SaferSkin[™] V2.0

User Manual





Integrative Skin Sensitization Assessment Tool

Supported by: EdelweissConnect

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 **SaferSkinTM**:

- 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) <u>Saferskin Service Bronze package</u>: User licence to SaferSkin[™] application and user support on the application use.
 - 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) <u>SaferSkin Service Gold package</u>: 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 SaferSkinTM in academic publications should be indicated as follows:

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

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GLOSSARY

| I doite It I trimp t | | |
|-----------------------------------|-------------------------|--------------------------------------------------------------------------------------|
| 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 TM | ¹ assay (KE2 | |
| EC1.5 | μΜ | 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 1. Terms associated with experimental values

Table 2. Terms associated with molecular descriptors

| Term | Unit | Description |
|-------------|------|---------------------------------------------------------------|
| LogD @ pH7 | / | Octanol/water partition coefficient at pH 7 calculated using |
| 6 1 | | OPERA QSAR model. |
| LogK | / | Octanol/Water partition coefficient, calculated using OPERA |
| LOGKOW | / | QSAR model. |
| Michael | | If a compound is a Michael acceptor, a post-prediction |
| WICHAEL | / | correction is performed to accommodate for the anti- |
| acceptor | | inflammatory effect of such chemicals. |
| Protein | 0/ | Percent of compound bound to the plasma proteins as |
| binding | %0 | predicted using OPERA QSAR model. |
| | | The highest skin sensitisation class for the compound and its |
| TIMES-SS | / | potential metabolites as predicted by TIMES-SS in silico |
| | | model. |
| Toxtree | / | Structural alerts as identified by Toxtree. |
| Vapour | Do | Vanour process of the compound |
| pressure | Pa | vapour pressure of the compound. |
| Water | | A queeue colubility et pH 7 coloulated using ODER A OS A P |
| solubility@ | Μ | Aqueous soluointy at pri / calculated using OPERA QSAR |
| pH7 | | model. |

1) Overview of SaferSkinTM 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 guideline¹. 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 KeratinoSensTM 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 guideline¹.
- Multiple Regression: A simple and robust machine learning model based on multiple linear regression that uses inputs from kDPRA, KeratinoSensTM and compound's vapour pressure to predict compound's potency as the pEC3 value in the LLNA assay².
- Neural Network: A machine learning model that uses three different types of *in vitro*, DPRA, KeratinoSensTM 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
 Sensitisation
 Sensitisation

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 SaferSkinTM 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

OPRA

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.

✤ KeratinoSensTM

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

OPERA⁷ (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 Nexus⁸ (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)

⁶

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

OECD QSAR ToolBox

The OECD QSAR Toolbox⁹ (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

ToxTree¹⁰ (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-SS¹¹ (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 203

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, KeratinoSensTM, 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 203 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 (<u>https://saferworldbydesign.com/saferskin/in-silico/skin-sensitization-app/app/</u>), you will automatically be granted access to the Demo version.

| SAFER | | | | | Login Signu | ەظر∣∾ | Cart Search Q |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|-----------------|---------|----------|---------------|-----------|-----------------|
| BY DESIGN | Consulting Desk | Solutions | Shop | About us | Our projects | News | Contact us |
| | | | | | | | |
| You are currently using the Demo version of the SaferSkin" application. If you wish to make predictions for your own molecules please sign in to enable thi | s feature.See <u>licensing opt</u> i | ons for more de | etails. | 3 | | | |
| | | | | | | | |
| MODEL DATA INPUT | | | | | PREDI | CTION | |
| 1 SUBMIT YOUR MOLECULE Draw or enter a SMILES of the molecule. You may also select from a list | t. | | | | М | ake a pre | ediction |
| Enter molecula identifier or Draw a molecula | Choose from test r | molecules | | | | | |
| SMILES Go | Choose non test | noiecules | ÷ | | | | |
| | | | | | | | |

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

8

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

| You are currently using the Demo version of the SaferSkin [™] application If you wish to make predictions for your own molecules please <u>sign in</u> to | o enable this feature. | See licensing optic | ons for more details. | Welcome | |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|----------------------------------------------------------------------------|-----------------------|
| | Our pricing plans | SAFERSKIN SOFTW Pricing Pla are crafted to suit your bud purements, offering you flexi | /ARE ans light and customized to your bility and affordability | Sign Up to Safer World by Design Safer World by Desig Email address* | to continue to gn. |
| | Bronze | Silver | Gold | Password* | ٢ |
| | | | | Continue Already have an account? Log in OR | |
| | | | | G Continue with Google | |

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.

| SAFER | | | | | Login Signup | ەقر ا | art Search Q | | | |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|----------------|----------|----------|--------------|-----------|----------------|-----------|----------------------------------------------------------|-------|
| W BY DESIGN | Consulting Desk | Solutions | Shop | About us | Our projects | News | Contact us | | V | |
| | | | | | | | | | Welcome | |
| | | | | | | | | Log in to | Safer World by Design to continue to World by Design. | Safer |
| You are currently using the Demo version of the SaferSkin" application. If you wish to make predictions for your own molecules please <u>sign in</u> to enable th | nis feature.See <u>licensing optic</u> | ons for more d | letails. | | | | | Ema | il address* | |
| | | | | | | | | Pas | sword* | 0 |
| MODEL DATA INPUT | | | | | PREDIC | NOITS | | Forgot | assword? | |
| 1 SUBMIT YOUR MOLECULE Draw or enter a SMILES of the molecule. You may also select from a list | st. | | | | Ма | ike a pre | diction | | Continue | |
| | | | | | | | | Don't ha | e an account? Sign up | |
| Enter molecule identifier or Draw a molecule or | Choose from test m | nolecules | | | | | | | OR | |
| SMILES Co | | | • | | | | | G | Continue with Google | |
| | | | | | | | | | | |

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.

| SAFER | | | Profile 🛓 눷 Cart St | | | | art Search Q |
|----------------------------------------------------------------------------------------------------|-----------------------|-----------|-----------------------|----------|--------------|-----------|----------------|
| BY DESIGN | Consulting Desk | Solutions | Shop | About us | Our projects | News | Contact us |
| | | | | | | | |
| You are currently using the Professional version of the SaferSkin [™] application. | | | | | | | |
| | | | | _ | | | |
| MODEL DATA INPUT | | | | | PREDIC | TION | |
| UBMIT YOUR MOLECULE Draw or enter a SMILES of the molecule. You may also select from a list. | | | | | Ma | ke a prec | liction |
| Enter molecule identifier or Draw a molecule Or | Choose from test mole | cules | | | | | |
| SMILES Go | | | | | | | |
| | | | | | | | |

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.

| 1 SUBMIT YOUR MOLECULE Draw or enter a SMILES of the molecule. You may also select from a list. |
|----------------------------------------------------------------------------------------------------|
| |
| X JSME Molecular Editor by Peter Ertl and Bruno Bienfait |
| Enter molecule identifier or Draw a molecule SMILES |

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.

| MODEL DATA INPUT | | | PREDICTION |
|---------------------------------------------------------------------------------------------------|----------------------------|---|-------------------|
| USUBMIT YOUR MOLECULE Draw or enter a SMILES of the molecule. You may also select from a list. | | | Make a prediction |
| Enter molecule identifier or Draw a molecule CC(CC1=CC+C(C=C1)C(C Co | Choose from test molecules | • | lot |

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.

| • | Wait few minutes for | × |
|---|----------------------|---|
| - | calculating | |

- 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 accept | ptor class (yes/no) need | to be entered by the user. |
|----------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| 2 ENTER EXPERIMENTAL VALUES AND MOLECULAR DES Enter available data for approaches of interest. | CRIPTORS | |
| OECD 2o3 OECD ITS Multiple regression Neuro | al network Bayesian network | |
| Model description: A Bayesian network model that uses the LLNA assay and estimate its confidence. The model | s three different types of in vitro, in chemico is capable of handling missing input parame | and in silico inputs to predict skin sensitisation potency class in ters. [7] |
| REVIEW MOLECULAR DESCRIPTORS Values are calculated based on validated QSAR model. Plea | ise modify the values if missing or you have t | better estimates. |
| * - Required for correct model calculation | | |
| logKow [*] ? | logD @ pH7* ? | Water solubility @ pH7 (M)* ? |
| 4.2 | 4.2 | 0.00015 |
| Prestala biadar (M) * (2) | | |
| Protein binding (%) · (r) | Michael acceptor (?) | |
| | | l |

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.

| MODEL DATA INPUT | | | | PREDI | CTION |
|----------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|-------------------------|-----------------------------------------------------------------|-------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Draw or enter a SMILES of the molecule. | You may also select from a ltat. | | | | Make a prediction |
| Enter molecule identifier or Draw a molecule CC(CC1+CC+CIC+C1)CIC | 20 | ar | Choose from test molecules | | 20+ |
| | | | | OECD 263 | |
| ENTER EXPERIMENTAL VALUES AND MOU Enter available data for approaches of in OECD 203 OECD ITS Multiple regress | ICULAR DESCRIPTORS arrold. Ion Neural network Bayestan network | | | OECD ITS Multiple re | the result of DPRA access to evaluate the composed annutanticon-semalizer. Macdatory parameters missing specific Macdatory parameters missing |
| | | | | Neural net | twork |
| Model description: A Bayesian network mo confidence. The model is capable of handle | del that uses three different types of in vitro, in chemico and in g missing input parameters. [7] | n silico inputs to pred | ct skin sensitisation potency class in the LLNA assay and estim | ate its Eavesian r | vetwork Weak sensitiser |
| VEW MOLECULAR DESCRIPTORS lues are calculated based on validated QSAR | model. Please modify the values if missing or you have better | estimates. | | | Franklinn confidence week |
| Required for correct model calculation | | | | | |
| okow* (7) | logD @ p#17* (?) | | Water solubility () pH7 (M) * (7) | | |
| 2 | 4.2 | | 0.00015 | | |
| | | | | | |
| otein binding (%)* (?) | Michael acceptor (7) | | | | |

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.

| OECD 2o3 OECD ITS Multiple regression | on Neural network Ba | yesian network |
|-------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|
| | | |
| Model description: A majority voting model sensitiser or not in the LLNA assay. This is or | hat uses three different typ he of the defined approache | es of in vitro and in chemico tests to predict whether a chemical is a skin s proposed by the OECD TG 497 guideline. [1] |
| ENTER IN VITRO VALUES Input data for assays associated with three key Recommended to improve prediction estimate. | events in the AOP for Skin s | ensitisation. |
| Note: Provide parameters of at least 2 of the 3 a | assays: DPRA, KeratinoSens | or h-CLAT |
| Covalent binding to skin proteins | Keratinocyte activatio | Dendritic cell activation |
| Percent of cysteine peptide DPRA depletion in the DPRA assay. | KeratinoSens™ | h-CLAT |
| DPRACys (% depleted) | EC1.5 (µM) (🗧 | CD54 (?) |
| | | |
| DPRALys (% depleted) (? | | сряе 🕜 🗲 |
| | | · • |

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).

| 2 ENTER E Enter av | XPERIMENTAL ailable data fo | VALUES AND MOLECUL r approaches of interes | AR DESCRIPTORS t. | |
|-------------------------------|-----------------------------------------|----------------------------------------------------|------------------------------------------|---------------------------------------------------------------------------------------------------------------------|
| OECD 2o3 | OECD ITS | Multiple regression | Neural network | Bayesian network |
| | | | | |
| Model desc and compo | ription: A sim und's vapour p | ple and robust machine pressure to predict comp | learning model bas bound's potency as | ed on multiple linear regression that uses inputs from kDPRA, KeratinoSens the pEC3 value in the LLNA assay. [2] |
| ENTER PHYS-C Mandatory for | HEM PROPERT making the pr | ry ediction. | | |
| * - Required f | or correct mod | del calculation | | |
| Vapour pressure | (Pa)* ? | | | |
| | | | | |
| L | | | 1 | |

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

| OECD ITS | | Neural Network | | Bayesian Network | |
|------------------------------------------------------------|-------------------------------------------------------------------------|----------------|-----------------------------------|--------------------|---|
| In silico Derek / OECD QSAR TB Sensitiser potency* ⑦ | In silico TIMES-SS Sensitiser po - Toxtree Structural ak | etency (?) | In silico TIMES-: Sensitise | SS er potency 🕐 | * |

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

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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 203 and ITS. Please check the label in the following table:

| OECD 203 | 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.

| DDEL DATA INPUT | | | PREDICTION |
|-------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
| SUBMIT YOUR MOLECULE Draw or enter a SMILES of the molecule. You may also select from a | list. | | Make a prediction |
| Enter molecule identifier or Draw a molecule CC(CC1=CC=C(C=C1)C(C Co | or | Choose from test molecules | 201 |
| ENTER EXPERIMENTAL VALUES AND MOLECULAR DESCRIPTORS Enter available data for approaches of interest. | | | OECD 203 Sensitiser OECD ITS 1B (Other Sensitizer) Multiple regression |
| ECD 2o3 OECD ITS Multiple regression Neural network | Bayesian network | | Strong sensitiser Neural network |
| Model description: A Bayesian network model that uses three differen sensitisation potency class in the LLNA assay and estimate its confider | t types of in vitro, in cl nce. The model is capa | hemico and in silico inputs to predict skin able of handling missing input parameters. [7] | Moderate sensitiser Bayesian network Weak sensitiser |
| IEW MOLECULAR DESCRIPTORS Jes are calculated based on validated QSAR model. Please modify the | values if missing or yo | u have better estimates. | Prediction confidence: weak |
| | | | |

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 203 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 SaferSkinTM (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.

| OECD 203 WEIG | T OF EVIDENCE INTEGRATED TESTING STRATEGY - DETAILED REPORT | |
|---------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|--|
| SAFETY ASSESSME | T GUIDE | |
| Sensitiser: Yes | | |
| Experimental values | | |
| Note: NA values are eithe Key Event 1 - Peptid | navailable or out of the experiments' applicability domain and therefore were neglected in the hazard assessment. | |
| DPRACys | 97.65 (% depleted) | |
| DPRALys | 9.7 (% depleted) | |
| Key Event 2 - Keratir | Sens™ Concentration yielding 1.5-fold (EC1.5) | |
| EC1.5 | 3.1563 µM | |
| Key Event 3 - Activa | n markers CD54 and CD86 in the h-CLAT | |
| CD54 | positive | |
| CD86 | negative | |
| | | |

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

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

| OECD ITS - DETAILE | ED REPORT |
|---------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| SAFETY ASSESSMEN Sensitisation category Battery score: 7 Experimental values | T GUIDE y: 1A (Strong) |
| Note: NA values are either u | navailable or out of the experiments' applicability domain and therefore were neglected in the hazard assessment. |
| Key Event 1 - Peptide r | reactivity |
| DPRACys | 97.65 (% depleted) |
| DPRALys | 9.7 (% depleted) |
| Key Event 2 - Keratinos KeratinoSens [™] assay; EC1.5 | Sens [™] Concentration yielding 1.5-fold (EC1.5); threefold (EC3) induction of Nrf2-dependent luciferase activity in the |
| EC3 | 14.7729 µM |
| Key Event 3 - ECETOC Sensitiser potency | skin sensitisation class for the compound as predicted by the Derek Nexus software; Sensitiser |

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.

| MULTIPLE REG | SION - DETAILED REPORT | |
|------------------------------------------------|------------------------------------------------------------------------------------------------------------------|--|
| SAFETY ASSESSN | GUIDE | |
| pEC3: 0.42 Sensitisation cate | 4 | |
| Experimental valu | | |
| Note: NA values are eith Key Event 1 - Cor1 | available or out of the experiments' applicability domain and therefore were neglected in the hazard assessment. | |
| Kmax | 0.073 1/mM/min | |
| Key Event 2 - Kera | ens™ Concentration yielding 1.5-fold (EC1.5); 50 % reduction in cell viability (IC50) in the KeratinoSens™ assay | |
| EC1.5 | 3.1563 µM | |
| IC50 | 50.8656 µM | |
| Vapour pressure: | 311 Pa | |

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.

| NEURAL NET | WORK - DETAILED |) REF |
|-------------------------------------------|--------------------------|--------|
| SAFETY ASSESS | MENT GUIDE | |
| Sensitisation cat | egorv: Strong sensiti | iser |
| pEC3: NA | egery: eachy sense | |
| Experimental val | ues | |
| | | |
| Note: NA values are e | ither unavailable or out | of th |
| Koy Event 1 De | | |
| DPRACvs | 97.65 (% depl | leted) |
| DPRALys | 9.7 (% depl | leted) |
| Key Event 2 - Ke | ratinoSons™ Concentr | ratio |
| EC1.5 | 3.1563 µM | auoi |
| Imax | 24 | |
| Kev Event 3 - Co | ncentrations vielding | 150 |
| marker CD54 in the | h-CLAT; 25 % reducti | ion (0 |
| EC150 | 1510.0918 ug | /mL |
| EC200 | 0.549 ug | /mL |
| CV75 | 1.827 ug, | /mL |
| Incilian predictio | n / outoomo | |
| inslico predictio | n / outcome | |
| _ | | |
| Toxtree: alert > 2 Presence of structu | ral alerts predictive of | f prot |
| TIMES-SS: Strop | r/Extreme | |
| Based on parent ch | emical and potential f | or Se |

Figure 19. Detailed report of neural network in SaferSkinTM (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.

| BAYESIA | N NETWORK INTEGRATED | D TESTING STRATEGY - | DETAILED REPORT | | |
|----------------|------------------------------------------|--------------------------------------|-----------------------------------------|---------------------------------------|-----------------|
| SAFETY AS | SSESSMENT GUIDE | | | | |
| EC3: (at the | 50th percentile): 2.984% | | | | |
| pEC3: (at th | ne 50th percentile): -0.693 | | | | |
| Sensitisatio | on category: 4 - Strong sensitizer | | | | |
| ocholdodd | | | | | |
| | | | | | |
| F | Prediction confidence: weak | | | | |
| | 50th percentile | 60th percentile | 70th percentile | 80th percentile | 90th percentile |
| pEC3 | -0.693 | -0.497 | 0.1 | 1.169 | 2.238 |
| EC3 | 2.984 | 1.9 | 0.479 | 0.0409 | 0.0035 |
| | | | | | |
| 4 | | | | | • |
| A potency pro | obability distribution based on explorin | g the 50th - 90th percentiles of the | e pEC3 value. | | |
| The y-axis rep | presents the posterior probabilities for | each classification category (non, | weak, moderate, strong). | | |
| The x-axis rep | presents the constant pEC3 ranges for | which each classification category | y falls (Non (< -1.9), Weak (-1.9, -1. | 1), Moderate (-1.1, -0.35), Strong (> | -0.35). |
| | | | | | |
| | | | | | |

Figure 20. Safety assessment guide of Bayesian network in SaferSkinTM (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.* 2015⁷)
 - 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.

| ior probabilit | ies | | | |
|-----------------------------------------------------------------------|--------------------------------------------------------------------|------------------------------------------------------------------|-------------------|--------------------|
| Non-sensitiser | Weak sensitiser | Moderate sensitiser | Strong sensitiser | |
| 0.265 | 0.265 | 0.272 | 0.197 | |
| osterior proba | abilities | | | |
| Non-sensitiser | Weak sensitiser | Moderate sensitiser | Strong sensitiser | |
| 0.00000887 | 0.272 | 0.381 | 0.346 | |
| osterior proba | bilities correc | ted for anti-inflan | nmatory action o | Michael acceptor |
| osterior proba olecule was not n ayes Factors Non-sensitiser | abilities correc narked as Michael Weak sensitiser | ted for anti-inflan acceptor. Moderate sensitiser | nmatory action o | f Michael acceptor |
| osterior proba olecule was not n ayes Factors Non-sensitiser | abilities correc narked as Michael Weak sensitiser 1.0354 | ted for anti-inflan acceptor. Moderate sensitiser 1.652 | Strong sensitiser | f Michael acceptor |

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

SaferSkin[™] Report _ _ _ _ 🖶 P REPORT SUMMARY MOLECULE 1,2-benzisothiazolin-3-one (proxel) O=C(NSc1cccc2)c12 Sensitiser Potency class pEC3 OECD 2o3 OECD ITS 1A (Strong) Multiple regression Strong sensitiser 0.4258 Neural network Strong sensitiser -0.6938* Bayesian network Yes Strong sensitiser * At the 50th percentile

12.2. Printing/Saving Report

Figure 22. SaferSkinTM 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.

| OECD 203 V | VEIGHT OF EVIDENCE INTEGRATED TESTING | | Print | |
|-------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|---|---------------|-------------|
| STRATEGY - | detailed report | | | |
| Safety Assess | ment Guide | | Destination | Save as PDF |
| Sensitiser: Yes | | | Deser | |
| Experimental v | alues | | Pages | All |
| Note: NA values are were neglected in t | either unavailable or out of the experiments' applicability domain and therefore the hazard assessment. | 3 | Layout | Portrait |
| Key Event 1 - Pep | tide reactivity | | l | |
| DPRACys | 14 (% depleted) | | More settings | |
| DPRALys | 0.7 (% depleted) | | | |
| Key Event 2 - Ker EC1.5 Key Event 3 - Act | titinoSens [™] Concentration yielding 1.5-fold (EC1.5) 2000 µM vation markers CD54 and CD86 in the h-CLAT | | | |
| CD54 | positive | | | |
| CD86 | negative | | | |
| OECD ITS - o Safety assess | regative etailed report ment guide | | | |
| Sensitisation categ Battery score: 5 | ory: 1B (Other Sensitizer) | | | |
| Experimental v | alues | | | |
| Note: NA values are were neglected in t | either unavailable or out of the experiments' applicability domain and therefore re hazard assessment. | | | Save |
| | | | | |

Figure 23. SaferSkinTM Application result report (example: proxel).