JRC EURL ECVAM Activities on Combined Exposure to Multiple Chemicals

Stephanie Bopp

JRC F3 Chemical Safety and Alternative Methods

EFSA MixTox Workshop October 2021

Communication on Chemical Mixtures 2012 and Chemical Strategy for Sustainability 2020

2012

- Review of new evidence since 2009 SoA report on mixtures
- Filling gaps identified

2020

EUROPEAN COMMISSION

Brussels, 31.5.2012
COM(2012) 252 final

COMMUNICATION FROM THE COMMISSION TO THE COUNCIL

The combination effects of chemicals

Chemical mixtures

COMMISSION STAFF WORKING DOCUMENT

Progress report on the assessment and management of combined exposures to multiple chemicals (chemical mixtures) and associated risks

Accompanying the document

COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS

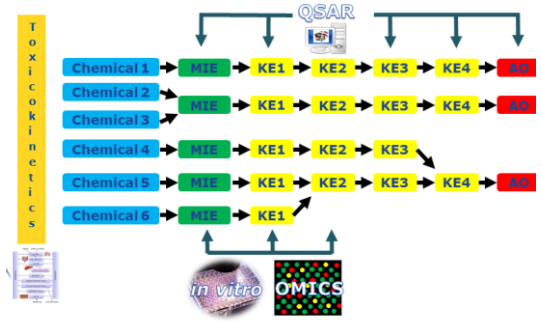
Chemicals Strategy for Sustainability
Towards a Toxic-Free Environment

{COM(2020) 667 final} - {SWD(2020) 225 final} - {SWD(2020) 247 final} -
{SWD(2020) 248 final} - {SWD(2020) 249 final} - {SWD(2020) 251 final}

Review of State of the Art

Regulatory Requirements

Kienzler et al 2014
Kienzler et al 2016



Scientific Methodologies and current practices

Bopp et al 2015
Desalegn et al 2019
Kienzler et al 2019
Pletz et al 2020
Pistollato et al 2020, 2021

Review of case studies

Bopp et al 2016

Scientific Advances and policy needs

Bopp et al 2018
Drakvik et al 2020

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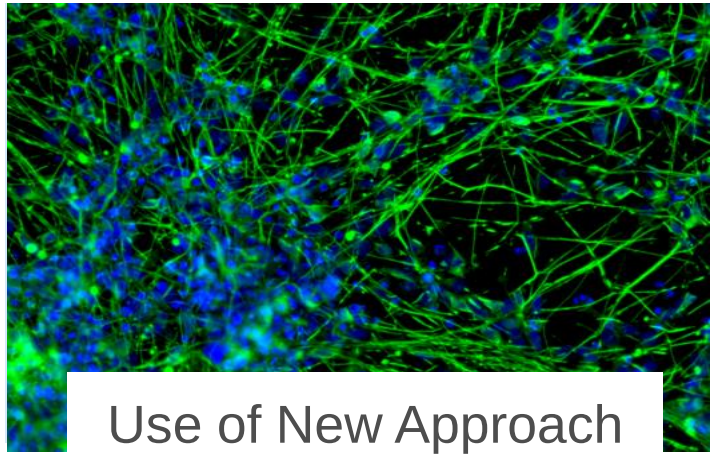
Addressing data and knowledge gaps

Sharing occurrence data

https://ipchem.jrc.ec.europa.eu/



Use of New Approach Methodologies for mixture risk assessment

Literature Review on Interactions

Addressing data and knowledge gaps

Exposure

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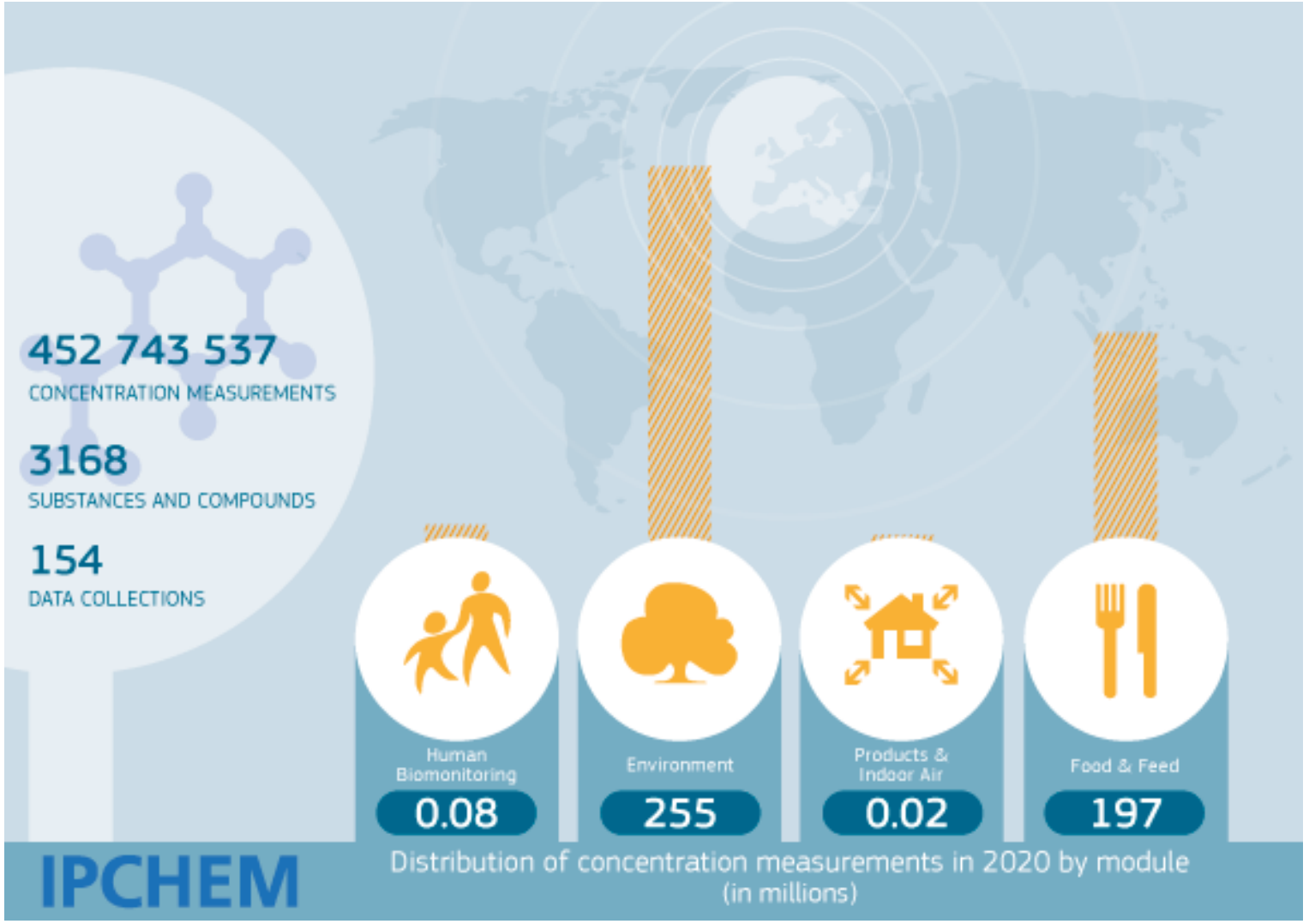
What is IPCHEM?

- Information Platform for Chemical Monitoring
- **Single access point** for **searching, accessing and retrieving chemical occurrence data** collected and managed in Europe and beyond

https://ipchem.jrc.ec.europa.eu/

What can you find in IPCHEM?



Harmonised & quality checked

Metadata & contact point

Share data publicly

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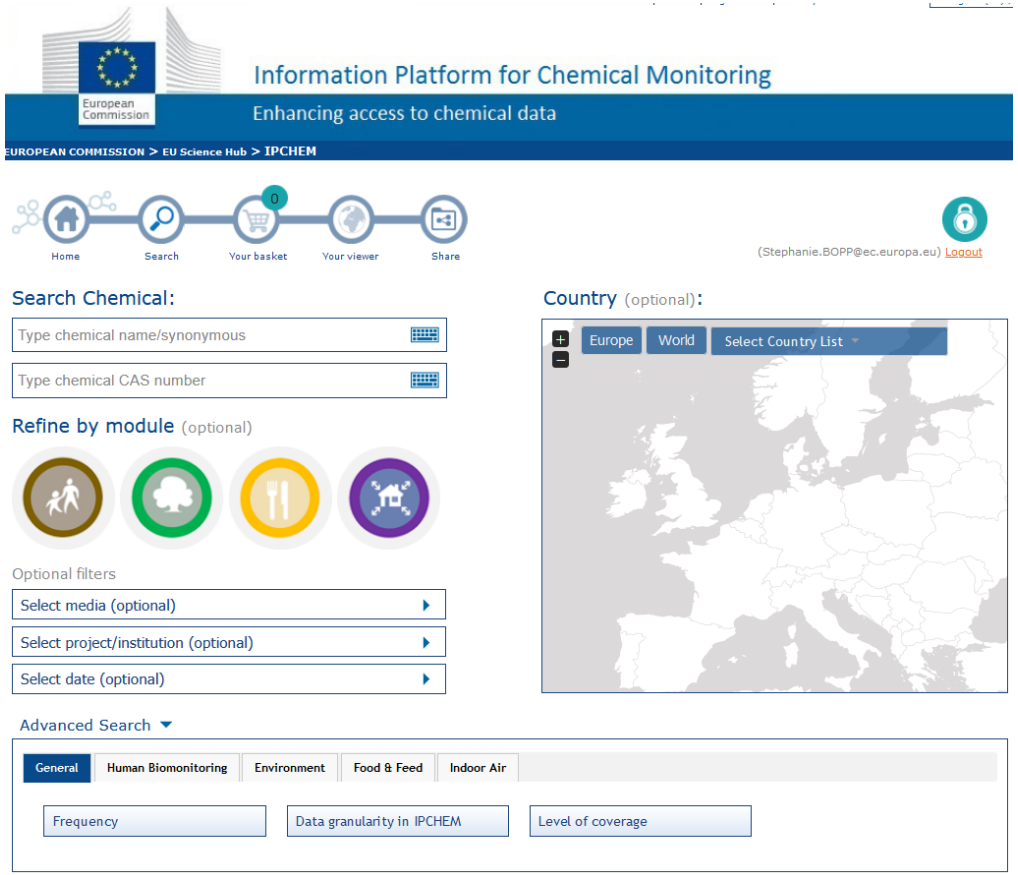
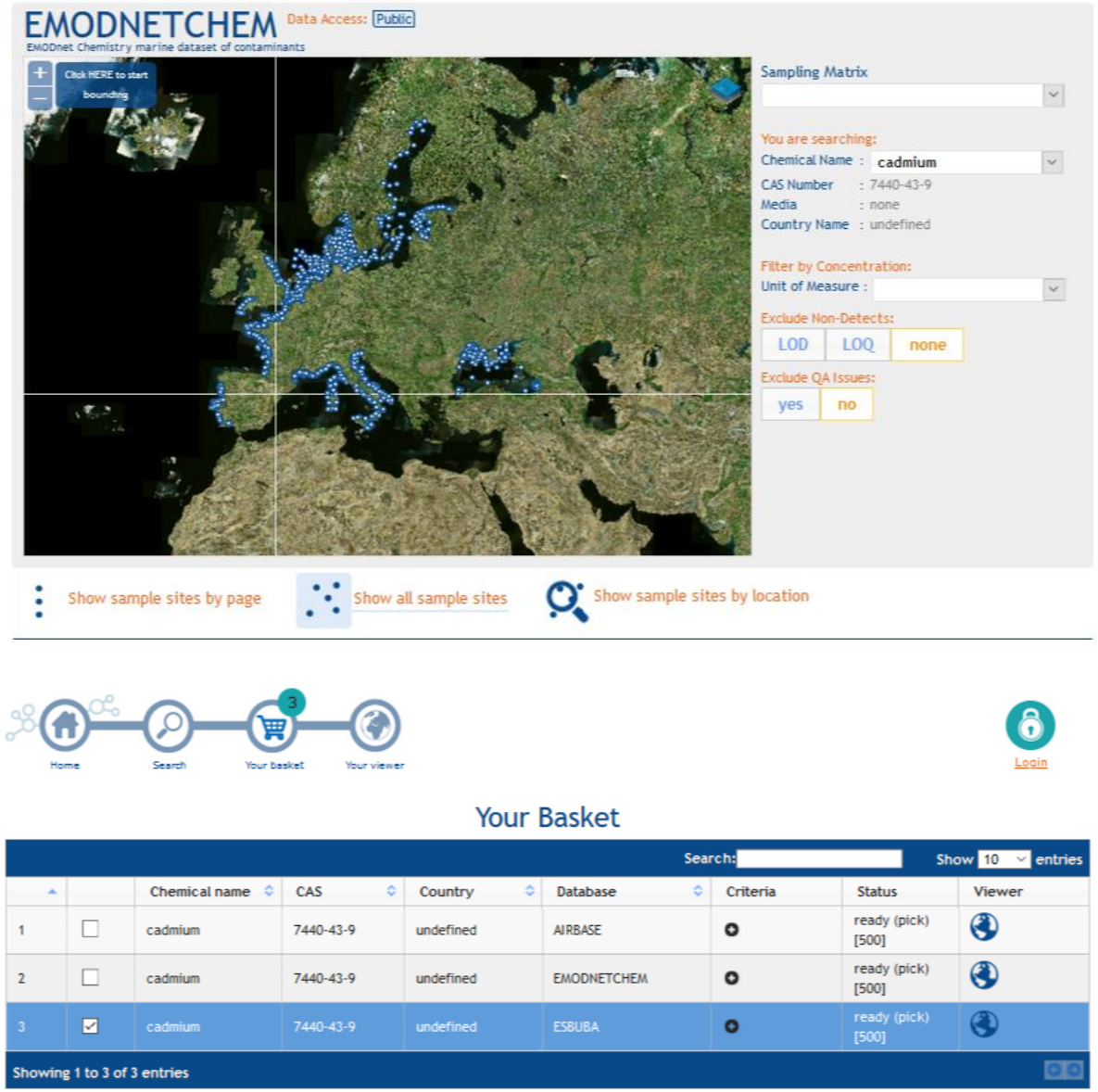
How to use IPCHEM?



Starting from a chemical or CAS number



Policy/scientific questions IPCHEM can help on

- Monitoring time and spatial trends
- Risk/impact indicators
- Monitoring compliance and targeting intervention
- Impact of regulatory intervention
- Regulatory risk assessments

- Explore aggregate and combined exposures

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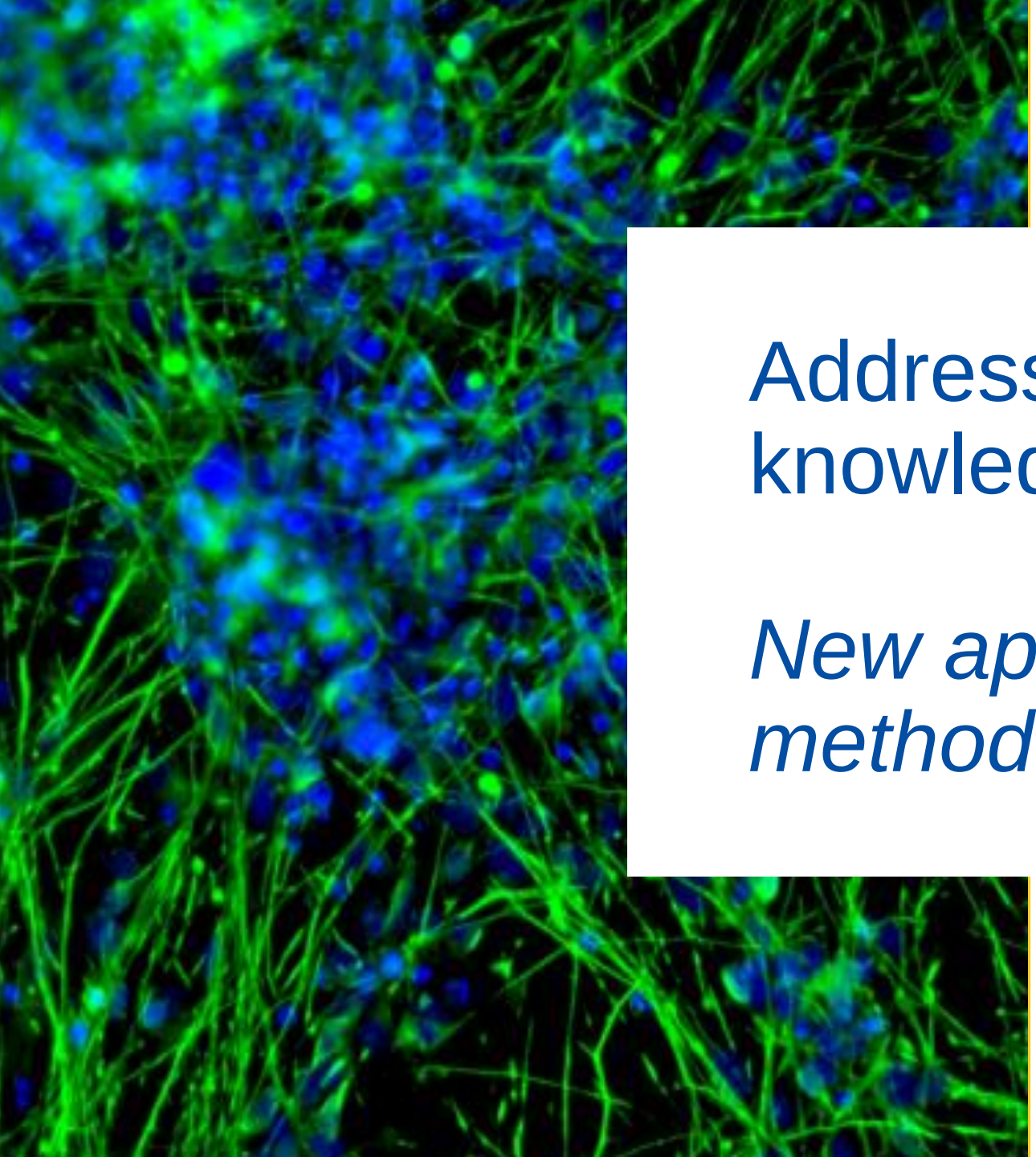

Addressing data and knowledge gaps

New approach methodologies (NAM)

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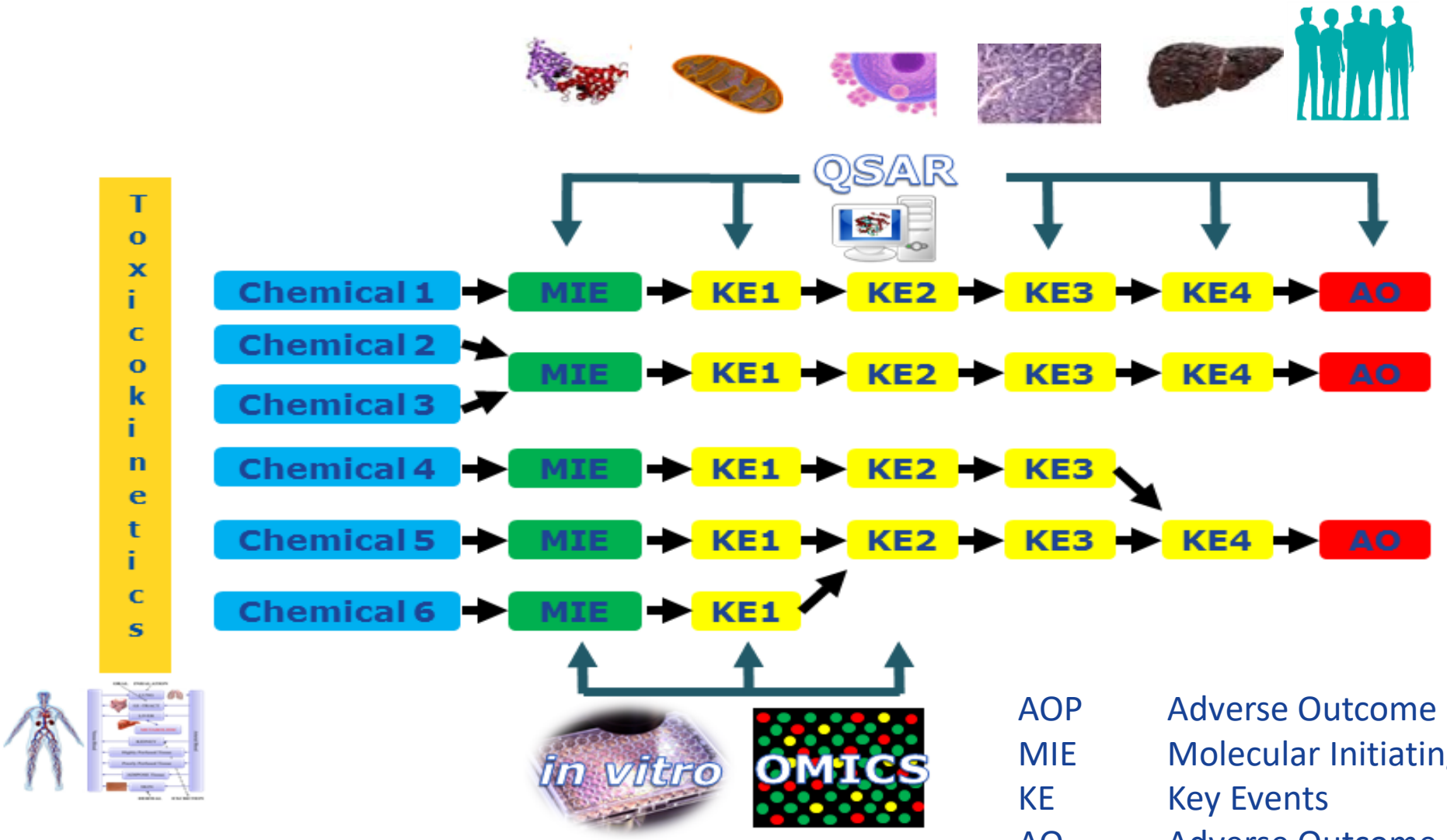
Use of New Approach Methodologies (NAM)



AOP	Adverse Outcome Pathway
MIE	Molecular Initiating Event
KE	Key Events
AO	Adverse Outcome

Bopp et al (2019) *Critical Reviews in Toxicology, 49*(2), 174-189. doi:10.1080/10408444.2019.1579169

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Developmental neurotoxicity (DNT) mixture study *Aims*

- Building on a **battery of *in vitro* assays** anchored to **common key events** (CKEs) identified in the DNT AOP network using human neuronal/glial culture to identify chemicals associated with **impairment of learning and memory in children**

- Determine whether chemicals combined at **individual non-neurotoxic concentrations** will produce **DNT effects in mixtures**

Chemical selection

1. Compounds known to cause cognitive impairment (AO)

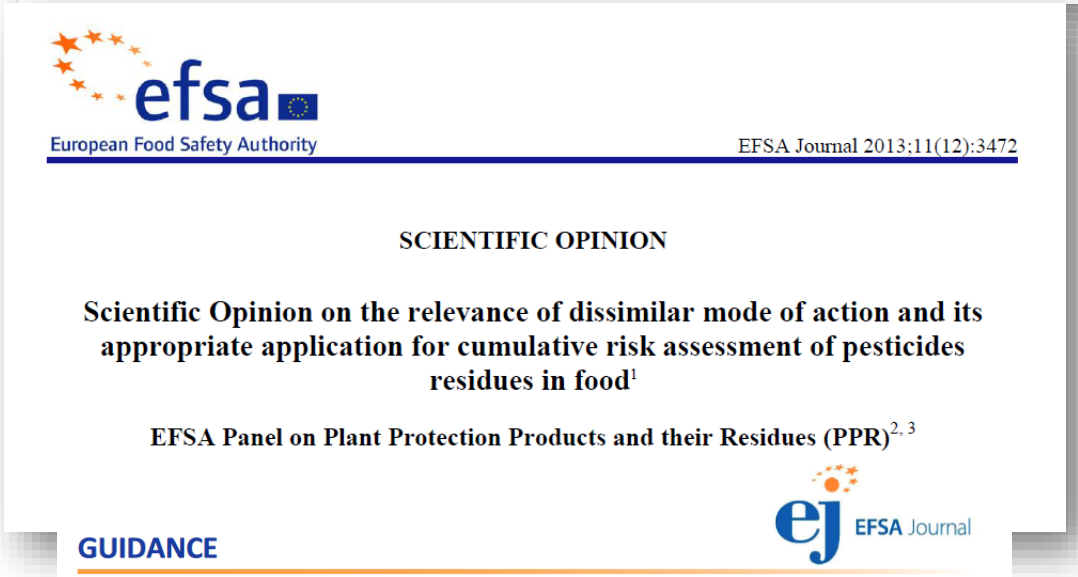
2. Compounds acting through identified common KEs in the AOPs

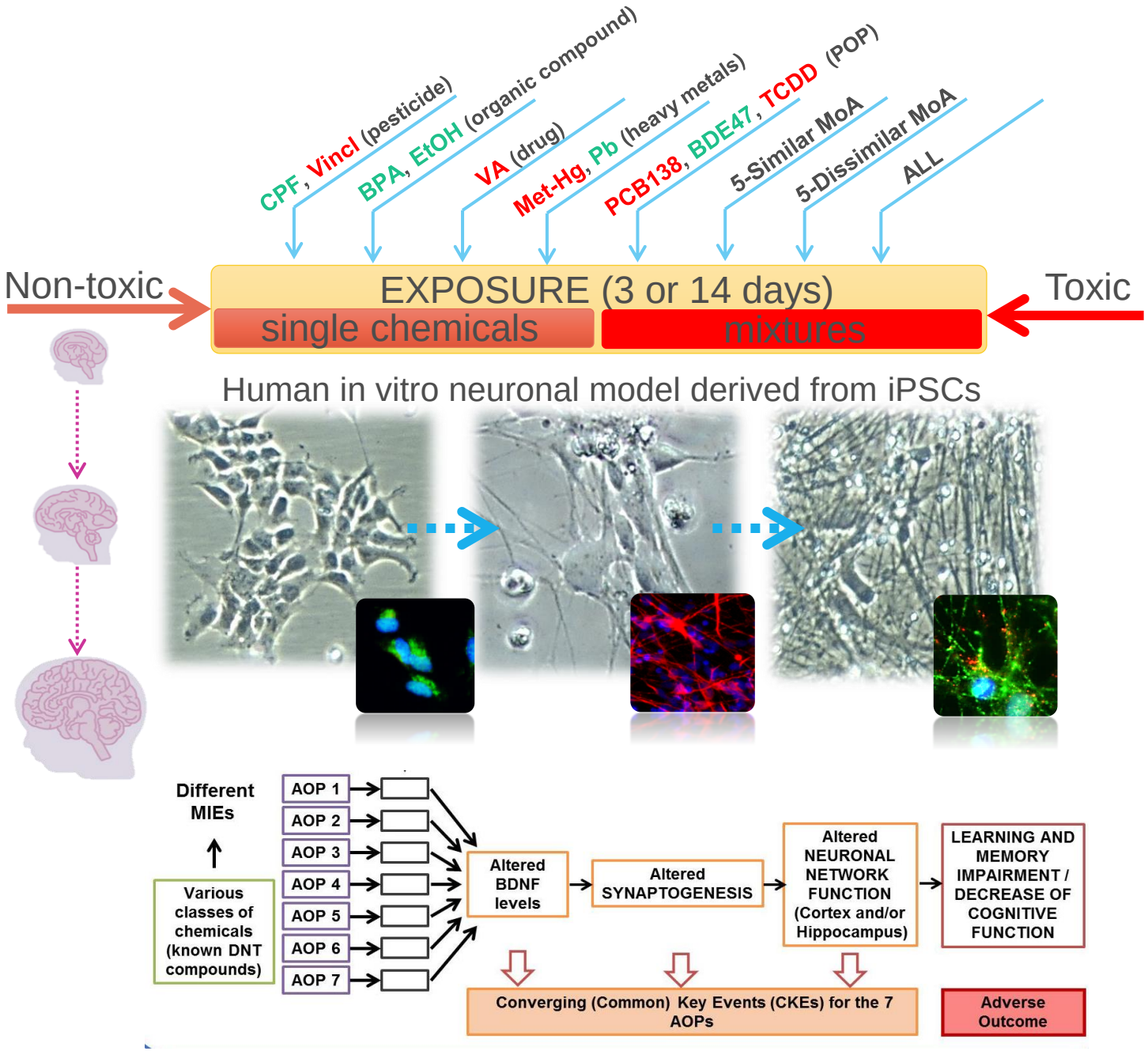
3. Compounds representing different classes (i.e., pesticides, industrial chemicals, heavy metals, POPs, and EDs)

4. Compounds found in human samples (e.g., breast milk, cord blood, urine, hair, umbilical cord plasma, brain tissues, maternal blood, or blood of children)

5. Compounds according to EFSA (2013) working through:

    - similar MoA
    - dissimilar MoA

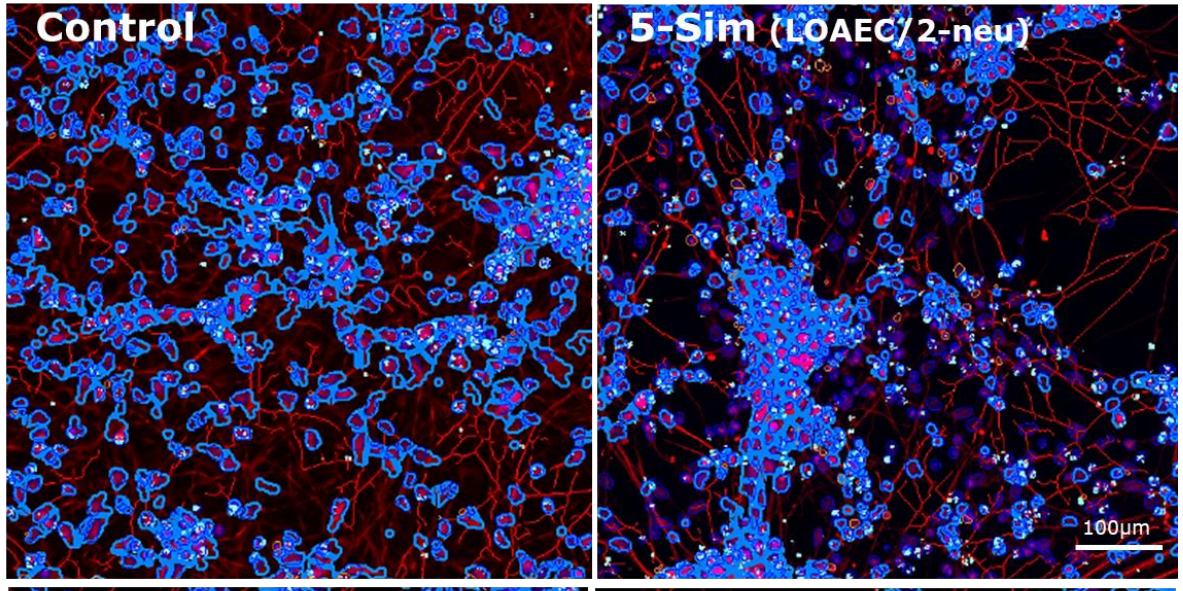




similar MoA - decreased BDNF level

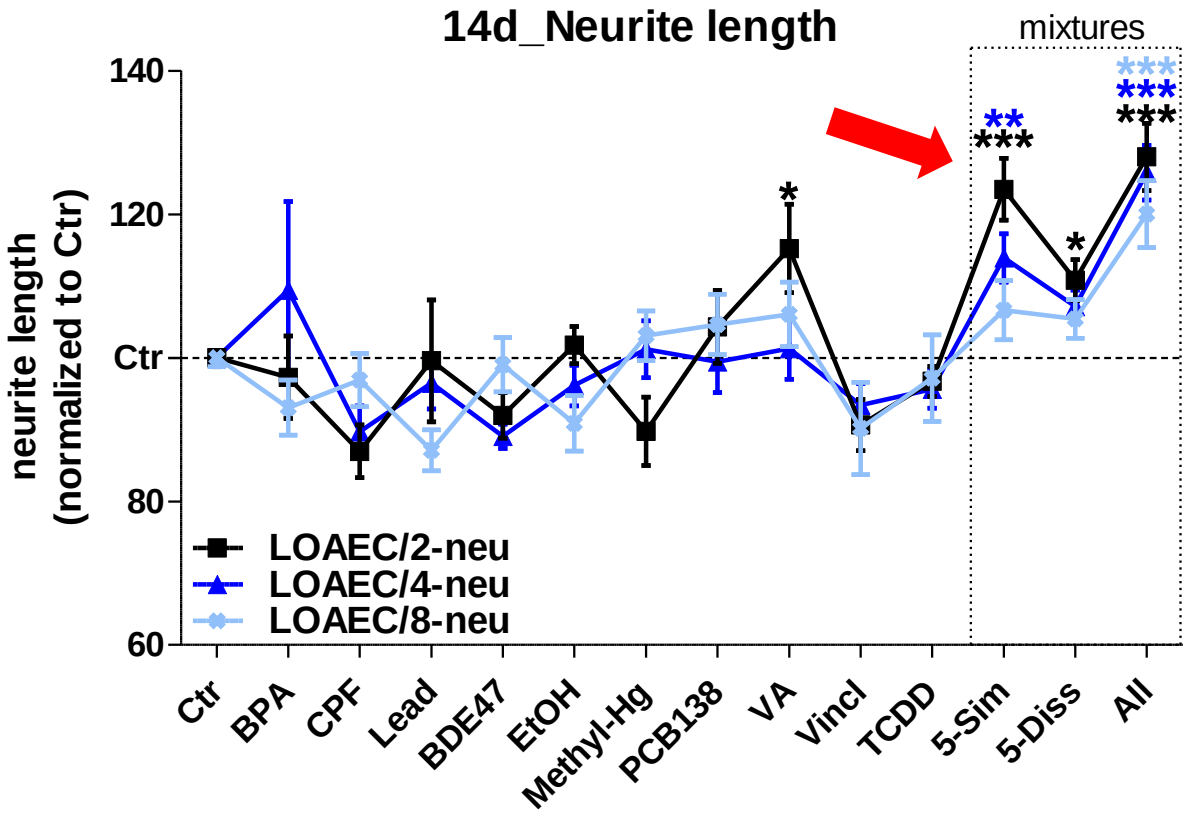
dissimilar MoAs

Mixtures' effects on neurite outgrowth



Masks: valid nuclei / selected cell bodies / neurites / branch points

β-III-Tub DAPI



→ Increase of neurite length by 5-Sim and 10-All mixtures

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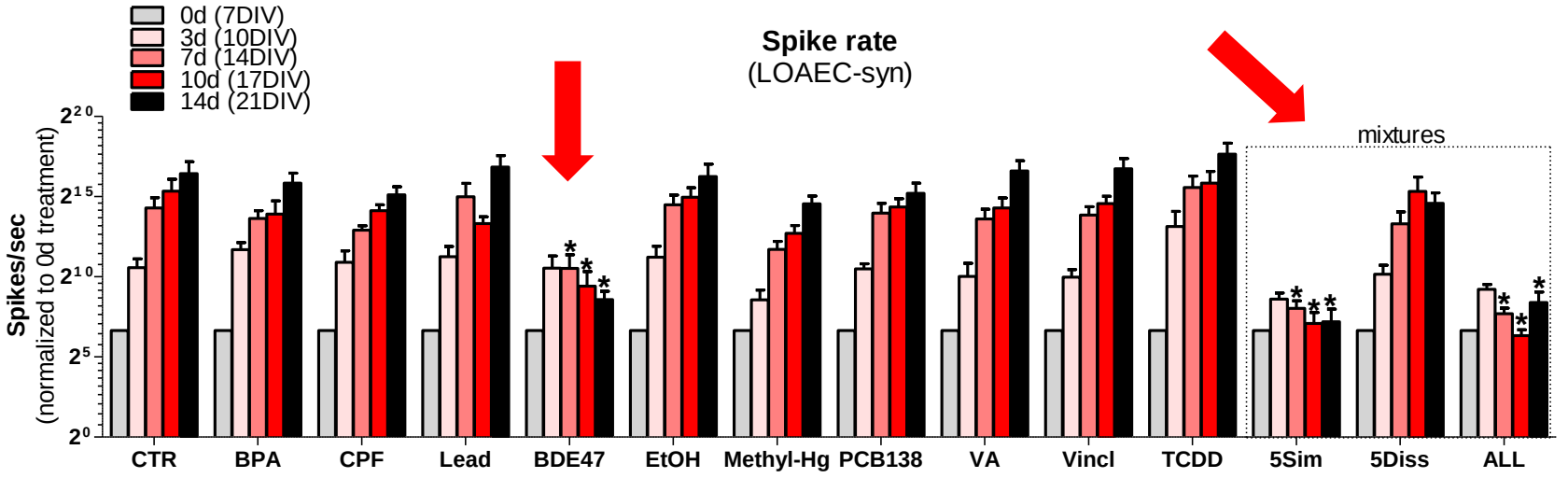
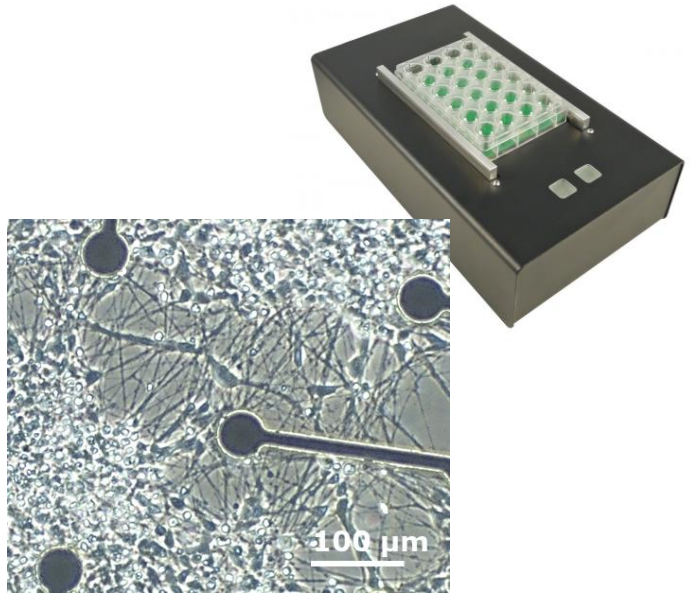
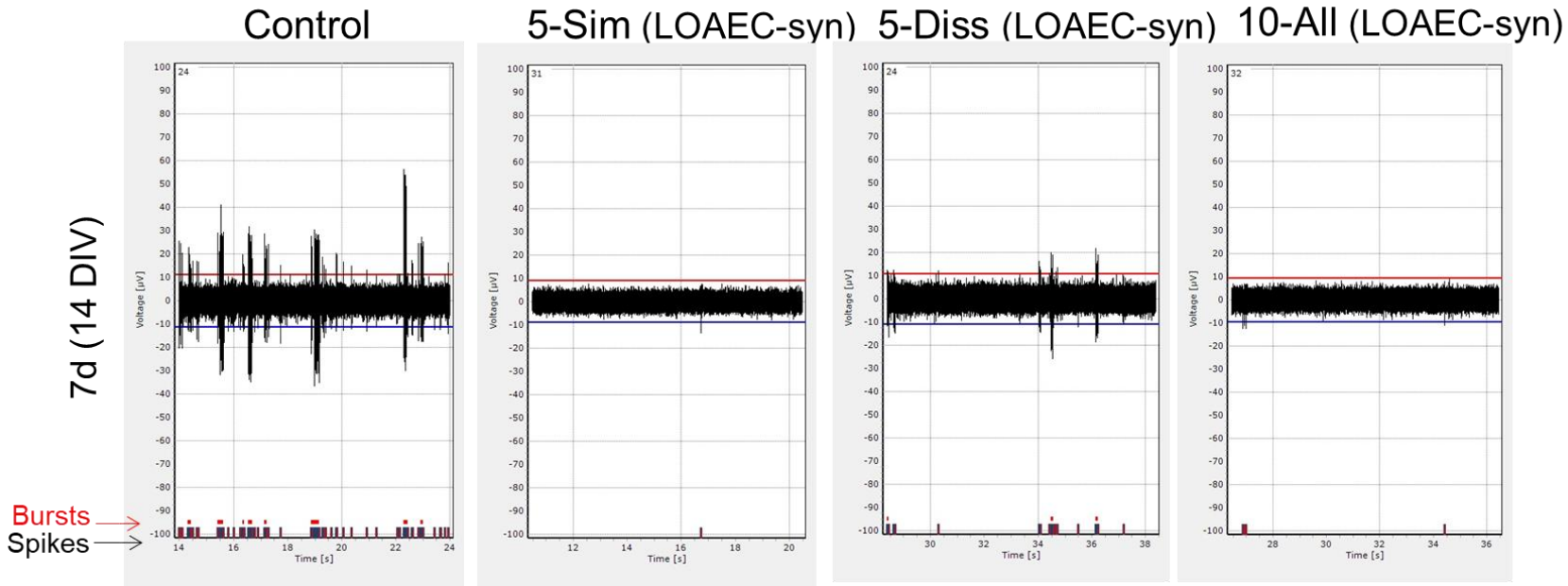
Mixtures' effects on electrical activity







→ ***Decrease of electrical activity by 5-Sim and 10-All***

→ ***BDE47 main driver of mixtures' effects***

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Conclusions DNT experimental study

- **Common Key Events** identified in DNT AOPs guided selection of the in vitro assays, permitting **mechanistic understanding** of toxicity

- Low concentrations (i.e., below LOAECs) of single chemicals (non-neurotoxic) become **neurotoxic in mixtures**, especially for the chemicals working through **similar MoA**

- Human iPSC-neurons/astrocytes exposed to chemical mixtures at low concentrations **reproduce some features described in neurodevelopmental deficits** (e.g., increased BDNF, higher % of neurons and astrocytes, decreased synapses, etc.)

Pistollato et al (2021) Reproductive Toxicology, 105, 101-119, https://doi.org/10.1016/j.reprotox.2021.08.007.

Addressing data and knowledge gaps

Interactions

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Do we need to worry about interactions?

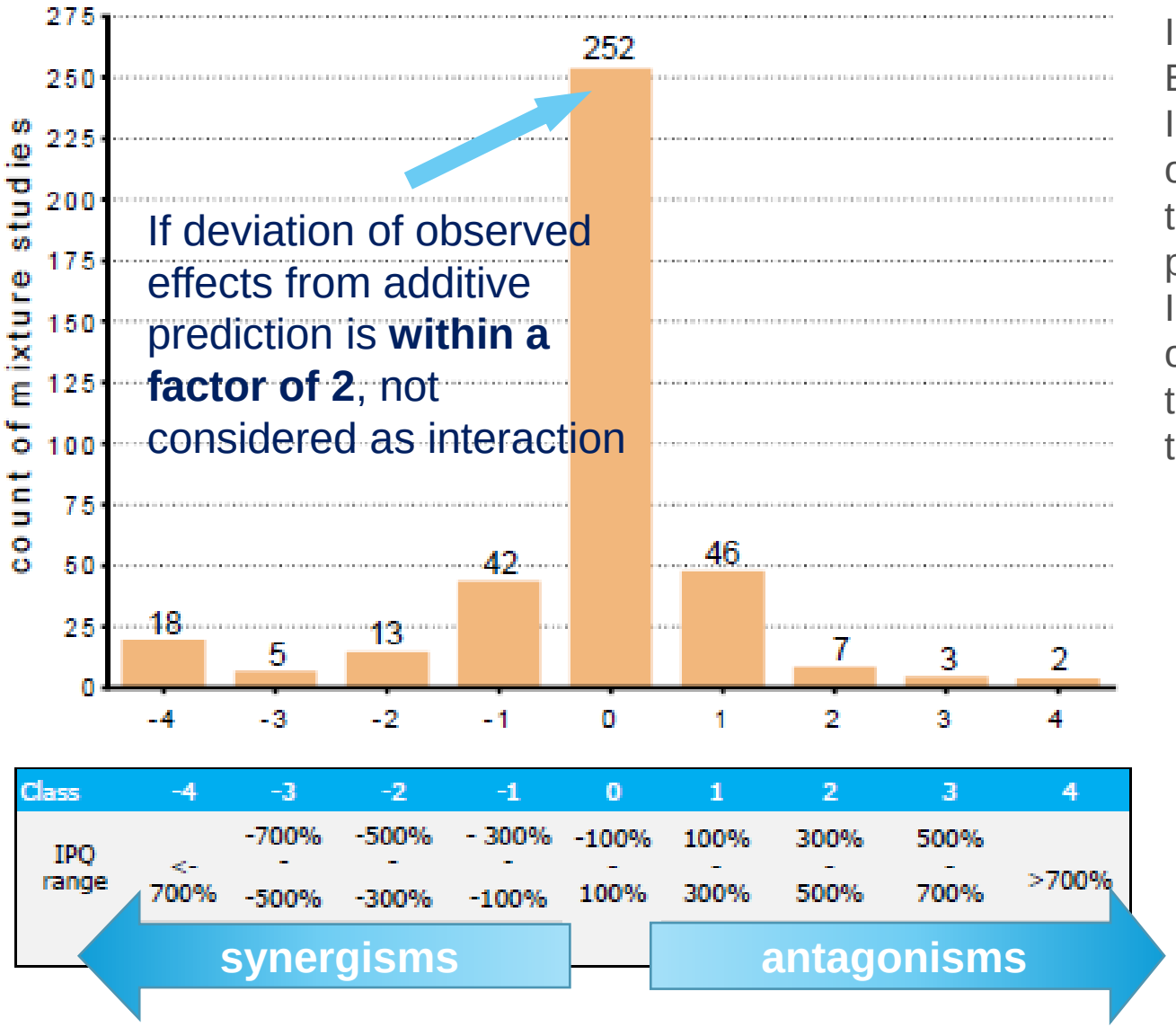
10790 papers

1205 papers
after abstract screening

761 papers
after full text screening

1220 experiments included

389 experiments re-appraised



IPQ=Index of Prediction Quality
Example:
IPQ = 50% means that the observed effect concentration of the mixture is 50% above the predicted value,
IPQ = -50% refers to an observed effect concentration of the mixture that is 50% below the predicted value

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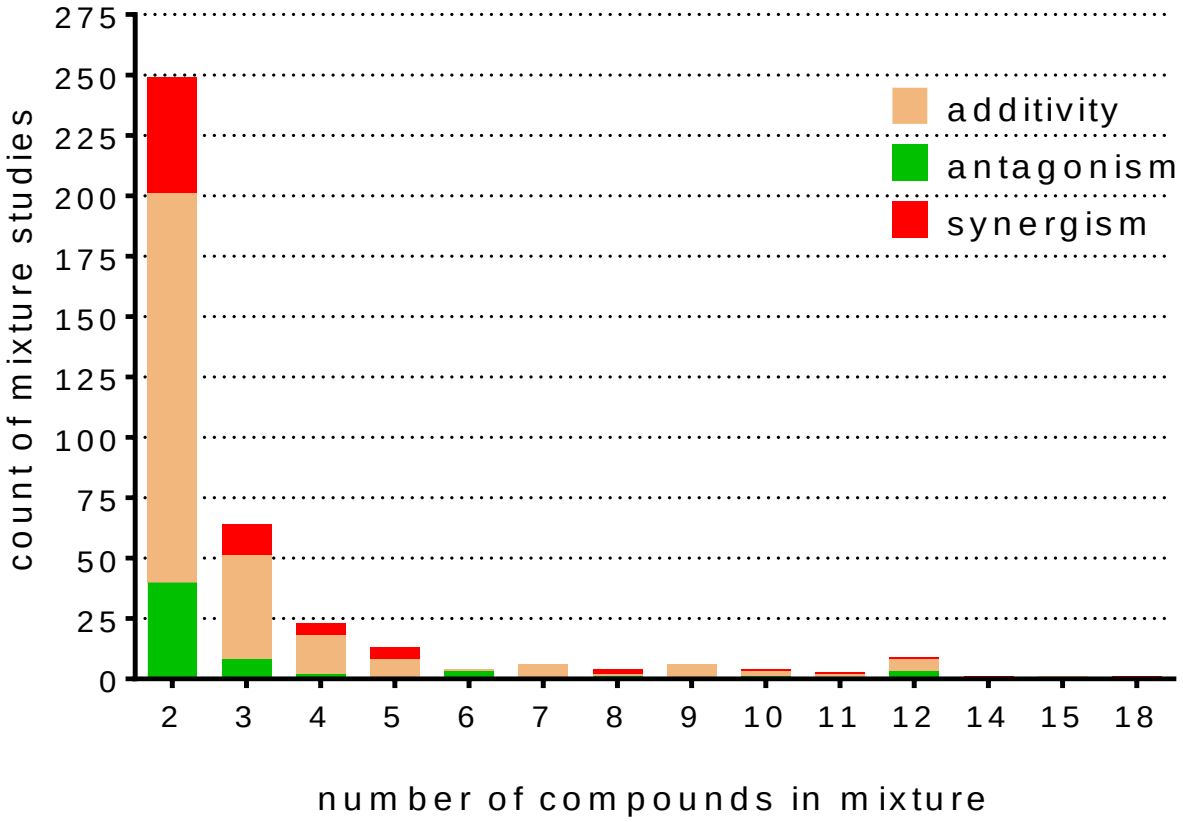
Do we need to worry about interactions?



Martin et al (2021) Ten years of research on synergisms and antagonisms in chemical mixtures: A systematic review and quantitative reappraisal of mixture studies, Env Int, 146, 106206, https://doi.org/10.1016/j.envint.2020.106206

Conclusions on Interactions (1)

- **65% of studies considered additive**
- synergisms as likely as antagonisms
- non-additivity slightly more frequent in ***in vitro*** **studies**
- deviations from additivity **mostly small**
- **large deviations** from additivity are more likely to occur as synergisms
- **strong synergisms** rare (effects at doses 50 to 100-times lower than expected based on dose addition)
- 47% of synergistic or antagonistic of higher **risk of bias** (compared to overall 38.5% for mixtures classified as additive, statistically not significant)
- **chemical groups** in the synergistic cases: number of cases with EDs (mechanistic basis is unclear) and cases for metals (CrVI, Ni with Cd), confirmation for chemicals such as triazines, azoles and pyrethroids

Conclusions on Interactions (2)

- No new evidence of synergisms at low doses, close to points of departure of the individual components
- Results confirm the utility of default application of the dose (concentration) addition concept for predictive assessments of simultaneous exposures to multiple chemicals
- Application of dose addition must however be complemented by an awareness of the synergistic potential of specific classes of chemicals
- (consider the classes of chemicals included in the underlying studies)

Thank you

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Brunel University team for work on interactions

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