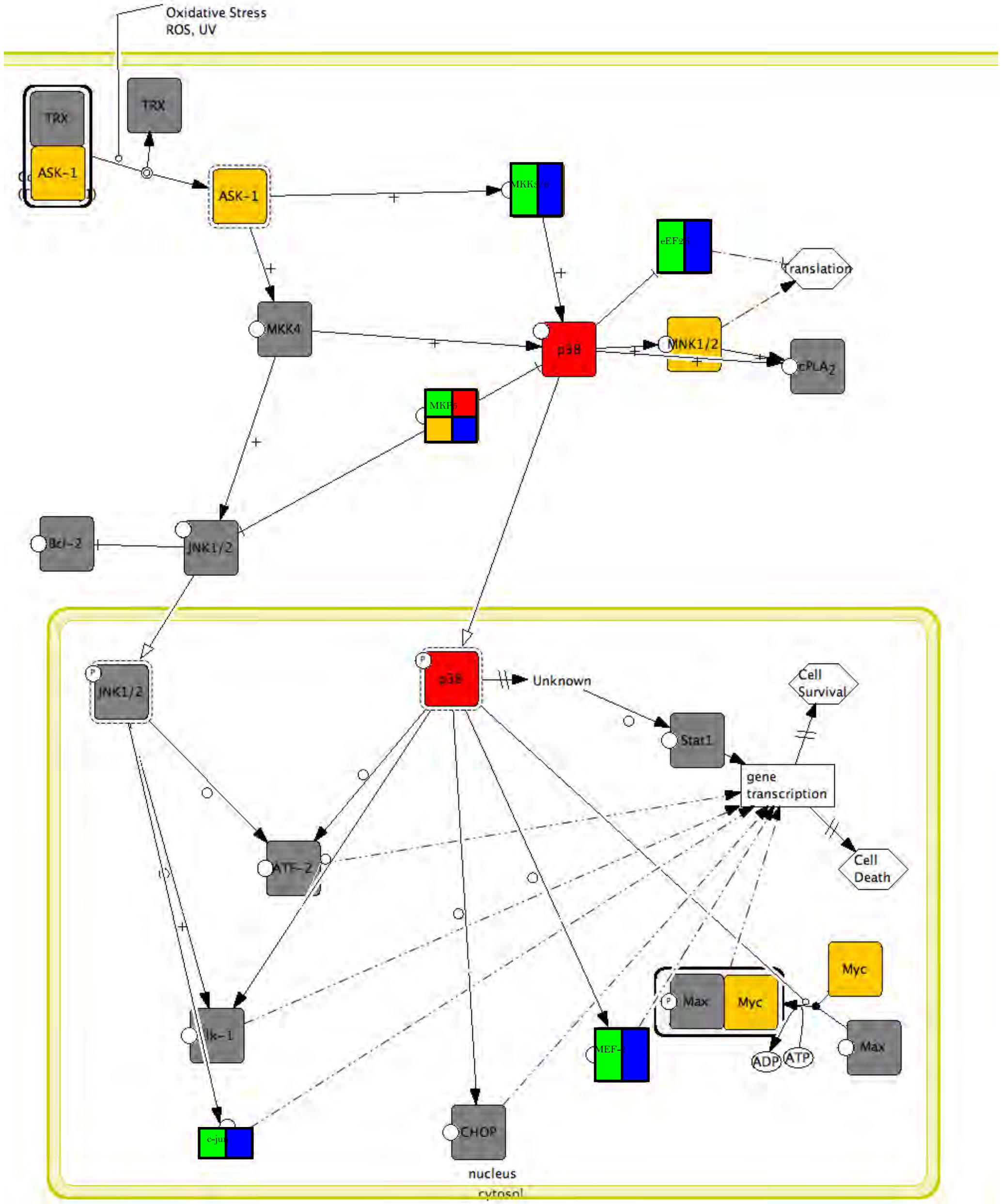
p53 Pathway: differential modulation of gene expression in A549 cells by Indoor Air Mix and components

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

Yellow*: components in more than one treatment*



Oxidative stress pathway



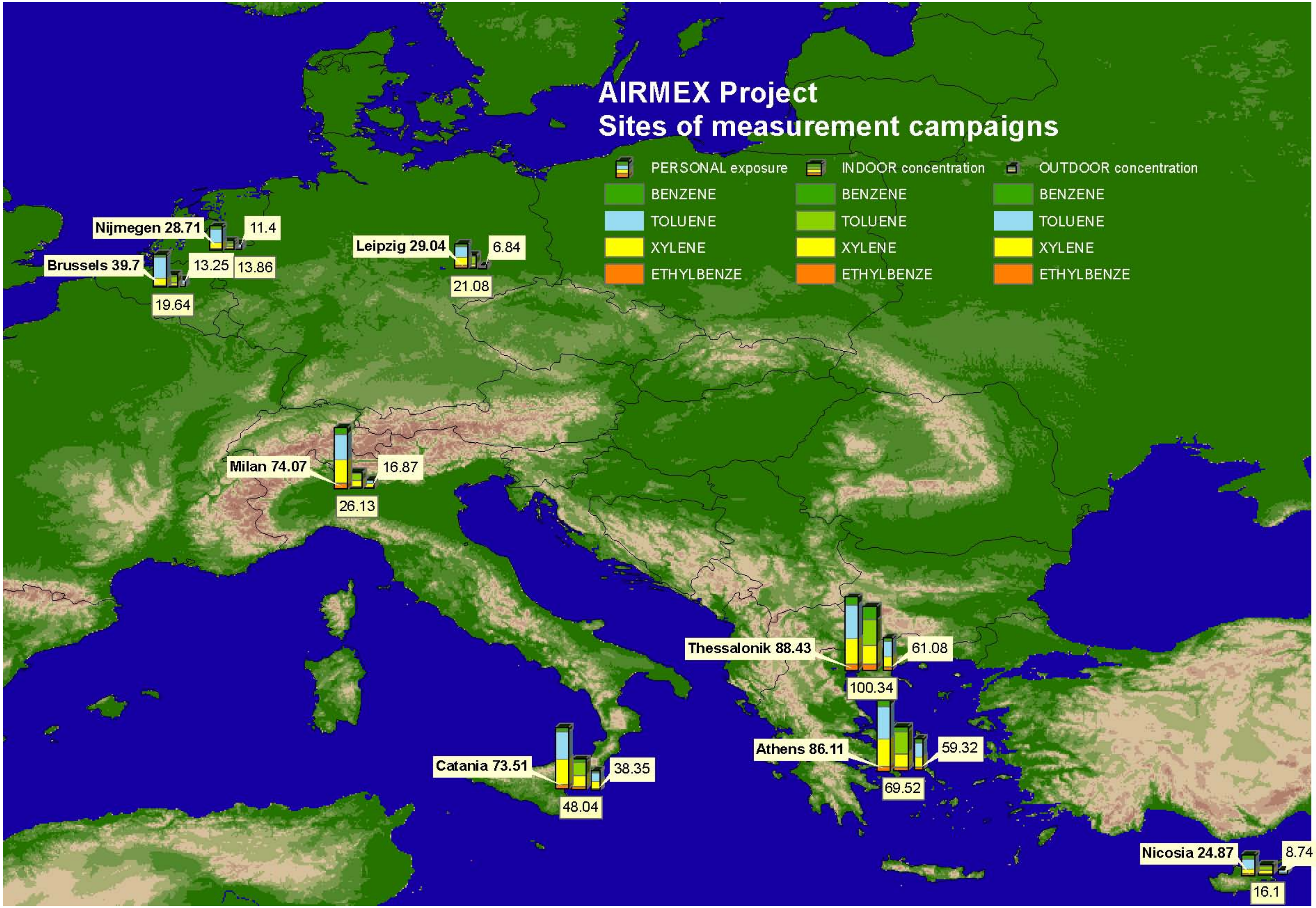
= Indoor Air Mix

= Formaldheyde

= Terpenes

= Aromatics

Environmental exposure: in-/outdoor/personal



BTEX *“in vitro” experiments*

- **Cells: A549**
- **Time of exposure: 4h and 24 h**
- **Doses: 10ng/L, 100ng/L, 10ug/L**

BTEX *“in vivo” experiments*

- **Tissue: Mouse Lung after i.tr.**
- **Time of exposure: 4h and 24 h**
- **Dose: 10 ug/L (=250ng/Kg)**

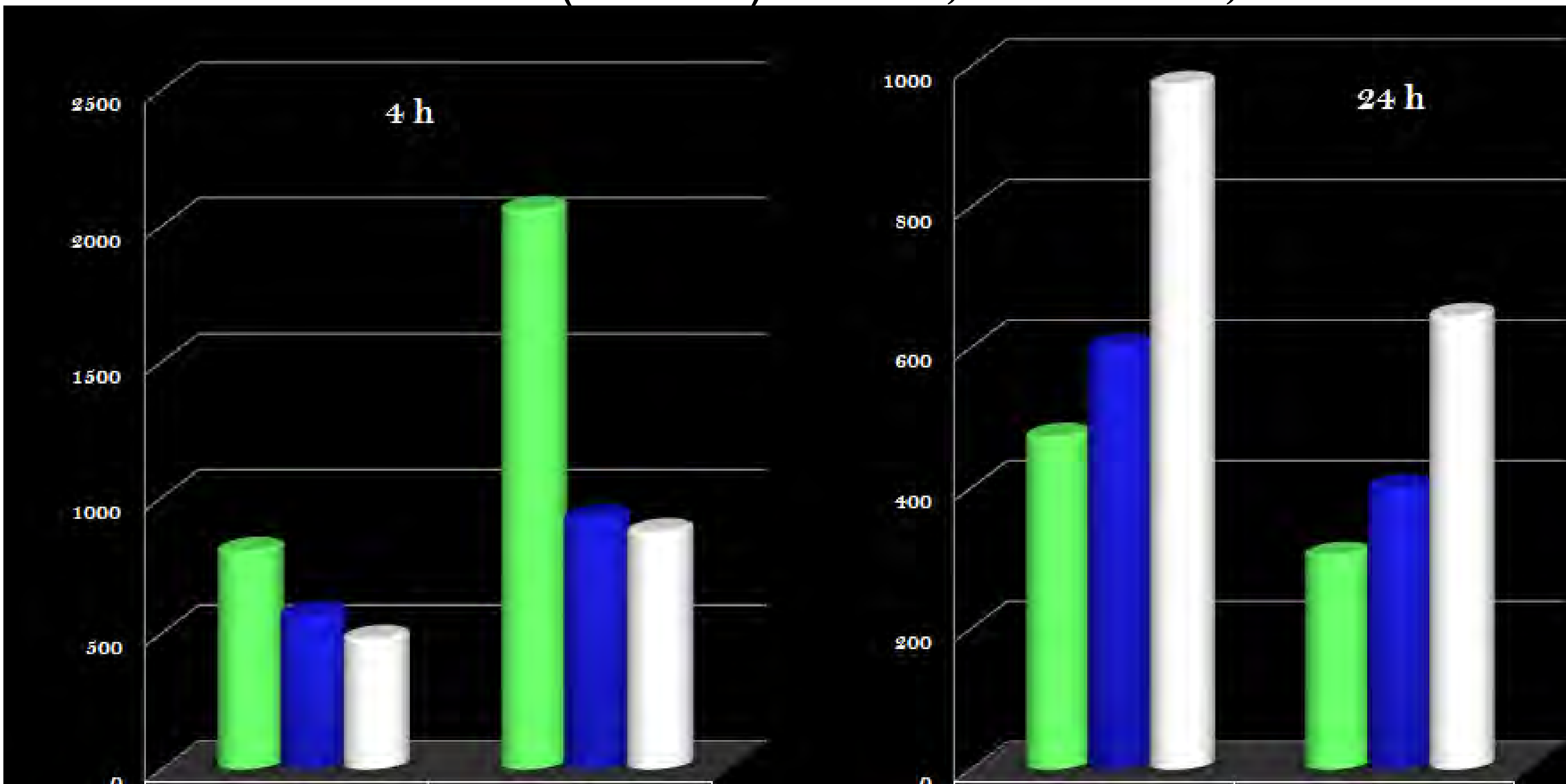
Mixture A:

- **20% Benzene**
- **40% Toluene**
- 10% Ethylbenzene
- **30% Xylene**

Mixture B:

- **10% Benzene**
- **60% Toluene**
- 10% Ethylbenzene
- **20% Xylene**

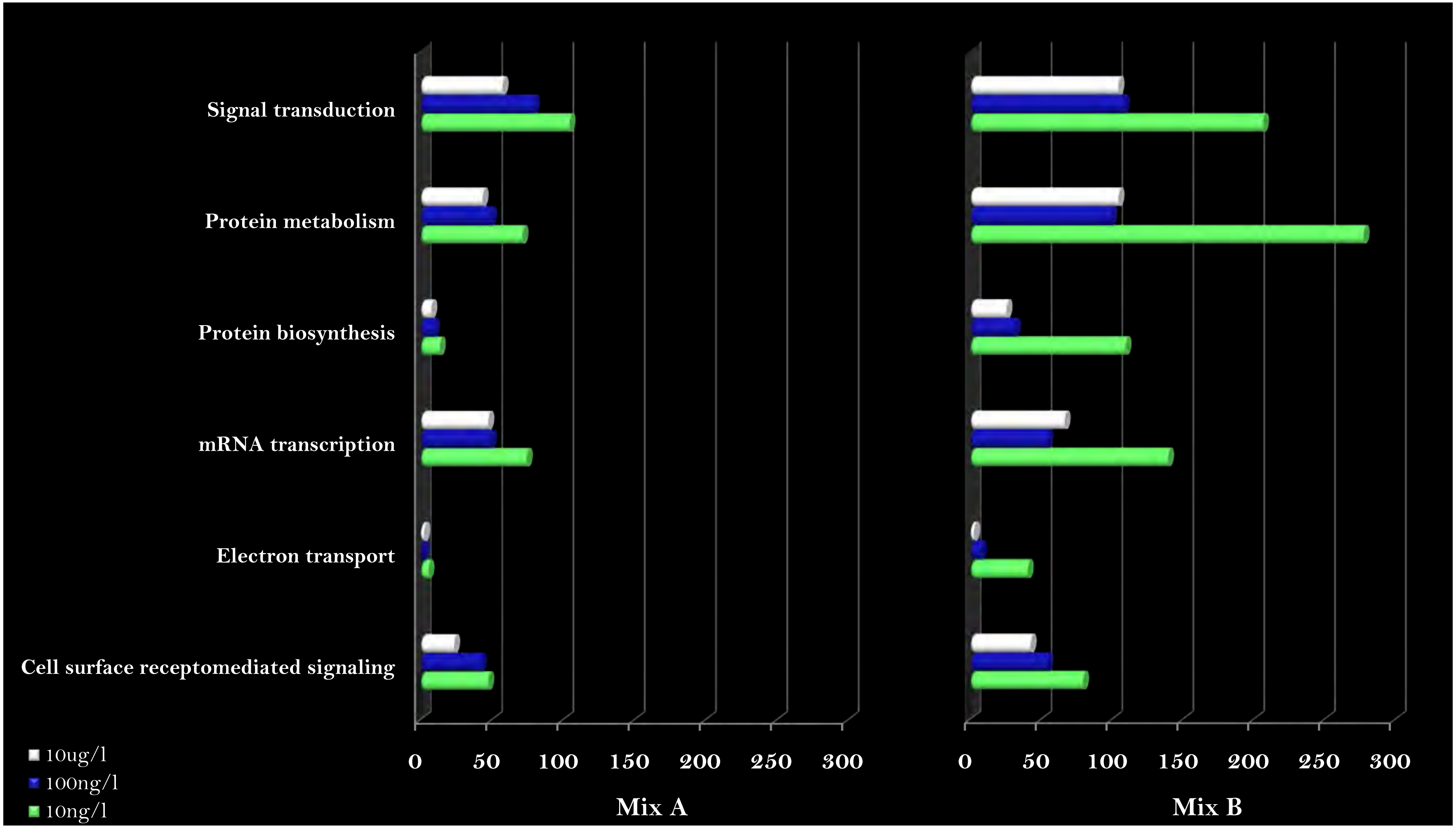
Number of modulated genes by different mixtures (A and B) in A549, 4h and 24 h, 2FC



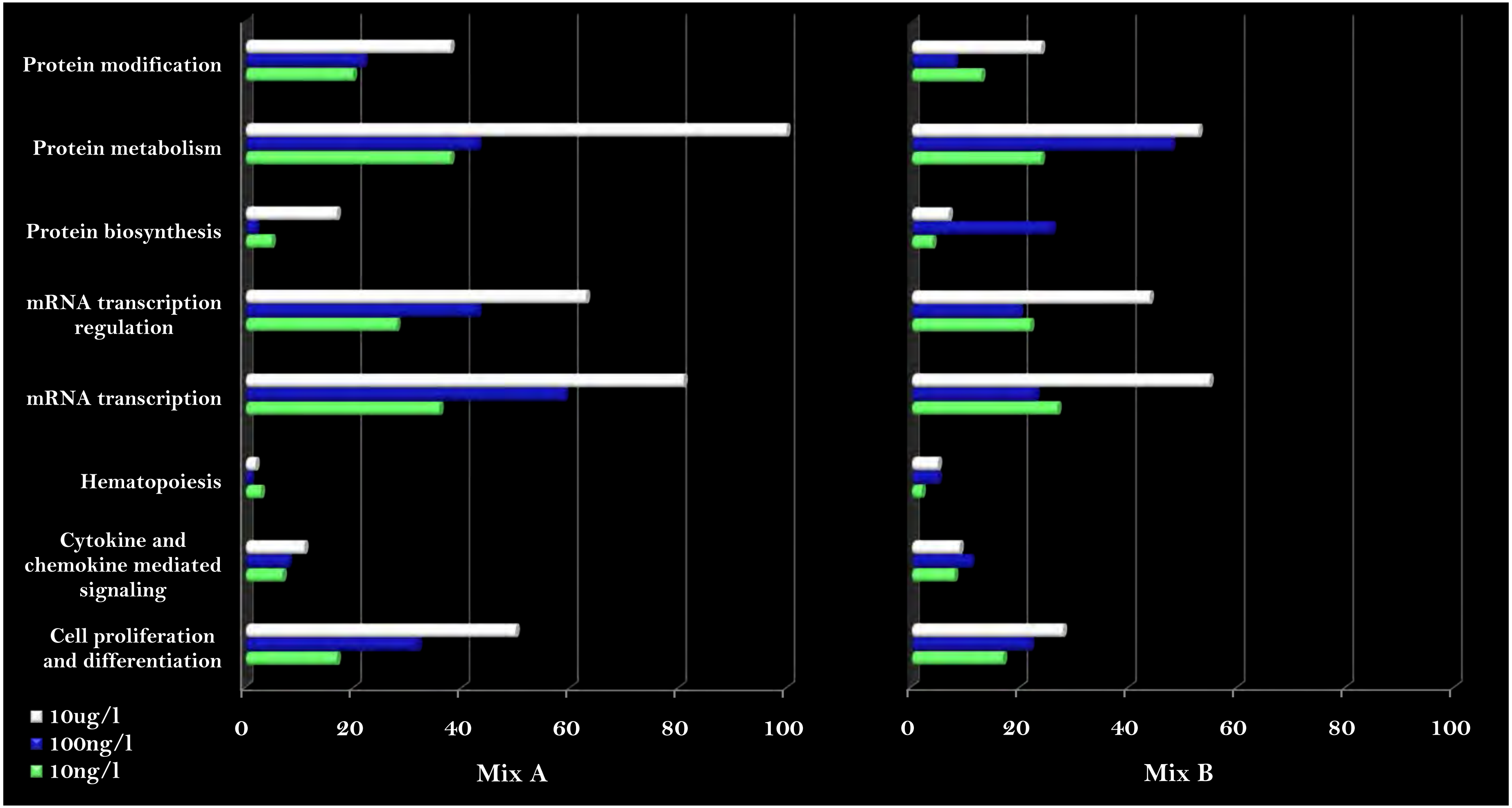
	Mix A	Mix B
10ng 4h	805	2061
100ng 4h	569	924
10ug 4h	481	874

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

Biological processes (pvalue ≤0.01), BTEX in A549 cells, Mix A and Mix B, 4h

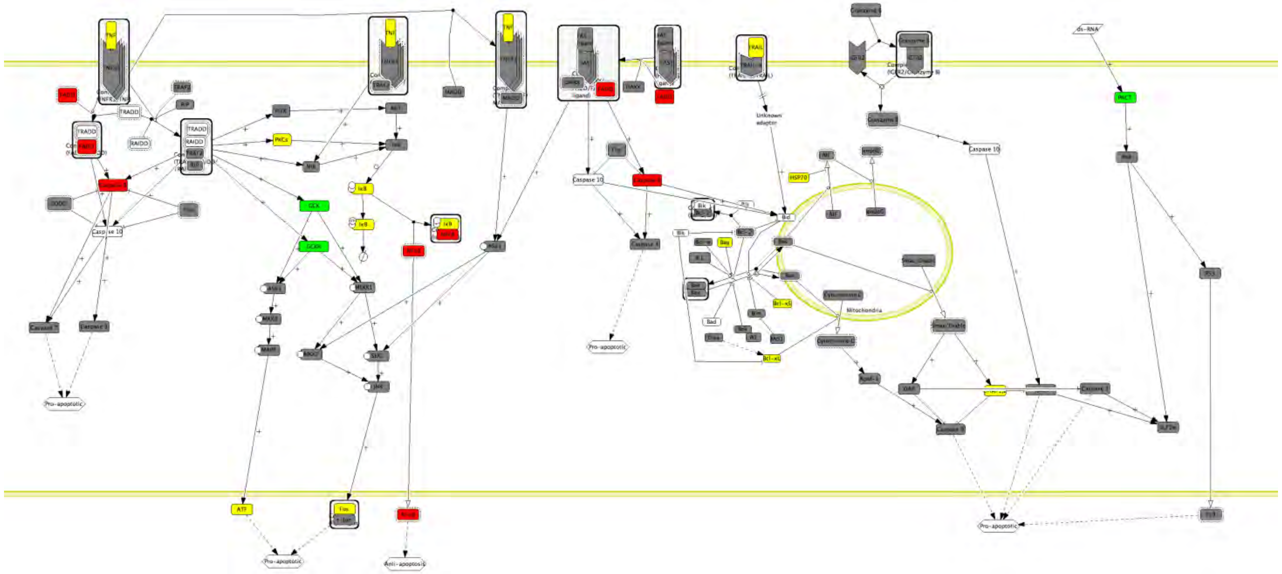


Biological processes (pvalue ≤0.01), BTEX in A549 cells, Mix A and Mix B, 24h

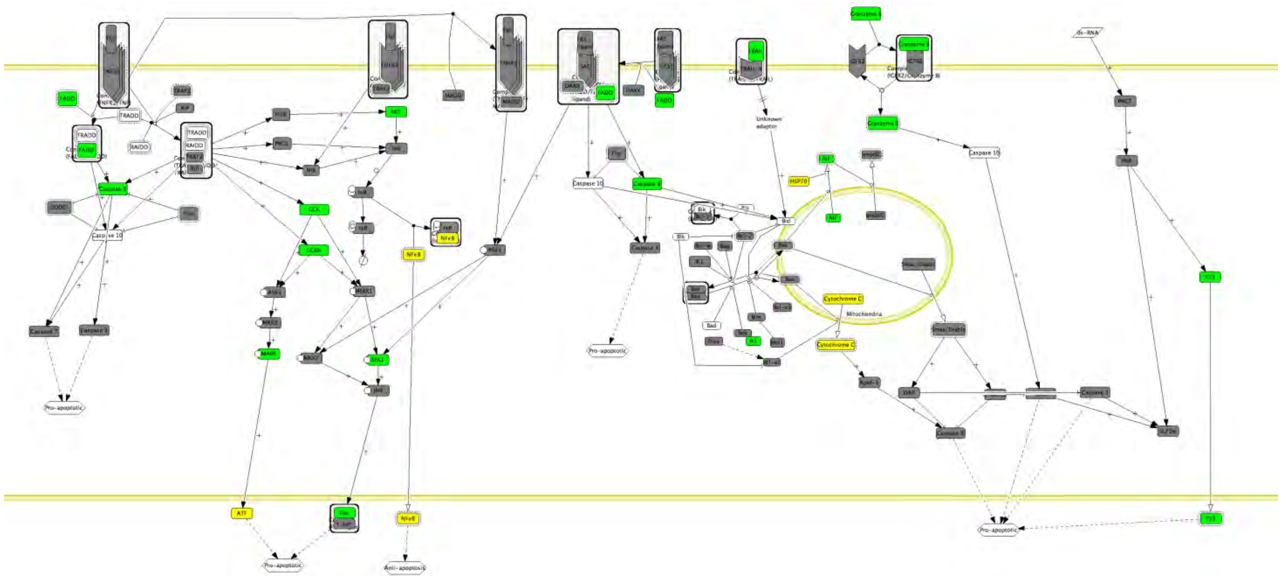


Apoptosis Signaling Pathway – BTEX, Mouse Lung (i.t.)

4h



24h

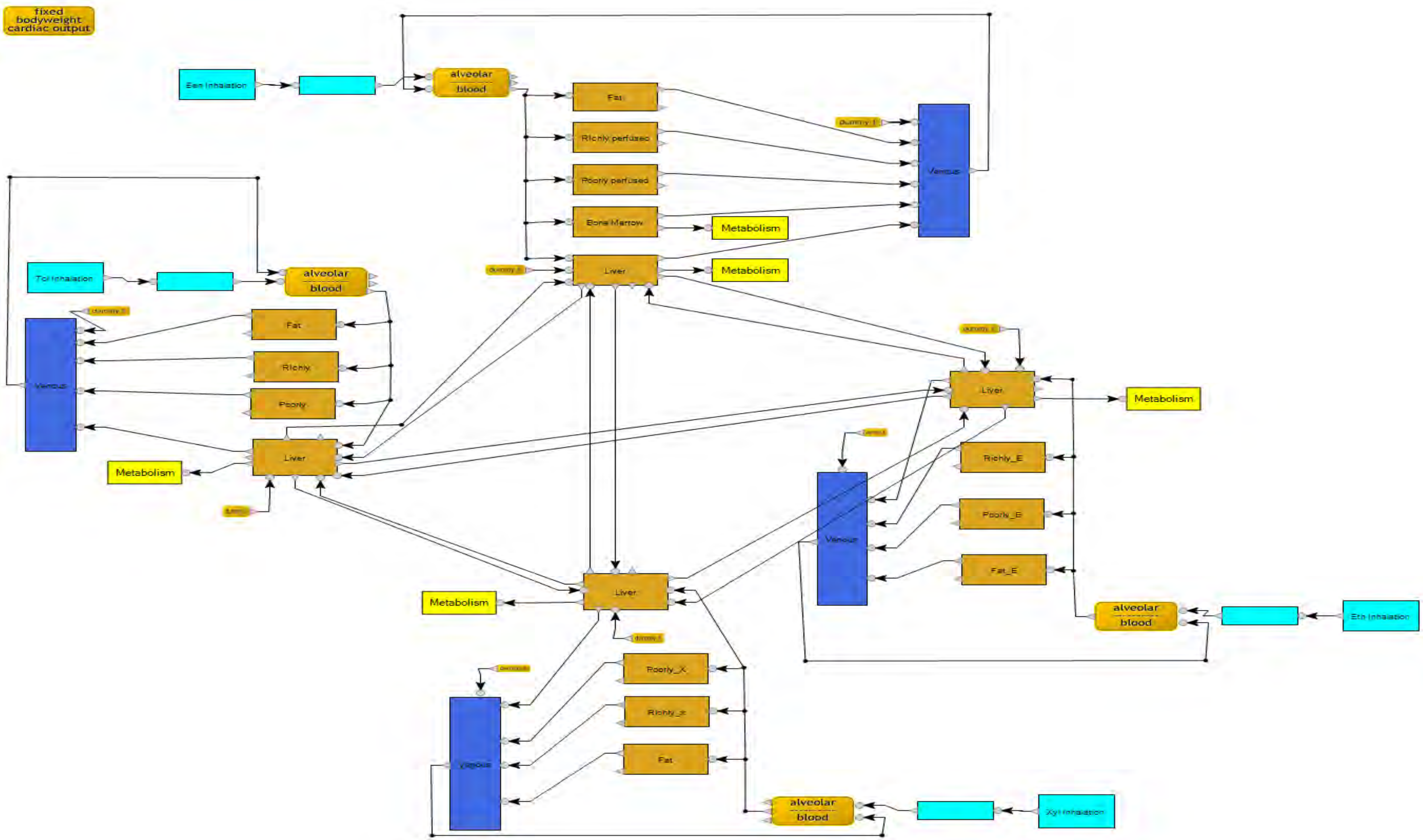


Mix A

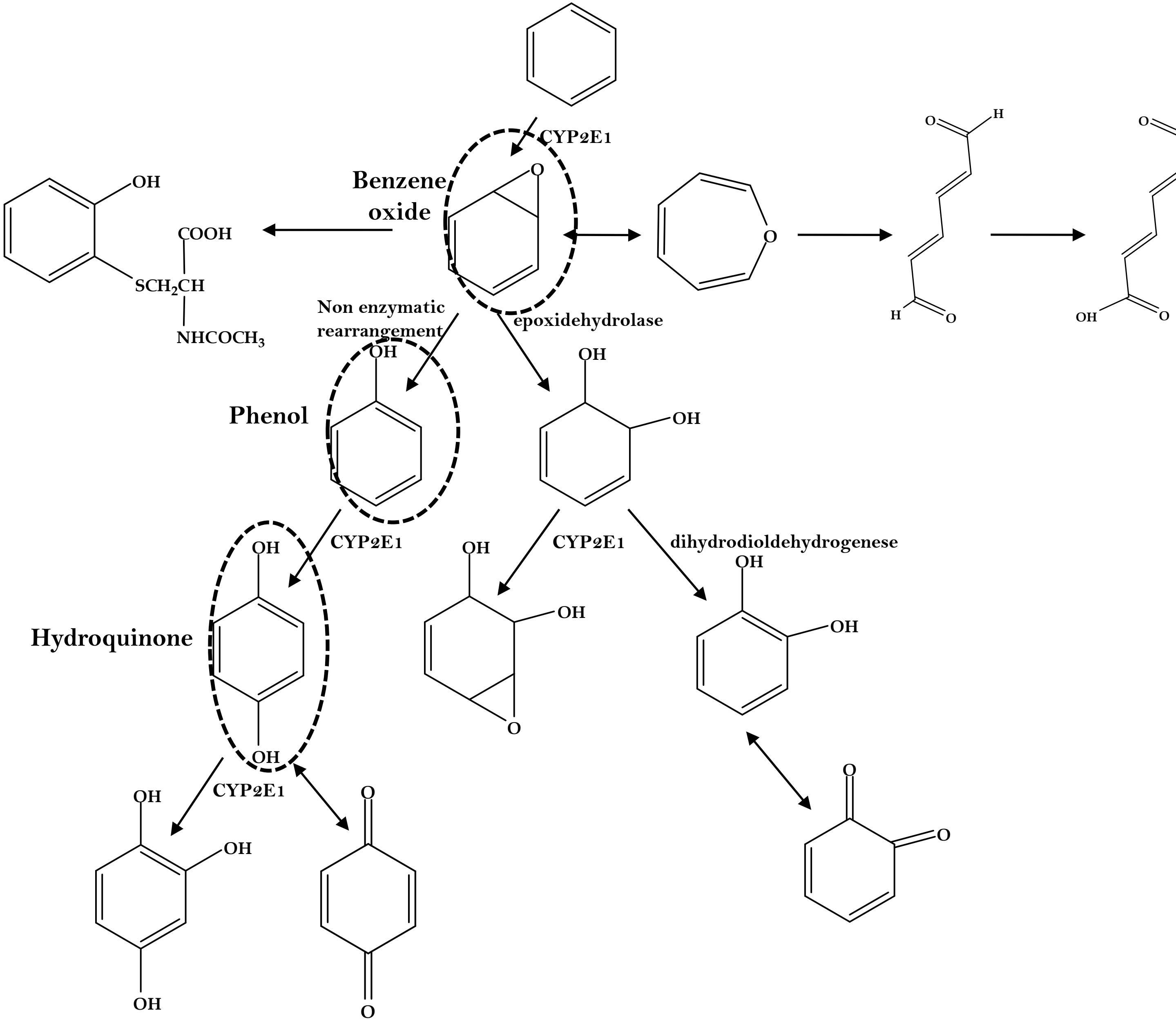
Mix B

Common

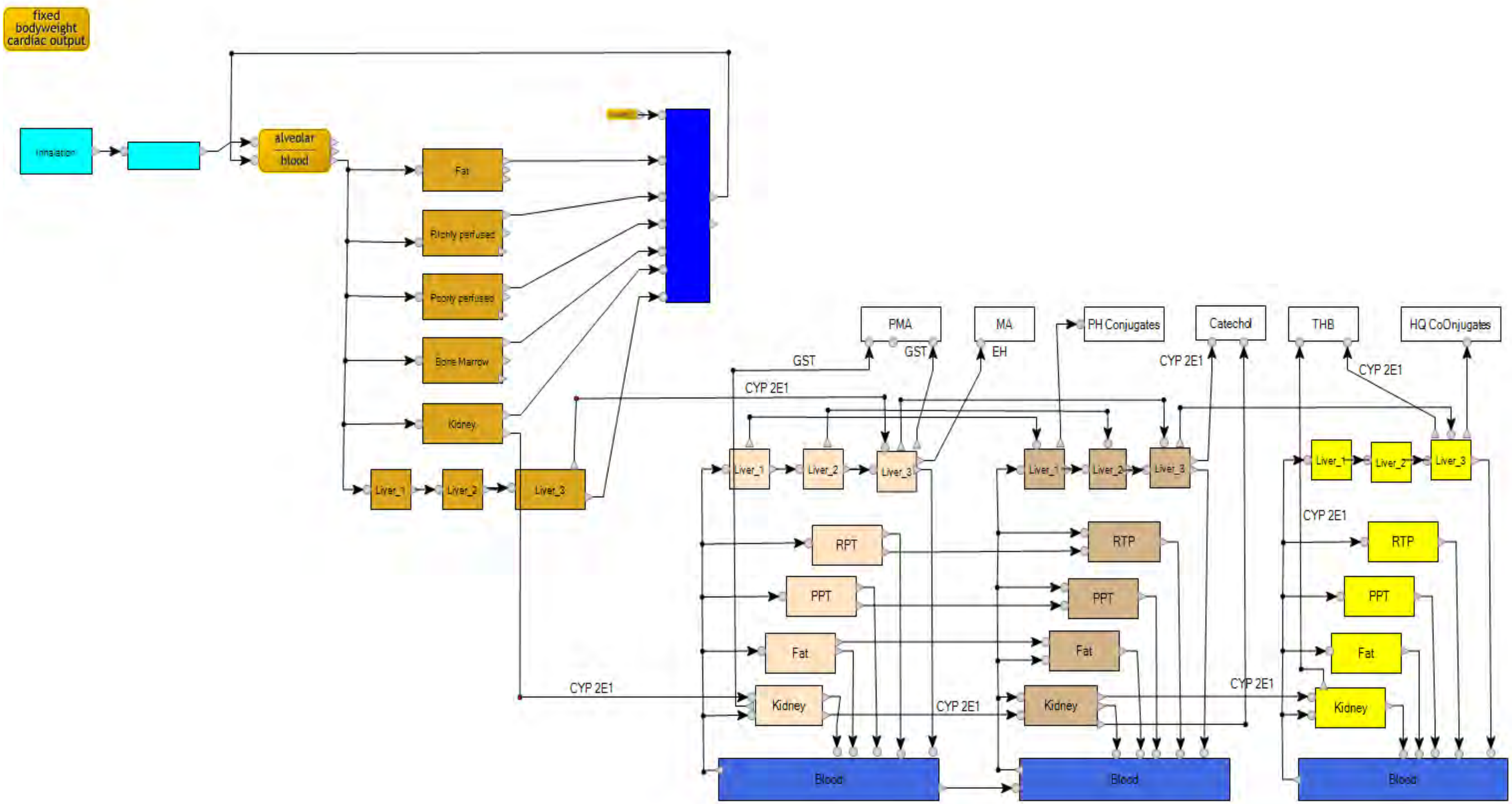
Linking with the physiome



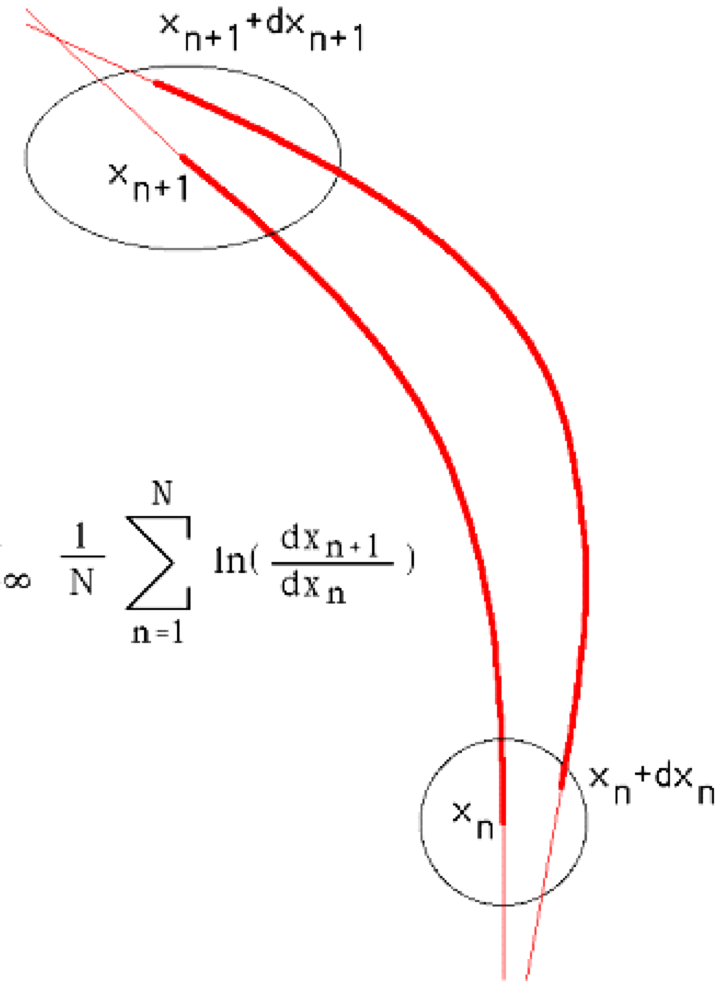
Benzene metabolism



PBPK Model for Benzene (with Metabolism)



Lyapunov exponent: system stability



$$\lambda = \lim_{N \to \infty} \frac{1}{N} \sum_{n=1}^{N} \ln\left(\frac{dx_{n+1}}{dx_n}\right)$$

If $\lambda > 1$ the system is chaotic and unstable

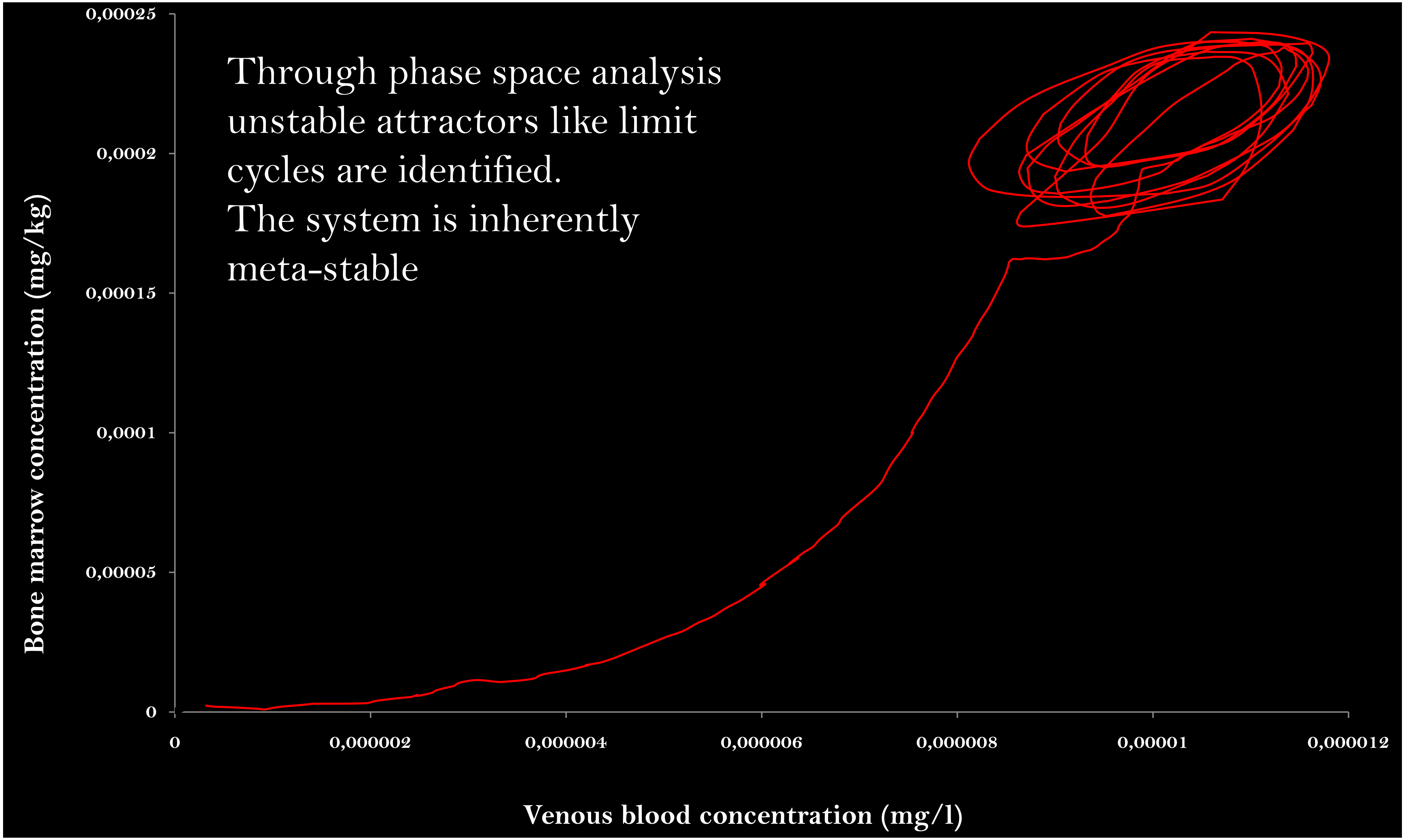
λ measures the sensitivity of the system to its initial conditions

If $\lambda < 1$ the system is attracted to a stable point or a stable periodic trajectory (limit cycle). This is a non conservative condition. The absolute value of λ is a metric of system sensitivity

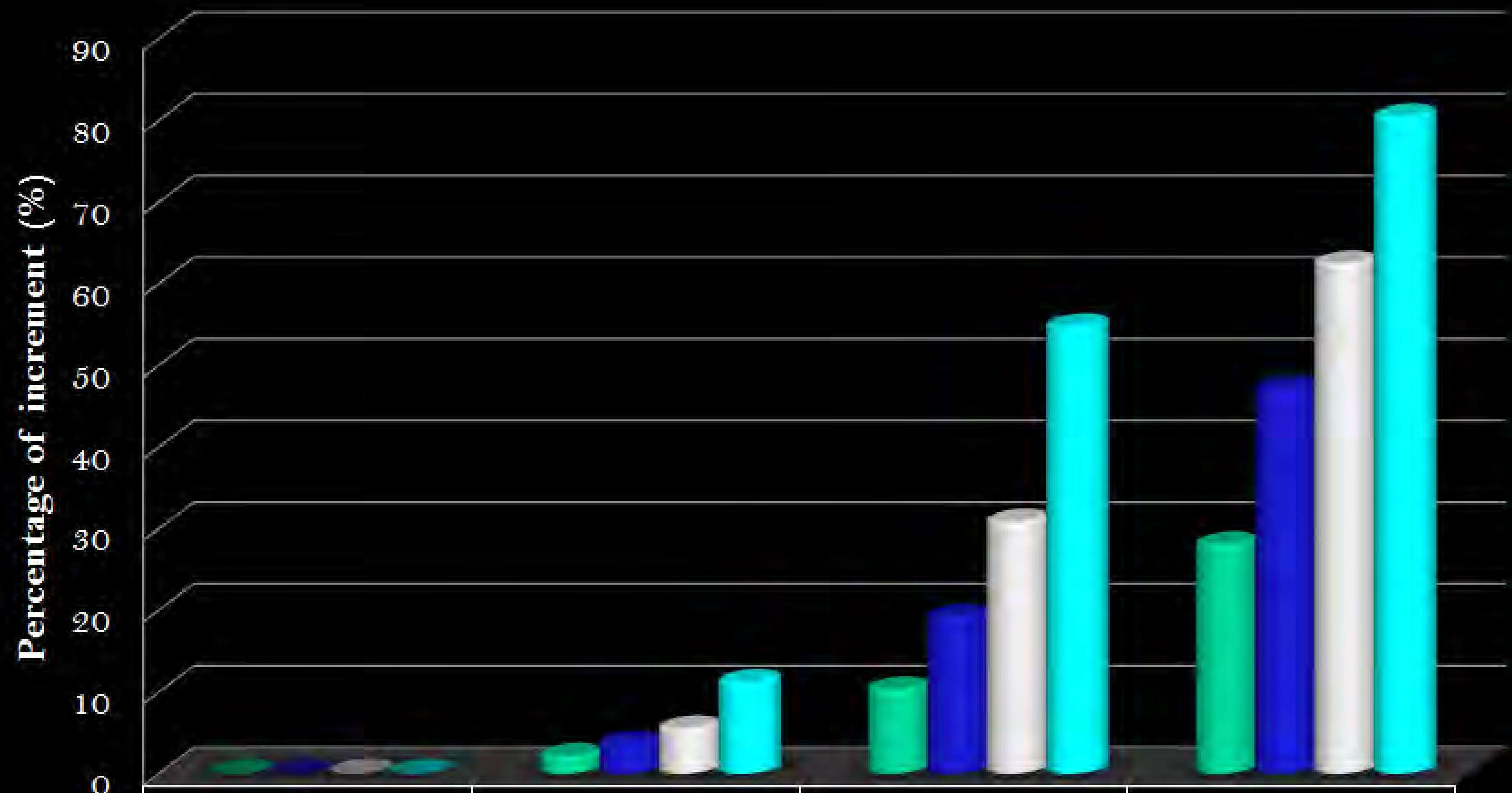
If $\lambda = 1$ the system is stable, conservative and at steady state

$\lambda_{BTEX} < 1$ Unstable system

Biological system dynamics: emergence of limit cycles

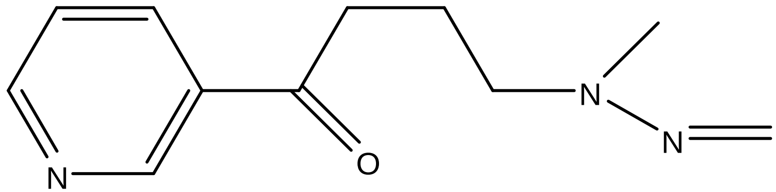


Increment in maximum bone marrow concentration of benzene for exposition to different mixtures of BTEX



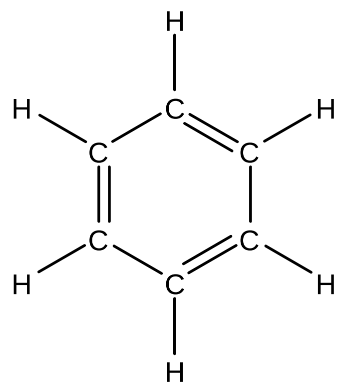
	B	B+T	B+T+X	B+T+X+E
TLV/3	0	2.2	10.4	28.1
TLV/2	0	3.7	19.2	47.2
TLV(2/3)	0	5.6	30.9	62.5
TLV	0	11.3	54.8	80.5

Health endpoint-triggered exposure assessment

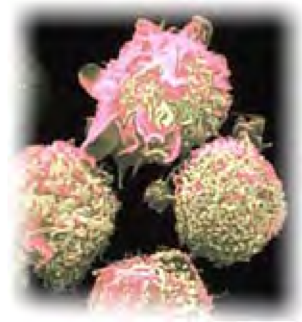


4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)

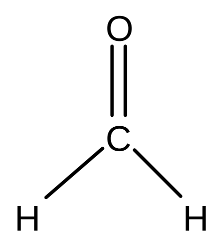
Lung cancer



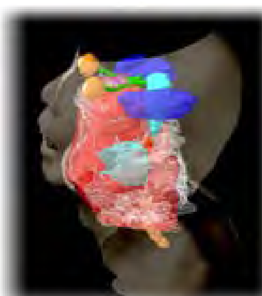
Benzene



Leukemia

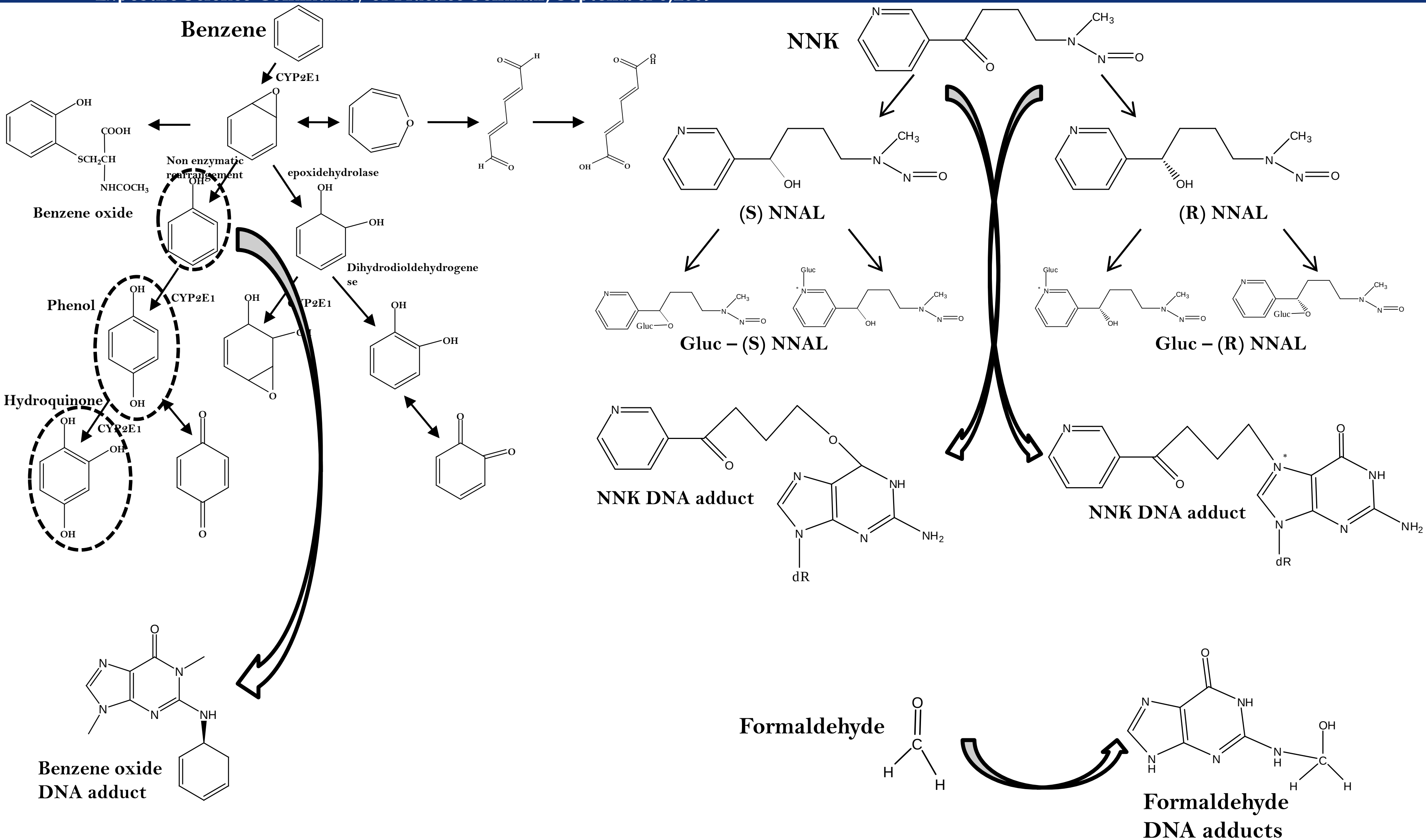


Formaldehyde



Nasopharyngeal cancer

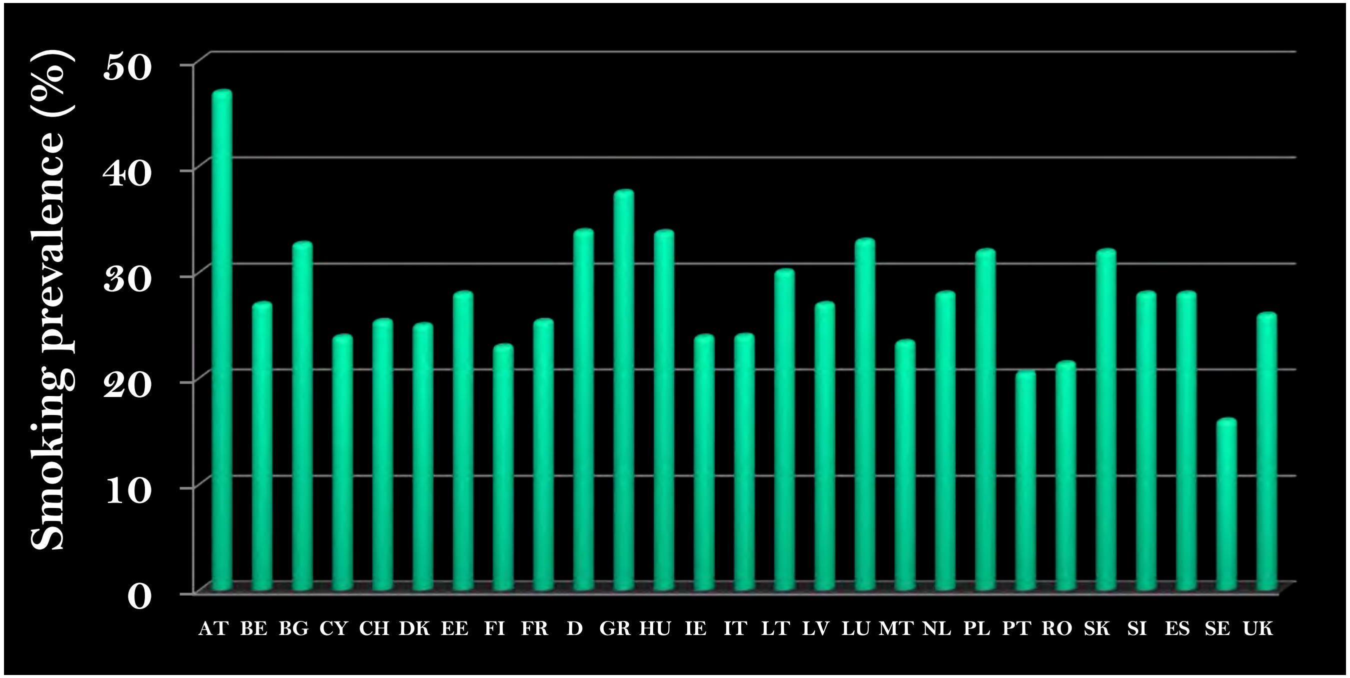
Benzene, NNK, Formaldehyde metabolism – DNA adducts formation



Data necessary for the EU-27 scale estimation

Average values of major smoke constituents in the sidestream smoke of 12 commercial cigarette brands assayed in the 1999 Massachusetts Benchmark Study using Massa-chusetts smoking parameters (IARC, 2004)

Constituent	Unit	Range	SS/MS ratio[a]
Ammonia	mg/cig.	4.0–6.6	147
1-Aminonaphthalene	ng/cig.	165.8–273.9	7.10
2-Aminonaphthalene	ng/cig.	113.5–171.6	8.83
3-Aminobiphenyl	ng/cig.	28.0–42.2	10.83
4-Aminobiphenyl	ng/cig.	20.8–31.8	5.41
Benzo[*a*]pyrene	ng/cig.	51.8–94.5	3.22
Formaldehyde	**µg/cig.**	**540.4–967.5**	**14.78**
Acetaldehyde	µg/cig.	1683.7–2586.8	1.31
Acetone	µg/cig.	811.3–1204.8	1.52
Acrolein	µg/cig.	342.1–522.7	2.53
Benzene	**µg/cig**	**71-134**	0.8
Propionaldehyde	µg/cig.	151.8–267.6	1.06
Crotonaldehyde	µg/cig.	62.2–121.8	1.95
Butyraldehyde	µg/cig.	138.0–244.9	2.68
Hydrogen cyanide	mg/cig.	0.19–0.35	0.77
Mercury	ng/cig.	5.2–13.7	1.09
Nickel	ng/cig.	ND–NQ	
Chromium	ng/cig.	ND–ND	
Cadmium	ng/cig.	122–265	1.47
Arsenic	ng/cig.	3.5–26.5	1.51
Selenium	ng/cig.	ND–ND	
Lead	ng/cig.	2.7–6.6	0.09
Nitric oxide	mg/cig.	1.0–1.6	2.79
Carbon monoxide	mg/cig.	31.5–54.1	1.87
'Tar'	mg/cig.	10.5–34.4	0.91
Nicotine	mg/cig.	1.9–5.3	2.31
Catechol	µg/cig.	64.5–107.0	0.85
Hydroquinone	µg/cig.	49.8–134.1	0.94
Resorcinol	µg/cig.	ND–5.1	
meta-Cresol + *para*-Cresol[b]	µg/cig.	40.9–113.2	4.36
ortho-Cresol	µg/cig.	12.4–45.9	4.15[c]
NNN	ng/cig.	69.8–115.2	0.43
NNK	**ng/cig.**	**50.7–95.7**	**0.40**
NAT	ng/cig.	38.4–73.4	0.26
NAB	ng/cig.	11.9–17.8	0.55
1,3-Butadiene	µg/cig.	81.3–134.7	1.30



- Smoking emissions (IARC)
- Smoking prevalence-population exposed to ETS (WHO)
- Time activity patterns
- Volumes of residences
- Indoor/outdoor air exchange rate

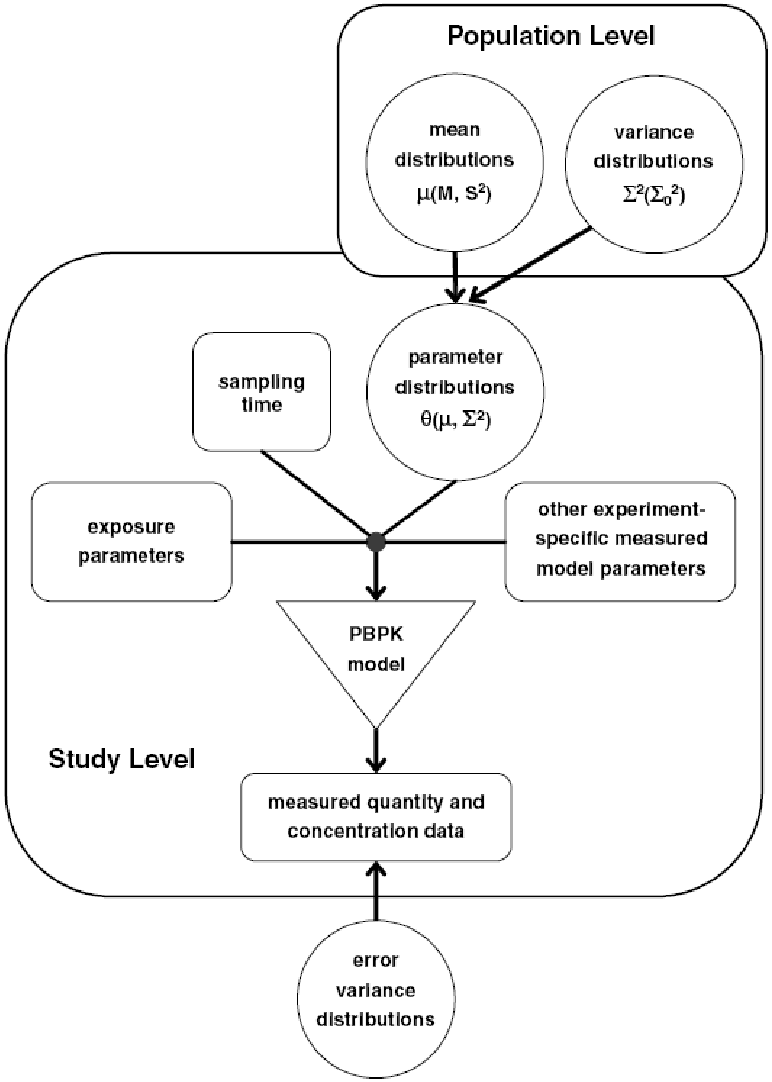
(EXPOLIS study)

Hierarchical population exposure model

Hierarchical population model used in Bayesian analysis (Bois *et al*, 1996).

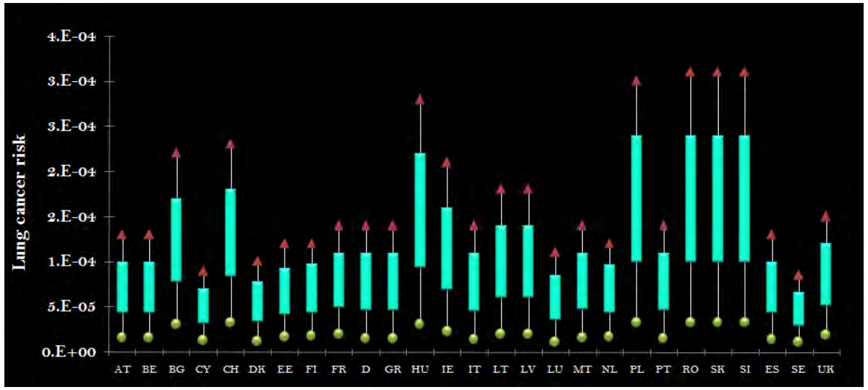
Circles represent distributions and squares/rectangles represent known entities.

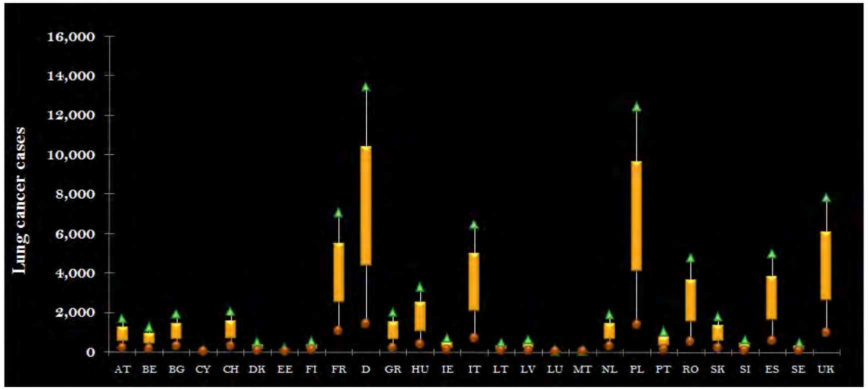
μ: prior mean distribution
Σ^2: prior variance distribution
θ: study level distributions for each of the parameters based on randomly selected values for the mean and variance from the population distributions μ and Σ^2

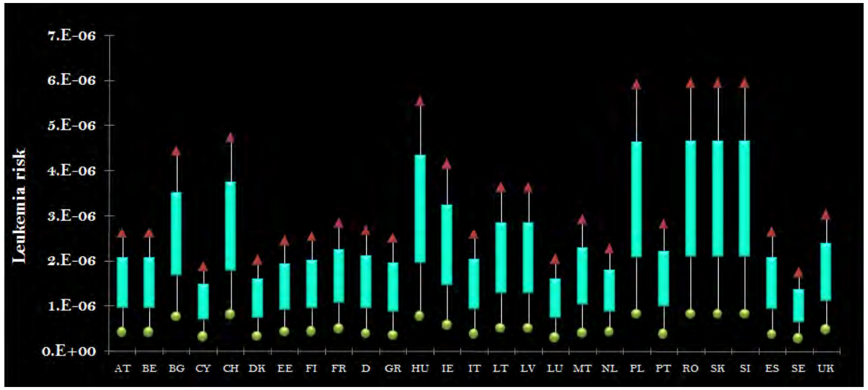


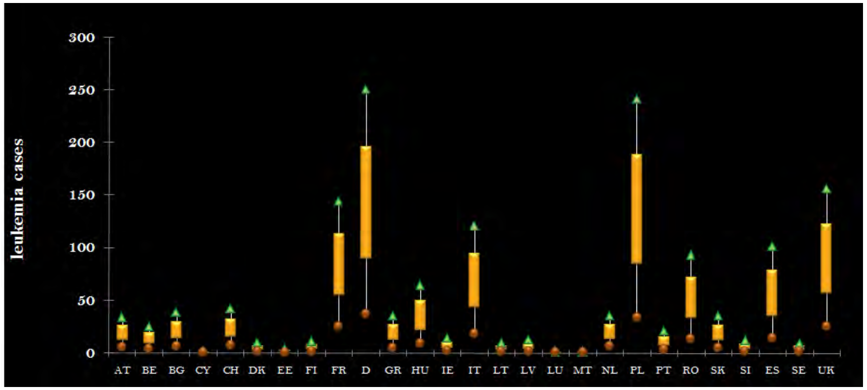
EU-27 cancer risk estimations

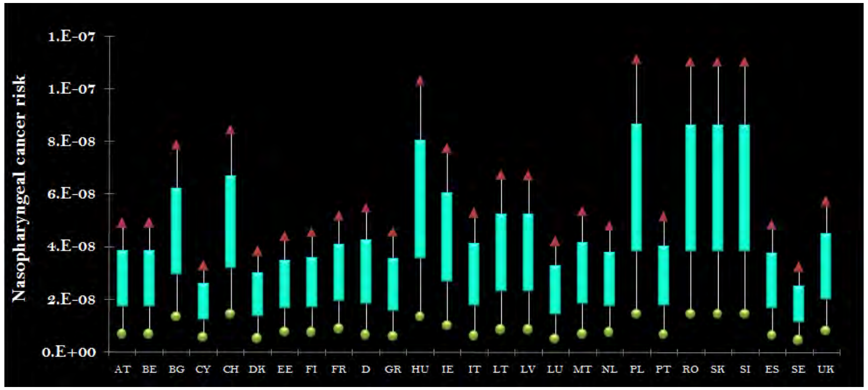
Individual risk-Expected lifetime cases

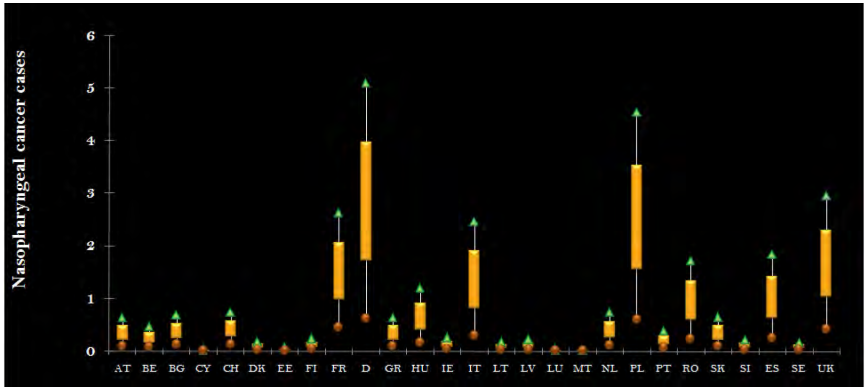






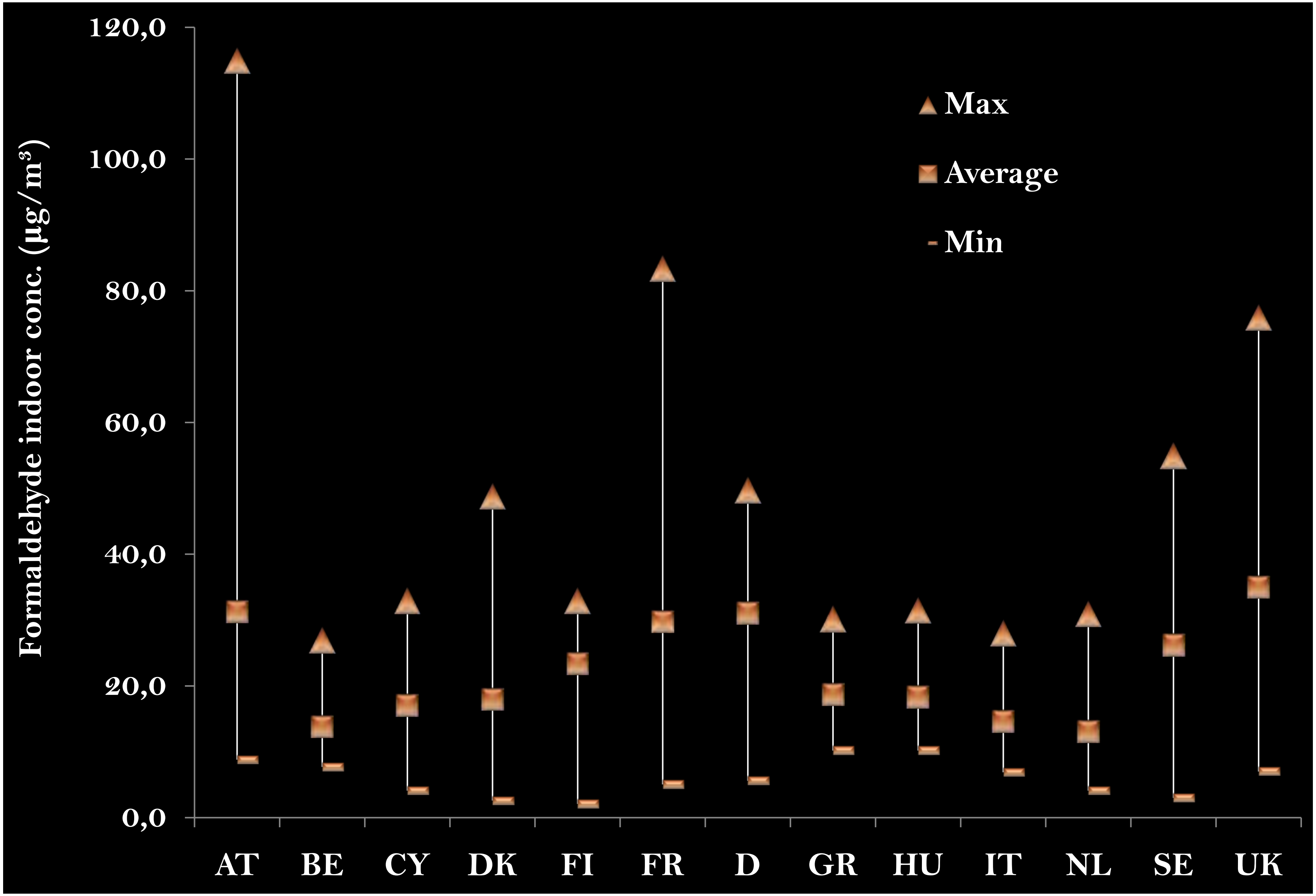




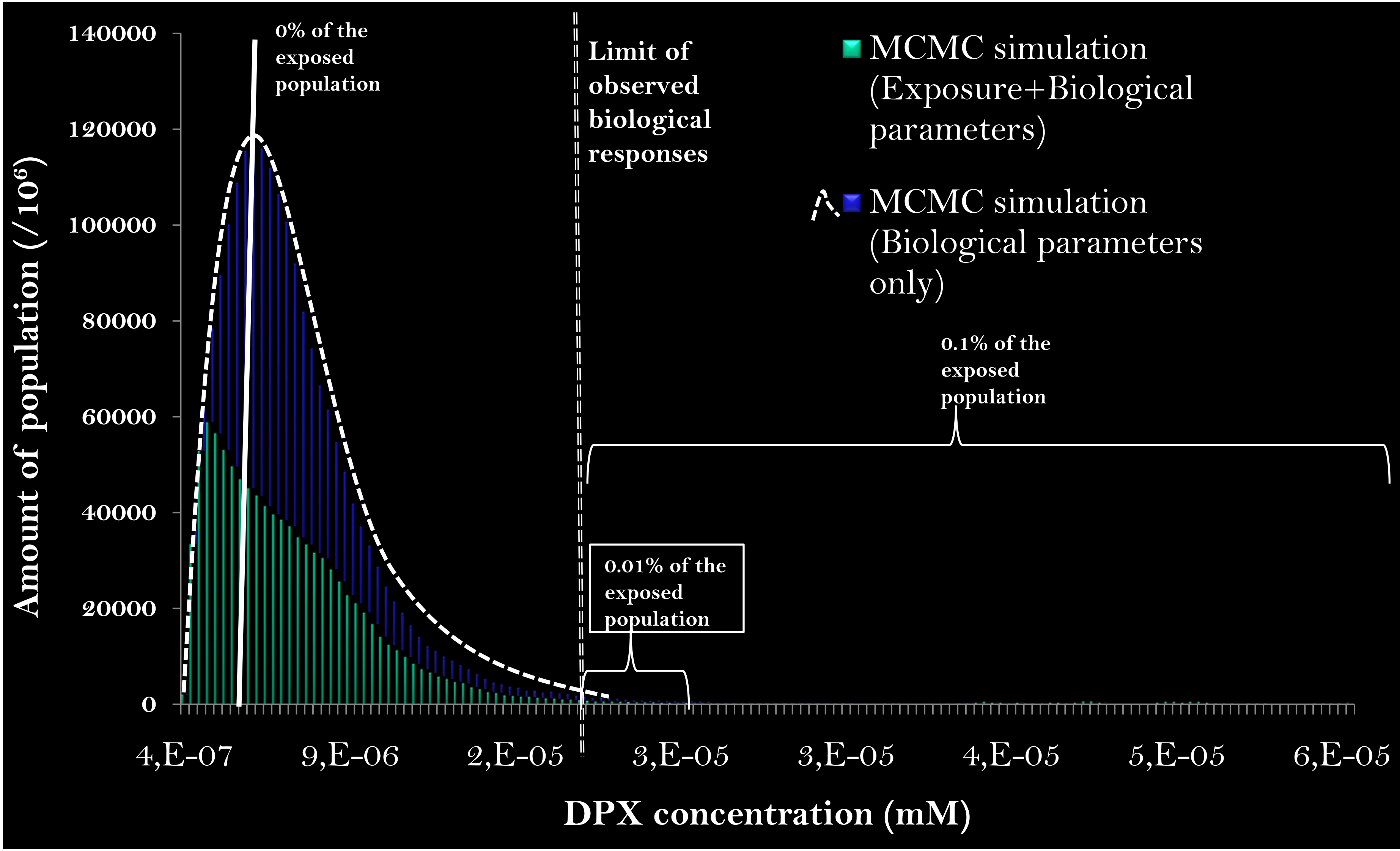


Aggregate exposure

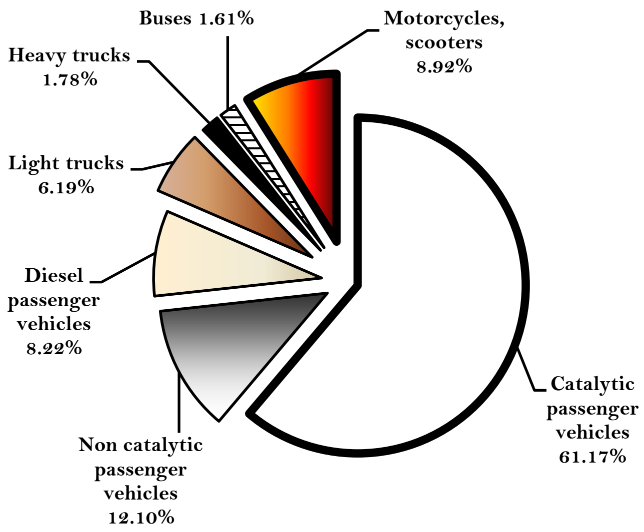
Formaldehyde exposure

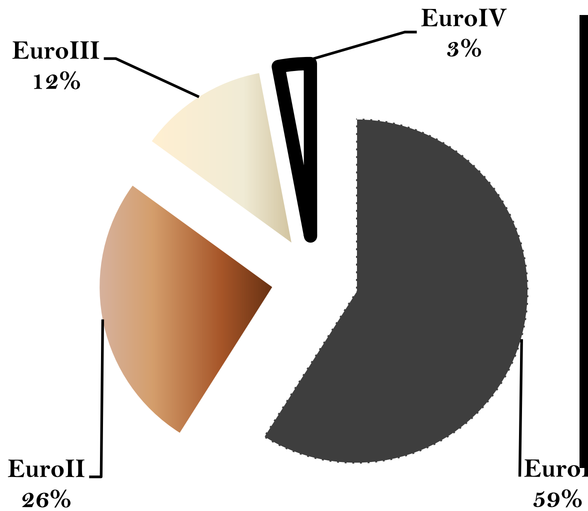


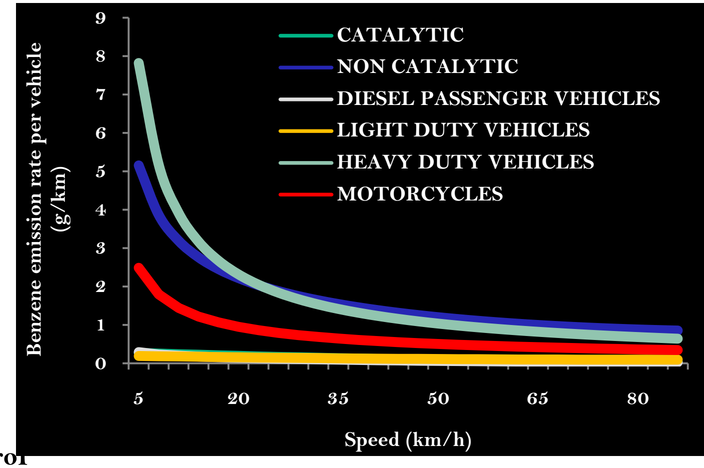
Formaldehyde exposure

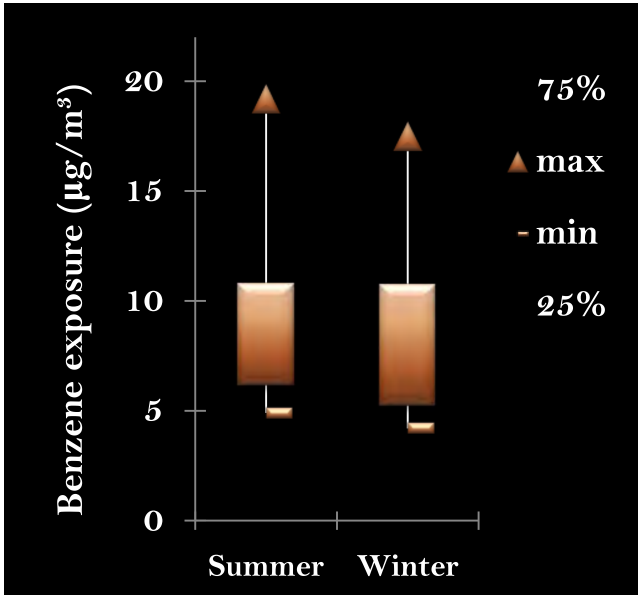


Aggregate exposure: the Benzene study



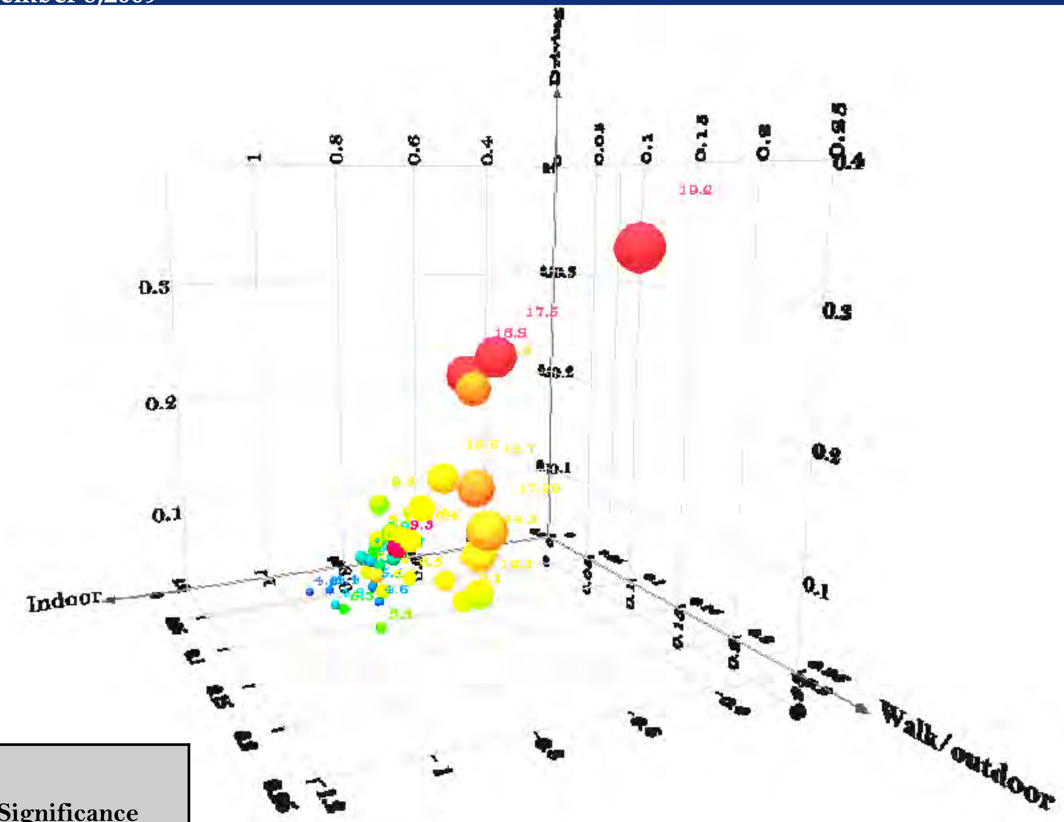






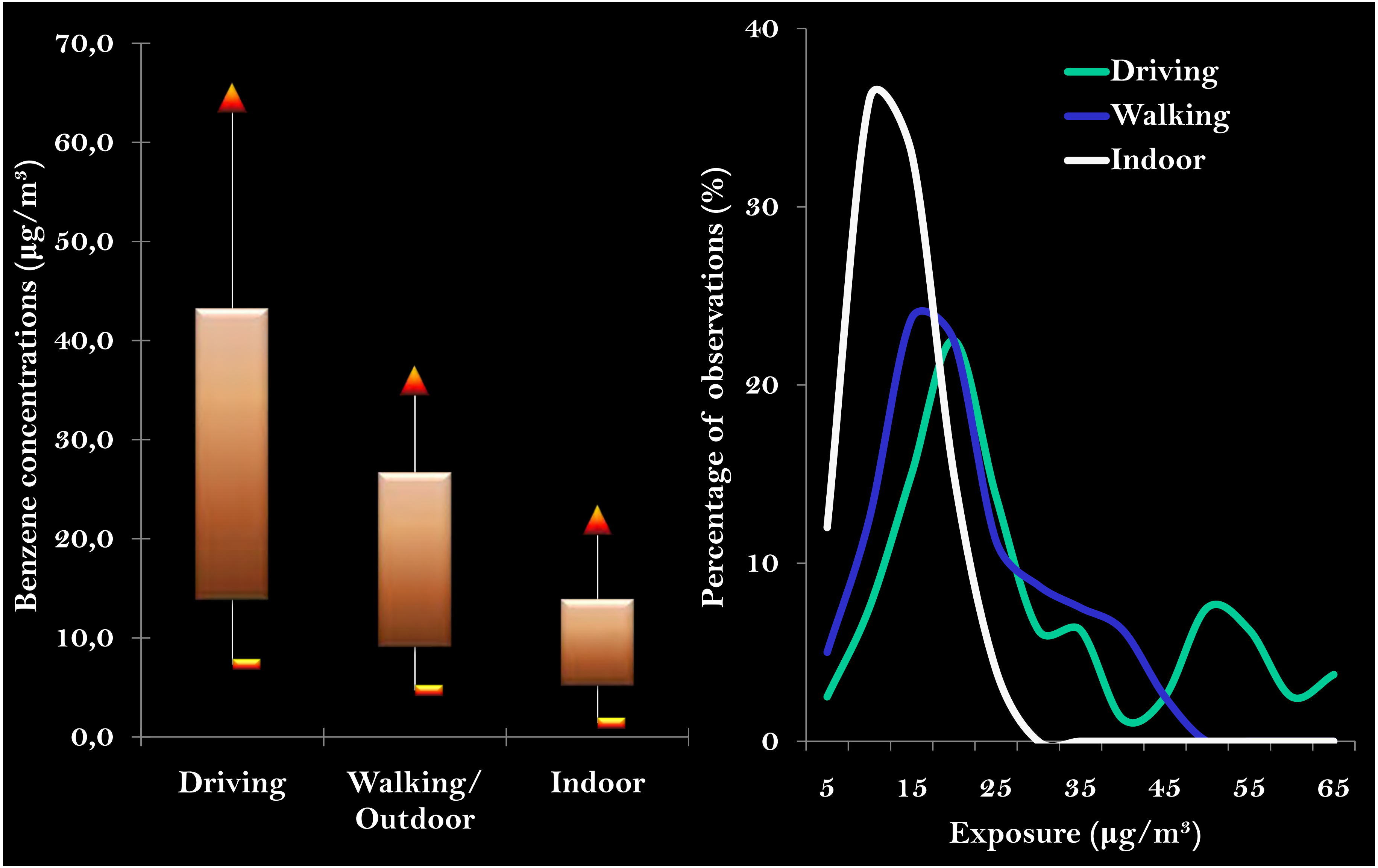
Activities time fraction

Size: Exposure
Color: ETS presence
X axis: Fraction of time spend Outdoor
Y axis: Fraction of time spend indoor
Z axis: Fraction of time spend driving



	Unstandardized Regression Coefficient		Standardized Regression Coefficient		Significance (>0.05)	
	Summer	Winter	Summer	Winter	Summer	Winter
Constant	3.1	2.3			0.02	0.67
Walking/Outdoor	-0.06	0.09	0.03	0.03	0.80	0.54
Driving	0.53	0.62	0.46	0.53	0.00	0.00
Ind.Loc. Zone 1	0.20	0.06	0.33	0.16	0.00	0.07
Ind. Loc. Zone 2	-0.04	-0.01	0.08	0.02	0.19	0.47
ETS presence	3.41	4.41	0.17	0.29	0.02	0.02

Active sampling measurements



Regression exposure modelling

$$E = \left(\frac{T_I}{T}\right)(11.1 + Sm \cdot 4.3 - Lz \cdot 4 - Tz \cdot 2.5 - Ws \cdot 0.6 + U_B \cdot 1.3) +$$

$$\left(\frac{T_D}{T}\right)(36.5 + Wc \cdot 11.9 - Lz \cdot 14.8 - Tz \cdot 3.72 - Ws \cdot 0.001 + U_B \cdot 2.5) +$$

$$\left(\frac{T_W}{T}\right)(22.2 - Lz \cdot 6.2 - Tz \cdot 1.1 - Ws \cdot 5.8 + U_B \cdot 2)$$

Sm: ETS presence (fraction 0 till 1)

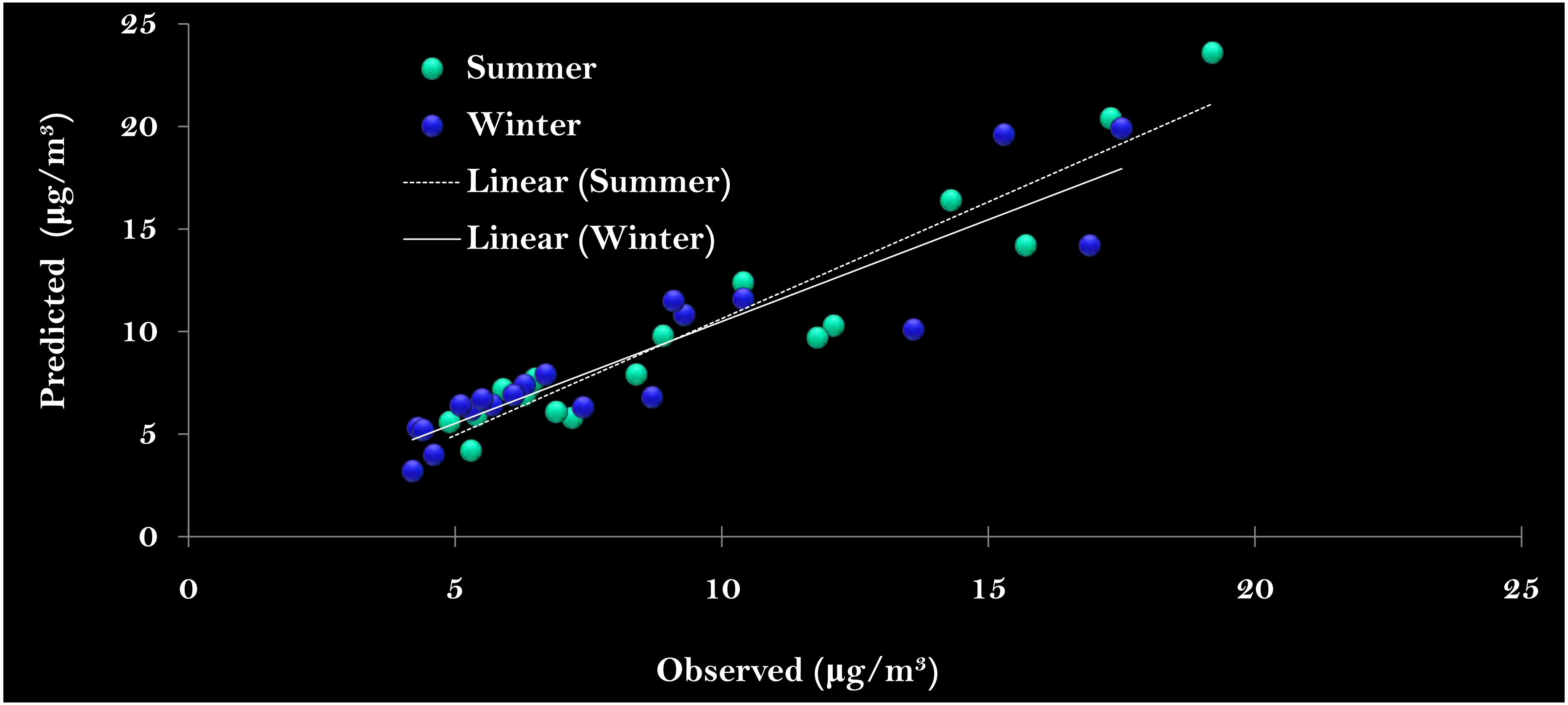
Wc: Closed (1) or open (0) windows during driving

Lz: Location zone (1-3)

Tz: Time zone (1-4)

Ws: Wind speed (m/sec)

U_B: Average urban benzene conc (µg/m³)

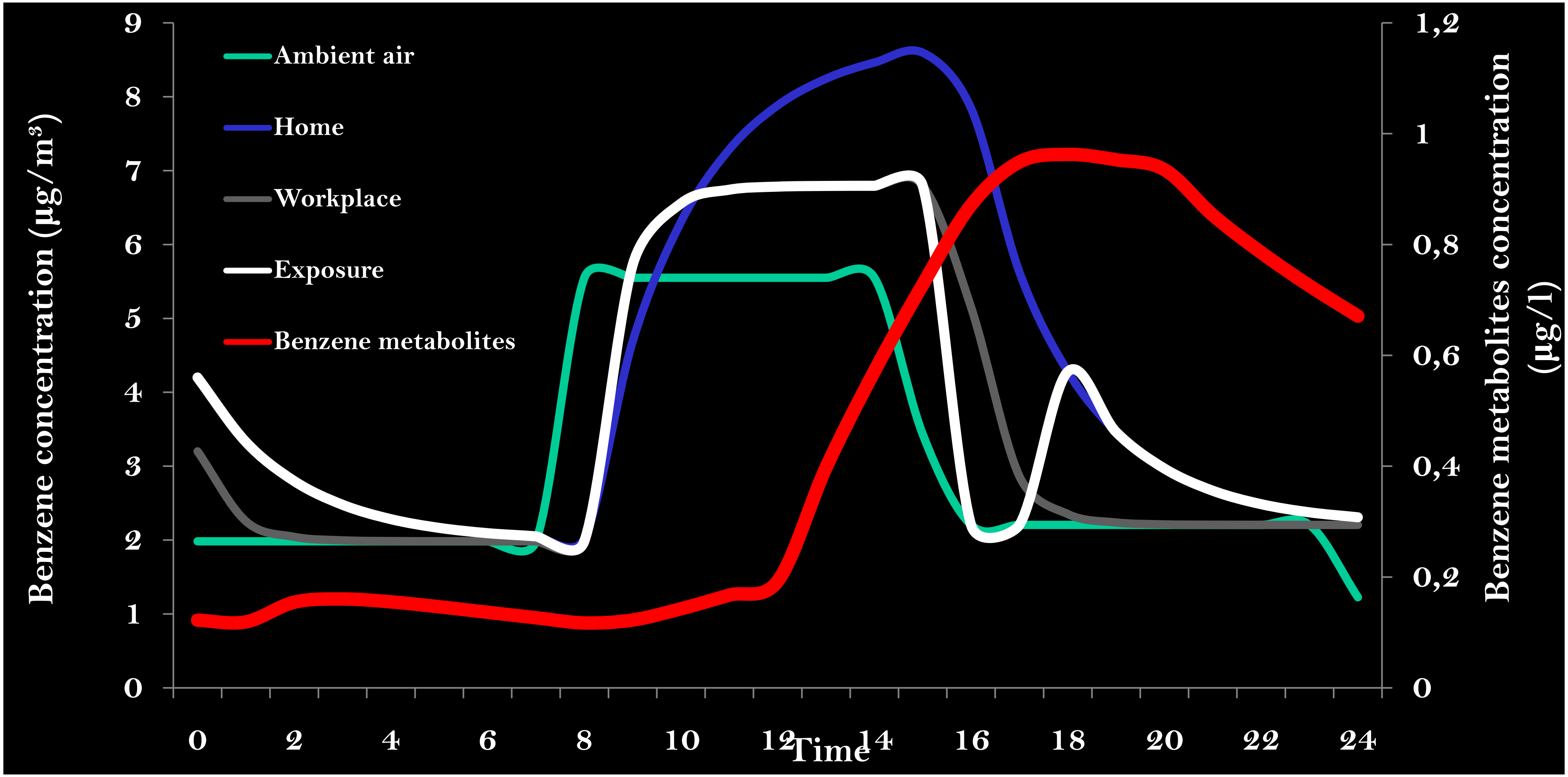


Leukemia risk estimation

- Considering the observed exposure levels, no acute effects from exposure to benzene are expected
-The interest is focused on the prolonged chronic exposure which is responsible for leukemia
- The estimated risk due to benzene exposure in the area under study is calculated considering:

- *Benzene exposure levels*
- *Benzene internal concentration*
- *Biologically effective dose of benzene metabolites in target tissue (bone marrow)*
- *Dose response relationship*
- *Susceptibility of the population considering that the enzymes (CYP2E1, quinone reductase NQO1, and myeloperoxidase) related to benzene metabolism are polymorphic*

oBenzene exposure during the day is not constant. Internal dose variation is exposure-dependent but not linearly linked to encountered microenvironment concentrations. Inhaled benzene and the produced metabolites are dynamically and continuously calculated through time (not just steady state estimations)



Advantages of Biology Based Exposure Assessment – mechanistic approaches

o Dose response relation takes into account the internal dose at the target tissue, which is the real exposure metric

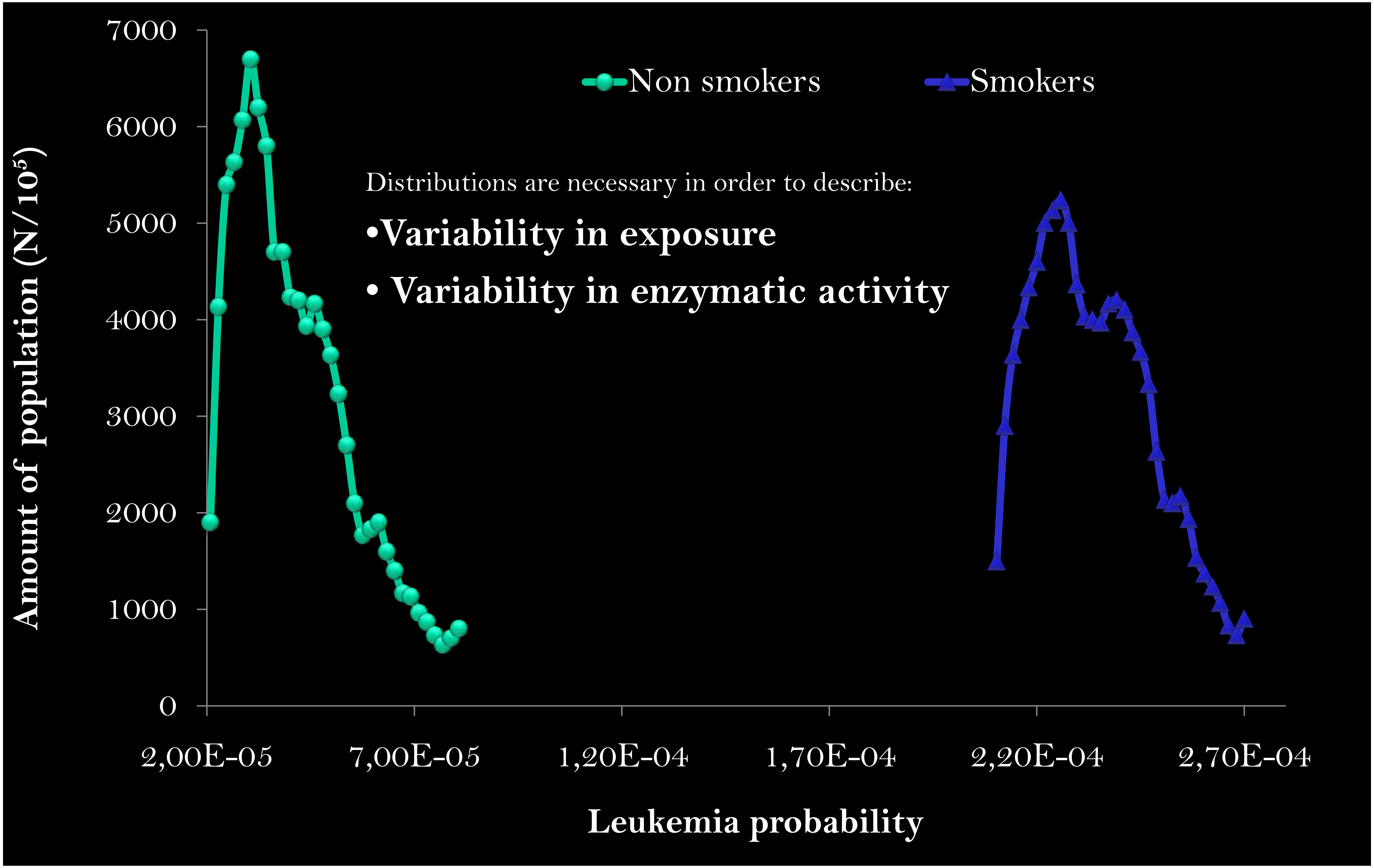
o Multiple pathways (air, water, food, consumer products) and routes of exposure (inhalation, oral) for the same pollutant can be incorporated into the PBTK/D model and provide a realistic aggregate exposure assessment

o Biology-based dose response is more representative for low exposure levels, since epidemiological approaches are based on extrapolations obtained by incidences that occurred at exposure levels 4-5 orders higher

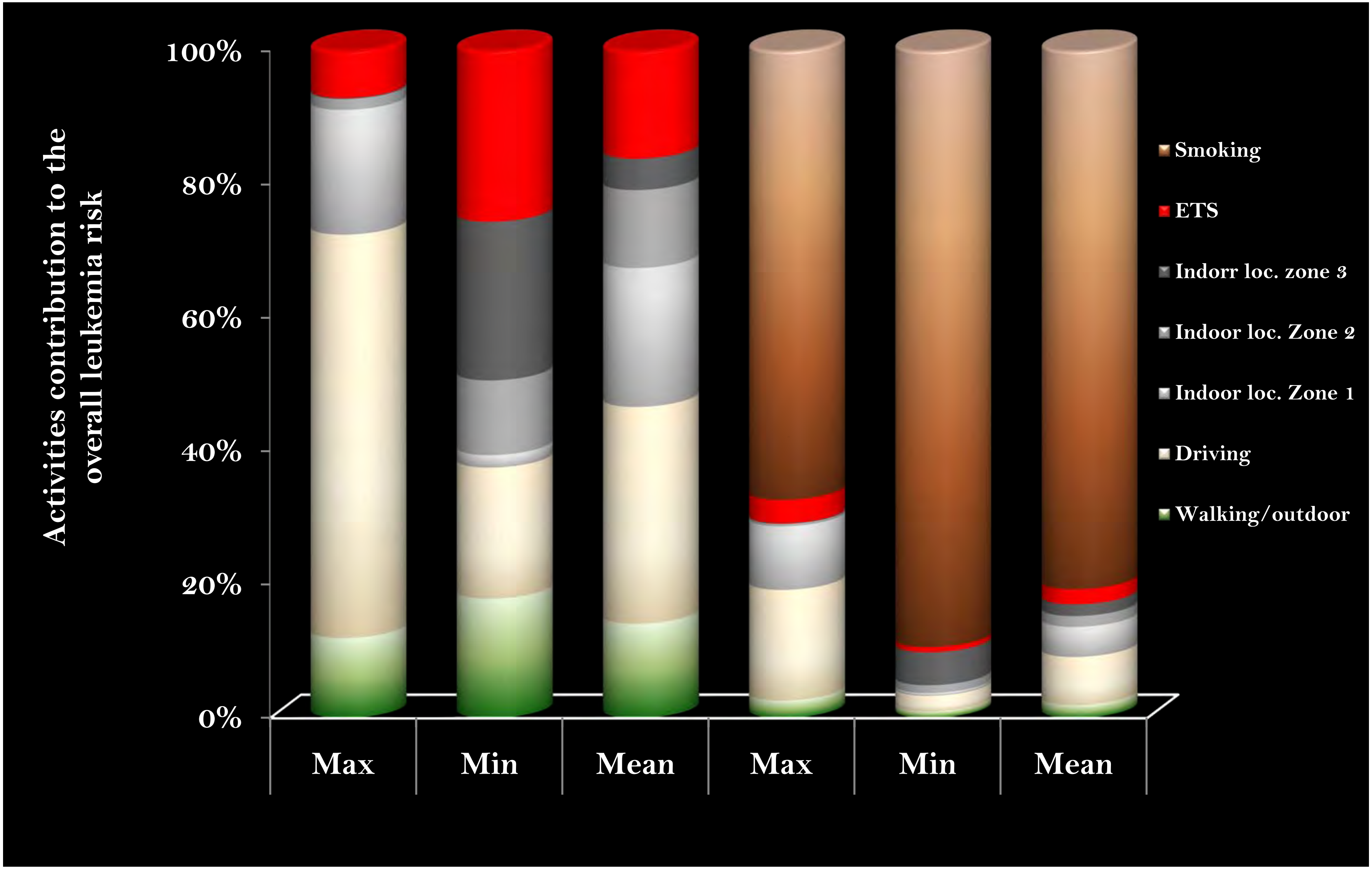
o Capturing both toxicokinetics, toxicodynamics and exposure dynamics allowed us to incorporate mechanistic knowledge on exposure assessment and thus improve on the validity and relevance of the dose-response relationship

o Traffic emissions and health endpoints are linked within a "continuous" mathematical frame allowing the exploration of alternative scenarios and the explicit incorporation of uncertainty and variability in the final risk estimates

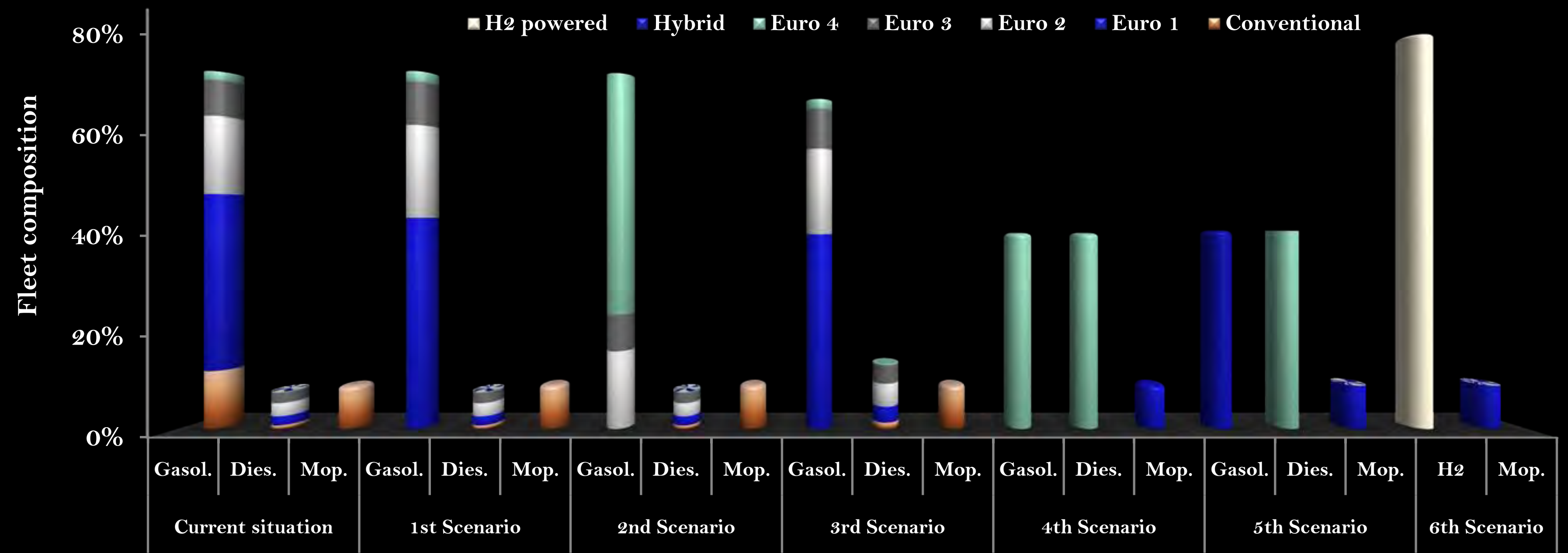
Leukemia risk estimation

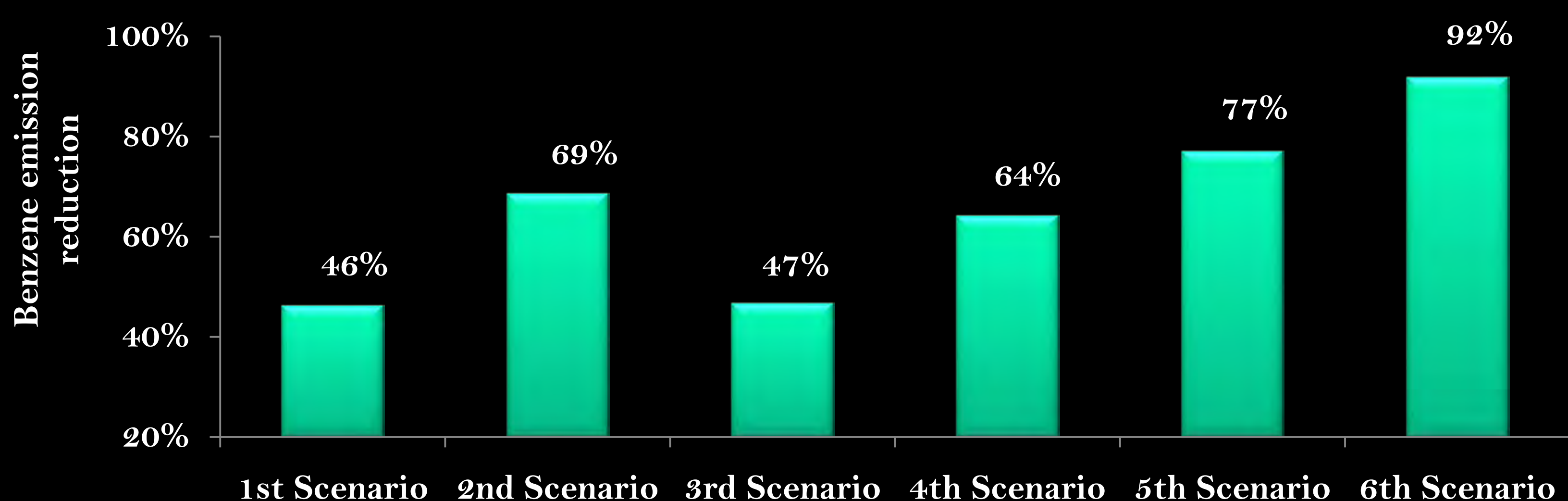


Risk attributable to the activities

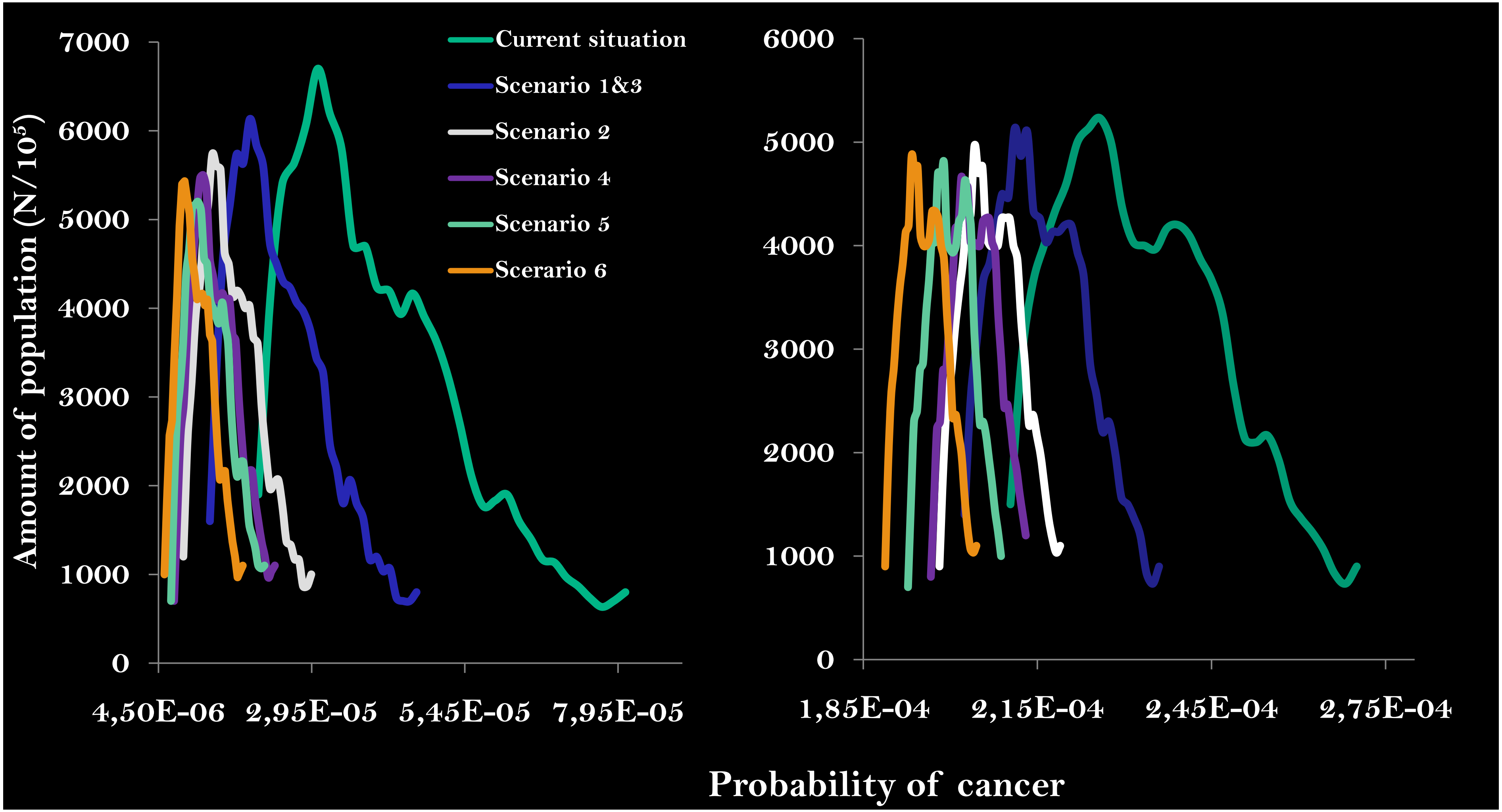


Traffic fleet composition under the "what if" scenarios

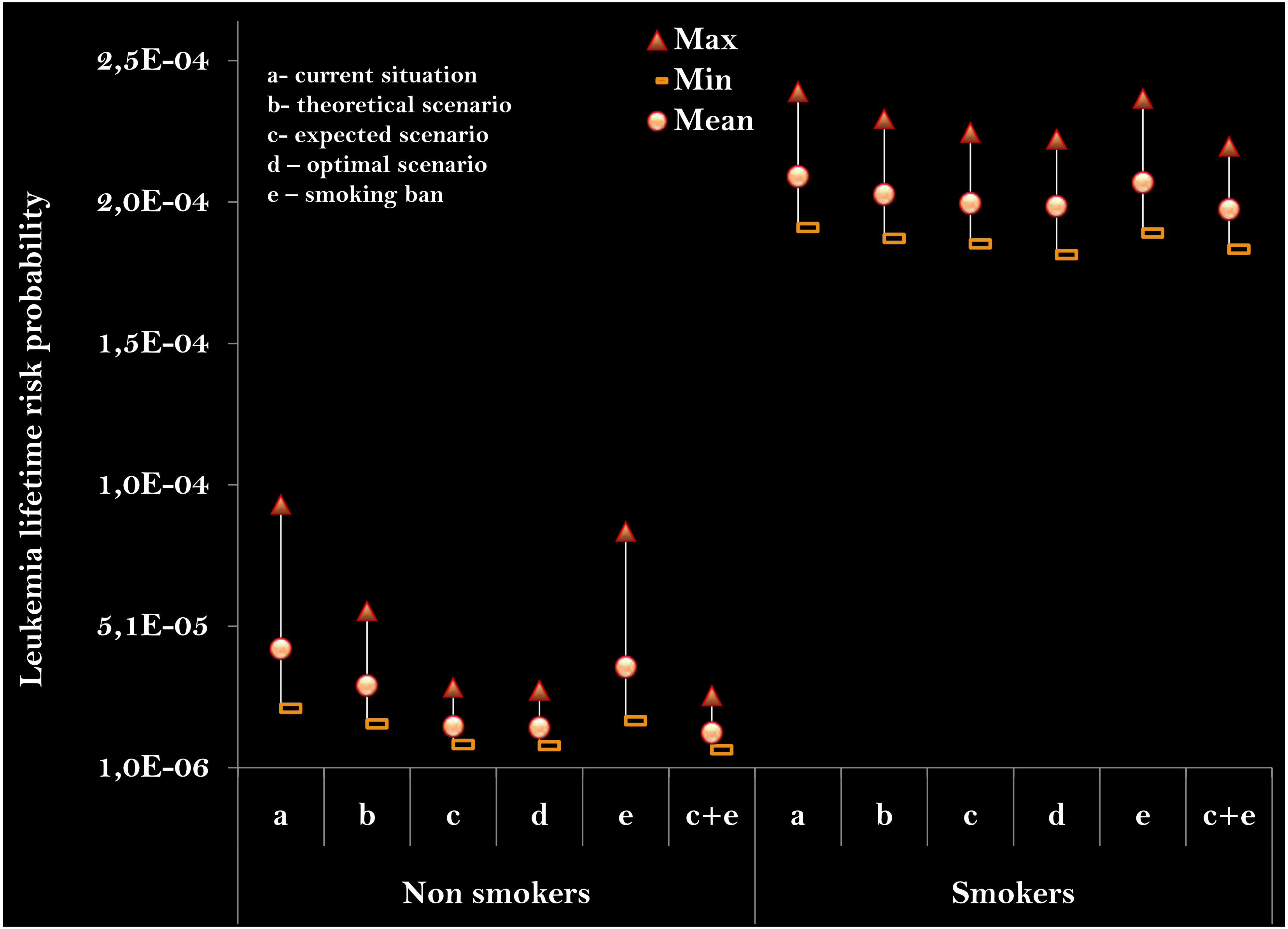




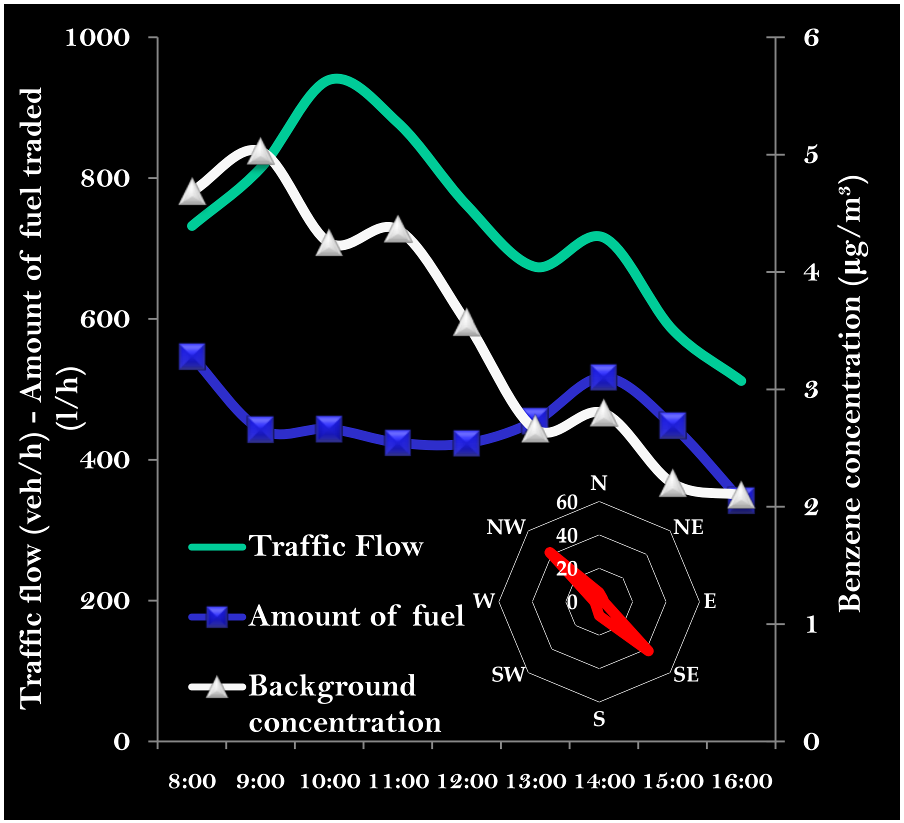
Risk mitigation

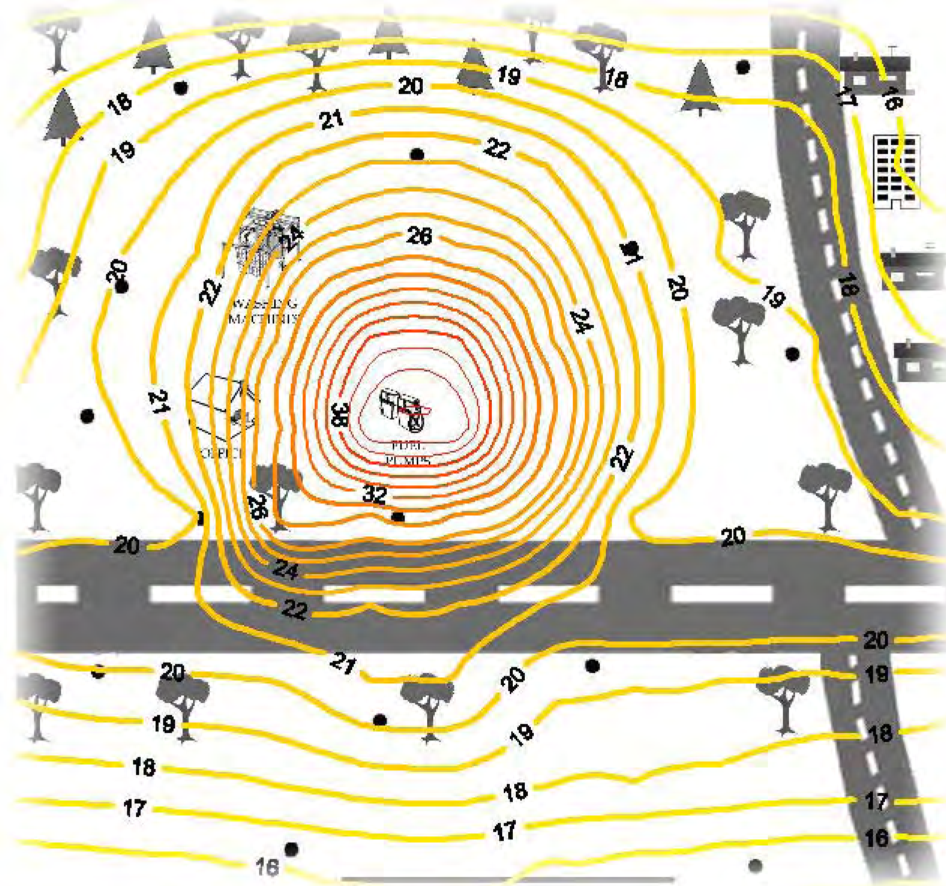


Aggregate risk mitigation



Gasoline station effect





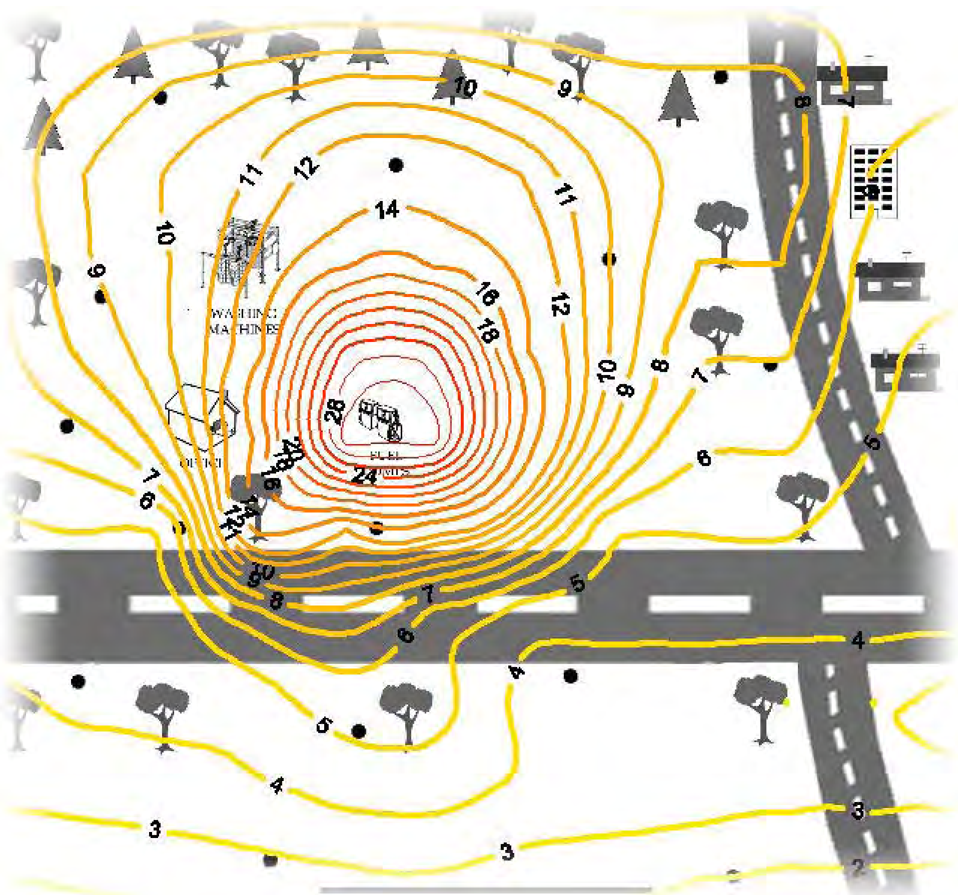
Measurements:

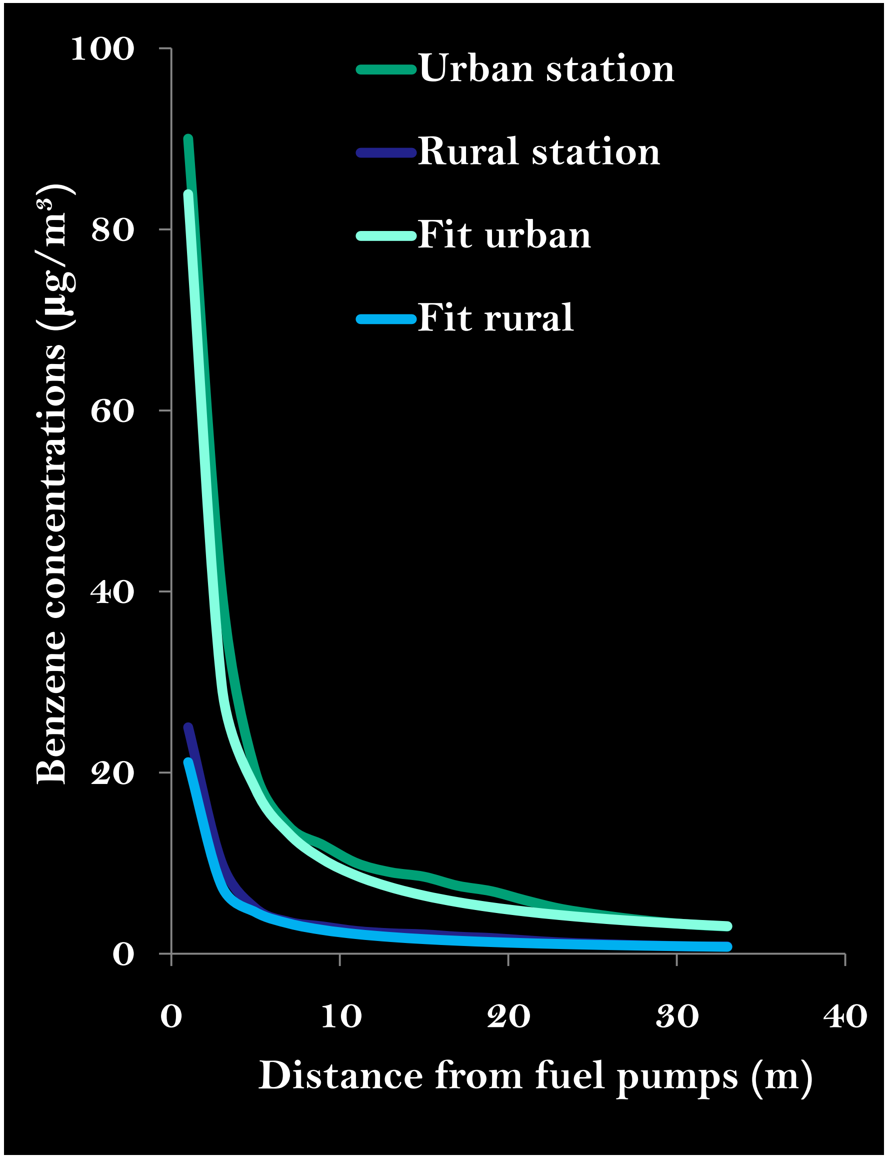
- benzene in 16 points around the gasoline station
- benzene urban background concentration
- benzene in a later point of the adjacent road (to optimize CALINE 4)
- traffic parameters of the adjacent road
- meteorological observations (wind, temp, humidity, cloudiness)
- fuel traded rate

} Modelling of the contribution of the adjacent street with CALINE4 model

$$C_{point\_i\_gas.station} = C_{point\_i\_measured} - C_{background} - C_{point\_i\_street}$$

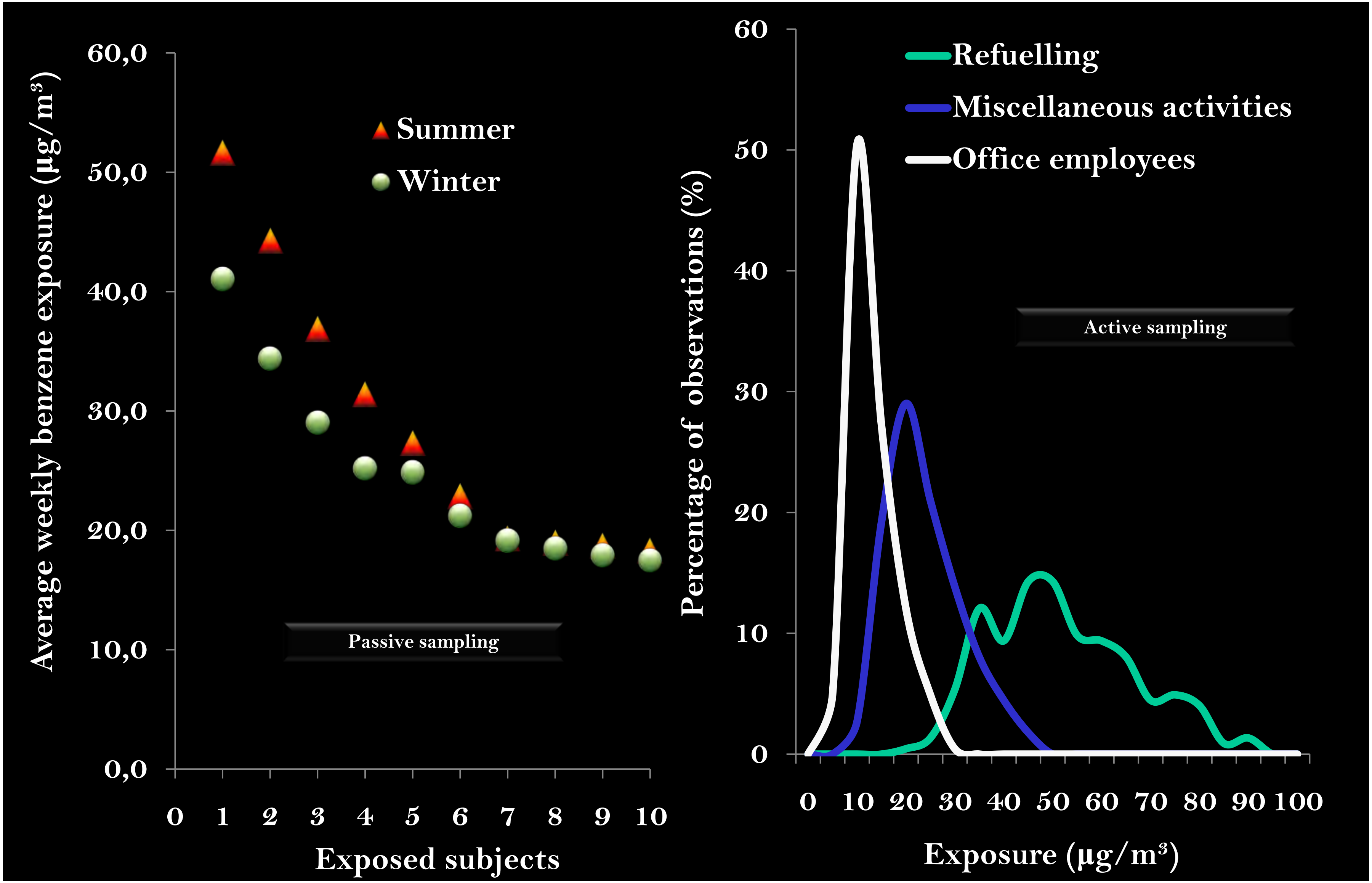
Gasoline station effect



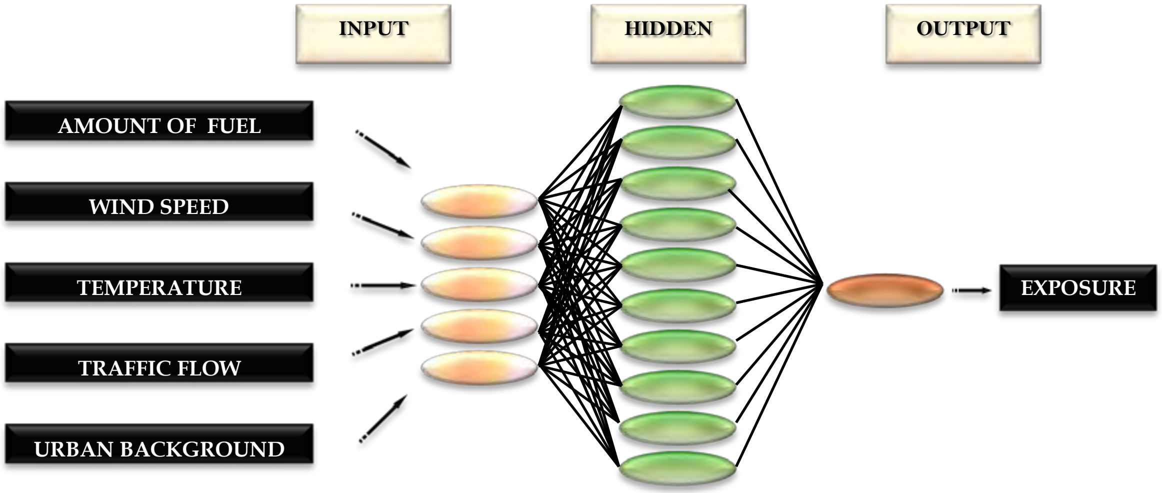


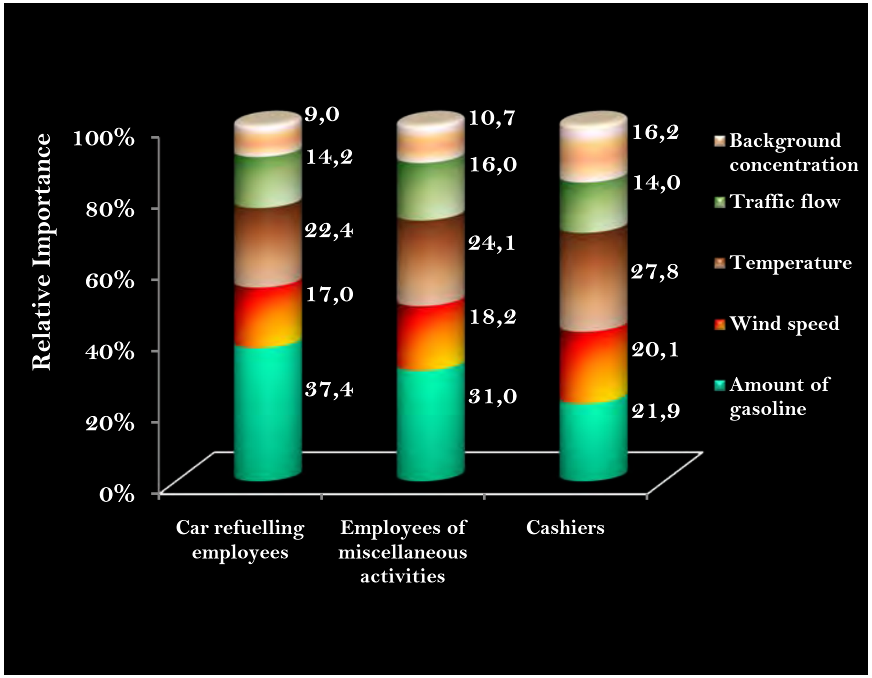
$$C_B = \frac{F^{0.713} \cdot T^{0.0298}}{D^{0.95} \cdot W^{2.55}}$$

Gasoline station employee exposure

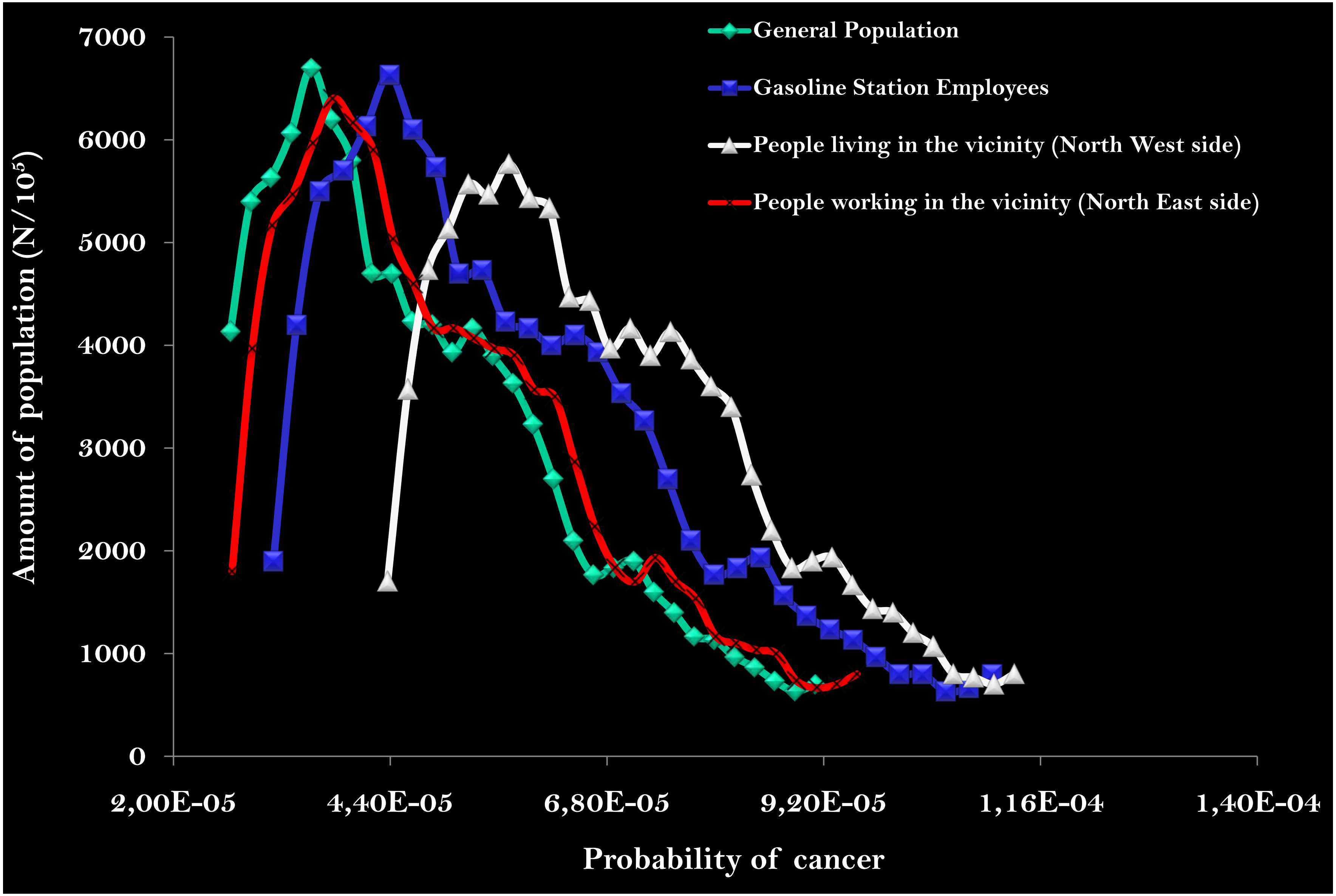


Exposure modelling by ANNs





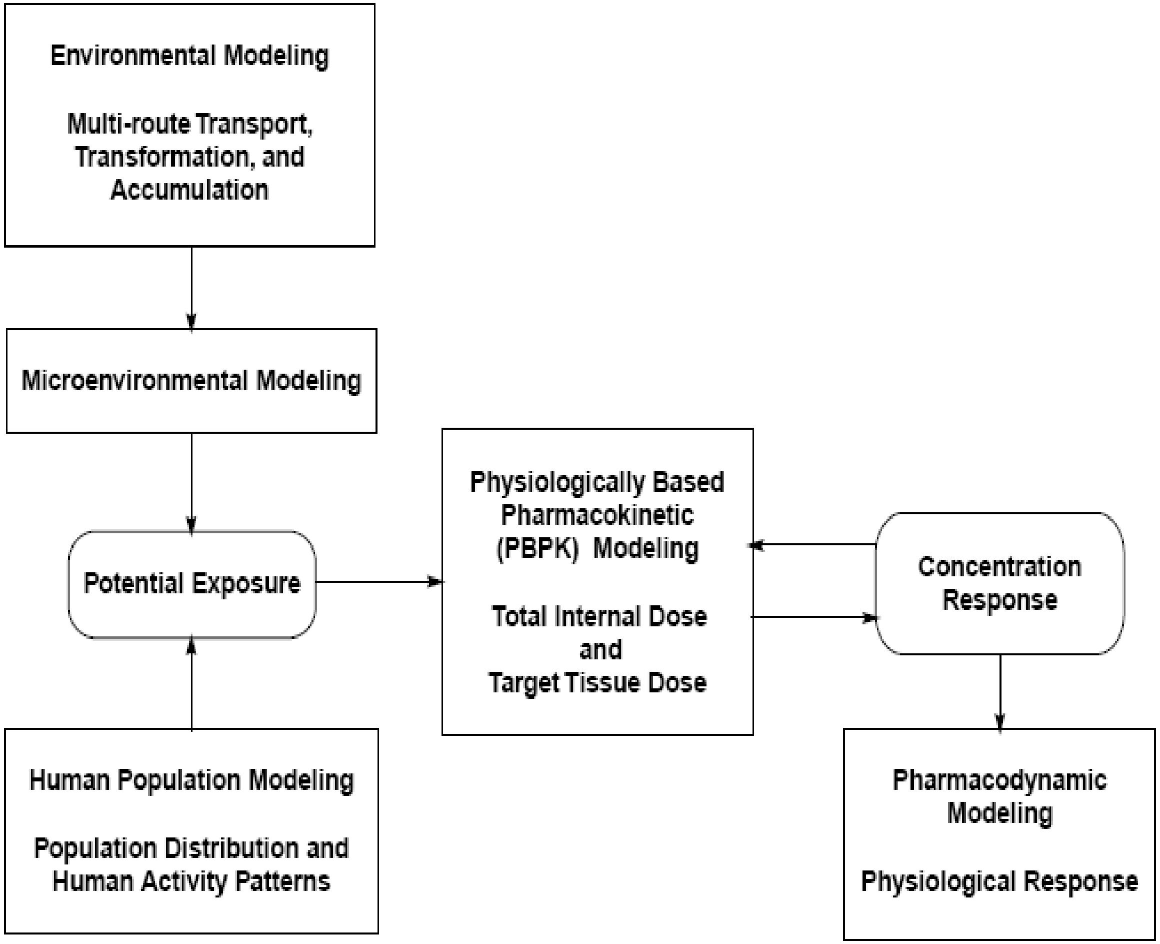
Gasoline station effect



Health impact assessment of policies: the case of Arsenic

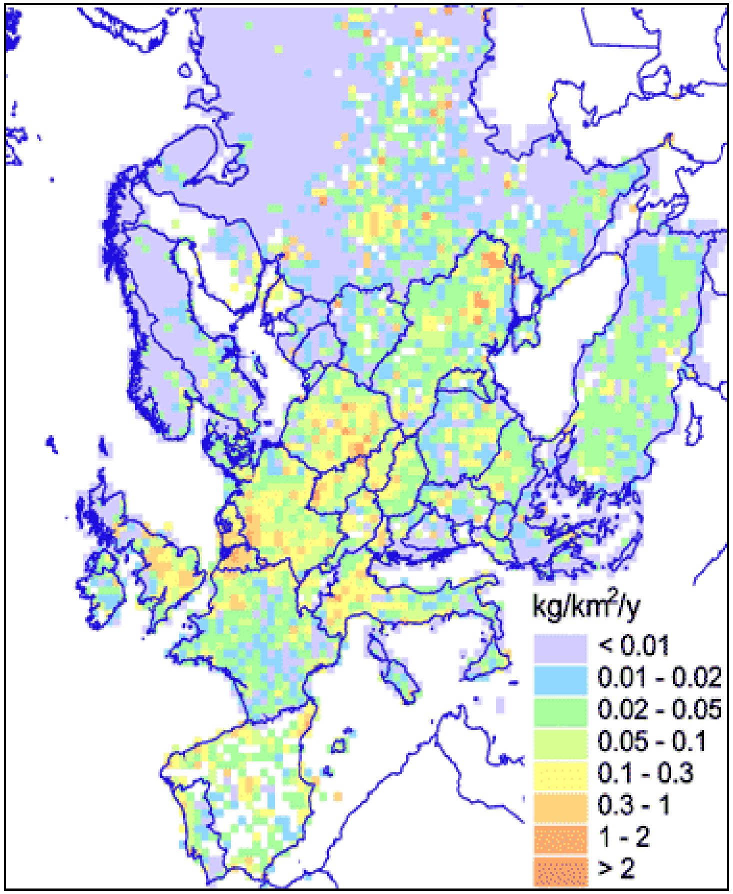
Sector	Specific policies considered
Large combustion plants	Baseline 2010: IPPC Directive – BREF on large combustion plants Large Combustion Plants Dir (2001) Baseline 2020: Emerging techniques MFTR 2020: Kyoto Protocol – Council Decision 2002/358/EC Directive 2001/77/EC – IGCC & supercritical polyvalent processes
Iron / Steel production	Baseline 2010: IPPC Directive – BREF on iron/steel production Baseline 2020 – emerging techniques in sintering, catalytic oxidation MFTR 2020 – new iron-making techniques: direct reduction/smelting reduction
Cement industry	Baseline 2010: IPPC Directive – BREF on cement and lime manufacturing Baseline 2020: FGD techniques, activated C filters for HM reduction MFTR 2010 = Baseline 2020 MFTR 2020 = all plants with HM reduction technologies
Petrol	Baseline 2010: Directives 98/70/EC and 2003/17/EC - Ban in use of leaded petrol - 5 mg Pb/l in unleaded petrol - high % of passenger vehicles comply with Euro 2000 and 2005 norms - high % of HDV comply with Euro III norm Baseline 2020: significant % of LPG cars and lot of HDV comply with Euro IV and V MFTR 2010 = Baseline 2020 + increase of % of LPG cars MFTR 2020: increase of share of electric/FC cars

Integrated risk assessment based on BED



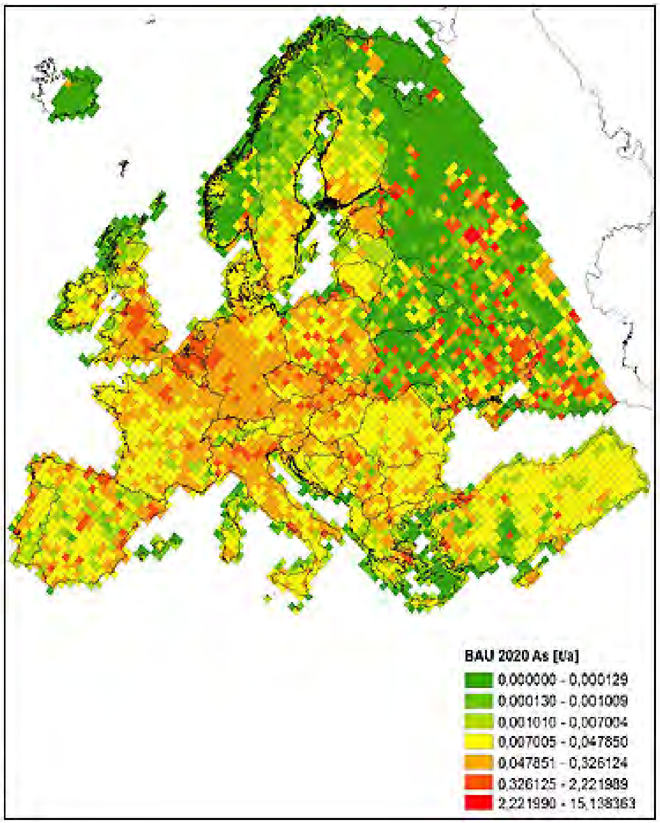
Modeling framework

- Stuttgart Emission Tool (SET) for country-specific emissions, by activity sectors
- MSCE-HM for transboundary transport across Europe
- WATSON for soil, water concentration and food-relevant exposure
- XtraFood for food contamination through plant uptake
- JRC BBDR platform and ISE for internal dosimetry and risk assessment
- VSL and contingent valuation functions for monetary cost assessment
- Quantification/reduction of uncertainty with MCMC

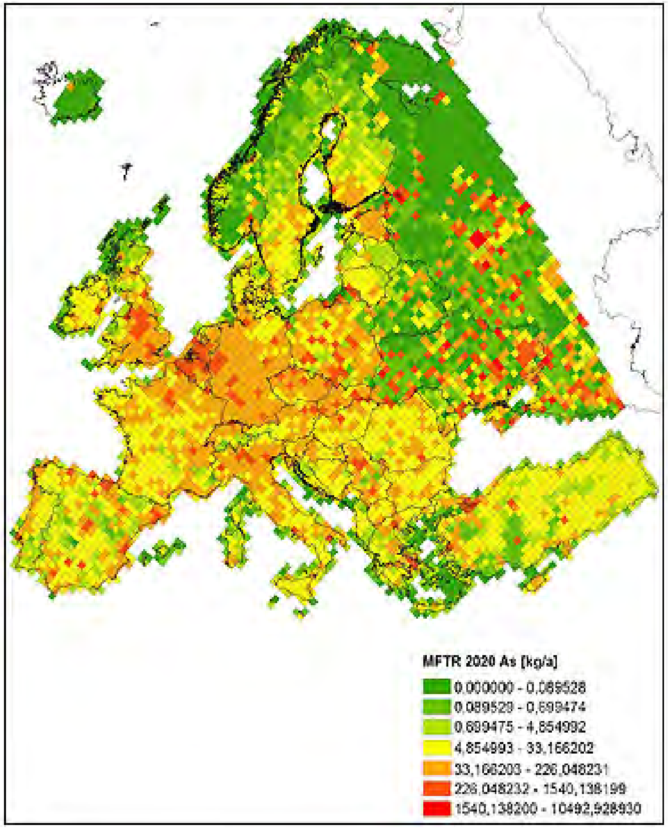


Spatial distribution of anthropogenic air emissions of arsenic in Europe for the year 2000 [kg/km^2/y].

(a)

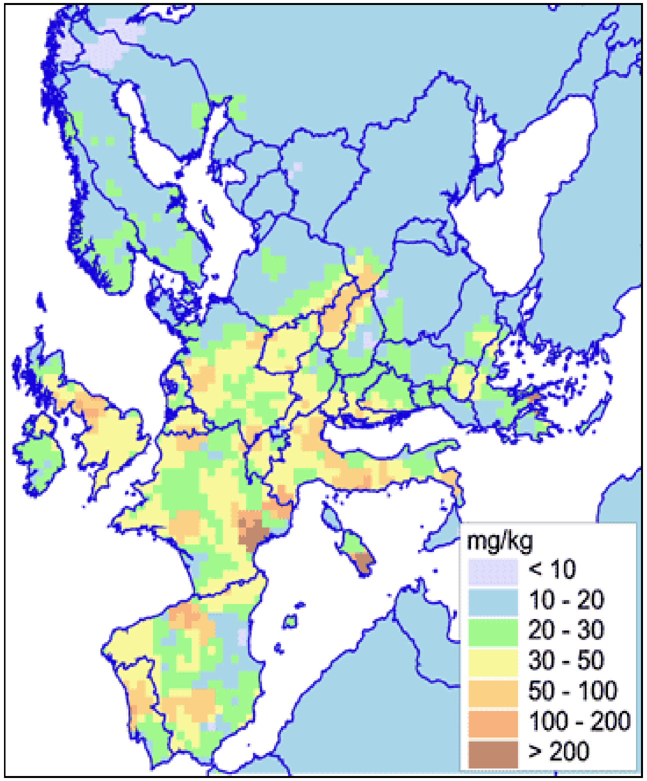


(b)

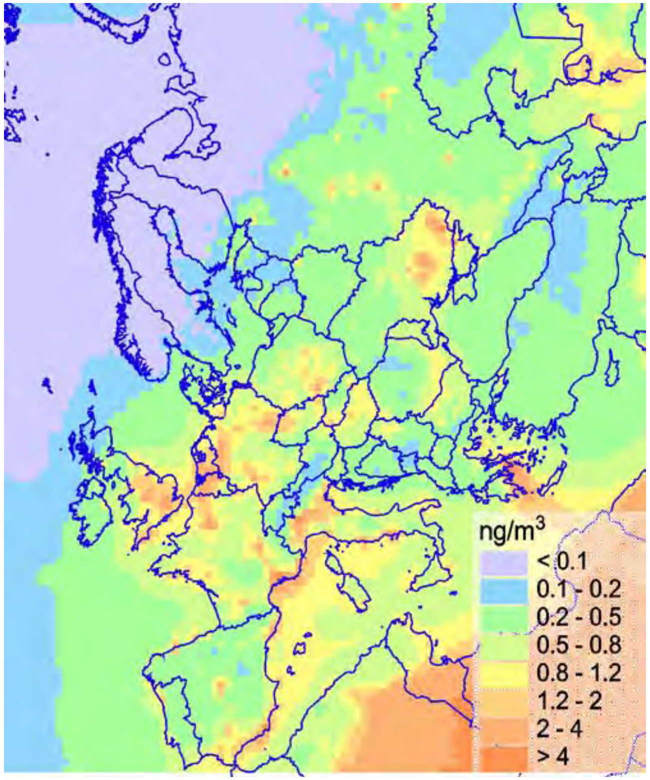


Spatial distribution of anthropogenic air emissions of arsenic in Europe (a) for the BAU scenario and (b) for the MFTR scenario projection of the year 2020 [t/y].

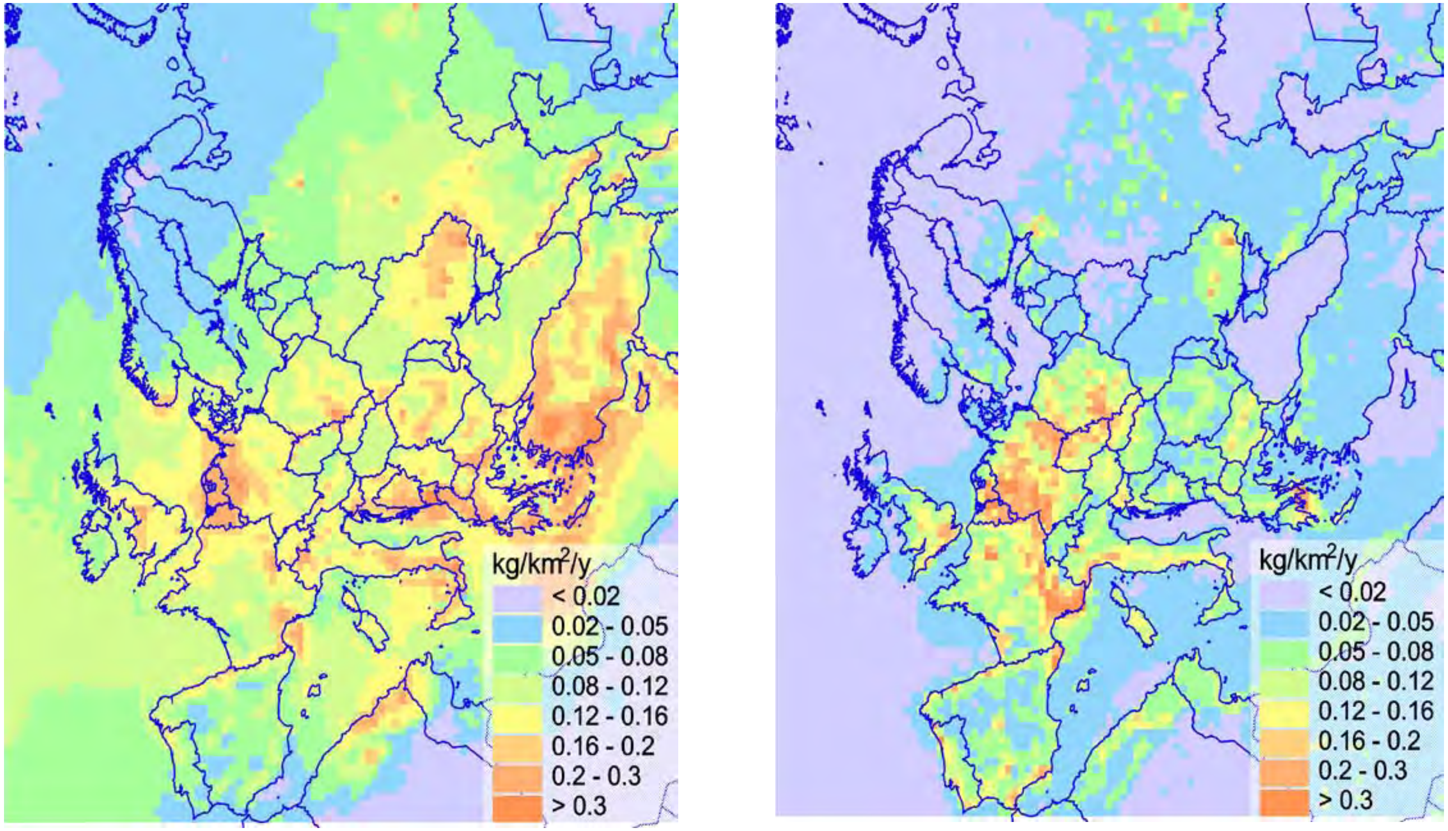
(a)



(b)

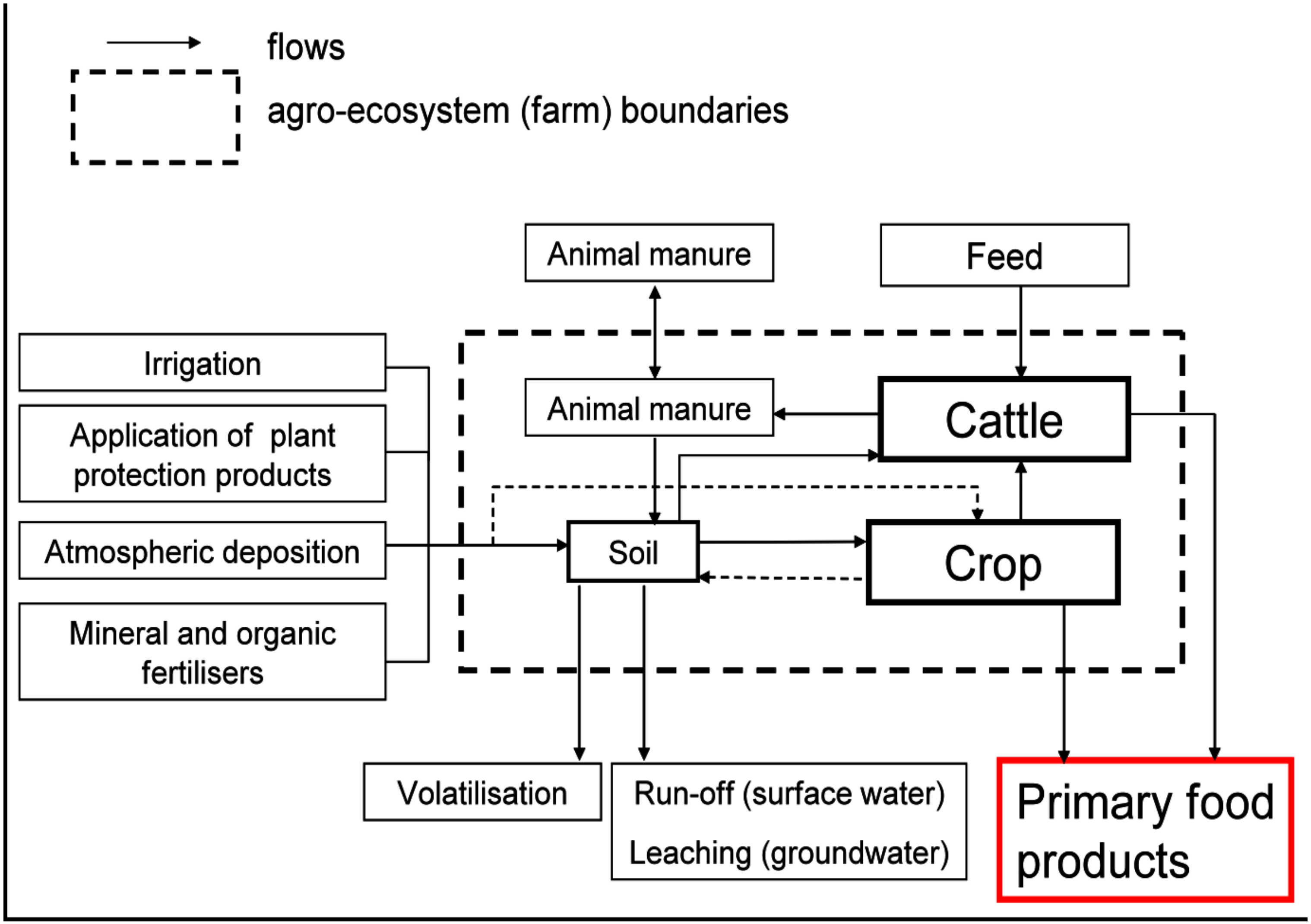


Spatial distribution of concentrations in European top-soils including adjacent territories [mg/kg] (a) and mean annual concentration in ambient air (b) for arsenic for the year 2000.

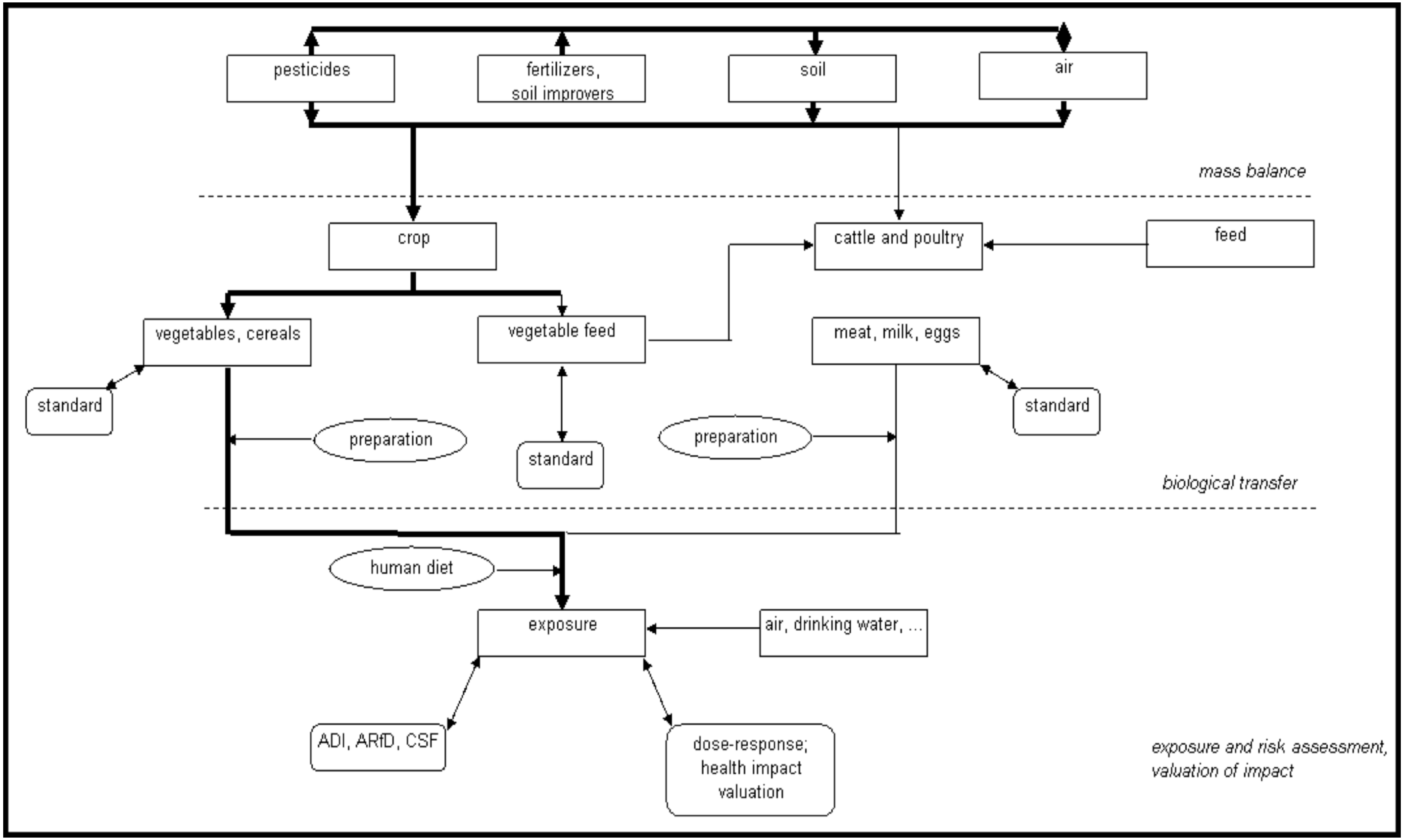


Spatial distribution of arsenic annual wet (a) and dry (b) deposition over Europe in 2000.

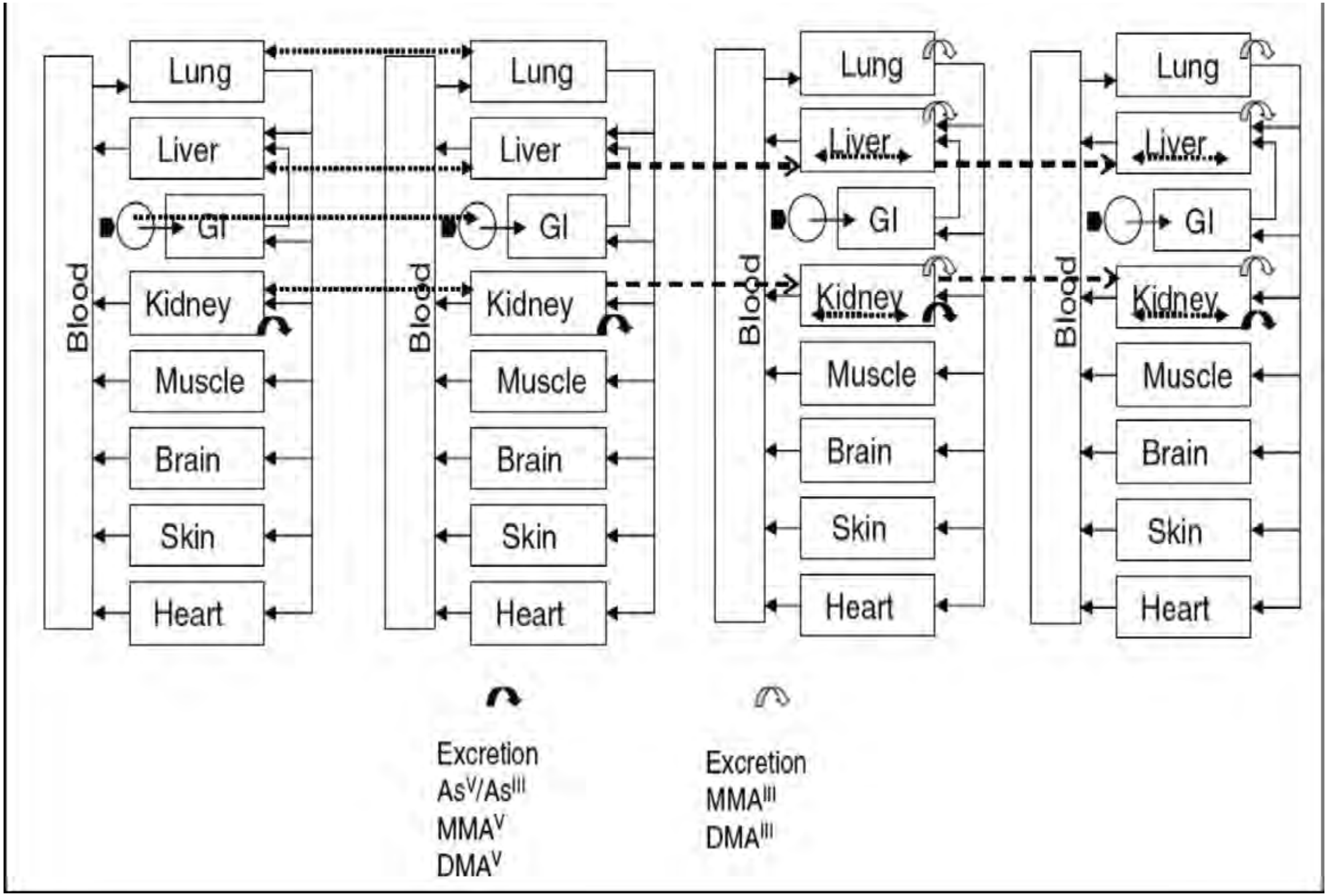
Contaminant flows in the food chain

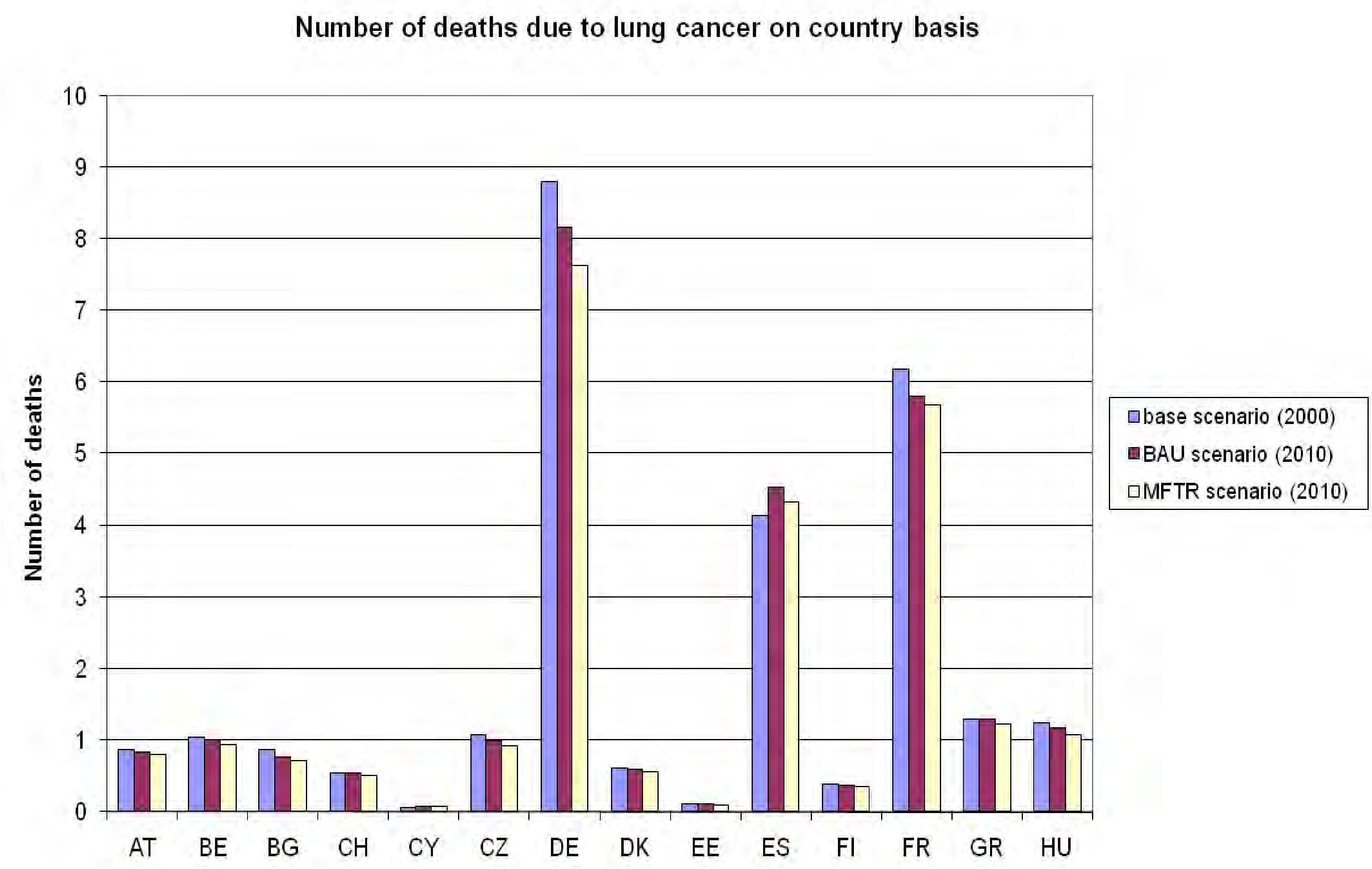


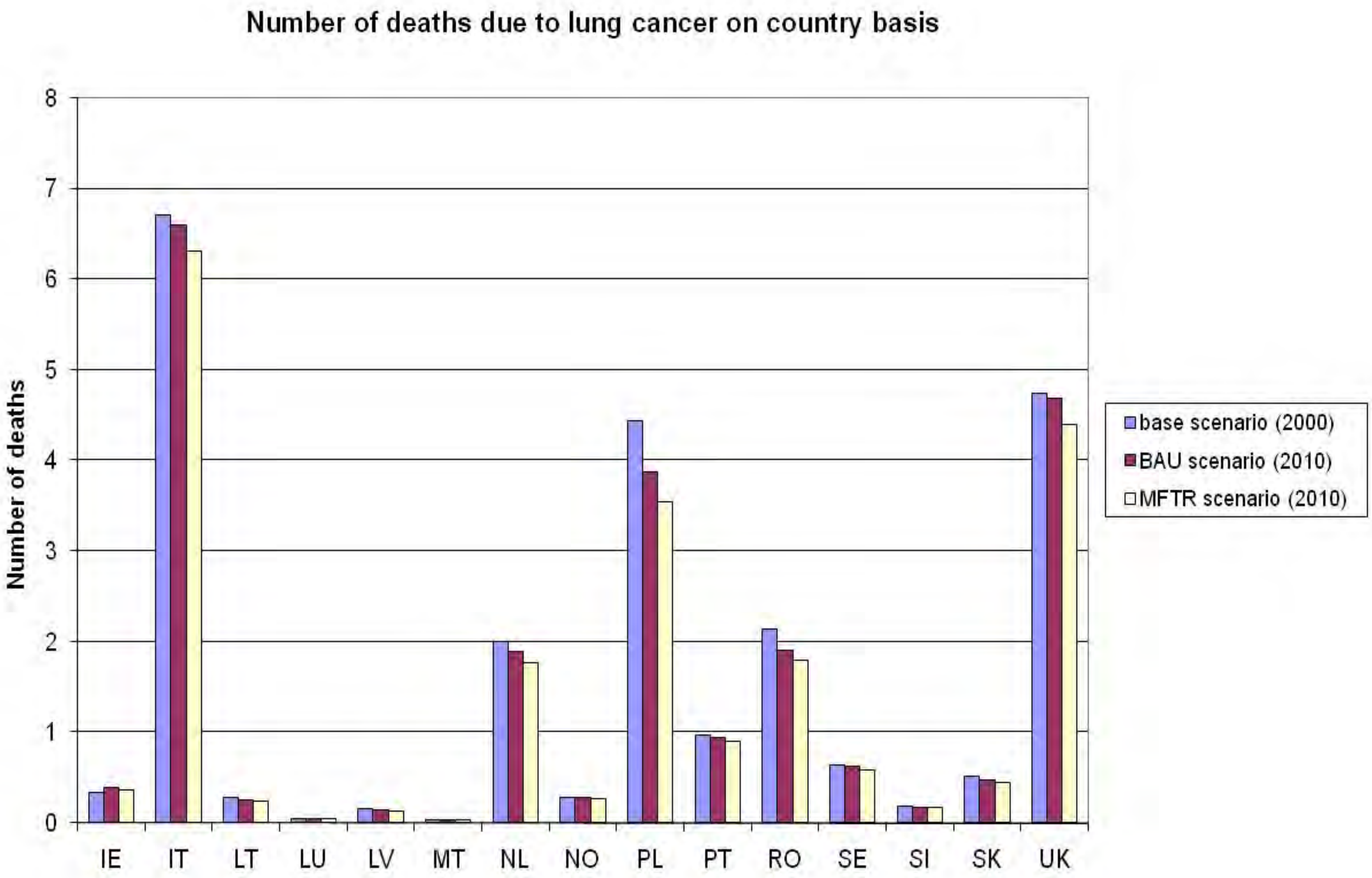
Human exposure routes via contaminated food



As PBPK/PD model



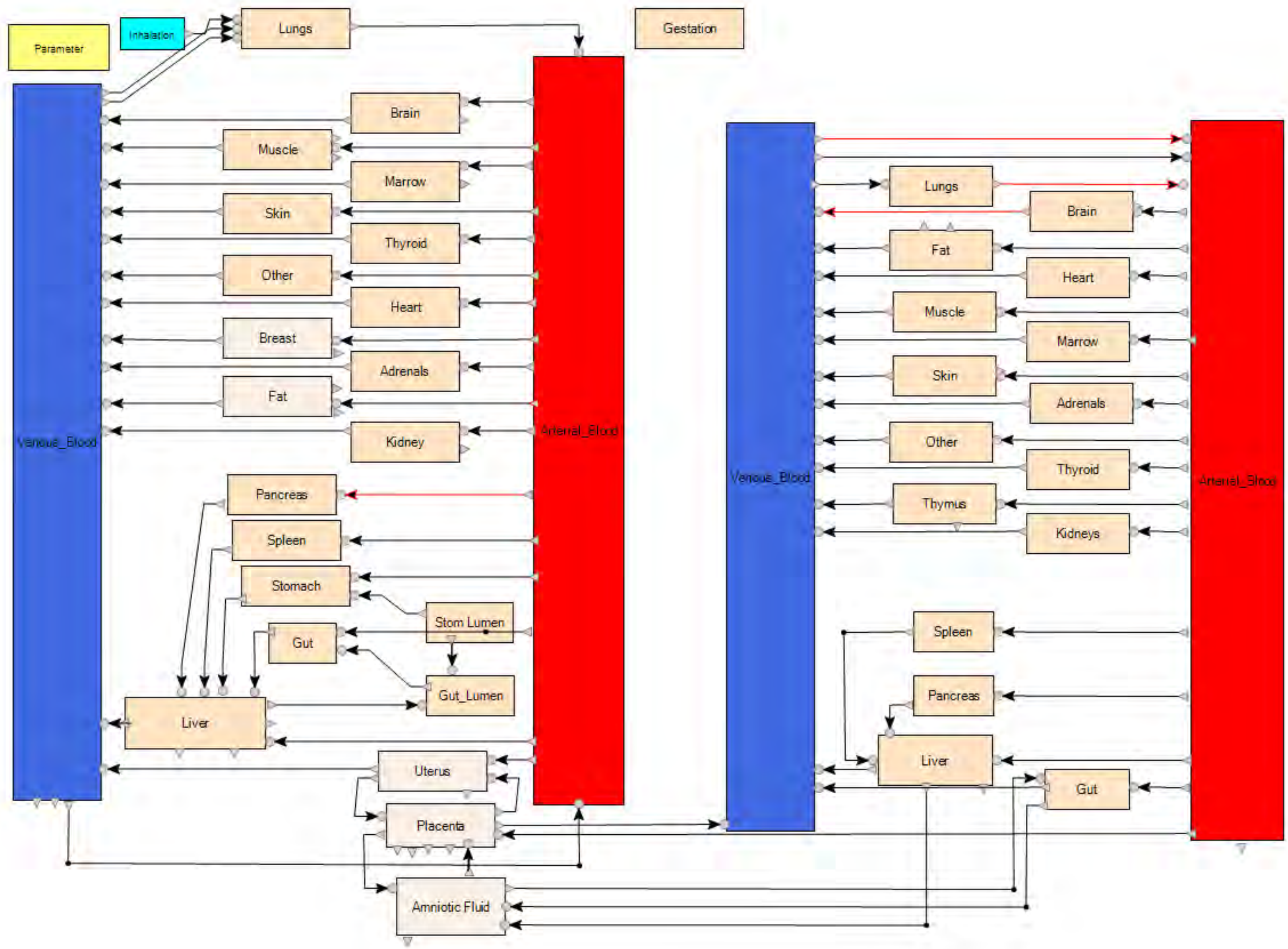

Number of deaths due to lung cancer on country basis
Number of deaths
10
9
8
7
6
5
4
3
2
1
0
AT
BE
BG
CH
CY
CZ
DE
DK
EE
ES
FI
FR
GR
HU
base scenario (2000)
BAU scenario (2010)
MFTR scenario (2010)


Number of deaths due to lung cancer on country basis
Number of deaths
0
1
2
3
4
5
6
7
8
IE
IT
LT
LU
LV
MT
NL
NO
PL
PT
RO
SE
SI
SK
UK
base scenario (2000)
BAU scenario (2010)
MFTR scenario (2010)

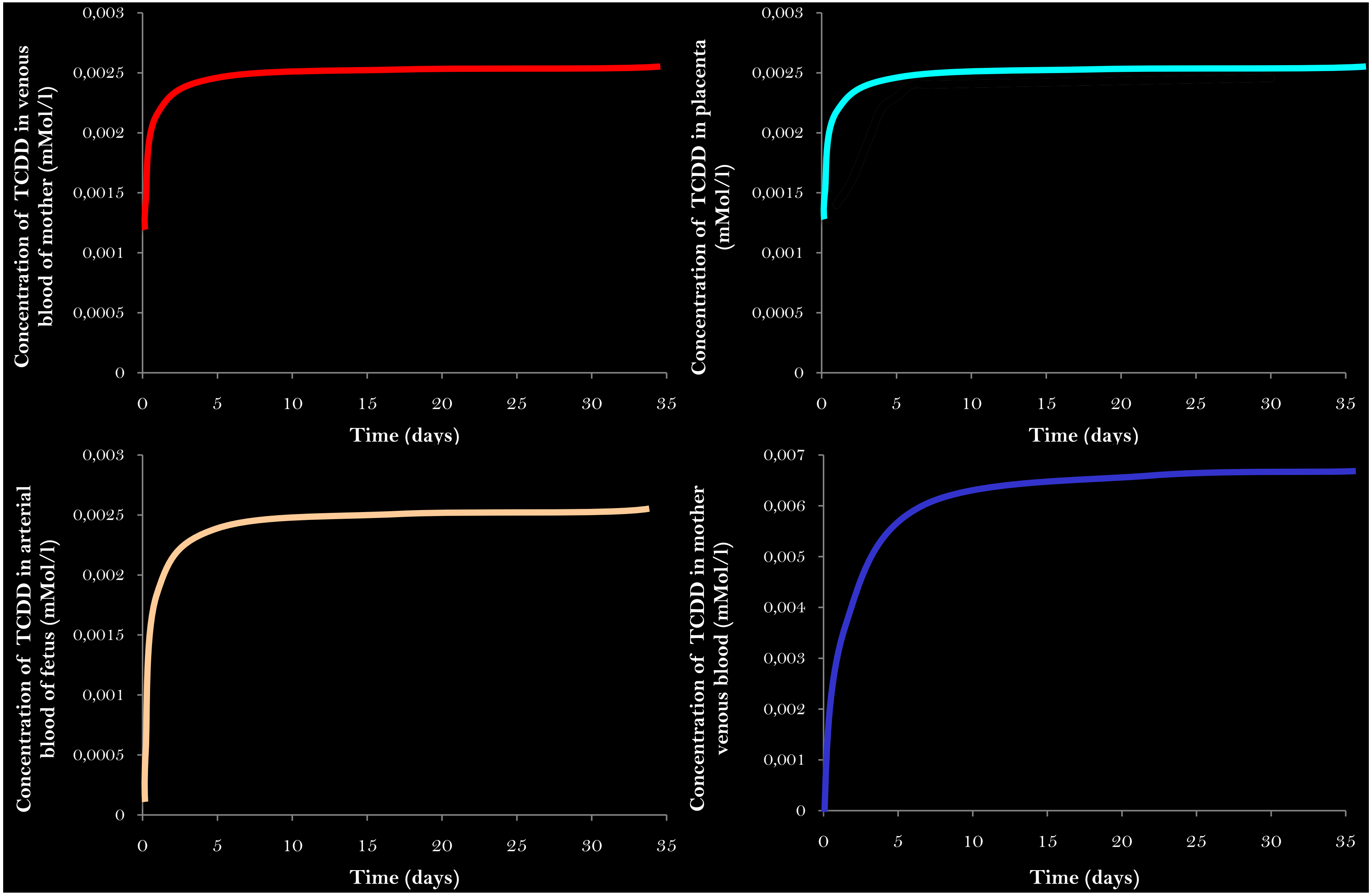
Lifetime exposure including:

- In utero exposure
- Newborn exposure
- Childhood exposure
- Adulthood exposure

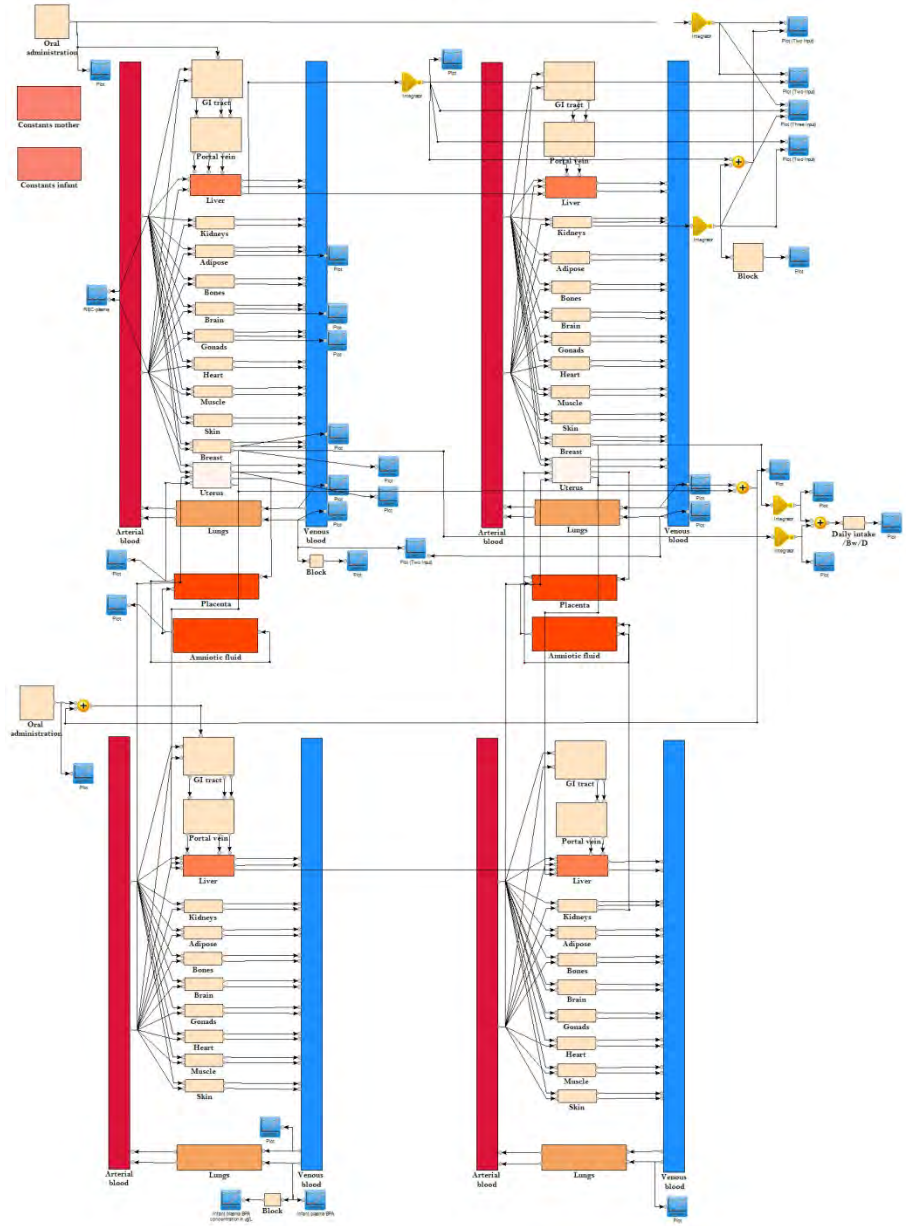
Mother-fetus model for 2-generation effects



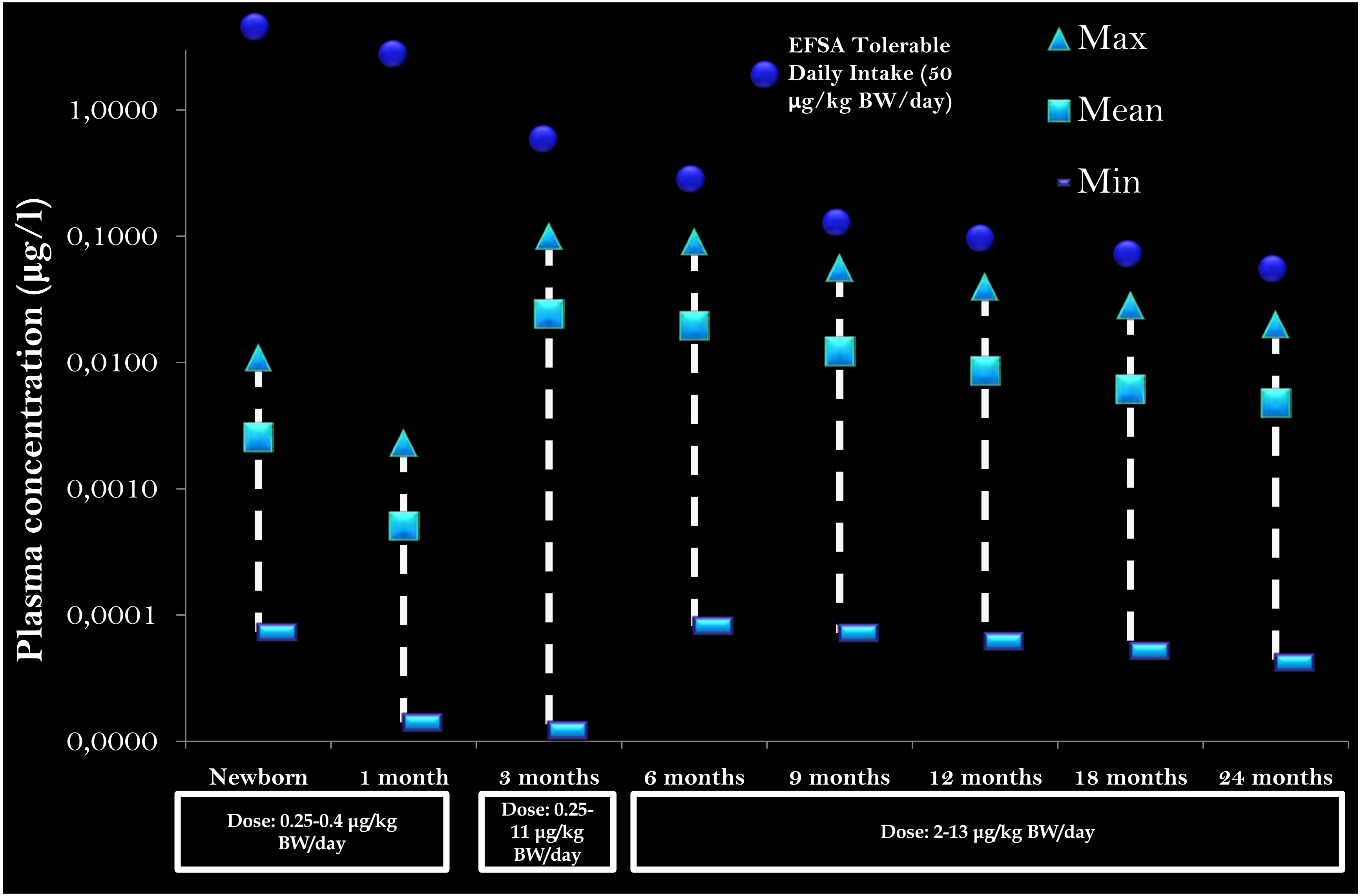
In utero exposure to dioxins



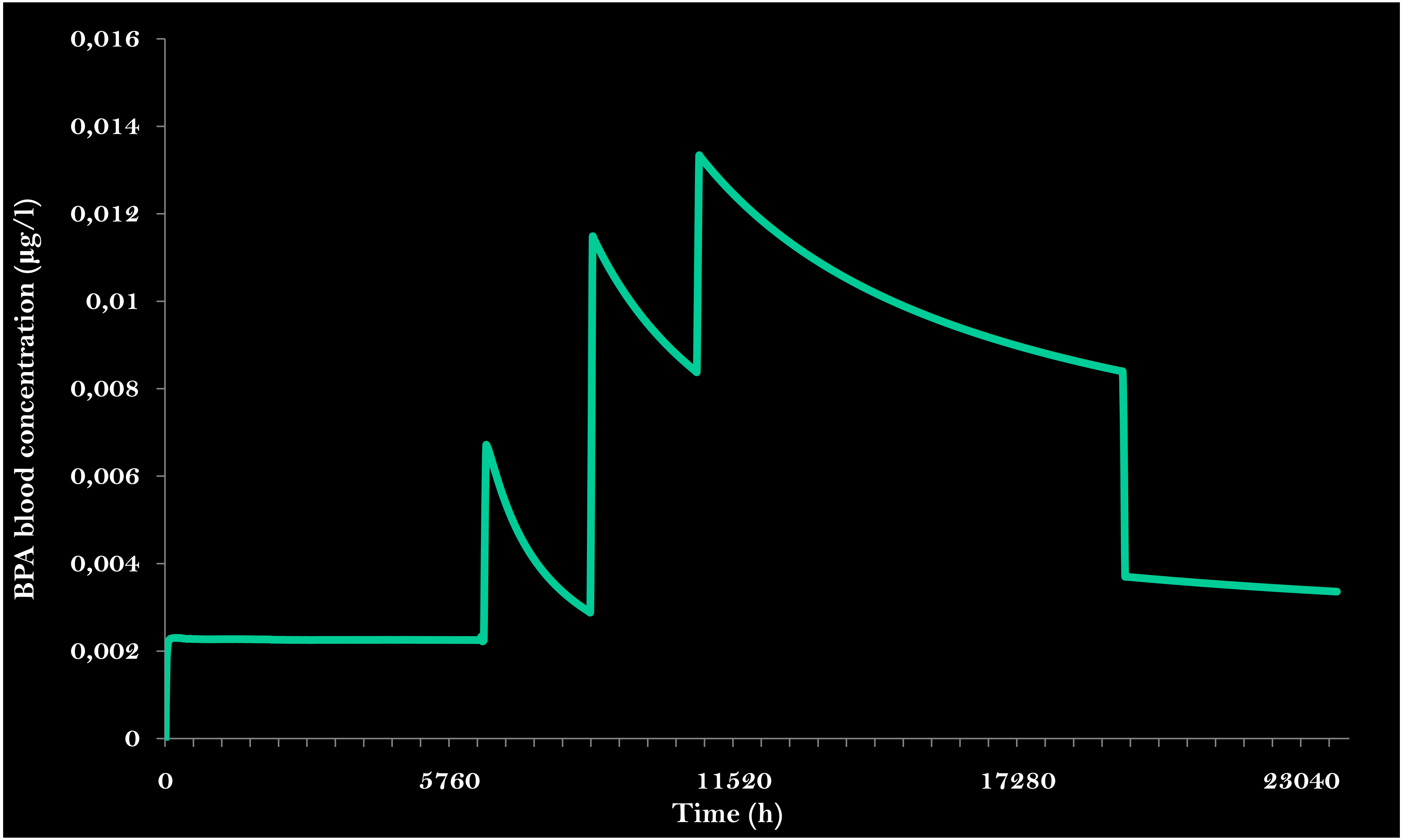
The case of Bisphenol A (BPA)



Bisphenol A (BPA)



Bisphenol A (BPA)



Gestation period (9 months)

Breast feeding (till 3rd month)

Bottle feeding from 6th to 9th month (7.5 µg/kg BW/d)

Bottle feeding from 9th to 18th month (13 µg/kg BW/d)

Bottle feeding from 18th to 24th month (5.3 µg/kg BW/d)

Conclusions (1/2)

Benefits to public health – improved risk assessment

- Expressomics allowed identification of gene expression profiles characterising exposure tochemicals alone and in co-exposure to other substances
- Gene expression profiles can be used as biomarkers of exposure to taking into account risk modifiers such as:
  - diet
  - gender
  - age
  - time length of exposure
- Whole genome micro-arrays allow reviewing all gene associations modulating physiological response and identifying end points specific to the most significant associations
- Bioinformatic data analysis holds great potential for building plausible mechanistic hypothesis on mechanism of action and exposure biomarker discovery

Towards the exposome:

- The exposome approach can be implemented coupling:
  - macro-/micro-environmental modeling
  - passive/active personal monitoring
  - human biomonitoring
  - expression biomarkers
  - physiologiy-based biokinetic modeling
  - systems biology modeling
- A tiered approach should be developed to use exposure information for toxicity prioritization:
  - Tier 1
    - exposure surrogates
    - sentinels of exposure
  - Tier 2
    - Full chain exposure assessment

Thank you for your attention

Acknowledgments

- JRC

A Gotti
S Karakitsios
I Liakos
F Camilleri
E Marafante
G CiminoReale
B Casatti
A Colotta
R Brustio
D Kotzias
J Barrero-Moreno
S Tirendi
O Geiss
A Katsogiannis

- IER

P Fantke
B Tirruchitampalam
U Kummer

- MSC-East

O Travnikov

- VITO

J Bierkens
R Torfs