Protocol for Generating Read-across Prediction for Skin Sensitization Hazard Using QSAR Toolboxv3.2

1. Input the target compound into QSAR Toolboxv3.2 by CASRN.
2. Profile the target compound for protein binding by OASISv1.2, protein binding by OECD, protein binding potency, and protein binding alerts for skin sensitization by OASIS v1.2.
3. If there are no protein binding alerts go back to input and generate auto-oxidation products and skin metabolites and then profile the products/metabolites for protein binding alerts. To generate auto-oxidation products and metabolites (which cannot be done simultaneously):
    a. Right click on SMILES structure and then right click to select "Multiplication," then "Metabolism/Transformation," and then "Auto-oxidation Products." Generating auto-oxidation products may take a little while, but afterwards, they appear in a tree form after clicking "[set]Auto-oxidation simulator."
    b. Select single component mode on the right of top banner for profiling the products (repeat #2).
    c. Generate skin metabolites by repeating #3a and #3b for metabolites: right click on SMILES structure and then right click to select "Multiplication," then "Metabolism/Transformation," and then "Skin metabolism simulator". The metabolites appear in a tree form after clicking "[set]Skin Metabolism simulator."

d. If any auto-oxidation products or metabolites have protein binding alerts, select the one with more alerts for the skin sensitization hazard analysis. If an auto-oxidation product and a metabolite have an equal number of protein binding alerts, select the auto-oxidation product by right clicking on it in the data matrix and then select "Focus," which allows the product/metabolite to represent the target chemical. The selected product/metabolite appears in a new data matrix. Go to #4.

e. **If neither the parent nor the auto-oxidation products nor the metabolites have protein binding alerts, the substance is negative**. No report can be generated for these.

4. For substances with protein binding alerts, retrieve data on *in vivo* skin sensitization endpoints for the target compound in the endpoint module. Use "Skin sensitization" and "Skin sensitization ECETOC" databases.

5. Go to the category definition module and use a structural profiler in this order of preference: US EPA new chemicals categories, OECD categories, organic functional groups, or structural similarity (use the default option, which is the Dice method) to look for analogs. If the substance is not classified by the first preference, go to the next. If the chemical is categorized in multiple categories, click "OR" at "combine profiles logically" in the dialog box to provide the largest possible group of analogs. Make sure "Skin sensitization ECETOC" and "Skin sensitization" are selected so that analogs are sought among substances with skin sensitization data. If there are multiple outcomes for a chemical, choose select one at the dialog box. It will use 1

representative of each different outcome (if there are 3 positives and 4 negatives for a substance it will use only 1 positive and one negative).

6. Fill data gap by read across. Select the in vivo skin sensitization cell under toxicity for the target substance. Select apply. Select the scale option for Skin sensitization II ECETOC (the lowest common denominator – positive and negative categories).

7. Use the default descriptor option of log Kow, which identifies the analogs closest to the target chemical.

8. Subcategorize: if all analogs are sensitizers or if all are nonsensitizers, there is no need to continue with subcategories. Go to #9. Otherwise, verify that analogs have the same mechanism/mode of action by opening the Select/Filter data menu on the right of the graph and re-profiling the identified analogs. Click on subcategorize and choose “Protein binding alerts for skin sensitization by OASISv1.2”. [Note: This profiler is in the endpoint-specific section of profilers and is different from the general mechanistic profiler called “Protein binding by OASISv1.2.”] This compares the mechanistic properties of the analogs with the target chemical. Eliminate the dissimilar chemicals by clicking on the “Remove” button. Those will be highlighted in green on the graph and in blue on the right of the subcategorization menu.

9. Accept the read across prediction.

    a. If an “unreliable” message is received (usually because the log Kow for the target chemical is outside the range for the analogs) or if a message indicates that there are too few data points to make a prediction, go back to #4 to add the ECHA CHEM database and then repeat the subsequent steps. **If adding**

the ECHA CHEM database fails to achieve a reliable prediction, make a "qualified" prediction based only on the structural category (i.e., without using the subcategorization step). If a qualified prediction cannot be made, a prediction cannot be made with this protocol. (NOTE: Some, but not all, predictions made with the ECHA CHEM database yield a message that so much proprietary data was used for the prediction that a report cannot be made. Thus, if subcategorization yields an unreliable prediction with or without the ECHA CHEM database, the prediction should be made without the ECHA CHEM database (and without subcategorization) so as to get a report.

b. If the prediction was made for a product/metabolite of the parent chemical of interest, assign prediction to parent by going back to data matrix of the target chemical (go to the input module). Click on "[set]Skin Metabolism simulator" or "[set]Auto-oxidation simulator," as appropriate. Then select the in vivo skin sensitization cell of the target chemical and then "Data Gap Filling." Click "Independent MOA" and then "Apply." Accept prediction and return to matrix. Final prediction for the parent is CI for component based independent mode. Select prediction in data matrix; right click on the prediction and then select report to generate a report.

c. If the prediction was not made for a product/metabolite, accept the prediction, return to matrix, and go to the report module. Double click the prediction of interest on the left panel and then click the create button to generate a report.