Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Read Across Workgroup: Towards Guiding Principles for Read-Across Applications

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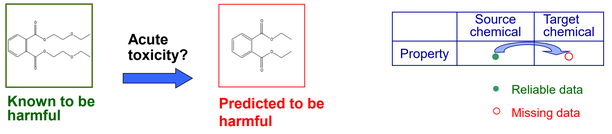
INTRODUCTION

In 2018, US Agencies established a read-across workgroup (RAWG) under ICCVAM to develop and implement a plan to build capacity in the development and application of read-across approaches and to harmonize them. Initially, the RAWG summarized current experiences and needs, as well as catalogued the different tools applied. Recent RAWG efforts have focused on developing a compendium of member agency read-across case studies to inform guiding principles for different read-across decision contexts. Case studies were intended to cover how read-across was applied currently and what new approaches member agencies were considering to refine future read-across applications, e.g. use of data such as ToxCast and systematic approaches such as Generalized Read-Across (GenRA). Here, two of the case studies that the RAWG discussed are presented. The first of these detailed the qualitative use of ToxCast data to characterize bioactivity similarity of a target substance and its candidate analogues (Lizarraga et al., 2019). The second case study was submitted to the OECD in 2018 and utilized a categorical approach to assess the potential for testicular toxicity based on metabolite formation. Chemicals were grouped based on expected metabolic pathways, and predictions were supported with *in vivo* data from structural and metabolic analogues. The RAWG discussed challenges such as how to reconcile the impact of different similarity contexts in a weight of evidence assessment and addressing residual uncertainty. It is envisaged that insights from these case studies will shape practical technical guidance to facilitate read-across applications across the various member agencies.

Read-across is a data gap filling technique whereby a chemical with existing data values is used to make a prediction for a 'similar' chemical.



Specific RAWG charge element:

- Develop a compendium of member agency read-across case studies and use it as a basis to inform guiding principles for different read-across decision contexts.
- Case studies under consideration should balance how read-across has been undertaken in the recent past and what new approaches are being considered in refining how read-across might be conducted in the future

CASE STUDY 1 – US EPA CPHEA

- US EPA's Center for Public Health and Environmental Assessment (CPHEA) use case illustrated the application of read-across for deriving screening-level provisional peer-reviewed toxicity values (PPRTVs).
- The development of PPRTVs is based on EPA methods and guidelines to provide toxicity information and health reference values for chemicals of interest to the US Superfund Program.
- When human or animal toxicity data for a target chemical are inadequate or unavailable, an expert-driven approach based on three similarity principles (i.e., structure, toxicokinetics and toxicodynamics) is used to identify and evaluate analogues for read-across.
- Analogues with existing health reference values are identified, and relevant information is evaluated for consistency and concordance, identifying data gaps and uncertainties.
- Suitable analogues are then selected based on a weight of evidence approach.
- Finally, a 'best' source analogue is selected, and it's point of departure adopted for use in the screening-level assessment of the target chemical.

Refs
Lizarraga et al., 2019, *Regul Toxicol Pharmacol* 103:301-313
Schultz et al., 2015, *Regul Toxicol Pharmacol* 72: 586-601
http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm

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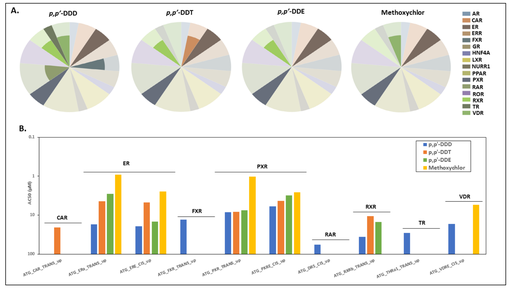
CASE STUDY 1 – US EPA CPHEA

- One of the target substances evaluated was p,p'-DDD (dichlorodiphenyldichloroethane).
- Bioactivity similarity comparisons based on ToxCast *in vitro* data showed similar responses between p,p'-DDD and its source analogues in human liver cells, including mitochondrial damage, cellular stress/cytotoxicity, and upregulation of xenobiotic-sensing nuclear receptors, relevant to the mechanisms of hepatoxicity for this group of chemicals.

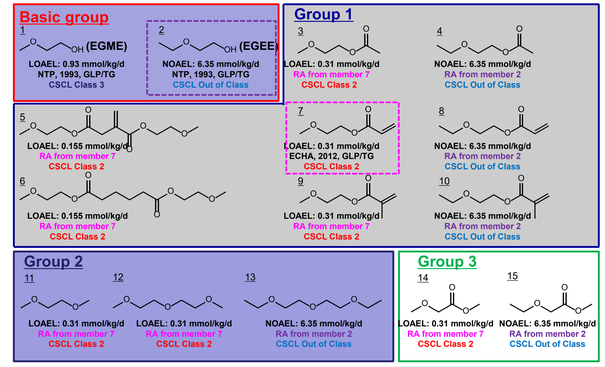


- Furthermore, the ToxCast data and *in silico* QSAR models suggested p,p'-DDD and its analogues exhibited estrogen receptor agonistic and androgen receptor antagonistic activities, which coincided with the reproductive and developmental effects observed in animals.

CASE STUDY 2 - JAPAN

- Integrated Approaches for Testing and Assessment (IATAs) for testicular toxicity of ethylene glycol methyl ether (EGME)-related chemicals submitted to the OECD IATA Case Studies Project to apply read-across in filling data gaps for screening level assessments required under the Japanese Chemical Substances Control Law (CSCL).
- It was hypothesized that EGME-related chemicals that were metabolized to methoxy- or ethoxyacetic acid induced testicular toxicity in rodents.
- The Japanese MITI inventory comprising 16,000 substances was loaded into the Hazard Evaluation Support System (HESS) read-across platform (https://www.nite.go.jp/en/chem/qsar/hess-e.html).
- Potential possible metabolites were generated using the Rat Cellular Metabolism Simulator. Chemicals that were predicted to be metabolized to EGME, ethylene glycol ethyl ether (EGEE) or methoxy- or ethoxy acetic acid were then searched for using a custom profiler that defined the structure of these chemicals as a boundary. The search resulted in 40 substances which were then evaluated, based on presence of common elements and availability of relevant toxicity data. The final set of 20 chemicals returned were then considered for categorization purposes.
- A categorical approach to assess potential for testicular toxicity was utilized as follows:
  - Group 1: esters of EGME and EGEE which are readily hydrolyzed to generate EGME and EGEE
  - Group 2: chemicals that form EGME or EGEE via oxidative O-dealkylation
  - Group 3: chemicals that are hydrolyzed to generate methoxy- or ethoxy acetic acid.
  - Other than EGME and EGEE, the simulator and additional literature search identified 13 chemicals that are predicted to be metabolized to methoxy- or ethoxy acetic acid, including eight chemicals in Group 1, three chemicals in Group 2, and two chemicals in Group 3. Data were gathered for EGME-related chemicals from published literature, and metabolite formation and testicular toxicity endpoints were summarized. These data supported the formation of a read-across category of chemicals that are metabolized to methoxy- or ethoxy acetic acid via hydrolysis or O-dealkylation. Data gaps were filled using read-across based on the predicted metabolite of each chemical.
- Overall uncertainty of the read-across was determined to be low since all source and target chemicals were expected to have common active metabolites and since core structural differences were minor.

CASE STUDY 2 - JAPAN



Additionally, toxicity profiles of the metabolites other than methoxy- or ethoxy acetic acid were collected and analyzed. Formation of additional toxic metabolites to the reproductive organ was not likely.

LEARNINGS FROM CASE STUDY 1

- The requirement for analogues to have health reference values for inclusion in the read-across analysis (according to EPA's current CPHEA approach) poses restrictions in the evaluation of chemical categories.
- The qualitative application of NAMs (i.e., *in vitro* ToxCast data) supported biological similarity comparisons and increased confidence in the selection of analogues for read-across.
- Analogues are often identified using structural similarity. Expanding the analogue search strategy to include toxicokinetic and toxicodynamic considerations could assist in expanding the pool of analogues and provide more appropriate means of grouping chemicals for read-across.
- More systematic and transparent approaches to evaluate and integrate heterogenous lines of evidence are needed to support read-across justifications and assess confidence.

LEARNINGS FROM CASE STUDY 2

- Gathering relevant data to construct the data matrix in order to substantiate the metabolism hypothesis was extremely resource intensive.
- The following issues were identified regarding the use of metabolism information in read-across:
  - How to assess similarity in a metabolic pathway
  - How other structurally diverse metabolites did not cause the toxic effects of interest.
- Uncertainty assessment was qualitative but guided by principles outlined by Schultz et al (2015).
- Assessment made use of bespoke tools (HESS) to identify analogues and evaluate them based on their simulated metabolism.
- OECD Template for reporting IATA was useful to structure the case study and rationale for the read-across.

NEXT STEPS

- Three additional RAWG case studies are under discussion. These will form the basis of a compendium where the case studies are summarized, and guiding principles extracted.