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Computational modelling and simulation for immunotoxicity prediction induced by skin sensitisers

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ABSTRACT

In many domains regulating chemicals and chemical products, there is a legal requirement to determine skin sensitivity to allergens. While many in vitro assays to detect contact hypersensitivity have been developed as alternatives to animal testing over the past ten years and significant progress has been made in this area, there is still a need for continued investment in the creation of techniques and strategies that will allow accurate identification of potential contact allergens and their potency in vitro. In silico models are promising tools in this regard. However, none of the state-of-the-art systems seems to function well enough to serve as a stand-alone hazard identification tool, especially in evaluating the possible allergenicity effects in humans.

The Universal Immune System Simulator, a mechanistic computational platform that simulates the human immune system response to a specific insult, provides a means of predicting the immunotoxicity induced by skin sensitisers, enriching the collection of computational models for the assessment of skin sensitization.

Here, we present a specific disease layer implementation of the Universal Immune System Simulator for the prediction of allergic contact dermatitis induced by specific skin sensitizers.

1. Introduction

Allergic contact dermatitis (ACD) represents a type IV, cell-mediated hypersensitivity reaction caused by an allergen coming in contact with the skin [1]. ACD has a prevalence of approximately 15–20 %, and accounts for 10–15 % of all occupational diseases [2]. ACD is caused mainly by the generation of $CD8^+$ Tc1/Tc17 and $CD4^+$ Th1/Th17 effector T cells as a result of repeated exposure of an allergen [3]. In ACD, resident epidermal cells, dermal fibroblasts, endothelial cells, and invading leukocytes interact with one another under the control of a network of cytokines and lipid mediators [4].

In the acquisition of ACD, two main phases are recognized: the induction (sensitization) phase and the subsequent elicitation phase (Fig. 1). The induction phase, also called sensitization or afferent phase, begins when vulnerable individuals have their initial skin contact with the allergen. The induction phase in humans usually occurs in 10–15 days, whereas in mice it takes 5–7 days. In order for a chemical to induce skin sensitization, several key steps must occur. Briefly, following the interaction between the hapten and the epidermal and dermal proteins and thus the formation of the complete antigen, the development of ACD then requires the activation of innate immune cells, including keratinocytes (KCs) necessary for maturation and migration of dendritic cells (DCs), and DCs, necessary for the activation of T cells. The acquisition of the specific immune response will take place at the level of draining lymph nodes, where DCs migrate and stimulate the activation of hapten-specific responsive T-cells and the generation of effector cells.

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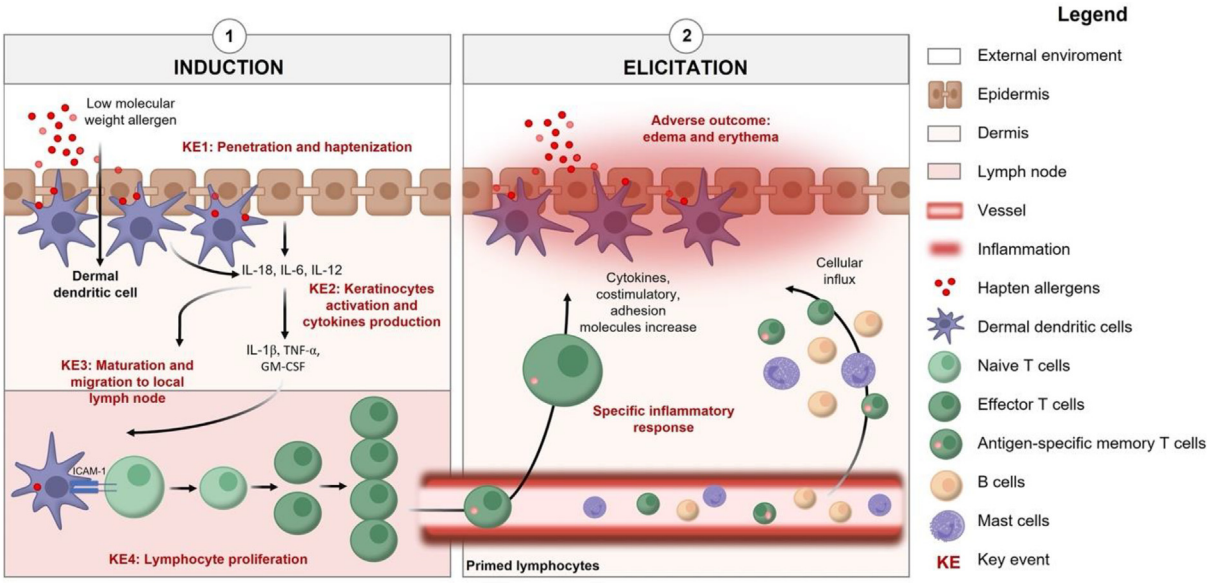

Fig. 1. The phases of allergic contact dermatitis (panel 1) and subsequent elicitation process (panel 2). The induction phase can be summarised in the 4 key events (KE) described in the skin sensitization Adverse Outcome Pathway. KE1 represents the penetration of low molecular weight allergen through the epidermis and the haptenization with skin proteins. This is followed by keratinocytes (KE2) and dendritic cells (KE3) activation, and their migration to the draining lymph nodes. Here, the presentation of the antigen to naïve T cells happens, and at the end of the process there is the lymphocyte T proliferation (KE4), in which antigen-specific clones differentiate and proliferate into effector T cells. In panel 2, the elicitation phase is described. In this phase, upon re-exposure to hapten, dermal dendritic cells, keratinocytes and mast cells are activated and produce various inflammatory mediators, which activate endothelial cells and cause inflammatory cell infiltration, including antigen-specific T cells. Antigen-specific effector T cells are activated, and produce pro-inflammatory cytokines and chemokines, resulting in oedema and erythema.

The cellular and molecular events that are associated with the induction of skin sensitization and the elicitation of ACD are, to some extent, well defined. The key events involved are formally described by the OECD in a document titled "*The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins*" with the goal of facilitating the development of methods and approaches addressing the relevant events [5]. This understanding allowed a great deal of progress in the development of new approach methodologies (NAM) to assess ACD. Testing strategies for skin sensitization based on the adverse outcome pathway (AOP) are currently preferred, and ten methods have been successfully validated addressing the first three key events (KE) of the skin sensitization AOP.

Chemical allergens are low molecular weight compounds (typically < 500 Dalton) haptens too small to be recognized by our immune system, as such, once absorbed through the stratum corneum, they must bind to self-macromolecules (e.g., heat shock proteins, chaperons, etc.) to form a complete antigen, which is considered the molecular initiating event or key event 1 (KE1). The complex formation is related to electrophilic reactivity and hydrophobicity of the allergen. As they are not immunogenic by themselves, they need to bind to skin proteins to create a complex that can trigger T-cell responses [6].

The induction of skin sensitization and the elicitation of allergic contact hypersensitivity reactions are dose-dependent phenomena, and in both instances, it is possible to determine threshold concentrations of allergen required for a response. The potency of a chemical contact allergy is defined in terms of such threshold values, then it follows that for weaker allergens, sensitization will require exposure to larger amounts than is necessary for sensitization to stronger allergens [7]. All contact allergens need to be absorbed, and skin absorption does not correlate with potency. Some allergens are strong sensitizers, able to induce an immune response in normal people; others are weaker sensitizers, and they can induce sensitization only in vulnerable individuals. During the induction phase, as mentioned above, four KEs have been identified [5]. The first one is the penetration of the hapten into the skin and its molecular interaction with dermal and epidermal proteins: the electrophilic residues of the haptens usually interact with the nucleophilic residues of the proteins; cysteine is the most common amino acid implicated in hapten–protein interactions, but lysine, methionine, tyrosine, and histidine are all involved [8]. The second key event is the activation of KCs, which leads to an inflammatory response as well as gene expression of cell signalling pathways. The third key event is represented by the activation of DCs and epidermal Langerhans cells (LCs) which, also interacting with the nearby keratinocytes, recognize the allergen-protein complex, take up the complex, move to skin-draining lymph nodes, and operate as antigen-presenting cells (APCs). Inflammatory cytokines, chemokines, and adhesion molecules are involved in these responses [2]. The fourth key event is represented by T-cell differentiation and proliferation in the lymph node.

The second main phase is the elicitation (Fig. 1- panel 2), which happens after a subsequent encounter with the same allergen with a delay of 12–48 h, and this is the phase in which the patient's inflammatory symptoms appear. During this phase, the hapten-protein conjugate is generated again, and LCs, DCs, and other APCs process the hapten-protein and expose it, in the specific case of DCs, as an MHC class II complex on the cell surface. Thus, allergen-specific memory T-cells are activated, and they induce the mobilisation of cytotoxic T-cells and other inflammatory cells from the blood as well as the release of inflammatory cytokines [5]. The effects are local inflammatory signs, such as redness, blisters, and papules, followed by itchy, scaling, dry, and burning skin, and they usually affect hands, arms, and face.

The assessment of skin sensitization is a key requirement in all regulated sectors [9]. While significant progress has been made in the last decade, and several alternative in vitro assays to detect contact hypersensitivity are available, there is a clear need for continued investment in the development of methods and approaches that will allow the correct identification in vitro of potential contact allergens and their potency, and in silico models are believed

to be central. Several quantitative structure–activity relationship (Q)SAR models and expert systems have been developed and are described in the literature, e.g., OECD toolbox, Toxtree, TOPKAT, Derek, TOPS-MODE [10,11].

None of the systems appear to perform sufficiently well to act as a standalone tool for hazard identification, with an external correct classification rate of 70 % for skin sensitization, but they may be extremely useful within a structured decision support system as part of a safety assessment strategy being well suited to assist in hazard assessment and chemical screening/prioritization [12]. Goebel et al. [13] provide an overview of published reactivity-based QSAR models and their use in non-animal safety assessment of skin sensitization. It is important to remember that such models have specific applicability domains and are not applicable to chemicals with undefined structures, mixtures, and substances containing metals.

The life sciences are increasingly infused by the innovations in information and communication technology. Computer modelling and simulation approaches are gaining a foothold in the discipline of immunotoxicology by the usage of specific computer-based (in silico) toxicity prediction tools for a wide range of chemical substances [14,15]. Recently, among them, the Universal Immune System Simulator (UISS), a mechanistic computational platform that simulates the human immune system response to a specific insult [16–18], may offer the opportunity to investigate the effects on vulnerable populations, like children, the elderly or immunocompromised people through individualized computer simulation. The UISS can also guide the long and winding pathway for the development or regulatory evaluation of a medicinal product or medical intervention.

In this study, we have extended the UISS simulation platform, previously developed to predict the immunotoxicity of PFAS chemicals through its module called UISS-TOX [19], to the skin sensitization domain using nickel as reference sensitizer and with the aim to simulate their effects on the immune system.

UISS-SENS, which represents the specific module of UISS for skin sensitizers, enriches the collection of computational models for the sustainable management of chemicals, in particular the ones used in the assessment of skin sensitization.

With the use of modelling and simulation platforms, immunotoxicology and chemical safety assessment could benefit from the progress made in computational immunology and predictive toxicology [19–21] about chemical safety assessment.

2. Material and methods

The UISS platform can be flexibly extended to simulate several biological and safety assessment scenarios. In particular, within predictive toxicology, the methodology applied in this work includes the following steps:

1. Development of the agent-based model of UISS- SENS applied to skin sensitizers and creation of the ACD conceptual model.
2. Retrospective validation at level 1 (population-based) of UISS-SENS.

The agent-based model (ABM) paradigm, on which UISS computational framework is based, allows simulation of the host immune system response to a general stimulus. Moreover, complex emergent behaviour can arise and lead to the simulation of non-coded dynamics. ABM and UISS have been extensively described. Interested readers can find additional information and more details in specialized literature [22–24]. One of the key steps in creating a new disease layer in the UISS, is to deeply understand the mechanism of action of the disease, considering all the entities involved and all the interactions they have with each other. With the aim of describing this, the first step is usually to build a conceptual model, which is a representative and complex model able to depict the main entities and interactions involved in the disease of interest. In UISS-SENS, we developed a specific conceptual model for ACD provoked by a generic skin sensitizer (also named "allergen"), describing in detail the consequent immune response cascade after the exposure throughout the three compartments involved: skin, blood, and lymph nodes (Fig. 2).

Generally speaking, the UISS is a multi-scale, multi-organ, three-dimensional agent based simulator of the human immune system. Multi scale means that it is able to merge events that occur at multiple levels (molecular, cellular and organ), eventually harmonizing time and space scales. Compartments are implemented in terms of lattice-space. In particular, the simulation space is represented as a $L \times L \times L$ cubic lattice, with periodic boundary conditions to the left and right side, while the top and bottom are represented by rigid walls. All entities (molecular and cellular) are allowed to move with uniform probability between neighbouring lattices in the grid with an equal diffusion coefficient (Brownian motion). Entities interact following a specific stochastic rule translated from biological knowledge. The presence of cytokines is also considered, and they can act by favoring or inhibiting the probability of the interaction. In particular all the interactions are implemented through a stochastic finite machine technique. Fig. 3 illustrates how a naïve B cell interacts with a hapten-protein conjugate, eventually leading the B cell into a MHC/II-protein-hapten presenting cell.

Table 1 depicts the actors/agents and how they are represented and interact in the model.

Most contact allergens are small, chemically reactive molecules. Since these molecules are too small to be antigenic themselves, contact sensitizers are generally referred to as haptens. Upon penetration through the epidermal horny layer, haptens readily bind to epidermal and dermal proteins to be recognized and processed by epidermal Langerhans Cells (LCs) and dermal dendritic cells (DCs). LCs and DCs are "professional" antigen-presenting dendritic cells (APC) in the skin, thus the protein-allergen complex binds to the major histocompatibility complex of class I (MHC-I) and class II (MHC-II) expressed by LCs in the epidermis and by DCs in the dermis. Furthermore, after exposure to a contact sensitizer, production of IL-1β from LCs is induced. In turn, IL-1β stimulates release of tumour necrosis factor-alpha (TNF-α), granulocyte–macrophage colony-stimulating factor (GM-CSF), and IL-8 from keratinocytes. Together, IL-1β, GM-CSF, and TNF-α facilitate the migration and maturation of LCs from the epidermidis towards the lymph nodes. In this context, keratinocytes go under oxidative stress. The antigen-protein complex also interacts with naïve B lymphocytes as well as naïve macrophages, activating them; active macrophages release IL-8, as well as active B lymphocytes both release IgM and interact with protein-allergen complex, so that they differentiate in B/MHC-II and then they migrate into the lymph node. Once activated, also the APCs migrate through the afferent lymphatic vessels to the paracortical region of the regional lymph nodes. Here they encounter naïve T lymphocytes to which they bind via ICAM-1 and LFA-3 and thanks to the co-stimulator B7-1 (CD80) and B7-2 (CD86). The binding determines the secretion of various cytokines: IL-1 secreted by APCs and IL-2 secreted by T lymphocytes are the most important ones; IL-2, through an autocrine mechanism, stimulates survival, proliferation, and differentiation of T lymphocytes. IL-18, secreted by keratinocytes, promotes the proliferation of naïve T lymphocytes, together with IL-12 and IL-6, both secreted by DCs. IL-4 and IL-1β co-stimulate the differentiation of naïve T lymphocytes into effector CD4+ T cells, memory CD4+ T cells, effector CD8+ T cells and memory CD8+ T cells. Effector CD8+ T lymphocytes interact with hapten/MHC-I complex and

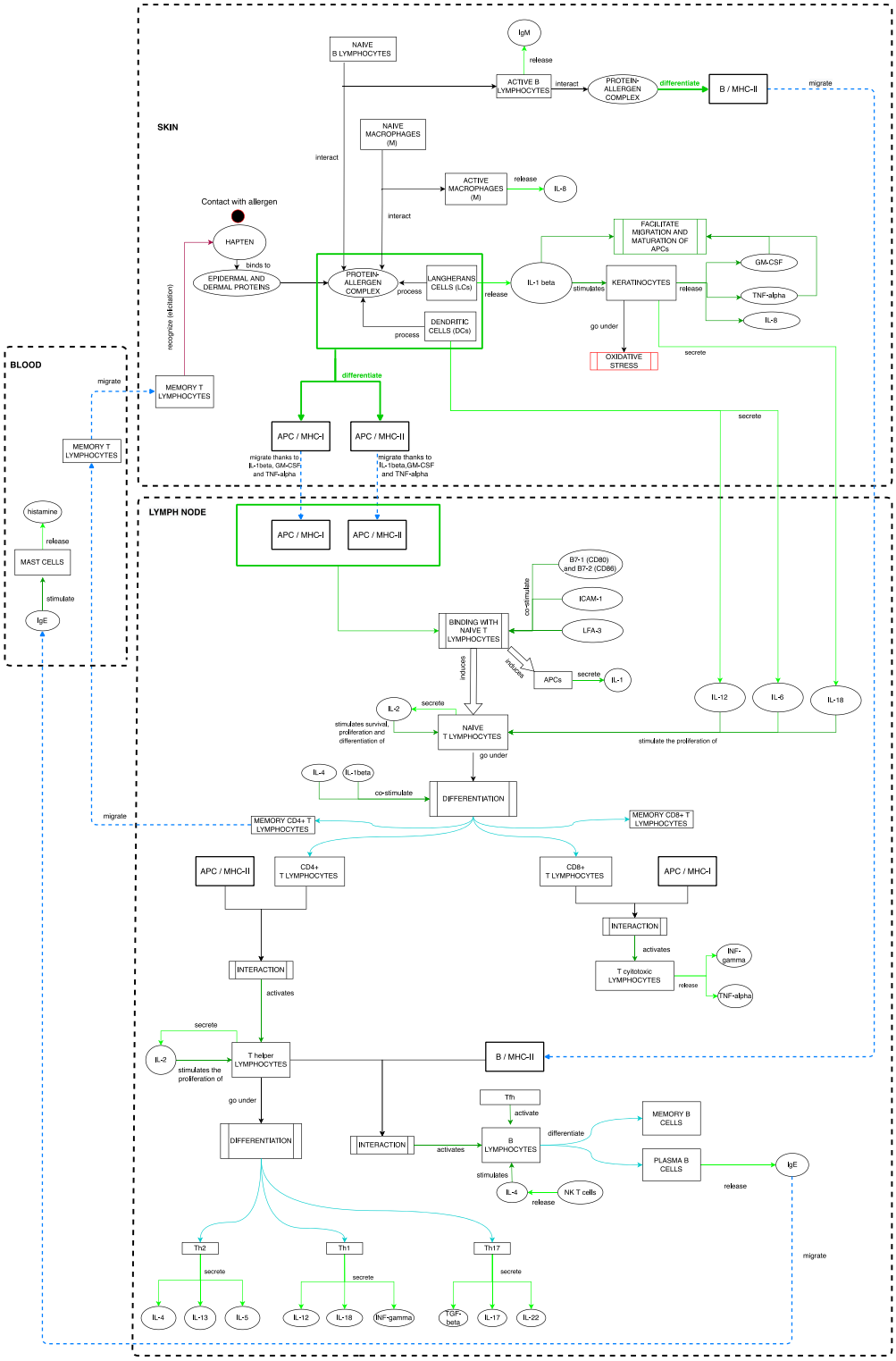

Fig. 2. Conceptual description of the main immunological entities and interactions involved in Allergic Contact Dermatitis. The sketch depicts the generic immune response after the exposure to a common allergen. The main compartments involved (skin, blood, and lymph nodes) are represented.

this activates T cytotoxic lymphocytes, which release INF-gamma and TNF-α. Effector CD4+ T lymphocytes interact with hapten/MHC-II complex and this activates T helper lymphocytes, which secrete IL-2, a cytokine that stimulates the proliferation of Th lymphocytes themselves. T helper lymphocytes differentiate into Th2, Th17 and Th17. Th2 lymphocytes secrete several cytokines, including IL-4, IL-13, and IL-5; Th1 lymphocytes secrete cytokines such as IL-12, IL-18, and INF-gamma; Th17 lymphocytes secrete TGF-β, IL-17, and IL-22. Memory lymphocytes migrate to blood and to the skin, where they recognize the hapten, after the second contact with the allergen, in a phase called 'elicitation'. In the lymph node T helper lymphocytes, before their differentiation, interact with B/MHC-II coming from the skin, and this interaction activates B lymphocytes. This process is stimulated by both T follicular helper

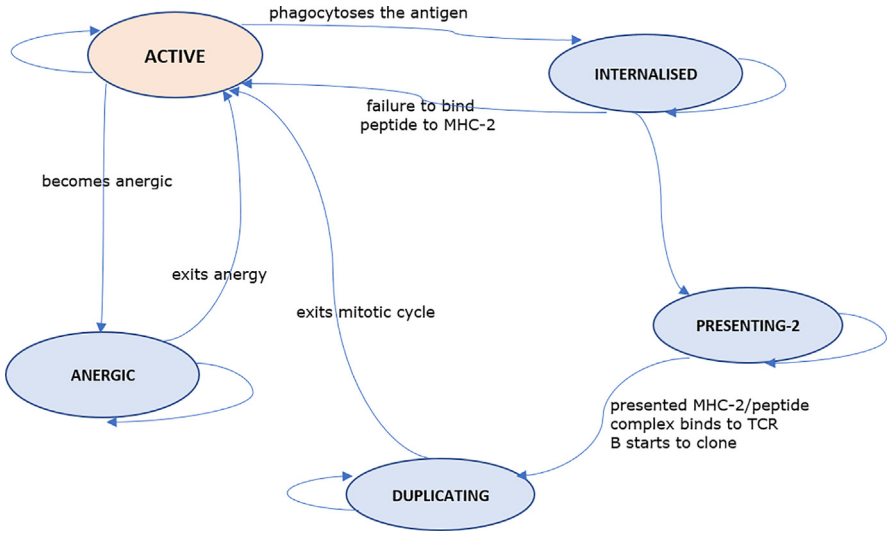

Fig. 3. Stochastic state machine illustrating the B – hapten-protein interaction and the cellular state change.

Table 1
Main actors of the UISS modeling and simulation platform.

	Cells	Small molecules	Large molecules
Who	B cells Cytotoxic T cells Helper T cells (TH1, TH2, TH17) Regulatory T cells NK M DC Neutrophils Eosinophils Mast cells Generic EP Keratinocytes cells KC Langerhans cells LC	IL-1 IL-2 IL-4 IL-5 IL-6 IL-8 IL-10 IL-12 IL-17 IL-21 IL-22 IL-23 IFN-γ TNF TGF-β GM-CSF Type 1-IFN (alpha, beta) Histamine D-signal Vit. D Chemokines	Antibody (Ab) Ig (M, D, G1, G2, E, A) Antigen (Ag) IC
Represented by	Discrete variables (agents)	Continuous variables	Discrete variables (no internal states)
Interaction based on	Binary strings (n bits)	Only concentration on the lattice site is needed	Binary strings (n bits)
How they move	Chemotaxis and random diffusion	Diffusion equation (parabolic PDE) $\frac{\partial C}{\partial t} = D\nabla^2 C - \lambda C$	Random diffusion

lymphocytes (Tfh) and by the IL-4 released by NK T cells. Active B lymphocytes differentiate in memory B cells and plasma B cells. The latter release Ig-E, and Ig-E migrate into the blood, where they stimulate the degranulation of mast cells and consequently the release of histamine.

3. Results and discussion

We retrospectively validated the UISS-SENS platform at the population level (Level 1) considering two experimental studies (one in vitro [25] and one in vivo [26]) coming from specialized literature dealing with the Nickel-mediated Allergic Contact Dermatitis.

3.1. Study 1

Summer et al. investigated the cytokine patterns in vitro to detect patients with contact allergy to nickel [25]. They collected blood samples from 30 patients: 15 of them were positive to nickel in a patch test, while the other 15 were negative in the nickel patch test. For six days they stimulated peripheral blood mononuclear cells (PBMC) isolated from the blood of patients positive to patch test with three different concentrations of $NiSO_4$: 7.5×10^{-6}mol/L $NiSO_4$, 1×10^{-5} mol/L $NiSO_4$ and 2.5×10^{-5} mol/L $NiSO_4$. They used as further controls both the metal antigen CoC_2 and peripheral blood mononuclear cells without stimuli (i.e., in medium alone). After patient blood samples were collected, the Lymphocyte Transformation Test (LTT) was performed. LTT is a method developed to verify a diagnosis of nickel allergy, being ACD mediated by T lymphocytes. They investigated cytokine patterns Th1-related (IFN-γ, IL-2 and IL-12), Th2-related (IL-4, IL-5 and IL-13) and Th17-associated IL-17. They noticed differences in cytokine production, especially for IL-5, IL-8, TNF-alpha and INF-gamma, but the most distinctive differences were found for IL-5 and IL-8. Results coming from this study show that in allergic patients an increase in IL-5 production is observed, such as a decrease in IL-8 production, although in the latter case the difference was not significant. Moreover, in allergic patients the ratio between IL-8 and IL-5 correlates positively for IL-5 and negatively for IL-8; on the other side, in non-allergic patients, the ratio between IL-8 and IL-5 correlates positively for IL-8 and negatively for IL-5.

We modelled in silico the same cohort of patients (15 allergic to Nickel and 15 non allergic ones) and we simulated through the Universal Immune System Simulator (UISS) the virtual copy of the patients treated with the concentrations of $NiSO_4$ used in [25]. We simulated the effect of the same three $NiSO_4$ concentrations on both allergic and non-allergic patients and we obtained specific in silico cytokine dynamics in which the concentration of interleukins (expressed in fg/μl) is shown over time. In silico results are in good agreement compared to the in vivo ones. In silico cytokines levels are measured in peripheral blood compartment.

After the stimulation with 7.5×10^{-6} mol/L of $NiSO_4$, an increase of IL-5 concentration in Ni allergic patients compared to

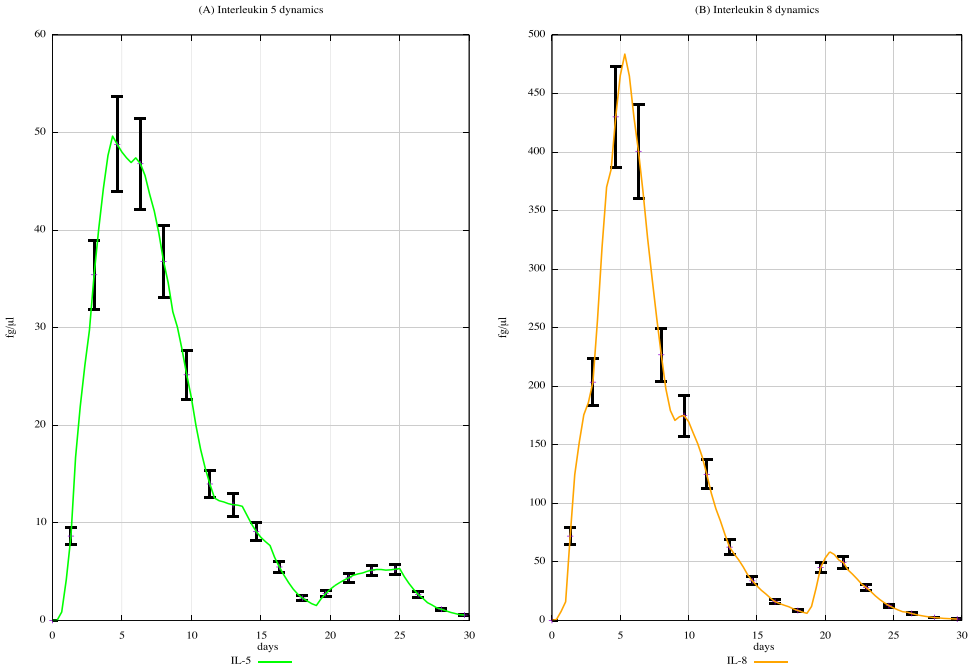

Fig. 4. Immune system dynamics for the Ni non-allergic virtual patients exposed to 7.5×10^{-6} mol/L of $NiSO_4$. UISS-TOX in silico prediction of IL-5 (panel A) and IL-8 (panel B) dynamics are represented.

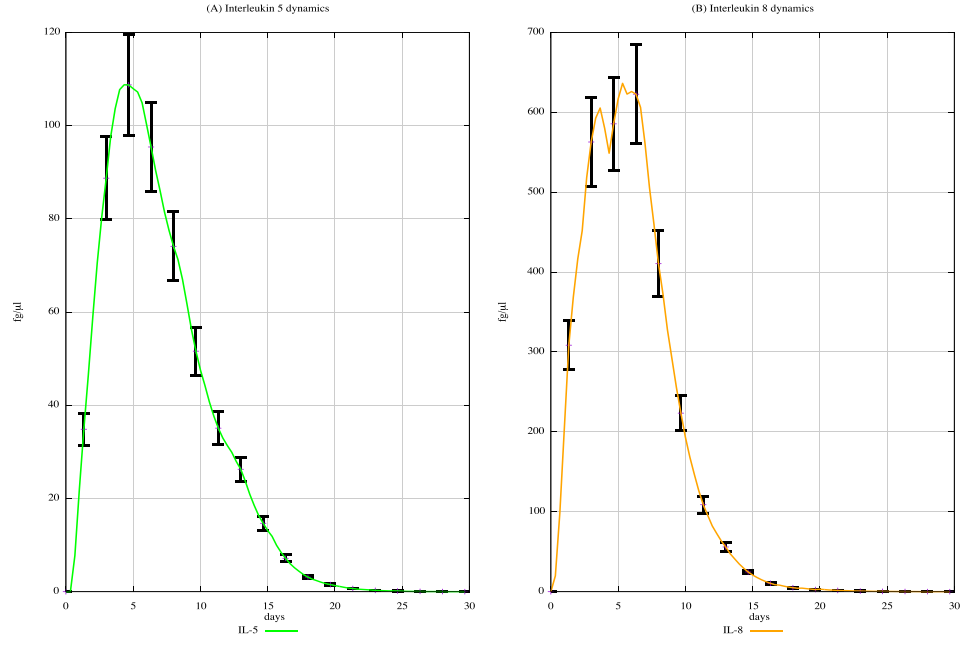

Fig. 5. Immune system dynamics for the Ni allergic virtual patients exposed to 7.5×10^{-5} mol/L of $NiSO_4$. UISS-TOX in silico prediction of IL-5 (panel A) and IL-8 (panel B) dynamics are represented.

non-allergic ones can be observed. Fig. 4-panel A, depicts the IL-5 dynamics in non-allergic patients in which a peak around 5000 fg/µl after 5 days from stimulation occurs; in allergic patients IL-5 dynamics (Fig. 5-panel A), a peak around 11,000 fg/µl after 5 days from stimulation is reported. For what concerns IL-8 production, no significant differences between allergic and non-allergic patients are detectable: in Fig. 4-panel B, which represents the IL-8 dynamics in non-allergic patients, a peak around 50,000 fg/µl after 5 days from stimulation is present, while in Fig. 5-panel B, reporting the IL-8 dynamics in allergic patients, a peak around 60,000 fg/µl after 5 days is observed. Furthermore, after stimulation with 7.5×10^{-6} mol/L of $NiSO_4$, an increase of

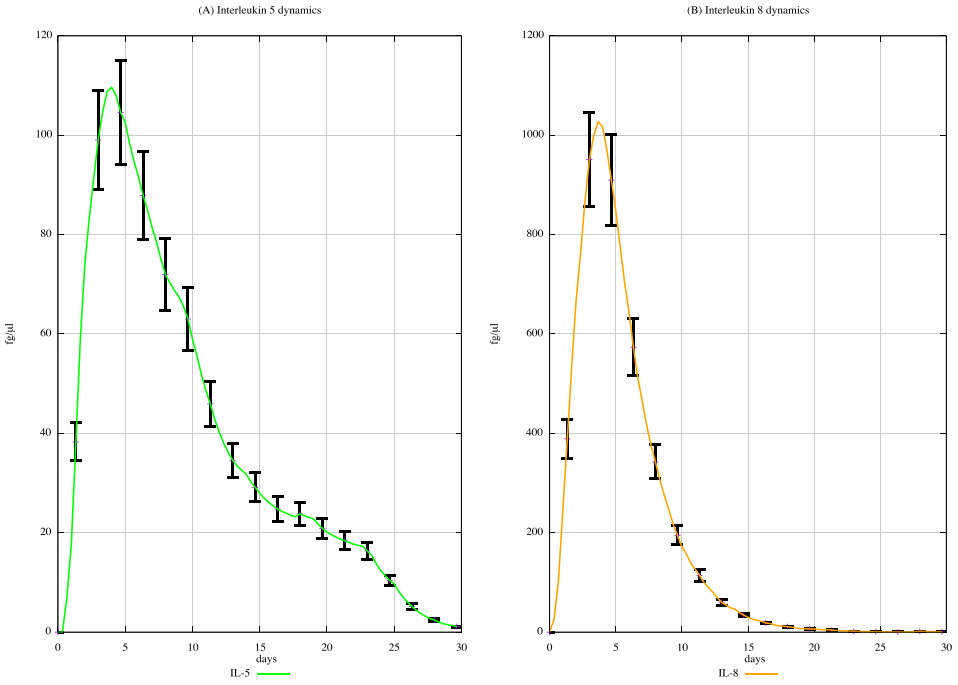

Fig. 6. Immune system dynamics for the Ni non-allergic virtual patients exposed to 1×10^{-5} mol/L of $NiSO_4$. UISS-TOX in silico prediction of IL-5 (panel A) and IL-8 (panel B) dynamics are represented.

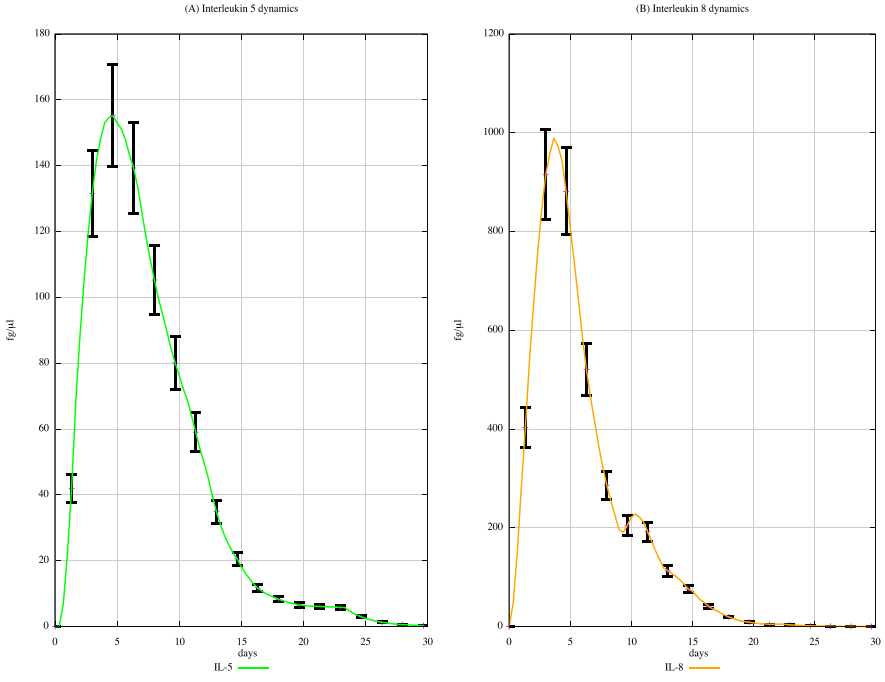

Fig. 7. Immune system dynamics for the Ni allergic virtual patients exposed to 1×10^{-5} mol/L of $NiSO_4$. UISS-TOX in silico prediction of IL-5 (panel A) and IL-8 (panel B) dynamics are represented.

IL-5 production in allergic patients compared to the non-allergic ones can be observed (Fig. 4-panel A and Fig. 5-panel A), as highlighted by Summer and co-authors' results.

Even after stimulation with 1×10^{-5} mol/L of $NiSO_4$, an increase in IL-5 concentration in Ni allergic patients can be observed. Fig. 6-panel A depicts the IL-5 dynamics in non-allergic patients, in which a peak around 11,000 fg/µl after 5 days from stimulation is present; in the scenario representing allergic patients (Fig. 7-panel A), a peak of IL-5 concentration around 15,500 fg/µl after 5 days can be observed. For what concerns IL-8 production, both in the figures representing IL-8 dynamics in non-allergic (Fig. 6-panel B) and allergic patients (Fig. 7-panel B), a peak around 100,000 fg/µl after 5 days from stimulation is shown. Thus, even after stimulation with 1×10^{-5} mol/L $NiSO_4$, an increase in IL-5

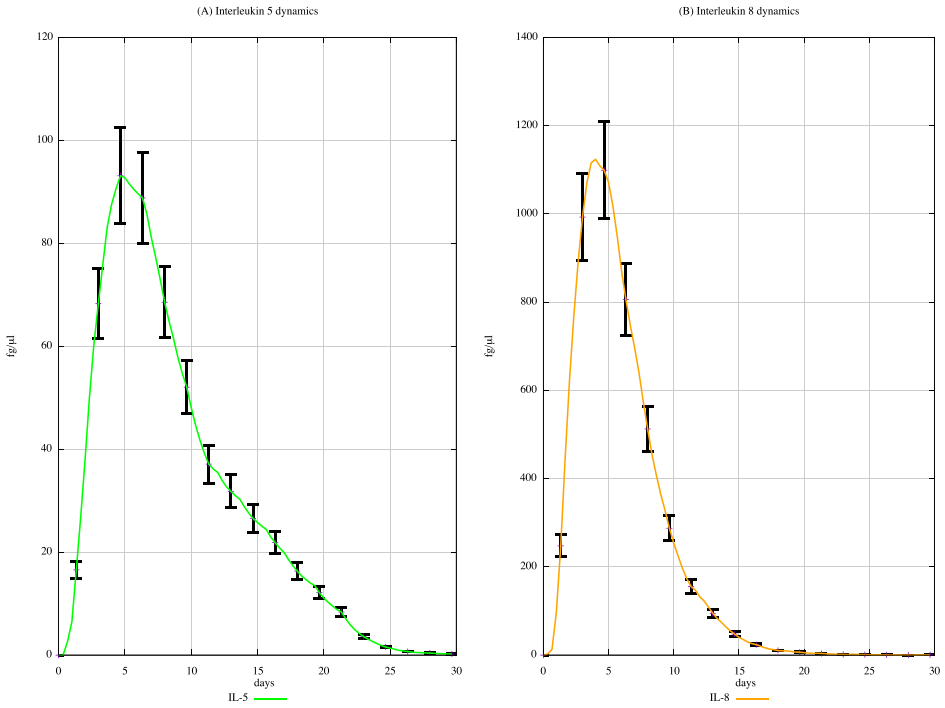

Fig. 8. Immune system dynamics for the Ni non-allergic virtual patients exposed to 2.5×10^{-5} mol/L of $NiSO_4$. UISS-TOX in silico prediction of IL-5 (panel A) and IL-8 (panel B) dynamics are represented.

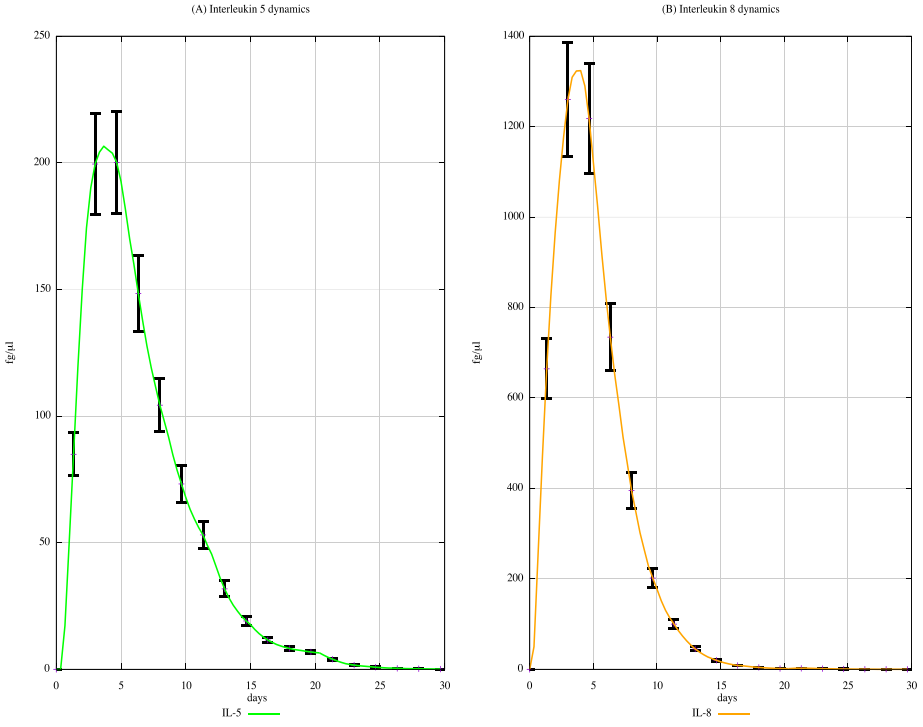

Fig. 9. Immune system dynamics for the Ni allergic virtual patients exposed to 2.5×10^{-5} mol/L of $NiSO_4$. UISS-TOX in silico prediction of IL-5 (panel A) and IL-8 (panel B) dynamics are represented.

production can be noticed, as well as there is not any significant difference in IL-8 production between allergic and non-allergic patients.

After stimulation with 2.5×10^{-5} mol/L of $NiSO_4$, a significant increase in IL-5 production in allergic patients compared to non-allergic ones can be observed. Fig. 8-panel A depicts IL-5 dynamics in non-allergic patients, in which a peak around 9000 fg/µl after 5 days from stimulation is present; in Fig. 9-panel A, representing allergic patients' scenario, a peak around 20,000 fg/µl after 5 days is shown. As for IL-8 production, Fig. 8-panel B depicts the IL-8

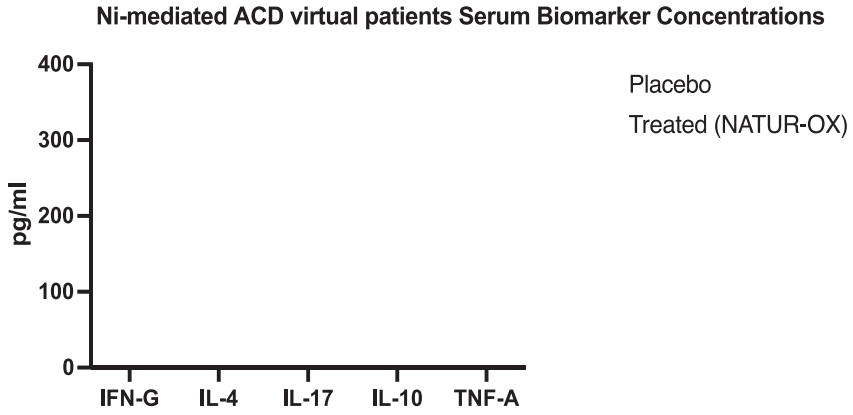

Fig. 10. Prediction of serum concentrations of IFN-γ, IL-4, IL-17, IL-10 and TNF-α in Ni allergic virtual patients treated with placebo compared to ones treated with NATUR-OX®.

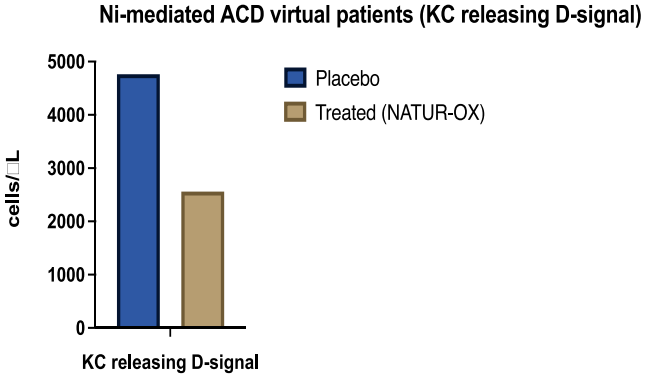

Fig. 11. Prediction of KC releasing D-signal in Ni allergic virtual patients treated with placebo compared to ones treated with NATUR-OX®.

dynamics in non-allergic patients in which a peak around 110,000 fg/μl after 5 days from stimulation is observable, while in the allergic patients' scenario (Fig. 9-panel B) a peak around 130,000 fg/μl is present. Thus, even after stimulation with 2.5×10^{-5} mol/L of $NiSO_4$ there is not any significant difference in IL-8 production, while a significant increase in IL-5 production can be observed.

In conclusion, after stimulation with each of the three $NiSO_4$ concentrations, we can observe an increase in IL-5 production in allergic patients compared to the non-allergic ones, in agreement with the in vivo results from Summer et al. Moreover, the in silico results show that the administration of a higher concentration of $NiSO_4$ leads to a proportional increase of IL-5 in allergic patients, correlating with severity of ACD.

3.2. Study 2

The clinical trial "*Red Grape Polyphenol Oral Administration to Women Affected by Nickel-mediated Allergic Contact Dermatitis (Grapolyphen)*" (NCT03902392) aimed to verify that the administration of polyphenols (NATUR-OX®) to patients with Nickel Allergic Contact Dermatitis is able to reduce peripheral biomarkers. It is an interventional, randomized and double-blind study. For three months, 300 mg of polyphenols was administered everyday (one NATUR-OX® capsule/day) to 18 patients and one placebo capsule/day to another 18 patients. Then, serum biomarker (IFN-γ, IL-17, IL-4, IL-10, PTX3 and NO) concentrations (pg/ml) were evaluated at the time of enrolment (T0) and at the end of the treatment (T1), using ELISA method. They demonstrated that concentration levels of IFN-γ, IL-17, IL-4, PTX3 and NO decreased at T1 in treated patients, while concentration levels of IL-10 increased, compared to T0 values. On the other hand, in placebo treated patients no modifications of biomarker levels were detected. Through UISS, we simulated virtual Nickel allergic patients treated with polyphenols and, analysing the serum biomarker concentrations, we obtained the same results observed in the clinical trial. We obtained specific dynamics depicting the differences in cytokine concentrations between patients treated with NATUR-OX® and patients treated with placebo. As depicted in Fig. 10, INF-γ and IL-17 levels are approximately double in subjects treated with placebo compared to subjects treated with NATUR-OX®; IL-4 and TNF-alpha levels are more than triple in patients treated with placebo than patients treated with NATUR-OX®. Additionally, the IL-10 concentration is higher in subjects treated with NATUR-OX® than in subjects with placebo: IL-10 is an anti-inflammatory cytokine; hence, the treatment increases its concentration.

Concerning the effects of NATUR-OX® on oxidative stress, through UISS we did not evaluate the levels of NO, but the D-signal released by keratinocyte cells (KCs). This represents a signal released by the cells when they are in a state of danger [27], for example under oxidative stress conditions. We obtained in silico confirmation that in those patients treated with NATUR-OX®, a significant decrease in KC releasing D-signal is observable in comparison to the patients taking a placebo (Fig. 11). This means that the treatment can reduce the oxidative stress of keratinocytes.

4. Conclusions

The need to assess the skin sensitizing properties of chemicals is fundamental in all regulated sectors. Considerable progress has been made in methodologies to detect contact hypersensitivity; however, none can be used to as a standalone tool for hazard identification and regulatory classification. Moreover, a framework capable of predicting the immune system dynamics induced by a skin sensitizer is missing.

In this work, we adapted the UISS modelling and simulation platform to simulate allergic contact dermatitis. Two case studies were used to illustrate the potential of this in silico model. Nickel was used as reference contact allergen, where an increase in IL-5 production in allergic patients was observed compared to non-allergic ones, in agreement with the in vivo observations. In addition, the simulations show that the administration of a higher concentration of $NiSO_4$ leads to a proportional increase of IL-5 in allergic patients, correlating with the severity of ACD. In the second

case study, the effect of NATUR-OX® on oxidative stress was simulated. Simulations obtained confirmed that patients treated with NATUR-OX® a significant decrease in KC releasing D-signal was observed in comparison with patients taking a placebo, indicating a treatment that can reduce the oxidative stress of keratinocytes.

To translate these results also for applications in chemical safety assessment, we need to determine whether a chemical is a sensitiser. For classification and labelling, we need to identify its potency (i.e. LLNA EC3 < 2 % or HMT 500 μg/cm^2) and for quantitative risk assessment a threshold of activation (i.e. LLNA EC3 value) needs to be reported. As the LLNA is based on the ability of a sensitiser to induce lymphocyte proliferation, we introduced this parameter in the UISS platform to adjust the proliferating activity of the lymphocytes. This allows UISS-SENS to be used as a general in silico platform for hazard characterization of sensitizing chemicals.

CRediT authorship contribution statement

Giulia Russo: Validation, Data curation, Writing – original draft, Writing – review & editing. **Elena Crispino :** Validation, Data curation, Writing – original draft, Writing – review & editing. **Martina Luini:** Resources, Writing - review & editing, Formal analysis. **Emanuela Corsini:** Resources, Writing – review & editing, Formal analysis. **Alicia Paini:** Resources, Writing – review & editing, Funding acquisition. **Andrew Worth:** Writing – review & editing, Funding acquisition, Project administration. **Francesco Pappalardo:** Conceptualization, Supervision, Methodology, Software , Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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