SaferSkin™ V2.0

User Manual

SAFER WORLD BY DESIGN

Supported by: EdelweissConnect

Integrative Skin Sensitization Assessment Tool

PREFACE

SaferSkin™ is a web-based application developed by Edelweiss Connect GmbH (EwC) and provided on the SaferWorldbyDesign platform (SWbD) as part of the solution area «SaferSkin» for supporting the determination of key safety properties of skin, including irritation, tolerance, penetration, metabolism, and sensitisation. The SaferSkin™ web application currently contains an integrative skin sensitisation assessment tool.

There are two version available for **SaferSkin™:**

- **Demo version** is designed for guest users and comes with certain restrictions, including limitations on adding new compounds, making predictions, and generating automated reports. Users can select from a list of test molecules familiarising themselves with how to complete all required sections. However, the predicted results will not be displayed.

- **Professional version** offers the capability to predict skin sensitisation using all available approaches and to automate the generation of reports. To access the professional version, one of the available packages must be selected:
  1) **Saferskin Service Bronze package**: User licence to SaferSkin™ application and user support on the application use.
  2) **Saferskin Service Silver package**: User licence to SaferSkin™ application, user support on the application use, and 16 hours of modelling consulting on customer problems.
  3) **SaferSkin Service Gold package**: User licence to SaferSkin™ application, user support on the application use, 16 hours of modelling consulting on customer problems, Cloud-based data management for *in vitro* data (EdelweissData™), and 4200 EUR ($5,000) of *in vitro* testing (current catalogue value).

SaferSkin™ can be used as a Demo version but for commercial or regulatory reporting activities the Professional version is required.

Citation of results obtained with SaferSkin™ in academic publications should be indicated as follows:

- SaferWorldbyDesign, Edelweiss Connect GmbH (2024). SaferSkin™ V2.0: Integrative Skin Sensitisation Assessment Tool. https://saferworldbydesign.com/saferskin/in-silico/skin-sensitization-app/app/

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GLOSSARY

Table 1. Terms associated with experimental values

Term	Unit	Description
kDPRA/DPRA assay (KE1)		
DPRACys	%	Cysteine peptide depletion
DPRALys	%	Lysine peptide depletion
K_{max}	1/mM/min	Peptide reactivity Cor1-C420 assay
KeratinoSens™ assay (KE2)		
EC1.5	µM	Concentration yielding 1.5-fold induction in Nrf2-dependent luciferase activity
IC50	µM	Concentration yielding 50% reduction in cellular viability
Imax	/	The maximum fold-induction of luciferase activity compared to the untreated control.
h-CLAT assay (KE3)		
EC150	µg/mL	Concentration yielding 150% induction of the cell surface activation marker CD86
EC200	µg/mL	Concentration yielding 200% induction of the cell surface activation marker CD54
CV75	µg/mL	Concentration yielding 25% reduction in cell viability
LLNA (KE4)		
Mw	Da	Molecular weight
EC3	/	Effective Concentration for a stimulation index of 3-fold
pEC3	/	Logarithmic transformation of the EC3.

Table 2. Terms associated with molecular descriptors

Term	Unit	Description
LogD @ pH7	/	Octanol/water partition coefficient at pH 7 calculated using OPERA QSAR model.
$LogK_{ow}$	/	Octanol/Water partition coefficient, calculated using OPERA QSAR model.
Michael acceptor	/	If a compound is a Michael acceptor, a post-prediction correction is performed to accommodate for the anti-inflammatory effect of such chemicals.
Protein binding	%	Percent of compound bound to the plasma proteins as predicted using OPERA QSAR model.
TIMES-SS	/	The highest skin sensitisation class for the compound and its potential metabolites as predicted by TIMES-SS *in silico* model.
Toxtree	/	Structural alerts as identified by Toxtree.
Vapour pressure	Pa	Vapour pressure of the compound.
Water solubility@ pH7	M	Aqueous solubility at pH 7 calculated using OPERA QSAR model.

1) Overview of SaferSkin™ Application V2.0:

The SaferSkin™ Application provides Integrated Approaches to Testing and Assessment (IATA) that assesses skin sensitisation potency based on the following five approaches:

- **'2 out of 3' Voting**: A majority voting model that uses three different types of *in vitro* and *in chemico* tests to predict whether a chemical is a skin sensitiser or not in the LLNA assay. This is one of the defined approaches proposed by the OECD TG 497 guideline[1]. The required inputs for this model include: (i) Cys and Lys protein depletion capacity of a compound measured during the DPRA assay, (ii) the 1.5 and 3 – fold sensitisation gene expression capacity of a compound at a given concentration observed during the KeratinoSens™ assay, and (iii) CD54 and CD86 dendritic cell activation capacity of a compound measured during the h-CLAT assay.

- **OECD ITS** (Integrated Testing Strategy): A decision tree and scoring model that uses DPRA, h-CLAT *in vitro* assays and *in silico* tools (OECD QSAR TB or Derek Nexus) to predict GHS skin sensitisation potency class. This is one of the defined approaches proposed by the OECD TG 497 guideline[1].

- **Multiple Regression**: A simple and robust machine learning model based on multiple linear regression that uses inputs from kDPRA, KeratinoSens™ and compound's vapour pressure to predict compound's potency as the pEC3 value in the LLNA assay[2].

- **Neural Network**: A machine learning model that uses three different types of *in vitro*, DPRA, KeratinoSens™ and h-CLAT assays and *in silico* inputs (TIMES-SS and/or ToxTree) to predict skin sensitisation potency as the pEC3 value in the LLNA assay [3,4,5,6].

- **Bayesian Network**: The model uses molecular descriptors for its classification of a compound into skin sensitiser or skin non-sensitiser, including the octanol/water partition coefficient ($logK_{ow}$), the octanol/water partition coefficient (logD), the water solubility, the protein binding capacity and if the compound is a Michael acceptor or not. To increase the prediction accuracy, the experimental values from

[1] OECD Guideline No. 497: Defined Approachs on Skin Sensitisation https://www.oecd-ilibrary.org/environment/guideline-no-497-defined-approaches-on-skin-sensitisation_b92879a4-en

[2] Natsch, A., Emter, R., Gfeller, H., Haupt, T., & Ellis, G. (2015). Predicting skin sensitizer potency based on in vitro data from KeratinoSens and kinetic peptide binding: global versus domain-based assessment. *Toxicological sciences : an official journal of the Society of Toxicology*, *143*(2), 319–332.

[3] Im, J. E., Lee, J. D., Kim, H. Y., Kim, H. R., Seo, D. W., & Kim, K. B. (2023). Prediction of skin sensitization using machine learning. *Toxicology in vitro : an international journal published in association with BIBRA*, *93*, 105690.

[4] Hirota, M. et al. (2015). Evaluation of combinations of in vitro sensitization test descriptors for the artificial neural network-based risk assessment model of skin sensitization. *Journal of applied toxicology : JAT*, *35*(11), 1333–1347.

[5] Kleinstreuer, N. C., et al. (2018). Non-animal methods to predict skin sensitization (II): an assessment of defined approaches . Critical reviews in toxicology, 48(5), 359–374.

[6] Hirota, M., Ashikaga, T., & Kouzuki, H. (2018). Development of an artificial neural network model for risk assessment of skin sensitization using human cell line activation test, direct peptide reactivity assay, KeratinoSens™ and in silico structure alert parameter. Journal of applied toxicology : JAT, 38(4), 514–526.

DRPA, KeratinoSens™ and h-CLAT assays as well as the *in silico* QSAR TIMES-SS result can be included.

2) Main Functions of SaferSkin™ Application V2.0:

- ❖ Predict the skin sensitisation potential of a single compound using five distinct approaches, two of which are defined approaches based on OECD Guideline 497 (2 out of 3 and the ITS), and three other approaches namely Multiple Regression, Neural Network, and Bayesian Network.
- ❖ Provide a confidence level for the skin sensitisation prediction.
- ❖ Calculate the molecular descriptors of compounds automatically.
- ❖ Generate comprehensive reports for all results obtained from the approaches. The report can be used for regulatory purposes and is printable and can also be saved as a PDF file.
- ❖ Provide transparency in various comparative model prediction processes.
- ❖ Support case studies comparing the results of different approaches.

3) Description of Inputs

3.1. *in vivo*

- ❖ **LLNA**

The local lymph node assay (LLNA) addresses KE4, activation and proliferation of antigen-specific T-cells. The concentration of a substance that is expected to cause a 3-fold increase in lymphocyte proliferation in the lymph nodes compared to the background level is known as EC3. The pEC3= log (Mw/(250*EC3(%))), a measure of potency was considered in the Bayesian network model. The pEC3 ranges for which each classification category falls: Non-sensitiser (< -1.9), Weak (-1.9, -1.1), Moderate (-1.1, -0.35), Strong (> -0.35).

3.2. *in vitro*

- ❖ **DPRA**

The direct peptide reactivity assay (DPRA) addresses KE1, which involves the binding of compounds to proteins. In the DPRA, a chemical's reactivity is measured based on its ability to bind to synthetic peptides containing either cysteine or lysine. Two parameters are represented: Cysteine peptide depletion (DPRACys) and Lysine peptide depletion (DPRALys).

❖ **kDPRA**

The kinetic direct peptide reactivity assay (kDPRA) is an enhanced version of the traditional DPRA by measuring the rate of peptide depletion over time. One parameter is represented: the maximum rate constant of peptide depletion by the test chemical (K_{max}), which quantifies the highest rate at which a chemical can react with a synthetic peptide containing either cysteine or lysine.

❖ **KeratinoSens™**

The KeratinoSens™ assay addresses KE2 known as the activation of keratinocytes by assessing the activation of the Keap1-Nrf2-ARE pathway. Three parameters are represented: (i) the concentration yielding 1.5-fold induction in Nrf2-dependent luciferase activity (EC1.5), (ii) the concentration yielding 50 % reduction in cellular viability (IC50), and (iii) the maximum fold-induction of luciferase activity compared to the untreated control (Imax).

❖ **h-CLAT**

The h-CLAT assay addresses the KE3 known as the dendritic cell activation. It quantifies changes in the expression of cell surface molecules (CD54 and CD86). Three parameters are included: (i) the concentration yielding 200% induction of the cell surface activation marker CD54 (EC200), (ii) the concentration yielding 150% induction of the cell surface activation marker CD86 (EC150), and (iii) the concentration yielding 25% reduction in cell viability (CV75).

3.3. *in silico*

❖ **OPERA**

OPERA[7] (v2.9) is a free and open-source suite of QSAR models providing predictions for physico-chemical properties of the tested molecules including: (i) the octanol-water partition coefficient of the tested compound at pH 7 (logD@pH7) reflecting how the compound's solubility in water changes due to its ionization state, (ii) the water solubility at pH 7, (iii) the octanol-water partition coefficient ($logK_{ow}$) at equilibrium, and (iv) the percent of compound bound to the plasma proteins (protein binding).

❖ **Derek Nexus**

Derek Nexus[8] (v6.3) is a modelling software that predicts EC3 values for compounds of interest. can classify compounds into Five potency classes can be derived based on this EC3 prediction: non-sensitisers, weak, moderate, strong, and extreme sensitisers. For the modelling we merged the classes of strong and extreme sensitisers into a single

[7] Mansouri, Kamel, Chris M. Grulke, Richard S. Judson, and Antony J. Williams. (2018). 'OPERA Models for Predicting Physicochemical Properties and Environmental Fate Endpoints'. Journal of Cheminformatics 10(1).92879a4-en

[8] Lhasa Limited. (2024). Derek Nexus [Software]. Available from https://www.lhasalimited.org/solutions/skin-sensitisation-assessment/ (accessed 12.06.2024)

class. We have included the support of inputs from Derek Nexus in the OECD TG 497 ITS model.

❖ **OECD QSAR ToolBox**

The OECD QSAR Toolbox[9] (v4.6) is a software application developed by the OECD to support the assessment of chemical hazards by using the Quantitative Structure-Activity Relationship (QSAR) approach.

❖ **ToxTree**

ToxTree[10] (v3.1.0) is an open-source software tool designed to assess the potential toxicity of chemicals through decision tree-based approaches.

❖ **TIMES-SS**

TIMES-SS[11] (TIssue MEtabolism Simulator for Skin Sensitisation, v2.32.1) is a computational model that uses mechanistic models that predict the skin sensitisation potential of chemicals.

4) Integrated Workflow of Scoring for 2o3/ITS

4.1. Decision tree of 2o3

The decision tree, outlined in OECD Guideline 497, provides a structured approach for assessing skin safety risks. This guideline thoroughly describes the utilisation of three standardised *in vitro* methods: DPRA, KeratinoSens™, and h-CLAT. Each of these assays is tailored to identify a specific molecular key event that triggers skin sensitisation. For the results to be considered conclusive, at least two of the three assays must yield clear outcomes. If this is not achieved, the results are deemed inconclusive, necessitating the inclusion of additional data for further evaluation.

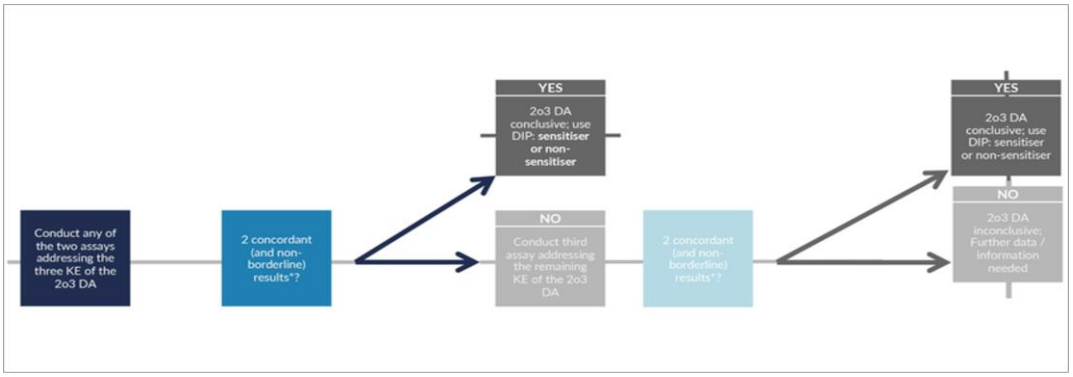


Figure 1. OECD decision tree of 2o3 defined approach.

[9] OECD. (2023). QSAR Toolbox version 4.6. Retrieved from https://qsartoolbox.org/download/ (accessed 12.06.2024)

[10] Ideaconsult Ltd. (2018). ToxTree version 3.1.0 [Software]. Retrieved from https://toxtree.sourceforge.net/ (accessed 12.06.2024)

[11] Laboratory of Mathematical Chemistry. (2023). TIMES-SS version 21.26. Retrieved from http://oasis-lmc.org/ (accessed 12.06.2024)

4.2. Standardisation of ITS scoring and potency

The OECD ITS employs a strategy where data from various sources are evaluated simultaneously. In this approach, a range of specific methodologies, including statistical and mathematical models, are used to transform the data from these diverse sources into a coherent prediction.

Score	h-CLAT MIT µg/mL	DPRA mean Cysteine and Lysine % depletion	DPRA Cysteine % depletion	*in silico*
3	≤10	≥42.47	≥98.24	Positive
2	>10, ≤150	≥22.62, <42.47	≥23.09, <98.24	
1	>150, ≤5000	≥6.38, <22.62	≥13.89, <23.09	
0	not calculated	<6.38	<13.89	Negative

Total Battery Score	Classification
6-7	UN GHS 1A
2-5	UN GHS 1B
0-1	Not classified

Figure 2. OECD standardisation of ITS scoring and potency. (MIT= Minimum Induction Threshold, GHS 1A: Strong sensitiser, GH1B: Other sensitiser).

5) Sign Up and Log In

To access only the Demo version, you can bypass the sign-up process.

By simply entering the page (https://saferworldbydesign.com/saferskin/in-silico/skin-sensitization-app/app/), you will automatically be granted access to the Demo version.

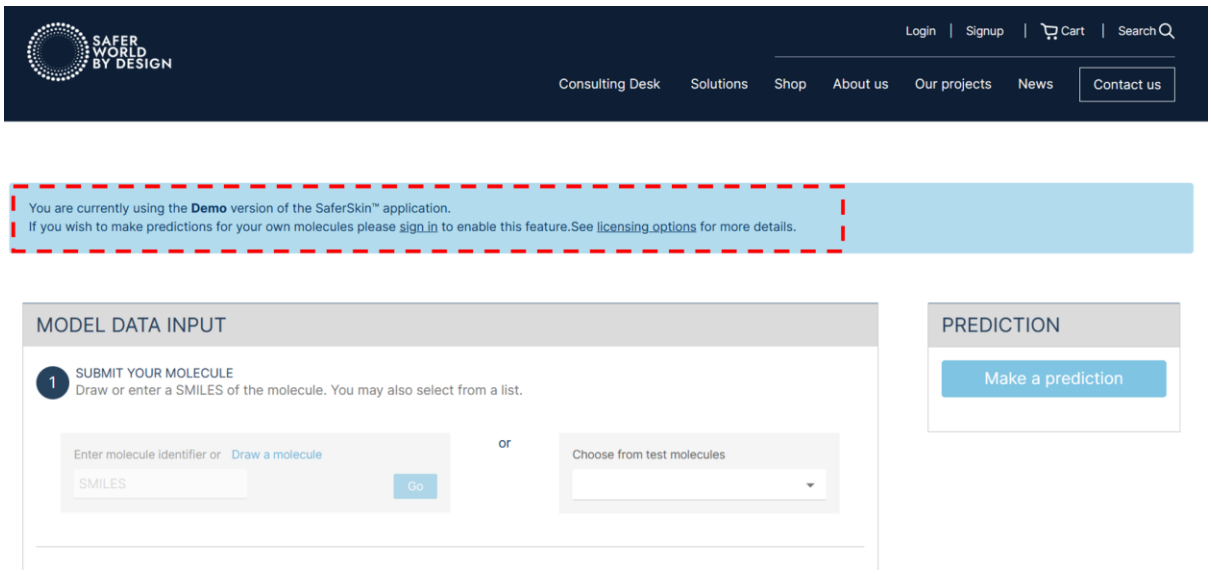


Figure 3. SaferSkin™ Application interface (Demo version).

To access the Professional version, sign up (enter the email and the password) and order the service package that best meets your needs.

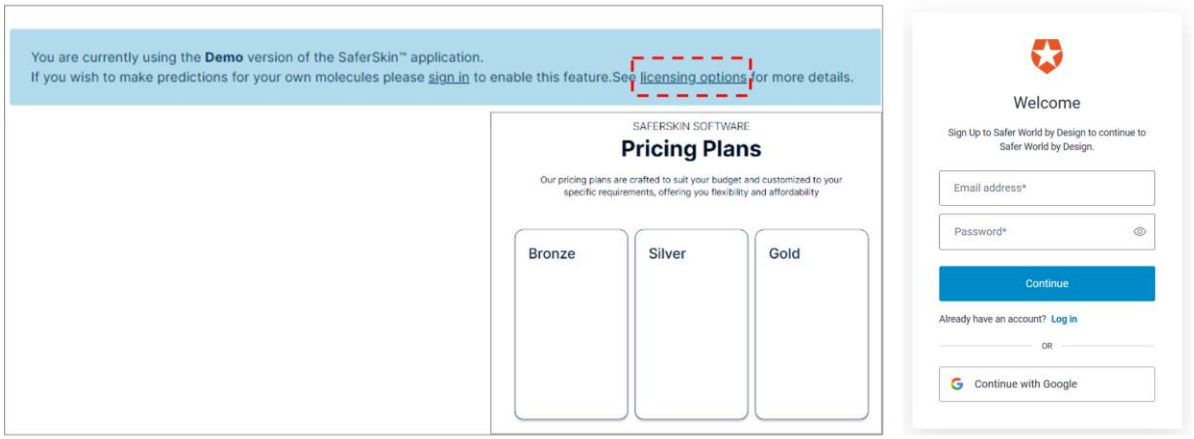


Figure 4. SaferSkin™ Application sign up and licensing screen.

Once you have ordered your package and contacted the SaferSkin representative, your account will be activated, granting you access to the Professional version.

To log in press the "Log In" button, enter your email address and password, then press the "Continue" button.

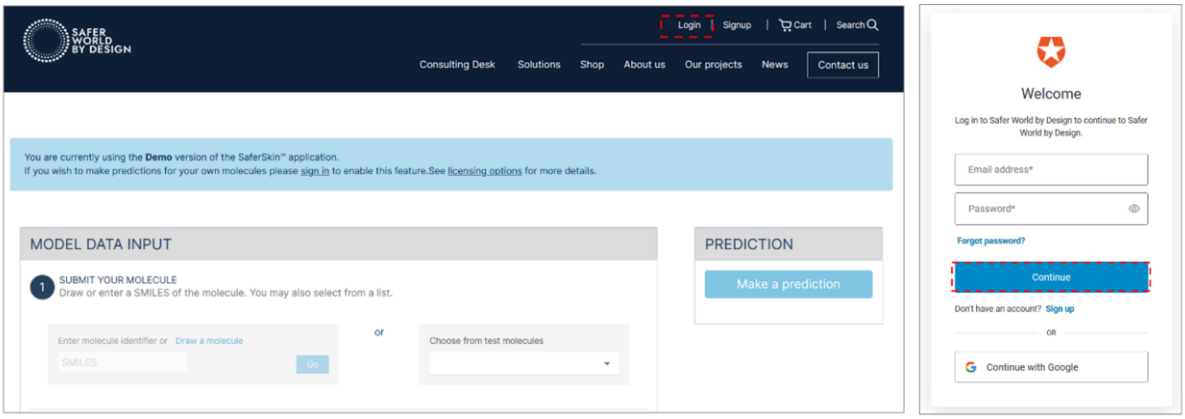


Figure 5. SaferSkin™ Application log in screen.

If you have forgotten your password, click on the "Forget password" link at the bottom of the page. Then, enter the email address associated with your account to receive an email with instructions on how to set a new password.

After Log in, the interface changes from the Demo to the Professional version, you will be able to access the profile section. Here, you can update your personal information. This section also includes details of your billing and orders.

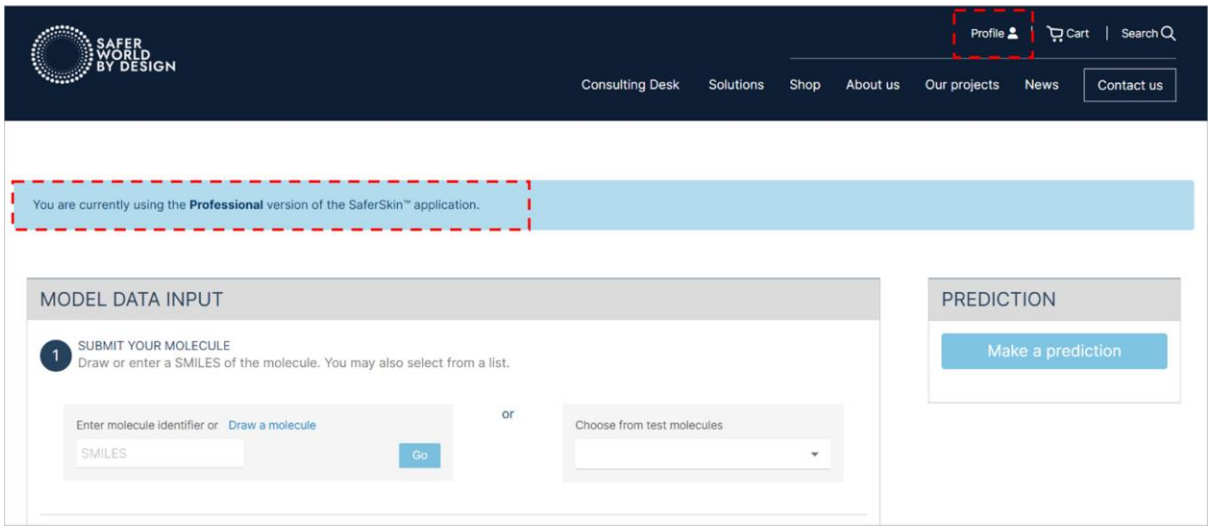


Figure 6. SaferSkin™ Application interface (Professional version).

6) Molecule Submission

The following steps pertain to the Professional version:

- Enter the SMILES of the molecule into the designed field or use the option to draw the structure.

- If you have an SDF file, simply drag and drop it into the drawing panel. This action will load the 3D structure of the molecule.

- Alternatively, before trying your molecule, you can select a molecule from the provided list to explore the application.

Note: Entry is limited to one single molecule at a time.

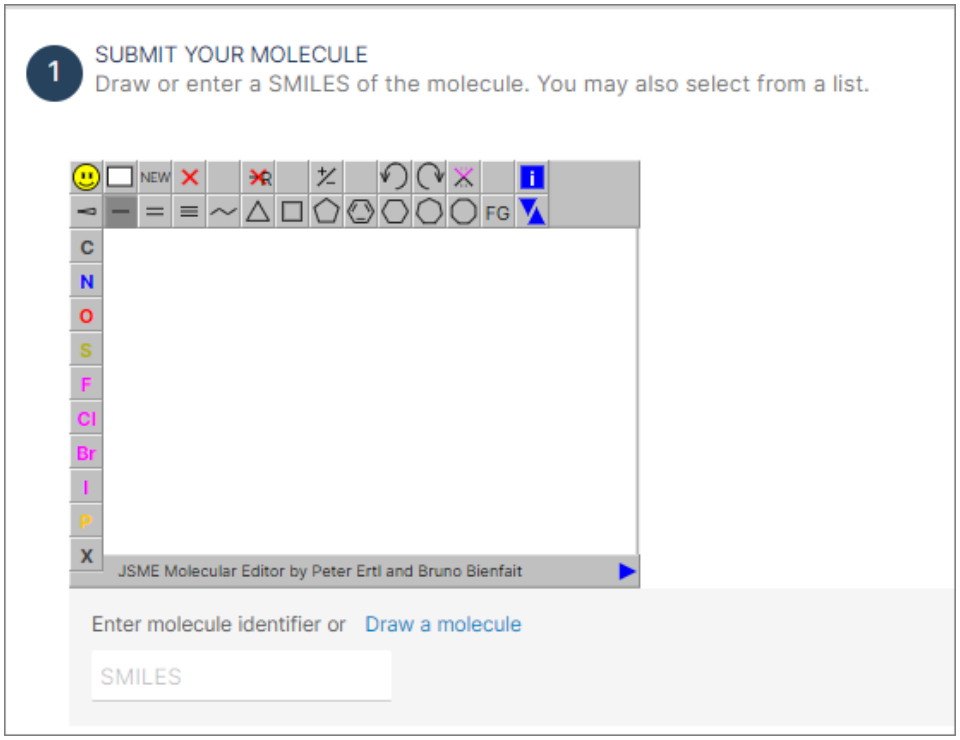


Figure 7. SaferSkin™ Application draw screen.

- Press the "GO" button to initiate the process.

- The chemical structure of the molecule in question will be displayed under the "Prediction" panel.

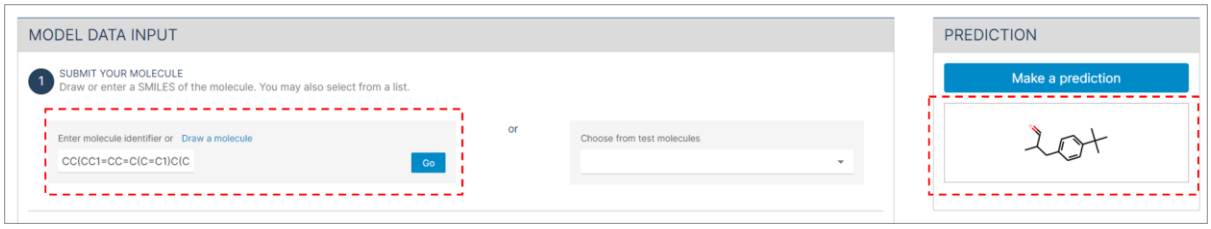
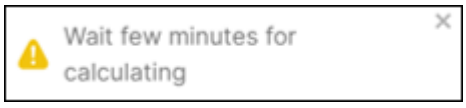


Figure 8. SaferSkin™ Application molecule submission screen (example: lilial).

7) Molecular Descriptors Generation

- After submitting the molecule, by pressing the Go button, the automatic generation of molecular descriptors will initiate.

- Allow a few minutes for the calculation of molecular descriptors to complete.



- The molecular descriptors will be displayed under the Bayesian Network section of the interface.

- If you have more accurate estimates or if there are missing pieces of information, you may modify the displayed values as needed.

Note: Michael acceptor class (yes/no) need to be entered by the user.

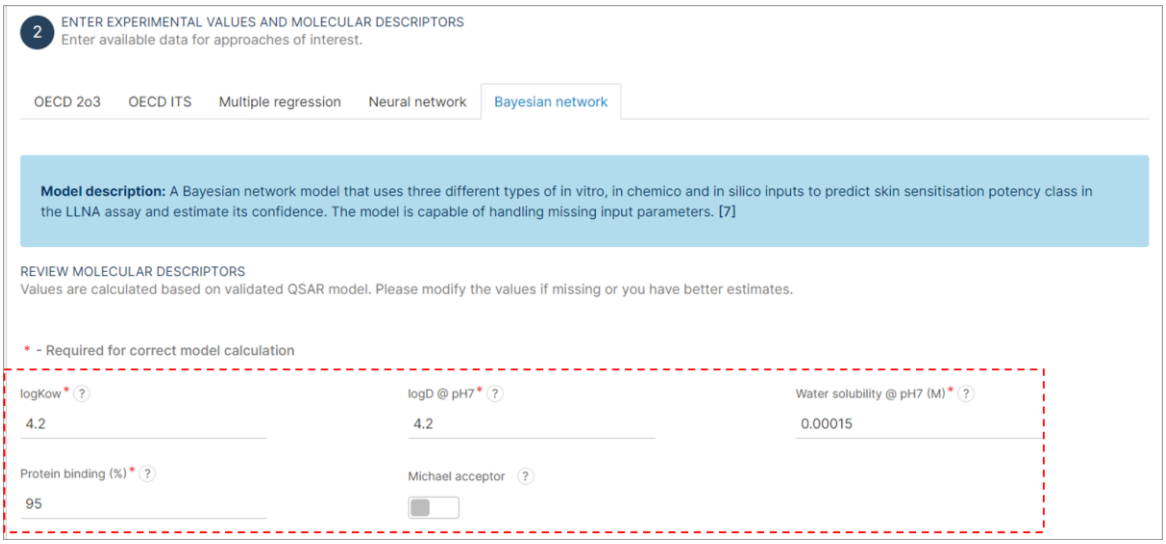


Figure 9. SaferSkin™ Application molecular descriptors screen (example: lilial).

- At this stage, you can use the molecular descriptors to predict the skin sensitisation potential of the molecule:

1. Press the "Make a prediction" button to initiate the prediction process.
2. Please be aware that the confidence level of this prediction is considered weak as only molecular descriptors are submitted.

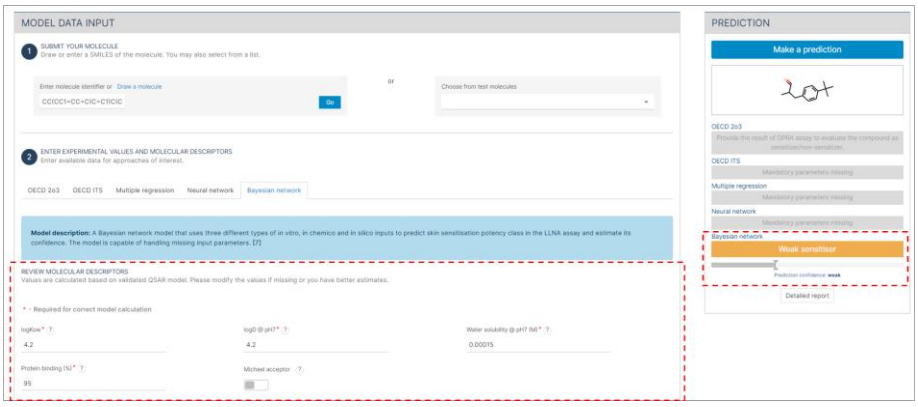


Figure 10. SaferSkin™ Application prediction screen based on molecular descriptors (example: lilial).

8) Experimental Values Input

If you have experimental values available from the validated alternative assays, follow the steps below to input them. These assays include:

- ❖ DPRA (Direct Peptide Reactivity Assay)
- ❖ KeratinoSens™
- ❖ h-CLAT (Human Cell Line Activation Test)

- Input the experimental values of each assay in the appropriate field.

- For detailed information about each assay and the expected input values: Click on the "?" buttons next to each assay entry field.

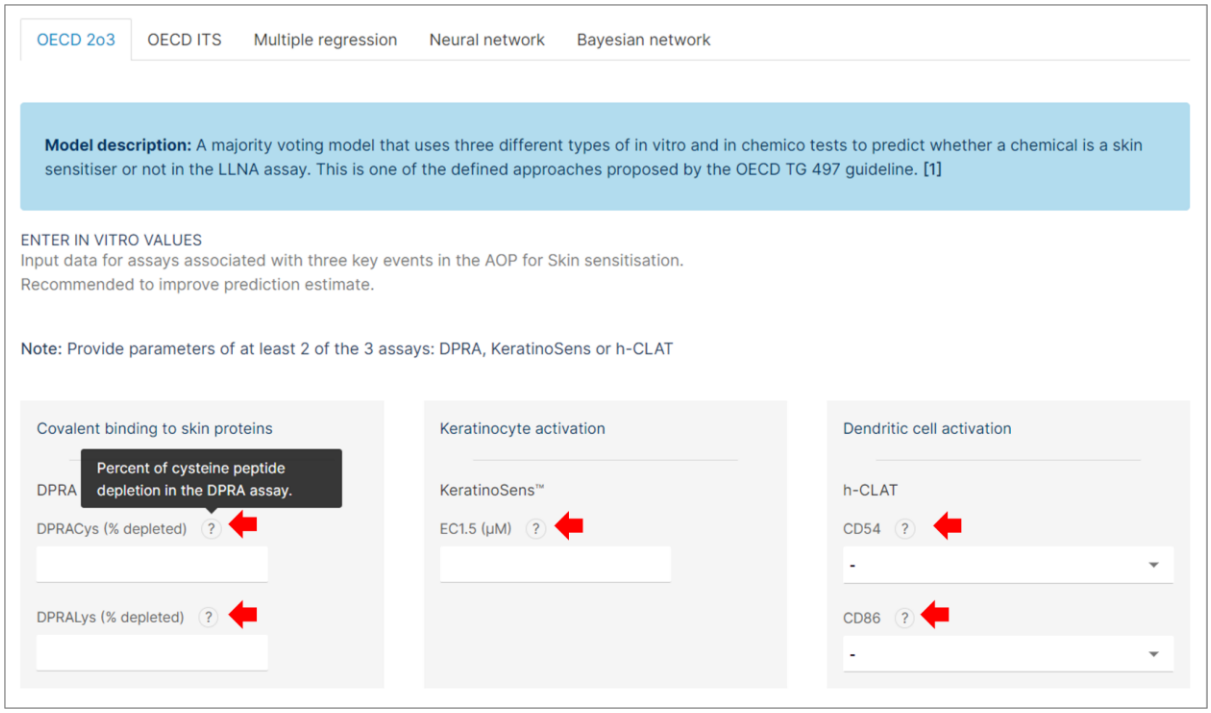


Figure 11. SaferSkin™ Application experimental values input screen.

- When values are entered for a specific assay in one interface, these values will be automatically filled in the corresponding assay field of any other related interface.

- ❖ *e.g*, Entering data for DPRACys (% depleted) in the "OECD 2o3" interface will result in the same data being auto-filled in the DPRACys (% depleted) field of "OECD ITS" interface.

Note: Entering all required experimental values is recommended as it will enhance the accuracy of the prediction.

9) Physico-Chemical Property Value Input

- Find the section of "Multiple regression" interface.

- Click on the field labeled as "Vapour Pressure". This field is mandatory and must be filled to proceed with the analysis. It is marked with an asterisk (*) to indicate its importance.

- Enter the numerical value of the vapour pressure as specified by the interface (Pa).

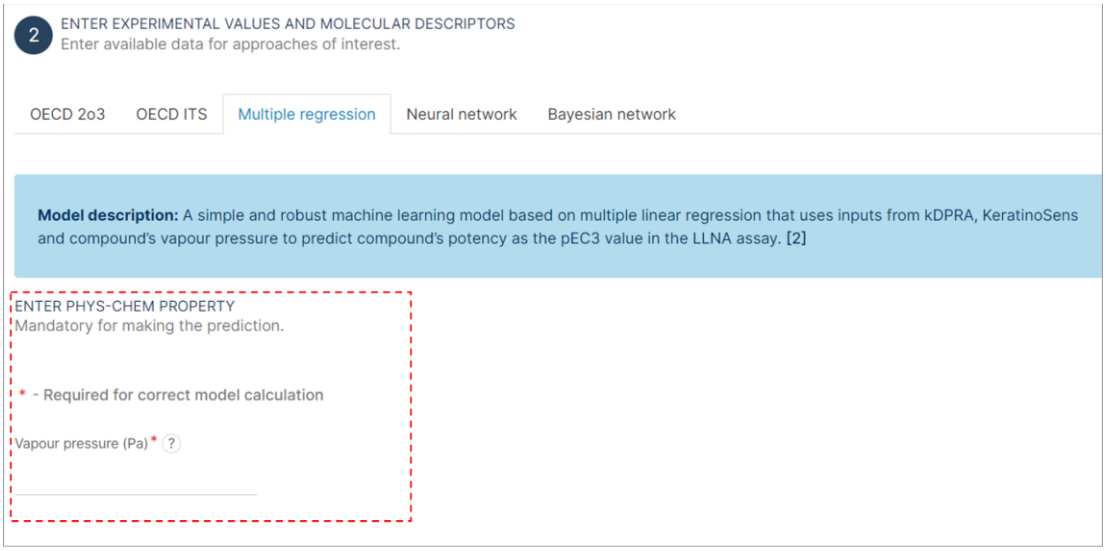


Figure 12. SaferSkin™ Application physico-chemical property value input screen.

10) *in silico* Data Input

- Choose from the following interfaces to input your data:

- ❖ OECD ITS (*in silico* input is mandatory)
- ❖ Neural Network
- ❖ Bayesian Network

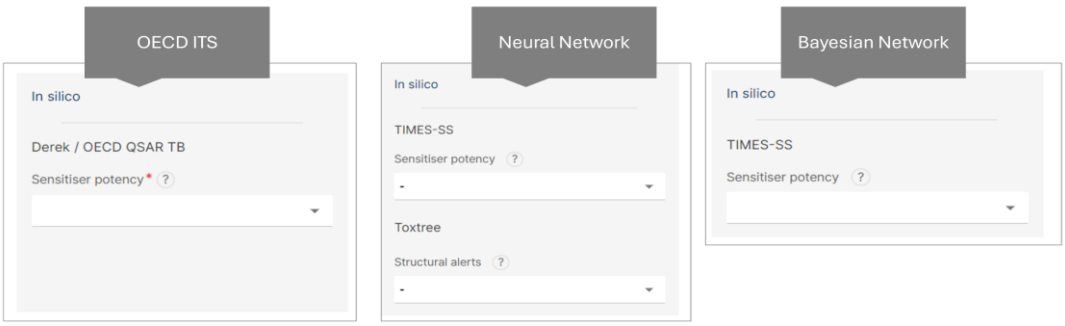


Figure 13. SaferSkin™ Application *in silico* input screen.

11) Prediction

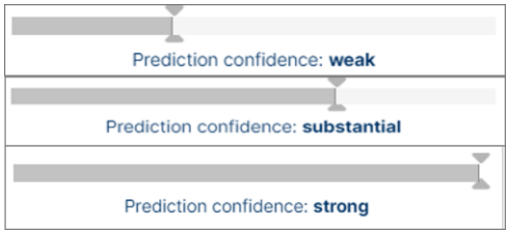
- Click on the blue "Make a prediction" button at the top.

- Check under all the sections for its prediction, labeled as "**Not a sensitiser**" (Green), "**Weak sensitiser**" (Light Orange), "**Moderate Sensitiser**" (Orange), "**Strong Sensitiser**" (Red).

The label differs for OECD 2o3 and ITS. Please check the label in the following table:

OECD 2o3	OECD ITS	Multiple regression	Neural network	Bayesian network
Not a sensitiser	Not a sensitiser	Not a sensitiser	Not a sensitiser	Not a sensitiser
Sensitiser	1B (Other Sensitiser)	Weak Sensitiser	Weak Sensitiser	Weak Sensitiser
	1A (Strong)	Moderate Sensitiser	Moderate Sensitiser	Moderate Sensitiser
		Strong Sensitiser	Strong Sensitiser	Strong Sensitiser

- Look at the "Prediction confidence" slider to understand the confidence level, which is marked as "weak", "substantial" or "strong".



- If the necessary values are not provided or are incomplete, it will not be possible to make predictions due to the missing mandatory section.

- Click on the "Detailed report" button at the bottom for an in-depth analysis.

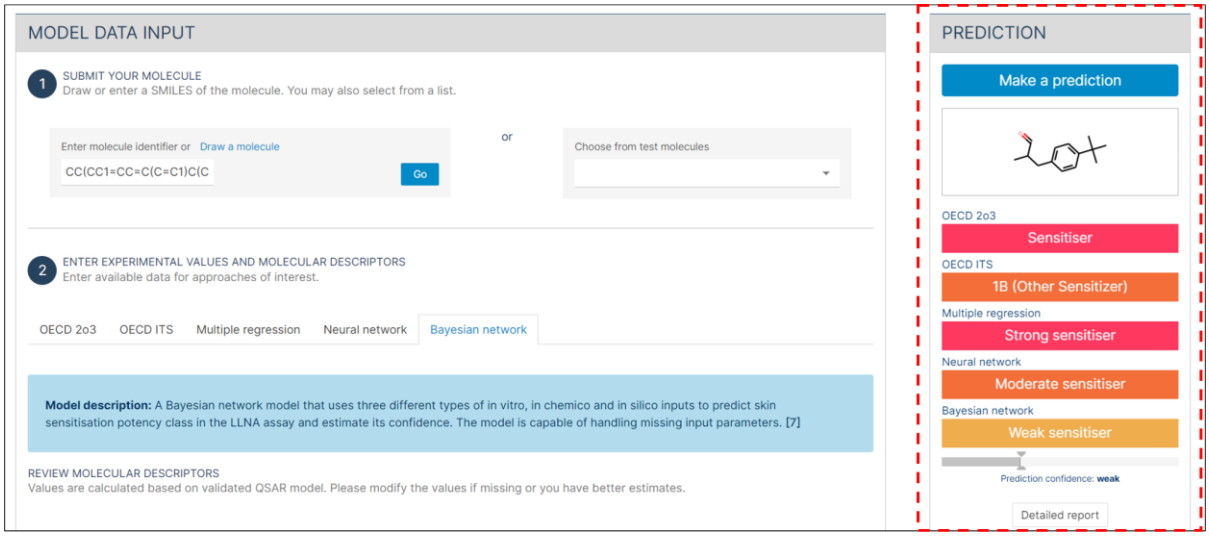


Figure 14. SaferSkin™ Application prediction input screen (example: lilial).

12) Report

12.1. Report Analysis

A summary of report is presented at first which includes the binary classification (sensitizer/not a sensitiser) for OECD DA 2o3 and the three-tier GHS potency classification (1A, 1B and not a sensitiser) for ITS, as well as the four-tier potency classification (not a sensitizer, weak sensitiser, moderate sensitiser, or strong sensitiser) available for Multiple regression, Neural network, and Bayesian network approaches.

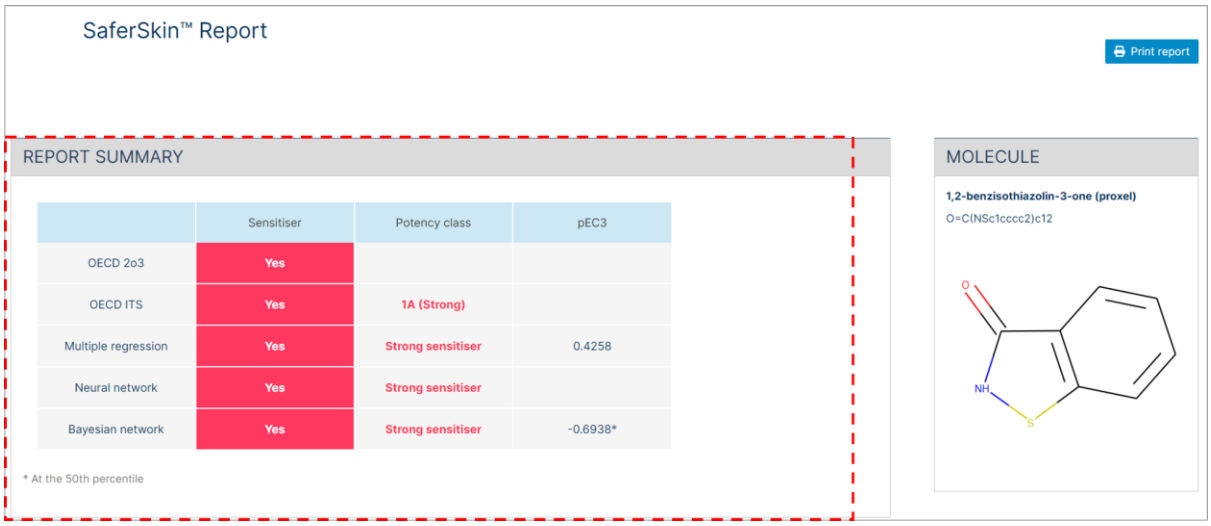


Figure 15. Summary report of the 5 different approaches in SaferSkin™ (example: proxel).

Following the summary report, a detailed report for each approach is presented. Each detailed report is composed of "Safety assessment guide" (*e.g.*, sensitisation category, EC3, pEC3),

"Input parameters" (experimental values, molecular descriptors, *in silico* prediction), and "Calculated values" section generated for the Bayesian network approaches.

Figure 16. Detailed report of OECD 2o3 in SaferSkin™ (example: proxel).

❖ The OECD ITS detailed report also includes a display of the battery score.

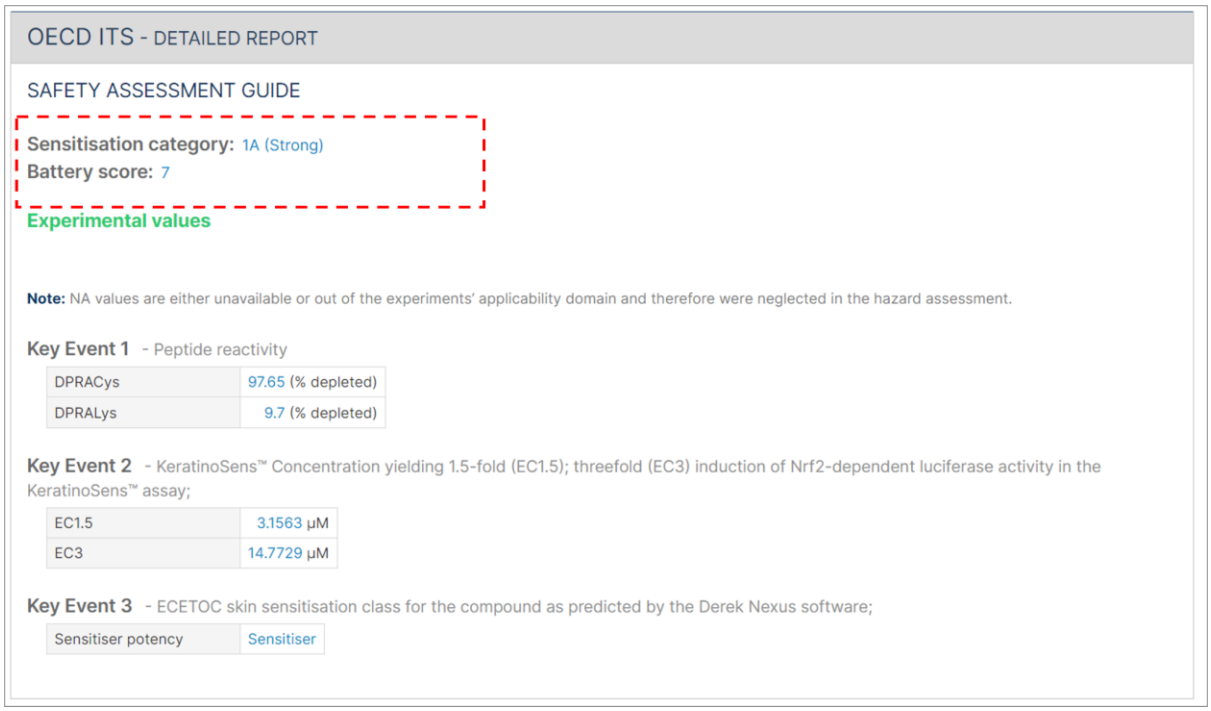


Figure 17. Detailed Report of OECD ITS in SaferSkin™ (example: proxel).

❖ The Multiple regression detailed report includes the pEC3 result and the sensitisation category. The sensitisation category (1 = Not a sensitiser, 2 = weak sensitiser, 3 = moderate sensitiser, or 4 = strong sensitiser) is derived from the pEC3.

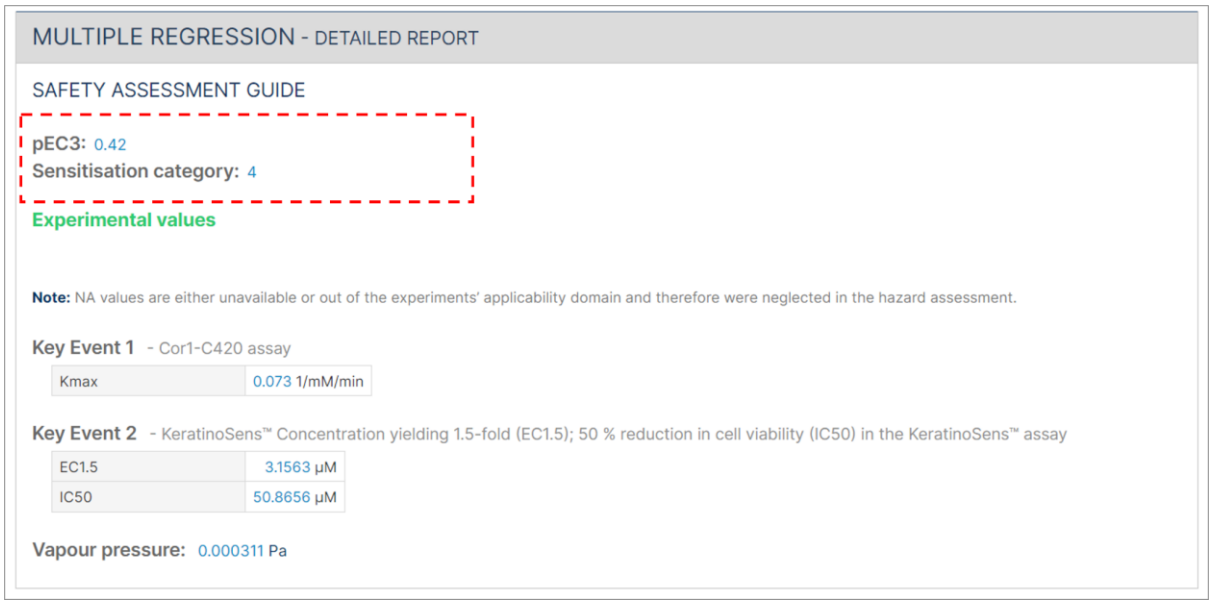


Figure 18. Detailed report of multiple regression in SaferSkin™ (example: proxel).

❖ In cases where not all required information is submitted, the safety assessment will indicate "NA" instead of providing a pEC3 value.

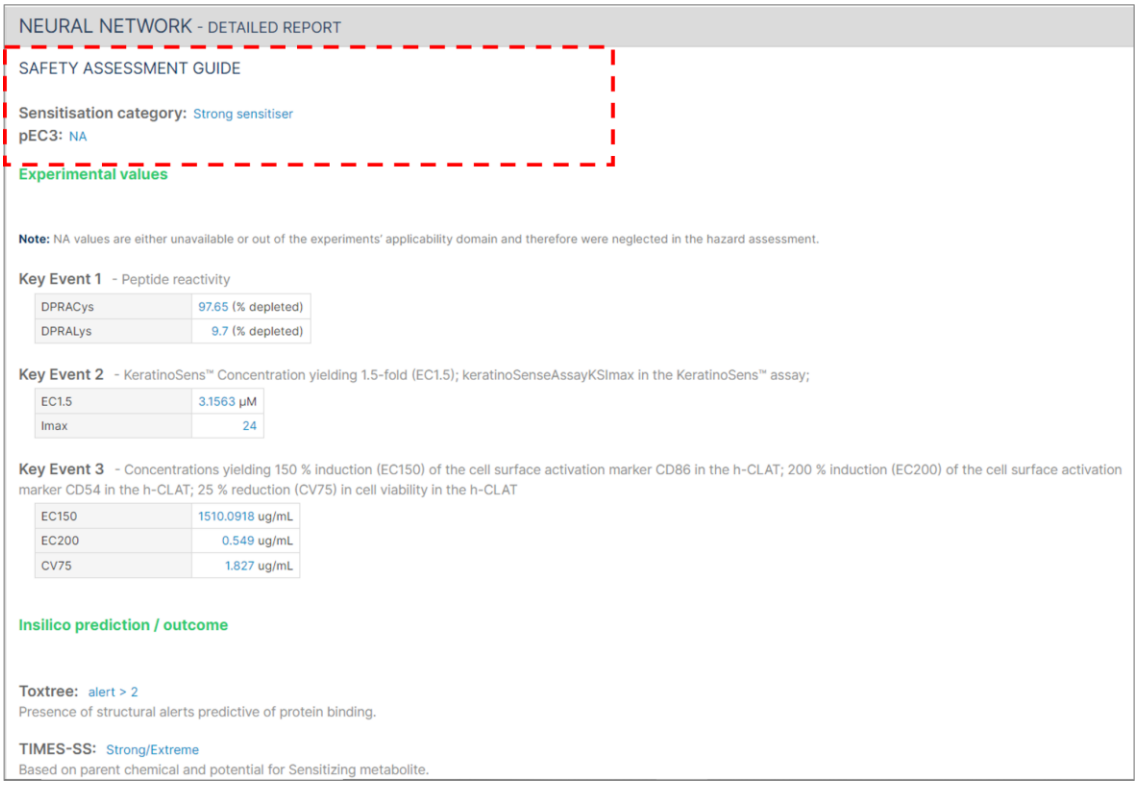


Figure 19. Detailed report of neural network in SaferSkin™ (example: proxel).

- The "Safety Assessment Guide" for the Bayesian network detailed report includes the EC3 and pEC3 at all the percentiles (50th, 60th, 70th, 80th and 90th). A potency probability distribution based on exploring the 50th - 90th percentiles of the pEC3 for the molecule is generated.

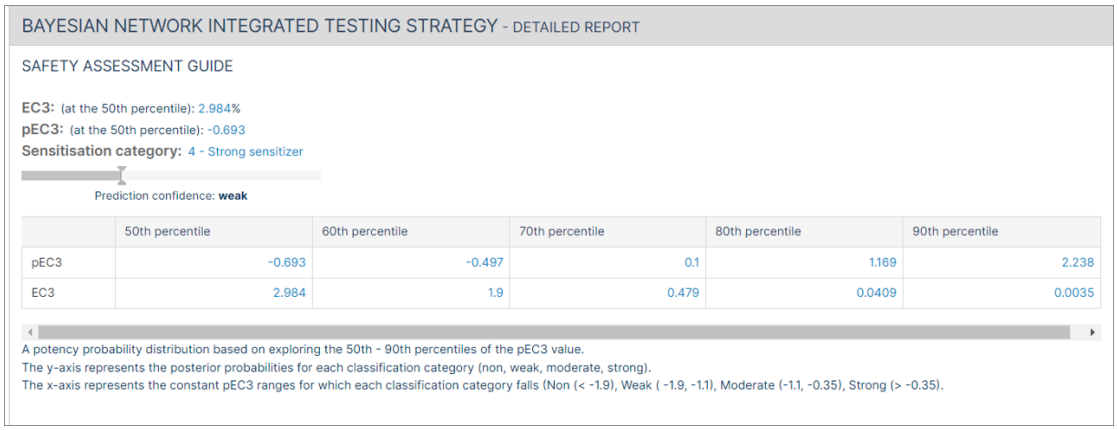


Figure 20. Safety assessment guide of Bayesian network in SaferSkin™ (example: proxel).

- The "Calculated values" section is specific to the Bayesian network model, which includes:

1. Prior Probabilities: The prior probabilities are always: Non= 0.2653, weak= 0.2653, moderate= 0.2721, Strong= 0.1973 (as calculated from Jaworska *et al.* 2015[7])

2. Posterior probabilities: The calculated posterior probabilities may change with each molecule prediction.

3. Michael acceptor correction: In the instance where a compound is designated as a Michael acceptor, the posterior probabilities are adjusted to account for the anti-inflammatory properties of the compound.

4. The Bayes factor is an indication of the strength of evidence for accepting the prediction. It quantifies the uncertainty to aid in decision-making.

5. The compound is classified based on the category with the highest Bayes factor. The larger the Bayes factor, the stronger the evidence to support a prediction. <1 = Negative (evidence supports an alternative); 1–3 = Weak; 3–30 = Substantial; >30 = Strong.

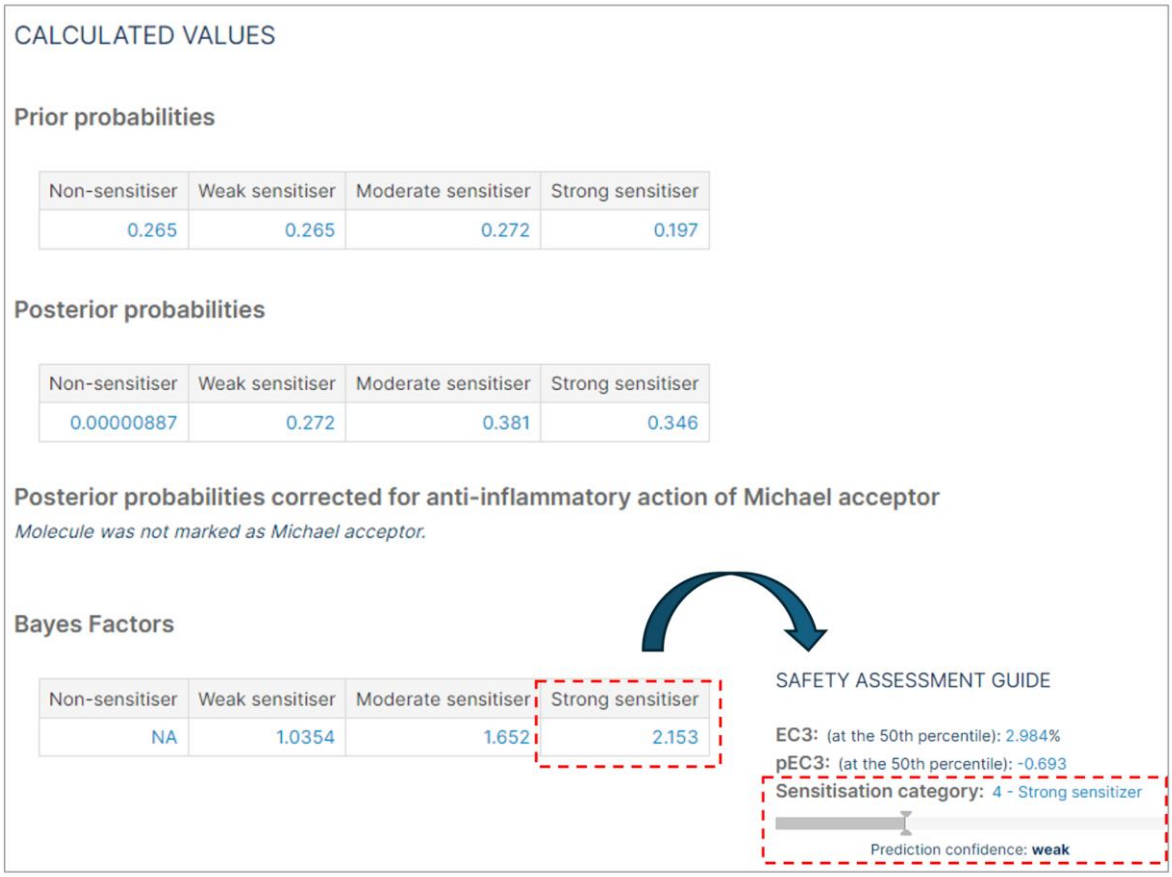


Figure 21. Calculated values section of Bayesian network in SaferSkin™ (example: proxel).

12.2. Printing/Saving Report

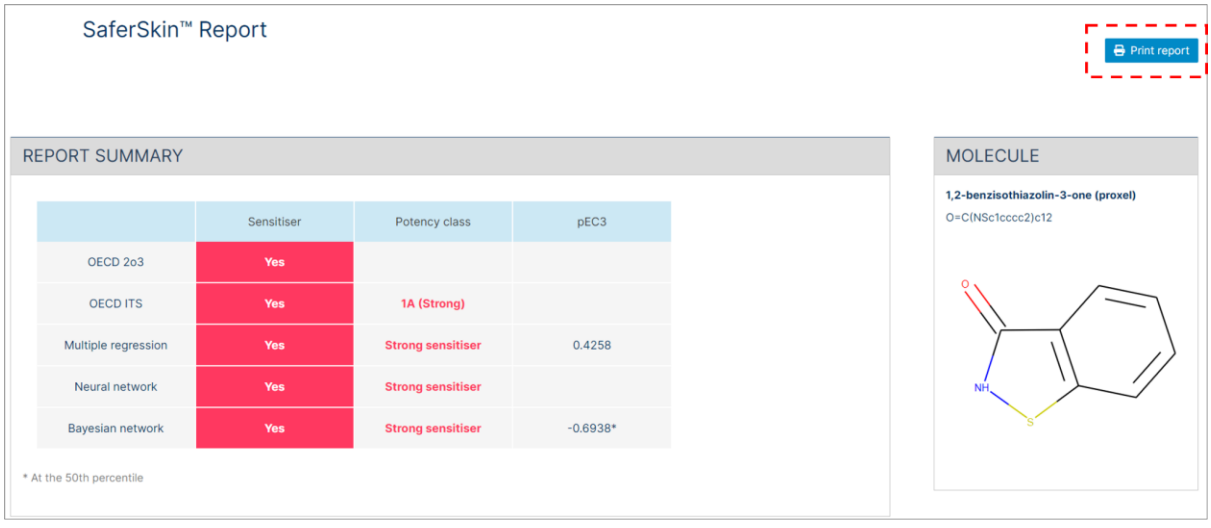


Figure 22. SaferSkin™ Application result report screen (example: proxel).

When you click the "Print Report" button, the result report will be generated and can be saved as a PDF or printed.

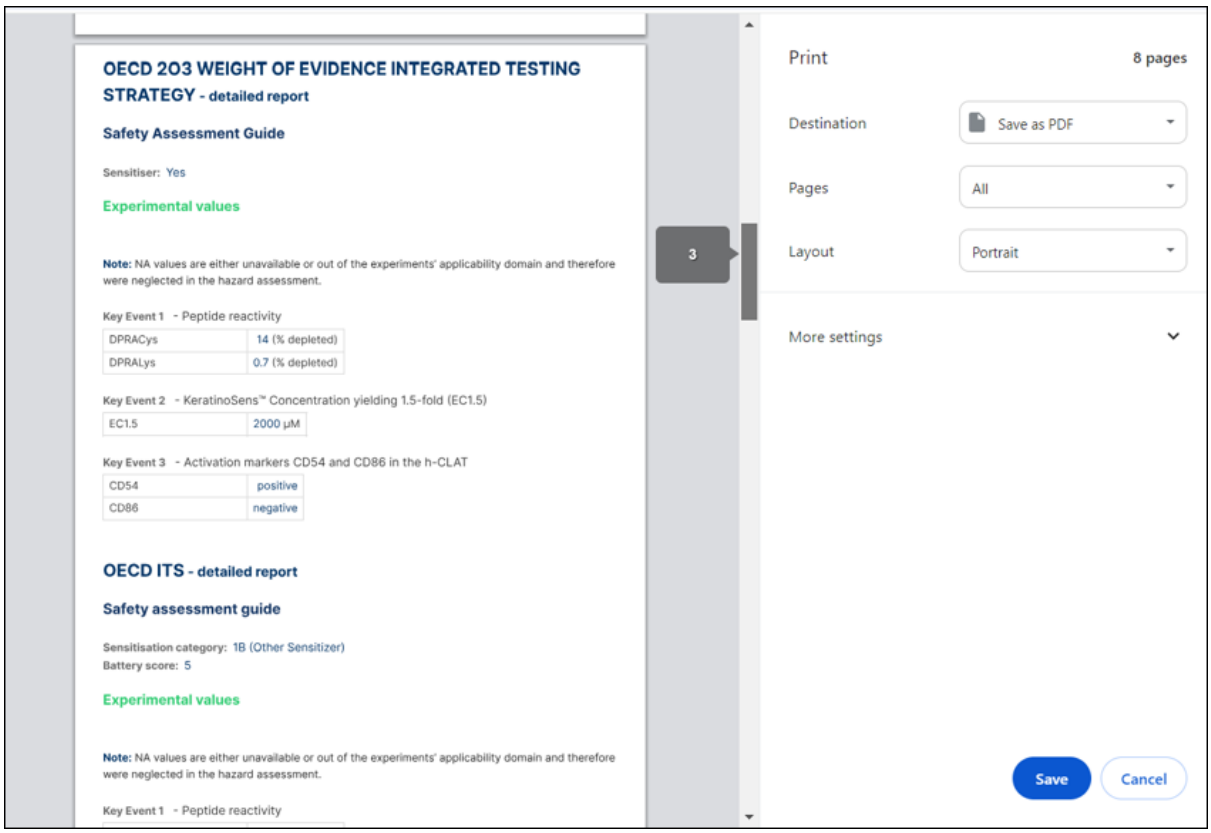


Figure 23. SaferSkin™ Application result report (example: proxel).