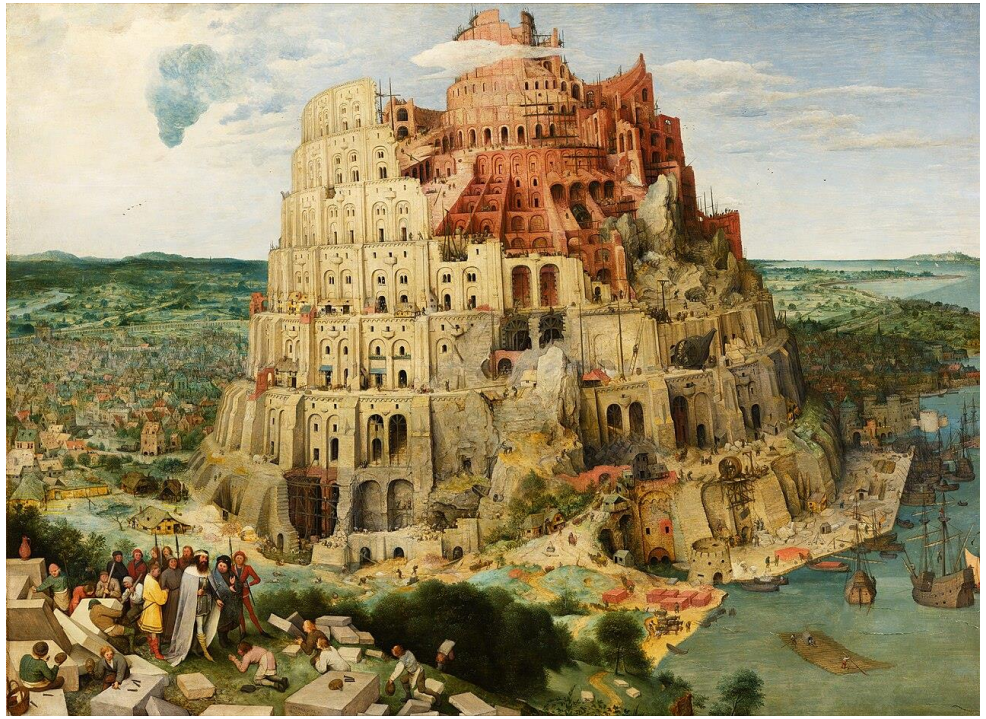
sciensano

healthy all life long

The use of new approach methodologies under REACH

By Birgit Mertens

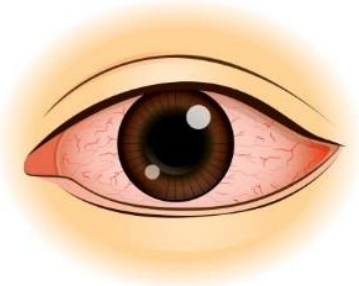
New Approach Methodologies



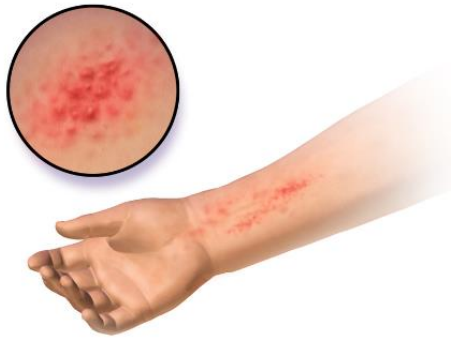
ECHA
EUROPEAN CHEMICALS AGENCY

Defines New Approach Methodologies (NAMs) as any technology, methodology, or approach that can provide ***information on chemical hazard and risk assessment without using intact animals****. This includes in vitro, in chemico, and in silico methods, as well as other non-animal approaches. NAMs aim to replace, reduce, or refine the use of animals in testing, adhering to the 3Rs principles.*

Use of NAMs under REACH *(human health)*



Eye irritation



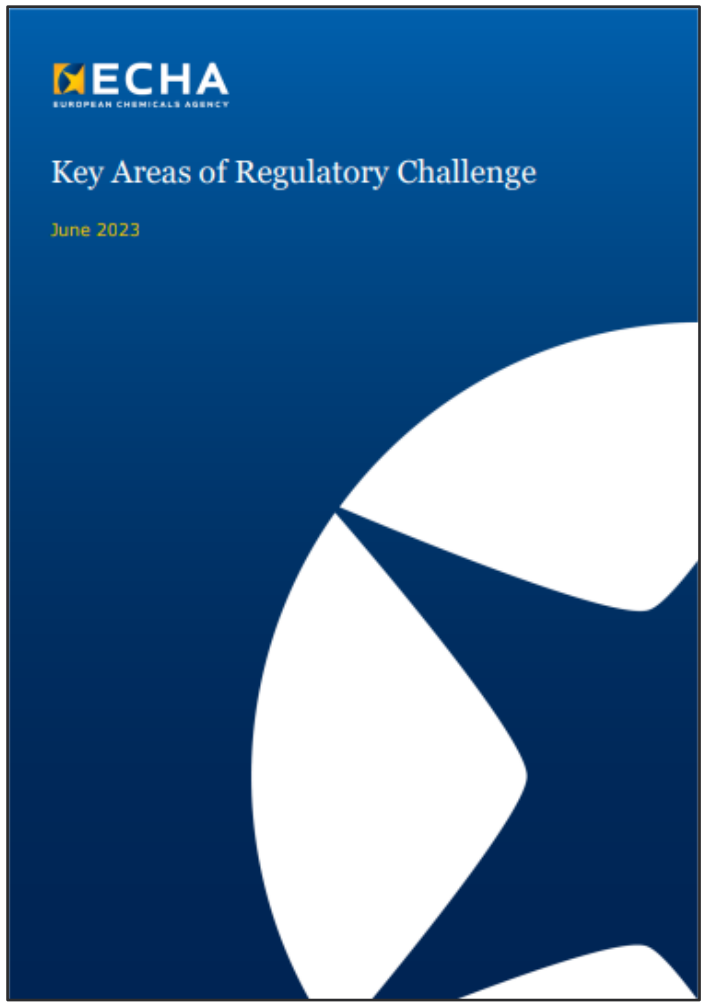
Skin irritation and sensitization

Mutagenicity

+ Read-across (supported by NAMs)

Limited use of NAMs – Several challenges in implementation of NAMs under REACH

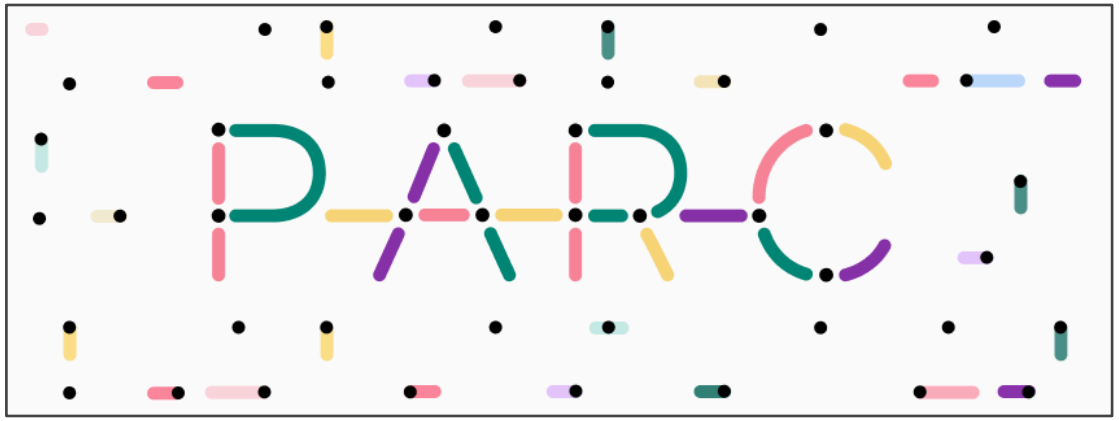
Key Areas of Regulatory Challenge (KARC)



- Hazard identification for critical biological effects that currently lack specific and sensitive test methods: i.e. developmental and adult neurotoxicity, immunotoxicity and endocrine disruption
- Chemical pollution in the natural environment (bioaccumulation, impact on biodiversity, exposure assessment);
- **Shift away from animal testing (read across under REACH, move away from fish testing, mechanistic support to toxicology studies e.g. carcinogenicity)**
- New information on chemicals (polymers, nanomaterials, analytical methods in support of enforcement)

Support and inspire PARC and the wider research community – *Updated in 2024 (e.g. kinetic info)*

Partnership to assess risks from chemicals

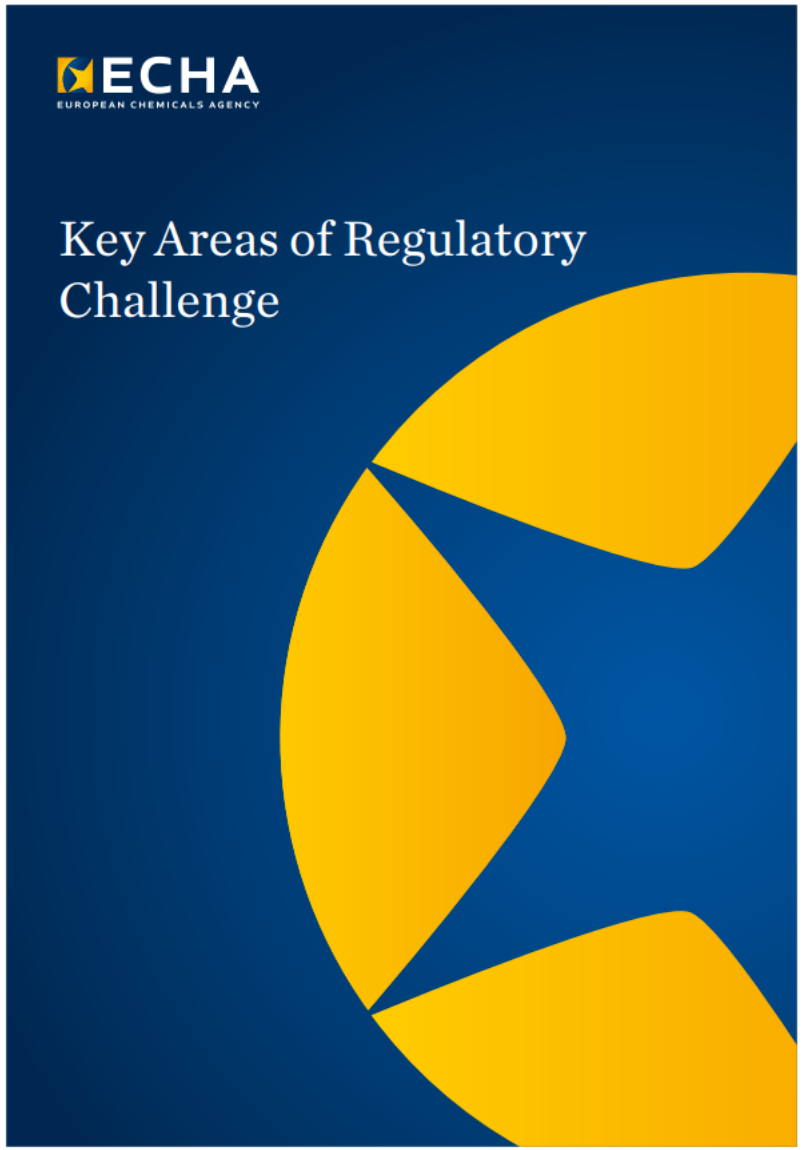


https://www.eu-parc.eu/

- Public-public partnership funded under the HorizonEurope call
- >200 partners
- 400 million euro (50:50 EC/member states)
- GOAL: to consolidate and strengthen the **EU's Research and innovation capacity for chemical risk assessmen**t to protect human health and the environment

STIMULATE TRANSITION TOWARDS NEXT GENERATION RISK ASSESSMENT

KARC – Read-across



2.3.1 Read-across and NAMs – Development of case studies

Why further research is needed:

Read-across can possibly substitute the need for (in vivo) data generation under REACH. NAMs may be used in the read-across justification to strengthen predictions regarding similarity of structural, toxicokinetic and -dynamic, and toxicological properties.

Regulatory needs:

Explore and demonstrate the possible use of NAMs in supporting read-across and build regulators' confidence with case studies.

Impact:

- Reduce the need for animal testing.
- Speed up identification and regulation of hazardous chemicals
- Reduce the costs.

Why the topic is relevant: Read-across is considered one of the main possible adaptations for more complex toxicological endpoints such as repeated dose toxicity, developmental and reproductive toxicity. This is presuming that a scientifically plausible hypothesis can be justified and used to derive a quantitative prediction for the targeted substances. Read-across is the most used adaptation to the standard information requirements in REACH and accounts for circa 23 % of all information requirements (all other adaptations: 14 %, experimental data: 31 %)[36].

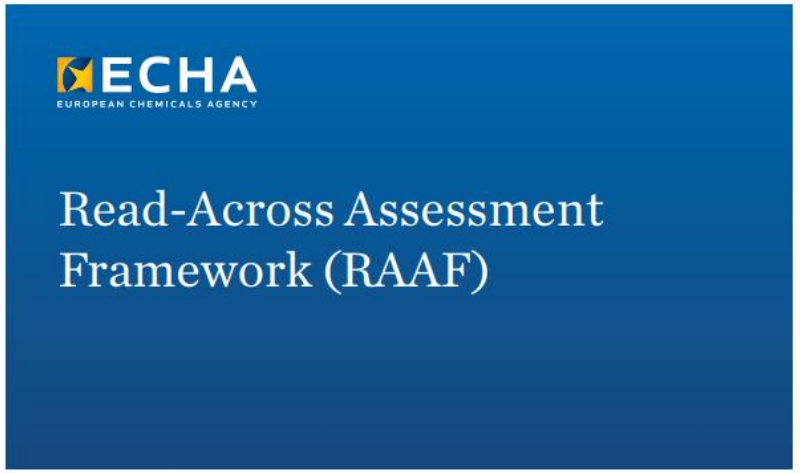
The read-across approach starts with identifying a structural/ physicochemical similarity between target (the substance for which one would like to better understand in hazard properties) and source (the substance for which information on a specific hazard property is available) substance, provided that similar structural characteristics lead to similar hazards. In addition, similarity should be demonstrated for the toxicokinetic and toxicodynamic properties of the target and source substance. Many read-across cases fail to demonstrate toxicokinetic and toxicodynamic similarities. Reasons for this include deficiencies in the quality of the source studies and lacking data to support predictions based on toxicokinetics. Also, there are often shortcomings in the hypothesis and justification of the toxicological prediction. And on top of that, the variation in the severity and type of the adverse outcome makes it often difficult to conclude on a "similar" toxicological hazard.

36 ECHAs summary report on alternatives to animal testing, 2023

Read-Across & Grouping

Read-across

- Structural similarity
- Prediction of an endpoint information for one substance (target substance), by using data from the same endpoint from (an)other substance(s), (source substance(s))
- Read-across hypothesis also needed



Many read-across cases fail to demonstrate similarities due to:

- **Deficiencies in the quality** of the source studies
- Lack of **data to support predictions**
- **Shortcomings** in the hypothesis and justification of the toxicological prediction and variation in the severity and type of the adverse outcome

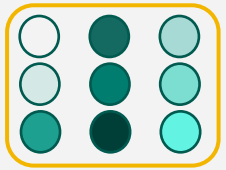
Read-Across & Grouping

Read-across

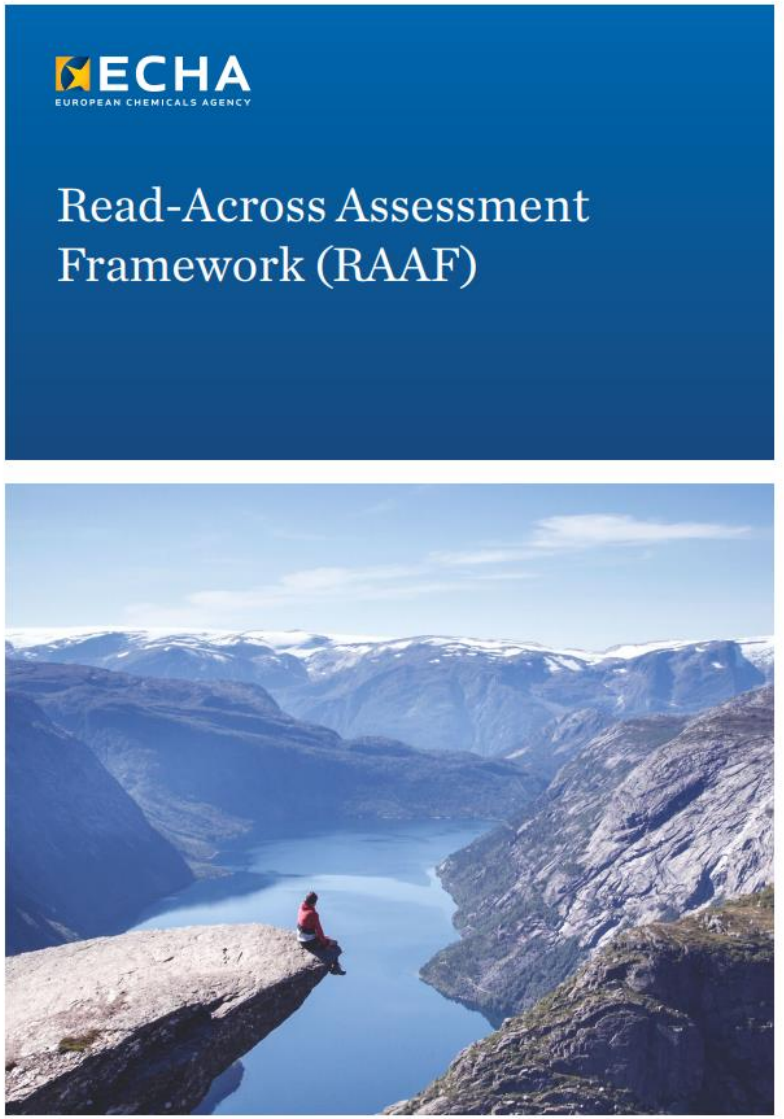
- Structural similarity
- Prediction of an endpoint information for one substance (target substance), by using data from the same endpoint from (an)other substance(s), (source substance(s))
- Read-across hypothesis also needed

versus

Grouping

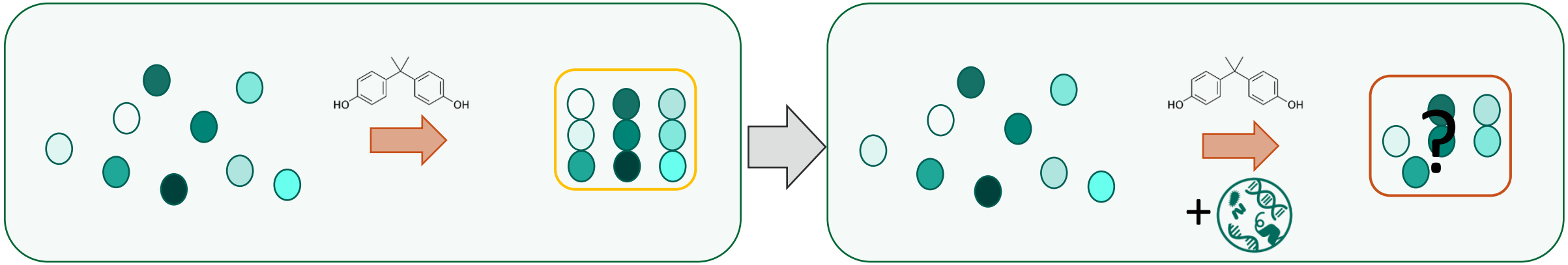


- Grouping of chemicals for prioritising chemicals for further regulatory action; mostly based on structural similarity



Read-Across & Grouping

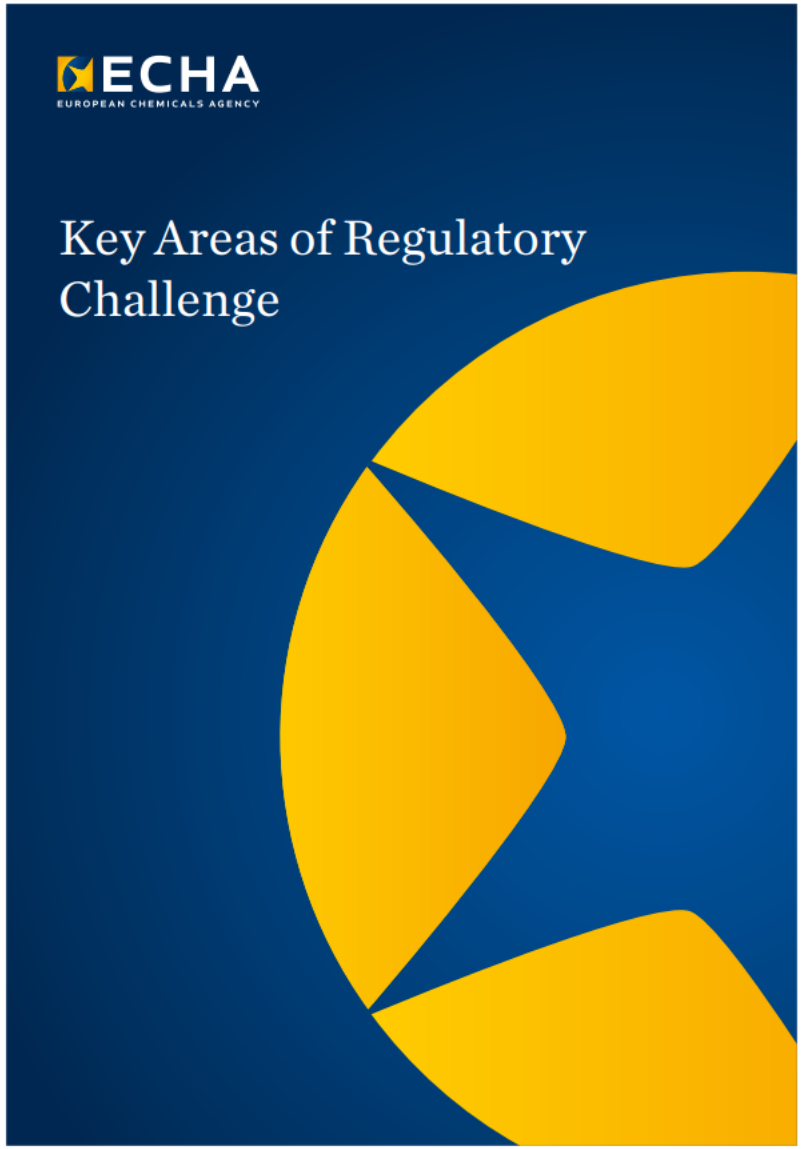
Integration of (transcript)omics data into read-across domain



Aim: To develop a workflow, easily applicable to partners to identify and explore groups of chemicals based on multiple different relevant sets of features including **molecular responses** (OMICs), **chemical structural information**, and other chemical properties

Case study is being performed with BPA alternatives (collaboration with ECHA)

KARC – Genotoxicity



2.3.5.2. Development of Adverse Outcome Pathways (AOPs) for specific modes of genotoxic or mutagenic action

Why the topic is relevant: Further research is needed to understand how different types of mutagenic substances act *in vivo* and identify the key steps leading to their genotoxic or mutagenic effects. This information could then be used to develop Adverse Outcome Pathways (AOPs) for specific modes of genotoxic or mutagenic action.

For instance, AOP 296 on "Oxidative DNA damage leading to chromosomal aberrations and mutations" has recently been developed by OECD and may be relevant to mutagenicity hazard assessment as indirect genotoxic effects caused by oxidative damage are assumed to be threshold effects, contrary to direct genotoxic effects. Therefore, safe levels of exposure could in principle be derived for substances causing indirect genotoxic effects after oxidative damage only, and specific risk management measures put in place. This AOP could be used to develop non-animal test methods specific for each of the AOP key events and possibly develop testing strategies or defined approaches under the OECD TG programme in the future.

Another potential AOP could be targeted at germ cell mutagenicity. Specifically, some research is needed to identify key factors or key events that determine whether a substance that is mutagenic/genotoxic in somatic cells *in vivo* will also be mutagenic/ genotoxic in germ cells. Further understanding of the key steps leading to germ cell mutagenicity *in vivo* would be valuable to develop non-animal test methods that could eventually replace animal testing and potentially lead to a revision of the GHS/CLP criteria.

Where it fits into the regulatory landscape: Although AOPs are not covered by the Mutual Acceptance of Data (MAD) principle, which allows the data generated under MAD to be accepted by authorities in any OECD member countries, they could be used to develop non-animal test methods specific for each of the AOP key events and possibly develop test guidelines, testing strategies or defined approaches under the OECD TG programme, which would be covered by MAD.

Short-term impact:

- further characterisation of the mode(s) of genotoxic or mutagenic action of a substance;
- better selection of the most appropriate *in vivo* follow-up test(s) based on the identified modes of genotoxic or mutagenic action.

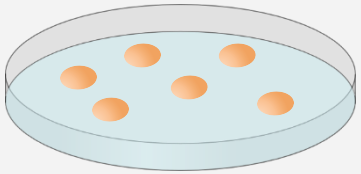
Long-term impact:

- development of non-animal test methods specific for each of the AOP key events;

Genotoxicity testing requirements under REACH

Always

Ames test

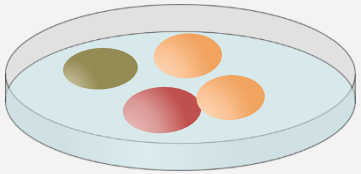


GENE MUTATIONS

≥ 10 tonnes/year

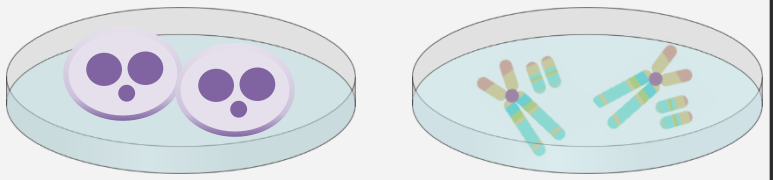
MLA/TK/HPRT

Only if other two tests are neg



GENE MUTATIONS

Micronucleus test *or* Chromosome aberration test



STRUCTURAL and/or NUMERICAL CHROMOSOME ABERRATIONS

In vivo comet assay

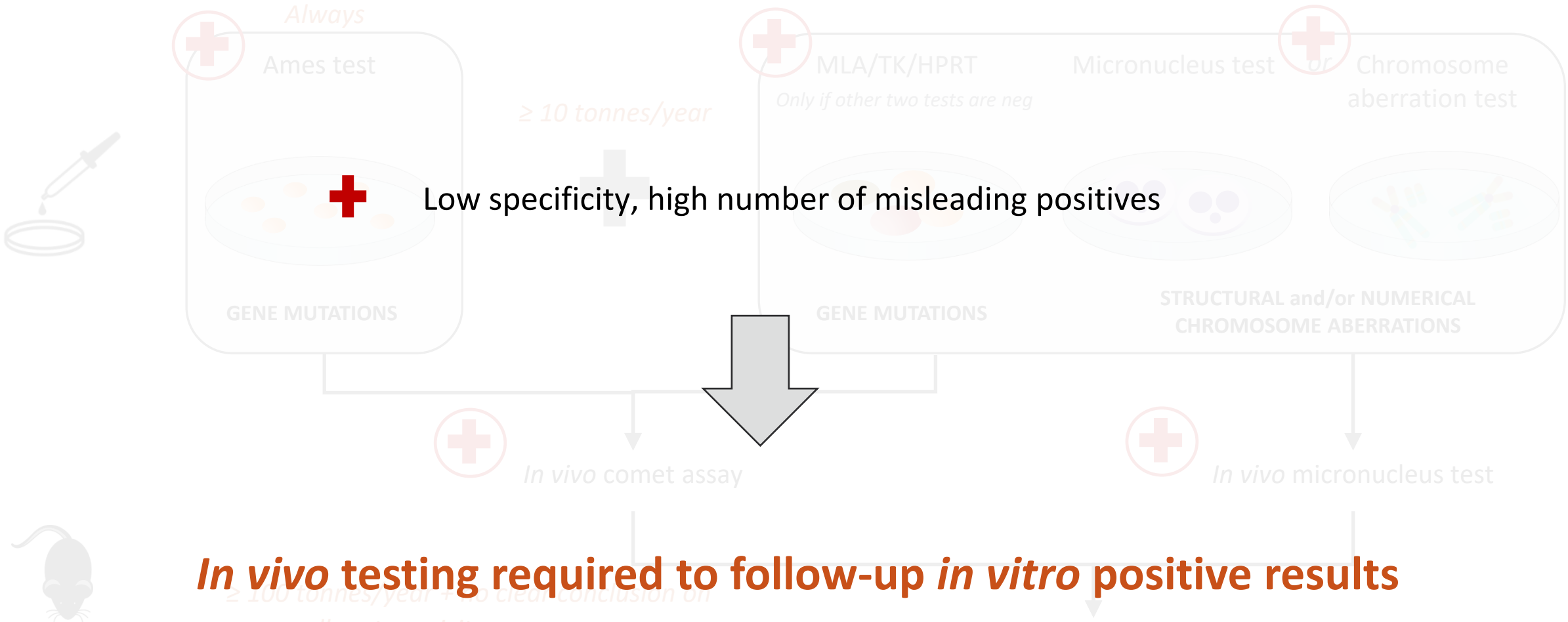
In vivo micronucleus test

≥ 100 tonnes/year + no clear conclusion on germ cell mutagenicity

In vivo gene mutation test in transgenic rodents
Mammalian spermatogonial chromosomal aberration test

MLA: Mouse Lymphoma Assay; TK: Thymidine Kinase; hprt: hypoxanthine-guanine phosphoribosyltransferase

Genotoxicity testing requirements under REACH



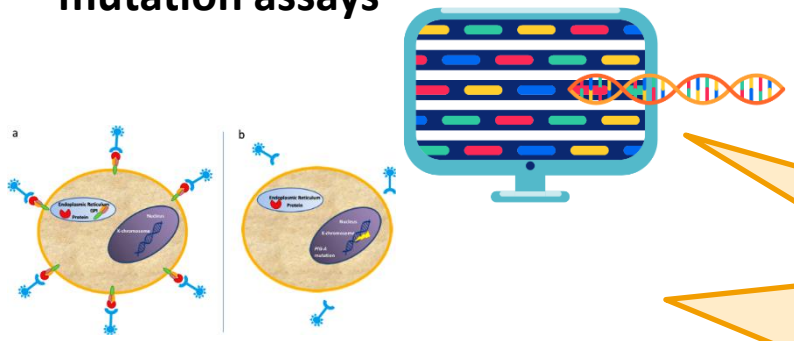
Low specificity, high number of misleading positives

***In vivo* testing required to follow-up *in vitro* positive results**

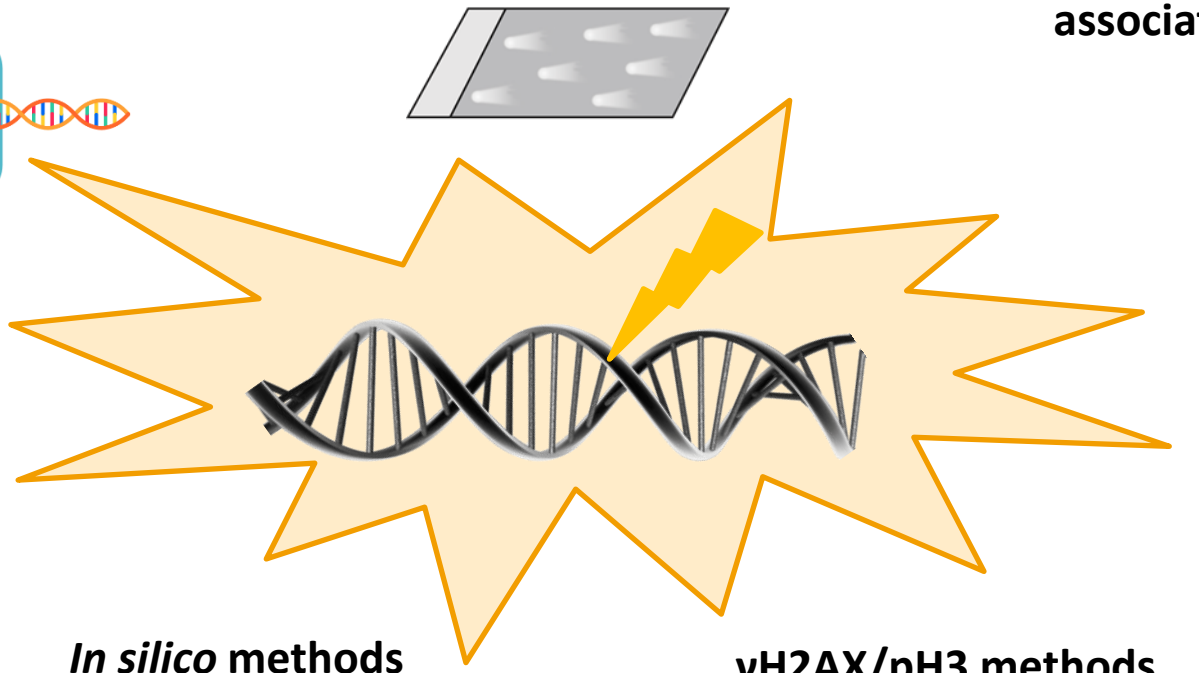
MLA: Mouse Lymphoma Assay; TK: Thymidine Kinase; hprt: hypoxanthine-guanine phosphoribosyltransferase

NAMs for Genotoxicity

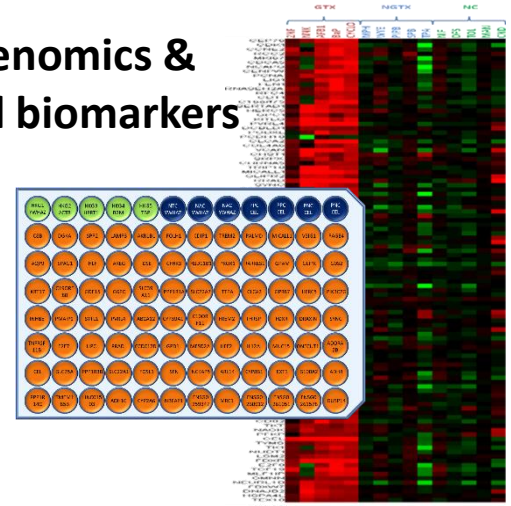
New mammalian gene mutation assays



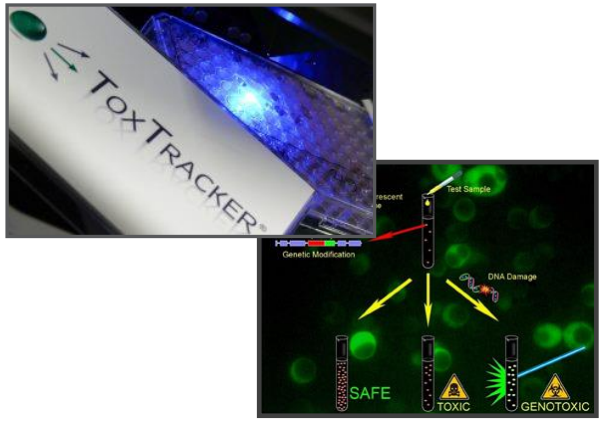
In vitro (modified) comet assay



Toxicogenomics & associated biomarkers

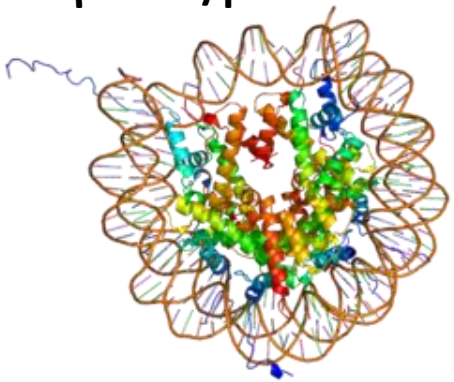


Reporter gene assays

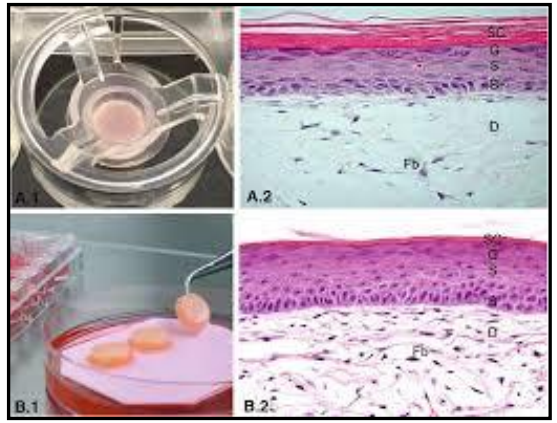


In silico methods

γH2AX/pH3 methods



Tests in 3D models

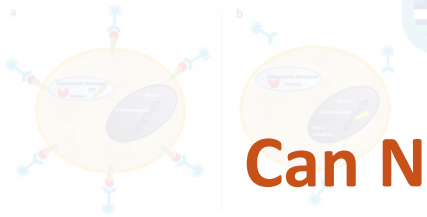


NAMs for Genotoxicity

New mammalian gene mutation assays

In vitro (modified) comet assay

Toxicogenomics & associated biomarkers



Can NAMs help to increase confidence in *in vitro* positive results?

Reporter gene assays

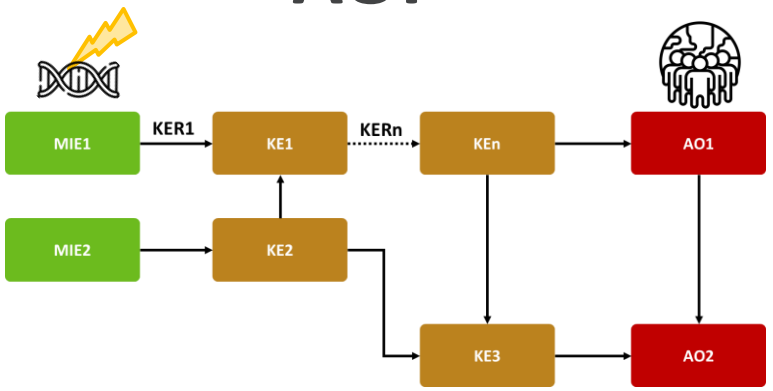
In silico methods

γH2AX/pH3 methods

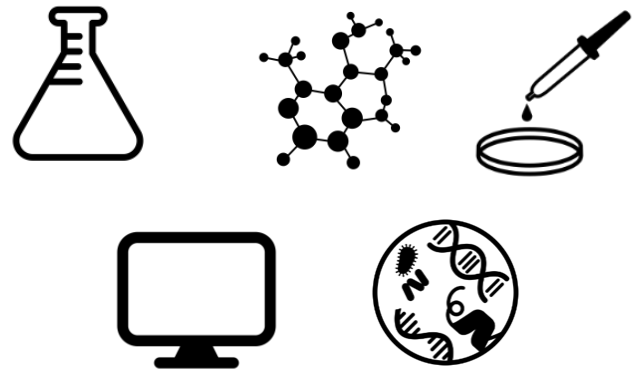
Tests in 3D models

IATA for Genotoxicity

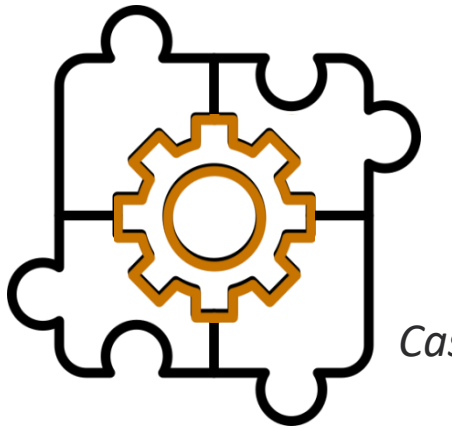
AOP



NAMs



IATA



Case study on classification (collaboration with ECHA)

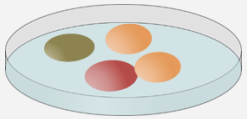
MIE: Molecular Initiating Event; KE(R): Key Event (Relationship); AO: Adverse Outcome; IATA: Integrated Approach to Testing and Assessment; NAMs: New Approach Methodologies

Methodology case study

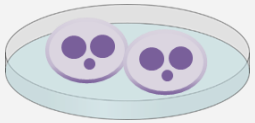
Harmonized classification status
'Traditional' genotoxicity data
Physicochemical properties
Commercial availability/price

Traditional tests

MLA/TK/HPRT



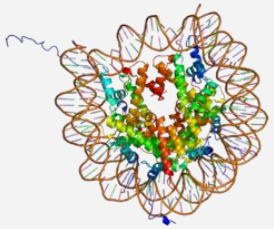
Micronucleus test



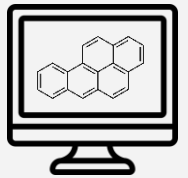
NAMs

Transcriptomic biomarkers

γH2AX/pH3 methods

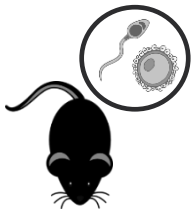
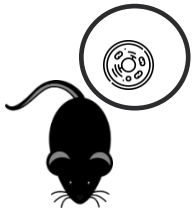
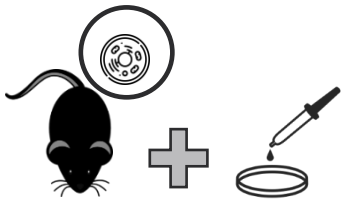


QSAR models



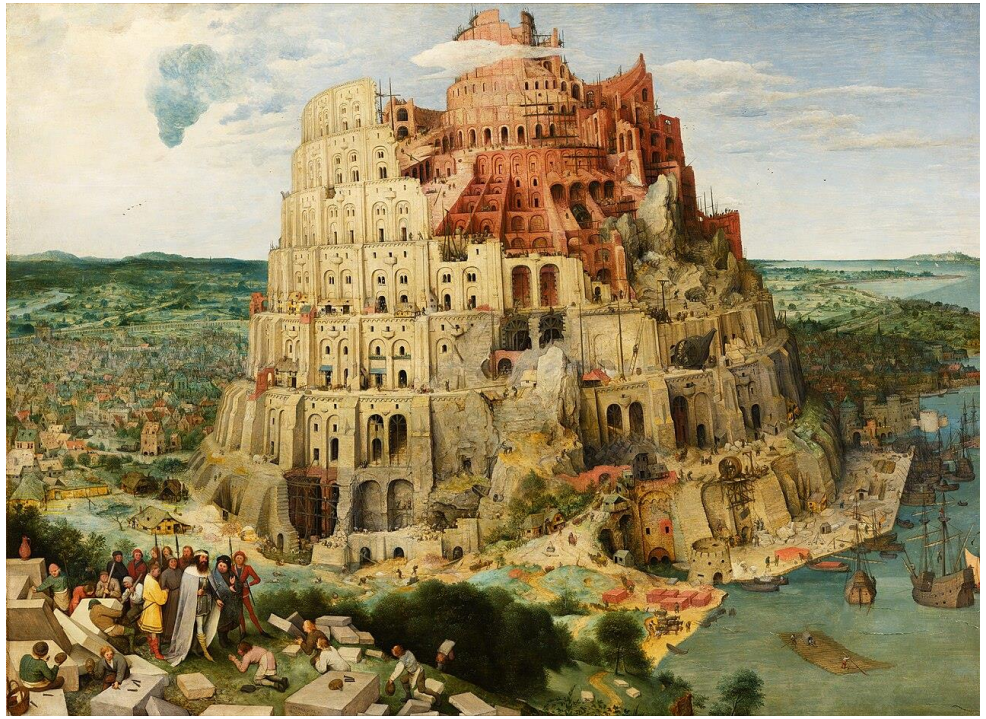
+ other assays (e.g. (modified) comet assay, MN/comet/γH2AX/pH3 test in 3D)

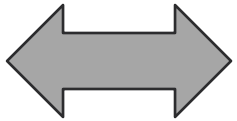
REACH and Classification & Labelling

Category 1 – *Substances **known to induce** heritable mutations **or to be regarded** as if they induce heritable mutations in the germ cells of humans*		**Category 2** – *Substances which cause concern for humans owing the possibility that the **may induce** heritable mutations in the germ cells of humans*
Category 1A	**Category 1B**	
Positive results from human epidemiological studies 	Positive results from *in vivo* heritable germ cell mutagenicity studies 	Positive *in vivo* somatic cell mutagenicity tests (mammals)  *OR* Other positive *in vivo* somatic cell genotoxicity tests supported by positive *in vitro* mutagenicity assays 

- Classification in **<u>Category 1 & 2</u>** for mutagenicity requires **<u>human or *in vivo* animal data</u>**

Need for collaboration!





Acknowledgements

Other PARC partners

- WU-TOX (NL), ISS (IT), IBTM (DE), UG-PL (PL), STAMI (NO), LIST (LU), BPI (GR), IRSN (FR), RIVM (NL), KWR (NL), NILU (NO), IRFM (IT), MUI (AT), NIPH (NO), WR (NL)

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