Exploring mechanistic toxicity of mixtures using PBPK modeling and computational systems biology

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Protecting People from Harmful Environmental Exposures

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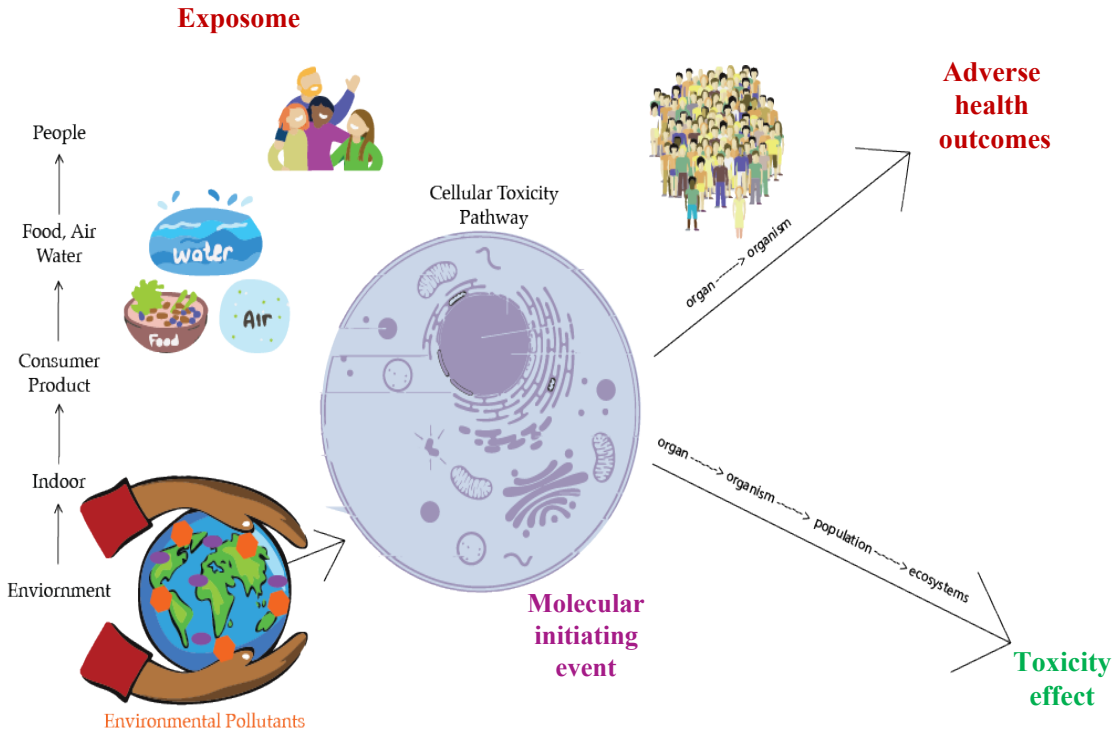
The findings and conclusions in this presentation are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.

Outline

- Background
- Single and Multiple Chemical Risk Assessment
- Current State of Affairs
- Methods
- Results and discussion
- Conclusions

Background

- Often toxicity assessment of a single chemical is conducted
- Three approaches can be used for mixtures risk assessment: Whole mixture, similar mixture and the components-based hazard index (HI)[ATSDR, 2018].



Modified from Kortenkamp et al., *Science* (2018). DOI: 10.1126/science.aat9219

HAZARD QUOTIENTS (HQ)

The HQ for each chemical component of a mixture is calculated for only its **critical** effect using its **exposure level (E)** and **health guidance value (HGV)**

$$HQ = E / HGV$$

Hazard Quotients (HQ) and Hazard Index (HI)

Component Based Assessments

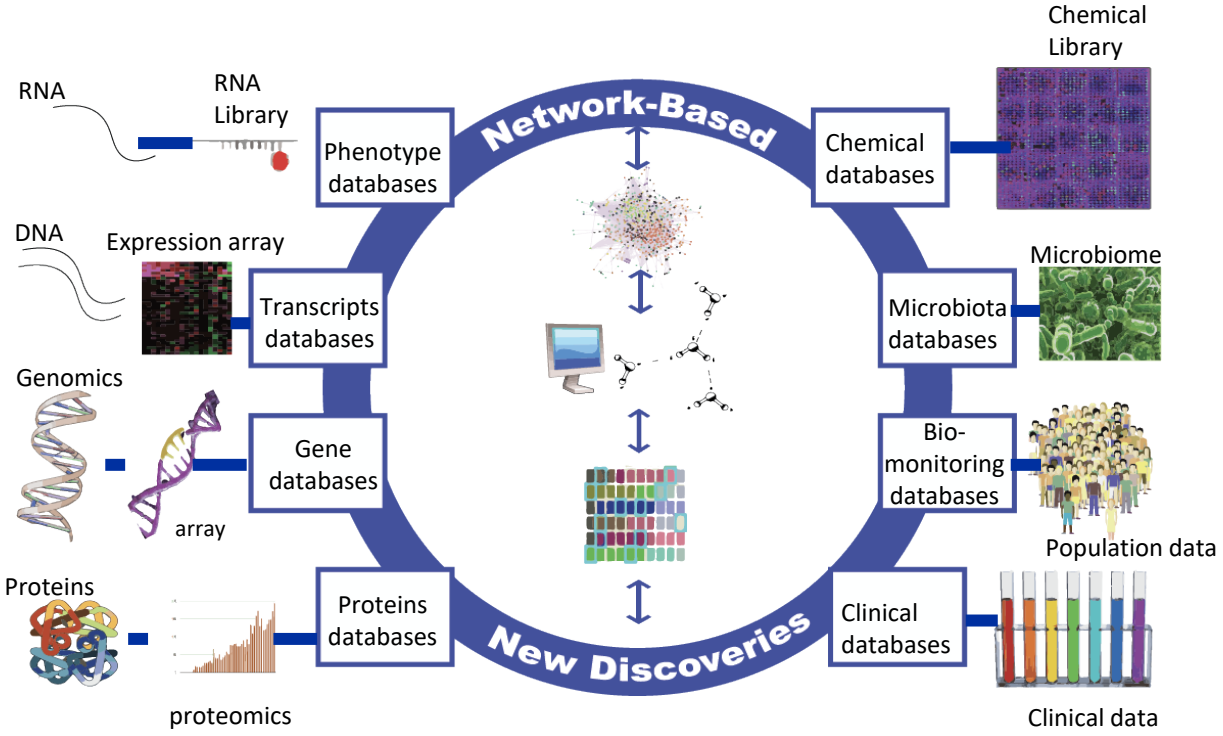
Hazard Index (HI) = $E_1 / HGV_1 + E_2 / HGV_2 + .. E_n / HGV_n$

HQ = E /HGV

Mixture Component	HGV	Exposure	Hazard Quotient
A	2	1	0.5
B	16	8	0.5
C	1	1	1.0
D	30	10	0.3
	HI (MIXTURE)		2.3

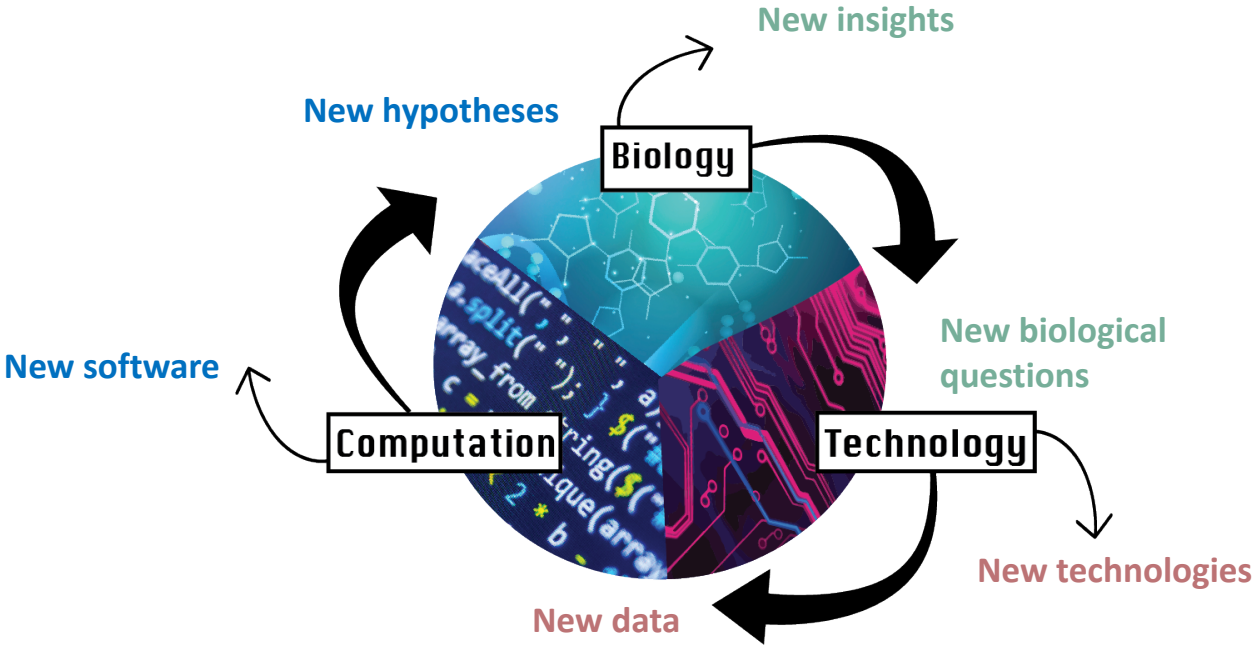
Current State of Affairs

- Integration of Data from Multiple Streams
- Systems Biology
- Adverse Outcome Pathways



Modified from resource: Leung et al., 2013. Brief Bioinform. 14(4):445-460

Science, Computation and Technology

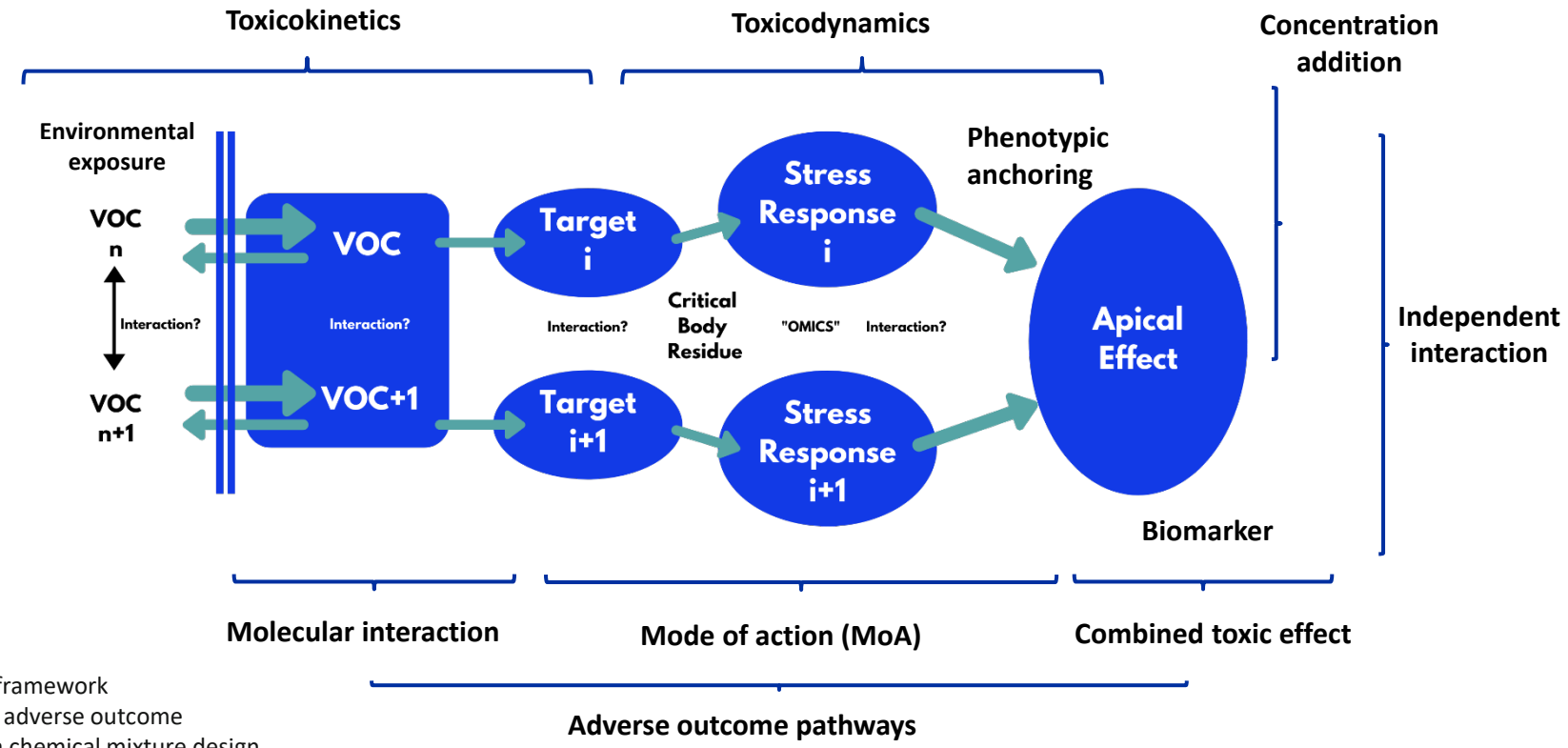


Modified from https://www.systemsbiology.org.au/

Toluene, Ethylbenzene and Xylene

- People are exposed to multiple chemicals.
- VOCs exposures may increase risks for cancer and exacerbate asthma and other adverse respiratory effects. However, the mechanistic understanding of these health effects is lacking.
- Epidemiological studies have reported adverse outcomes to VOCs exposure even if no biological and occupational exposure limits are exceeded.

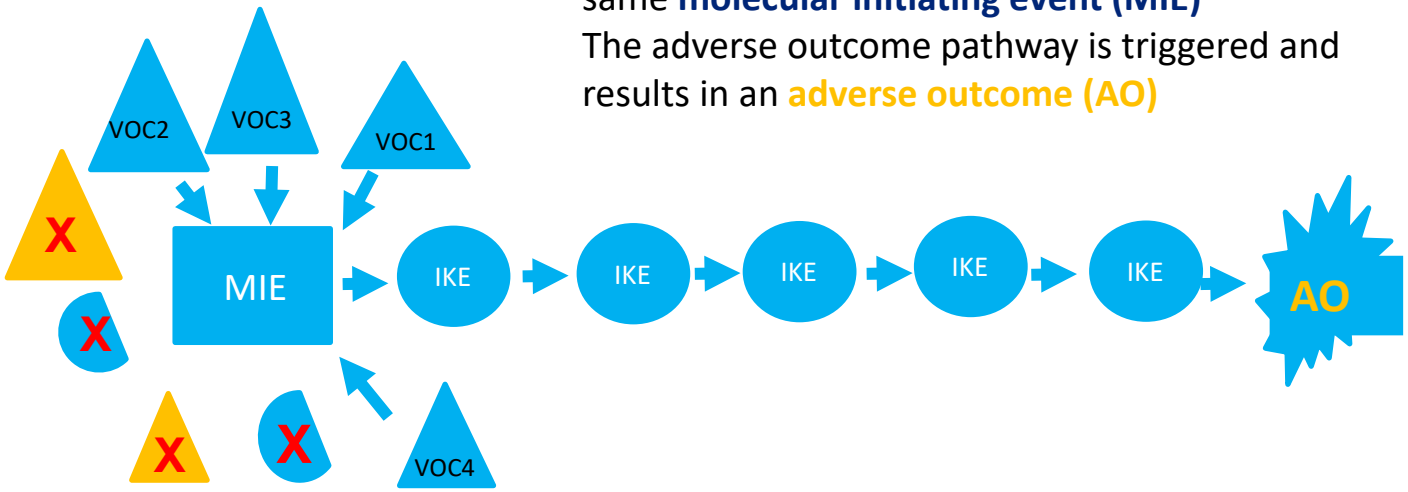
Toxicology Pathways for Chemical Mixtures



Conceptual framework showing the adverse outcome pathway in a chemical mixture design (adapted from Beyer et al. 2013)

Grouping VOCs Combinations by Toxicity Using Adverse Outcome Pathways (AOPs)

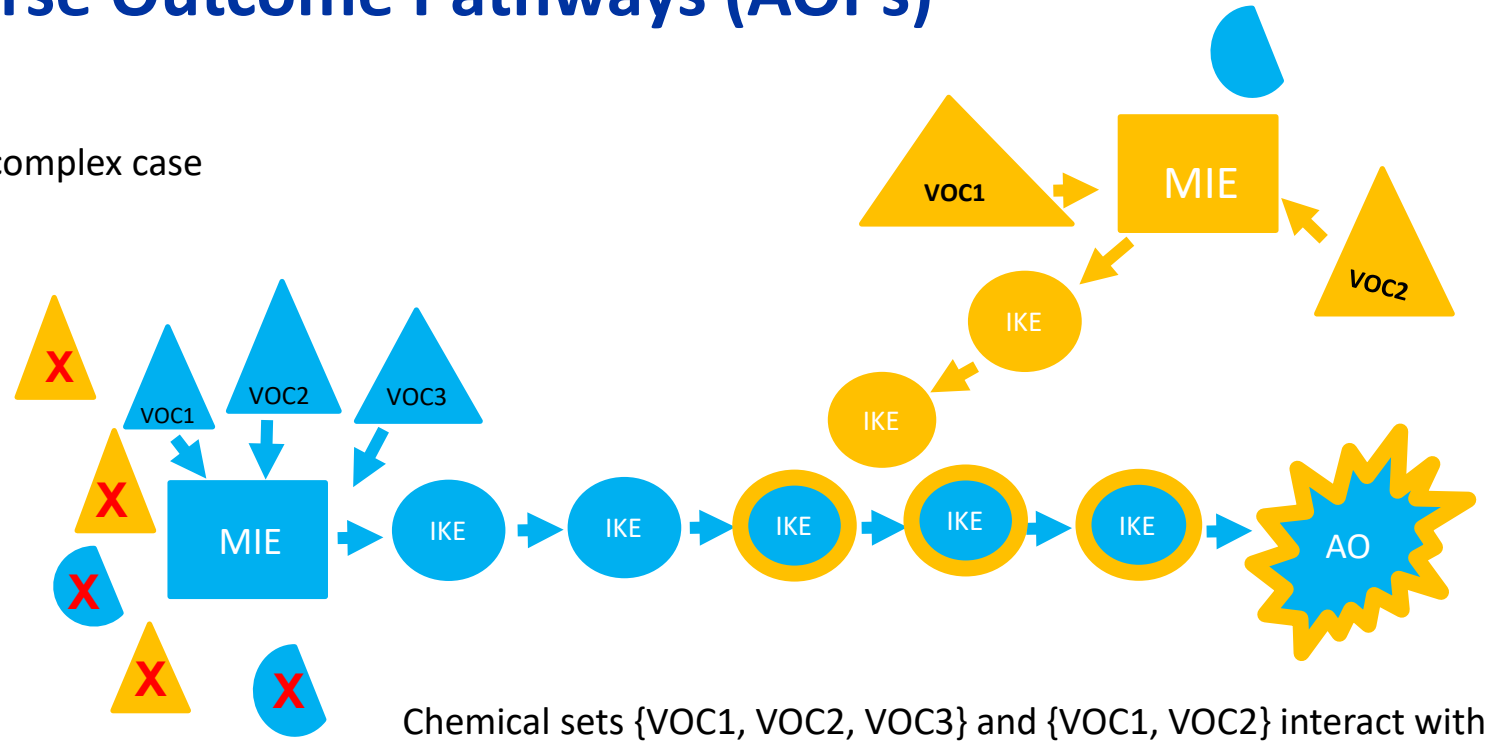
VOC1, VOC2, VOC3, VOC4 all interact with the same **molecular initiating event (MIE)**
The adverse outcome pathway is triggered and results in an **adverse outcome (AO)**



The simplest case: one pathway one endpoint one **MIE** and a linear series of **Intermediate Key Events (IKEs)**

Grouping VOCs Combinations by Toxicity Using Adverse Outcome Pathways (AOPs)

A more complex case



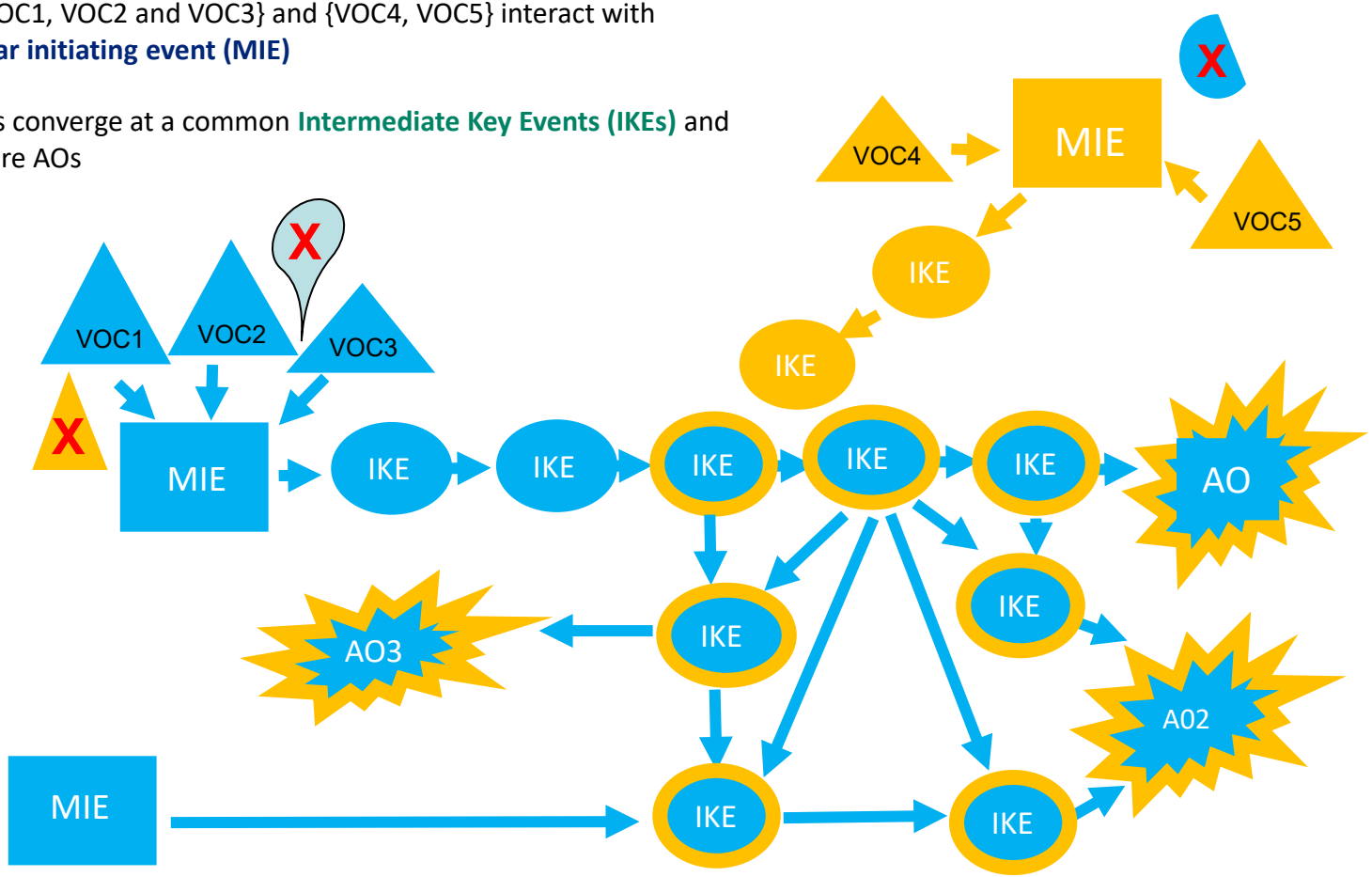
Chemical sets {VOC1, VOC2, VOC3} and {VOC1, VOC2} interact with different **molecular initiating event (MIE)**

The MIE pathways converge at a common **Intermediate Key Events (IKEs)**

A more more complex case

Chemicals sets {VOC1, VOC2 and VOC3} and {VOC4, VOC5} interact with different **molecular initiating event (MIE)**

The MIE pathways converge at a common **Intermediate Key Events (IKEs)** and lead to one or more AOs



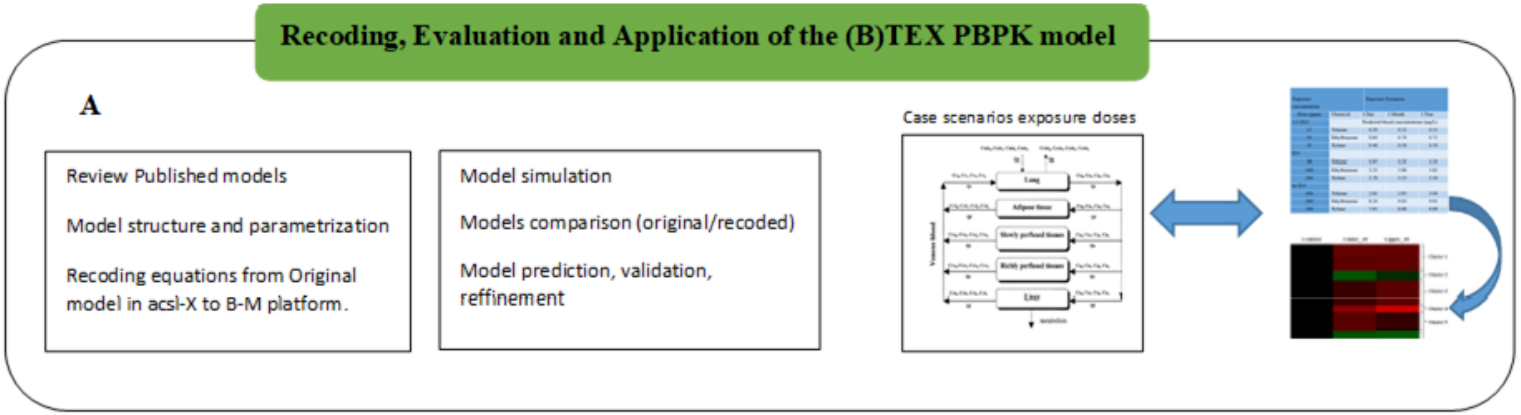
Aims

- **Share** a recoded PBPK model for toluene, ethylbenzene and xylene (TEX).
- **Explore** the use of chemical data from various sources to hypothesize the potential mechanisms of toxicity of mixture.
- **Introduce** a framework for testable hypotheses to address chemical mixture data gaps.

Methods

Two-pronged approach

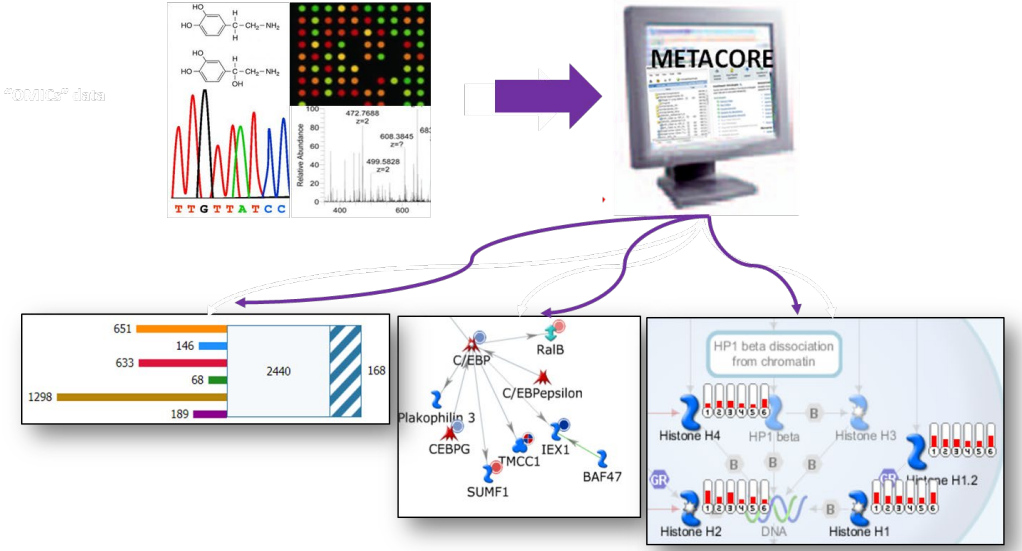
- Recoded available PBPK model using Berkeley Madonna software and assessed its reproducibility.



Methods

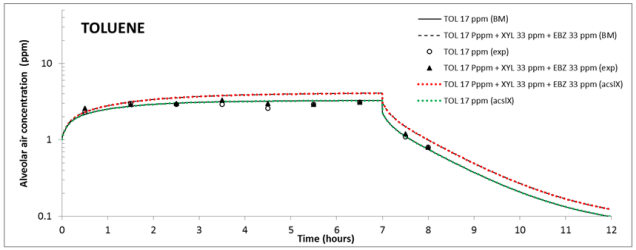
Two-pronged approach

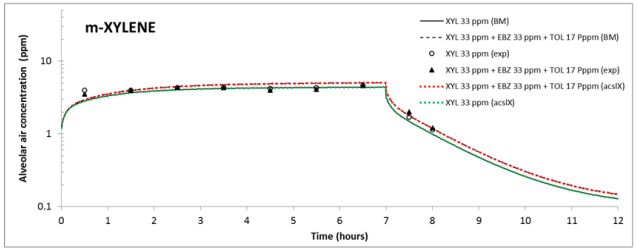
- Used toxicogenomic databases, systems biology tools, and published gene expression data to gain insights into the potential biological pathways affected by exposure to TEX mixtures.

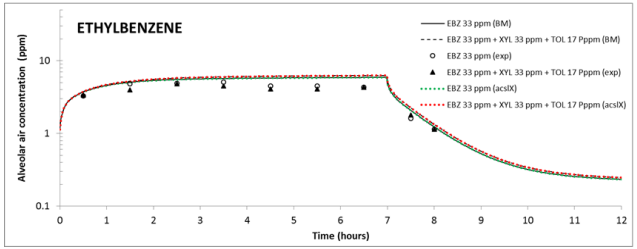


Recoding, Evaluation and Application of the PBPK Model

Comparison between original (Haddad et al., 1999) and recoded PBPK model simulations and experimental data for toluene, ethylbenzene and xylene alone (green line) and in combination (orange line).







PBPK model mixture was used to calculate biological hazard indexes (BHIs) for 8-hour exposures to varying simulated mixtures of the three chemicals.

$$BHI = \sum_{i-1}^{n} \frac{SCi}{BEIi}$$

SC*i* is the simulated venous blood concentration of the component chemical (*i*) and BEI*i* is the biological exposure index or blood concentration of the component chemical in a healthy person

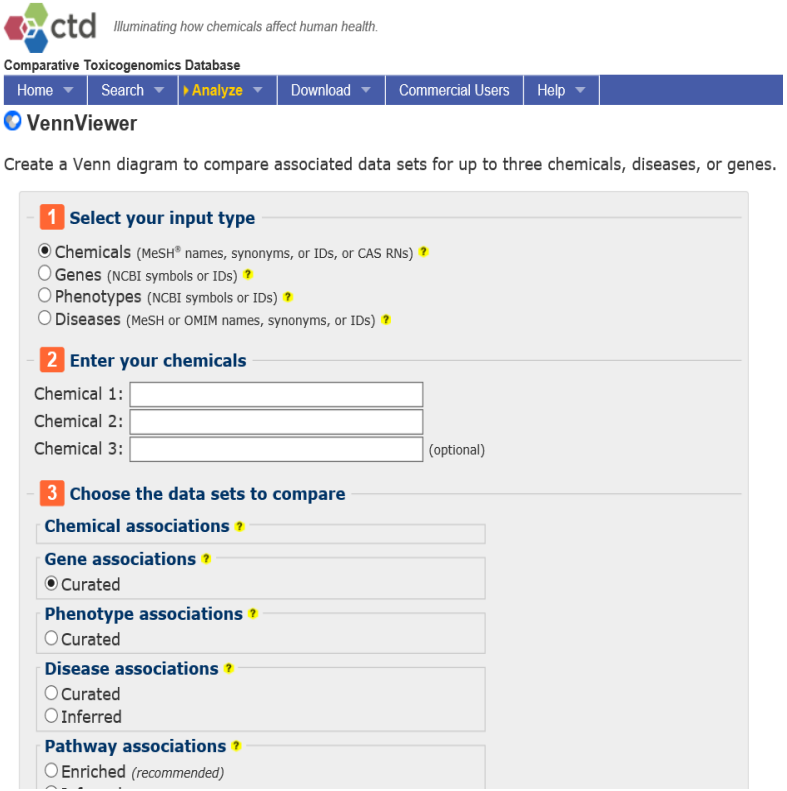
The BHIs were subsequently compared with exposure concentration-based hazard indices (HI) values for each mixture.

$$HI = \sum_{i-1}^{n} \frac{Ei}{HGVi}$$

E*i* is the exposure level of the chemical

Exposure Concentration (ppm)			HI	Venous blood concentration (mg/L)			BHI
T	E	X		T	E	X	
5	40	50	1	0.08	0.87	0.94	1.04
40	10	10	1	0.55	0.20	0.15	1.05
20	45	15	1	0.34	0.98	0.27	1.11
16.5	33	33	0.99	0.27	0.70	0.59	1.06
8	50	30	0.96	0.14	0.11	0.55	1.00
10	30	30	0.80	0.15	0.60	0.48	0.80
20	20	20	0.80	0.28	0.40	0.31	0.80

Comparative Toxicogenomics Database (CTD)



1) Top curated genes for each of the three VOCs (toluene, xylene and ethylbenzene).

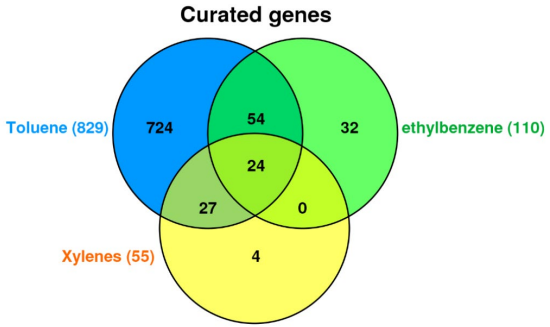
2) Genes in common to them.

3) Top interacting curated diseases in common to all the individual VOCs.

Davis et al. The Comparative Toxicogenomics Database: update 2021. Nucleic Acids Res. 2020 Oct 17

Comparative Toxicogenomics Database and MetaCore™

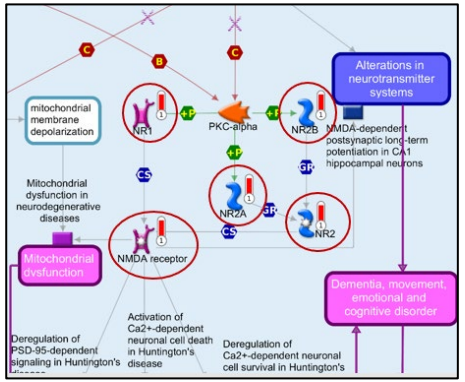
https://ctdbase.org/

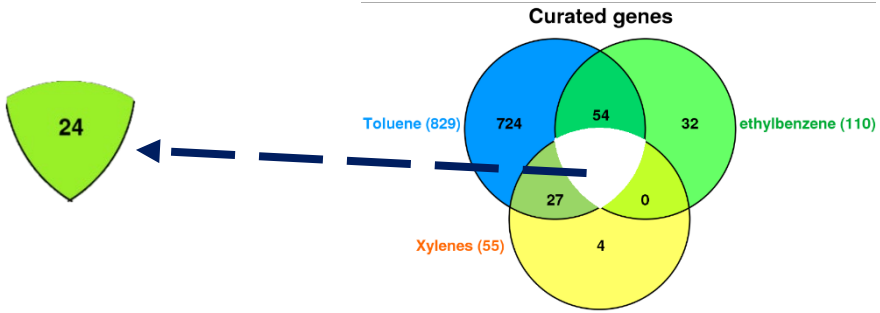


MetaCore version 6.36 build 69400

Pathway enrichment analysis

Understand the biological impact of their data by visualizing the intersection of their dataset to curated ontologies which are ranked by significance based on p-value.

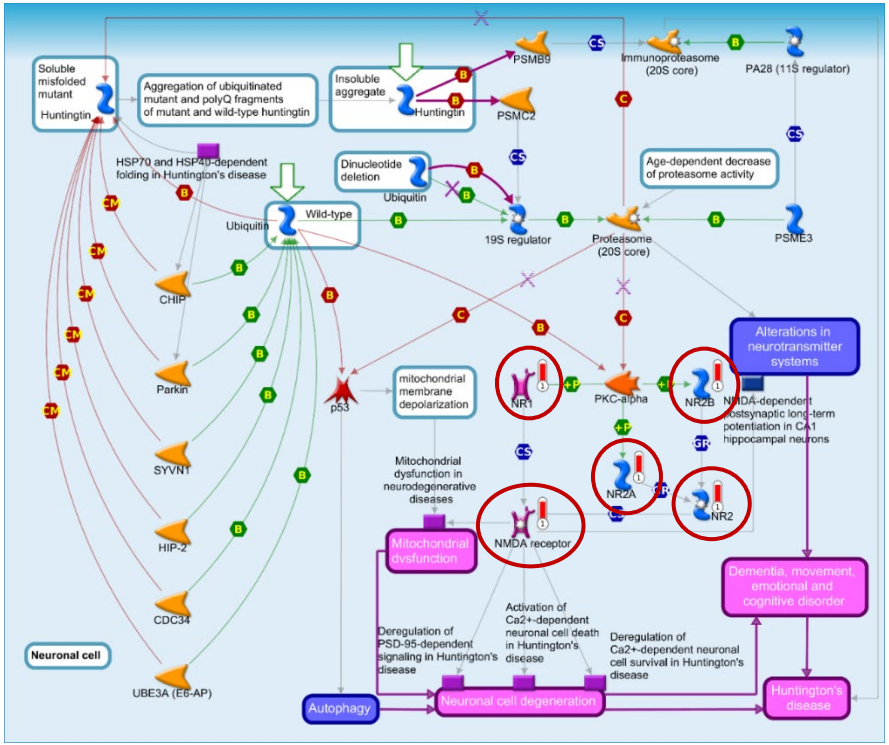




Chemical-gene-disease interactions using Comparative Toxicogenomics Database (CTD)

	Gene Symbol	Gene names	Gene roles
1	**ACE**	angiotensin I converting enzyme	neuronal metabolism, hematopoiesis, digestion and reproduction
2	**BMP8B**	bone morphogenetic protein 8b	energy balance regulation in both the hypothalamus and brown adipose tissue
3	**CARMIL3**	capping protein regulator and myosin 1 linker 3	membrane-associated functions related to actin assembly and signaling
4	**CAT**	catalase	signaling in cell proliferation, apoptosis, carbohydrate metabolism, and platelet activation
5	**CDCP2**	CUB domain containing protein 2	autoimmune diseases such as encephalomyelitis, multiple sclerosis and inflammatory arthritis
6	**CLCN4**	chloride voltage-gated channel 4	pathogenesis of neuronal disorders
7	**CYP1A1**	cytochrome P450 family 1 subfamily A member 1	formation of various types of human cancer
8	**DPT**	dermatopontin	cell adhesion
9	**ENTPD1**	ectonucleoside triphosphate diphosphohydrolase 1	vascular homeostasis
10	**FEZ2**	fasciculation and elongation protein zeta 2	normal axonal bundling and elongation within axon bundles
11	**FLVCR1**	feline leukemia virus subgroup C cellular receptor 1	erythropoiesis
12	**GRIN1**	glutamate ionotropic receptor NMDA type subunit 1	long term potentiation
13	**GRIN2A**	glutamate ionotropic receptor NMDA type subunit 2A	long-term potentiation, an activity-dependent increase in the efficiency of synaptic transmission
14	**GRIN2B**	glutamate ionotropic receptor NMDA type subunit 2B	brain development, circuit formation, synaptic plasticity, and cellular migration and differentiation
15	**HIST2H3A**	histone cluster 2 H3 family member a	gene expression and regulation, DNA repair, chromatin condensation, cell cycle progression,
16	**HSPA5**	heat shock protein family A (Hsp70) member 5	chromosome segregation, and apoptosis
17	**KLF4**	Kruppel like factor 4	monitoring protein transport through the cell
18	**NAMPT**	nicotinamide phosphoribosyltransferase	epidermal barrier function
19	**OTX1**	orthodenticle homeobox 1	metabolism, stress response and aging
20	**PAPLN**	papilin, proteoglycan like sulfated glycoprotein	brain and sensory organ development
21	**PTGS2**	prostaglandin-endoperoxide synthase 2	organogenesis
22	**RAB11FIP1**	RAB11 family interacting protein 1	prostanoid biosynthesis, inflammation and mitogenesis
23	**RP9P**	RP9 pseudogene	endocytic sorting, trafficking of proteins and epidermal growth factor receptor (EGFR), and transport between the recycling endosome and the trans-Golgi network
24	**WASHC2C**	WASH complex subunit 2C	pre-mRNA splicing

Chemical-gene-disease interactions using Comparative Toxicogenomics Database (CTD)



https://portal.genego.com/

- Proteasomes are depleted and altered degradation of tumor protein (p53), protein kinase C-alpha (PKC-alpha), and mutant Huntingtin.

↓

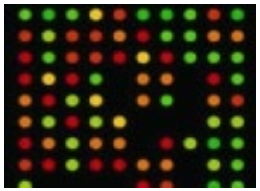
- Activation of N-methyl-D-aspartate subclass of ionotropic glutamate receptor (NMDA receptor).

↓

- Mitochondrial dysfunction, alterations in neurotransmitter systems, and neuronal cell death.

Enrichment analysis on lung-specific ontologies for TEX microarray data using MetaCore™

Gene Expression data:
Blood samples
- Unexposed
- Short term VOCs (<10yrs)
- Long term VOCs (>10yrs)

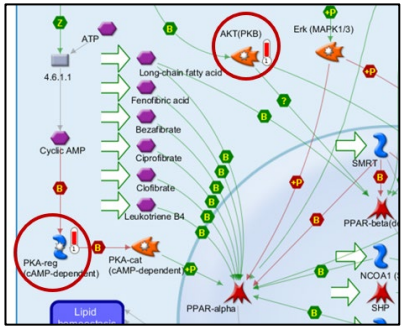


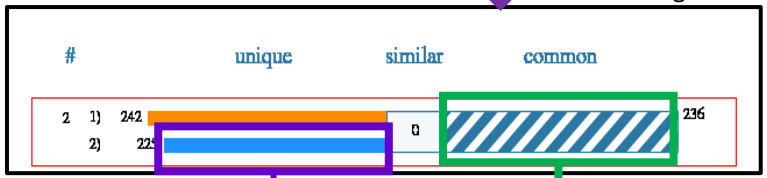
GSE68906
(Hong et.al 2016)

MetaCore version 6.36 build 69400

System's Toxicology module
(Lung ontology)

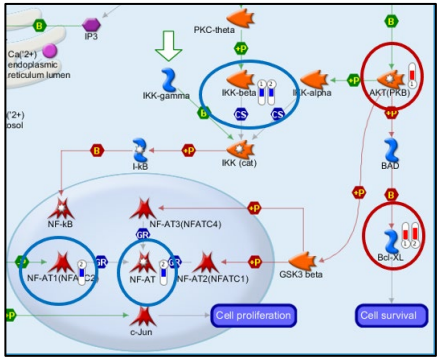
List of statistically significant genes:
- Short term VOCs vs Unexposed
- Long term VOCs vs Unexposed





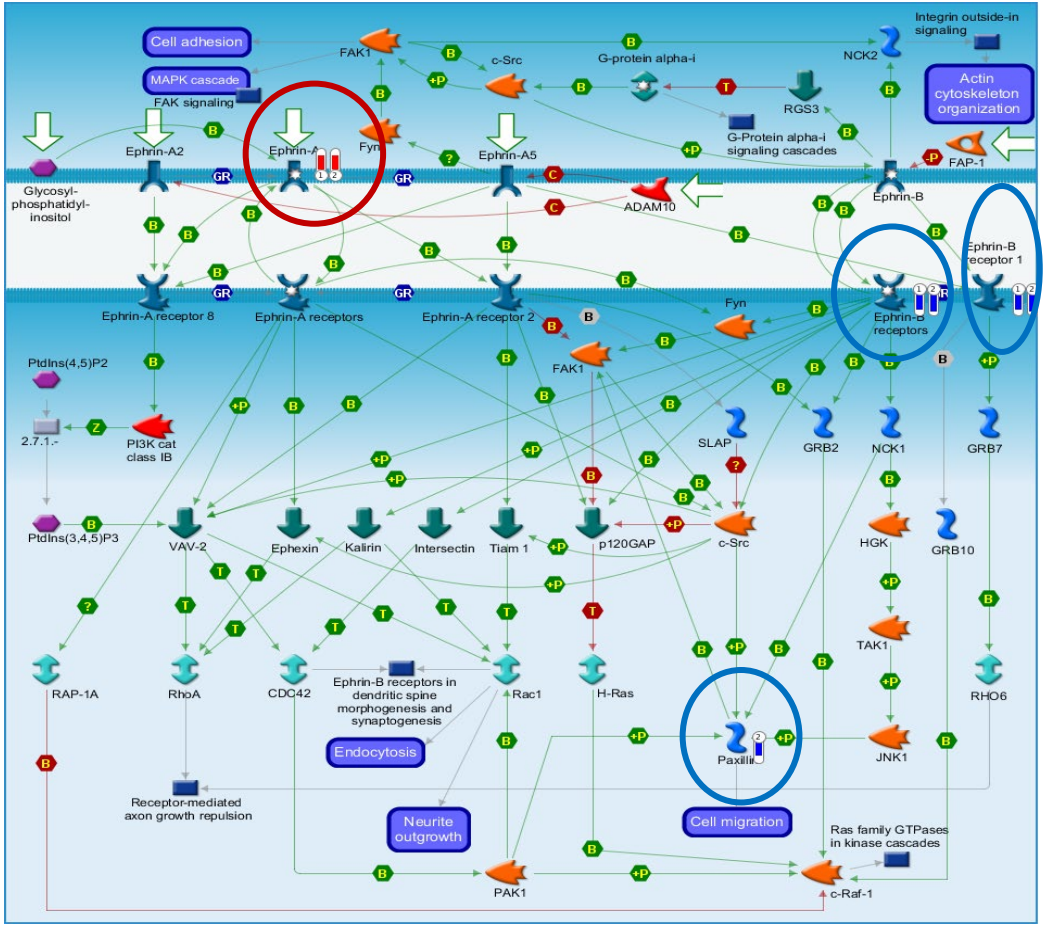
Pathways impacted by genes unique to long-term exposure

Pathways impacted by genes common to short and long-term exposure

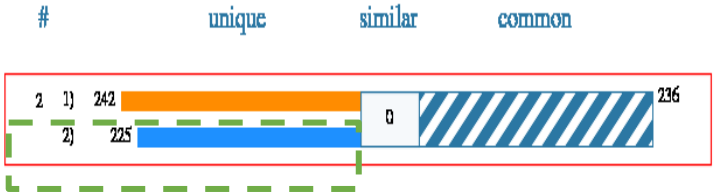


Common genes for short- and long- term exposure

- Cell adhesion-Ephrin signaling was the top scored map by 'common' set.
- TEX short-term (1) and long-term exposure (2) common gene expression data are linked to and visualized on the maps as thermometer-like figures.
- Upward thermometers (**red**) indicate up-regulated signals and downward thermometers (**blue**) indicate down-regulated expression levels of the genes.

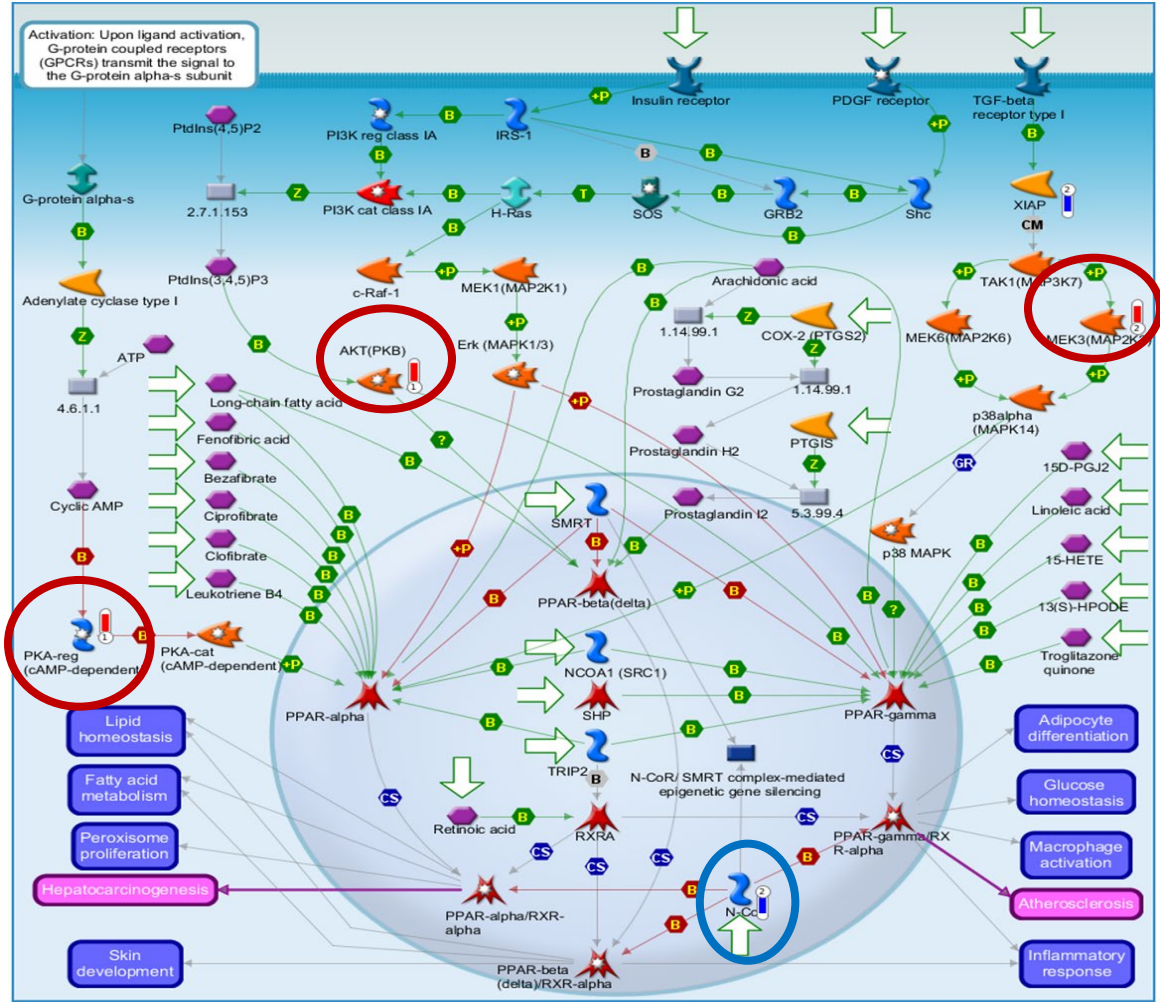


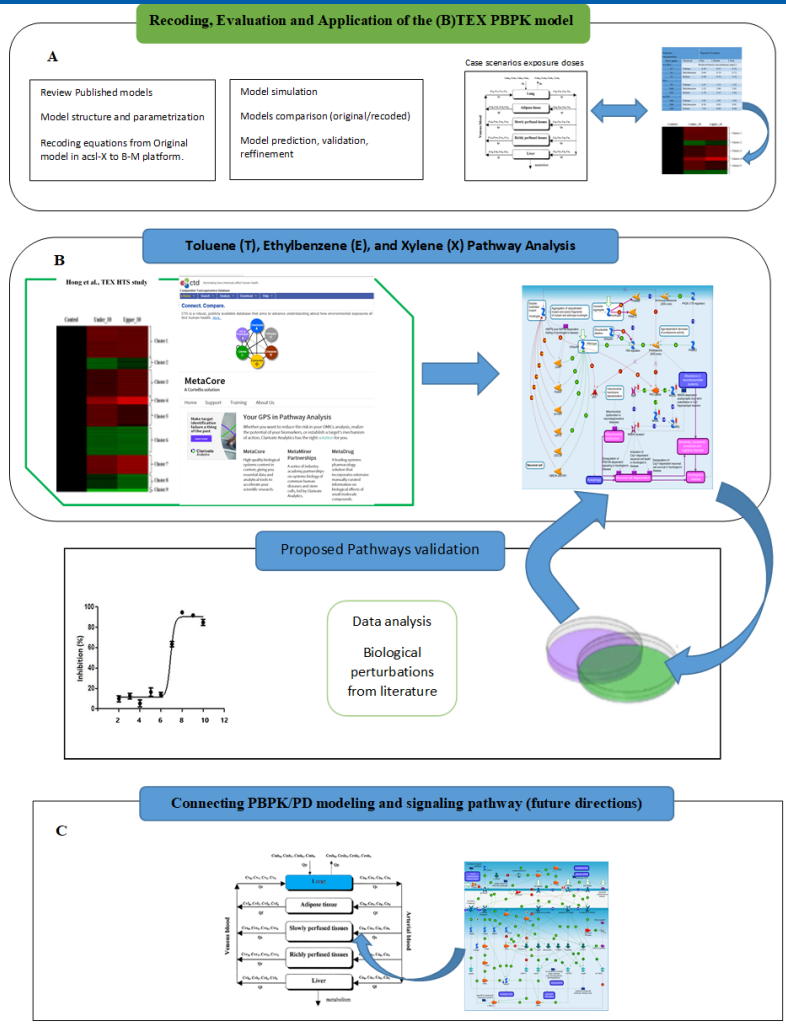
Unique genes for the experiments are marked as colored bars, short-term: **orange bar** and long-term: **blue bar**.



Top scored PPARs pathway map impacted by differentially expressed genes on long-term exposures to TEX.

Upstream interactors (MEK3, AKT and PKA-reg) of these PPARs transcription factors were shown to be upregulated in the long-term exposure group.





- Enrichment analyses suggests that exposure to TEX mixture may result in disruption of biological pathways such as Ephrin and PPARs.
- Disruption of these proposed pathways could translate to adverse respiratory and neurological outcomes, depending on exposure durations.
- Future experimental evaluation of the pathways are needed to explore the proposed hypotheses.

Conclusions

- A conceptual framework that employs PBPK modeling, toxicogenomics, and systems biology to reveal mechanistic insights into the toxicity of TEX mixtures was presented.

- These proposed pathways, Ephrin and PPARs, are supported by experimental data from multiple laboratories and have not been examined sufficiently to date by an integrated research laboratory.

- Future experimental evaluation of these pathway maps might lead to the development of new predictive markers of TEX effects that could translate into new disease prevention and clinical use strategies.

Acknowledgement

- Claude Emond
- Eva McLanahan
- Shivanjali Joshi-Barr
- Moiz Mumtaz

Thank you for your attention!

Do you have questions?

Patricia Ruiz

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