Internal Exposome

Rob Stierum, PhD, ERT.

TNO

on behalf of all Exposogas partners

About TNO

- **Organisation:**
  - TNO, the Netherlands Organisation for applied scientific research TNO, was founded by law in **1932** to **enable business and government to apply knowledge**. As an organisation regulated by public law, we are **independent**: not part of any government, university or company.
- **Mission:**
  - TNO **connects people** and **knowledge** to create **innovations** that boost the **competitive strength** of industry and the **well-being** of society in a **sustainable way**.

Complex exposures at home/ at work

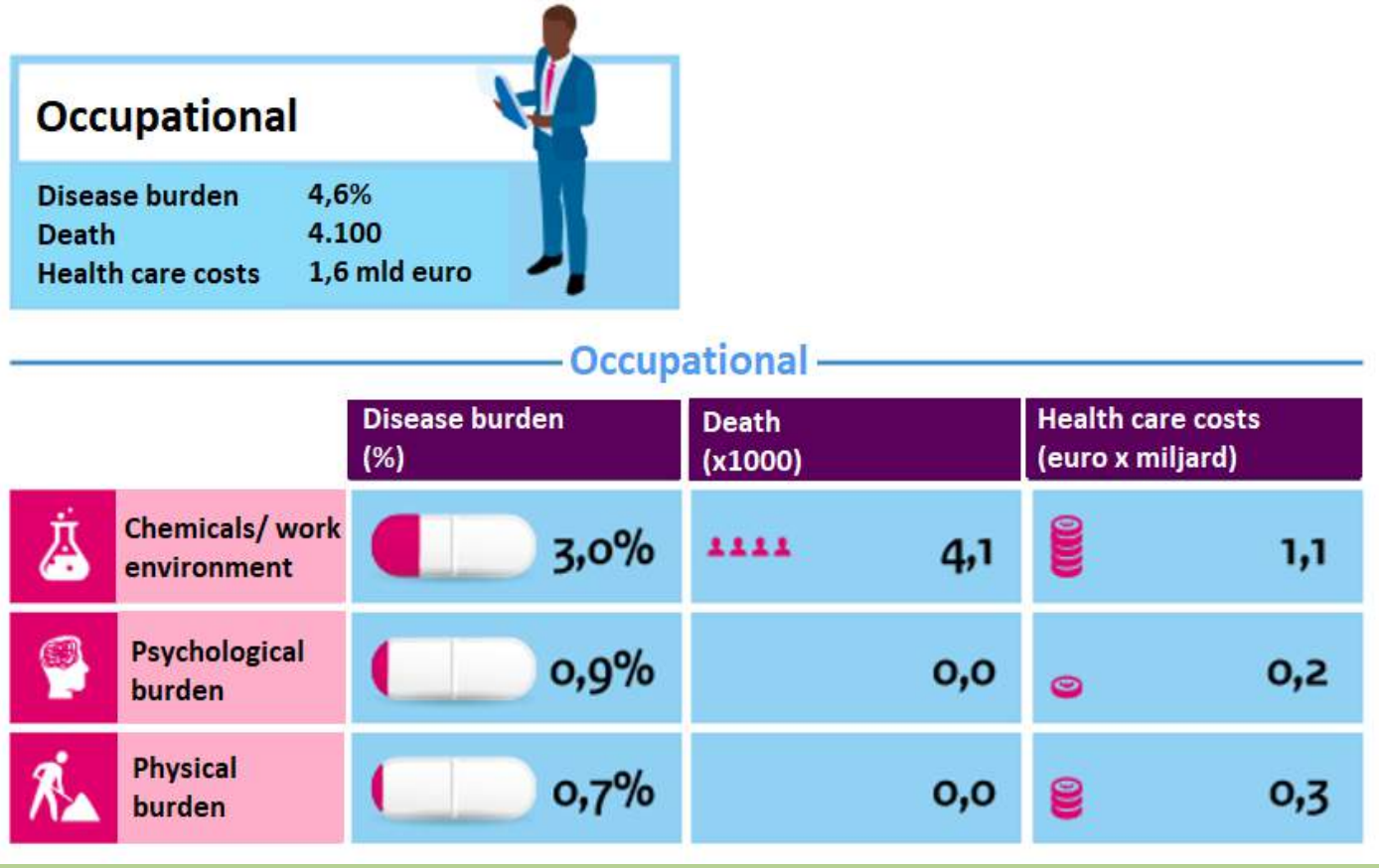
- Lack of knowledge on the associations between risk factors and health is limiting effective prevention

- The burden of disease due to
  - occupational risks: 4.7%
  - environmental factors: 5.7%

of the total in the Netherlands

Necessity to consider occupational exposure in relation to health

- **Impact of occupational diseases:**



First, to get a feel for chemicals

- hazard, exposure and risk
- some lions and rabbits

hazard, no exposure = no risk

hazard plus exposure = risk

Still, hazard plus exposure = risk?

Not yet, as internal exposure is needed (biting through your skin and beyond!)

Exposure/dose level matters. More lions →increased exposure →increase in risk

Paracelsus (1493–1541), founder of modern toxicology, “The dose makes the poison”.

chemical	LD50 mg/kg body weight	gram intake at 70 kg body weight
water	140000000	9800
glucose	35000000	2450
Sodiumchloride	3700000	259
Potassiumiodide	300000	21
Arsenic trioxide	45000	3.15
Potassium cyanide	10000 2450	0.7
Sulfur mustard	3000	0.21
Strychnin	500	0.035
Sarin	20	0.0014
Tedrodotoxin	5	0.00035
Ricin	0.02	0.0000014
Tetanus toxin	0.0001	0.000000007
Botulin toxin	0.00003	2.1E-09

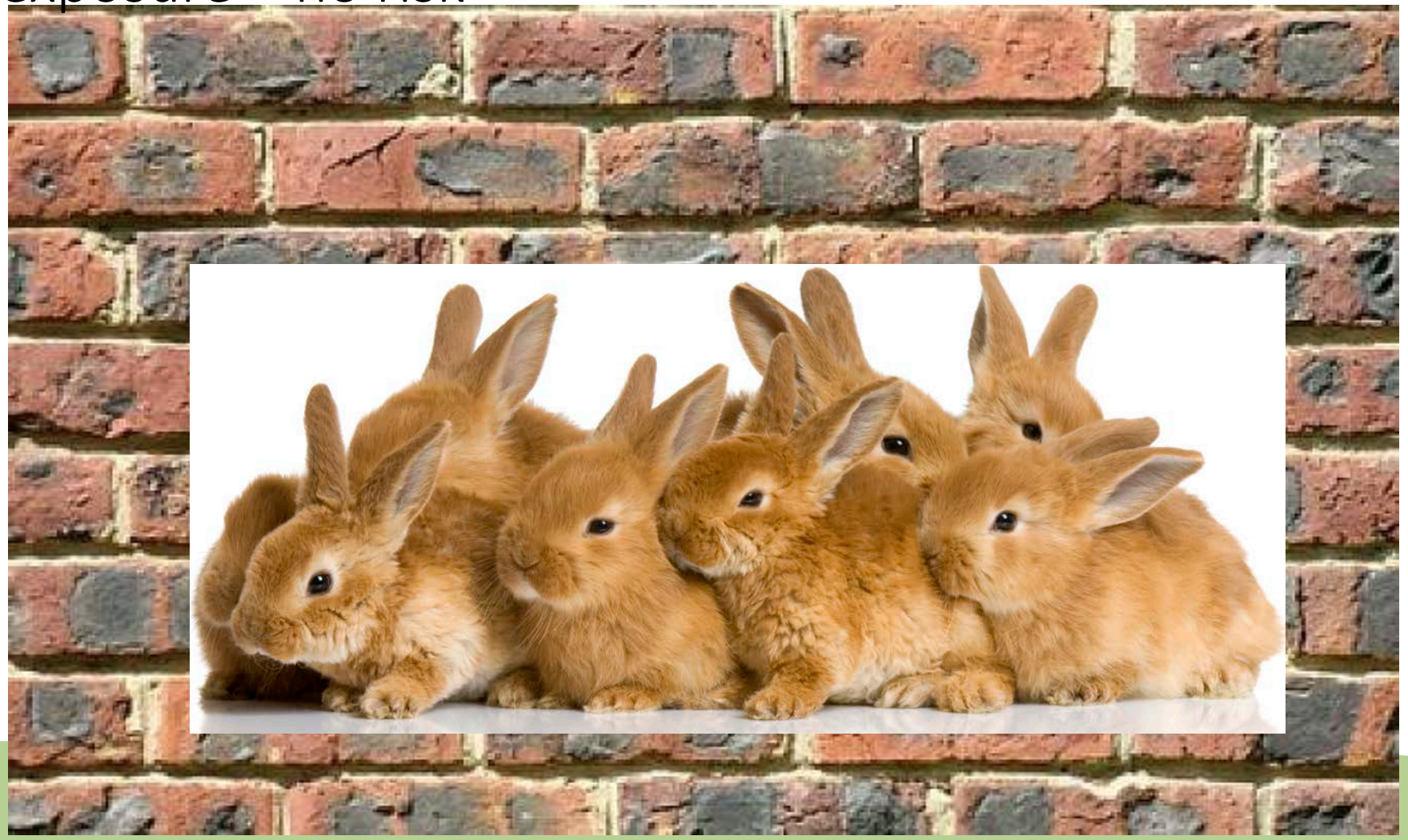
~10 liter of water

730 tablets of

LD50 = dose level at which mortaility occurs in 50% of the population

Exposogas

And then the rabbits. No (lethal) hazard in presence of exposure = no risk



Some “psychology”

- Just the fact that a chemical is present, does not mean it is harmful to humans, at the amount that is present

- In addition, perception of risks comes into play:

  The more uncontrollable and unobservable risk are

  the more severely risks are perceived

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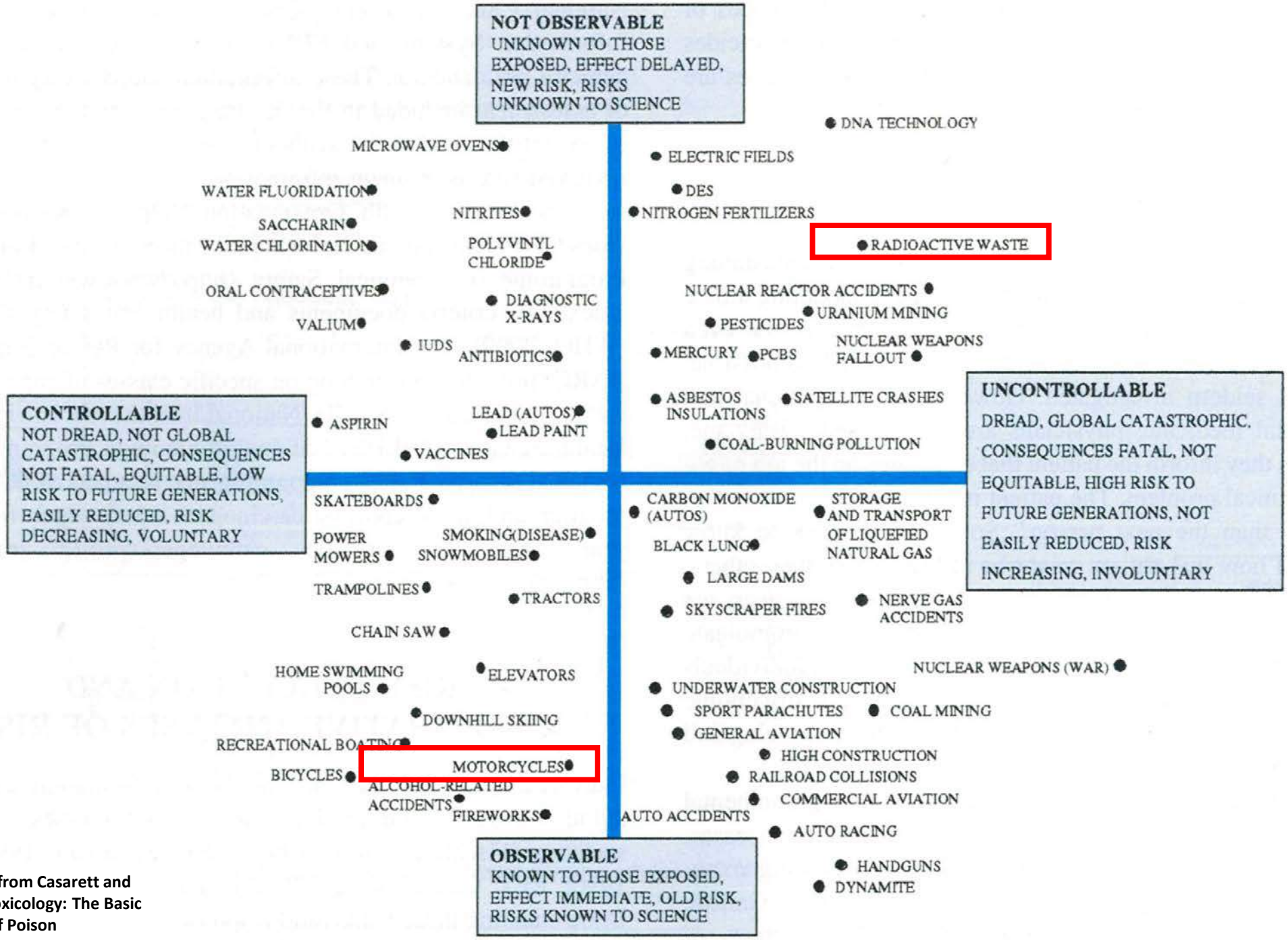


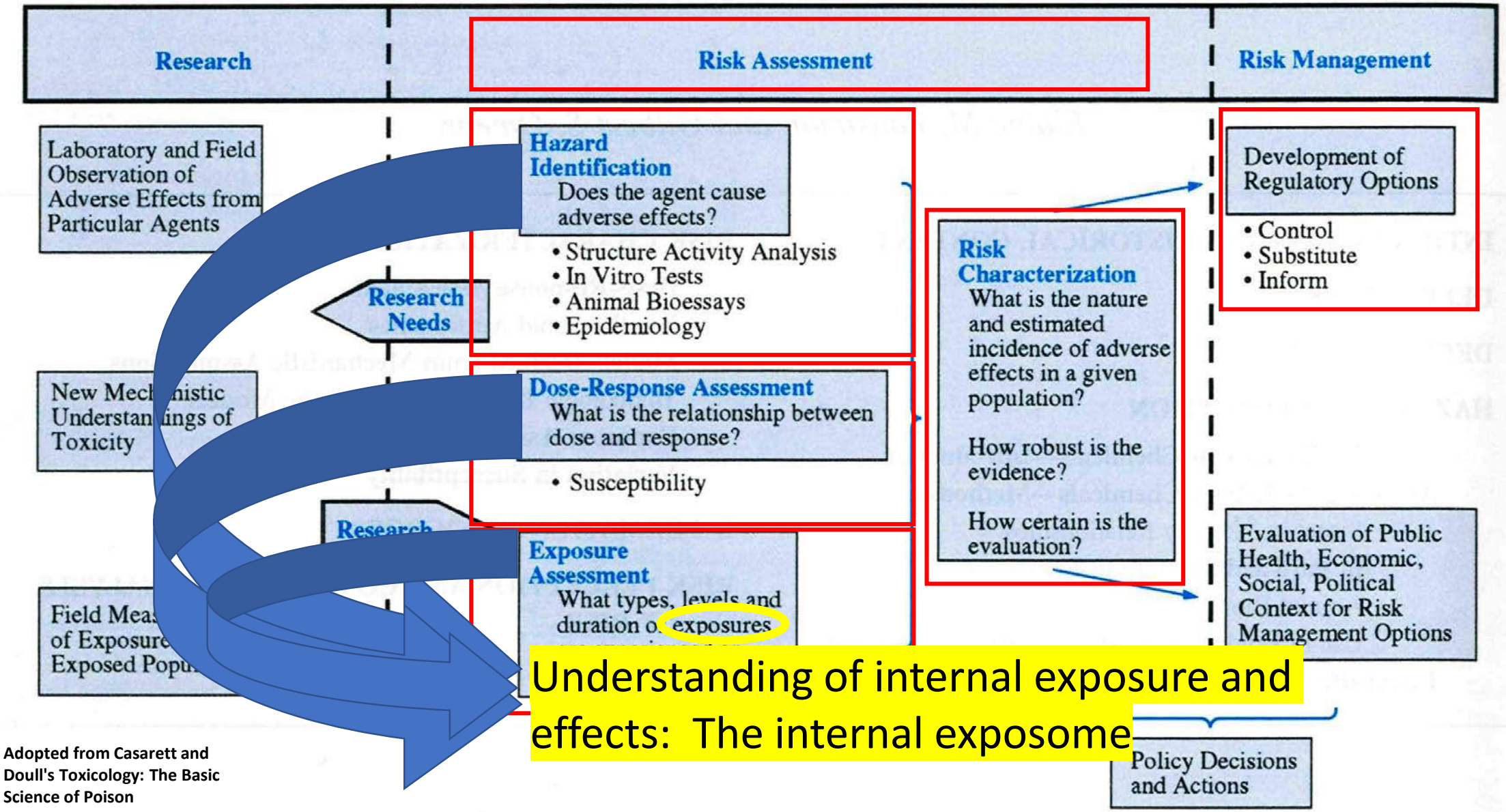
Adopted from Casarett and Doull's Toxicology: The Basic Science of Poison

osogas

For health risk management, thresholds to ensure maximum exposure levels need to be in place

- Acceptable daily intake (nutrition)
- Occupational exposure limit (profession)
- maximum concentration in air (environmental threshold)
- Maximum daily dose (drugs)
- And maximum possible exposures should not exceed these
- Risk assessment needed!

To define a hazard, possible foreseeable internal effects (binting of lions)



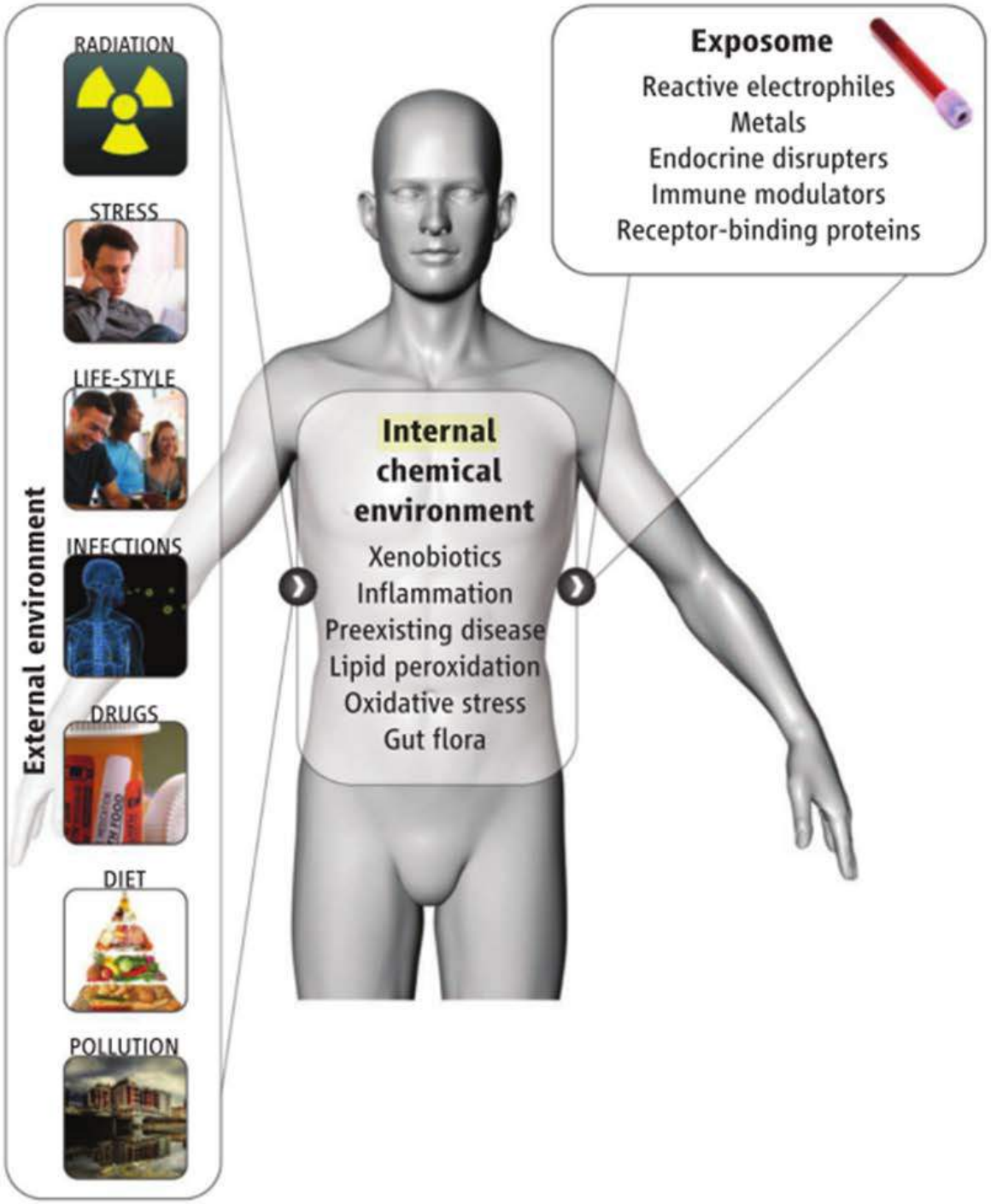
Understanding of internal exposure and effects: The internal exposome

Adopted from Casarett and Doull's Toxicology: The Basic Science of Poison

Definition of the internal exposome

The internal exposome is composed of all stressors once present in the body:

- chemicals from air, water, soil, food,
- Breakdown products (metabolites) from these
- endogenous chemicals produced by inflammation, oxidative stress, lipid peroxidation, infections, gut flora, and other natural processes



From Environment and Disease Risks Stephen M. Rappaport and Martyn T. Smith Science 330 (6003), 460-461.

Definition of the internal exposome

The internal exposome is composed of all stressors once present in the body:

- Can be measured in biofluids/excreta (blood, urine, sputum, stool, exhaled breath etc.)
- Can yield signatures of internal exposure

Definition of the internal exposome

The internal exposome is composed of all stressors once present in the body:

- Can provide indications for early health effects
- Can help to assist in ensuring risk management
  - Detecting exposure and effects in humans
  - Proposing biological effect levels
    - blood levels at which an effect is expected
  - Detecting the effect of exposure reducing measures

Why characterizing the internal exposome?

- In relation to external exposome
  - If suspected/known exposures: Is a chemical/stressor actually entering into the body?
  - If unknown exposures: which chemicals/stressors are persons exposed to?
  - Totality of external exposures is taken into account
  - Is somebody truly exposed?
  - Use internal exposome/markers to predict the extent of possible external exposure?
  - Use to define efficacy of exposure reducing measures
  - How many subjects are exposed? Group based risk assessment

Why characterizing the internal exposome?

- In relation to possible health effects
  - If the stressors/chemical is identified within the internal exposome
    - Estimation of hazards using existing toxicological data, using epidemiological data
  - Estimation of (individual) metabolism/metabolic processes: are potentially hazardous metabolites formed?
  - Individual susceptibility
  - Quantitative determination of internal exposure in relation to early markers of health and disease effects
    - E.g. binding of a chemical to DNA or a protein
    - Early cytogenetic changes

within the internal exposome

- Important to identify *hazardous* molecules
  - all molecules within the exposome are in a way hazardous, depending on type of hazard and extent of exposure (and exposure route)

within the internal exposome

- Prioritize at which exposure *levels* health effects are to be expected
  - External exposures in relation to external exposure treshholds (Occupational exposure limit, ADI)
  - Internal exposures in relation to external exposure levels
  - Internal exposures in relation to internal Biological Effect Levels

Internal exposome

Quantification

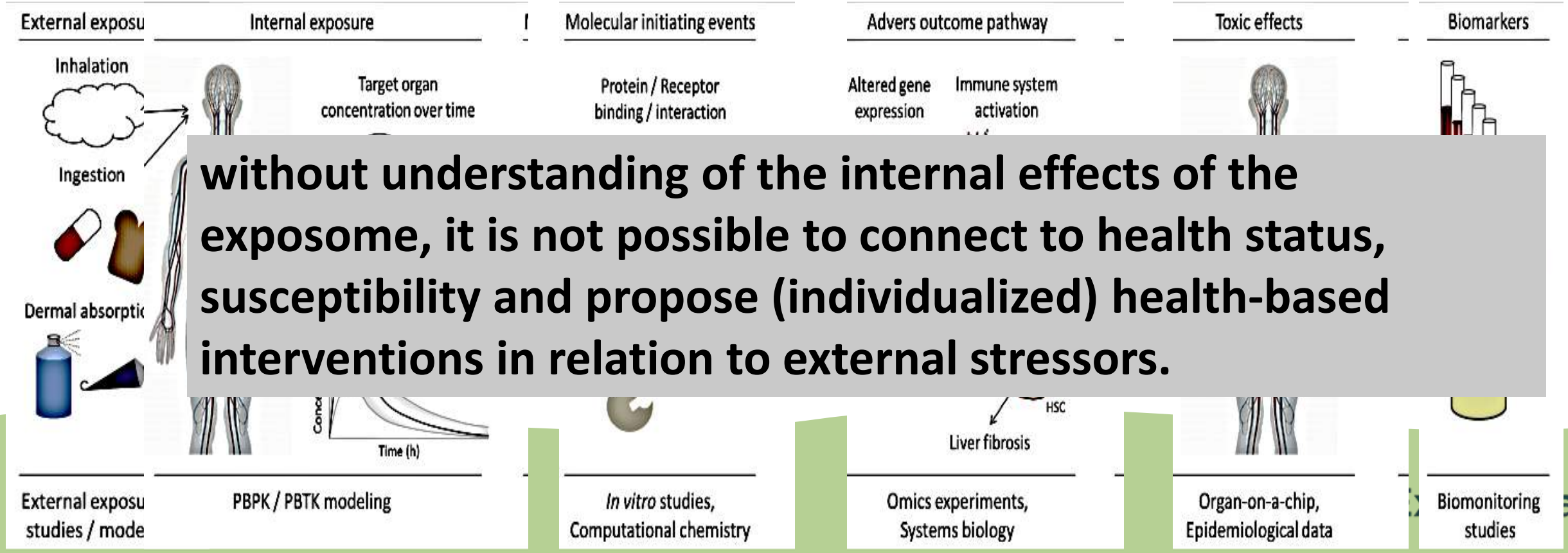
What/how much gets in

which biomolecule is target

activation of pathways/ biology

prediction health effect

Biomarkers to detect internal exposure and health effect



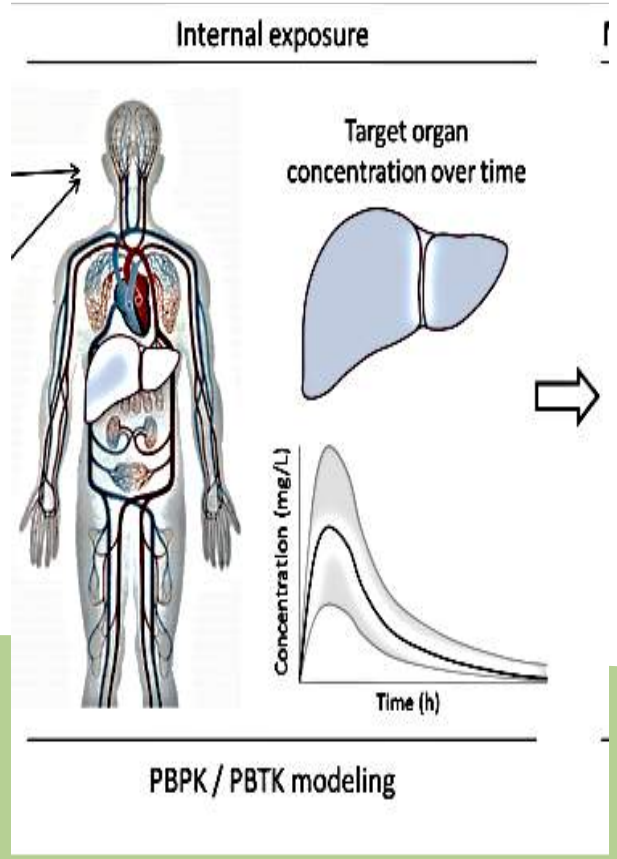
without understanding of the internal effects of the exposome, it is not possible to connect to health status, susceptibility and propose (individualized) health-based interventions in relation to external stressors.

Some technology examples along this paradigm

- Ongoing work at TNO , with partners
- No need to understand all details
- To give a flavour of possibilities to prepare for the group assignment

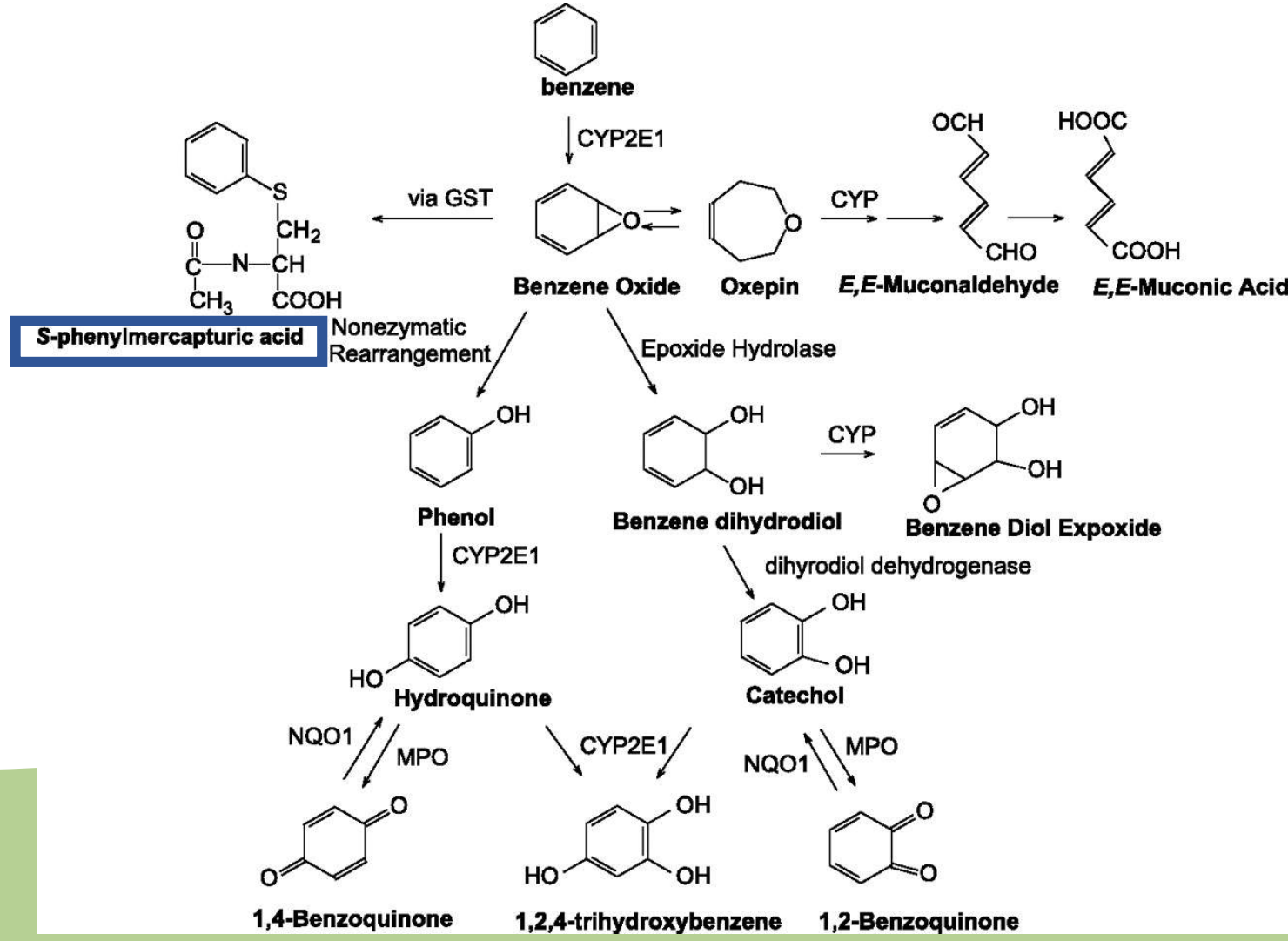
What/how much gets in? I: Biomonitoring

What/how much gets in



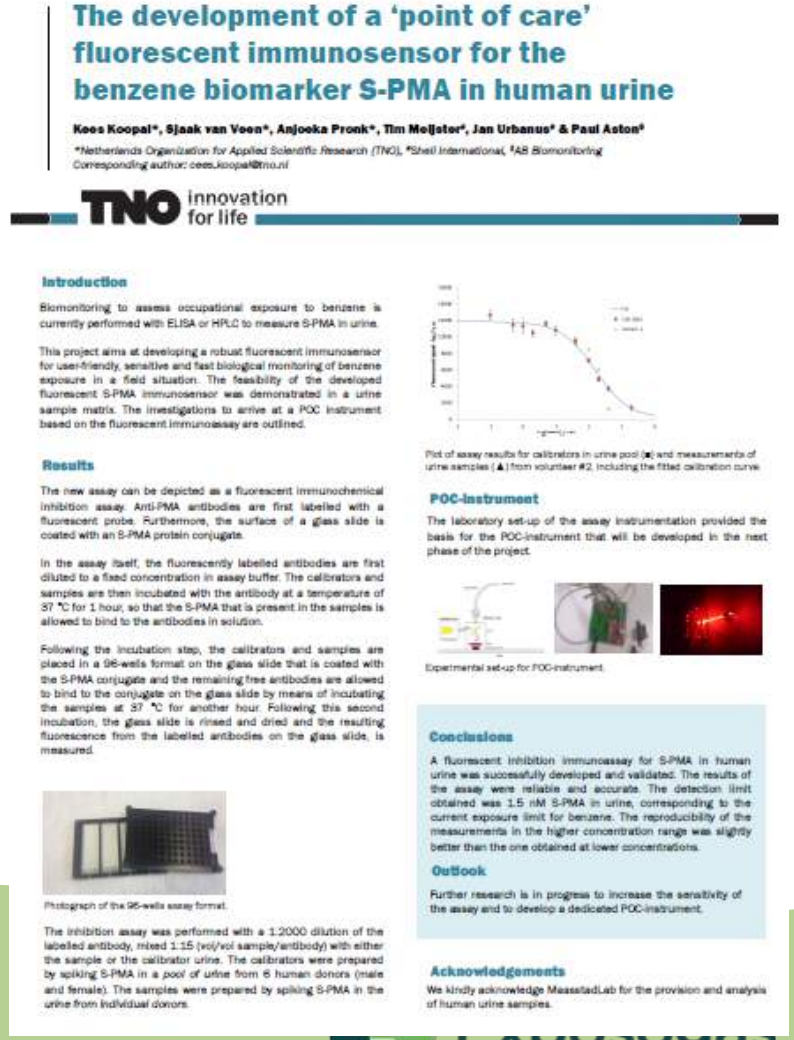
An example: benzene

- Relevant for occupational setting (petrochemical industry)



Detection of SPMA, a metabolite of benzene

- developing a robust fluorescent immunosensor for user-friendly, sensitive and fast biological monitoring of benzene exposure in a field situation.



The development of a 'point of care' fluorescent immunosensor for the benzene biomarker S-PMA in human urine

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*Netherlands Organization for Applied Scientific Research (TNO), *Shell International, *AB Biomonitoring

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TNO innovation for life

Introduction

Biomonitoring to assess occupational exposure to benzene is currently performed with ELISA or HPLC to measure S-PMA in urine.

This project aims at developing a robust fluorescent immunosensor for user-friendly, sensitive and fast biological monitoring of benzene exposure in a field situation. The feasibility of the developed fluorescent S-PMA immunosensor was demonstrated in a urine sample matrix. The investigations to arrive at a POC instrument based on the fluorescent immunoassay are outlined.

Results

The new assay can be depicted as a fluorescent immunochemical inhibition assay. Anti-PMA antibodies are first labelled with a fluorescent probe. Furthermore, the surface of a glass slide is coated with an S-PMA protein conjugate.

In the assay itself, the fluorescently labelled antibodies are first diluted to a fixed concentration in assay buffer. The calibrators and samples are then incubated with the antibody at a temperature of 37 °C for 1 hour, so that the S-PMA that is present in the samples is allowed to bind to the antibodies in solution.

Following the incubation step, the calibrators and samples are placed in a 96-wells format on the glass slide that is coated with the S-PMA conjugate and the remaining free antibodies are allowed to bind to the conjugate on the glass slide by means of incubating the samples at 37 °C for another hour. Following this second incubation, the glass slide is rinsed and dried and the resulting fluorescence from the labelled antibodies on the glass slide, is measured.

Photograph of the 96-wells assay format.

The inhibition assay was performed with a 1:2000 dilution of the labelled antibody, mixed 1:15 (vol/vol sample/antibody) with either the sample or the calibrator urine. The calibrators were prepared by spiking S-PMA in a *pool of urine* from 6 human donors (male and female). The samples were prepared by spiking S-PMA in the urine from individual donors.

Plot of assay results for calibrators in urine pool (●) and measurements of urine samples (▲) from volunteer #2, including the fitted calibration curve.

POC-instrument

The laboratory set-up of the assay instrumentation provided the basis for the POC-instrument that will be developed in the next phase of the project.

Experimental set-up for POC-instrument.

Conclusions

A fluorescent inhibition immunoassay for S-PMA in human urine was successfully developed and validated. The results of the assay were reliable and accurate. The detection limit obtained was 1.5 nM S-PMA in urine, corresponding to the current exposure limit for benzene. The reproducibility of the measurements in the higher concentration range was slightly better than the one obtained at lower concentrations.

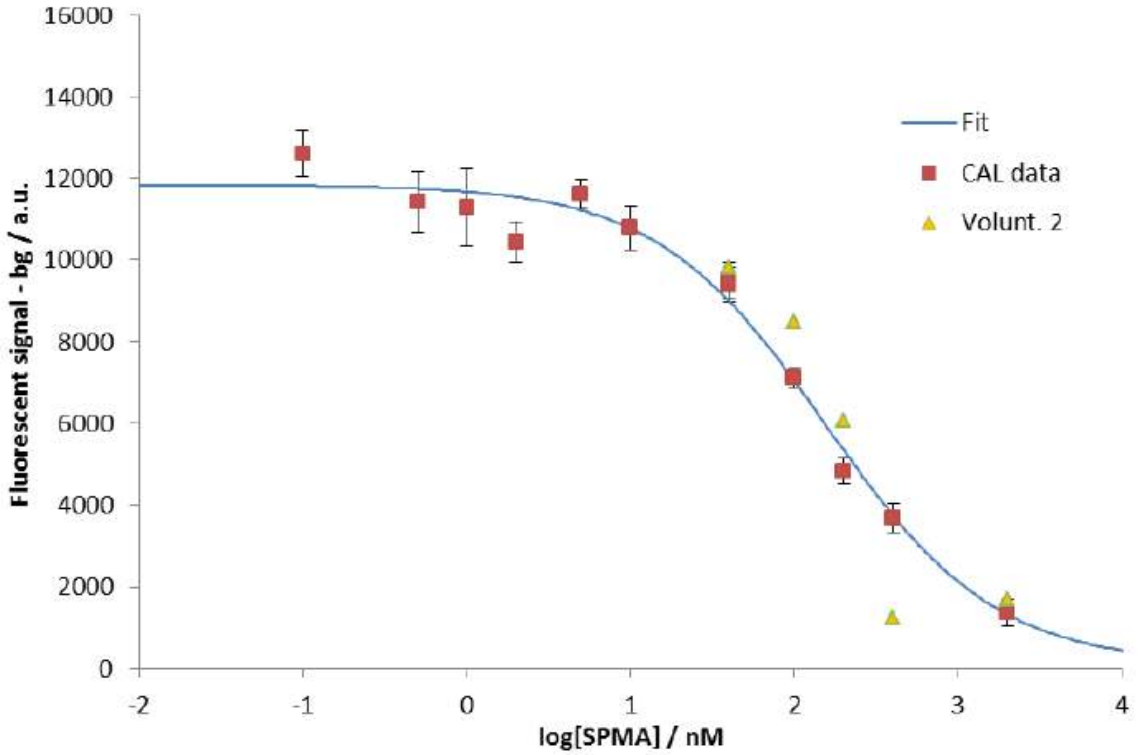
Outlook

Further research is in progress to increase the sensitivity of the assay and to develop a dedicated POC-instrument.

Acknowledgements

We kindly acknowledge MeasetadLab for the provision and analysis of human urine samples.

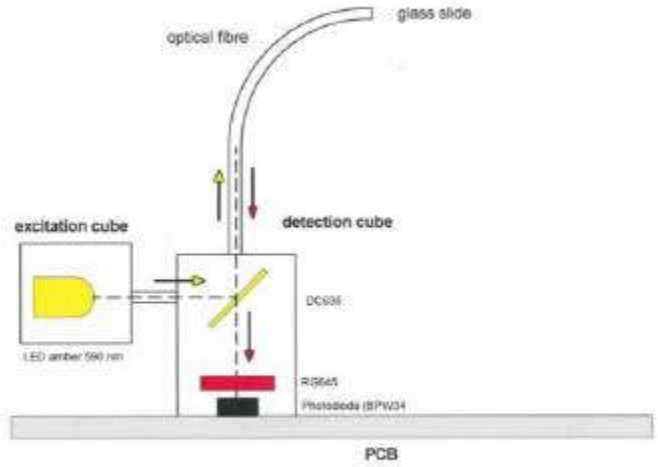
fluorescent inhibition immunoassay for S-PMA in human urine was successfully developed and validated. The detection limit obtained was 1.5 nM S-PMA in urine, corresponding to the current exposure limit for benzene.

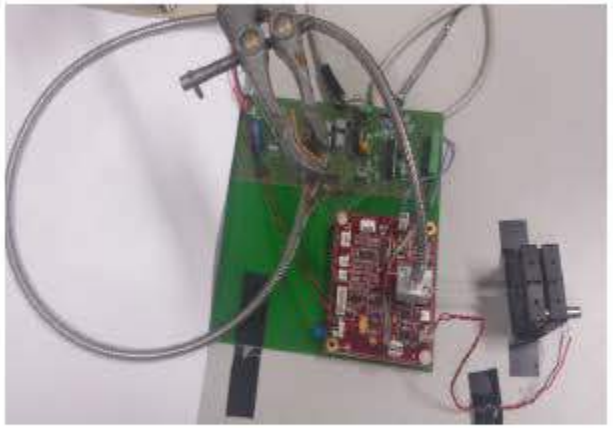


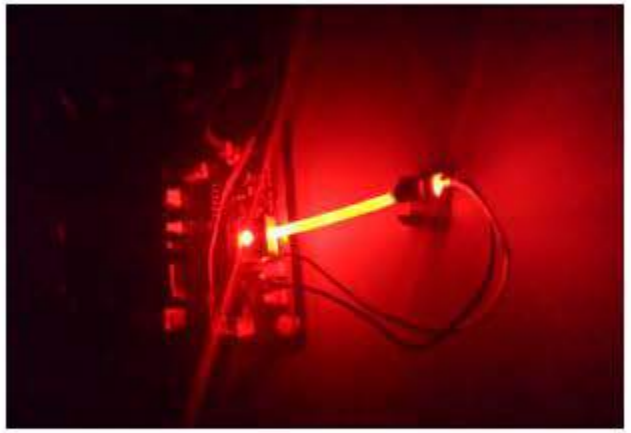
Plot of assay results for calibrators in urine pool (■) and measurements of urine samples (▲) from volunteer #2, including the fitted calibration curve.

Point of care assay

- The feasibility of the developed fluorescent S-PMA immunosensor was demonstrated in a urine sample matrix. The investigations to arrive at a POC instrument based on the fluorescent immunoassay are outlined.







Experimental set-up for POC-instrument.

Future fluid sensing

Wearables – sweat analyser

A possibility

- Sweat. Monitoring sweat as a key biometric is not new. Sweat contains biomarkers like sodium, glucose, and proteins that can be collected and measured noninvasively using sensors. Sweat has been used to monitor other conditions like cystic fibrosis, but now sweat can be used to monitor nutritional deficiencies, ion imbalances, elevated glucose levels and inflammation that industrial workers experience. Sweat can even tell a doctor if your medicines are not working properly.
- https://www.forbes.com/sites/jenniferhicks/2017/04/29/how-this-wearable-smart-patch-analyzes-your-sweat-to-monitor-your-body/#68ae7c244b02

https://www.kenzen.com/patch

Gao, W. *et al*. Nature http://dx.doi.org/10.1038/nature16521 (2016)

Biomarkers are suitable for internal exposure estimation, quantification may depend upon dose and time of exposure! Meaning….?

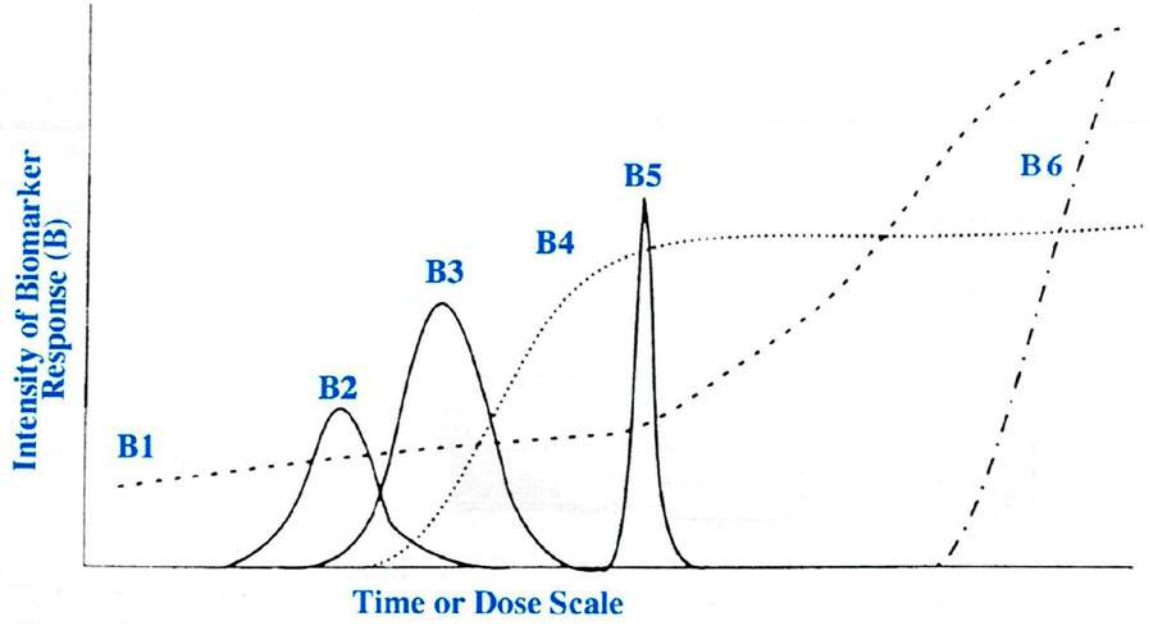
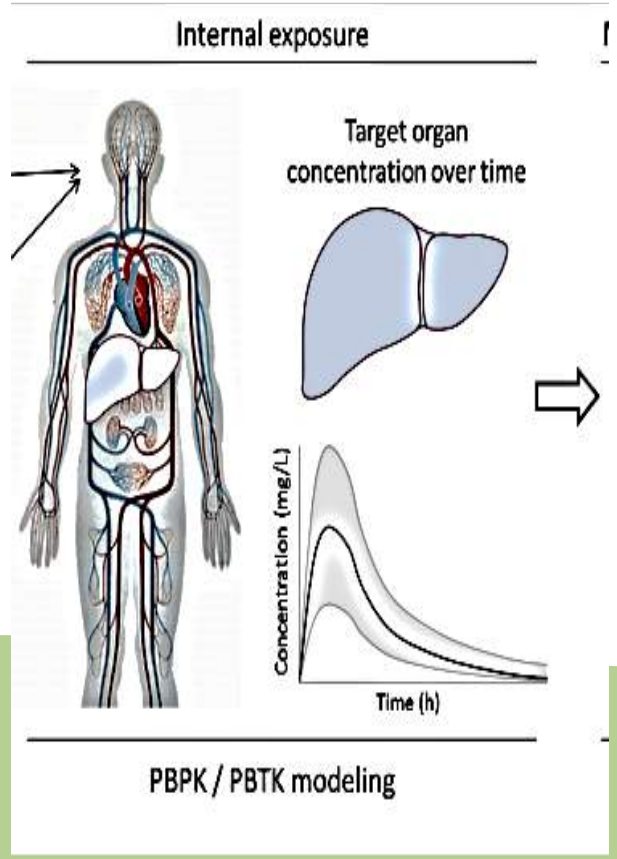


Figure 4-6. Hypothetical biomarker response relationships.

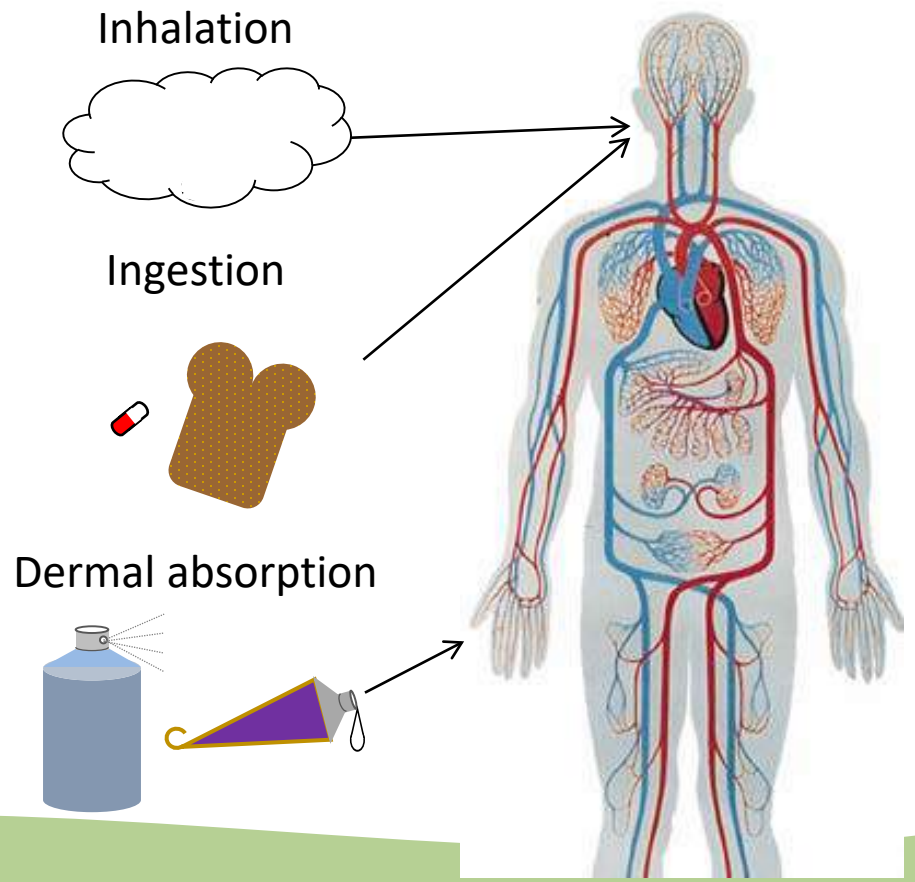
This figure illustrates a variety of dose-related biomarker responses shown as biomarker intensity (B) plotted against dose or time. The text expands upon the potentially complex relationships between biomarkers of early effect versus toxicity that can occur when very sensitive molecular biomarkers are utilized for toxicity assessment. (Adapted from Waterfield and Timbrell, 1999, and Depledge, 1993.)

What/how much gets in? II PBPK modelling

What/how much gets in



TNO's generic physiologically-based toxicokinetic-toxicodynamic (PBTK-TD) modeling tool

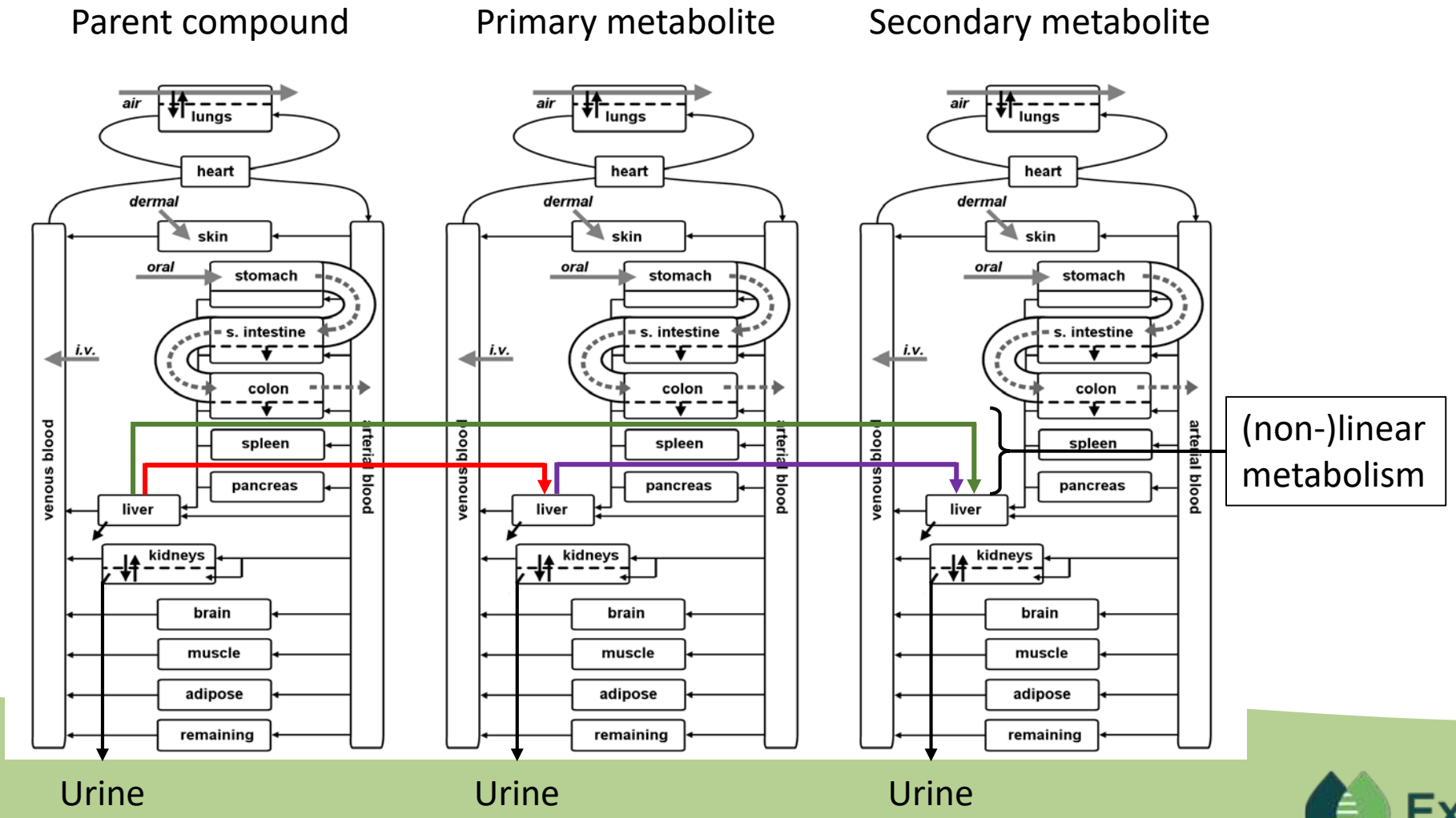


Goals:

1) To predict blood and organ concentrations (toxicokinetics) and the toxic effects (toxico-dynamics) of chemicals in humans based on in silico, in vitro and in vivo data
2) In vitro-in vivo and animal to human extrapolation of toxicity data
3) To establish safe exposure limits and appropriate safety measures for new chemicals

TNO's generic PBTK-TD modeling tool

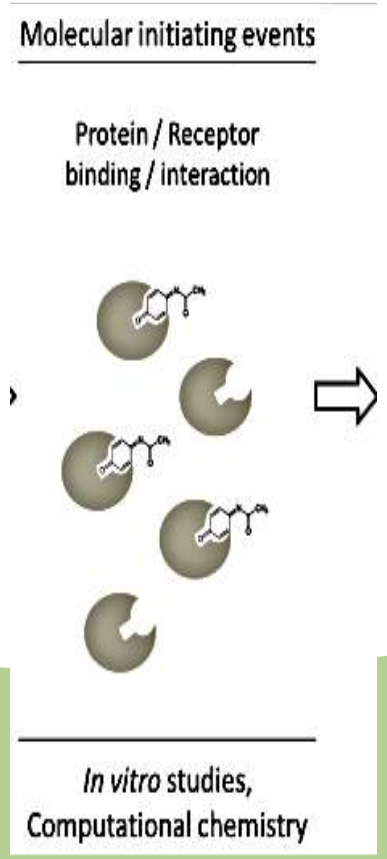
Liver metabolism:

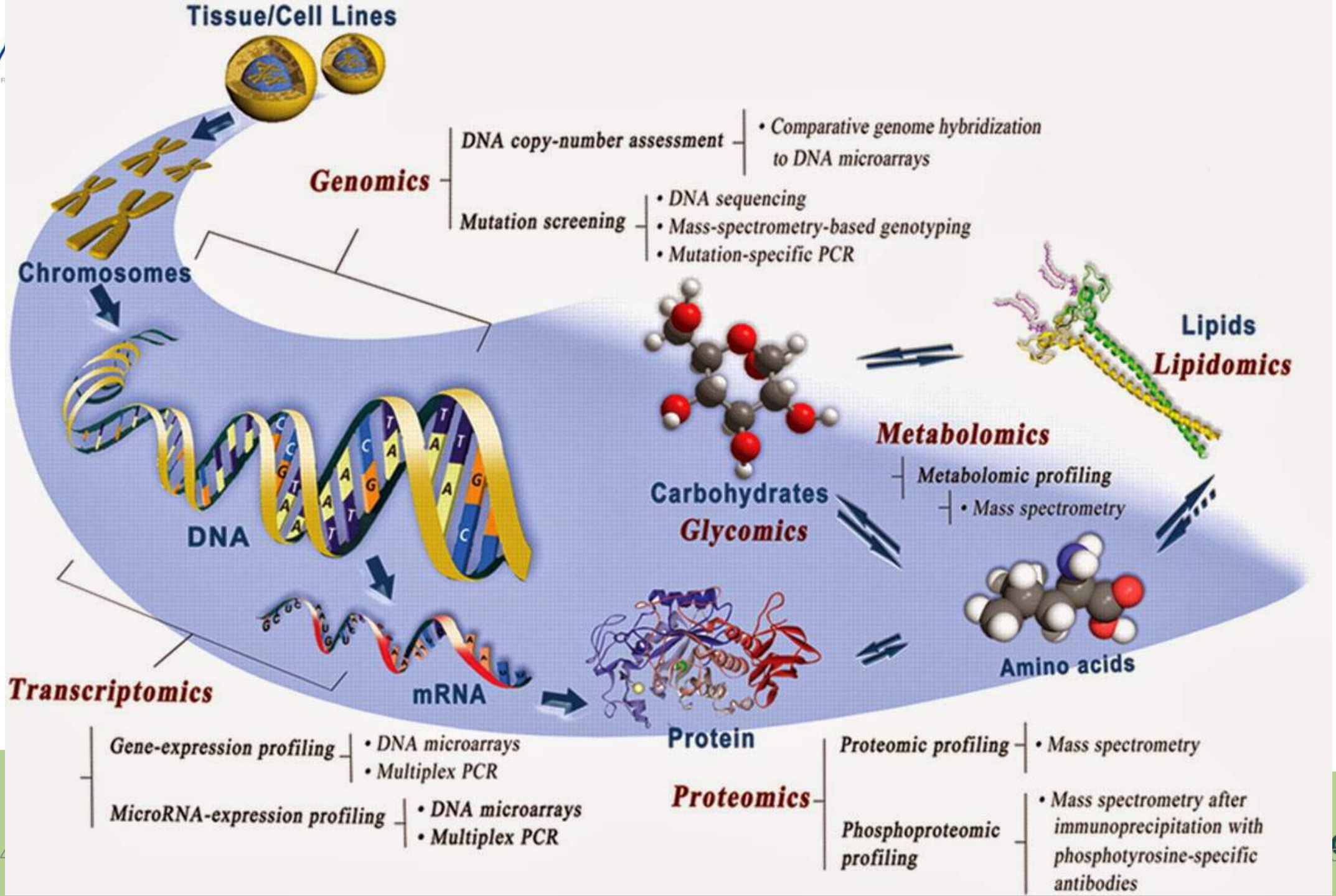


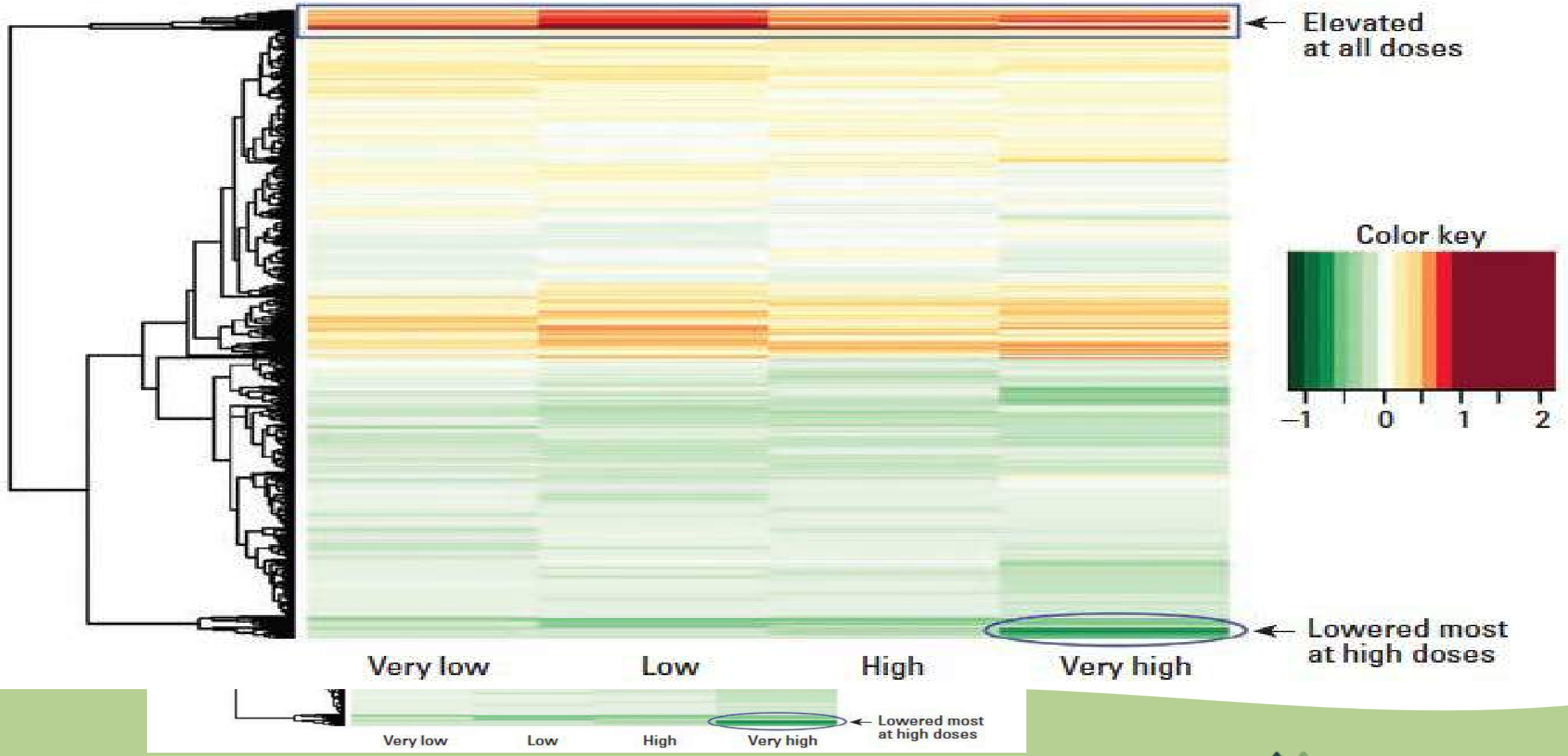
PBPK model prediction of concentration of benzene metabolites in bone marrow

Which biomolecule is target: I: comparison with omics data

which biomolecule is target




Tissue/Cell Lines
Chromosomes
Genomics
DNA copy-number assessment
• Comparative genome hybridization to DNA microarrays
Mutation screening
• DNA sequencing
• Mass-spectrometry-based genotyping
• Mutation-specific PCR
DNA
Transcriptomics
mRNA
Gene-expression profiling
• DNA microarrays
• Multiplex PCR
MicroRNA-expression profiling
• DNA microarrays
• Multiplex PCR
Protein
Proteomics
Proteomic profiling
• Mass spectrometry
Phosphoproteomic profiling
• Mass spectrometry after immunoprecipitation with phosphotyrosine-specific antibodies
Carbohydrates
Glycomics
Metabolomics
Metabolomic profiling
• Mass spectrometry
Lipids
Lipidomics
Amino acids



McHale et al 2010 EHP

Table 4. Potential biomarkers of benzene exposure based on gene expression ratios relative to unexposed controls.

			Benzene exposure category							
			Very low ($n = 29$)		Low ($n = 30$)		High ($n = 11$)		Very high ($n = 13$)	
Probe ID	Symbol	Definition	Ratio	p-Value[a]	Ratio	p-Value[a]	Ratio	p-Value[a]	Ratio	p-Value[a]
5090327	*SERPINB2*[b]	serpin peptidase inhibitor, clade B, member 2	2.47	0.002	5.19	0.000	3.03	0.005	3.39	0.001
2370524	*TNFAIP6*	tumor necrosis factor, alpha-induced protein 6	2.26	0.000	2.94	0.000	1.72	0.030	2.13	0.000
6590338	*IL1A*[b]	interleukin 1, alpha	2.00	0.001	3.03	0.000	2.36	0.000	2.53	0.000
1260746	*KCNJ2*	potassium inwardly-rectifying channel, subfamily J	1.97	0.000	2.54	0.000	2.09	0.000	1.56	0.012
2230131	*PTX3*[b]	pentraxin-related gene, rapidly induced by IL-1 beta	1.80	0.000	2.30	0.000	1.62	0.003	1.81	0.000
5860333	*F3*	coagulation factor III (thromboplastin, tissue factor)	1.73	0.003	2.83	0.000	1.78	0.034	2.41	0.001
1410189	*CD44*[b]	CD44 antigen (Indian blood group)	1.64	0.000	1.76	0.000	1.64	0.005	1.78	0.000
2470100	*CCL20*	chemokine (C-C motif) ligand 20	1.63	0.005	2.30	0.000	1.59	0.041	2.11	0.000
4880717	*ACSL1*	acyl-CoA synthetase long-chain family member 1	1.63	0.001	1.79	0.000	1.59	0.010	1.68	0.002
1470682	*PTGS2*[b]	prostaglandin-endoperoxide synthase 2	1.60	0.000	1.98	0.000	1.68	0.003	1.75	0.000
1770152	*CLEC5A*	C-type lectin domain family 5, member A	1.57	0.009	2.26	0.000	1.78	0.014	2.26	0.000
4060674	*IL1RN*	interleukin 1 receptor antagonist	1.55	0.003	2.26	0.000	1.54	0.020	1.61	0.004
7320646	*PRG2*	proteoglycan 2, bone marrow	1.37	0.011	1.83	0.000	1.5	0.007	1.69	0.000
650709	*SLC2A6*	solute carrier family 2, member 6	1.36	0.005	1.72	0.000	1.5	0.000	1.60	0.000
2900286	*GPR132*	G protein-coupled receptor 132	1.34	0.047	1.87	0.000	1.6	0.003	1.80	0.000
3710379	*PLAUR*	plasminogen activator, urokinase receptor	1.29	0.035	1.80	0.000	1.6	0.002	1.58	0.001

Genes shown are up- or down-regulated ≥ 1.5-fold relative to unexposed controls at three or four doses.
[a]FDR-adjusted p-value (Benjamini and Hochberg 1995). [b]Genes that have p-values ≤ 0.005 at all four doses.

McHale et al 2010 EHP

Exposogas

Omics and aggregated exposure pathways

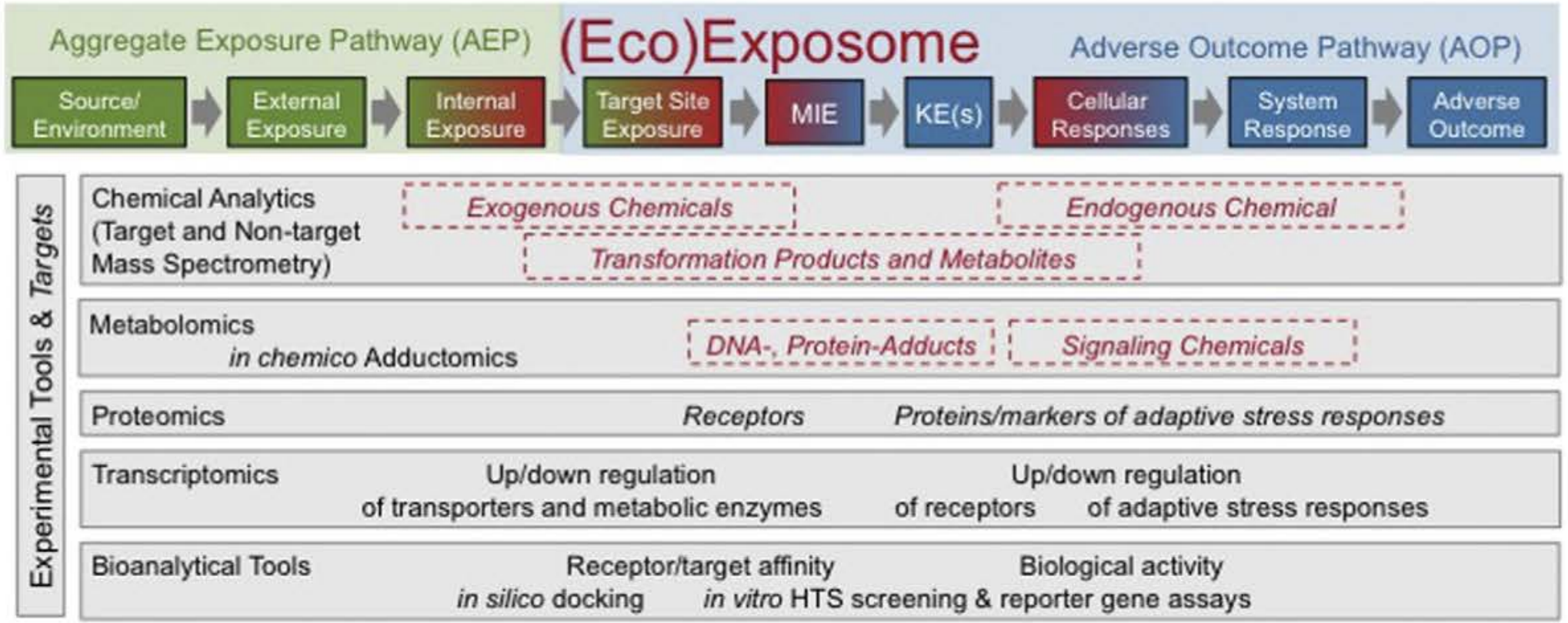
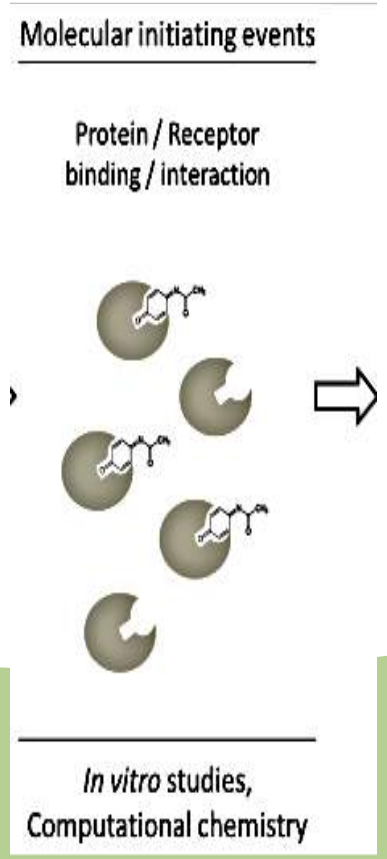


Fig. 3. Interface between the (eco)exposome (in red), the aggregate exposure pathway (AEP, green) and adverse outcome pathway (AOP, blue). The red dashed boxes represent chemical components of the exposome. The AEP/AOP concept allows one to disentangle key events and allocate them to steps from the source of exposure to adverse effects. The grey boxes indicate experimental methods to quantify the chemical components of the exposome and the biological components of the AOP. Figure partially adapted from Teeguarden et al. (2016).

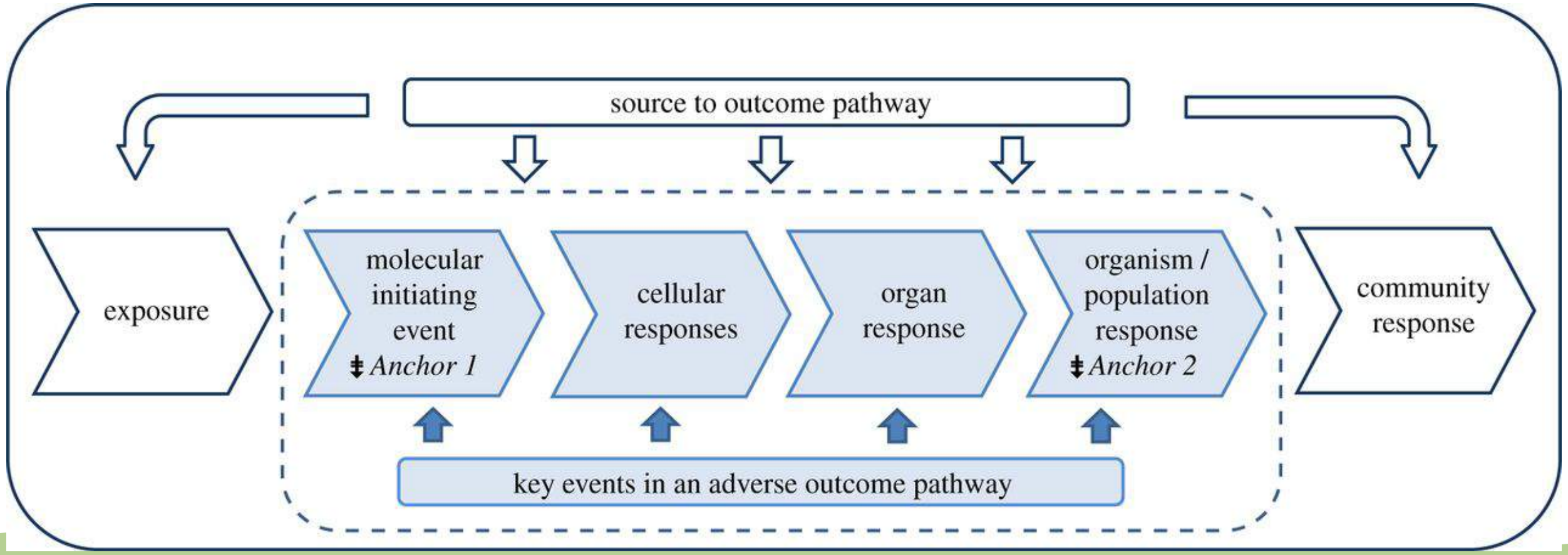
Which biomolecule is target: II: Biomolecular data computing framework for prediction of molecular effect

which biomolecule is target



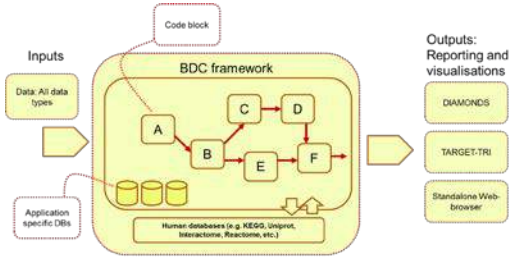
Exposogas

ADVERSE OUTCOME PATHWAY



BDC work-flow for AOP generation

Comparative toxicogenomics database as starting point



A→B→C→D→F is one work-flow, and A→B→E→F is another work flow.
- Both workflows use A, B, and F as common work blocks.
- Databases are commonly used in both workflows

CTD

Code block: Get Association Chemical – Genes – Phenotype – Disease

Code block: Phenotype to GO: SlimGo terms

Code block: SlimGo terms to Pathways (e.g. KEGG pathways)

Code block: Generate AOP(networks) MIE → KEs → AO

Code block: Pathways to BEL statements

Code block: BEL statements to INDRA statements

Code block: Generate AOP(networks) MIE → KEs → AO

Code block: BEL statements to cause-effect models

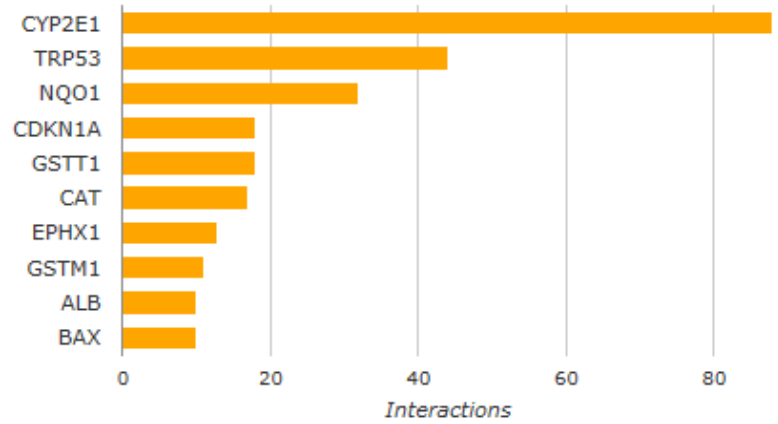
Code block: INDRA to mechanistic models

Qualitative AOPs

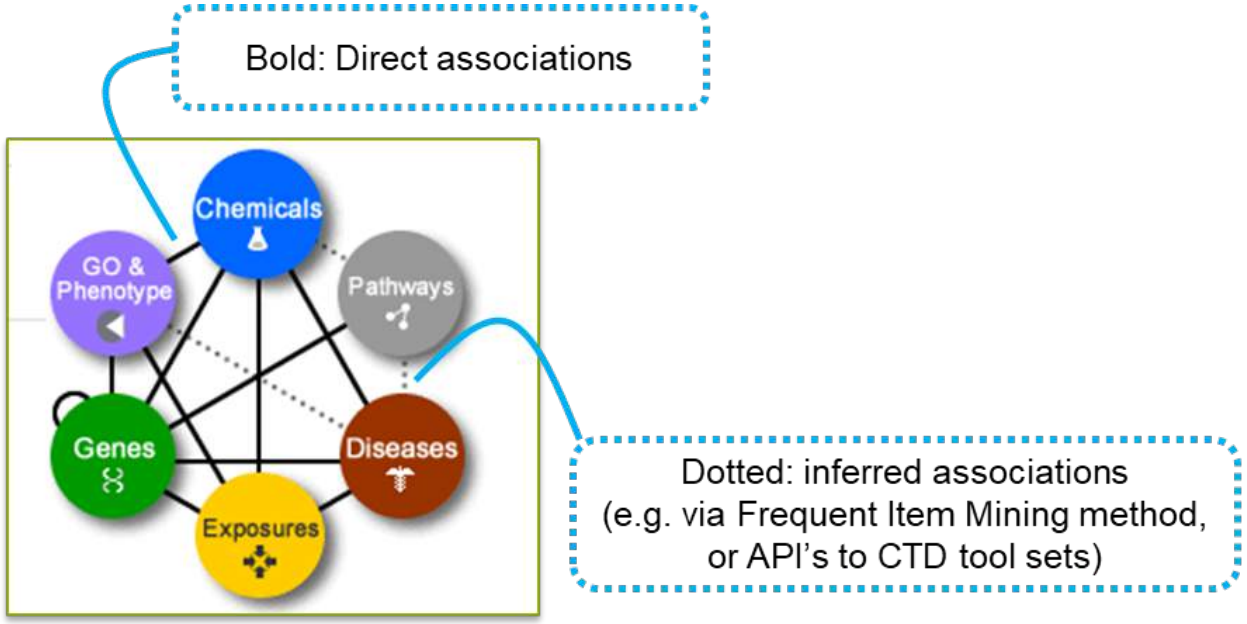
Quantitative AOPs

Exposogas

Benzene

Name	**Benzene**
CAS Type 1 Name	Benzene
Equivalent Terms	Benzol | Benzole | Cyclohexatriene
CAS Registry Number	71-43-2
Definition	Toxic, volatile, flammable liquid hydrocarbon byproduct of coal distillation. It is used as an industrial solvent in paints, varnishes, lacquer thinners, gasoline, etc. Benzene causes central nervous system damage acutely and bone marrow damage chronically and is carcinogenic. It was formerly used as parasiticide.
Structure	
Top Interacting Genes	

Case study: Benzene
computational approaches to develop Qualitative AOP network generation



Comparative Toxicogenomics Database

exposure health outcome

[Chemical – GO & Phenotype (Slim GO) – Disease] associations are extracted

Biological processes or functions

Exposogas

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Internal exposome modelling

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