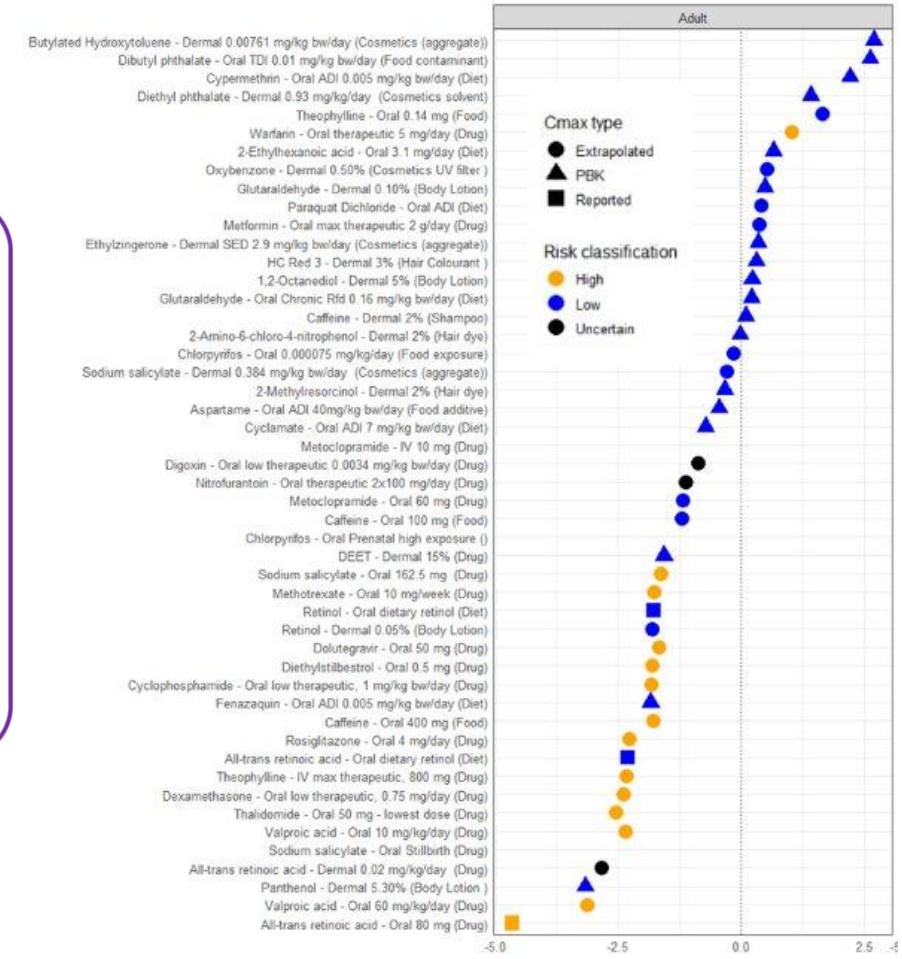
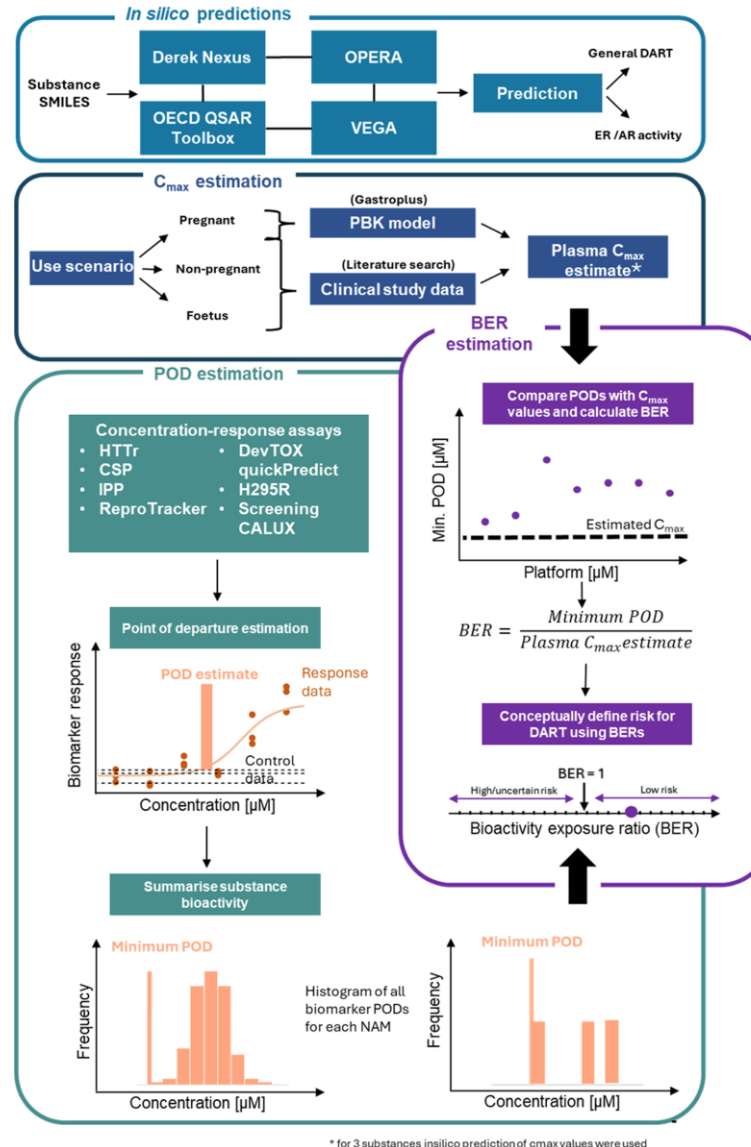
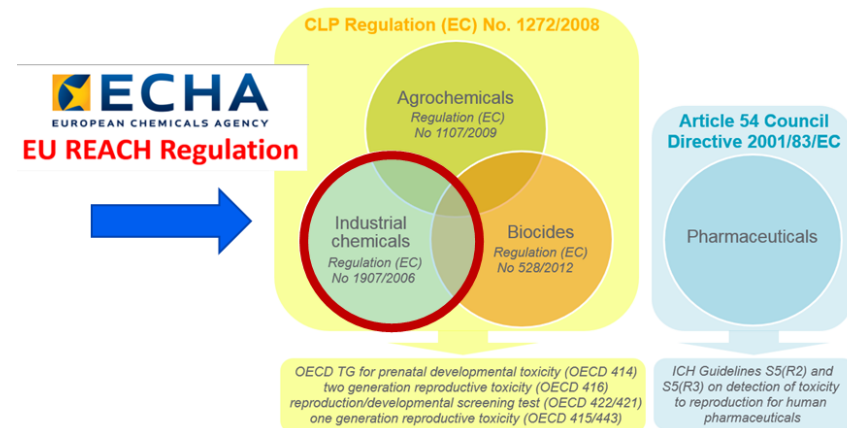


Dose response modelling and transferability of ReproTracker – an industry perspective



A next generation approach for DART risk assessment

Unilever



Evaluating ReproTracker in an NGRA framework

BMD
Express2
Build 3

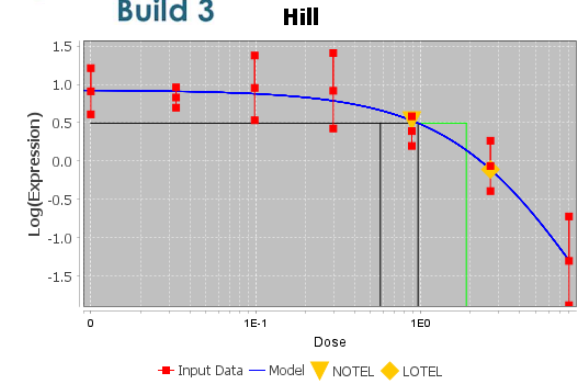
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1. Evaluate ReproTracker suitability for integration into NGRA
2. Assess the impact of dose response modelling choices on points of departure
3. Assess biological relevance and characterise protectiveness of DART risk



Animal-free developmental toxicity predictions

QC and
normalisation



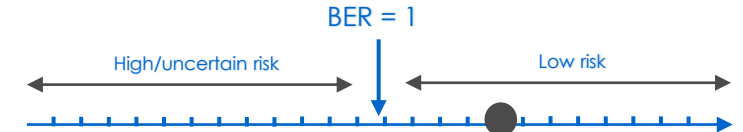
66 compounds
78 exposure scenarios

Calculate exposure scenario
Bioactivity exposure ratios (BER)

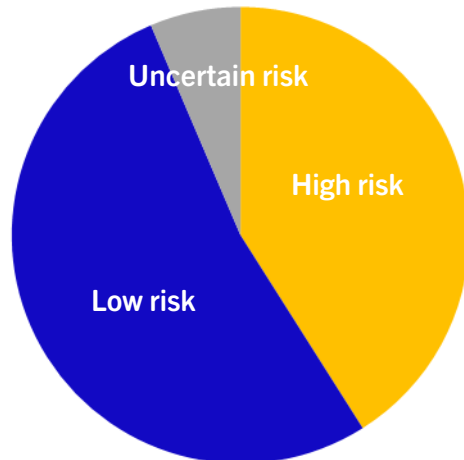
$$BER = \frac{\text{Minimum } POD}{\text{Plasma } C_{max} \text{ estimate}}$$

Bioactivity exposure ratio (BER)

Conceptually define risk for
DART using BERs



Risk classifications based on
what would be **traditionality**
considered **high or low risk**
based on **animal or human**
toxicology studies



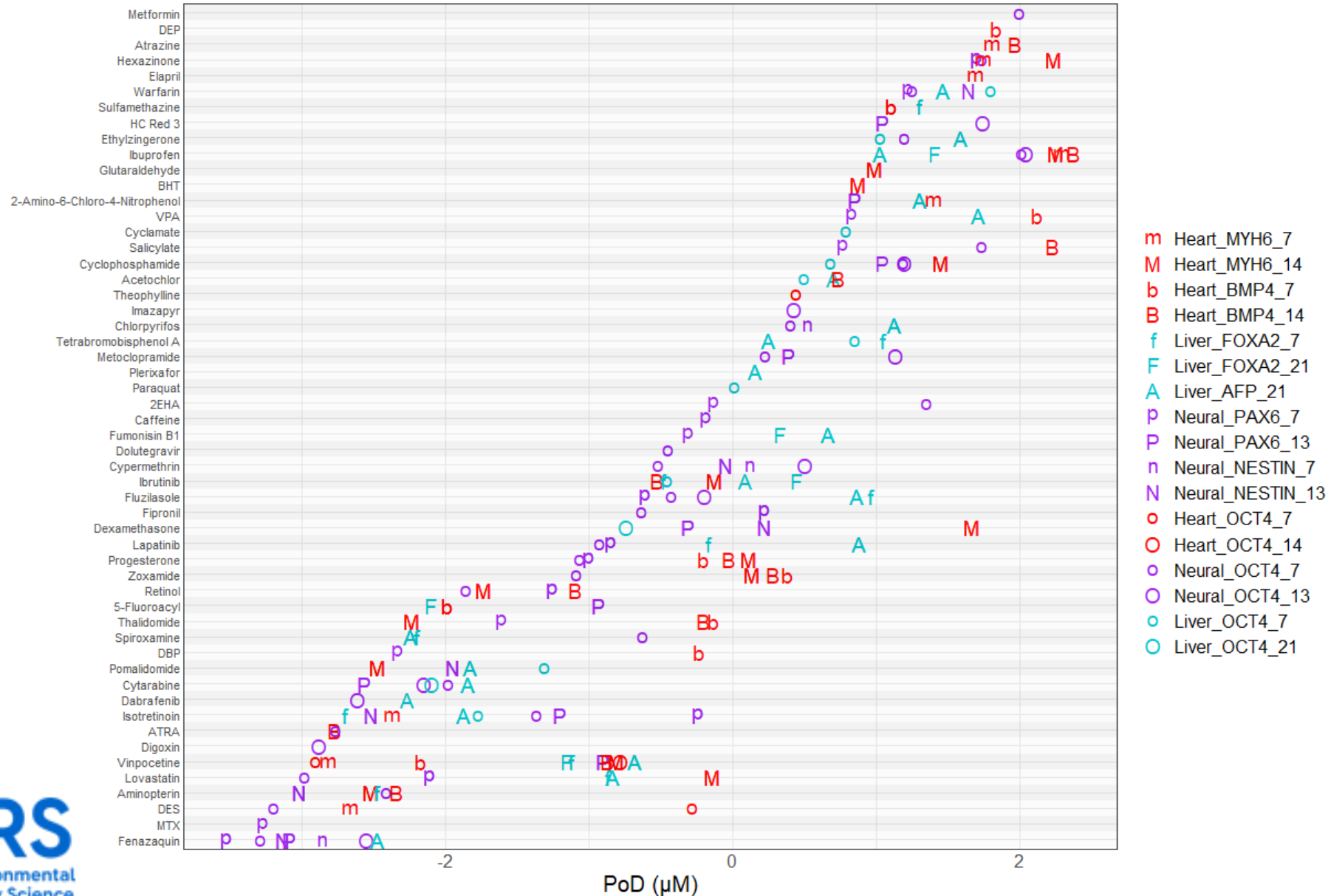
Internal exposure – clinical & PBK
estimates

Determining ReproTracker Bioactivity

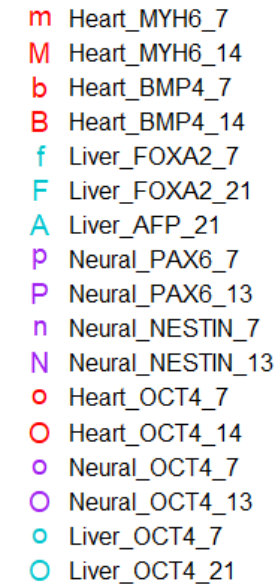
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Toxys Classification applies a weight of evidence approach considering the down regulation of:

- **BMP4 D7**
- **MYH6 D14**
- **FOXA2 D7**
- **AFP D21**
- **PAX6 D7 & D13**
- **NESTIN D13**



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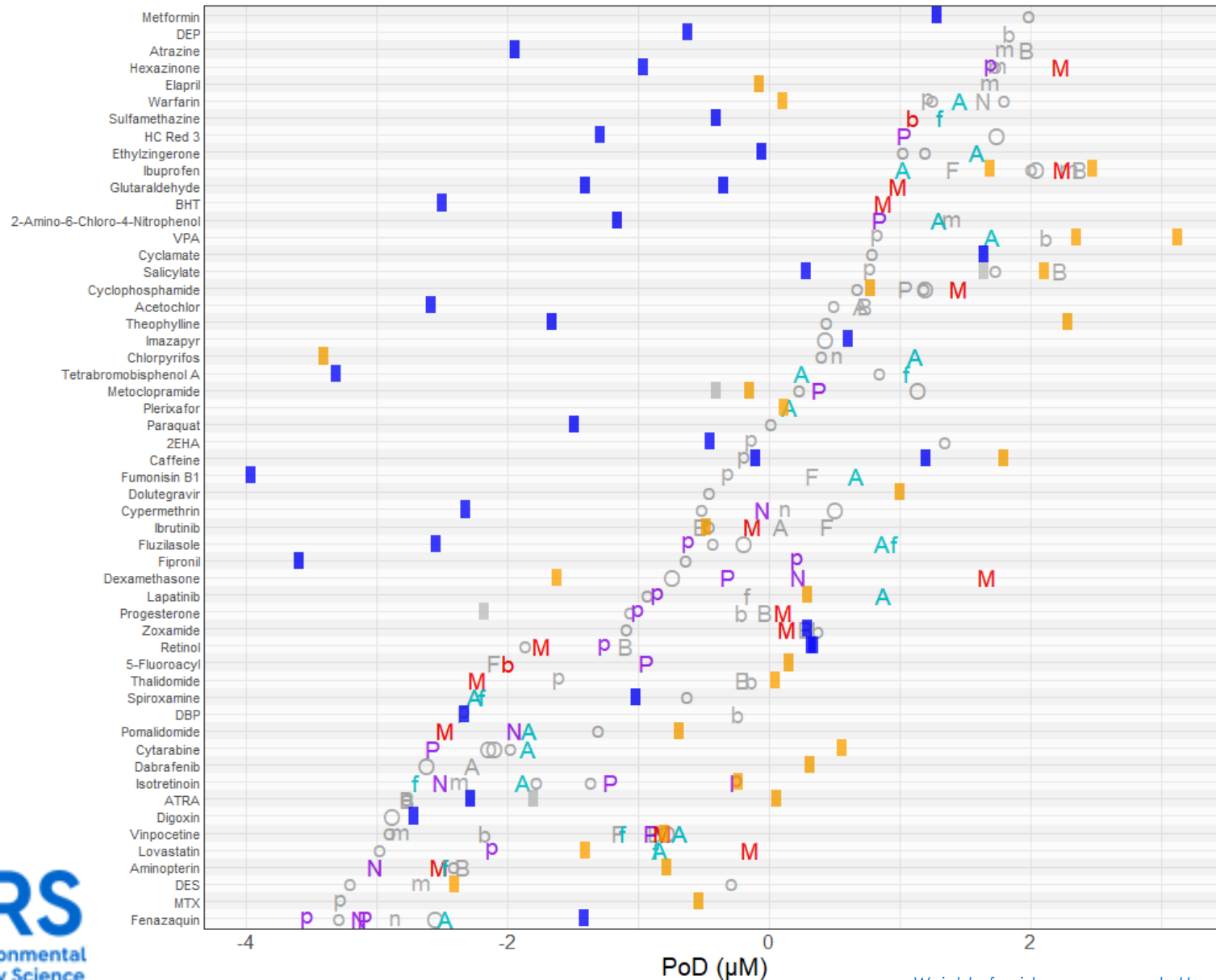
- **BMP4 D7**
- **MYH6 D14**
- **FOXA2 D7**
- **AFP D21**
- **PAX6 D7 & D13**
- **NESTIN D13**

All biomarkers · up & down ·
every timepoint

Key biomarkers-timepoints only · down-regulation only

Determining ReproTracker Bioactivity

Unilever



Toxys Classification applies a weight of evidence approach considering the down regulation of:

- **BMP4 D7**
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- **PAX6 D7 & D13**
- **NESTIN D13**

LOOSE criteria - wide net

All biomarkers · up & down · every timepoint

**STRICT criteria –
targeted for dev tox
hazard**

Key biomarkers-timepoints only · down-regulation only

Determining ReproTracker Bioactivity

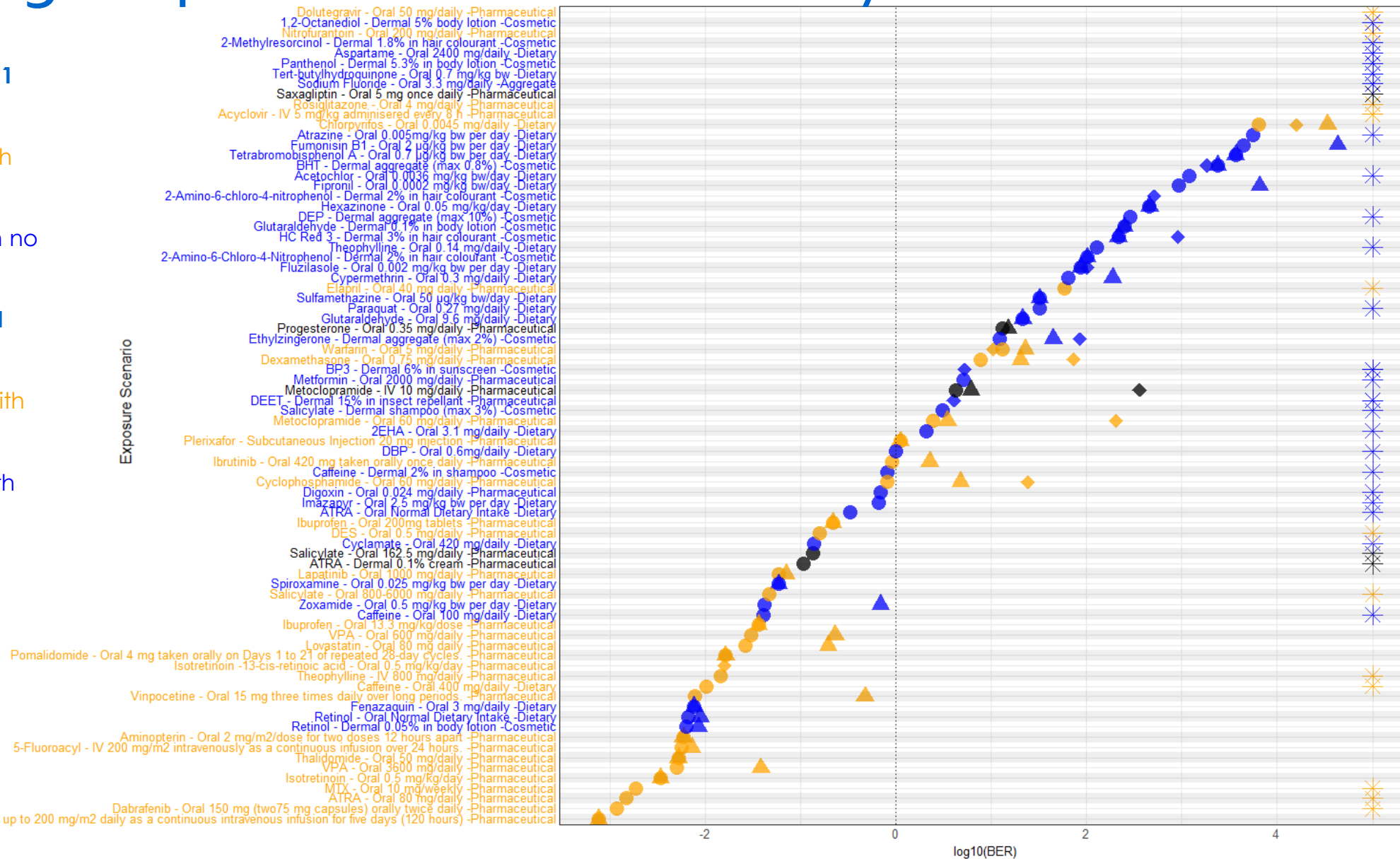
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BMD-loose - With BER threshold = 1

- 22/32 high risk protectiveness (BER<1)
 - 4 high risk scenarios with no pods
- 21/41 low risk utility (BER>1)
 - 8 low risk scenarios with no pods

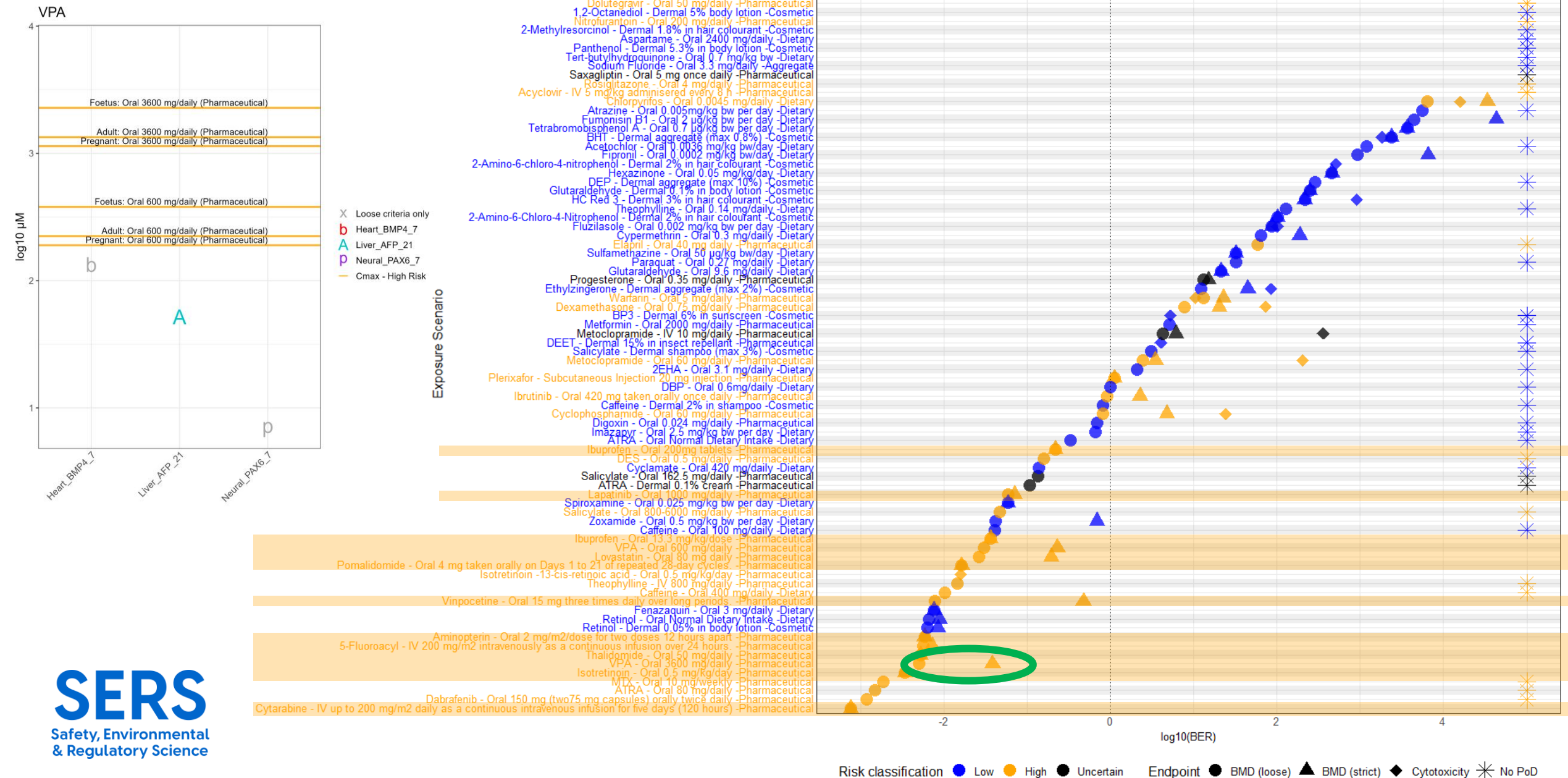
BMD-strict - With BER threshold = 1

- 13/32 high risk protectiveness (BER<1)
 - 12 high risk scenarios with no pods
- 13/41 low risk utility (BER>1)
 - 18 low risk scenarios with no pods



Protected High Risk Scenarios

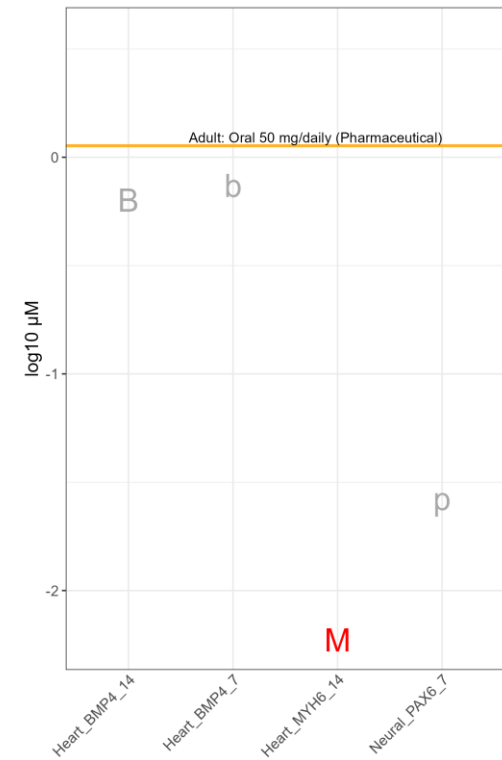
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Protected High Risk Scenarios

Unilever

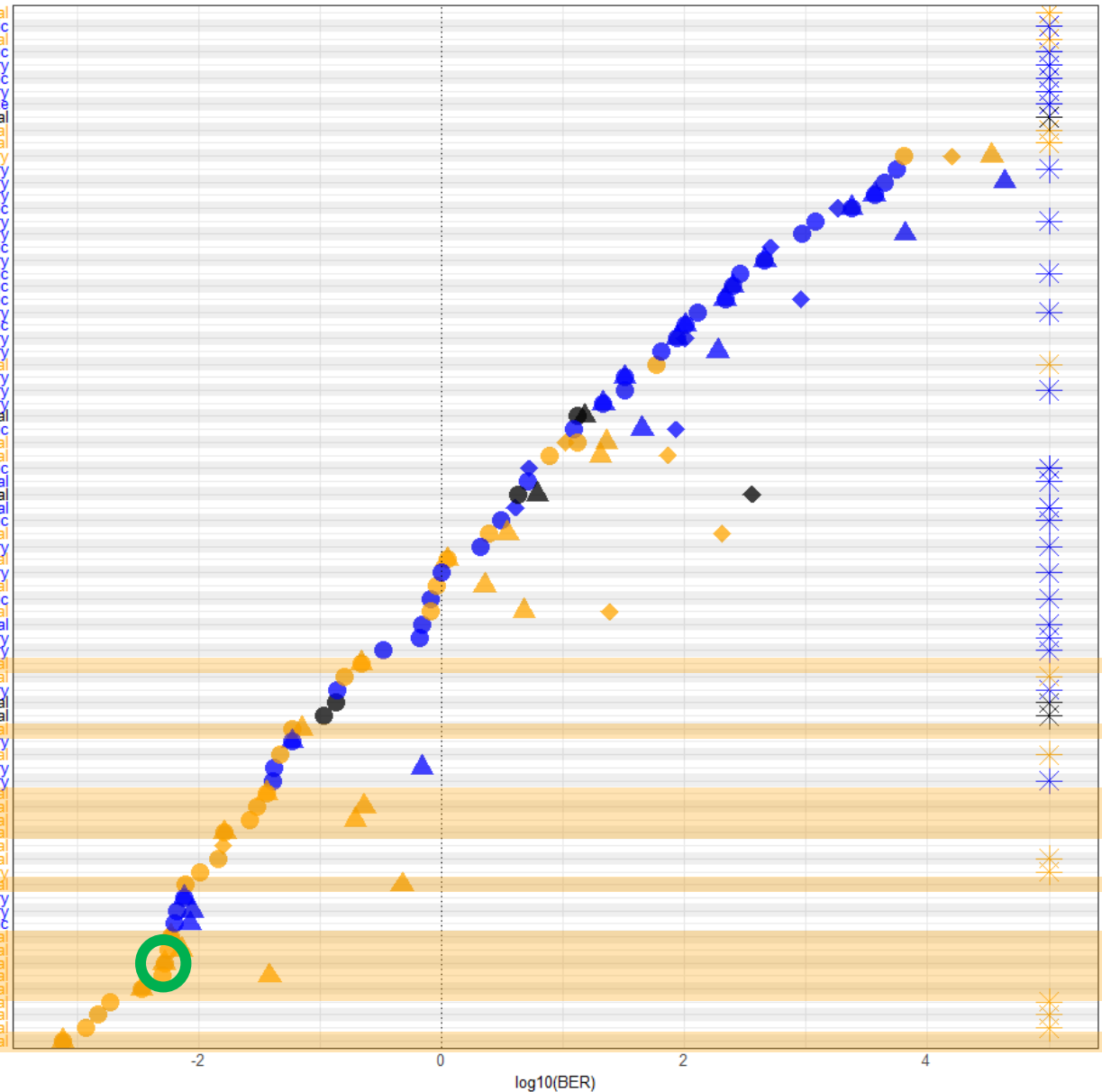
Thalidomide



× Loose criteria only
M Heart_MYP6_14
b Heart_BMP4_7
B Heart_BMP4_14
p Neural_PAX6_7
— Cmax - High Risk

Exposure Scenario

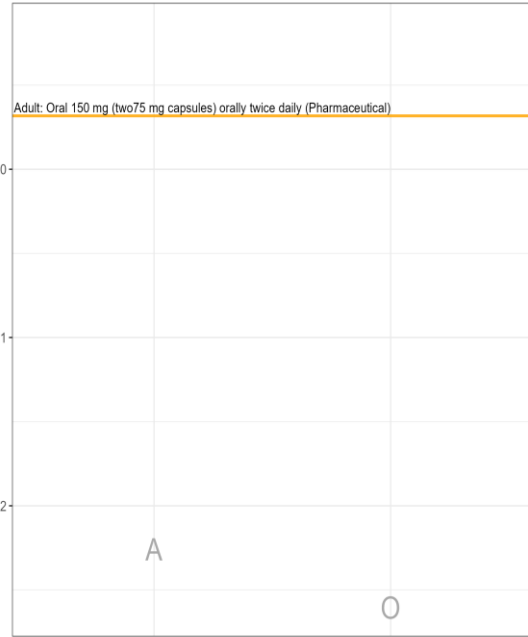
Dolutegravir - Oral 50 mg/daily - Pharmaceutical
1,2-Octanediol - Dermal 5% body lotion - Cosmetic
Nitrofurantoin - Oral 200 mg/daily - Pharmaceutical
2-Methylresorcinol - Dermal 1.8% in hair colourant - Cosmetic
Aspartame - Oral 2400 mg/daily - Dietary
Panthenol - Dermal 5.3% in body lotion - Cosmetic
Tert-butylhydroquinone - Oral 0.7 mg/kg bw - Dietary
Sodium Fluoride - Oral 3.3 mg/daily - Aggregate
Saxagliptin - Oral 5 mg once daily - Pharmaceutical
Rosiglitazone - Oral 4 mg/daily - Pharmaceutical
Acyclovir - IV 5 mg/kg administered every 8 h - Pharmaceutical
Chlorpyrifos - Oral 0.0045 mg/daily - Dietary
Atrazine - Oral 0.005 mg/kg bw per day - Dietary
Fumonisin B1 - Oral 2 μg/kg bw per day - Dietary
Tetrabromobisphenol A - Oral 0.7 μg/kg bw per day - Dietary
BHT - Dermal aggregate (max 0.8%) - Cosmetic
Acetochlor - Oral 0.0036 mg/kg bw/day - Dietary
Fipronil - Oral 0.0002 mg/kg bw/day - Dietary
2-Amino-6-chloro-4-nitrophenol - Dermal 2% in hair colourant - Cosmetic
Hexazinone - Oral 0.05 mg/kg/day - Dietary
DEP - Dermal aggregate (max 10%) - Cosmetic
Glutaraldehyde - Dermal 0.1% in body lotion - Cosmetic
HC Red 3 - Dermal 3% in hair colourant - Cosmetic
Theophylline - Oral 0.14 mg/daily - Dietary
2-Amino-6-Chloro-4-Nitrophenol - Dermal 2% in hair colourant - Cosmetic
Fluzilazole - Oral 0.002 mg/kg bw per day - Dietary
Cypermethrin - Oral 0.3 mg/daily - Dietary
Elapril - Oral 40 mg daily - Pharmaceutical
Sulfamethazine - Oral 50 μg/kg bw/day - Dietary
Paraquat - Oral 0.27 mg/daily - Dietary
Glutaraldehyde - Oral 9.6 mg/daily - Dietary
Progesterone - Oral 0.35 mg/daily - Pharmaceutical
Ethylzingerone - Dermal aggregate (max 2%) - Cosmetic
Warfarin - Oral 2 mg/daily - Pharmaceutical
Dexamethasone - Oral 0.75 mg/daily - Pharmaceutical
BP3 - Dermal 6% in sunscreen - Cosmetic
Metformin - Oral 2000 mg/daily - Pharmaceutical
Metoclopramide - IV 10 mg/daily - Pharmaceutical
DEET - Dermal 15% in insect repellent - Pharmaceutical
Salicylate - Dermal shampoo (max 3%) - Cosmetic
Metoclopramide - Oral 60 mg/daily - Pharmaceutical
2EHA - Oral 3.1 mg/daily - Dietary
Plerixafor - Subcutaneous Injection 20 mg injection - Pharmaceutical
DBP - Oral 0.6 mg/daily - Dietary
Ibrutinib - Oral 420 mg taken orally once daily - Pharmaceutical
Caffeine - Dermal 2% in shampoo - Cosmetic
Cyclophosphamide - Oral 60 mg/daily - Pharmaceutical
Digoxin - Oral 0.024 mg/daily - Pharmaceutical
Imazapyr - Oral 2.5 mg/kg bw per day - Dietary
ATRA - Oral Normal Dietary Intake - Dietary
Ibuprofen - Oral 200mg tablets - Pharmaceutical
DES - Oral 0.5 mg/daily - Pharmaceutical
Cyclamate - Oral 420 mg/daily - Dietary
Salicylate - Oral 162.5 mg/daily - Pharmaceutical
ATRA - Dermal 0.1% cream - Pharmaceutical
Lapatinib - Oral 1000 mg/daily - Pharmaceutical
Spiroxamine - Oral 0.025 mg/kg bw per day - Dietary
Salicylate - Oral 800-6000 mg/daily - Pharmaceutical
Zoxamide - Oral 0.5 mg/kg bw per day - Dietary
Caffeine - Oral 100 mg/daily - Dietary
Ibuprofen - Oral 13.3 mg/kg/dose - Pharmaceutical
VPA - Oral 600 mg/daily - Pharmaceutical
Lovastatin - Oral 80 mg daily - Pharmaceutical
Pomalidomide - Oral 4 mg taken orally on Days 1 to 21 of repeated 28-day cycles - Pharmaceutical
Isotretinoin -13-cis-retinoic acid - Oral 0.5 mg/kg/day - Pharmaceutical
Theophylline - IV 800 mg/day - Pharmaceutical
Caffeine - Oral 400 mg/daily - Dietary
Vinpocetine - Oral 15 mg three times daily over long periods - Pharmaceutical
Fenazaquin - Oral 3 mg/daily - Dietary
Retinol - Oral Normal Dietary Intake - Dietary
Retinol - Dermal 0.05% in body lotion - Cosmetic
Aminopterin - Oral 2 mg/m2/dose for two doses 12 hours apart - Pharmaceutical
5-Fluorouracil - IV 200 mg/m2 intravenously as a continuous infusion over 24 hours - Pharmaceutical
Thalidomide - Oral 50 mg/daily - Pharmaceutical
VPA - Oral 3600 mg/daily - Pharmaceutical
Isotretinoin - Oral 0.5 mg/kg/day - Pharmaceutical
MTX - Oral 10 mg/weekly - Pharmaceutical
ATRA - Oral 80 mg/daily - Pharmaceutical
Dabrafenib - Oral 150 mg (two 75 mg capsules) orally twice daily - Pharmaceutical
Cytarabine - IV up to 200 mg/m2 daily as a continuous intravenous infusion for five days (120 hours) - Pharmaceutical



Only Protective using the Loose Criteria

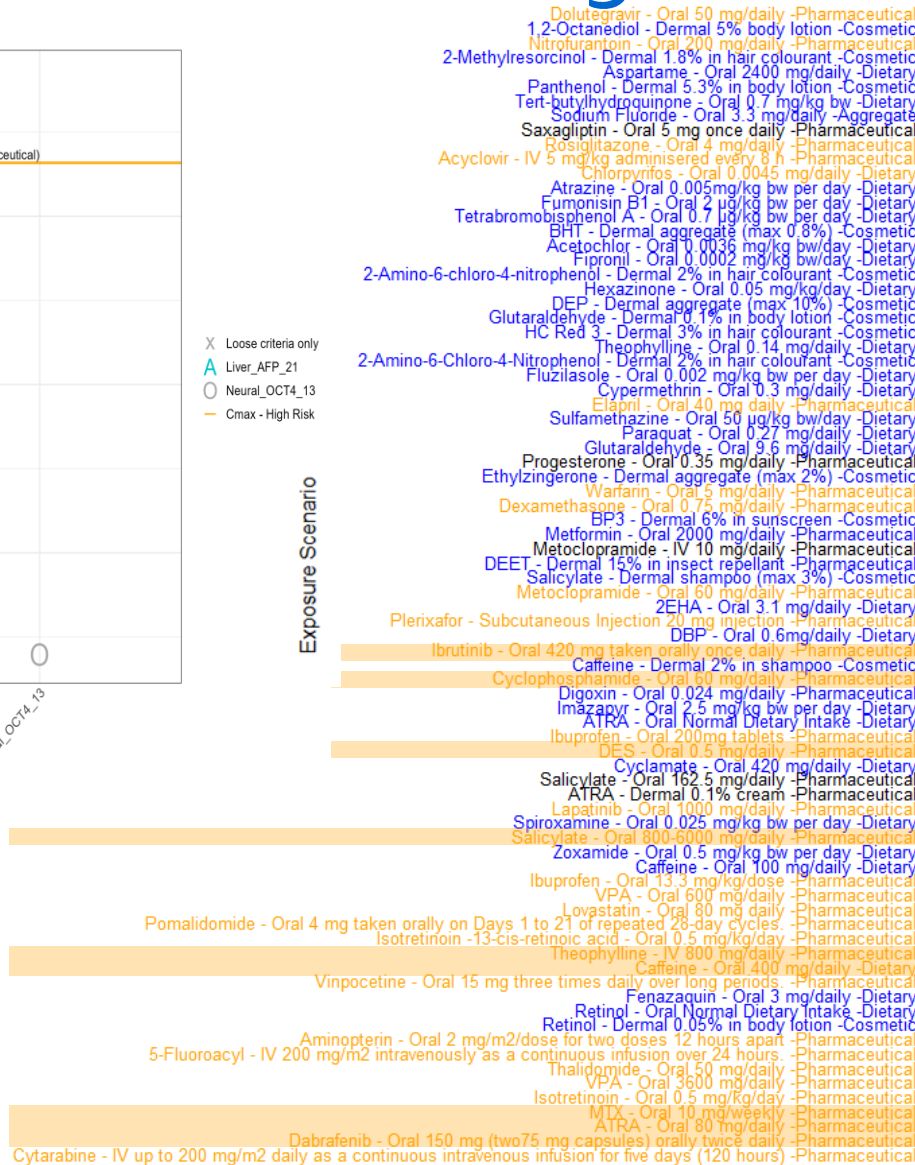
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Dabrafenib



X Loose criteria only
A Liver_AFP_21
O Neural_OCT4_13
— Cmax - High Risk

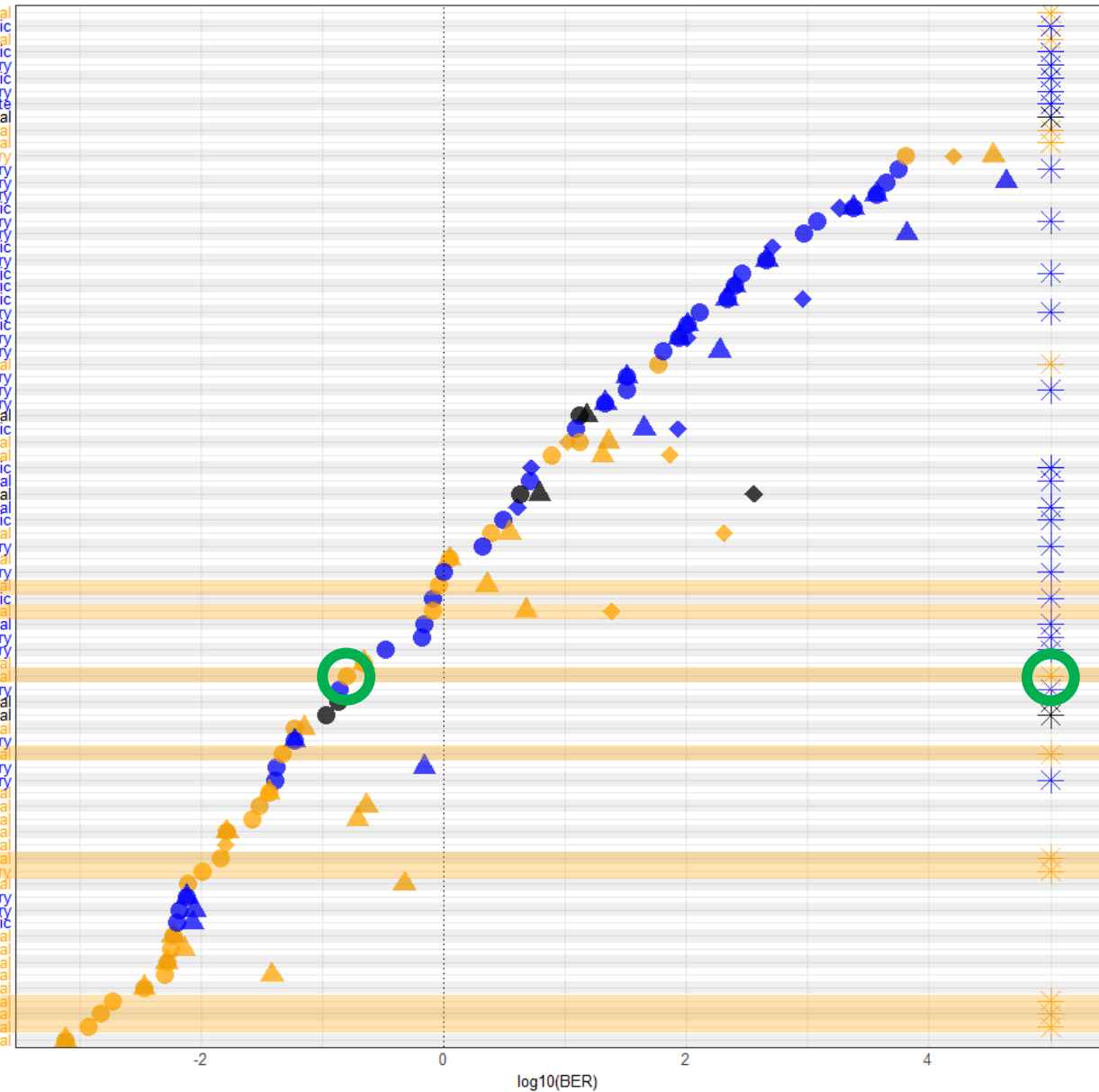
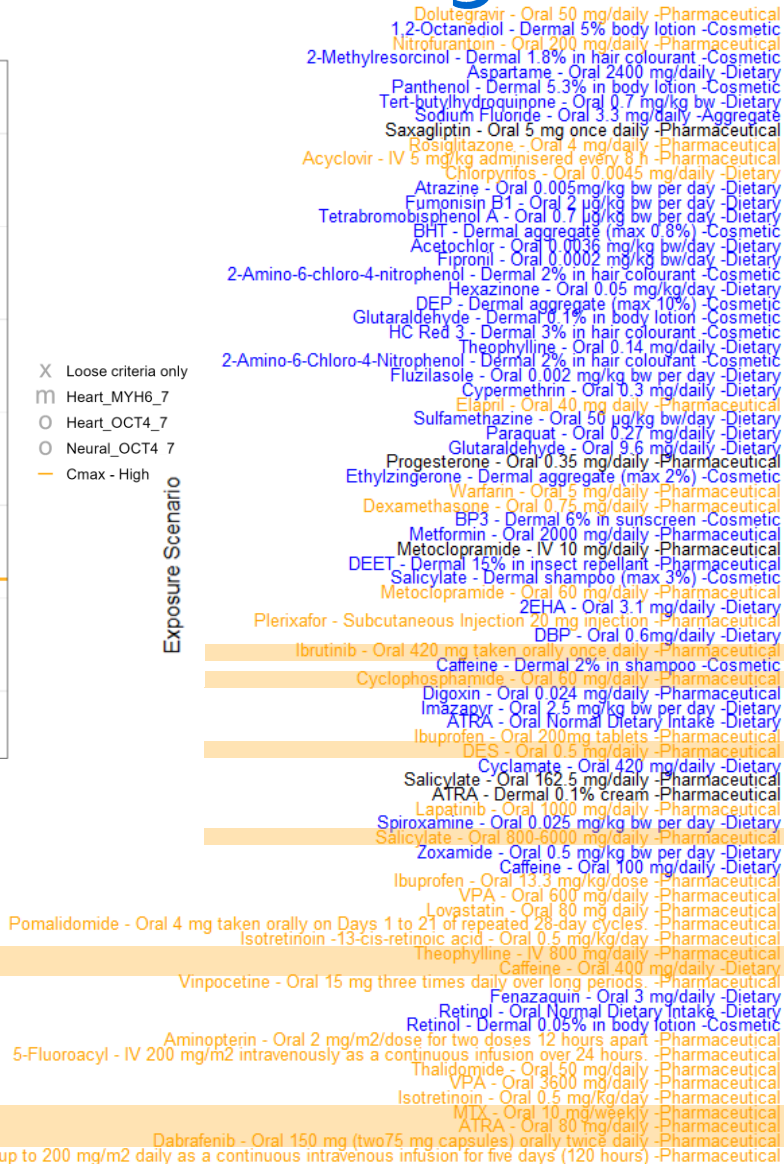
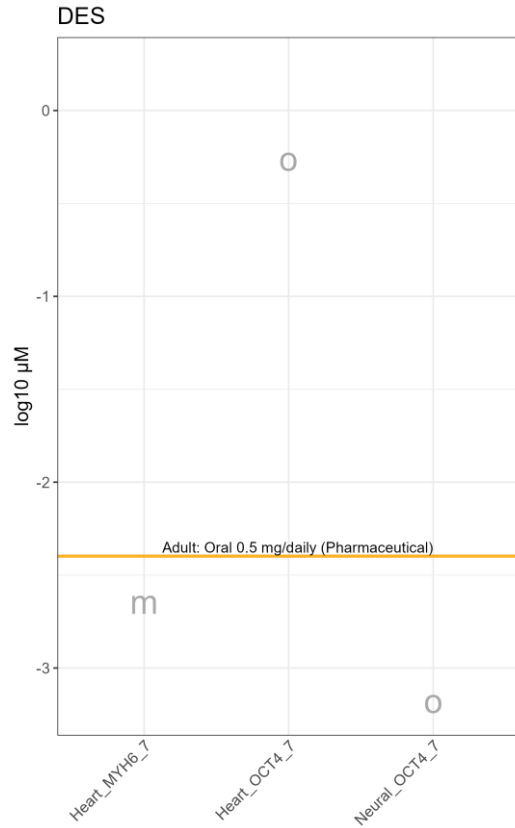
Exposure Scenario



Risk classification ● Low ● High ● Uncertain Endpoint ● BMD (loose) ▲ BMD (strict) ◆ Cytotoxicity ✱ No PoD

Only Protective using the Loose Criteria

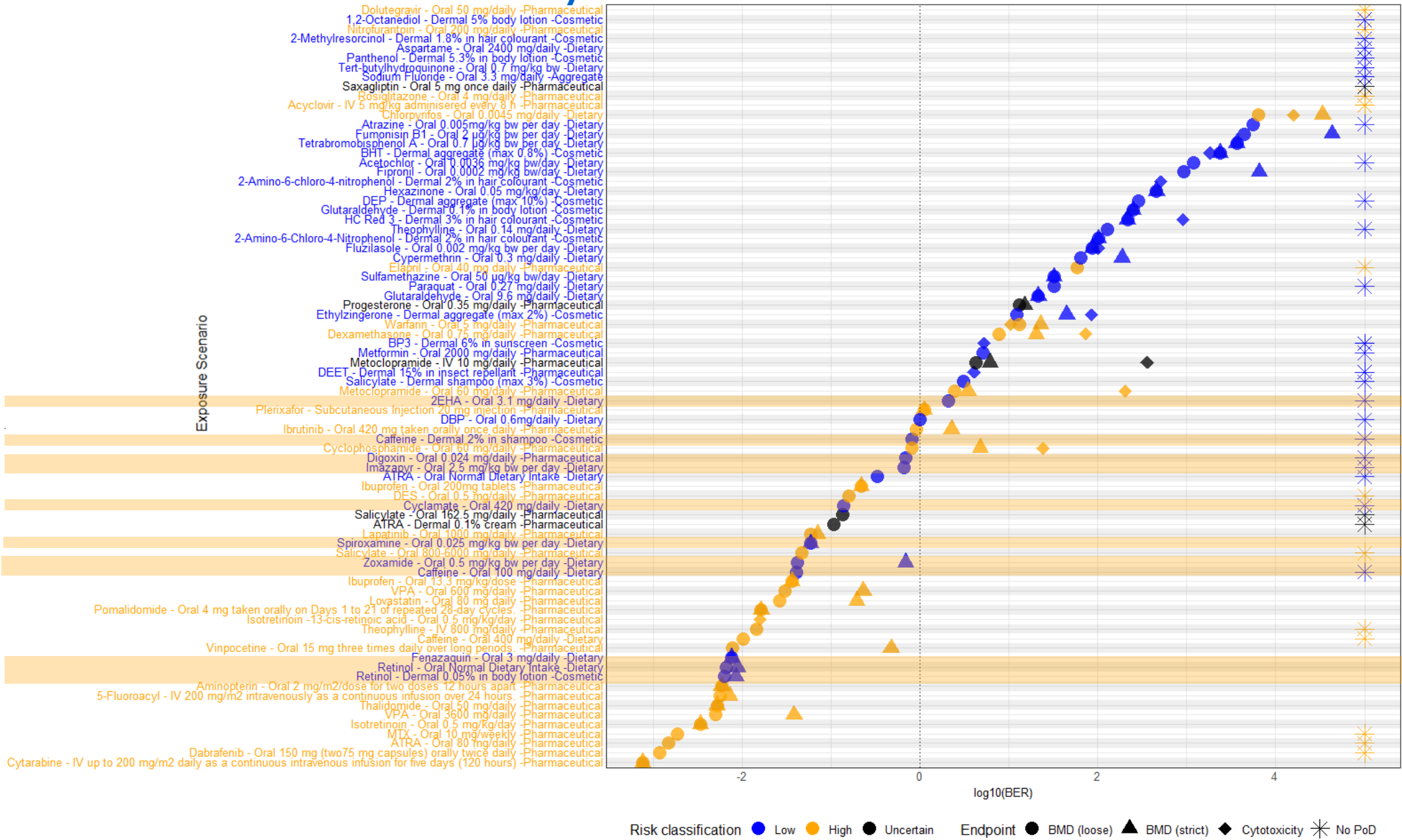
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Risk classification ● Low ● High ● Uncertain Endpoint ● BMD (loose) ▲ BMD (strict) ◆ Cytotoxicity ✱ No PoD

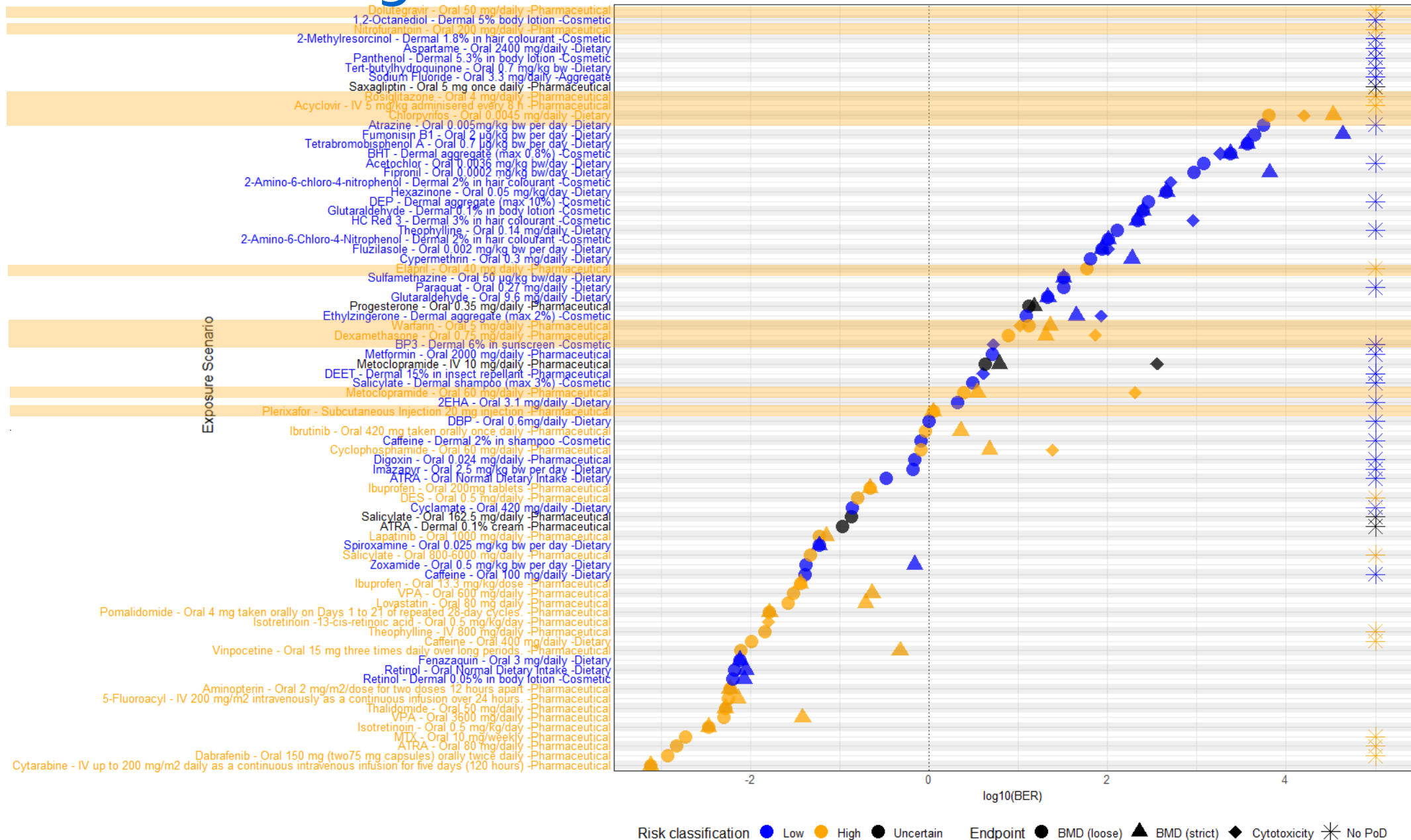
Is The Loose Criteria Overly Protective?

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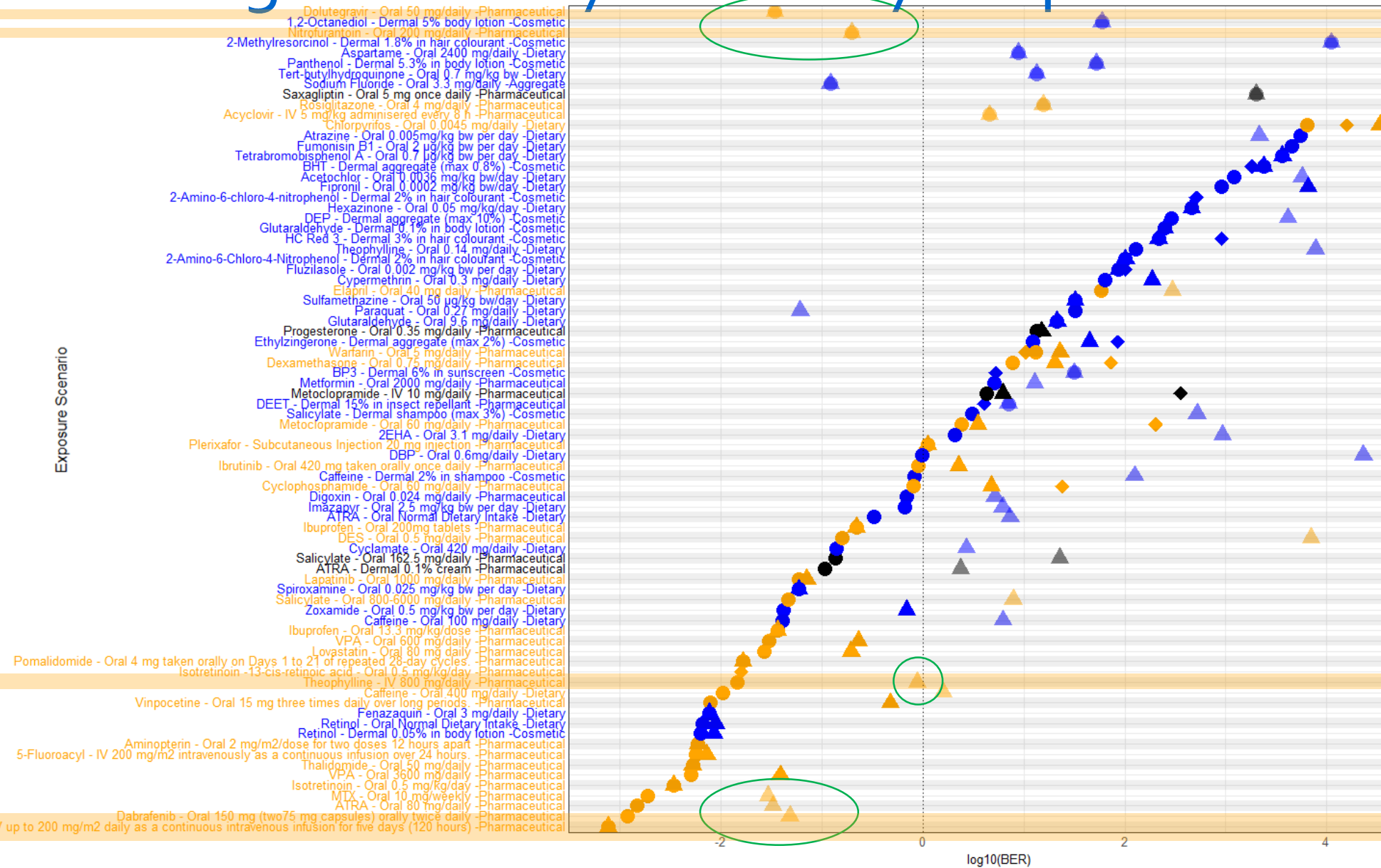


What Are We Missing

Unilever



What Are We Missing – Does cytotoxicity help? *Unilever*



Concluding points

- The ReproTracker assay can be successfully adapted for dose–response modelling within an NGRA framework
- Using all bioactivity measurements (loose criteria) increases protectiveness, but at the cost of utility
- With an initial BER threshold of 1, ReproTracker alone can provide up to 75% protection for DART risk

Next Steps & Considerations

- Explore a Bayesian approach to derive PoDs that jointly model time and concentration
- Evaluate the reproducibility of ReproTracker through a multi-lab study
- Develop an approach to integrate exposure estimates with bioactivity variability and uncertainty
- Define a robust and protective BER threshold for future applications

Thank you & Acknowledgements

Unilever

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Jose Manuel Horcas Nieto

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Matt Dent

SERS

Safety, Environmental
& Regulatory Science