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ANIMAL-FREE SAFETY ASSESSMENT COLLABORATION

Integrating in vitro data to establish a Margin of Exposure

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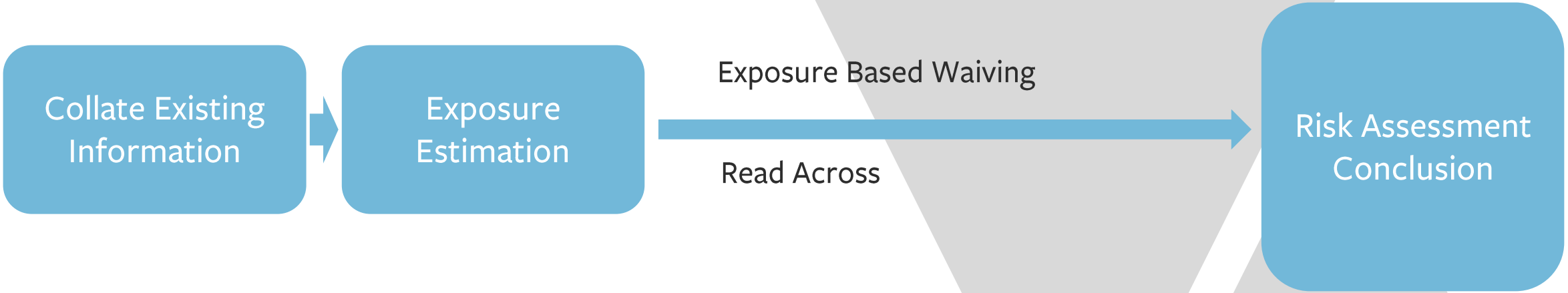
UNILEVER

WORLD CONGRESS ON ALTERNATIVES AND ANIMAL USE IN THE LIFE SCIENCES ¦ 27 AUGUST 2021

Risk assessment decision-making where data are available



Safety decisions can be made at this stage:

- In cases where the applied dose exposure estimates show that the consumer is only exposed to negligible amounts of the compound of interest. i.e. they are below the threshold of toxicological concern.
- For compounds where there are relevant toxicological data available it is possible to conclude at this stage or build a robust read across case based on literature evidence.

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Risk assessment decision-making where data needs to be generated

*NGRA is defined as **an exposure-led, hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure **safety without the use of animal testing***

Dent et al 2018. Computational Toxicology Volume 7, August 2018, Pages 20-26

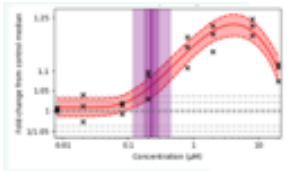
ICCR International Cooperation on Cosmetics Regulation

Collate Existing Information

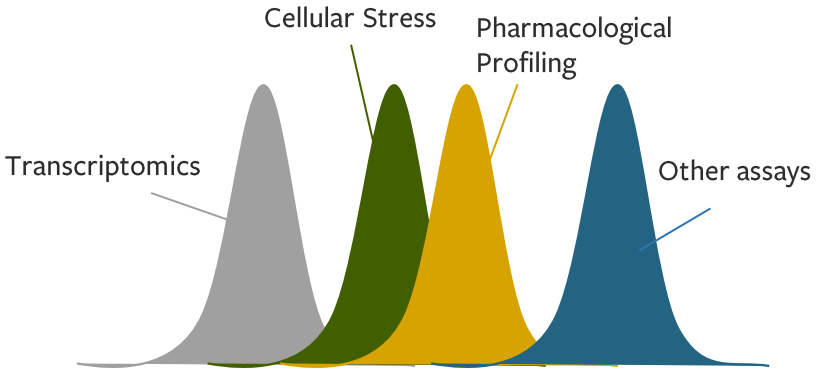
- Literature searching
- In Silico Screening

Exposure Estimation

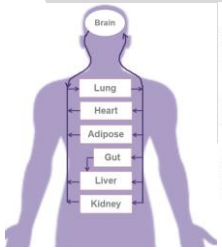
Biological activity characterisation

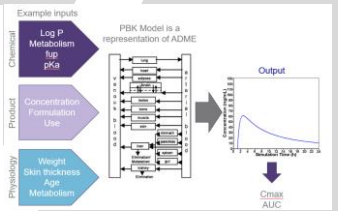


Concentration response modelling to obtain Points of Departure **(PoD)** from the data.

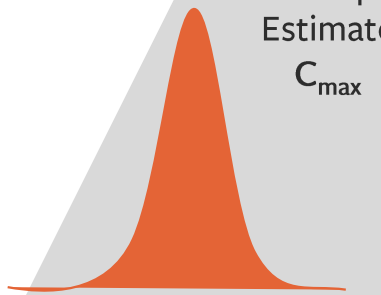


Exposure Refinement



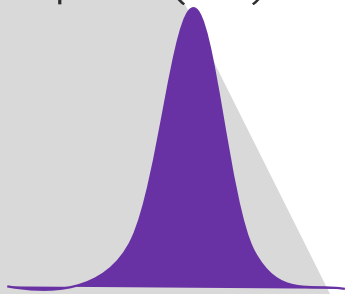


Internal Exposure Estimate C_{max}



Risk Assessment Conclusion

Calculation of the Margin of Exposure (MoE)



The MoE is defined as the ratio of the PoD and the relevant C_{max} estimate

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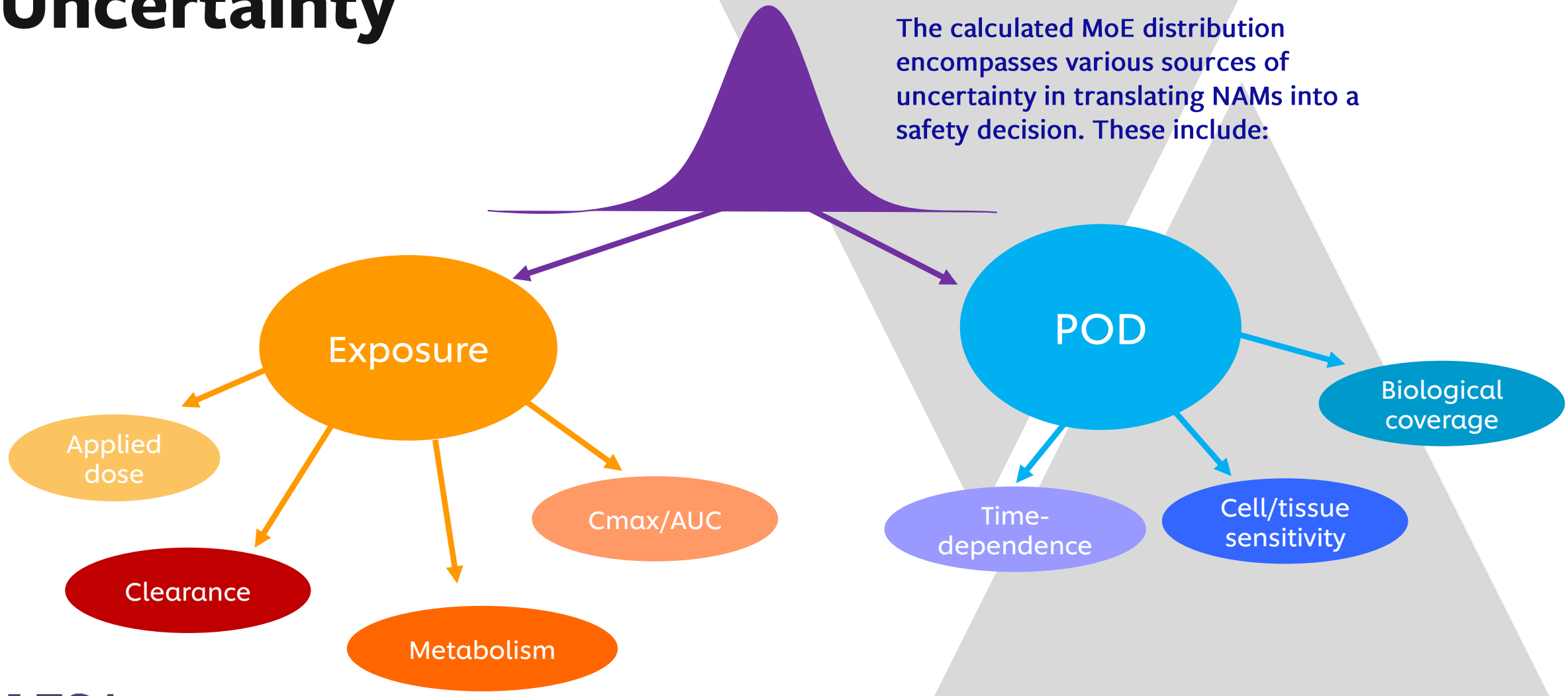
NGRA: Characterising Uncertainty

The calculated MoE distribution encompasses various sources of uncertainty in translating NAMs into a safety decision. These include:



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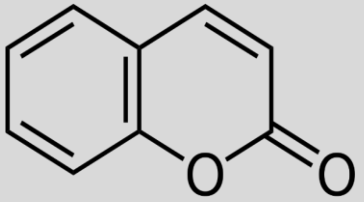
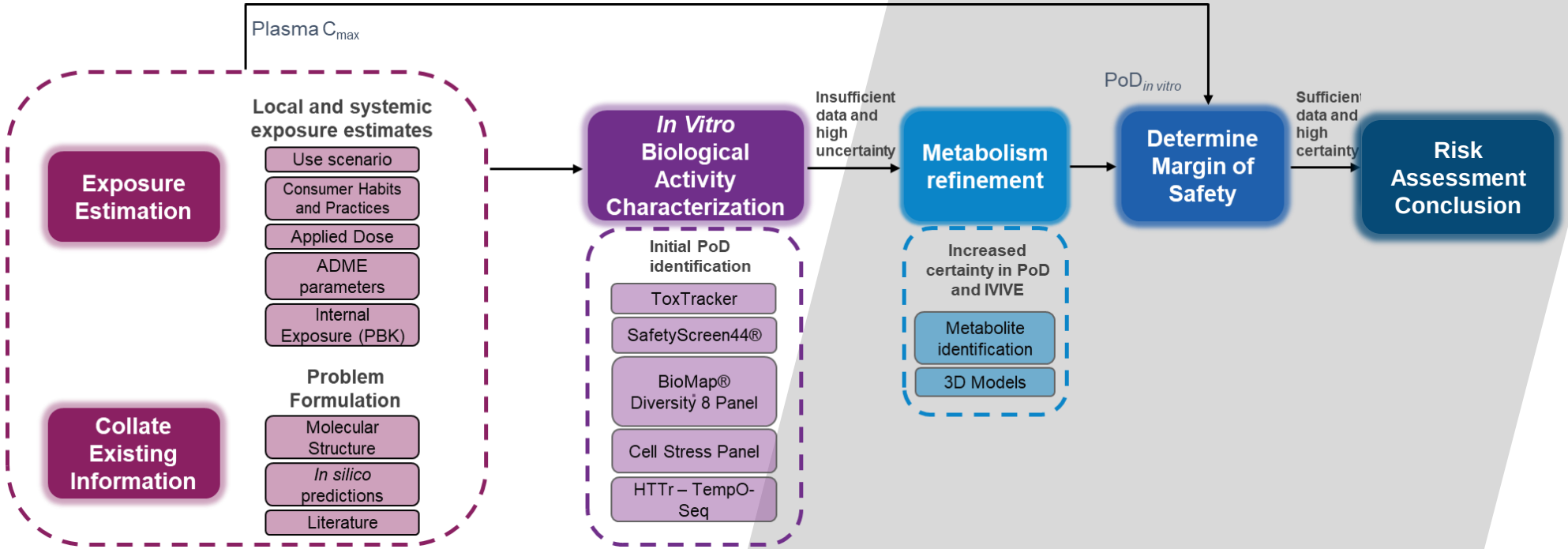
A case study approach – human health safety assessment required for…

0.1% COUMARIN IN FACE CREAM (NEW FRAGRANCE)





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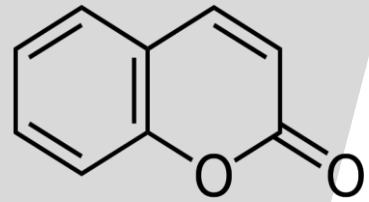
Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252



Plasma C_{max}

Exposure Estimation

- Plasma Cmax obtained (range 0.002- 0.02 µM) from PBK models
- Stability assays indicated coumarin rapidly metabolized mainly via CYP2A6

Collate Existing Information

- Gentox alerts for parent
- Reactive metabolites predicted.
- 90-100% coumarin predicted to be freely available *in vitro*
- Low bioactivity in ToxCast and Pubchem: binding to Carbonic Anhydrases and MAO-A/B reported

***In Vitro* Biological Activity Characterization**

Initial PoD identification

- ToxTracker
- SafetyScreen44 ®
- BioMap® Diversity 8 Panel
- Cell Stress Panel
- HTTr – TempO-Seq

Insufficient data and high uncertainty

Metabolism refinement

Increased certainty in PoD and IVIVE

- Metabolite identification
- 3D Models

$PoD_{in\ vitro}$

Determine Margin of Safety

Sufficient data and high certainty

Risk Assessment Conclusion

Low risk conclusion based on the margin of safety calculations.

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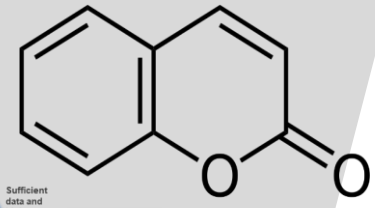

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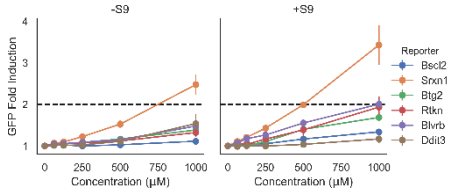
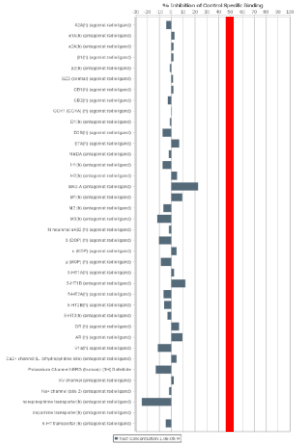
ToxTracker



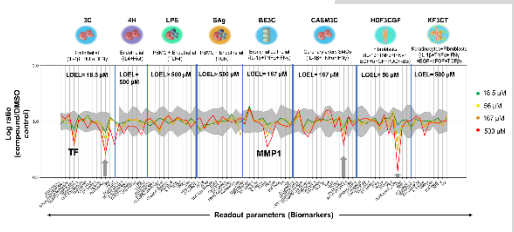
Coumarin was negative in ToxTracker, but reactive metabolite(s) could induce DNA lesions secondary to oxidative stress

SafetyScreen44 ®



All binding and enzymatic assays were negative at the screening concentration of 10 µM.

BioMap® Diversity 8 Panel

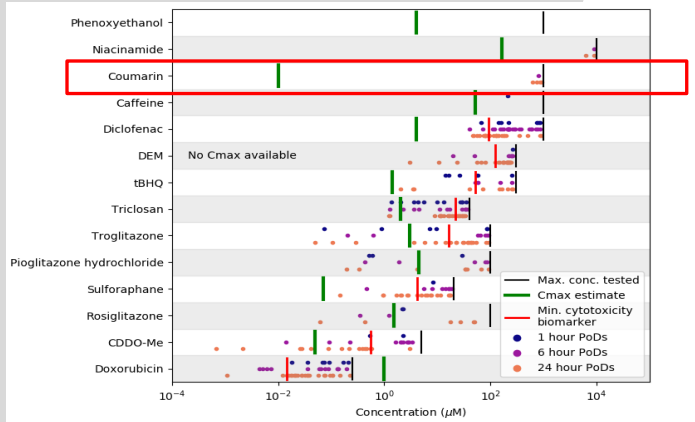


Data suggested that coumarin has no immunomodulatory effects at relevant concentrations and is not an anti-inflammatory compound.

HTTr – TempO-Seq

Concentration response analysis was performed on the results and multiple PoDs at both gene and pathway level were derived using several published methods.

Cell Stress Panel



Concentration response analysis of coumarin showed low bioactivity in the cell stress panel.

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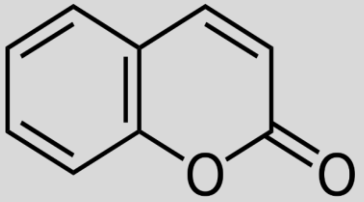

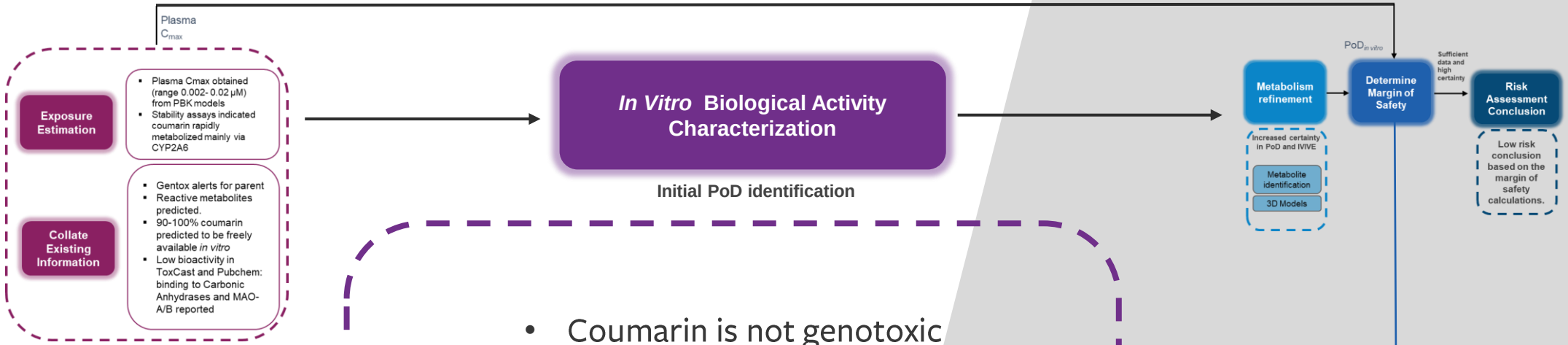

- Coumarin is not genotoxic
- No immunomodulation potential at relevant exposures
- Low bioactivity
- PoD range: 31-912 µM

Preliminary MoE distribution determined as: 706 - 96738

However, due to the prediction of reactive metabolites and the lack of metabolic competency in the cell lines used, the confidence in this MoE distribution was low.

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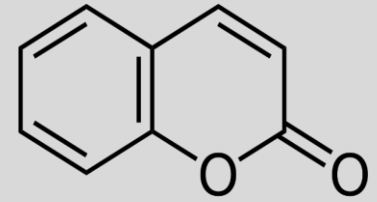

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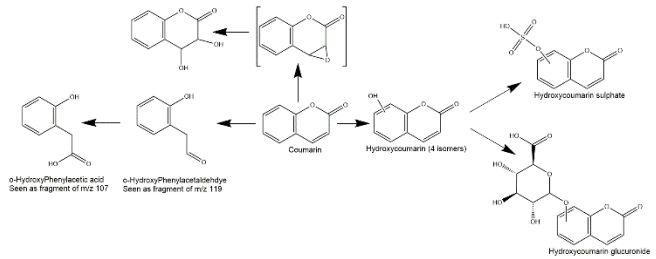
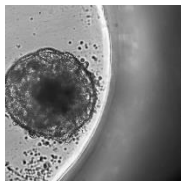
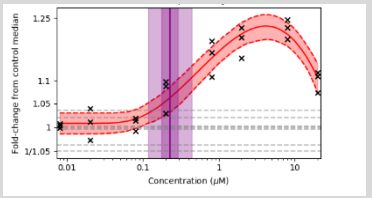


Coumarin is preferentially detoxified to hydroxycoumarin

3D Models

- Low bioactivity also found in a metabolically competent cell model (HepaRG 3D) in the Cell Stress and HTTr assays
- PoDs range: 41-871 µM – comparable to the 2D cells.





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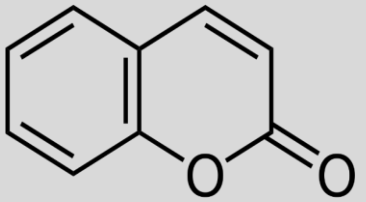
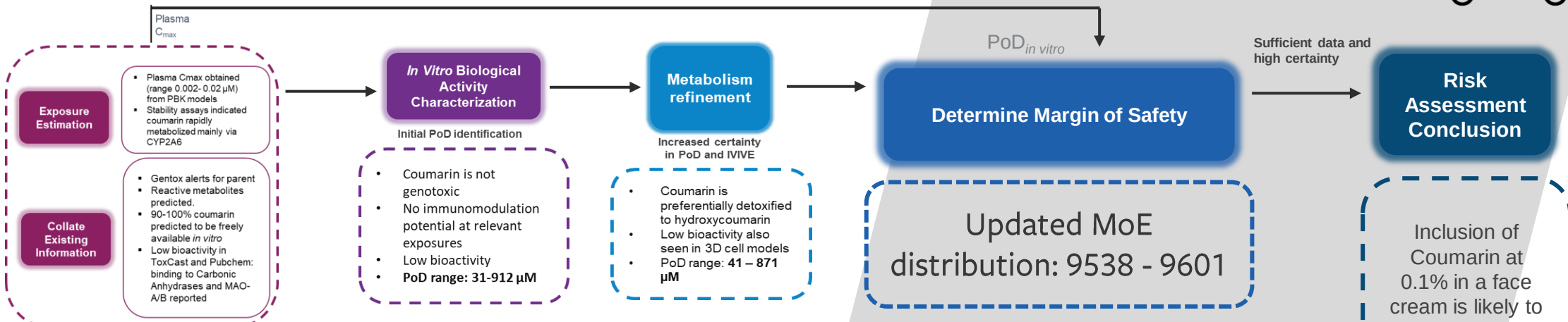
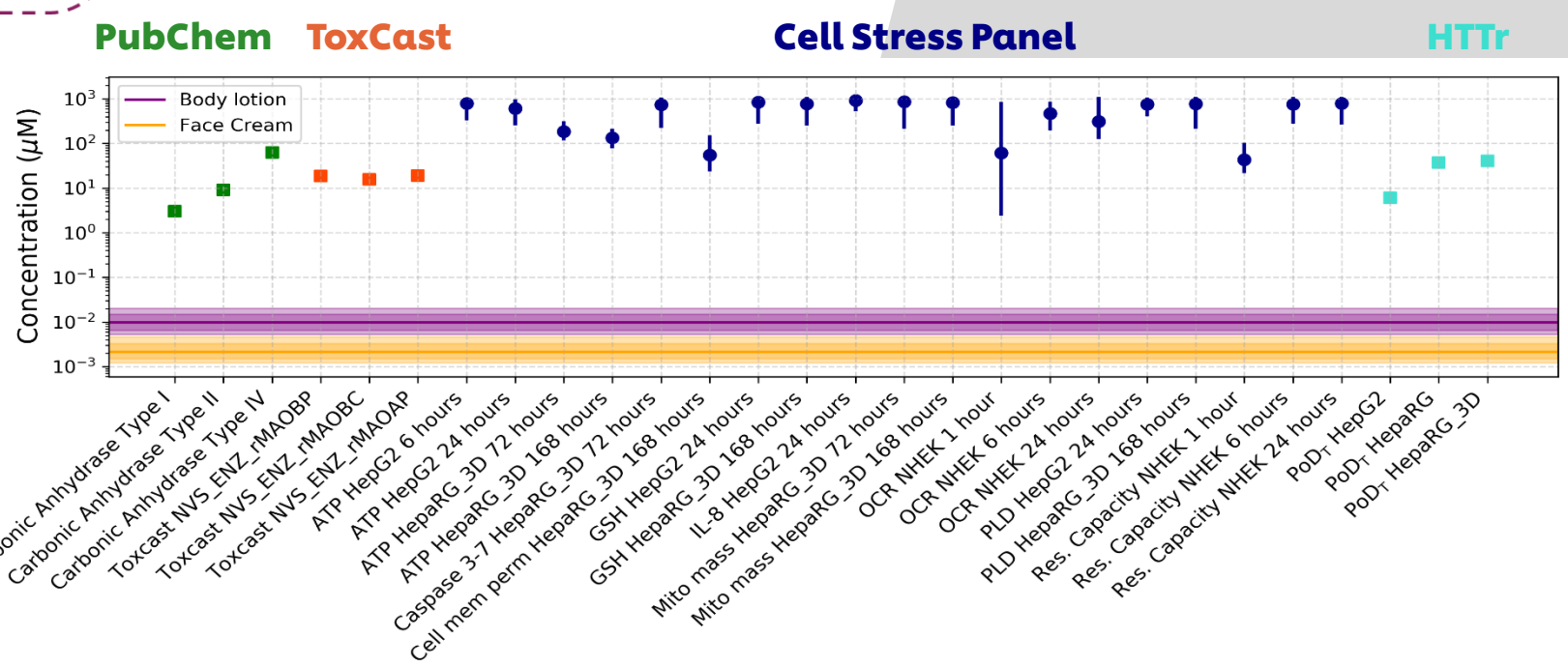
Using a weight of evidence to make a safety decision







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A NEXT GENERATION RISK ASSESSMENT CASE STUDY FOR COUMARIN IN HYPOTHETICAL COSMETIC PRODUCTS

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Concluding remarks:

- The types of data that will need to be generated will be specific to the risk assessment questions being asked.

- The approach taken must follow the principles of NGRA and be:
  - Exposure-Led
  - Hypothesis Driven
  - Human Relevant
  - Designed to Prevent Harm

- Determination of a margin of exposure can be done by comparing the internal consumer exposure estimates with *in vitro* points of departure and factoring in sources of uncertainty.

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Thank You!

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