**Supplemental Material: Integrating TTC with High Throughput Exposure for Risk Based Prioritization**

**1. Summary of Tiered risk-based prioritization programs.**

In Australia, NICNAS (2016) has an Inventory Multi-Tiered Assessment and Prioritisation (IMAP) program which prioritizes substances by consideration of a matrix of exposure and hazard (. The three IMAP tiers are: Tier 1- identification of chemicals which, using exposure data, do not require further consideration; Tier 2- identification of relevant data, and preparation of a brief report to characterize the likely risks for chemical identified from exposure data; and Tier 3- examination of whether appropriate risk management measures already exist, and whether the available data are sufficient to justify relevant risk management measures.

The Canadian Chemicals Management Plan (CMP) consists of a 3-phase risk assessment initiative (Health Canada, 2017). In Phase 1, Canadian authorities conducted a screening level risk-based triage (categorization) of approximately 23,000 chemicals in commerce culminating in 4,300 substances being identified as of highest priority. Phase 2 consists of screening level and in depth risk evaluation of the 4,300 substances identified for further attention by a 2020 deadline. CMP phase 3 involves evaluating 1,550 substances – the last of the 4,300 identified under Canada’s prioritization process as requiring health and ecological assessments.

In the EU, substances that may have serious and often irreversible effects on human health and/or the environment have the potential to be identified as substances of very high concern (SVHCs). If a substance is identified as a SVHC, it is added to the Candidate List for eventual inclusion in the Authorisation List. SVHCs are identified based on the hazard properties of a substance. There are three main types of hazard properties that are used in the identification process:

1. substances meeting the criteria for classification as carcinogenic, mutagenic or toxicity for reproduction category 1A or 1B in accordance with the CLP Regulation
2. substances which are persistent, bioaccumulative or toxic (PBT) or very persistent, very bioaccumulative (vPvB) according to REACH Annex XIII or
3. substances on a case by case level that cause an equivalent level of concern as CMR or PBT/vPvB

Note there are also risk-based prioritization criteria that are used to identify substances for evaluation, i.e. those substances that should be included in the CoRAP, the community rolling action plan. These consider both hazard and exposure aspects. There are several different scenarios covering human health, environmental and endocrine effects (ECHA, 2016; 2017).

**2. Research Programs Related to Toxicity Testing in the 21st Century**

In the U.S., US EPA initiated the Toxicity Forecaster (ToxCast) program, and then formed the interagency Tox21 consortium involving the EPA, the National Institutes of Health, and the Food and Drug Administration. The ToxCast program and Tox21 consortium have evaluated over 8,000 chemicals using a broad range of in vitro high-throughput screening (HTS) assays to identify potential hazards through interactions with proteins, pathways, and cellular processes. See <https://www.epa.gov/chemical-research/toxicity-forecasting>.

One of the striking aspects of the EU REACH regulation is that it mandated that animal testing should be used as a last resort to address the information requirements and called for alternative approaches to be exploited in accordance with the adaptations laid out in Annex XI. This generated significant momentum resulting in a number of activities to develop alternatives approaches that could be considered for regulatory purposes. These efforts led to the establishment in 2004 of the OECD Principles for the Validation of (Q)SARs (<http://www.oecd.org/chemicalsafety/risk-assessment/validationofqsarmodels.htm>) followed by an OECD guidance document <http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2007)2> which provided a framework for (Q)SARs to be used to address information requirements as supplemental or in lieu of traditional experimental data. In addition, OECD developed a guidance document (GD No. 211) that describes information to be provided by method developers for non-guideline in vitro methods to help facilitate understanding of data quality and potential utility in regulatory applications (OECD 2014).

A number of EU funded research programs had REACH as a strong focus – shifting towards applied research that was aligned with specific regulatory purposes. EU Programs such as the Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Test Information (OSIRIS; <http://www.seurat-1.eu/>) focused on development of integrated testing strategies for endpoints (including skin sensitization), a key component for how REACH information requirements were anticipated to be met. The SEURAT-1 program (which was co-funded by Cosmetics Europe) explored strategies to replace repeated dose toxicity studies. The SEURAT-1 program was a particular example that included a significant read-across component. Templates for structuring and reporting of read-across predictions were developed and applied to a set of repeated dose toxicity case studies to compare and contrast traditional read-across as well as read-across integrating new approach methodologies (NAM) (Berggren et al, 2015; Schultz et al., 2015; Schultz and Cronin, 2017 and references within). The EUToxRisk program (http://www.eu-toxrisk.eu/), the successor to SEURAT, also has a strong focus on exploring how new approach methodologies (NAM) can be used to support read-across justifications through a series of different case studies. EU ToxRisk is focusing on advancing human cell –based assays linked to mechanistic understanding of adverse effect pathways to conduct safety evaluations and assess potential risks.

Within the OECD, use of HTS and AOPs have been a major focus of study particularly under the auspices of the Task Force on Hazard Assessment (now termed Working Party on Hazard Assessment WPHA) where AOP-informed IATA case studies (OECD 2017a) are being developed by different member countries. Many of case studies published or in progress to date have had a read-across focus but these have utilized HTS outcomes as a means of providing additional mechanistic information. Another focus is the OECD AOP work program itself (OECD 2017b) which is developing AOPs for inclusion into the AOP Wiki (https://aopwiki.org/) and the AOP knowledge base (http://aopkb.org/)), as well as investigating the extent to which HTS assays as well as other non-guideline tests can be mapped to key events within AOPs. Indeed, OECD Guidance Document No. 211 (OECD 2014) describes how such assays should be described and documented.

References

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**Analyzing the dataset through the TTC workflow as outlined in Figure 2**

Chemical structures were available for 7699 substances in the dataset as extracted from DSSTox within the EPA CompTox dashboard (https://comptox.epa.gov/dashboard).

Filters were used to identify and exclude dioxin-like substances, metals, mixtures, salts, high potency carcinogens, and steroids from the set of 7699 substances. Inorganics, bioaccumulative substances, steroids were identified using the Kroes et al (2004) workflow module contained within Toxtree v2.5 (Ideaconsult Ltd), the OECD Toolbox’s ‘structure type’ profiler and Leadscope structural features ([www.leadscope.com](http://www.leadscope.com)).

Using the starting dataset of 7699 substances, application of the Kroes exclusions within Toxtree removed 414 substances. Applying the steroids filter using the Leadscope structural feature hierarchy removed a further 211 substances. A further 271 substances which were metals, inorganics or mixtures were identified using the Toolbox “structure type’ profiler and removed. High potency carcinogen alerting groups identified using the *in vitro* mutagenicity alerts in the ISS profiler within the OECD Toolbox v3.4 removed 8 further substances (4 nitroso, 3 azoxy and 1 aflatoxin substance). Overall, 904 substances out of the starting 7966 structures were not applicable to be evaluated by the TTC approach.

The remaining set of 6795 substances applicable for consideration of the TTC were then evaluated further to determine which TTC value was most appropriate. 1853 substances contained a structural alert for genotoxicity using the *in vitro* mutagenicity alerts in the ISS profiler within the OECD Toolbox v3.4. Organophosphates (OP) were identified using a specific SMARTS pattern encoded within Toxtree as a new module (taking the same SMARTS pattern as included in the Kroes workflow) whereas carbamates were identified as structural features using Leadscope’s structural feature hierarchy. 102 substances met the criteria for categorization as organophosphates or carbamates (no organophosphates were identified). The remaining 4840 substances were evaluated using the Cramer structural classes decision tree in Toxtree v2.5. Of these, 3214 substances were categorised as Cramer III, 32 substances were categorised as Cramer II, and 1294 substances were categorised as Cramer I.

**Re-analyzing the dataset using Kroes et al workflow within Toxtree**

As noted in the manuscript, a more straightforward approach might have been to take full advantage of the Kroes workflow as implemented in the Toxtree application. From our findings, this was not practically feasible to do in a batch form for several thousand substances as an exposure level needs to be identified upfront for each substance. Although there is a means by which this can be “silenced” in the tool, our experience found that this prevented the workflow from being processed to completion and many incorrect assignments resulted given no substance in the entire 7699 set was assigned in any of the Cramer structural classes or was correctly identified as an OP or carbamate. This prompted separate filters to be created though these were found to be overly conservative in some cases based on the lack of OPs and number of steroids and carbamates being identified in the original analysis.

In reviewing the output file for the Kroes workflow, we found that certain elements could be extracted to address some of the TTC categories but that it was necessary to parse out some of the specific rules – carbamates, steroids and to devise a new rule for OPs. All of these could be done as using the existing rules in the Kroes workflow simply re-saved as new modules in the Toxtree platform itself. This did require some manipulation of the original output file, some reprocessing through these new modules and use of the Cramer module itself.

To investigate the outcomes from a modified approach that relied upon one tool – Toxtree to process the file rather than 3 tools as used in the original analysis, the following steps were undertaken.

Steps followed were:

1. Process a sdf of the dataset through the Kroes workflow in Toxtree
2. Parse out substances that require a compound specific risk assessment – Nominally N/A to TTC
3. Subset from these substances those that flag for being High Potency genotoxic carcinogens based on the outcomes to Q3 in the workflow (Q2YQY3)
4. Parse out the substances from the NA substances those that resulted in a Yes to Q1.
5. Reprocess the substances from Q1Y through a new module to identify OPs [using an existing rule in Kroes]
6. Parse out substances that flag for an alert for genotoxicity
7. Reprocess this set to identify steroids and carbamates through new modules [using existing rules in Kroes]
8. Exclude the steroids and carbamates set from the subset of substances flagged with a genotoxicity alert
9. Combine the steroid set with the remaining compound specific risk assessment set but excluding the OPs to derive the total of substances for which it was not appropriate to use TTC for.
10. Process the remaining dataset after removal of substances NA or flagging an alert for genetox or an OP/carbamate through the Cramer Toxtree module

For each of the respective sets of categories, a count of the number of substances that exceeded the Total median or UCI was performed using the dplyr in R.

The outputs of this re-analysis resulted in the following revised tables 2 and 3.

**Table 2. Results of filtering the dataset based on structures to categorize substances for application of structural category-specific TTC values**.

|  |  |
| --- | --- |
| TTC category | # of chemicals |
| Total dataset | 7968 |
| Dataset with available structures | 7699 |
| TTC is not appropriate | 231 |
| TTC is appropriate | 5378\* |
| Genotoxic chemicals | 1827 |
| AChEIs | 263 |
| Cramer class III | 3957 |
| Cramer class II | 165 |
| Cramer class I | 1253 |

#3 substances were corrupted during Cramer class processing hence final number is 5375

**Table 3. Risk-Based Prioritization Results Based on the HTE: TTC Exposure to Activity Ratio Method**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TTC category | Number of chemicals | TTC (µg/kg-day for 60 kg adult) | Percentage of Substances Exceeding the TTC | |
| UCI Exposure Value (number of chemicals) | Median Exposure Value (number of chemicals) |
| Cramer class III | 3957 | 1.5 μg/kg-day | 1% (51) | 0 |
| Cramer class II | 165 | 9.0 μg/kg-day | 0 | 0 |
| Cramer class I | 1253 | 30 μg/kg-day | 0 | 0 |
| AChEIs | 263 | 0.3 μg/kg-day | 3% (8) | 0 |
| Genotoxic alerts | 1827 | Kroes 0.0025 μg/kg-day | 94% (1723) | 4% (77) |
| ICH 0.025 μg/kg-day | 19% (341) | 1% (22) |

Although some of the numbers of some of the categories in Table 2 have changed, there was no significant impact in the outcomes in Table 3 – the number of substances exceeded the exposures are very comparable as that in the original analysis. Our insights did not change despite the differences in the tools applied.

Prioritizing large numbers of substances presents some challenges using existing software for TTC. Users should be pay careful attention to the output produced. The learnings gained from processing this file through the Kroes workflow in Toxtree will be described in more detail in a subsequent manuscript.

The R code, associated R data file as well as the specific Toxtree schemes to identify OPs, carbamates and steroids are provided as part of this supplementary information.