In Silico Eco/Tox Prediction Using Triad of QSAR model, Toolbox read-across and expert knowledge. Application for skin sensitization

H. Ivanova[1], G. Dimitrova[1], K. Kirilov[1] and O. Mekenyan[1]

[1]*Laboratory of Mathematical Chemistry, Burgas State University "Prof. D-r Assen Zlatarov", Burgas, Bulgaria*

INTRODUCTION

For centuries, animals have been used in scientific research to investigate biological processes and to assess potential toxicity risks in humans. The European Union's (EU's) 2013 ban on both testing and marketing cosmetics with animal-tested ingredients, including for skin sensitization, eliminated animal testing for cosmetic purposes and requires the use of alternative methods. In silico systems can reduce the need for (animal) testing, increase human safety and support critical decisions. They are increasingly being cited in regulatory guidance documents and it is generally recognized that no single alternative method will be able to provide a one-to-one replacement of tests, but information from a combination of techniques is required.

The aim of the current work is to propose a general scheme combining Quantitative Structure-Activity Relationship (QSAR) predictions, read-across (RA) analogues approach and expert knowledge for providing mechanistically reliable predictions. The combined approach is called "QSAR Triad" and will be demonstrated for predicting skin sensitization potential of a case study chemical.

MATERIALS AND METHODS. WORKFLOW

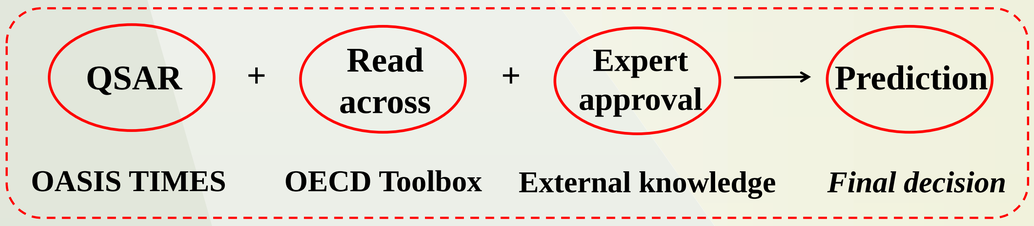


Figure 1. General scheme for mechanistically reliable QSAR predictions

The proposed scheme for evaluating (eco)toxic endpoints includes three general stages/pillars:

🔎 ***Pillar I*** includes a QSAR prediction from the OASIS TIMES which could be justified by:

- ✓ Identification of analogues supporting the prediction,
- ✓ Identification of analogues supporting the simulated metabolic pathway/active metabolite,
- ✓ Toxicokinetic considerations of the adduct formation,
- ✓ Quantify the extend of damage that could be caused to macromolecules (i.e., skin proteins).

🔎 ***Pillar II*** relies on a RA prediction obtained in OECD QSAR Toolbox and is based on:

- ✓ Selection of mechanistically and structurally similar analogues using the available knowledge incorporated within the so-called profilers,
- ✓ Calculation of *Alert performance* which is used to estimate the percentage of positive and negative analogues sharing the same mechanism(s) of interaction/MoA as the target.

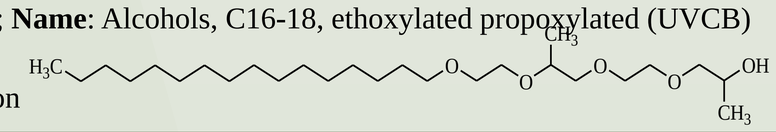
🔎 ***Pillar III*** is associated with the expert approval of the results obtained in Stages I and II, which is an important step for verification of final prediction based on external knowledge.

RESULTS. CASE STUDY

The proposed workflow will be demonstrated with the following case study chemical:

🔎 Target: **CAS#** 68002-96-0; **Name**: Alcohols, C16-18, ethoxylated propoxylated (UVCB)

🔎 2D representative:



🔎 Endpoint: Skin sensitization

CASE STUDY: STAGE I. QSAR PREDICTION

The analysis associated with Stage I of the workflow is done using the TIMES Skin sensitization models (TIMES-SS) available in the OASIS TIMES software.

Non-kinetic TIMES-SS model:

- ❑ The model is developed combining LLNA and GPMT data.
- ❑ **Prediction:** The target chemical is predicted as a Weak/Non sensitizer according to an alert for *Hydroperoxides* identified in the simulated autoxidation products.
- ❑ **Metabolism:** The tissue-specific metabolism of target chemical is simulated by the TIMES-SS model and the hydroperoxides are highlighted automatically by the system (Fig.2).

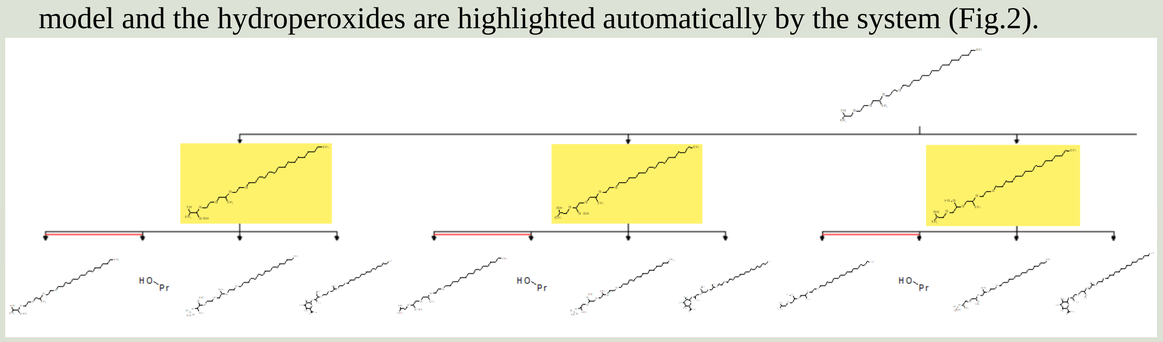


Figure 2. Skin metabolism simulated by TIMES-SS model

- ❑ **Analogues supporting prediction:** Analogues are searched within the training set of TIMES-SS model using a combination of: *OFG* (applied on parent) **AND** *Protein binding alerts for skin sensitization* (applied on parent and simulated metabolites) profilers. The search results in identification of one analogue with Negative experimental data (Fig. 3).
- ❑ **Analogues supporting metabolism**: To further justify the prediction, analogues are searched within the database with documented autoxidation reactions using the *US-EPA New Chemical Categories* profiler. The search results in identification of two analogues confirming the formation of hydroperoxides in similar structures (Fig.3).

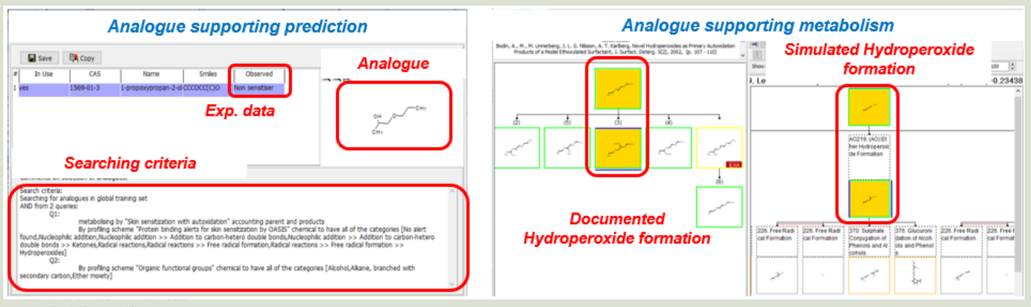


Figure 3 Identification of analogues supporting prediction and metabolism

CASE STUDY: STAGE II. READ ACROSS

Several RA scenarios were applied to analyze the predictions for different skin sensitization endpoints, data from *Guinea Pig Maximization Test* (GPMT) and *Local Lymph Node Assay* (LLNA) are considered separately.

Scenario 1. GPMT:

- ✓ ***Target endpoint*** is defined to consider GPMT data.
- ✓ ***Primary grouping*** (i.e., selection of analogues) is done using structure-based profiler *US-EPA New Chemical Categories;* the target is classified as a *Nonionic Surfactant.*
- ✓ ***Alert performance*** is calculated in advance using Positive/Negative data scale (Fig. 4).
- ✓ ***Subcategorizations*** are performed to reduce the inconsistency among analogues.
- ✓ ***Prediction*** is Negative based on two structurally and mechanistically similar analogues (Fig. 4).

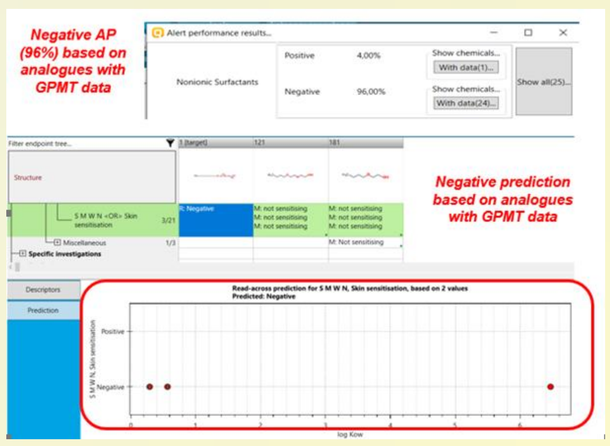


Figure 4. Prediction based on GPMT data

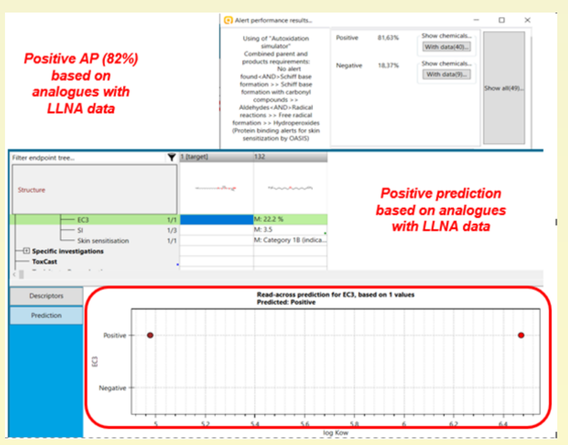


Figure 5. Prediction based on LLNA data

Scenario 2. LLNA:

- ✓ ***Target endpoint*** is defined to consider LLNA data, only
- ✓ ***Primary grouping*** (i.e., selection of analogues) is done using mechanistic-based profiler *Protein binding alerts for skin sensitization by OASIS* in combination with *Autoxidation simulator*
- ✓ ***Alert performance*** is calculated in advance using Positive/Negative data scale (Fig. 5).
- ✓ ***Subcategorizations*** are performed to reduce the inconsistency among analogues.
- ✓ ***Prediction*** is Positive based on one structurally and mechanistically similar analogue (Fig. 5).

Summary: *The RA analysis clearly shows that depending on the used test, the outcomes vary from Negative (GPMT) to Positive (LLNA), which is consistent with the exp. data of the analogues and AP estimations.*

CASE STUDY: STAGE III. EXPERT

There already exists literature debating potential false positives in the LLNA compared to older guinea pig outcomes. As supporting evidence for the argument that high logP chemicals have a higher tendency to give false positives, studies have been cited in which lipophilic unsaturated fatty acids and ethoxylated alcohol surfactants were found to be positive in the LLNA but negative in the GPMT [1]. These apparent LLNA false positives have been discussed in an earlier paper [2] and here is the conclusion:

"The LLNA positives all have alerts for autoxidation, and therefore should be considered as potential pro-haptens. The LLNA offers a greater opportunity for these non-directly reactive compounds to express their potential to induce sensitization. Thus, the LLNA results are not false positives, but simply reflect the differences in protocols between the LLNA and GPMT."

CONCLUSIONS

1. The suggested reliability workflow was used to predict the skin sensitization of a target chemical (ethoxylated alcohol). According to different stages, the target is predicted as:
   a. Weak/Non sensitizer (Negative) based on TIMES-SS QSAR model combining GPMT and LLNA data
   b. Negative based on RA with GPMT data
   c. Positive based on RA with LLNA data
2. The workflow (i.e., the QSAR Triad) is applicable for any (eco)toxicological endpoint with known mode of action and clear mechanisms of interaction with macromolecules.
3. The workflow is assumed as being of high importance especially in the cases of conflicting results, negative or out-of-domain prediction and could be used for regulatory purposes.

REFERENCES:

[1] N. Natsch, *Regul. Toxicol. Pharmacol.* (**2023**), 10.1016/j.yrtph.2023.105333

[2] D.W. Roberts, *Regul. Toxicol. Pharmacol.*, 80 (**2016**), pp. 260-267.