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Developing Reference Chemical Lists for Validation of *In Vitro* Assays

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OECD EAGMST, Agenda Item 10

June 21 2019

Motivation

- There is an increasing desire to use new approach methods (NAMs) for chemical hazard and dose response characterization.
- Many *in vitro* assays are available, but few have been validated for use in regulatory decisions.
- Key to validation is the availability of reference chemicals
- For high-throughput *in vitro* assays, multiple reference chemicals should be used, covering:
  - varying chemical classes that are active and inactive
  - a range of potencies
  - chemicals that are likely to cause false positive results due to assay interference

Problem Statement

- Currently, only a few *in vitro* assays of regulatory-relevance have more than one or two accepted reference chemicals (e.g., estrogen and androgen receptors, skin sensitization, genotoxicity).
  - These reference chemical lists were developed through a consensus, expert-driven approach based on surveys and evaluation of the literature.
  - Development of lists took multiple years to develop.

- A more rapid, semi-automated approach to developing reference chemical lists is desirable.

Reference Chemical: Working Definition

- Target assays are ones that measure activity against
  - a specific target or molecular mechanism (e.g. estrogen receptor, mitochondrial activity), and
  - mode (e.g. agonist, antagonist)

- A reference chemical for such an assay is one:
  - that measures activity against the target or molecular mechanism
  - for the specified mode
  - that gives consistent results (active vs. inactive, consistent potency)
  - across multiple different assays (usually run in different laboratories), with different cell types and assay readout technologies

Proposed Approach

- EPA has developed a two-pronged approach to developing reference chemical lists:

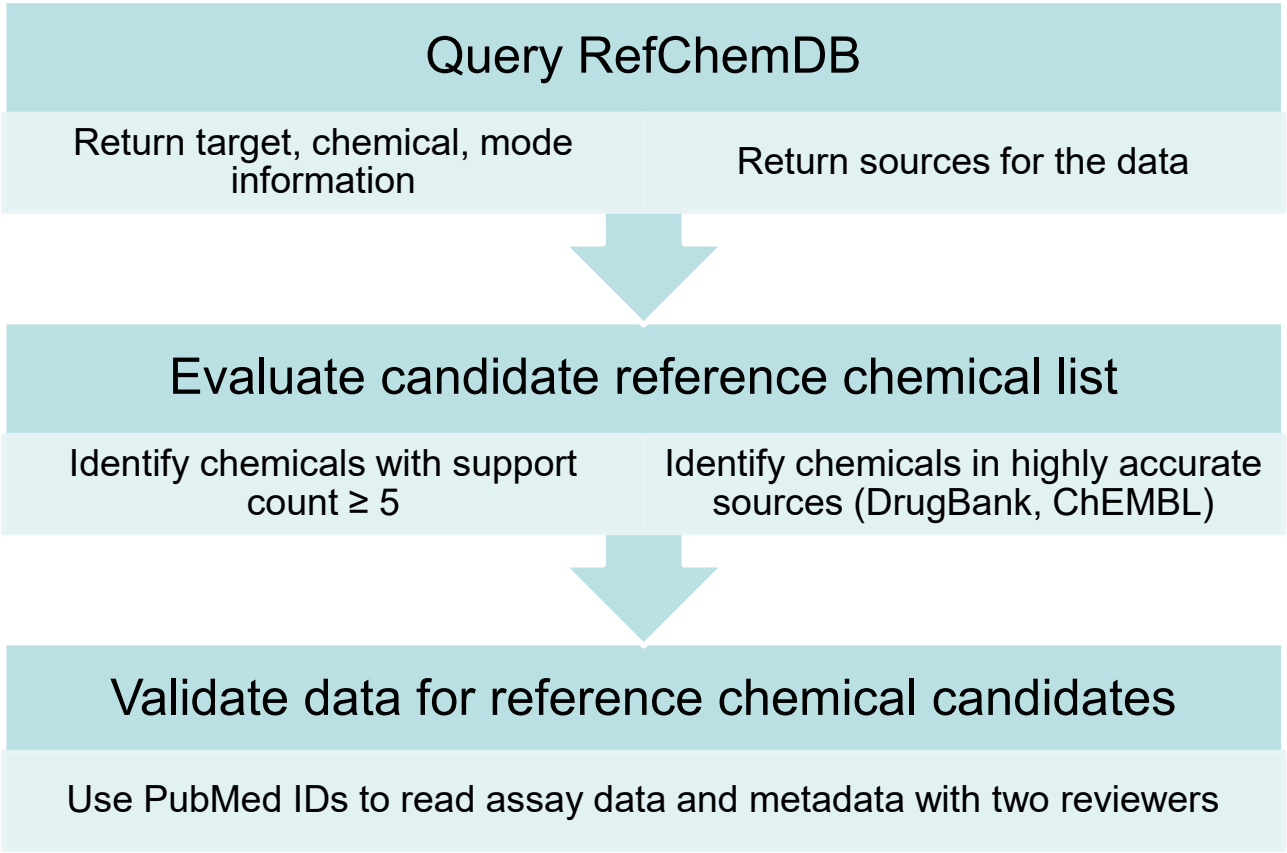
1. Cases where there is significant testing data in the literature

2. Cases where there is little to no data in the literature

1. Cases where there is significant testing data in the literature

- Available literature has been automatically compiled into a database RefChemDB
  - Target, mode, source reference
- Key metric of data availability is “support”
  - Support = number of references linking chemical to target and mode
- Key finding
  - if there are at least 5 reports linking the chemical, target and mode, manual curation will usually confirm this linkage
  - If there are only 1 or 2 such reports, manual curation will often find that the linkage is false
- Approximately 150 targets have 5 or more active reference chemical candidates

Manual Curation is Still Required to Arrive at Final Reference Chemical Set



Result: Peer-reviewed publication with reference chemical list for the target

2. Cases where there is not significant testing data in the literature

- Redundant Assay Screening Process (RASP)

1. Initial assay is developed and a large number of chemicals are screened (Assay 1)
2. Multiple other assays for the same target are developed (Assays 2-N)
3. Selected positive and negative chemicals from Assay 1 are screened in Assays 2-N
4. Chemicals that behave consistently in Assays 1-N become reference chemicals

Thoughts on RASP

- The process is resource intensive
  - may need development of up to 5 assays per target
  - Different cell types and assay readout technologies

- Will likely require support from national or international bodies

- However, retesting against this new set of reference chemicals will provide a performance-based validation for Assays 1-N

Questions to EAGMST

- Would EAGMST provide input or participate in further development of these approaches for developing reference chemical lists?
  - Case studies
  - Development of guidance

References

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