Internal Doses to STOT RE classification (ID2STOT)

STOT RE classification based on concentrations of bioactivity referenced by internal dose.

Team: **ID2STOT**
Anastasia Weyrich, Varun Giri, Susanne Kolle, Karin Wiench, Eric Fabian, Robert Landsiedel
BASF SE

We create chemistry

Idea

External dose limits for the classification of **STOT RE** → Translation → Substance-specific corresponding internal dose limits (ID_{limit}) → Usage → Qualification of the concentration of bioactivity (**CoB**) in a set of defined **NAMs**

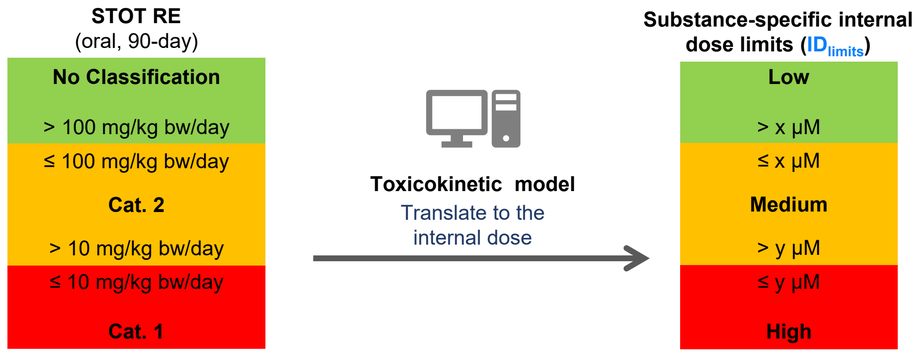
The approach is using the STOT RE classification system, which is **an existing and globally recognized regulatory classification system**.
This is expected to support the transition from the existing classification systems and regulatory acceptance of classifications based on the results of NAM testing.
The categories of the STOT RE classification system (1, 2 and no classification) are transferred directly into the levels of concern (high, medium and low).
The approach enables determining if the activity(ies) observed with NAMs are linked to a toxicological endpoint at a **dose which is relevant for classification under the current CLP**.
In contrast to the external dose limits for the STOT RE classification the ID_{limits} **are substance-specific** due to differences in toxicokinetic.
In contrast to the prescribed schema of the EPAA Designathon, the ID2STOT approach **does not conduct two independent assessments of bioactivity and potential systemic availability.**
Instead, both, bioactivity and bioavailability, are merged into one parameter, the ID_{limit}. These reflect the systemic availability of the substance and the bioactivity.
The resulting ID_{limits}, are directly used to assess the relevance for classification of the bioactivity concentrations obtained from NAMs.

Step 1a Extrapolation of *in vivo* to *in vitro* dose (IVIVE)

Toxicokinetic models
- Models of varying complexity available: static model → simple kinetic models → multi-component PBTK models
- Substance-specific input parameters: physiochemical parameters, intrinsic hepatic clearance (experimental), plasma protein binding and permeability through Caco-2 cells (Papp) (experimental or in silico), etc.
- Physiological input parameters

Key questions on complexity and possible modifications
- Which exposure scenario should be addressed?
- What are the defined toxicological targets and are these sufficiently well described in the model used?
- Does the substance undergo metabolism?
- Human vs rat models?
- How complex does the model need to be?

Result: Internal dose derived from GHS STOT RE class limits → ID_{limit}



Step 1b NAM based assessment of bioactivity

***in vitro* & *in silico* NAMs** associated with adverse outcomes relevant for systemic toxicity
Quantitative NAMs providing a concentration-response relationships → Concentration of Bioactivity (**CoB**)
NAMs that address the same endpoint are each organized into **endpoint groups**
- **Ideal:** clearly defined set of endpoints (specific and unspecific systemic toxicity endpoints described by AOPs), which are fully describing toxicity potential of a substance and are fully covered by NAMs
- **Reality:** often mechanisms are unknown / are known but are not fully covered by NAMs
- **2 solution approaches:**
  - Screening of known NAMs for their association with systemic toxicity
  - Development of NAMs for specific AOPs

Determination of the overall CoB per endpoint group: Most sensitive response or weighted/modelled response or mechanism-driven approach?

Endpoint-group 1	
***In vitro/silico* NAM assay**	**CoB [µM]**
Assay 1.1	67.25
Assay 1.2	100.54
Assay 1.3	50.43
…	…
Overall	50.43

Endpoint-group 2	
***In vitro/silico* NAM assay**	**CoB [µM]**
Assay 2.1	657.89
Assay 2.2	305.67
Assay 2.3	407.23
…	…
Overall	534.24

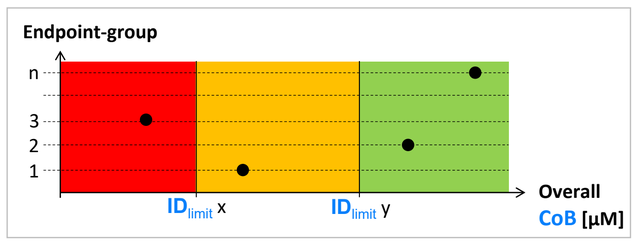
Endpoint-group 3	
***In vitro/silico* NAM assay**	**CoB [µM]**
Assay 3.1	34.54
Assay 3.2	4.67
Assay 3.3	6.73
…	…
Overall	6.89

Endpoint-group n	
***In vitro/silico* NAM assay**	**CoB [µM]**
Assay n.1	235.63
Assay n.2	437.98
Assay n.3	56.98
…	…
Overall	300.56

Step 2 Assessment of the bioactivity CoBs in reference to ID_{limits}

Comparison of the bioactivity determined for the endpoint groups from Step 1b with the ID_{limits} defined based on the STOT RE limits in Step 1a to assess the level of toxicological concern

Overall **CoBs** are used to **assign each endpoint group to one of the three levels of concern**



Step 3 Substance classification

Combination of the levels of concern determined in Step 2 per endpoint group into an overall assessment, considering the **relevance of the bioactivities present for the overall systemic toxicity**

Lowest concentration of bioactivity is not per se the most relevant one!

Different types of toxicity:
- **Unspecific**: substances is active in many NAMs (usually at rather high concentrations)
- **Specific**: one or few specific bioactivities (usually at low concentrations)

Uncertainty

Each component of the concept contributes its own uncertainty.

Uncertainty (ID_{limits}) + Uncertainty (**CoB**) → Confidence of classification

Uncertainty (ID_{limits}) - PBPK-model:
- type of model, experimental and in silico determination of the parameters

Uncertainty (**CoB**) - *in vitro* assays
- quality of the assay, number of concentrations and replicates tested, distribution of replicates, noise, adaptation of the mathematical model used to determine the **CoB**, etc.

Uncertainty (**CoB**) - *in silico* assays
- applicability domain, nearest neighbours and model validation

Next steps & open questions

Iterative testing process based on a data-rich chemicals of the reference list
These limits were designed for animal testing, are they applicable to more human-relevant NAM-testing? How to account for species differences?
Is **CoB** directly (only by toxicokinetic translation) related to the adverse outcome / hazard?

How much data is enough? How sensitive is the classification because of inaccuracies in determining ID_{limits} and the missing data for bioactivity, **CoB**.

How customized should the model be to specific substances to obtain reliable ID_{limits}? How should metabolism be modelled in?

Is an adjustment of the nominal assay concentration necessary (towards actual concentrations vs. nominal concentrations)?

What is the most appropriate way of grouping endpoints, specifically when these are measured in completely different assays?
Are all relevant adverse outcomes covered with the existing NAMs?

Increase precision and confidence in toxicokinetic modelling for forward dosimetry

Define sets of NAMs critical for the STOT RE endpoints