Secondary Intelligence™ as a tool for interpreting the human relevance of in vitro pharmacological profiling data in next generation risk assessment.

Kathryn Wolton, **Maria Baltazar, Sophie Cable, Iris Muller, Ans Punt.**
Safety, Environmental & Regulatory Science, Unilever, Colworth Science Park, UK.
kathryn.wolton@unilever.com

Next generation risk assessment (NGRA) integrates exposure and bioactivity estimates derived from new approach methodologies (NAMs) for safety assessments without animal testing.

In vitro pharmacological profiling (IPP) is commonly used in the pharmaceutical industry to avoid off-target effects (Brennan et al., 2024). In the context of NGRA, we, and others (Burbank et al.,2024) use IPP to determine if bioactivity at key targets occurs at physiologically relevant concentrations and could therefore represent a safety concern for a particular human exposure of interest. The Unilever IPP panel consists of 75 molecular targets including GPCRs, enzymes, ion channels and nuclear hormone receptors, each known to be associated with adverse effects or toxicity. As part of our evaluations of our non animal NGRA frameworks for systemic and reproductive toxicity (Cable et al., 2024 and Muller et al., 2025 under review) we have generated both Ki and functional IC50 data across all 75 targets for a large number of compounds. We have also calculated internal plasma C_{max} (unbound/free- Cu) using PBK modelling for specific exposures of interest for each of the compounds. In this piece of work we will focus on only 5 compounds, each with a defined and specific exposure scenario with associated Cu.

4-hexylresorcinol	BP-4	Caffeine	Diethyl Phthalate	Metoclopramide
Throat lozenge Oral 2.4 mg per 2 hours 10 days	Sunscreen Dermal 450 mg 2 x day 10 days	Beverage Oral 200 mg 2 x day 10 days	Aggr. Cosmetic Dermal 55.8 mg 8h	Pharmaceutical Oral 10 mg daily 10 days
Cu = 0.02 µM	Cu = 0.01 µM	Cu = 0.03 µM	Cu = 0.07 µM	Cu = 0.13 µM

Figure 1A

4-HR	IC50 (M)	Ki (M)
5-HT2B (h)	3.60E-06	1.80E-06
NET (h)	7.40E-06	5.50E-06
COX1(h)	3.10E-07	N.A.
COX2(h)	1.80E-06	N.A.

BP4	IC50 (M)	Ki (M)
PXR (h)	3.60E-05	2.50E-05
PDE4D2 (h)	9.20E-05	N.A.

Caff	IC50 (M)	Ki (M)
A2A (h)	6.10E-06	5.00E-06

DEP	IC50 (M)	Ki (M)
A2A (h)	2.30E-04	1.90E-04
MAO-A	1.50E-05	8.90E-06
5-HT2B (h)	1.40E-06	6.90E-07
PDE4D2 (h)	2.70E-05	N.A.

Met	IC50 (M)	Ki (M)
ACHE (h)	3.70E-05	N.A
HTR1B (h)	2.20E-05	9.70E-06
alpha1A (h)	8.20E-05	4.10E-05
HTR1A (h)	3.30E-06	1.70E-06
alpha2A (h)	7.40E-06	3.30E-06

Met	IC50 (M)	Ki (M)
D1 (h)	1.00E-05	4.00E-06
HTR3A (h)	1.70E-06	1.20E-06
HTR2B (h)	2.30E-07	1.10E-07
HTR2A (h)	2.60E-06	1.90E-06
M2 (h)	6.90E-05	4.80E-05
D2 (h)	6.50E-08	2.60E-08
norepineph	1.80E-04	1.40E-04
COX1	1.0E-04 M	N.A

Figure 1B

Figure 1A) Unbound/free plasma concentrations for the 5 exposure scenarios. For each compound we want to risk assess we first establish the route and nature of the anticipated human exposure, and we generate diverse in vitro ADME parameters for each chemical. Internal exposure estimates were generated using PBK models developed using Gastroplus 9.8. (Simulation Plus, Lancaster, CA, United States) following a tiered framework outlined in Moxon et al. (2020), separated into different levels of complexity and refinement based on the input parameter. The summary table details the high-level description of the given exposure scenario for each chemical, as well as the output of the PBK modelling in terms of a plasma C_{max} (unbound, and therefore free for receptor/enzyme interaction) for each chemical exposure scenario. ***Figure 1B) Active hits for each compound across 75 targets.*** Each chemical was run in a screening phase across the 75 targets (single conc, 2 replicates) to determine which targets to follow up with a dose response experiment (seven concs, 2 replicates) to generate IC50/Ki values as described in Cable et al., 2024 and Muller et al., 2025 (under review). For each of the five compounds the associated IC50/Ki values for each 'hit' are summarised in Molar (M) concentrations.

An open question when interpreting IPP data for NGRA is how large the bioactivity exposure ratio (BER) needs to be for each target to ensure safety? How can Secondary Intelligence™ help with decision making?

One way to address this question is to benchmark BERs against known 'bad actors' i.e. compounds with known activity/exposure relationships leading to adverse events/toxicity. Secondary Intelligence™ is a tool developed by Certara which enables users to compare activity/exposure relationships for compounds of interest with established benchmarks. For each compound, the ratio between the Cu and its Ki (or functional IC50) at each receptor is calculated. If this ratio falls within or above the range of the reference drugs targeting that receptor, the test compound is considered to have a 'high' likelihood of interacting with the receptor at the Cu of interest. Secondary Intelligence™ also provides the user with a summary of the associated toxicity/adverse events associated with such an interaction to help inform further testing necessary to complete the risk assessment. We could already calculate the BER or margin of safety for each target for each compound, however, we were left with an open question – is this BER protective against harm? How large does the bioactivity exposure ratio (BER) need to be for each target to ensure safety? Should the margin between activity and exposure be different for different targets? In this work we investigated if Secondary Intelligence™ could help address these outstanding questions.

Figure 2

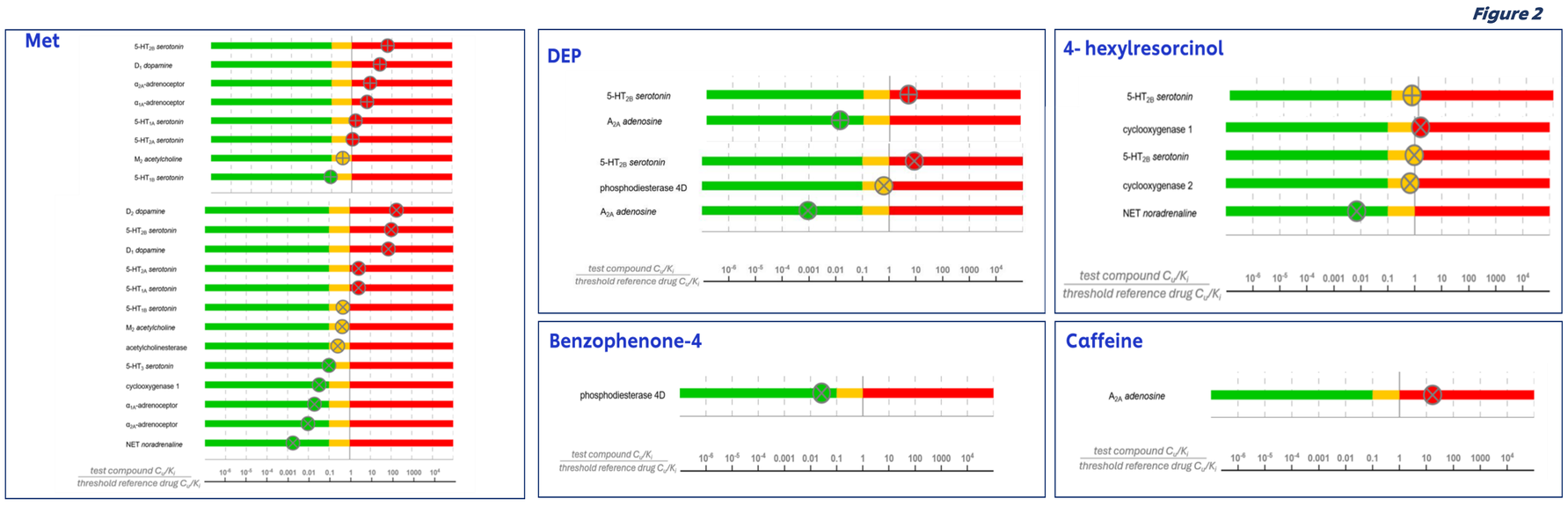

Figure 2) Compound specific plots of the ratio between Cu and Ki plotted for each target against the reference range of established benchmarks with associated adverse events/toxicity. The reference range of known drugs targeting each receptor is given in red. If the ratio of the compounds Cu/Ki falls within or above (1 or above) the range of the reference drugs targeting that receptor, the test compound is considered to have a 'high' likelihood of interacting with the receptor at the Cu of interest. Conversely, if this ratio falls below the range of the known reference drugs targeting that receptor, the compound is considered to have a 'low' likelihood of interacting with the receptor at the Cu of interest (0.1 or below). Between 0.1 and 1, this would be classed as 'medium' likelihood and for the purposes of risk assessment would also be considered by us as a 'flag' to follow up further. As functional data might not be available, information on each functional mode is presented, where appropriate. In some cases some targets are known to be modulated in only one mode. For each compound, the results are shown for both agonism (circle with +) and antagonism (circle with X) where available.

Figure 3A

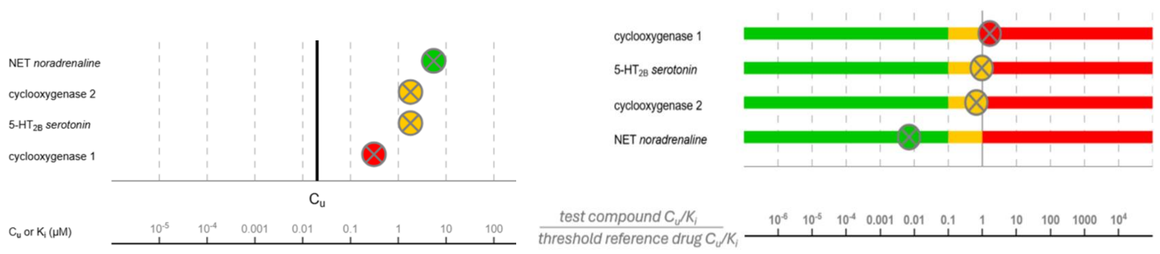

Figure 3B

Receptor	Mod	Risk	Main organ/organ system(s) affected	Liability of most concern	Commonest associated side effects	Expected safety pharmacology outcomes	Translatable biomarker	ADL	Note
cyclooxygenase 1	⊗	H	GIT	Gastrointestinal bleeding	Abdominal pain; Anaemia; Asthenia; Dermatitis; Gastrointestinal bleeding; Nausea; Vomiting	Altered GIT function	Blood/plasma prostaglandin E2; Faecal blood	-	-
5-HT2B serotonin	⊗	M	CVS	Cardiovascular effects (potential)	Cardiovascular effects (potential)	Cardiovascular effects (potential)	-	-	No selective drugs with relevant clinical data
cyclooxygenase 2	⊗	M	CVS; GIT; RES	Myocardial infarction; Stroke	Dizziness; Dyspnoea; Insomnia; Nausea; Peripheral oedema; Vomiting	Altered GIT function; Dyspnoea; ↓ Locomotor activity	Blood/plasma prostaglandin I2	-	-

Receptor	Mod	Risk	Main organ/organ system(s) affected	Liability of most concern	Commonest associated side effects	Expected safety pharmacology outcomes	Translatable biomarker	ADL
5-HT2B serotonin	⊕	H	CNS; CVS; RES	Cardiac valvulopathy; Pulmonary hypertension	Dizziness; Dyspnoea; Nausea	-	-	-
D1 dopamine	⊕	H	CNS; CVS; RES	Blood pressure decreased	Agitation; Blood pressure decreased; Dyspnoea; Heart rate increased; Intraocular pressure increased; Pulmonary oedema	↓ Blood pressure; ↓ Body temperature; ↑ Locomotor activity	Blood pressure	Yes
α2A-adrenoceptor	⊕	H	ANS; CNS; CVS	Somnolence	Blood pressure decreased; Dizziness; Dry mouth; Heart rate decreased; Mydriasis; Somnolence	↓ Activity; ↓ Blood pressure; ↓ Body temperature; ↓ Heart rate; ↑ Pupil diameter	Pupil diameter; Sedation score	Yes

Figure 3A) Benchmarking using Secondary Intelligence™ provides human relevant information for decision making in NGRA. In this example we have used the data for 4-hexyresocrinol to highlight the added value for decision making/contextualisation of risk using the tool. Plotted on the left are the Ki values for each target against the Cu for 4-HR. The visualisation clearly shows that all bioactivity against each target occurs at higher concentrations than the plasma Cu. This can be calculated as a bioactivity exposure ratio, sometimes also referred to as a margin of exposure or margin of safety. In this case as the modulation at each receptor occurs at higher concentrations than the plasma Cu (circles to the right of the line) and as the BERs/MoE are all above ten, further follow up might not be initiated. However, you can see from the graph to the right, despite the BERs/MoE for each target being above ten, the tool has flagged that benchmark data exists which shows that based on existing knowledge, the interaction of the receptor at the Cu of interest is considered high for Cox 1, and medium likelihood for 5HT2B and Cox 2, and that such interaction is associated with adverse effects in human. Therefore, for these targets, a BER/MoS of 10 may not be sufficiently protective of human health and would require further investigation. ***Figure 3B) Summary of the adverse events/toxicity associated with receptor interaction .*** For each interaction that the tool ranks as having high or medium likelihood of occurring, Secondary Intelligence™ provides users with a summary of the known safety liabilities, side effects and expected toxicology of such an interaction, based on expertly curated knowledge across various data sources such as regulatory submissions (FDA/EMA), clinical trial reports etc. In addition, information on each benchmark is available to the user, for further querying. Together the information could be used in a second tier of risk assessment to address any concerns in a hypothesis driven and bespoke manner.

Conclusions

- From this small pilot study, Secondary Intelligence™ offers additional value to risk assessors who use in vitro pharmacological profiling data as part of a NAM based NGRA.
- By easily and quickly benchmarking exposures and potencies to substances with known safety liabilities, risk assessors can clearly identify potentially higher risk exposures – those that would require further follow up work to make a safety decision.
- By also including information on the known safety liabilities of such interactions, it provides a steer as to where to focus future efforts - whilst saving time and resource compared to a systematic literature review.
- Further case studies are needed to explore how to follow up flags identified through use of the tool, in a second tier of a hypothesis driven risk assessment.

Literature

1) Brennan et al., The state of the art in secondary pharmacology and its impact on the safety of new medicines. Nature Reviews Drug Discovery. 2024 May 21; 23, 525–545.
2) Burbank et al., In vitro pharmacologic profiling aids systemic toxicity assessment of chemicals. Toxicology and Applied Pharmacology. 2024. 492. Volume 492, 117131.
3) Cable et al., Advancing systemic toxicity risk assessment: Evaluation of a NAM-based toolbox approach. 2025 March. Toxicological Sciences. Volume 204. 1-17.
4) Muller et al., An Advancement in Developmental and Reproductive Toxicity (DART) Risk Assessment: Evaluation of a Bioactivity and Exposure-Based NAM Toolbox. 2025. Under Review.

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