For our Environment

Umwelt Bundesamt

WORKSHOP Methodologies for prioritising hazardous chemicals in European waters: the state of play and the need for improvement

# Tox-Box: an enhanced health-related approach for risk assessment of drinking water in Germany

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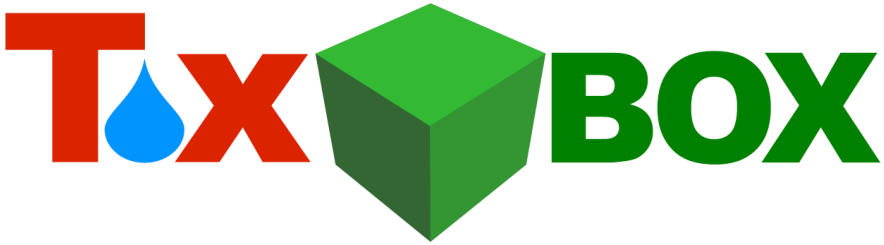
Fachgebiet II 3.6 / Toxikologie des Trink- und Badebeckenwassers




**past:** theoretical background

**present:** project approach

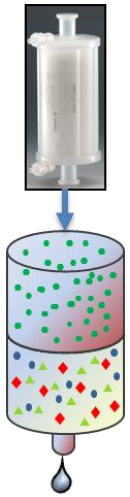
**future:** implementation

## Module Endocrine Effects

TP 11: Incos Boté, Nieder-Olm
TP 12: RWTH, Aachen

## Module Exposure

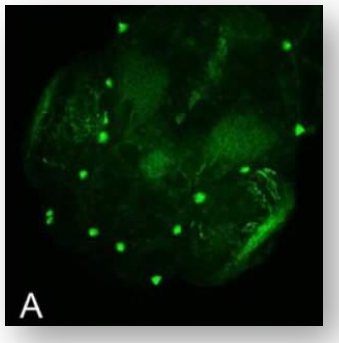
TP 1: RheinEnergie, Köln
TP 2: UFZ, Leipzig



TOXBOX

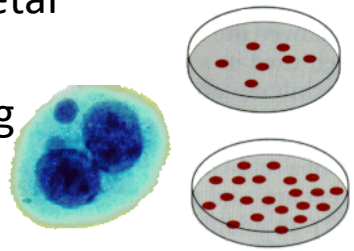
## Module Neurotoxicity

TP 9: UBA, Bad Elster
TP 10: Universität Heidelberg



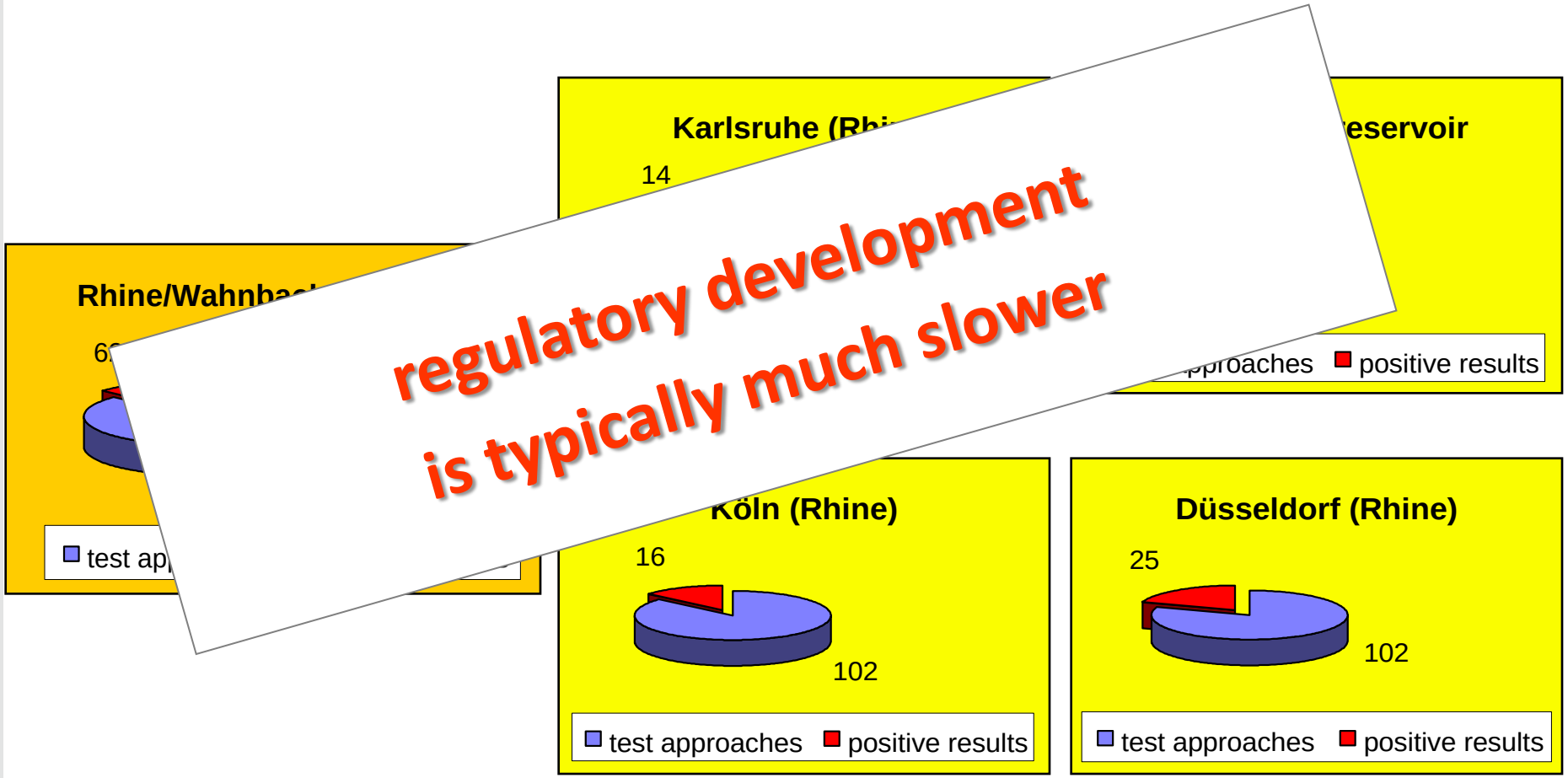

## Module Genotoxicity

TP 3: RheinEnergie, Köln
TP 4: DLR, Köln
TP 5: Hydrotox GmbH, Freiburg
TP 6: DIfE, Nuthetal
TP 7: DLR, Köln
TP 8: UFZ, Leipzig



## Coordination

# Rate of positive findings in the research program "Rhine/Wahnbach reservoir“ (1995 – 1999)



# Theoretical background

Recommendation of the C
Agency after consult

**Practical approach to guarantee toxicological safety and regulatory reliability**

w of health of the presence in
ances that are not (yet) possible or only partially possible to evaluate“

# Health related indication values (HRIV)

| HRIV [µg/l] |
| --- |
| 0.01 |
| 0.1 |
| 0.3 |
| 1 |
| 3 |

bioassay

**Critical toxic endpoints**
(genotoxicity, neurotoxicity, endocrine disruptors)

**knowledge of the MoA**

**+**

**Increasing data base**

# TOX-BOX activities

→ screend ~ 65 compounds

→ compared the sensitivity

→ developing statistical tools for analyzing the resulting data and for compound priorization

→ identifying „fingerprints“ potentially indicative of adverse effects

# Critical Issues

→ adequate metabolism

→ non adverse versus adverse effects

→ biological plausibility

→ conceptual validation

# Module Genotoxicity

## In vitro short-term tests

Bacterial assay

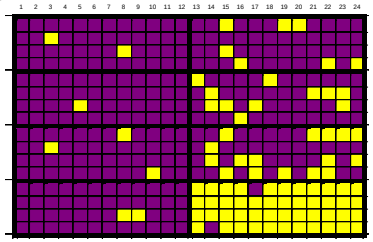
Ames I

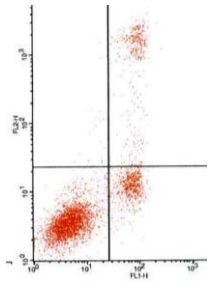
multiple endpoints ✓

adequate metabolism

Comet assay

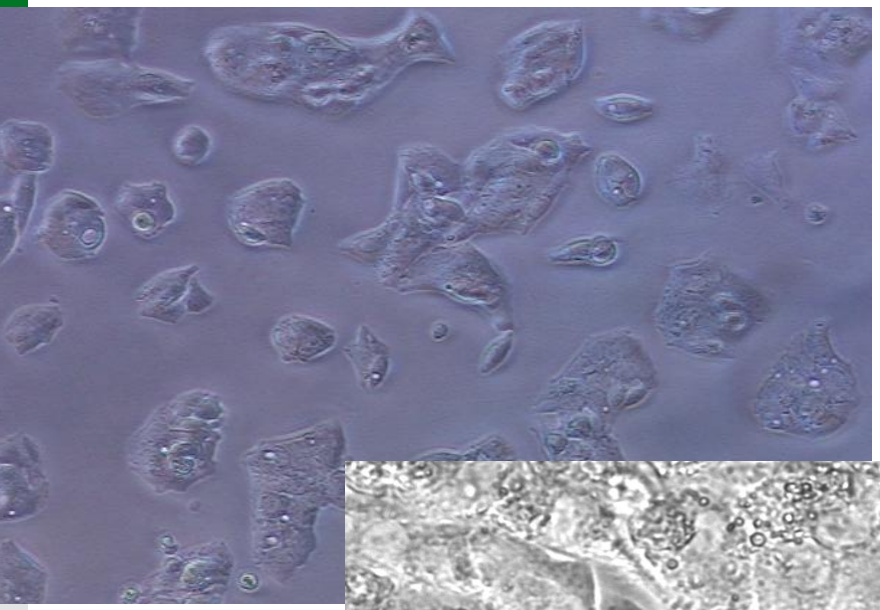
Micronuclei



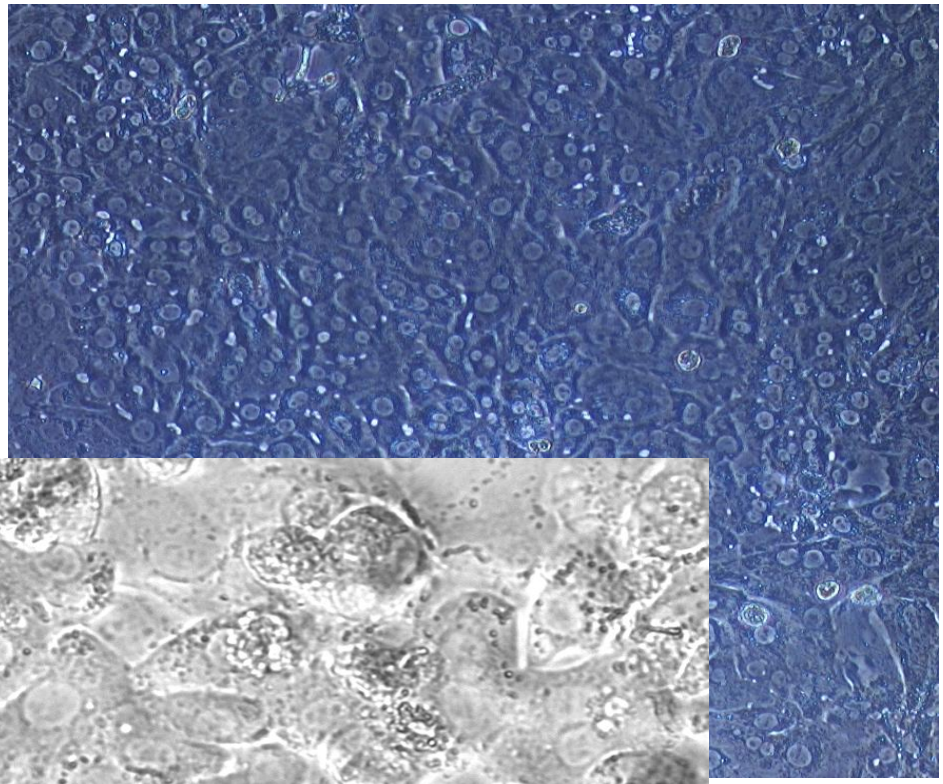


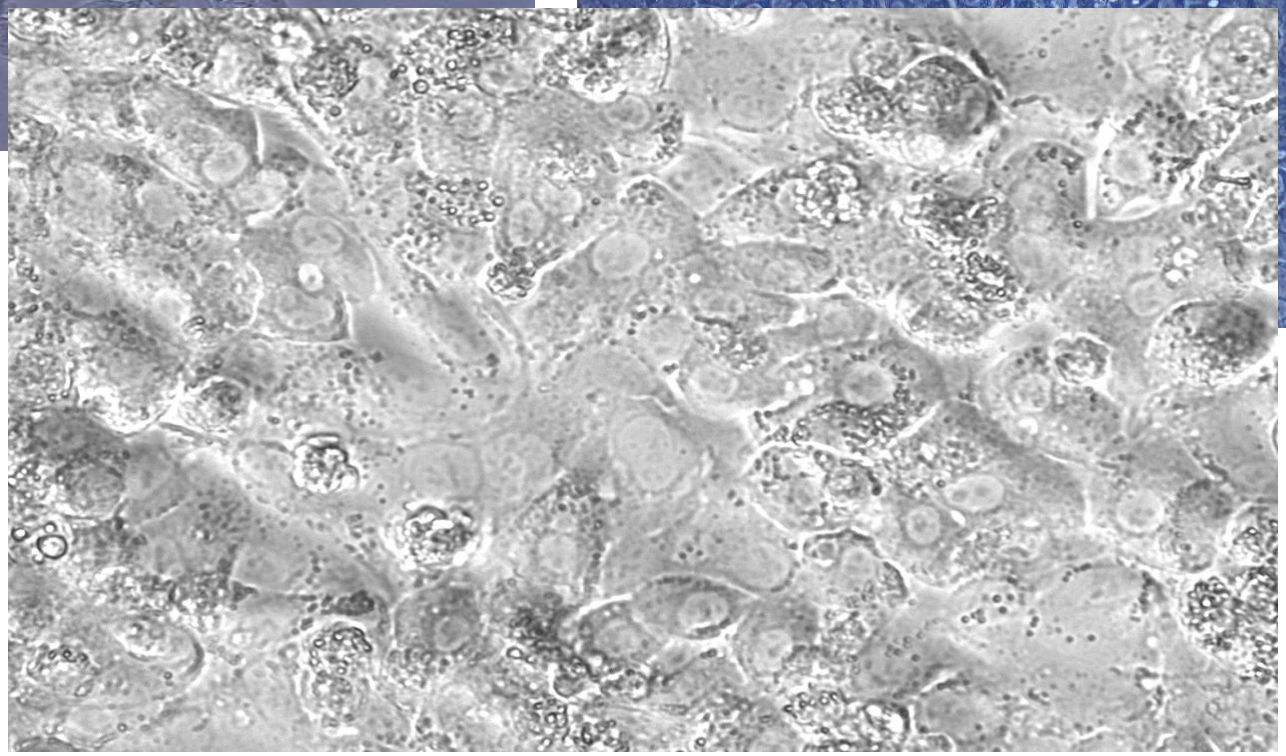
FACS

# Adequate metabolism



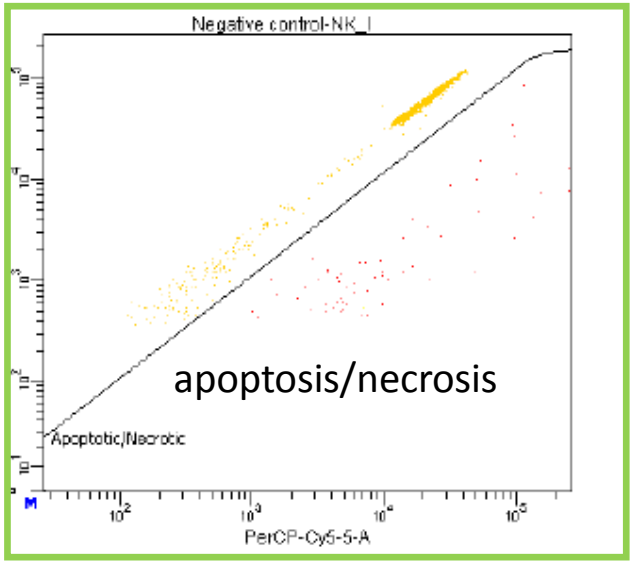
HepG2

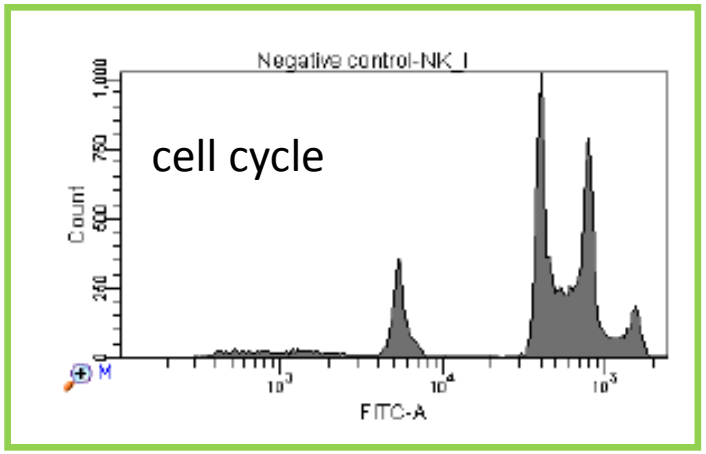


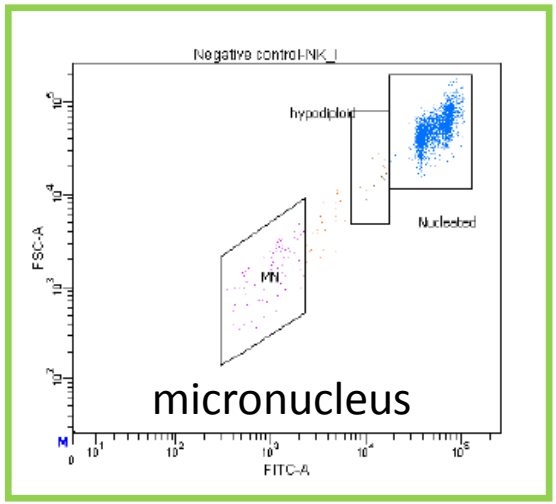


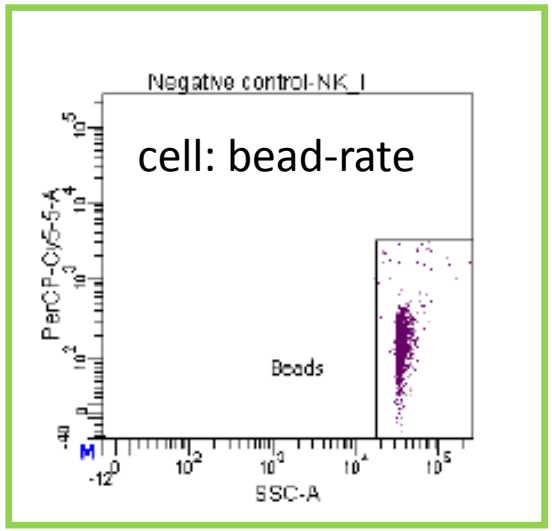
HepaRG™-cells

# Multiple endpoints

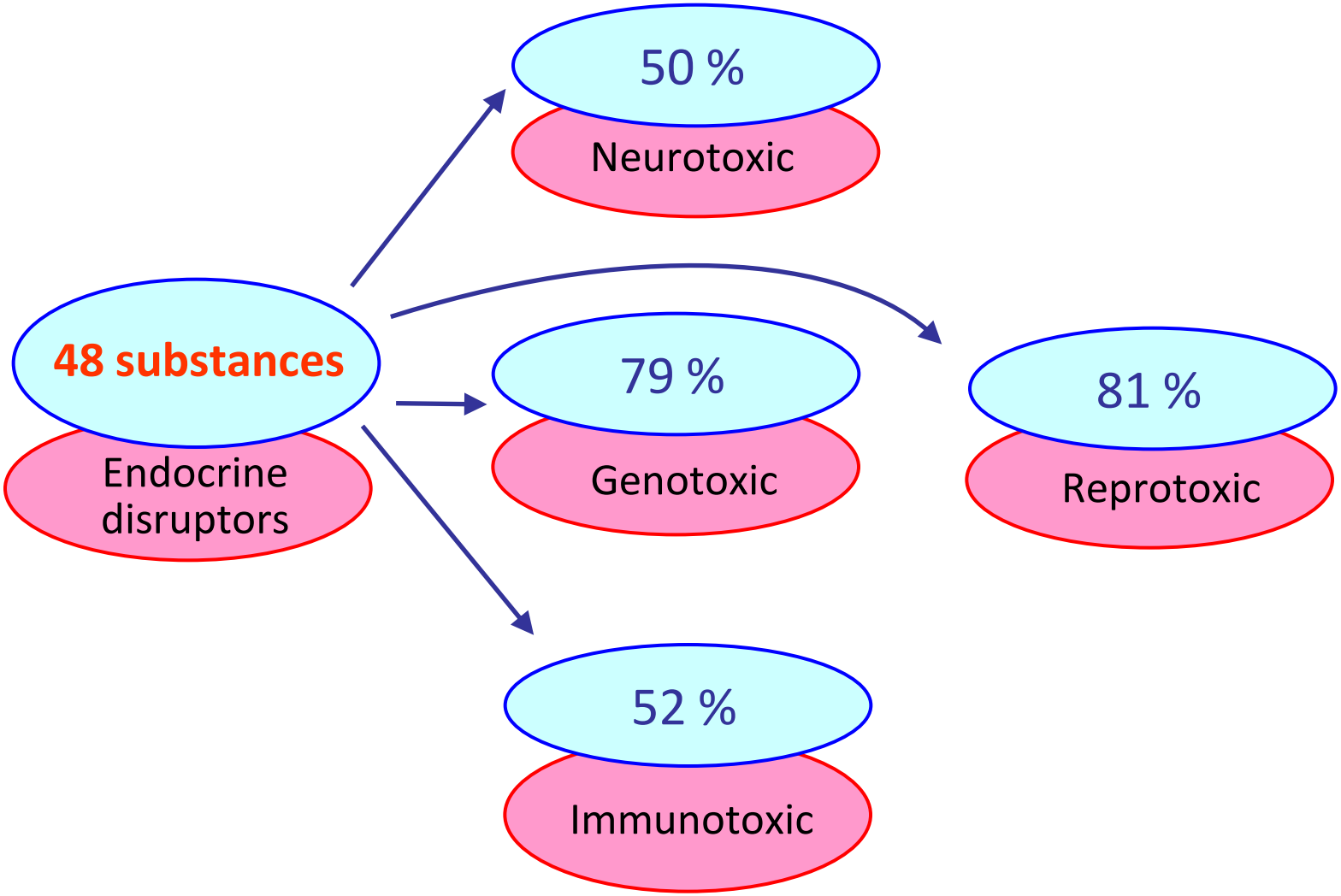


# Multiple endpoints




Literatur: *J. Toxocol. Environ. Health Part B,* 7 (2004) 1-24

# An integrated multidisciplinary approach

ROS

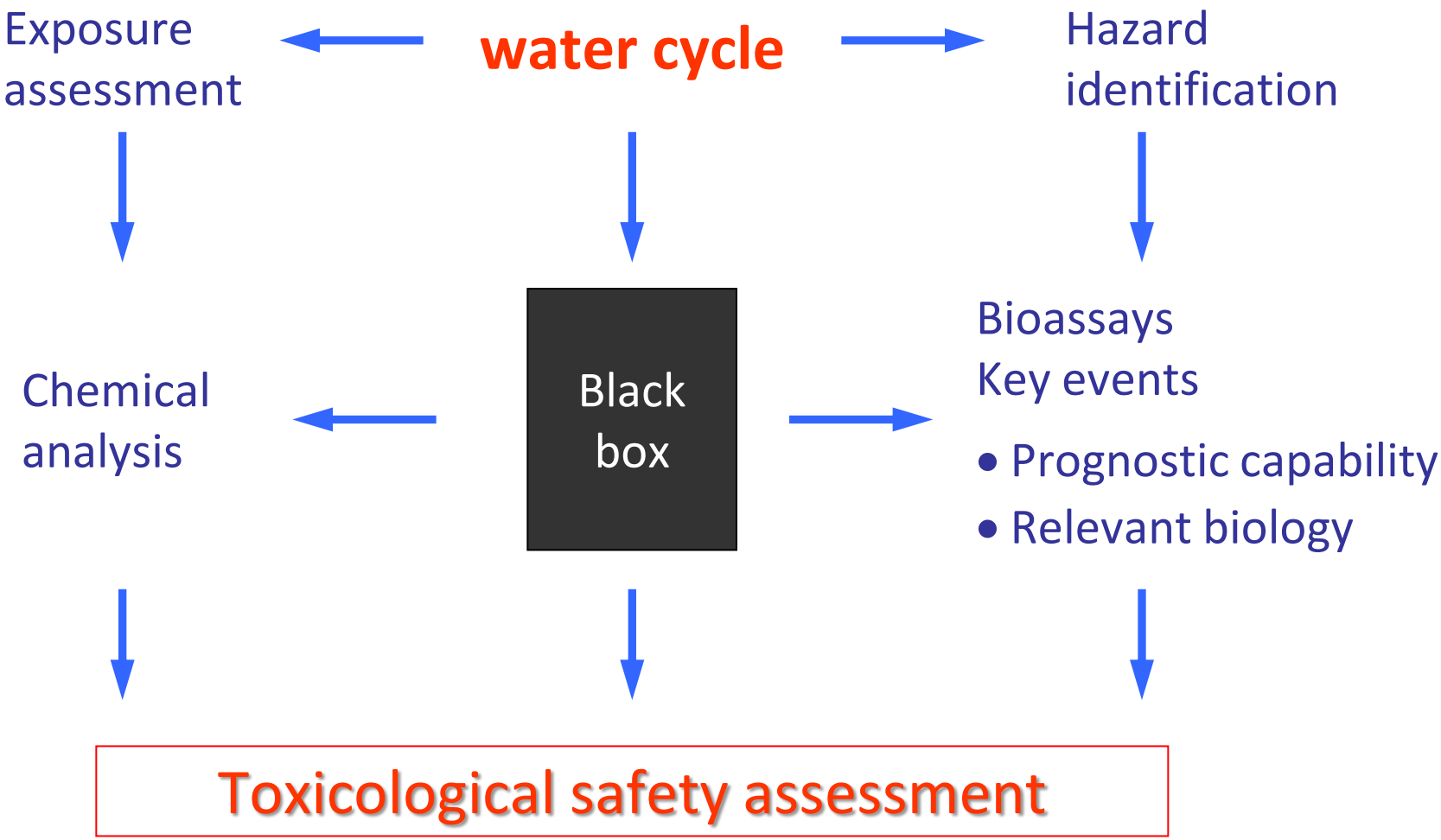
- early distress signal
- not any det

- robust

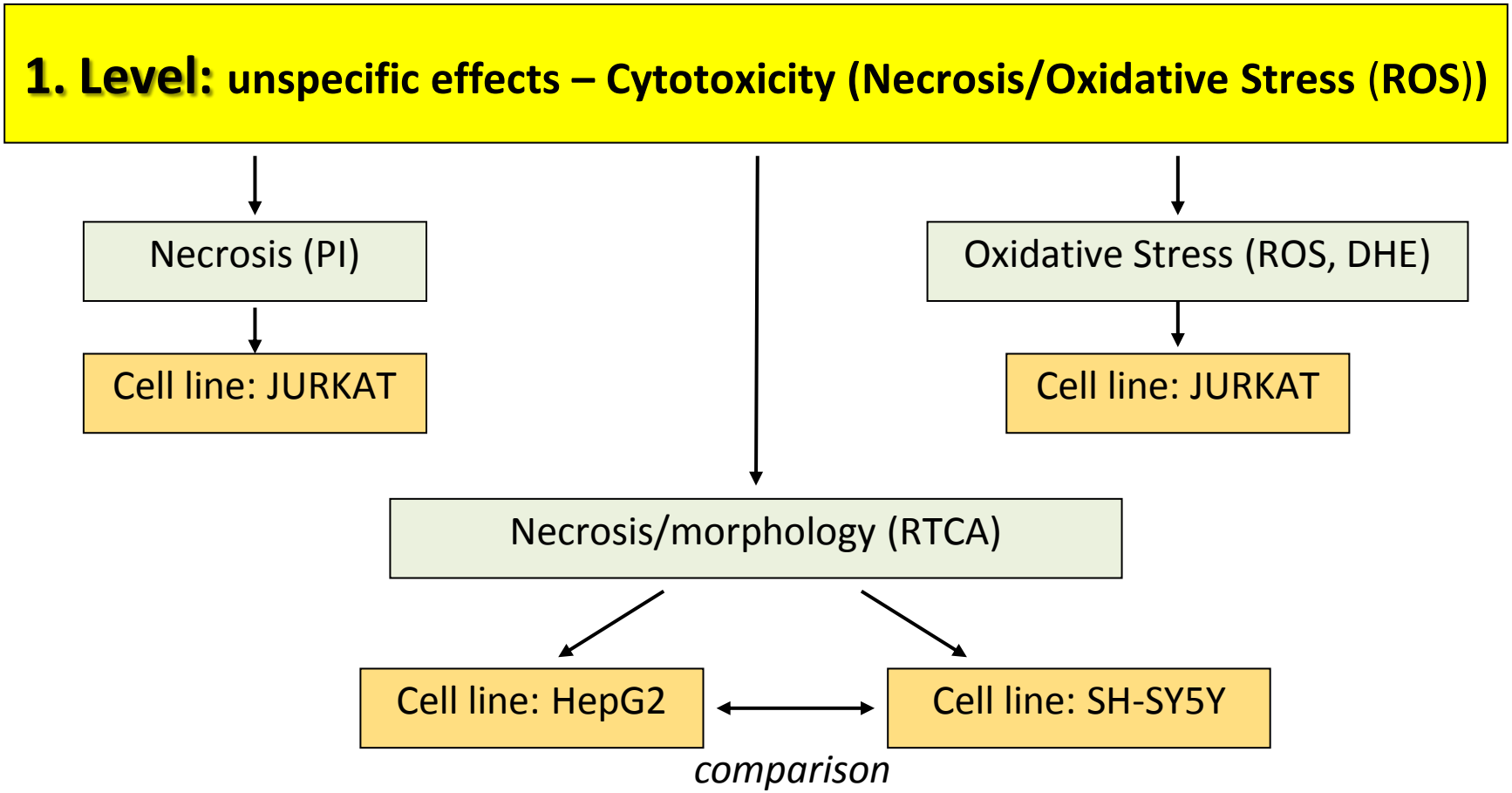
EARLY WARNING !

Identification of acute hazard potential

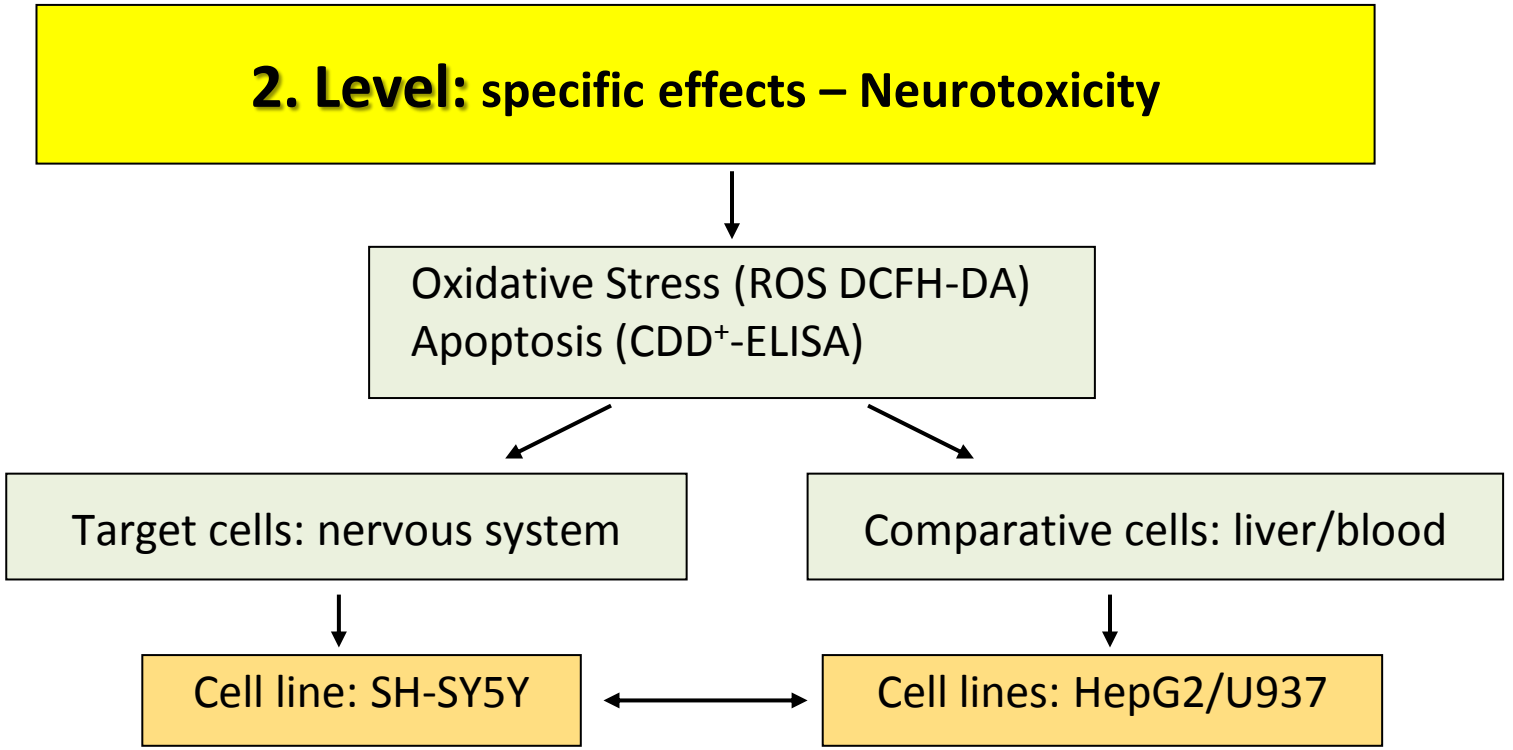
# Theoretical concept



# Modul Neurotoxicity

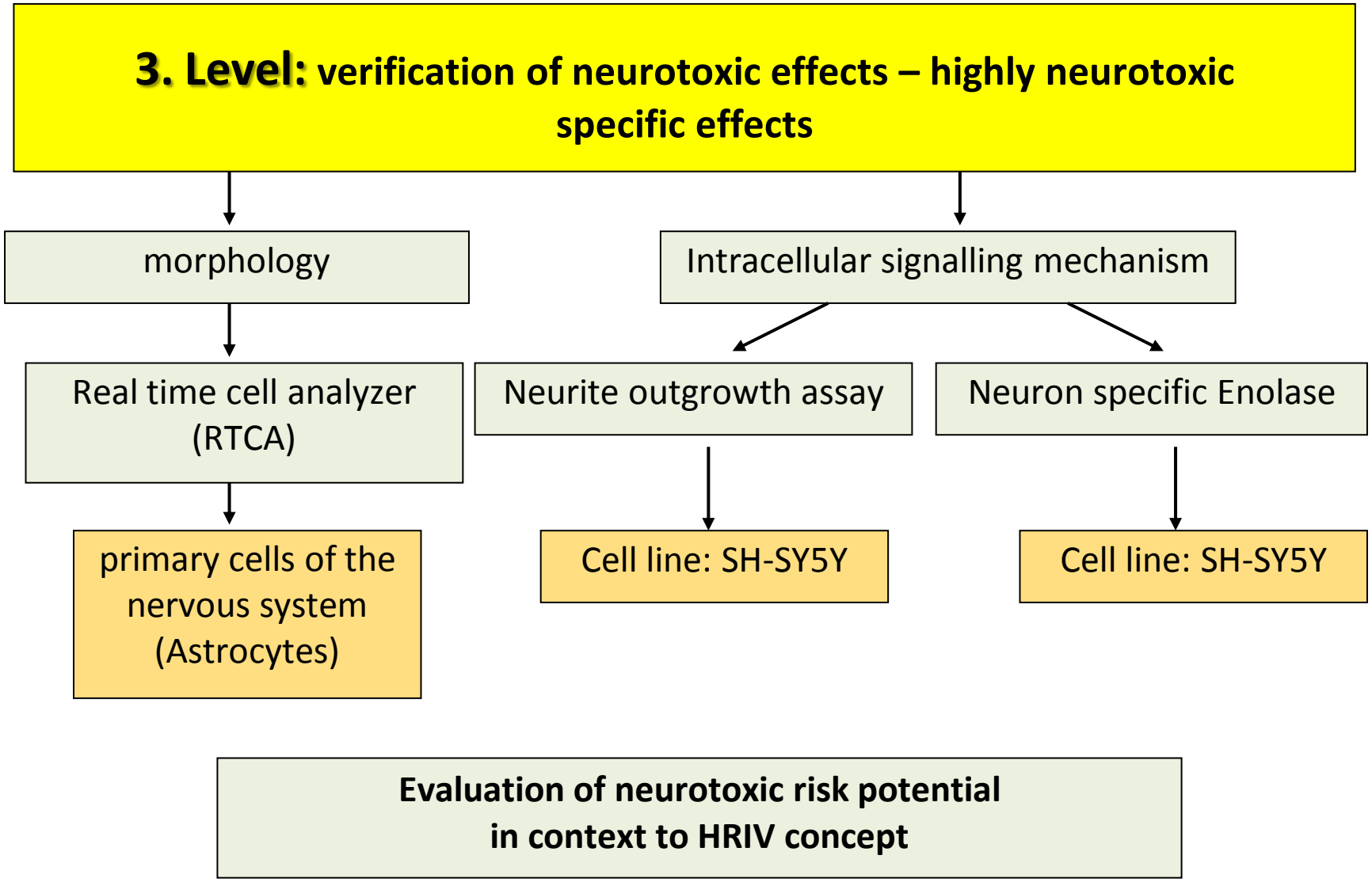



- Propidium Iodid (PI) Necrosis Test – to exclude cytotoxic concentrations
- Oxidative Stress (ROS) – of major importance for nervous system cells

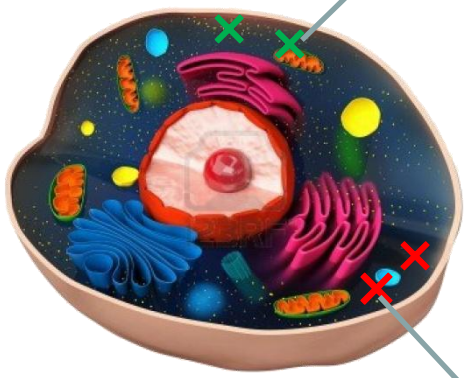



**Assessment**

same effects on cells of target tissue and comparative cells
→ *no neurotoxicity*
strong effect on target tissue cells and small on comparative cells
→ **suspicion for neurotoxicity**


3. Level: verification of neurotoxic effects – highly neurotoxic specific effects
morphology
Intracellular signalling mechanism
Real time cell analyzer (RTCA)
Neurite outgrowth assay
Neuron specific Enolase
primary cells of the nervous system (Astrocytes)
Cell line: SH-SY5Y
Cell line: SH-SY5Y
Evaluation of neurotoxic risk potential in context to HRIV concept

# Fingerprints



normal signal (X)

changed signal (X)

**Mode of Action**

| | | |
|---|---|---|
| signal 1 | X | |
| signal 2 | X | |
| signal 3 | | X |
| signal 4 | | X |

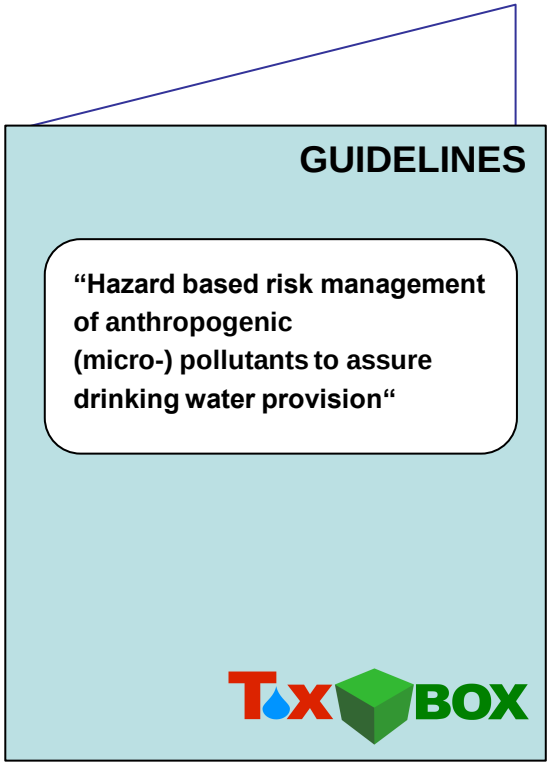
$HRIV_1$

| | |
|---|---|
| | X |
| X | |
| X | |
| | X |

$HRIV_2$

# TOX-BOX

- Measurement of substance-specific effects
- Fast and reliable results
- Establish test battery
- Create guidelines for evaluation



# Thank you for your attention

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