Supplemental Information on Changes from ITS-2 to ITS-2 Lipid and Moving Toward Open-Source

This document provides information on the refinements made to the Bayesian network integrated testing strategy for skin sensitization (ITS-2) since its original publication (Jaworska et al. 2013). The refined model implemented with commercial software is referred to as ITS-2 lipid and the refined model implemented in R is referred to as open-source (OS) ITS-2 lipid.

First, the refinements to both models include the correction of errors in the experimental data from the direct peptide reactivity assay for the following chemicals:

- Benzoic acid (training set)
- Imidazolidinyl urea (test set)

Second, the method for calculating the bioavailability parameters was changed to improve the transparency and consistency of the predictions. Specifically, these changes include eliminating the skin diffusion pathway for polar substances from the calculation and revising the prediction strategy for physico-chemical properties.

In ITS-2, both lipid and polar skin diffusion pathways were used for the bioavailability calculations in an MS Excel version of the Kasting skin penetration model (Dancik et al. 2013). The bioavailability calculations for the lipid diffusion pathway are publicly accessible on the National Institute for Occupational Safety and Health website (http://www.cdc.gov/niosh/topics/skin/finiteSkinPermCalc.html). The polar skin diffusion pathway module remains under development and is not publicly available. Accordingly, to produce an entirely transparent package, we focused on the lipid pathway in the OS model. However, for comparison, we also implemented the ITS-2 commercial software version with the lipid pathway

We also adopted a new prediction strategy for physico-chemical properties to promote accessibility and transparency. The new strategy is as follows:

- Log K_{ow}
  - Use the EPI Suite™ v. 4.1 measured value (http://www.epa.gov/opptintr/exposure/pubs/episuite.htm). If not available, use the ACD/Labs v. 12.0 predicted value

- Water solubility, S_w
  - If the chemical is not ionized at pH 7 (neutral water pH), use the EPI Suite measured value at 25°C. If not available, use ACD/Labs v. 12.0 intrinsic solubility value
  - If the chemical is ionized at pH 7, use ACD/Labs v. 12.0 intrinsic solubility value. The rationale behind this is that Kasting's lipid pathway model assumes that only the neutral fraction of the chemical penetrates the skin.
- Vapor pressure, P_{vp}
  - When the P_{vp} measured at 25°C (mP_{vp}[25°C]) is available in EPI Suite, use it and two additional parameters (calculated vapor pressure at 25°C and at 32°C) to calculate the final vapor pressure for the skin temperature at 32°C
    - Final $P_{vp}(32°C) = mP_{vp}(25°C) \cdot cP_{vp}(32°C) / cP_{vp}(25°C)$
  - When P_{vp} measured at 25°C is not available in EPI Suite, use the calculated EPI Suite P_{vp} for 32°C, $cP_{vp}(32°C)$ only
- Use the measured melting point from EPI Suite. If not available, use the predicted value
- Use density, strongest acid and/or base pKa value(s), MW (molecular weight), and log D, which is the pH dependent octanol:water partition coefficient (optional if more than one acidic or more than one basic pKa value impacts transport through the skin) from ACD/Labs v. 12.0

Although the changes to log K_{ow} values are minor and pertain mostly to lipophilic chemicals, there are substantial differences in water solubility inputs. In ITS-2, S_w values were obtained from a variety of sources (e.g., Aquasol database, Syracuse Research Corporation, EPI Suite, the Merck Index, ChemGold) or calculated from the Jain & Yalkowsky correlation (Jain and Yalkowsky 2001), which has a much smaller applicability domain than the ACD model.

The performance of the commercial ITS-2 lipid model is summarized in **Tables 1** and **2**. When compared to the original ITS-2 model (Jaworska et al. 2013), the prediction of moderate sensitizers is improved.

Table 1. Confusion Matrix for LLNA Potency Category Predictions by ITS-2 Lipid for the Training Set of 124 Substances.

	Observed Potency Category			
Predicted Potency Category	**1 (36)**	**2 (28)**	**3 (35)**	**4 (25)**
1 (32)	29	1	1	1
2 (26)	3	21	2	0
3 (35)	3	4	24	4
4 (31)	1	2	8	20

Abbreviations: ITS-2 lipid = integrated testing strategy for skin sensitization 2, implemented using commercial software and the lipid diffusion pathway for diffusion through the skin; LLNA = murine local lymph node assay.

The numbers in parentheses show the number of chemicals predicted or observed in each category. Categories are based on LLNA potency: 1 = nonsensitizer; 2 = weak sensitizer (EC3 ≥10%), 3 = moderate sensitizer (1% ≤ EC3 <10%), 4 = strong/extreme sensitizer (EC3 <1%). Shaded cells show correct category predictions.

Table 2. Confusion Matrix for LLNA Potency Category Predictions by ITS-2 Lipid for the Test Set of 21 Substances.

	Observed Potency Category			
Predicted Potency Category	**1 (6)**	**2 (5)**	**3 (5)**	**4 (5)**
1 (7)	6	1	0	0
2 (5)	0	4	1	0
3 (5)	0	0	4	1
4 (5)	0	0	1	4

Abbreviations: ITS-2 lipid = integrated testing strategy for skin sensitization 2, implemented using commercial software and the lipid diffusion pathway for diffusion through the skin; LLNA = murine local lymph node assay.

The numbers in parentheses show the number of chemicals predicted or observed in each category. Categories are based on LLNA potency: 1 = nonsensitizer; 2 = weak sensitizer (EC3 ≥10%), 3 = moderate sensitizer (1% ≤ EC3 <10%), 4 = strong/extreme sensitizer (EC3 <1%). Shaded cells highlight the correct category predictions.

The performance of the OS ITS-2 lipid was slightly better than the commercial model and is shown in **Tables 3** and **4** for the training and test sets, respectively. When compared to the ITS-2 lipid model, the OS ITS-2 lipid correctly predicted more nonsensitizers, weak sensitizers, and moderate sensitizers, but fewer strong/extreme sensitizers in the training set (compare **Table 3** for the OS ITS-2 lipid to **Table 1** for ITS-2 lipid). For the test set, OS ITS-2 lipid mispredicted three substances, as did the ITS-2 lipid (compare **Table 4** to **Table 2**). However, OS ITS-2 lipid (**Table 4**) underpredicted the potency of all three substances, while the commericial ITS-2 lipid underpredicted 2/3 mispredicted substances (**Table 2**).

Table 3. Confusion Matrix for LLNA Potency Category Predictions by OS ITS-2 Lipid for the Training Set of 124 Substances

	Observed Potency Category			
Predicted Potency Category	**1 (36)**	**2 (28)**	**3 (35)**	**4 (25)**
1 (36)	31	2	1	2
2 (27)	3	22	2	0
3 (35)	1	3	26	5
4 (26)	1	1	6	18

Abbreviations: LLNA = murine local lymph node assay; OS ITS-2 lipid = integrated testing strategy for skin sensitization 2, implemented using R software and the lipid diffusion pathway for diffusion through the skin.

The numbers in parentheses show the number of chemicals predicted or observed in each category. Categories are based on LLNA potency: 1 = nonsensitizer; 2 = weak sensitizer (EC3 ≥10%), 3 = moderate sensitizer (1% ≤ EC3 <10%), 4 = strong/extreme sensitizer (EC3 <1%). Shaded cells highlight the correct category predictions.

Table 4. Confusion Matrix for LLNA Potency Category Predictions by OS ITS-2 Lipid for the Test Set of 21 Substances.

	Observed Potency Category			
Predicted Potency Category	**1 (6)**	**2 (5)**	**3 (5)**	**4 (5)**
1 (7)	6	1	0	0
2 (5)	0	4	1	0
3 (5)	0	0	4	1
4 (4)	0	0	0	4

Abbreviations: LLNA = murine local lymph node assay; OS ITS-2 lipid = integrated testing strategy for skin sensitization 2, implemented using R software and the lipid diffusion pathway for diffusion through the skin;

The numbers in parentheses show the number of chemicals predicted or observed in each category. Categories are based on LLNA potency: 1 = nonsensitizer; 2 = weak sensitizer (EC3 ≥10%), 3 = moderate sensitizer (1% ≤ EC3 <10%), 4 = strong/extreme sensitizer (EC3 <1%). Shaded cells highlight the correct category predictions

References

Dancik Y, Miller MA, Jaworska J, Kasting GB. 2013. Design and performance of a spreadsheet-based model for estimating bioavailability of chemicals from dermal exposure. Adv Drug Deliv Rev 65: 221–236.

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