



**AFRL-RH-WP-TR-2023-0037**

**SKIN SENSITIZATION AMONG MAINTAINERS/FABRICATORS:  
USE OF NEW APPROACH METHODS TO IDENTIFY CHEMICAL  
SENSITIZERS AND DETERMINE PRELIMINARY CANDIDATE  
SURFACE LIMITS**

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| <b>14. ABSTRACT</b><br>Allergic contact dermatitis (ACD) is an immunologic reaction to a dermal chemical exposure that, once triggered in an individual, will result in an allergic response following subsequent encounters with the allergen. Air Force (AF) epidemiological consultations have indicated that AF aircraft structural maintenance workers may experience ACD at elevated rates compared to other occupations. This project aims to better characterize the potential link between ACD and chemical exposure during AF operations by 1) evaluating the sensitization potential of priority AF-relevant chemicals using new approach methodologies, and 2) developing preliminary candidate surface guidelines (PCSGs, i.e., guidelines for the maximum concentrations of chemicals on workplace surfaces to prevent induction of dermal sensitization) for chemicals that are identified as sensitizers. Surface guidelines for known sensitizers were derived from available local lymph node assay (LLNA) and quantitative experimental human results in previously published literature. New approach methods (NAMs) including <i>in chemico</i> and <i>in vitro</i> assays (k-DPRA and KeratinoSens <sup>TM</sup> ) and <i>in silico</i> models (Derek Nexus <sup>®</sup> and OECD QSAR Toolbox) were leveraged into an integrated approach to predict sensitizer status for 15 chemicals with unknown sensitization potential and four chemicals (limonene, xylene, benzaldehyde, methylglyoxal) with existing LLNA data. LLNA effective concentration values (EC3) were predicted from NAM data via the method of Natsch & Gerberick (2022). The derived EC3 values were used to calculate PCSGs, which can be used to compare to the presence of these chemicals on work surfaces to better understand the risk of Airmen, and workers in general, from developing ACD from occupational exposures. |                         |                                |                                              |                                                                          |                                                        |
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## 1.0 SUMMARY

Allergic contact dermatitis (ACD) is an immunologic reaction to a dermal chemical exposure that, once triggered in an individual, will result in an allergic response following subsequent encounters with the allergen. The induction of the initial immunologic response, termed sensitization, has a dose-response relationship and a threshold of exposure below which induction is not expected to occur, known as a No Expected Sensitization Induction Level (NESIL). Air Force (AF) epidemiological consultations indicated that AF aircraft structural maintenance workers may experience ACD at elevated rates compared to other occupations. Developing surface guidelines from NESILs for AF-relevant chemicals, and periodically verifying high-contact surfaces are below these levels, can be implemented as a control to prevent dermal sensitization of AF workers to contact allergens.

A number of methods have been developed to experimentally classify skin sensitizers and determine sensitizer potency. The mouse local lymph node assay (LLNA) is the gold standard assay for classifying skin sensitizers, and it has also been used to define potency of skin sensitizers under the Globally Harmonized System (GHS) for Classification and Labeling. However, the recent publication of an Adverse Outcome Pathway (AOP) and an Organisation for Economic Cooperation and Development (OECD) Guidance Document on the Integrated Approach to Testing and Assessment (IATA) for ACD has permitted the use of new approach methodologies (NAMs) to replace the traditional animal tests for evaluation of sensitization potential of chemicals. Under the IATA, several Defined Approaches (DAs) outline specific non-animal testing approaches that can be used to identify sensitizing chemicals. The “2 out of 3” DA uses the Direct Peptide Reactivity Assay (DPRA), KeratinoSens™, and human Cell Line Activation Test (h-CLAT) assays, which address the first three key events of the skin sensitization AOP, in a weight of evidence assessment to classify chemicals as skin sensitizers or non-sensitizers. Effective concentration values (EC3), which were traditionally derived from LLNA studies, can also be predicted from *in chemico* and *in vitro* data using a linear regression model developed by Natsch and Gerberick (2022a). Together, these methods provide an opportunity for rapid assessment of the potential induction of ACD by dermal exposure to chemicals used in the process of aircraft maintenance by eliminating time- and cost-intensive animal testing.

The goal of the current project was to better characterize the potential link between ACD and chemical exposure during AF operations by: 1) evaluating the sensitization potential of priority AF-relevant chemicals using NAMs, and 2) developing preliminary candidate surface guidelines (PCSGs, i.e. guidelines for the maximum concentrations of chemicals on workplace surfaces to prevent induction of dermal sensitization) for chemicals that are identified as sensitizers. An initial literature review was conducted to identify existing information on sensitizer status for 125 AF-relevant chemicals that were selected based on their prevalence in use across AF fabrication and maintenance shops. Surface guidelines for literature-identified sensitizers were derived from LLNA results or experimental human effect levels for chemicals with that quantitative data available in previously published literature. *In silico* models (Derek Nexus® and OECD quantitative structure-activity relationship (QSAR) Toolbox) were used to screen for potential sensitizers among those with insufficient information on sensitization potential in the literature and prioritize them for additional testing. The peptide depletion rates obtained from the kinetic version of the DPRA (k-DPRA) and the fold induction of antioxidant response element (ARE) genes obtained from the KeratinoSens™ assay were used to predict sensitization potential for

literature-identified sensitizers without quantitative data and *in silico*-identified sensitizers. The characterization of sensitization potential obtained from the NAMs, along with the predicted EC3 values, literature data, and *in silico* results, were combined into a weight of evidence approach to determine sensitizer status for each of the chemicals that were evaluated. PCSGs for all chemicals identified as sensitizers were calculated using appropriate adjustment factors for interspecies differences, interindividual variability, exposure conditions, and/or translation of new approach methodology (NAM) results to human effects.

The literature review preliminarily assigned 21 chemicals as sensitizers, 38 chemicals as non-sensitizers, and 58 chemicals as lacking information to determine sensitizer status. Eight chemicals were removed from consideration for low dermal exposure potential because they were light gases. Of the 58 chemicals lacking information on sensitizer status, *in silico* screening identified seven as potential sensitizers that were prioritized for NAM assays. NAM assays were also performed on four chemicals (d-limonene, xylene, benzaldehyde, methylglyoxal) that had literature LLNA EC3 values to provide a general evaluation of the quality of the NAM-based testing strategy. A total of 19 chemicals were tested using k-DPPRA and KeratinoSens™ assays (11 literature-identified potential sensitizers, 7 *in silico*-identified potential sensitizers, and 1 non-sensitizer according to literature review) to further evaluate sensitization potential and predict EC3 values. For the four chemicals with existing LLNA data indicated above, the predicted EC3 values were 26.8 percent (%), 80.8%, 51.9%, and 3.6%, respectively, which generally agreed with literature values.

Literature EC3 values categorized five chemicals as GHS Subcategory 1A strong sensitizers: glyoxal, methylglyoxal, formaldehyde, hexamethylene diisocyanate, and hexavalent chromium. Three chemicals (benzaldehyde, beryllium, nickel) had existing LLNA EC3 values that classified them as GHS Subcategory 1B sensitizers. The chemicals crotonaldehyde, acrolein, methacrolein, and 1-naphthylamine were identified as GHS Subcategory 1A sensitizers by NAM-derived EC3 values, which were 0.4%, 0.5%, 0.5%, and 1.5%, respectively. A weight of evidence assessment also categorized all the above chemicals as sensitizers. Similarly, epichlorohydrin and acrylonitrile were determined to be sensitizers, with the predicted EC3 values categorizing both as GHS Subcategory 1B. Methyl isobutyl ketone, propionaldehyde, d-limonene, and xylene were determined to be non-sensitizers by the weight of evidence.

A weight of evidence assessment categorized the remaining chemicals (bisphenol-A, n-phenyl-1-naphthylamine, p-tert butylphenol, naphthalene, valeraldehyde, m-tolualdehyde, and o-tolualdehyde) as sensitizers. There was non-concordance of the results of NAM assays for each of these chemicals, possibly because they were only, at most, moderate to weak sensitizers according to their NAM-predicted EC3 value. However, all but three (m-tolualdehyde, o-tolualdehyde, and valeraldehyde) of these seven chemicals had evidence of sensitization potential in the literature. The lack of NAM concordance indicated that future testing with the h-CLAT assay may be necessary to more definitively make an assessment of sensitizer status for these chemicals. PCSGs were calculated for all chemicals identified as sensitizers with quantitative experimental data from the literature or derived from the NAM assays.

PCSGs were derived for 22 chemicals and the sensitization potential was determined for the majority of 125 AF-relevant chemicals through a combination of literature review, *in silico*

modeling, and *in chemico/in vitro* assays. This project provides information on what chemicals and concentrations can potentially induce sensitization among workers and demonstrates a method to develop surface guidelines for AF-relevant chemicals with potential for inducing ACD in a more efficient manner than traditional animal-testing approaches.

## 2.0 INTRODUCTION/BACKGROUND

### 2.1 Allergic Contact Dermatitis: Clinical Presentation, Mechanism of Induction, and Sensitizer Categorization

#### Clinical symptoms

Contact dermatitis (CD) is characterized by inflammation of the skin and the formation of pockets of intercellular fluid in the epidermis (Marks *et al.*, 2001). Occupational contact dermatitis (OCD) can be broken into two distinct types: irritant contact dermatitis (ICD) and ACD. ICD, accounting for approximately 80% of OCD, is caused by a physical disruption of the epidermis and dermis (Marks *et al.*, 2001). Common irritants include long-chain hydrocarbons like those found in jet fuel (Contestable, 2017), soaps and other detergents, and solvents (Bains *et al.*, 2019). ICD can be resolved through application of medicaments/ointments and preventing prolonged contact with the irritant.

ACD is a Type IV hypersensitivity immune response with lifelong consequences due to permanent sensitivity to an allergen (Kimber, 2002a). Prior to the development of symptoms, sensitization is induced in a susceptible individual. This induction period is characterized by exposure to a sufficient concentration of an allergen, usually expressed as a mass per surface area of skin micrograms ( $\mu\text{g}$ ) per square centimeter ( $\text{cm}^2$ ). Both repeated doses at low concentrations and single, high dose exposures have been reported to cause sensitization. However, the current consensus is that induction to ACD follows a dose-response model and that a NESIL can be derived as a threshold of exposure below which induction will not occur (van Loveren *et al.*, 2008; Rees *et al.*, 1990; White *et al.*, 1986).

ACD normally presents 24-48 hours after a sensitized individual has contacted an allergen (Marks *et al.*, 2001). The location of the irritation can assist in identifying points of exposure, but because skin sensitivity and thickness varies across the body, the area presenting the most severe reaction may not be the initial point of contact (Marks *et al.*, 2001). For example, allergy to paraphenylenediamine in hair dye results in inflammation on the scalp and face, but if dye were washed out in a shower, irritation may also show on the back. Clinicians must take careful note and fully comprehend worker practices to understand how an irritant may be transferred from one place on the body to another (Marks *et al.*, 2001). Itching is an early sign of ACD, whereas those suffering from ICD report stinging sensations (Marks *et al.*, 2001). If extensive exposure to either irritants or allergens occur, the skin responds more vigorously and it becomes virtually impossible to determine the cause by visual inspection alone (Marks *et al.*, 2001). Instead, a clinician must rely on occupational history, skin sample histology, allergen patch test results, and expert judgement to determine if an allergen has caused the response (Marks *et al.*, 2001).

Once induction has occurred, workers must avoid further contact with the allergen to prevent eliciting an allergic response. After the immune system has been primed through induction, a lower

dose of an allergen can elicit a reaction than it took to cause sensitization. Thus, ACD can lead to duty-limiting allergies.

#### Mechanism for ACD Induction

As the largest organ by surface area, the skin presents a significant route of exposure for chemicals. The skin acts as a barrier to protect the rest of the body from foreign intrusions such as chemicals, biological agents (viruses, bacteria), ultraviolet radiation, and physical stresses (Honari and Maibach, 2014). The line of defense against these exogenous dangers is the epidermis, which is principally comprised of layers of keratinocytes.

Keratinocytes originate at the basal layer in the epidermis and over 28 days migrate to the topmost layer of the stratum corneum (Fenner and Clark, 2016). By the time keratinocytes leave the stratum granulosum and enter the bottom-most layer of stratum corneum, they have died (Fenner and Clark, 2016). As several layers of these dead keratinocytes build up, they create a “brick and mortar” structure where the non-viable keratinocytes form “bricks” held together by the “mortar”, an intercellular matrix principally composed of keratin and lipids (Stefaniak *et al.*, 2011). Chemicals that cause ACD must diffuse across the stratum corneum to reach the viable epidermis. Chemical diffusion through the stratum corneum can occur through 1) intercellular lipid pathway (through the “mortar”), 2) trans-cellular permeation (through the “brick”), or 3) along the skin structures such as hair follicles (Stefaniak *et al.*, 2011). As the “brick and mortar” structure contains only 5-20% water, compared to the viable epidermis layer which contains 70%, hydrophilic substances are less likely to pass through and initiate sensitization (Stefaniak *et al.*, 2011). Other factors that influence the passage of chemicals through the stratum corneum include skin integrity, environmental conditions, molecule size, and properties of the molecule (Table 1).

In general, hydrophobic chemicals are more likely to penetrate the stratum corneum and factors such as reduced integrity of the skin barrier, the presence of solvents, and other environmental factors can increase diffusion across the skin. However, other mechanisms of accessing the viable epidermis (e.g., along skin structures such as hair follicles) can cause more hydrophilic chemicals to act as sensitizing agents as well (Fitzpatrick, 2016).

**Table 1. Determining factors in whether a low molecular weight chemical successfully permeates across the stratum corneum**

| <b>Factor Impacting Likelihood to Cross the Stratum Corneum</b> | <b>Direction of Impact</b> |
|-----------------------------------------------------------------|----------------------------|
| Hydrophilic chemical ( $\log K_{ow} < 1$ )                      | Decrease                   |
| Hydrophobic chemical ( $\log K_{ow} > 1$ )                      | Increase                   |
| High molecular weight ( $>500$ Daltons)                         | Decrease                   |
| Extended contact time between skin and chemical                 | Increase                   |
| Solvents are present                                            | Increase                   |
| Skin is damaged/disrupted                                       | Increase                   |
| Underlying medical condition prevents normal skin function      | Increase                   |
| High humidity in the environment                                | Increase                   |
| Elevated temperatures                                           | Increase                   |

Once an allergen moves from the stratum corneum into the viable epidermis, it covalently binds with a skin carrier protein to form a hapten complex (Griem, 2003). Within the viable epidermis, Langerhans Cells (LC) act as the vanguard of the immune system, scavenging for hapten complexes. Once an LC has captured a hapten complex, it moves through the epidermis by afferent lymph vessels to the local draining lymph node in the dermis (Stefaniak *et al.*, 2011). While en route to the local draining lymph node, LCs mature into dendritic cells (DCs) (Wong *et al.*, 2015). At the local draining lymph node, the DCs present the hapten to naïve T-lymphocytes (Stefaniak *et al.*, 2011). If the hapten is recognized, the T-lymphocytes differentiate into memory T-cells (Stefaniak *et al.*, 2011). These memory T-cells create clones and await re-exposure to the allergen (Wong *et al.*, 2015). At this point, the allergic mechanism has been induced and subsequent exposures will elicit an ACD response.

## **2.2 Impact on Global, United States, and Air Force Worker Populations**

### *Global Impact of ACD*

Worldwide, 20% of the population is likely to develop ACD to at least one substance during their lifetime (Alinaghi *et al.*, 2019; Peiser *et al.*, 2012). In a meta-analysis of epidemiology studies, researchers found the prevalence of allergies in the general population in Europe and North America as: 11.4% (95% Confidence Interval (CI): 9.4-13.5%) to nickel, 2.7% (95% CI: 2.1%-3.4%) to cobalt, 1.8% (95% CI: 1.3-2.6%) to chromium, 1.2% (95% CI: 0.9-1.5%) to formaldehyde, and 1.2% (95% CI: 1.0-1.5) to *p*-tert-butylphenol formaldehyde resin (Alinaghi *et al.*, 2019).

In a Finnish cohort, 605 participants diagnosed with occupational hand eczema (OHE) seven years prior responded to a follow up questionnaire to determine the continuing impact their diagnosis had on their lives (Mäлкönen *et al.*, 2010). As a direct response to their OHE, only 8% of study population reported no change to their work (Mäлкönen *et al.*, 2010). The remaining population reported a combination of changes to their task or job (54%), a change in occupation (34%), or that they left the workforce through unemployment or retirement as a result of their OHE (25%) (Mäлкönen *et al.*, 2010). As participants were allowed to select all changes that occurred in the intervening seven years, some overlap in categories was observed, but an underlying trend of significant impact to working life was evident.

### *Current Understanding of the Impact of ACD in the Air Force*

In 2019, the United States AF School of Aerospace Medicine (USAFSAM) Epidemiology Consult Service Division examined records from 2010 – 2017 to evaluate the relationship between occupational hexavalent chromium exposure and seven potential health outcomes: lung cancer, asthma, nasal septum perforation, epistaxis, ACD (referred to as contact dermatitis in the report), ICD (referred to as non-allergic skin irritation in the report), and skin ulcers. Two AF Specialty Codes (AFSCs) known to have hexavalent chromium exposure were selected as the populations of interest: 2A7X3 (aircraft structural maintenance) and 2A7X5 (low observable aircraft structural maintenance). The control populations came from two non-exposed AFSCs: 2T0X1 (traffic management) and 2T2X1 (air transportation). Of the studied health outcomes, elevated risk was only found for ACD (incidence rate ratio (IRR): 4.67, 95% CI: 2.29 – 9.49) and ICD (IRR: 2.15, 95% CI: 1.24 – 3.75).

In 2020, the Epidemiology Consult Service Division reviewed the health records of USAF maintainers and fabricators from 2015 – 2019 to determine the incidence rates of dermatitis among five AFSCs: Aerospace Maintenance (2A), Fuels (2F), Missile and Space Systems Maintenance (2M), Transportation and Vehicle Management (2T), and Civil Engineering (3E). Nearly 10,000 cases met the study’s definition for acute dermatitis, which included International Classification of Disease codes (ICD-9 and ICD-10) for both allergic and ICD. In 2015, cases were evaluated using ICD-9 codes, but ICD-10 codes were used beginning in 2016. In 2016 through 2019, all studied AFSCs saw an increase in incidence over the previous year. The five-year average incidence by AFSC is shown in Table 2.

**Table 2. The average incidence of acute dermatitis by AFSC from 2015-2019**

| <b>AF Specialty Codes (AFSCs)</b>          | <b>Incidence per 1000 persons</b> |
|--------------------------------------------|-----------------------------------|
| Aerospace Medicine (2A)                    | 21.5*                             |
| Fuels (2F)                                 | 20.2*                             |
| Missile and Space Systems Management (2M)  | 15.0                              |
| Transportation and Vehicle Management (2T) | 20.5*                             |
| Civil Engineering (3E)                     | 21.8                              |

\*Estimated from a figure in Epidemiology Consult Service Division memorandum

The authors identified limitations in the available data that limited their ability to characterize the exposure-response relationship for the health effects that were studied. For the 2019 study, validating exposures was the primary limitation. From the data available to the consult service, it was not possible to determine if hexavalent chromium exposure was entirely from their job or if 1) secondary, environmental exposure to hexavalent chromium occurred, 2) hexavalent chromium exposure pre-dated their work in the defined AFSCs, or 3) chemicals other than hexavalent chromium were responsible for the elevated risk of developing dermatitis. In the 2020 study, the primary limitations were that 1) it was assumed an AFSC was an adequate proxy for exposure when it is known that exposures vary greatly by task and worker, 2) the cases were identified solely through the use of TRICARE, so if medical help was sought outside the system it wasn’t captured, and 3) no outside comparison group was evaluated to determine if the incidence of dermatitis within these AFSCs was significantly elevated. Nonetheless, the two studies provide qualitative evidence of an occupational contribution to dermatitis in AF personnel, even if there is insufficient data for the quantitative evaluation of the exposure-response relationship for specific chemicals or tasks (AFSCs)

Occupational allergens fall into general categories based on their mechanism of action: 1) low molecular weight (LMW) allergens (chemicals), 2) high molecular weight allergens (proteins), and 3) metals (Anderson *et al.*, 2017). Common LMW chemicals of interest to the AF include formaldehyde, isocyanates, epoxies and common metals including hexavalent chromium, nickel, and cobalt (Alinaghi *et al.*, 2019; Anderson *et al.*, 2017; Cashman *et al.*, 2012; Filon *et al.*, 2021).

While proteins cover a wide spectrum of potential allergens, the most commonly cited reactions in the AF occur from exposure to latex gloves, wheat flour, and to a lesser degree, handling laboratory animals (Anderson *et al.*, 2017). The current study focused on LMW and metal allergens as a source of occupational risk.

### 2.3 Prevention and Mitigation Strategies

Opportunities exist to prevent the induction of sensitization among workers. Viewed through the lens of hierarchy of controls, the most effective strategies include reformulating process chemicals to remove sensitizers completely (elimination) and substituting less potent sensitizers for more potent ones. While most effective, these methods also represent significant investments in time, manpower, and money without promise of feasibility or practicality. For example, hexavalent chromium is a potent human sensitizer commonly found in corrosion control operations (Carlton, 2003a; Bennett *et al.*, 2016; Bennett *et al.*, 2018). Efforts to find a replacement or substitute metal are being investigated by the AF Life Cycle Management Center (LCMC), but no clear solution has been identified. While hexavalent chromium is a single chemical out of the thousands used on military installations, the challenges associated with elimination and substitution are common across chemicals with well-established uses. It is often necessary to consult with experienced safety professionals or industrial hygienists, ideally those who are familiar with both the health effects of the products and the processes used in the workplace. Some substitutions that ostensibly appear less toxic may result in comparable or additional risk due to the process used.

Engineering controls are midway on the effectiveness scale of controls and still require significant implementation time. There are some processes using hexavalent chromium for which 1) controls do not yet exist, 2) existing controls do not adequately reduce the level of exposure (Carlton *et al.*, 2003b; England and Carlton, 1998), and 3) a change in occupational exposure limit or prescribed sampling methodology means a previously adequate control no longer meets the desired performance criteria (Carlton, 2003c).

While ranked lowest on the hierarchy of controls due to the reliance on worker compliance, personal protective equipment (PPE) against dermal exposures is currently available and used within the AF. Among PPE options, gloves, encapsulating suits, and barrier creams have all been shown to reduce exposure incidence of contact dermatitis by preventing direct allergen-to-skin contact (Saary *et al.*, 2005).

Ranked above PPE in the hierarchy of controls, administrative controls are concerned with the policies around work such as scheduling, duration of tasks, and housekeeping. Surface guidelines, or recommendations for limiting surface concentrations of a chemical below levels where induction of sensitization is expected to occur after dermal contact, can be used to inform these controls. Quantitative risk assessment recommendations that can be used to establish surface guidelines have been published for some fragrances and other ingredients in consumer products (Api *et al.*, 2014; Api *et al.*, 2020; Basketter and Safford, 2016). Recommendations for deriving surface guidelines for occupational allergens have been outlined by Naumann and Arnold (2019) and Fukushima, *et al.* (2022). These methods form the basis for developing exposure recommendations, referred to as PCSGs. PCSGs are proposed guidelines that would require review and approval by appropriate scientific and policy-setting organizations prior to adoption and implementation. Periodically verifying surfaces are below the PCSG level is a method that can

potentially be rapidly implemented to prevent dermal sensitization of AF workers to contact allergens.

The PCSG concept is predicated on having human-relevant dose-response data for sensitizing chemicals. For widely used chemicals, especially those used in cosmetics, human subject data may be available. However, for most chemicals, including those used in industrial processes or in consumer products, data have traditionally been obtained primarily through *in vivo* testing in laboratory animals. However, *in vitro* assays in human cell culture models and *in silico* QSAR models, such as those used in this current study, are becoming more widely accepted as replacements for *in vivo* testing. Currently, there is a lack of *in vivo* or *in vitro* data for chemicals that are commonly used within AF operations that are not widely used in civilian industries or consumer products.

## 2.4 Human *In Vivo* Skin Sensitization Test Methods

### Human Repeat Insult Patch Test (HRIPT)

The HRIPT was pioneered by John Draize in 1944 to establish induction thresholds for skin sensitization (Bormann and Maibach, 2021). In his original study, 200 subjects (50% female) were exposed to 0.5 g or 0.5 milliliters (mL) of a potential allergen on 1 in<sup>2</sup> patches that were placed on the back or upper arm (Draize *et al.*, 1944). The patches were left in place for 24 hours, then removed, and the reaction of the skin noted (Draize *et al.*, 1944). The following day, 48 hours after the first patch was placed, a new patch was placed in the same position and the reaction of the skin at the site read 24 hours later (Draize *et al.*, 1944). This pattern continued with a new patch placed on a subject every other 24 hours for 10 patches (Draize *et al.*, 1944). After the tenth patch was removed, participants were given a 10–14-day rest period (Draize *et al.*, 1944). At the conclusion of the rest period, a final patch was applied and left for 24 hours, then removed and any reaction recorded (Draize *et al.*, 1944).

The drawback of this test is that heterogeneity in the study design makes direct comparison between labs difficult. Differences in patch size and placement, occlusion status, allergen delivery vehicle, population size and demographics, allergen concentrations, patch duration all contribute the variability seen across studies testing the same chemicals (Bormann and Maibach, 2021). Despite this variability, however, general trends in the number of participants that experience induction at a particular concentration are indicative of allergic potential and potency.

Compared to other human data on sensitization, this type of study is unique in that it studies induction, not elicitation among the previously sensitized individuals. Given the ethical concerns surrounding the purposeful induction of a lifelong sensitivity in subjects, the HRIPT is used sparingly today (Basketter, 2009) and has more recently been referred to as the “Confirmation of No Induction in Humans” (CNIH) test for HRIPTs specifically performed to confirm a skin sensitization response (Na *et al.*, 2022).

### Human Maximization Test (HMT)

The HMT was designed to assess potency of contact allergens in humans (Kligman, 1966). The test placed the chemical of interest on the skin of the forearm with complete occlusion using a patch for five days of 48-hour periods with one day of rest in between each (Kligman, 1966). Pretreatment of the site with a 5% sodium lauryl sulfate solution for 24-hours prior to the test

article application was also sometimes used to cause a mild inflammatory response that would promote sensitization (Kligman, 1966). A 48-hour exposure to a challenge patch of the test chemical would occur up to two weeks following the induction exposures (Kligman, 1966). The test recommendations were eventually updated because of excessive irritancy to sodium lauryl sulfate, which included fewer exposures during induction and lower concentrations during the challenge test (Kligman and Epstein, 1975). While the HMT was able to provide semi-quantitative human evidence of potency based on the percentage of volunteers sensitized, as with the HRIPT, it has largely been replaced by animal and new approach methodology testing due to ethical concerns.

## **2.5 *In Vivo* Laboratory Animal Skin Sensitization Test Methods**

### **Guinea Pig Maximization Test (GPMT)**

The GPMT uses a semi-quantitative scale for estimating the sensitizing strength of tested chemicals by exposing guinea pigs to different doses of the test chemical via dermal application (Andersen *et al.*, 1995; Basketter *et al.*, 1997). The test uses Freund's complete adjuvant to potentiate skin sensitization. The test lacks a quantitative endpoint, so the analysis of group responses is a subjective assessment by the researcher. The test was adopted in OECD guideline 406 (OECD, 2022c). Current dermal sensitizer research no longer relies on the GPMT, but published historical data can be used to identify chemicals as sensitizers.

### **Buehler Test**

The Buehler test is a non-adjuvant *in vivo* testing method in guinea pigs. It has been used to provide information on the sensitization potential of a material under exaggerated, but relevant exposure conditions (Robinson *et al.*, 1990). Similar to the GPMT, observed responses are recorded on a graded semi-quantitative scale, and therefore it can only provide limited quantitative information on potency, which is dependent on dose level selection (OECD, 2022c). Because it is a non-adjuvant method, it is less sensitive than the GPMT. The Buehler test has been incorporated into OECD guideline 406 (OECD, 2022c).

### **Local Lymph Node Assay (LLNA)**

The LLNA test is an *in vivo* mouse assay that measures a chemical's ability to induce a proliferating response of specialized T-lymphocytes in the draining lymph nodes of the mouse ear following repeated dermal application to the area around the ear (Kimber *et al.*, 2002b). The method is based on the use of radioactive labeling of proliferating T cells, as described in OECD Testing Guideline number 429 (OECD, 2010). The quantitative result of the test is the EC3 value, which reflects the concentration at which the proliferation response is sufficiently induced (Basketter *et al.*, 1999). The EC3 value, which is typically reported as a percent concentration in the applied substance, is inversely related to sensitizer potency (Basketter *et al.*, 1999).

A newer version of the LLNA uses the measurement of 5-bromo-2-deoxyuridine (BrdU) using an enzyme-linked immunosorbent assay (ELISA) or flow cytometry as an indicator of cell proliferation. This method is called LLNA:BrdU-ELISA, or Flow Cytometry (FCM) when the flow cytometry method is employed. It is listed as OECD Testing Guideline number 442B (OECD, 2018). A test material is considered a potential sensitizer if the test results in a greater than 1.6-fold induction of cell proliferation using the ELISA method, or a 2.7-fold induction of cell proliferation using the flow cytometry method (OECD, 2018).

When compared to human dose-response data, EC3 values derived from the LLNA were consistently useful for predicting human relevant potency categories (Basketter *et al.*, 2001; Gerberick *et al.*, 2001; Griem *et al.*, 2003; Ryan *et al.*, 2000). EC3 values have been used to derive surface limits (or surface guidelines) that define the maximum allowable concentrations of chemicals on workplace surfaces in order to prevent induction of dermal sensitization (Naumann and Arnold, 2019).

EC3 values have also been incorporated into skin sensitizer potency assessment by the GHS of Classification and Labelling of Chemicals (GHS) and the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC). GHS describes all sensitizers as Category 1 and further subdivides the category by potency. Strong (Subcategory 1A) or other (Subcategory 1B) sensitizers, are distinguished using an LLNA EC3 threshold value of 2% (Table 3). Chemicals that are not considered sensitizers are labeled as “not classified” (United Nations, 2021). ECETOC categorizes sensitizers as weak, moderate, strong, or extreme using threshold EC3 values that range from 10% to 0.1% (Table 3) (ECETOC, 2003).

**Table 3. GHS and ECETOC categories for skin sensitizer potency**

| GHS     |                      | ECETOC  |                     |
|---------|----------------------|---------|---------------------|
| EC3 (%) | Category             | EC3 (%) | Category            |
| ≤2      | 1A-Strong sensitizer | ≥10     | Weak sensitizer     |
| >2      | 1B-Other sensitizer  | ≥1-<10  | Moderate sensitizer |
|         |                      | ≥0.1-<1 | Strong sensitizer   |
|         |                      | <0.1    | Extreme sensitizer  |

## 2.6 New approach methods (NAMs) used to Determine Skin Sensitization Potential

Recently, with the global transition to more human relevant *in vitro* models, large-scale international efforts have been underway to replace traditional animal assays for skin sensitization with *in chemico*, *in vitro* and *in silico* methods.

NAM testing approaches are based on the scientific consensus of the biological responses leading to the adverse health effect – or AOP – for skin sensitization/ACD (OECD, 2012). The complete AOP for ACD is shown in Figure 1. The key events of the AOP cover the mechanism of effect described in section 2.1. To date, no single assay or *in silico* model can recapitulate all four key events in the human AOP, but tests have been developed for the first three key events in the pathway (Figure 1). *In silico* methods have also been developed that use QSAR models to predict whether chemicals are likely to be sensitizers, the potency of sensitizers, and even EC3 values.

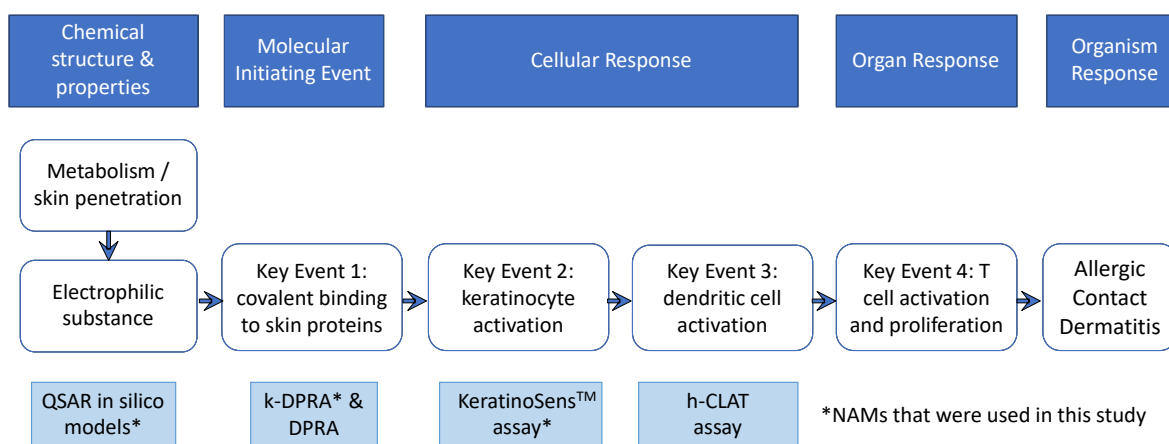


**Figure 1. Skin sensitization Adverse Outcome Pathway and test methods**

The DPRA addresses the first key event of the AOP. In the assay, the reaction of a test article towards a synthetic peptide containing cysteine and/or lysine is evaluated to determine the ability of that test article to electrophilically react with nucleophilic components of skin proteins. Depletion of a peptide containing both residues is considered a positive response if the mean depletion is at least 6.38% (OECD, 2021). If only cysteine depletion is being measured, depletion greater than 13.89% is considered a positive result (OECD, 2021). A kinetic version of the test (k-DPRA) can be performed to measure maximum reaction rate, with a logarithm of the maximum reaction rate constant ( $\log k_{\max}$ ) of  $\geq -2$  indicating that a test article is a UN GHS Subcategory 1A (strong) skin sensitizer (OECD, 2022a). Test articles with a  $\log k_{\max}$  less than this value are non-Subcategory 1A, but further categorization cannot be made from the k-DPRA test alone (OECD, 2022a).

The KeratinoSens™ assay addresses keratinocyte activation, the second key event of the AOP. KeratinoSens™ cells (Givaudan, Switzerland) have been propagated as a reporter cell line. The KeratinoSens™ cells are transfected immortalized human keratinocytes (HaCaT) that include a 56-base-pair insertion containing the ARE, sequence from the Aldo-Keto Reductase Family 1 Member C2 (AKR1C2) gene, a simian virus 40 (SV40) promoter, the luciferase gene in the vector plasmid GL4 (pGL4) from Promega, and a stable insertion allowing for cells to be selected in the presence of Geneticin (G418). The signaling pathway with the repressor protein Kelch-like ECH-associated protein 1 (Keap1) and the transcription factor Nuclear Factor-Erythroid Factor 2-Related Factor 2 (Nrf2), which binds to the ARE / electrophile-responsive element, has been shown to be a reliable cellular endpoint to detect skin sensitizers *in vitro* (Natsch and Emter, 2008; Natsch *et al.*, 2009). The induction of luciferase directly indicates the activation of ARE-dependent genes. The assay is considered positive when there is a greater than 1.5 fold luciferase induction over controls at cell viabilities of greater than 70% and test article concentrations of less than 1000 micromolar ( $\mu\text{M}$ ) in at least two valid trials (OECD, 2021).

The human cell-line activation or h-CLAT test characterizes the activation of DCs, addressing the third key event of the AOP. The test assays the upregulation of CD54 and/or CD86 as an indication of activation of antigen presenting cells and has been shown to be predictive of human sensitizing potential (Nukada *et al.*, 2011). Greater than 2-fold induction of CD54 or a  $> 1.5$ -fold induction of

CD86 compared to controls at cell viabilities of >50% is considered a positive response (OECD, 2021).

*In silico* methods have been shown to be highly predictive in determining sensitizer status (Golden *et al.*, 2021). Derek Nexus<sup>®</sup> (Lhasa Ltd., United Kingdom) is a commercial *in silico* modeling software based on structural alerts. Derek Nexus<sup>®</sup> evaluates the functional groups of a chemical of interest most likely to induce skin sensitization and compares those groups to all records in its database. If an exact match is found, all data collected for the chemical is presented with published sources. If an exact match cannot be found, Derek Nexus<sup>®</sup> provides predicted sensitization properties and EC3 values based on an assessment of functional groups of the most similar chemicals (i.e., “nearest neighbors”). Each prediction is accompanied by a Tanimoto score, which is an indication of how close a match the chemical of interest is to its neighbors.

The OECD QSAR Toolbox is a free *in silico* method that uses structural alerts to categorize target chemicals by their potential toxicological response. These features can be used to retrieve measured data for a target chemical or fill data gaps through automated workflows for skin sensitization that predict sensitizer status and EC3 values based on structural comparisons of the chemical of interest to those in the database with human, GPMT, and LLNA data.

## **2.7 Using NAMs to for Skin Sensitizer Hazard Identification and Potency Prediction**

The OECD has developed a guidance document on using NAMs for Hazard Identification (assigning a chemical a status of “sensitizer” or “non-sensitizer”) and Potency Prediction (OECD, 2021). Several possible workflows, or “DAs” have been explicitly defined for these purposes, which incorporate multiple NAMs that address key events in the skin sensitization AOP into an integrated assessment. Weight of evidence approaches that incorporate human, *in chemico*, *in vitro*, *in silico*, and animal data into an integrated assessment can also be used to make a determination of sensitizer status.

OECD Guideline 497 includes two different DAs that incorporate *in vitro* and/or *in silico* NAMs associated with the AOP listed in Figure 1. The DAs have been shown to be comparable, if not better, than *in vivo* methods in predicting whether chemicals are skin sensitizers (OECD, 2021). The “2 of 3” DA is used to assign sensitizer status according to positivity in two out of three of the DPRA, KeratinoSens<sup>™</sup>, and h-CLAT assays (Figure 2).

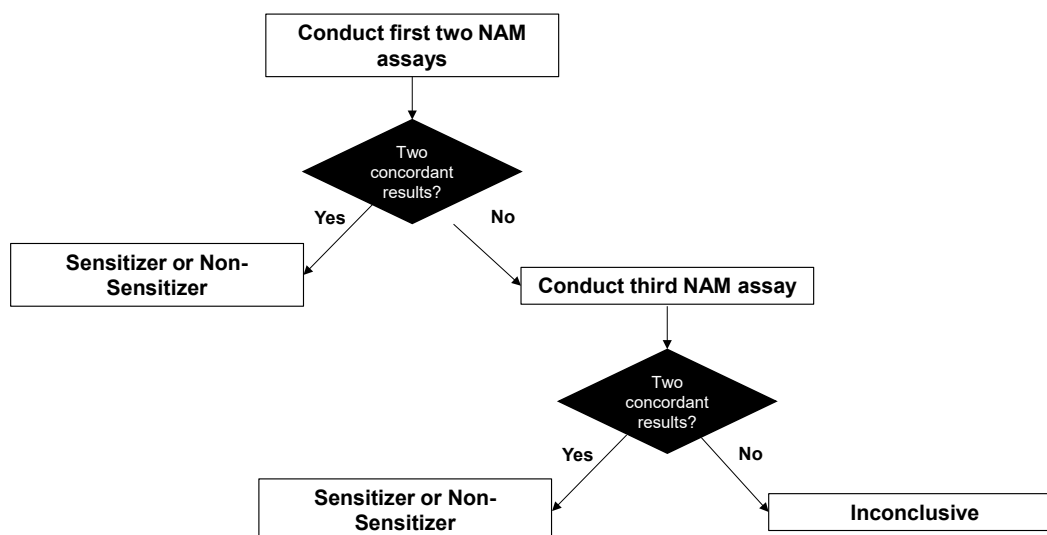


**Figure 2. OECD Guideline 497 “2 of 3” defined approach**

Two Integrated Testing Strategies (ITSv1 and ITSv2) in OECD Guideline 497 can also be used to determine sensitizer status. These strategies assign a 0-3 score for the DPRA, h-CLAT, and Derek<sup>®</sup> (ITSv1) or OECD Toolbox (ITSv2) results (Table 4). The scores are then summed to determine if chemicals are sensitizers that fall within one of the two GHS subcategories or are non-sensitizers (Table 4).

**Table 4. OECD Guideline 497, Integrated Testing Strategy (ITS) defined approach**

| Score                                                                                                 | <i>In silico</i> | DPRA                 | h-CLAT*     |
|-------------------------------------------------------------------------------------------------------|------------------|----------------------|-------------|
|                                                                                                       | ITSv1: DEREK     | Cysteine % Depletion | MIT (µg/mL) |
|                                                                                                       | ITSv2: OECD-T    |                      |             |
| 3                                                                                                     | N/A              | ≥98.24               | ≤10         |
| 2                                                                                                     | N/A              | ≥23.09, <98.24       | >10, ≤150   |
| 1                                                                                                     | Positive         | ≥13.89, <23.09       | >150, ≤5000 |
| 0                                                                                                     | Negative         | <13.89               | No result   |
| Total Score with all three assays = 6-7: UN GHS 1A<br>2-5: UN GHS 1B   0-1: Not classified            |                  |                      |             |
| Total Score with only DPRA and <i>in silico</i> = 3-4: UN GHS 1**<br>2: UN GHS 1B   0-1: Inconclusive |                  |                      |             |

\*h-CLAT was not performed as a component of this study \*\*additional categorization into GHS Subcategory 1A or 1B for chemicals with a score of 3-4 from only DPRA and *in silico* testing is not possible without additional h-CLAT data

The weight of evidence approach to determining sensitizer hazard is advantageous compared to the OECD guideline methods in that it is less reliant on specific data and allows for inclusion of all available data (human, animal, computational, *in chemico*, *in vitro*) on a sensitization potential (Na *et al.*, 2022). It can also be used to assign sensitizer potency categories (Na *et al.*, 2022). However, it also relies significantly on expert judgement that inherently involves a level of subjectivity that is eliminated in the OECD guideline methods.

NAM assays have also been used to predict EC3 values that are comparable to LLNA-derived EC3 values in order to evaluate sensitizer potency and determine a point of departure (Natsch and Gerberick, 2022a). Five regression equations were derived from an empirical comparison of EC3 values for ~200 chemicals to specific parameters obtained from the results of those chemicals in NAM assays (k-DPRA, KeratinoSens<sup>TM</sup>, h-CLAT) and their vapor pressure (Natsch and Gerberick, 2022a). Each equation incorporates a different set of these parameters, with each generally performing comparably in predicting LLNA EC3 values (Natsch and Gerberick, 2022a). The variety of regression equations and parameters used in each allows for flexibility in combining the “2 of 3” DA assays with the k-DPRA assay to be able to efficiently determine hazard, potency, and point of departure for a potential skin sensitizer (Natsch and Gerberick, 2022b)

## 2.8 Project Objective/Overview

The objective of this project was to better characterize the potential link between ACD and chemical exposure during AF operations by: 1) evaluating the sensitization potential of 125 AF-relevant chemicals, and 2) using quantitative experimental data from the literature and NAMs to develop PCSGs for chemicals that were identified as sensitizers. Figure 3 provides a visual overview of the study design. The literature was searched for information on the sensitizer status of each chemical, and the chemicals were preliminarily categorized into sensitizers, non-sensitizers, and chemicals for which insufficient data existed to determine sensitizer status. PCSGs for chemicals identified as sensitizers were derived from available quantitative experimental human data and LLNA EC3 values from previously published literature.

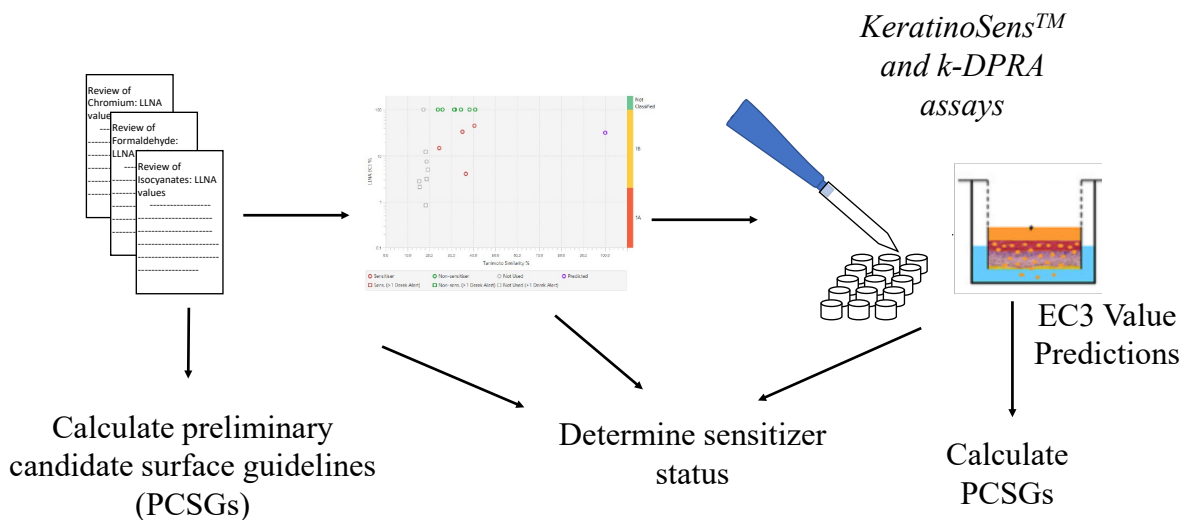


Figure 3. Overview of study design

Sensitizer status (sensitizer vs. non-sensitizer) was determined for chemicals without sufficient quantitative literature information using a weight of evidence approach that integrated results from *in vitro*, *in chemico*, and *in silico* NAMs. *In silico* models were used to screen for potential sensitizers among the chemicals with insufficient literature information available to make a

determination on their status. Chemicals that were predicted to be sensitizers by the *in silico* model and chemicals that were identified as sensitizers but lacked LLNA results were tested using NAM assays that addressed key events in the skin sensitization AOP. The results of the NAM assays and *in silico* models were then incorporated into an integrated approach with available qualitative literature information to predict sensitizer status. The NAM assay results were also used to predict EC3 values according to the methods of Natsch and Gerberick (2022a), which were then used to develop PCSGs.

## 3.0 METHODS

### 3.1 Identification of Air Force Relevant Chemicals and Literature Review for Sensitizer Status

The process to identify AF-relevant chemicals was previously described by Steele *et al.*, (2021), and is briefly summarized here. Researchers compiled candidate chemicals by 1) reviewing Enterprise Environmental, Safety, and Occupational Health Management Information System (EESOH-MIS) usage reports across the AF, 2) extracting confirmed exposures from AF occupational field assessments, 3) examining the Occupational Safety and Health Administration (OSHA) expanded standard list tracked at Robins AF Base (AFB), and 4) referencing the five most common allergens reported worldwide (Alinaghi et al, 2019).

To understand potential occupational exposures to these chemicals in the AF, the chemical usage in 2020 from ten representative bases was compiled from EESOH-MIS. Each base was selected to serve as a surrogate for chemical use in a Major Command (MAJCOM). Wright-Patterson AFB was chosen for AF Materiel Command (AFMC). In each report, line items were aggregated by chemical abstract service (CAS) number and the total weight consumed in 2020 was calculated. The top 100 items from each base were compiled in another spreadsheet.

In parallel to developing a list of chemicals used by base according to EESOH-MIS, chemicals identified during F-35 engine runs in a hardened aircraft shelter (HAS) and during cockpit sampling in F-22s were obtained from AFRL scientists. These lists were viewed as complementary to EESOH-MIS data as they contained the identities of combustion byproducts so the compounds identified may not have been in the original product or on the safety data sheet (SDS).

The combined EESOH-MIS data, HAS list, and F-22 list represented 882 unique chemicals. The chemical hazard evaluation tool (CHET), which is used by the bioenvironmental career field consulting branch (USAFSAM/OEC) for site assessments, was used to winnow down the list of chemicals to a testable number. For chemicals that appeared in the CHET, those which had health effect ratings of less than two via inhalation, dermal contact, or ingestion were removed. For those chemicals that did not appear in the CHET, the health effects reported in the haz-map.com database were consulted.

From the remaining list, chemicals were removed if 1) they were only used at two or fewer bases and did not overlap with the HAS list, 2) were one of the 21 entries for petroleum distillates after the decision was made to select a representative distillate, or 3) were on the F-22 list but not on the HAS list or used at more than two bases.

The final chemical list contained the 76 chemicals identified in the HAS list and the top 40 chemicals used across the AF by weight used in 2020 (Steele *et al.*, 2021). This list was sent out for peer-review within to other AF researchers. Additional chemicals suggested for evaluation were 1) trimethylsilanol, 2) 1-decene dimer, a known component of polyalphaolefin (PAO) coolant, 3) elemental carbon, and 4) PIP3.1, an organophosphate additive in engine oil.

A list of chemicals sampled at Robins AFB as part of the OSHA expanded standards was also consulted. As this list straddled the line between research exposure assessment and components listed in an SDS, the expanded standards were reviewed. Unique items were added to the list of chemicals to consider for deriving PCSGs.

Finally, in a recent meta-analysis of epidemiology studies, researchers found the prevalence of allergies in the general population in Europe and North America as: 11.4% (95% CI: 9.4-13.5%) to nickel, 2.7% (95% CI: 2.1%-3.4%) to cobalt, 1.8% (95% CI: 1.3-2.6%) to chromium, 1.2% (95% CI: 0.9-1.5%) to formaldehyde, and 1.2% (95% CI: 1.0-1.5) to p-tert-butylphenol formaldehyde resin (Alinaghi et al, 2019). Those allergens not already on the candidate chemical list were added.

As a first pass method for determining sensitizer status, the CAS number for each chemical on the candidate list was used to search several databases with historical data, including the chemical in the SkinSensDB database (Wang, 2017), the National Toxicology Program, Interagency Center for the Evaluation of Alternative Toxicological Methods, Integrated Chemical Environment database (NICEATM ICE), the OECD eChemPortal, as well as the curated lists of sensitizers maintained by the American Conference of Governmental Industrial Hygienists (ACGIH), National Institute for Occupational Safety and Health (NIOSH), and OSHA. Each chemical was marked as a sensitizer, non-sensitizer, or lacking sufficient data to determine sensitizer status. Some chemicals initially included in the candidate list were removed from consideration because they were unlikely to be dermal exposure concerns.

### **3.2 *In Silico* Predictions – Derek Nexus<sup>®</sup> and OECD Toolbox**

Chemicals identified as sensitizers by initial screening and chemicals with insufficient data in the literature to determine sensitizer status were analyzed using Derek Nexus<sup>®</sup> and the OECD QSAR toolbox. The purpose of this *in silico* screening was to identify the chemicals most likely to be sensitizers and prioritize them for testing with NAM assays.

In Derek Nexus<sup>®</sup>, the categorical assignments for skin sensitization were evaluated. Predictions were performed using knowledge base Derek KB 2022 1.0, species set to mammal, and did not account for tautomeric forms of compounds or individual components of mixtures. If a structural alert was indicated at a reasoning level of equivocal, plausible, probable, or certain, then the chemical was considered a potential sensitizer. Any chemicals that were indicated to be non-sensitizers, or alerts with reasoning levels below equivocal, were considered non-sensitizers. Derek Nexus<sup>®</sup> also provided predicted EC3 values based on a weighted average of 10 nearest neighbor chemicals (as defined by Tanimoto score) with skin sensitization alerts. However, validation of the predictivity of these EC3 values compared to experimentally-derived EC3 values

is limited (Canipa et al., 2017) relative to assay data. Therefore, the EC3 values were reported, but were not used in further PCSG calculations.

In the OECD QSAR Toolbox, chemicals were analyzed using the automated workflow for skin sensitization. Chemicals were considered potential sensitizers if they had positive test values in the database or predicted values derived by the software. In some cases, multiple measured values existed for a single chemical, and a determination was made by the researcher whether the measured values could be categorized as “positive” or “negative” based on the level of data (human vs. animal), potency indicated by measured values, and amount of positive vs. negative results. An “inconclusive” determination was assigned when evidence wasn’t sufficiently one-sided to allow for a “positive” or “negative” determination to be made. In some cases, there was insufficient data available to make a prediction, and/or the chemicals fell outside the domain of applicability of the model (e.g., metals). The OECD Toolbox also predicted EC3 values, however, no published comparisons of these data with experimentally-derived values were found. Therefore, the EC3 values were reported, but not used in further PCSG calculations.

### 3.3 *In Chemico and In Vitro Assays – k-DPRA and KeratinoSens™*

#### Kinetic Direct Peptide Reactivity Assay (k-DPRA)

The k-DPRA assay calculates the rate of depletion [ $k_{\max}$  (molar concentration<sup>-1</sup>seconds<sup>-1</sup>)] of a synthetic peptide via the reaction of a given test article with cysteine residues of that peptide. As described below, the unit of the kinetic rate constants are changed to their logarithmic values for most calculations and are referred to as unit-free and always referred to as logarithm of the rate constant measured. The method follows the procedures outlined in the OECD Guideline 442C, “Assays addressing the AOP key event on covalent binding to proteins” (OECD, 2022a). The kinetic rates of cysteine depletion were used to discriminate UN GHS subcategory 1A skin sensitizers from those not categorized as subcategory 1A (non-subcategory 1A). A log  $k_{\max}$  result of -2.0 was used as a threshold (Table 5), with a borderline range of -1.93 to -2.06. The overall depletion results were also extrapolated to the non-kinetic version of the DPRA assay (OECD, 2022a) and were used to determine positivity in the assay indicative of skin sensitization potential, with cysteine depletion greater than 13.89% (borderline range of 10.56-18.47%) following 24 hours incubation with a test article at a concentration of 5 millimolar (mM) considered a positive result.

**Table 5. UN GHS subcategory designation based on k-DPRA reaction rate constant**

| Reaction rate                          | k-DPRA Prediction                                                      |
|----------------------------------------|------------------------------------------------------------------------|
| $\log k_{\max} \geq -2.0$              | UN GHS subcategory 1A                                                  |
| Non-reactive or $\log k_{\max} < -2.0$ | Not Categorized as subcategory 1A <sup>^</sup><br>(non-subcategory 1A) |

<sup>^</sup>Further information is needed to discriminate UN GHS subcategory 1B from UN GHS No Category (OECD, 2021).

Prior to the assay, the solubility of each test article at a concentration of 20 mM in acetonitrile, or potential of hydrogen (pH) 7.5 phosphate buffer was determined. The solvent was selected for each test article based on its ability to dissolve the test article completely, or into a homogenous suspension, as determined by visual inspection. Acetonitrile is a well-characterized solvent in the assay that does not adversely impact results (Wareing *et al.*, 2020). Five concentrations of the test article (5.0, 2.5, 1.25, 0.625, and 0.3125 mM) were incubated with the synthetic cysteine peptide (0.667 mM, in pH 7.5 phosphate buffer) for six incubation times ( $10 \pm 0.5$ ,  $30 \pm 3$ ,  $90 \pm 5$ ,  $150 \pm 10$ ,  $210 \pm 10$ , and  $1440 \pm 15$  minutes) at 22.5 to 27.5 degrees Celsius ( $^{\circ}\text{C}$ ). Cinnamic aldehyde (in acetonitrile) was used as the positive control (PC), and was tested at the same five concentrations as the test articles. Negative controls (NCs) containing the solvent and peptide solution, buffer controls (BCs) containing the pH 7.5 phosphate buffer and solvent, and sample controls (SCs) containing the pH 7.5 phosphate buffer and test article/positive control were also tested in each assay. For pH 7.5 phosphate buffer-soluble substances, an appropriate amount of acetonitrile was added so that the composition of the samples of acetonitrile-soluble substance and pH 7.5 phosphate-buffer soluble substances was the same during the test.

After the respective incubation time, the reaction was stopped by addition of the fluorescence dye monobromobimane (mBrB). Highly reactive and non-fluorescent, mBrB rapidly reacts with unbound cysteine moieties of the model peptide to form a fluorescent complex. The remaining non-depleted peptide concentration was determined thereafter by fluorescence measurement using a photometer (TECAN® Infinite M Nano + Plate Reader and Magellan™ Tracker Software). Fluorescence was measured with an excitation filter of 390 nanometers (nm) and an emission filter of 480 nm.

The mean fluorescence values of the NC samples at each timepoint were calculated and corrected by subtracting the mean values of the BC samples for that run. The test article and PC values were corrected by subtraction of the corresponding SC values. For each sample of test article and PC:

Equation 1: 
$$\text{Relative } dp = \left(1 - \frac{\text{Corrected Sample Value}}{\text{Corrected Mean VC}}\right) \times 100\%$$

Where depletion (dp) was in units of %. For the test article, the arithmetic mean and standard deviation (SD) of the three replicates were calculated per exposure time. Depletion values were also converted to  $\ln(100\text{-dp})$  for kinetic analysis. The linear slope and correlation values were generated from plots of  $\ln(100\text{-dp})$  versus concentration (mM) at each timepoint using the equation:

Equation 2: 
$$\ln[(100 - dp)_{\text{conc}}] = \ln[(100 - dp)_0] - (k_{\text{obs}} \times \text{conc})$$

With the slope values for individual time points corresponding to the observed reaction kinetic constant (k observed ( $k_{\text{obs}}$ ),  $[\text{mM}^{-1}]$ , pseudo-first order rate constants) (Roberts and Natsch, 2009). From the k observed value of each timepoint, the correlation over all concentrations was calculated as follows:

Equation 3: 
$$k = k_{\text{observed}} \times \frac{1000}{(60 \times \text{timepoint [min]})}$$

Where the reaction kinetic constant ( $k$ ) was in units of  $M^{-1}s^{-1}$ . The decimal logarithm of  $k$  ( $\log k$ ) was calculated for each time point with a correlation  $> 0.9$ , with the  $\log k_{\max}$  (highest  $k$  value) and the respective time point reported.

In some cases where the concentration response was not linear, but clear depletion was noted, the rate constant could not be calculated by the slope method as the regression coefficient was  $r < 0.9$ . Alternatively, rate constants were calculated based on individual depletion values according to the formula:

Equation 4: 
$$k = \frac{\left[ \ln\left(\frac{100}{(100-dp)}\right) \right]}{[E]t}$$

Where  $dp$  was depletion in %,  $E$  was the concentration of test article in  $M$ , and  $t$  was the incubation time in seconds.

The assay was considered valid when the  $\log k_{\max}$  of the PC at 90-minute exposure was within the range of  $-1.75$  to  $-1.40$ . If no  $\log k_{\max}$  was obtained at 90 minutes, then the  $\log k_{\max}$  of the PC at 150-minute exposure was taken into account and was required to be within the range of  $-1.90$  to  $-1.45$ . Additionally, the assay was only considered valid if the variance of the 12 vehicle control values of a plate was  $< 12.5\%$  for 5 of the 6 time points.

#### KeratiNoSens™

The KeratiNoSens™ assay is designed to evaluate the induction of gene expression via the Nrf2-Keap1 pathway in transfected HaCaT keratinocytes using a luciferase reporter gene. The assay was considered positive for a test article being a potential sensitizer when there was a  $>1.5$ -fold greater luciferase induction at concentrations lower than  $1000 \mu M$  and cell viabilities of  $>70\%$  relative to the vehicle control for at least two valid trials. The cytotoxicity of the test articles was evaluated based on the NAD(P)H-dependent microsomal enzyme reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (Berridge, *et al.*, 1996) by the KeratiNoSens™ cells. The procedures were based on those in the OECD guideline 442D, “In vitro skin sensitization assays addressing the AOP key event on keratinocyte activation” (OECD, 2022b), which is a modification of the procedures previously described by Natsch and Emter (2008).

All test articles were diluted in dimethyl sulfoxide (DMSO), an organic solvent that is commonly used for a wide range of *in vitro* assays. Standard test article concentrations were 2000, 1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.81, 3.91, 1.95, and  $0.977 \mu M$ . Acrolein was tested across the same range, except with a concentration of  $0.488 \mu M$  replacing the  $2000 \mu M$  concentration because of cytotoxicity at low concentrations being noted in an early trial that was rejected due to dilution issues. Inadvertent incorrect dilution of some test articles led to them being tested on a weight per volume (w/v) basis at concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.781, 0.391, and  $0.195 \mu g/mL$ . Bisphenol A, p-tert-butylphenol, d-limonene, naphthalene, xylene, 1-naphthylamine, and benzaldehyde were tested at w/v-based concentrations, resulting in molar concentrations greater than the standard concentration range for all but bisphenol A, while all others were tested at standard concentrations. Cinnamic aldehyde was used as a positive control and tested at concentrations of 64, 32, 16, 8, and  $4 \mu M$ .

Cryopreserved KeratinoSens™ cells purchased from Givaudan were thawed and subsequently cultured in cell culture flasks. Upon reaching 60 to 90% confluence, the cells were subcultured in 96-well plates at a concentration of 10,000 cells per well and incubated for  $24 \pm 1$  hours at standard culture conditions ( $37 \pm 1$  °C humidified air containing  $5 \pm 1\%$  carbon dioxide (CO<sub>2</sub>)). The day following seeding, the cells were checked for appropriate growth, uniformity, and confluence. The media was then removed from the culture plates and cells were re-fed with 1% Dulbecco's Modified Eagle Medium (DMEM containing 1% fetal bovine serum). Cells were then incubated at standard culture conditions until treatment.

For each definitive trial, the KeratinoSens™ cells were treated with the positive control and test articles for approximately  $48 \pm 1$  hours under standard culture conditions and assessed for luciferase induction and MTT cytotoxicity. Each plate included 6 solvent control wells (cells exposed to 1% DMSO only). If an intermediate solvent was used (e.g., water), the concentration of the intermediate solvent was the same as was used with the test article treatment. Each plate also had a blank well that did not contain cells. After the treatment, culture plates were allowed to cool to room temperature before removal of treatment medium and addition of Dulbecco's calcium- and magnesium-free phosphate-buffered saline. Promega ONE-Glo Reagent was added, and plates were allowed to incubate in the dark at room temperature for 5 to 45 minutes before relative light units (RLU) were measured using a luminometer (SpectraMax, Molecular Devices) at a wavelength of 570 nm (RLU<sub>570</sub>).

MTT reduction was also measured in KeratinoSens™ cells treated with the test article and positive control. After  $48 \pm 1$  hours of incubation of the cells with the test article or positive control at standard culture conditions, 200 microliters (μL) of 1% DMEM containing 0.59 milligrams (mg)/mL MTT was added to each well. After  $4 \pm 0.3$  hours, the MTT solution was decanted and 200 μL of 10% sodium lauryl sulfate was added to each well. After an overnight incubation, the absorbance at 570 nm (OD<sub>570</sub>) was measured for each well with a Molecular Devices VersaMax™ plate reader.

For each well, the percent viability was calculated as the ratio of the corrected OD<sub>570</sub> of the test or control sample to the corrected OD<sub>570</sub> of the appropriate solvent control. Corrected values were obtained by subtracting experimental OD<sub>570</sub> values from the OD<sub>570</sub> value of the blank well.

Equation 5: 
$$\% \text{ Cell Viability} = \frac{\text{Corrected OD}_{570} \text{ of Test or Control Article}}{\text{Mean Corrected OD}_{570} \text{ of Solvent Control}} \times 100$$

Cytotoxicity response curves were plotted with the % viability on the ordinate and the test article or positive control concentration on the abscissa. IC<sub>30</sub> and IC<sub>50</sub> values (the concentrations resulting in 30% and 50% reductions in viability, respectively) were calculated for each test article by linear interpolation from the two consecutive concentrations where one concentration resulted in percent viability greater than 70% and 50%, and one concentration resulted in percent viability less than 70 and 50%, respectively. The IC<sub>30</sub> value was used in the evaluation of the test results as the highest concentration for which gene induction was evaluated. If an IC<sub>30</sub> or IC<sub>50</sub> value was not achieved at the highest concentration tested, the results were presented as greater than the highest dose tested.

To calculate the fold gene induction (over the controls), the corrected RLU<sub>570</sub> value of each well was divided by the mean corrected RLU<sub>570</sub> value of the solvent control wells. Corrected values were obtained by subtracting experimental RLU<sub>570</sub> values from the RLU<sub>570</sub> value of the blank well. An average fold gene induction for each test article at each concentration was determined using the three replicate plates within each definitive trial.

$$\text{Equation 6: Fold Induction} = \frac{\text{Corrected RLU}_{570} \text{ Value of Test Article or Positive Control Concentration}}{\text{Corrected Mean RLU}_{570} \text{ Value of Solvent Control}}$$

The average fold gene induction at each concentration was used to calculate the EC<sub>1.5</sub> value (the concentration inducing luciferase gene activity 1.5-fold above solvent control values) via linear interpolation for each definitive trial. In cases where a biphasic curve was observed, or where the gene induction response curve crossed the threshold more than once, the values with the lowest concentration with a gene induction above 1.5 and cellular viability greater than 70% were used. A mean EC<sub>1.5</sub> value for each test article was calculated by averaging the EC<sub>1.5</sub> values of the valid definitive trials. The maximal induction of gene activity (I<sub>max</sub>) was also determined for each test article. The I<sub>max</sub> for each test article in each definitive trial was defined as the largest average fold induction value for that test article.

Validity of each trial was determined by a number of conditions. Average luciferase gene induction by the positive control (cinnamic aldehyde) was required to cross the 1.5 threshold in at least one dose in each definitive trial. Furthermore, each definitive trial was considered valid if the cinnamic aldehyde luciferase gene induction fell within 2 SDs of the lab's historical mean (updated approximately every 3 months). Each trial was also assessed (but not necessarily deemed a valid trial) using similar criteria as outlined in the ring trial (Natsch, *et al.* 2011). The criteria included that the SD of the luminescence readings of the solvent controls for the luciferase plates for all of the definitive trials (3 plates per trial) were < 20%. Additionally, the criteria stated that cinnamic aldehyde was required to be significantly positive, result in an EC<sub>1.5</sub> value less than 64 µM, and produce a >2-fold induction at 64 µM.

### 3.4 Approach to Assign the Sensitizer Status of Chemicals

A weight of evidence approach was used for assignment of each chemical as a sensitizer or non-sensitizer (i.e., hazard identification). The assessment incorporated evidence from qualitative and quantitative human or animal data in the literature, NAM-predicted EC<sub>3</sub> values, k-DPRA-predicted GHS subcategory and peptide depletion, KeratinoSens<sup>TM</sup> results, and *in silico* model results. Human data from the literature was given priority when it existed. Animal data (primarily LLNA EC<sub>3</sub> values), was also considered in the determination of sensitizer status based on the strength of the published literature. Overall peptide depletion results obtained from the k-DPRA experiment were extrapolated to the DPRA, and combined with the KeratinoSens<sup>TM</sup> results to assess sensitizer status according to the “2 of 3” DA methods (OECD, 2021). However, because h-CLAT was not performed the sensitivity was limited because there was a potential for equivocal findings for chemicals with non-concordant results in the two assays. In some cases, it was possible to derive NAM-predicted EC<sub>3</sub> values or the k-DPRA-predicted subcategories even though the criteria for designating sensitizer vs. non-sensitizer were not met under the “2 out of 3” DA (i.e., assay results were inconclusive or differed across the two assays). In these cases, expert judgment was used to incorporate consideration of the k-DPRA-predicted GHS subcategory, the NAM-predicted EC<sub>3</sub>s, and the published literature into the weight of evidence-based designation of

sensitizer status. *In silico* predictions were also considered in the assessment, but were given less weight when sufficiently close analogues of the chemicals of interest were not present in the basis of the prediction.

### 3.5 Evaluating Sensitizer Potency and Predicting EC3 Values from Assay Results

EC3 values were predicted from k-DPRA and Keratinosens<sup>TM</sup> assay results using the methods derived by Natsch and Gerberick (2022a). Linear transformations were used to linearize logarithmic k-DPRA and KeratinoSens<sup>TM</sup> parameters into normalized values spanning a range of zero (for no effect) to a positive value of maximal effect:

Equation 7:  $\log EC_{1.5, \text{norm}} = -1 \times \log (EC_{1.5, \text{KeratinoSens}^{\text{TM}}}) + \log (4000)$

Equation 8:  $\log IC_{50, \text{norm}} = -1 \times \log (IC_{50, \text{KeratinoSens}^{\text{TM}}}) + \log (4000)$

Equation 9:  $\log k_{\text{max}, \text{norm}} = \log k_{\text{max}} + 3.5$

Vapor pressure (VP) in Pascals (Pa) was also normalized by the equation relating vapor pressure to half-life in the LLNA vehicle (Natsch *et al.*, 2015), with resultant VP<sub>norm</sub> values below one being set to zero:

Equation 10:  $\log VP_{\text{norm}} = \log (VP) - 1$

The normalized parameters were then related to pEC3 using the equation:

Equation 11:  $pEC3 = 0.42 + 0.40 \times \log k_{\text{max}, \text{norm}} + 0.15 \times EC_{1.5, \text{norm}} + \dots$   
 $\dots - 0.36 \times \log IC_{50, \text{norm}} - 0.21 \times \log VP_{\text{norm}}$

that was derived from a multiple linear regression analysis of pEC3 values calculated from normalized k-DPRA, KeratinoSens<sup>TM</sup>, and VP parameters against LLNA-sourced pEC3 values (Natsch and Gerberick, 2022a). pEC3 was related to EC3 by the equation:

Equation 12:  $pEC3 = \log (\text{molecular weight}/EC3)$

### 3.6 Calculation of Preliminary Candidate Surface Guidelines

Human No Observed Effect Level (NOEL) and Lowest Observed Effect Level (LOEL) values and EC3 values from LLNA animal study data from published literature were used as points of departure (PoD) for PCSG calculations for the nine chemicals where such data was available. For all other chemicals, NAM-derived EC3 values were used as the PoDs based on the methods of Natsch and Gerberick (2022b).

Human NOEL values were sourced from the maximum test levels with no induction of sensitization tested in published HRIPT or HMT studies (Na *et al.*, 2022). Human LOEL values

were derived from the dose per unit skin area leading to a sensitization incidence of 5% (DSA<sub>05</sub>) in the HRIPT or HMT (Schneider and Akkan, 2004).

PCSGs calculated from EC3s required conversion from w/v % into a surface concentration applied in the LLNA assay in units of  $\mu\text{g}/\text{cm}^2$ . As described in Equation 13, w/v (%) was converted into  $\mu\text{g}/\text{mL}$ , then multiplied by the amount applied to the ears ( $0.025 \text{ mL} \times 2 \text{ ears}$ ), then divided by the ear surface area ( $2 \text{ cm}^2$ ) (Naumann and Arnold, 2019). Metals are typically tested as compounds in the LLNA, and therefore the EC3 surface concentrations for metals needed adjusted by multiplying by the mass fraction of that metal in the compound.

$$\text{Equation 13: } EC3 \left( \frac{w}{v} \right) \rightarrow EC3 \left( \frac{\mu\text{g}}{\text{mL}} \right) \times 0.025 \frac{\text{mL}}{\text{ear}} \times 2 \frac{\text{ears}}{\text{mouse}} \div 2 \text{ cm}^2 = EC3 \left( \frac{\mu\text{g}}{\text{cm}^2} \right)$$

PCSGs were derived from the PoDs using Equation 14, which applied adjustment factors to account for uncertainty in translating experimental data into human responses and actual exposure conditions, then dividing by ten to convert the PCSGs to units of  $\text{mg}/100 \text{ cm}^2$ . Three adjustment factor values (Appendix C1) were multiplied together to derive the composite adjustment factor indicated in Equation 14: an adjustment factor for translation to human effects (AF<sub>WOE</sub>), an adjustment factor for interindividual variability (AF<sub>IV</sub>), and an adjustment factor for exposure considerations (AF<sub>E</sub>). The derivation of the adjustment factors, much of which followed the methods described in four recent publications (Naumann and Arnold, 2019; Fukushima *et al.*, 2022; Api *et al.*, 2020; Natsch *et al.*, 2018), is detailed in the following paragraphs.

$$\text{Equation 14: } PCSG \left( \frac{\text{mg}}{100 \text{ cm}^2} \right) = \frac{\text{Point of Departure} \left( \frac{\mu\text{g}}{\text{cm}^2} \right)}{\text{Composite adjustment factor} \times 10}$$

The AF<sub>WOE</sub> was variable depending on the source of the PoD used to calculate the PCSG and the weight of evidence available for each chemical (Appendix C2). The adjustment factor was assigned a value of one for human NOEL PoDs and three for human LOEL PoDs. A value of three was comparable to the average of ratios (3.3 with a SD of 2.8) of 33 human LOEL to NOEL values for HRIPT/HMT tests reported in Na *et al.*, (2022). Nauman and Arnold (2019) compared the ratio of mouse LLNA data to HRIPT data across a wide range of skin sensitization potencies and recommended an adjustment factor of six for translating LLNA EC3 data to human effects. Other literature has argued for an interspecies adjustment factor of 15 based on error in the LLNA to human data correlation (Bil *et al.*, 2017), whereas some have argued that the correlation of LLNA data to human data suggests that there is not a routine need for an interspecies adjustment factor (Baskettter *et al.*, 2018). Using a default AF<sub>WOE</sub> of six for LLNA-derived EC3s, with the option of lowering it to three if close structural analogues of a chemical had similar or lower potency in human testing, was determined to be a reasonably conservative compromise between the various approaches.

An AF<sub>WOE</sub> of ten was used for PoDs sourced from EC3 values calculated from NAM assays. An adjustment factor range of two to ten has been proposed for translating EC3 values derived from NAM assays into human NOELs (Nastch *et al.*, 2018). The adjustment factor used for a specific chemical depends on the consistency of that value with the weight of evidence of the sensitization potential of that chemical and its close structural analogues (Nastch *et al.*, 2018). An adjustment factor of six was used instead of ten if close structural analogues of a chemical had comparable

LLNA EC3 values. No chemicals in this study with a NAM-sourced PoD had sufficiently close structural analogues with quantitative human data or other evidence to support a lower AF<sub>WOE</sub>.

The adjustment factor for interindividual variability was set to ten (AF<sub>IV</sub>=10) based on the large human individual variability in induction of sensitization (Api *et al.*, 2020). Naumann and Arnold (2019) did not define an adjustment factor for interindividual variability, however, it was an outlier in the literature because it was the only publication that did not define an AF<sub>IV</sub> out of seven quantitative risk assessments for dermal sensitization (Fukushima *et al.*, 2022). Although an adjustment factor for vehicle/matrix effects on dermal penetration of the chemical of interest is sometimes used (Naumann and Arnold, 2019), it was not applied within the current effort, because even in consumer products, an adjustment factor of one is recommended unless the product contains known penetration enhancers (Api *et al.*, 2020).

The AF<sub>E</sub> = 3 reflects the middle range of values recommended for consumer products in a number of published sources, as described by Naumann and Arnold, (2019). Recommendations for risk assessments for dermal sensitization from cosmetics sometimes incorporate both an adjustment factor of three for repeated use of a product, and another factor of three for use on body areas, such as the hands, that are more prone to inflammation (Api *et al.*, 2020). While both scenarios apply to the occupational setting, only one adjustment factor of three was used to represent both exposure considerations because of the differences in accidental intermittent exposure in the workplace from the deliberate repeated dermal application of cosmetics.

## 4.0 RESULTS

### 4.1 Summary of Literature Review

The 125 chemicals of interest to the AF were preliminarily placed into four categories based on literature data: (1) sensitizers, (2) non-sensitizers, (3) insufficient data to determine sensitizer status, and (4) removed from consideration. A total of 21 chemicals were determined to be sensitizers and 38 chemicals were determined to be non-sensitizers through the review of the literature. There was insufficient data in the literature to determine sensitizer status for 58 chemicals. A total of eight chemicals were removed from consideration because they were light gases that showed no evidence of sensitization potential. A categorized list of the 125 chemicals is provided in Appendix A.

### 4.2 AF-Relevant Chemicals with Experimental Human and Animal Potency Data in the Literature

The literature was reviewed for LLNA EC3 values and human NOEL/LOEL data for the 21 chemicals that were defined as sensitizers. EC3 values were obtained for ten of those chemicals and are listed in Table 6. Xylene, which was considered a non-sensitizer in the literature, also had an existing LLNA EC3 value of 96%. Some chemicals, including d-limonene, glyoxal, formaldehyde, cobalt, and HMDI had multiple EC3 values in the literature. The median of the literature EC3 values was used for these chemicals (Table 6). Human NOEL values were obtained from HRIPT/CNIH tests and human LOEL values were obtained from DSA<sub>05</sub> values calculated from the results of HMT testing.

**Table 6. Chemicals with human NOEL/LOEL and mouse LLNA EC3 values in the literature**

Literature-derived human NOEL/LOEL and mouse LLNA EC3 values are listed for each chemical, with the median value used for chemicals with multiple EC3 values. References associated with each EC3 value are listed at the bottom of the table. “N/A” indicates that no NOEL/LOEL data was found.

| CAS#                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Chemical            | Preliminary Assignment from Lit Review | LLNA EC3 (%)        | LLNA EC3 (µg/cm <sup>2</sup> ) | Human NOEL/LOEL (µg/cm <sup>2</sup> ) | Human NOEL/LOEL Source  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|----------------------------------------|---------------------|--------------------------------|---------------------------------------|-------------------------|
| 5989-27-5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | d-limonene          | Sensitizer                             | 30 <sup>a,b,c</sup> | 7500                           | NOEL, 10000 <sup>o</sup>              | HRIPT/CNIH              |
| 1330-20-7                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Xylene              | Non-Sensitizer                         | 96 <sup>d</sup>     | 24000                          | N/A                                   | N/A                     |
| 100-52-7                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | benzaldehyde        | Sensitizer                             | >25 <sup>d</sup>    | >6250                          | NOEL, 590 <sup>o</sup>                | HRIPT/CNIH              |
| 78-98-8                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | methylglyoxal       | Sensitizer                             | 0.4 <sup>e</sup>    | 105                            | N/A                                   | N/A                     |
| 107-22-2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | glyoxal             | Sensitizer                             | 1.1 <sup>e,f</sup>  | 275                            | LOEL, 345 <sup>p</sup>                | DSA <sub>05</sub> , HMT |
| 50-00-0                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | formaldehyde        | Sensitizer                             | 0.4 <sup>g,h</sup>  | 100                            | NOEL, 37 <sup>o</sup>                 | HRIPT/CNIH              |
| 7440-41-7                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | beryllium           | Sensitizer                             | 8.6 <sup>d</sup>    | 185                            | LOEL, 11 <sup>p</sup>                 | DSA <sub>05</sub> , HMT |
| 7440-02-0                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | nickel              | Sensitizer                             | 3.5 <sup>i</sup>    | 481                            | LOEL, 28 <sup>p</sup>                 | DSA <sub>05</sub> , HMT |
| 822-06-0                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | HMDI                | Sensitizer                             | 0.02 <sup>j,k</sup> | 4.3                            | N/A                                   | N/A                     |
| 18540-29-9                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | hexavalent chromium | Sensitizer                             | 0.06 <sup>l</sup>   | 5.1                            | N/A                                   | N/A                     |
| 7440-48-4                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | cobalt              | Sensitizer                             | 0.6 <sup>m,n</sup>  | 68                             | LOEL, 313 <sup>p</sup>                | DSA <sub>05</sub> , HMT |
| <sup>a</sup> Warbrick <i>et al.</i> , 2001; <sup>b</sup> Christensson <i>et al.</i> , 2008; <sup>c</sup> ECHA, 2004; <sup>d</sup> Basketter <i>et al.</i> , 2014; <sup>e</sup> Anderson <i>et al.</i> , 2007; <sup>f</sup> Patlewicz <i>et al.</i> , 2002; <sup>g</sup> Basketter <i>et al.</i> , 2003; <sup>h</sup> Basketter <i>et al.</i> , 2001; <sup>i</sup> Kolle <i>et al.</i> , 2013; <sup>j</sup> Urbisch <i>et al.</i> , 2015; <sup>k</sup> Ohtake <i>et al.</i> , 2018; <sup>l</sup> Kimber <i>et al.</i> , 1995; <sup>m</sup> Ikarashi <i>et al.</i> , 1992; <sup>n</sup> Basketter and Scholes, 1992; <sup>o</sup> Na <i>et al.</i> , 2022; <sup>p</sup> Schneider and Akkan, 2004 |                     |                                        |                     |                                |                                       |                         |

### 4.3 *In silico* Evaluation of AF-Relevant Chemicals

The 21 chemicals preliminarily identified as sensitizers in the literature search and 58 chemicals with insufficient literature information to determine sensitizer status were evaluated for sensitization potential using Derek Nexus and the OECD Toolbox. This *in silico* evaluation was used to screen the chemicals and prioritize NAM assay testing. Chemicals were selected for further testing if they were predicted to be sensitizers by either *in silico* model (listed in Table 7). *In silico* results for chemicals with quantitative experimental human or animal data are also listed in Table 7. The *in silico* results of chemicals that were not selected for additional testing are listed in Appendix B. The majority of the chemicals with insufficient literature data to preliminarily determine sensitizer status were deemed to be non-sensitizers by both models. Compounds containing carbonyl functional groups were predominantly identified as potential sensitizers by the *in silico* models.

**Table 7. *In silico* results for chemicals prioritized for further testing with *in chemico/in vitro* assays**

All chemicals chosen for NAM assay testing were identified as potential sensitizers in the literature or from *in silico* modeling results. A selection of known sensitizers with LLNA EC3 data (Table 6) are also provided for comparison with *in silico*-predicted values. The Derek Nexus<sup>®</sup> output designates whether a chemical is predicted to be a sensitizer along with an associated level of reasoning for that prediction (*e.g.*, probable, plausible, equivocal), or if a chemical is not predicted to be a sensitizer. The OECD Toolbox (OECD-T) output designates whether a chemical is or is not expected to be a sensitizer based on measured results in its database or model predictions. “ND” indicates that no result could be returned. “Inconclusive” indicates that information supported both sensitizer and non-sensitizer status for a chemical. Gray cells: sensitizer, black cells: non-sensitizer, white cells: insufficient data or inconclusive results for sensitizer status.

| CAS#       | Chemical                 | Preliminary Assignment from Lit Review | Derek Nexus <sup>®</sup> (Reasoning Level) | OECD-T Measured (Assignment) | OECD-T Predicted (Assignment) |
|------------|--------------------------|----------------------------------------|--------------------------------------------|------------------------------|-------------------------------|
| 5989-27-5  | d-limonene               | Sensitizer                             | Probable                                   | Sensitizer                   | Sensitizer                    |
| 1330-20-7  | xylene                   | Non-Sensitizer                         | Non-Sensitizer                             | Non-Sensitizer               | Non-Sensitizer                |
| 100-52-7   | benzaldehyde             | Sensitizer                             | Equivocal                                  | Inconclusive                 | Non-Sensitizer                |
| 78-98-8    | methylglyoxal            | Sensitizer                             | Plausible                                  | ND                           | Non-Sensitizer                |
| 107-22-2   | glyoxal                  | Sensitizer                             | Probable                                   | Sensitizer                   | Sensitizer                    |
| 50-00-0    | formaldehyde             | Sensitizer                             | Plausible                                  | Sensitizer                   | Sensitizer                    |
| 7440-41-7  | beryllium                | Sensitizer                             | Plausible                                  | ND                           | ND                            |
| 7440-02-0  | nickel                   | Sensitizer                             | Plausible                                  | ND                           | ND                            |
| 822-06-0   | HMDI                     | Sensitizer                             | Probable                                   | Sensitizer                   | Sensitizer                    |
| 18540-29-9 | hexavalent chromium      | Sensitizer                             | Plausible                                  | ND                           | ND                            |
| 7440-48-4  | cobalt                   | Sensitizer                             | Plausible                                  | ND                           | ND                            |
| 80-05-7    | bisphenol A              | Sensitizer                             | Equivocal                                  | Sensitizer                   | Non-Sensitizer                |
| 98-54-4    | p-tert-butylphenol       | Sensitizer                             | Equivocal                                  | Inconclusive                 | Non-Sensitizer                |
| 90-30-2    | n-phenyl-1-naphthylamine | Sensitizer                             | Non-Sensitizer                             | Sensitizer                   | Non-Sensitizer                |
| 91-20-3    | naphthalene              | Sensitizer                             | Non-Sensitizer                             | ND                           | Sensitizer                    |
| 134-32-7   | 1-naphthylamine          | Sensitizer                             | Equivocal                                  | Non-Sensitizer               | ND                            |
| 4170-30-3  | crotonaldehyde           | Sensitizer                             | Plausible                                  | ND                           | Sensitizer                    |
| 107-13-1   | acrylonitrile            | Sensitizer                             | Probable                                   | Sensitizer                   | Sensitizer                    |
| 106-89-8   | epichlorohydrin          | Sensitizer                             | Plausible                                  | ND                           | Sensitizer                    |
| 107-02-8   | acrolein                 | Insufficient Data                      | Plausible                                  | ND                           | Sensitizer                    |
| 78-85-3    | methacrolein             | Insufficient Data                      | Plausible                                  | ND                           | Sensitizer                    |
| 108-10-1   | methyl isobutyl ketone   | Insufficient Data                      | Non-Sensitizer                             | Non-Sensitizer               | Sensitizer                    |
| 620-23-5   | m-tolualdehyde           | Insufficient Data                      | Equivocal                                  | ND                           | Sensitizer                    |
| 123-38-6   | propionaldehyde          | Insufficient Data                      | Plausible                                  | Non-Sensitizer               | Sensitizer                    |
| 110-62-3   | valeraldehyde            | Insufficient Data                      | Plausible                                  | ND                           | Sensitizer                    |
| 529-20-4   | o-tolualdehyde           | Insufficient Data                      | Equivocal                                  | ND                           | ND                            |

Derek Nexus<sup>®</sup> predictions of sensitization potential agreed with 19 of 22 chemicals that were identified as sensitizers in the literature (Table 7, Appendix B). OECD-T agreed with the sensitization potential of 11 of 16 of these chemicals, but was unable to make predictions on six chemicals because of their status as metals or lack of data. Some of the OECD-T measured results disagreed with the OECD-T predictions, however, there was limited measured data available for many of these chemicals, and more abundant data on structurally similar chemicals may have weighed more strongly in the predictions than the limited measured data associated with the chemical itself.

#### 4.4 NAM Assay Test Results

The 19 chemicals in Table 8 were selected for testing in the NAM assays. Xylene, d-limonene, benzaldehyde, and methylglyoxal, which have existing LLNA EC3 values, were selected to compare NAM results to a traditional *in vivo* approach and get a general evaluation of the quality of the NAM-based strategy. KeratinoSens<sup>™</sup> and k-DPRA assays were performed with each chemical to provide the data needed to determine sensitizer status and calculate EC3 values.

The k-DPRA data was extrapolated to the DPRA test criteria as one factor in evaluating sensitizer status. Seven of the nineteen chemicals (methylglyoxal, 1-naphthylamine, crotonaldehyde, acrylonitrile, epichlorohydrin, acrolein, methacrolein) had overall peptide depletion that met the criteria of positivity defined for the DPRA test. These chemicals all depleted the amount of synthetic cysteine peptide by at least 13.89% after 24 hours of incubation (Table 8). Six of these chemicals also had a sufficiently rapid  $k_{\max}$  values to be designated as GHS Subcategory 1A (“strong”) sensitizers. Only epichlorohydrin did not have a sufficiently rapid  $k_{\max}$  for designation as a GHS Subcategory 1A sensitizer of those that were positive based on the DPRA criteria.

**Table 8. k-DPRA percent peptide depletion and reaction rate**

Results of k-DPRA testing are described as the maximum reaction rate constant ( $\log k_{\max}$ ), the timepoint of the reaction associated with  $\log k_{\max}$ , and percent peptide depletion at 24 hours of incubation of the synthetic peptide with 5mM of each chemical. The chemicals were designated as GHS 1A sensitizers if  $\log k_{\max}$  was  $\geq -2$ . Positive control results for cinnamic aldehyde associated were valid for all trials (A1-A4 and B1-B5). NR indicates that the chemical was non-reactive in the assay, resulting in the timepoint being non-applicable (N/A).

| CAS#      | Chemical Name            | Trial | $\log k_{\max}$ | Timepoint (min) | % Peptide Depletion (5mM @ 24h) | GHS Subcategory |
|-----------|--------------------------|-------|-----------------|-----------------|---------------------------------|-----------------|
| 5989-27-5 | d-limonene               | A1    | NR              | N/A             | 1.0                             | Non-GHS 1A      |
| 1330-20-7 | xylene                   | A3    | NR              | N/A             | 1.0                             | Non-GHS 1A      |
| 100-52-7  | benzaldehyde             | A4    | NR              | N/A             | 4.6                             | Non-GHS 1A      |
| 78-98-8   | methylglyoxal            | B1    | -0.87           | 10              | 39.2                            | GHS 1A          |
| 80-05-7   | bisphenol A              | A1    | NR              | N/A             | 2.2                             | Non-GHS 1A      |
| 98-54-4   | p-tert-butylphenol       | A1    | NR              | N/A             | 1.0                             | Non-GHS 1A      |
| 90-30-2   | n-phenyl-1-naphthylamine | A2    | NR              | N/A             | 4.0                             | Non-GHS 1A      |

| CAS#                           | Chemical Name             | Trial | log<br>k <sub>max</sub> | Timepoint<br>(min) | % Peptide<br>Depletion<br>(5mM @ 24h) | GHS<br>Subcategory |
|--------------------------------|---------------------------|-------|-------------------------|--------------------|---------------------------------------|--------------------|
| 91-20-3                        | naphthalene               | A3    | NR                      | N/A                | 7.3                                   | Non-GHS 1A         |
| 134-32-7                       | 1-naphthylamine           | A3    | -1.12                   | 10                 | 21.0                                  | GHS 1A             |
| 4170-30-3                      | crotonaldehyde            | A4    | -0.15                   | 10                 | 91.9                                  | GHS 1A             |
| 107-13-1                       | acrylonitrile             | A4    | -1.48                   | 210                | 99.0                                  | GHS 1A             |
| 106-89-8                       | epichlorohydrin           | B3    | -2.23                   | 1440               | 91.8                                  | Non-GHS 1A         |
| 107-02-8                       | acrolein                  | B2    | 0.71                    | 10                 | 99.0                                  | GHS 1A             |
| 78-85-3                        | methacrolein              | B5    | 0.06                    | 10                 | 96.0                                  | GHS 1A             |
| 108-10-1                       | methyl isobutyl<br>ketone | B2    | NR                      | N/A                | 1.4                                   | Non-GHS 1A         |
| 620-23-5                       | m-tolualdehyde            | B4    | NR                      | N/A                | 1.0                                   | Non-GHS 1A         |
| 123-38-6                       | propionaldehyde           | B3    | NR                      | N/A                | 1.0                                   | Non-GHS 1A         |
| 110-62-3                       | valeraldehyde             | B3    | NR                      | N/A                | 1.0                                   | Non-GHS 1A         |
| 529-20-4                       | o-tolualdehyde            | B4    | NR                      | N/A                | 1.0                                   | Non-GHS 1A         |
| Batch A<br>Positive<br>Control | Cinnamic<br>Aldehyde      | A1    | -1.62                   | 90                 | 59.5                                  | GHS 1A             |
|                                |                           | A2    | -1.42                   | 30                 | 65.0                                  |                    |
|                                |                           | A3    | -1.54                   | 30                 | 73.4                                  |                    |
|                                |                           | A4    | -1.58                   | 90                 | 72.5                                  |                    |
| Batch B<br>Positive<br>Control | Cinnamic<br>Aldehyde      | B1    | -1.64                   | 90                 | 66.5                                  | GHS 1A             |
|                                |                           | B2    | -1.42                   | 30                 | 65.0                                  |                    |
|                                |                           | B3    | -1.47                   | 30                 | 71.2                                  |                    |
|                                |                           | B4    | -1.71                   | 210                | 68.9                                  |                    |
|                                |                           | B5    | -1.73                   | 150                | 61.7                                  |                    |

A total of 14 of 19 chemicals were positive in the KeratinoSens™ assay as activators of ARE-dependent genes in keratinocytes. These chemicals induced a 1.5-fold increase in luciferase activity, which was representative of ARE-dependent gene expression, at cell viabilities of greater than 70%. In Table 9, the chemicals were considered positive in the assay if they had at least two trials with EC<sub>1.5</sub> values for induction of luciferase activity lower than IC<sub>30</sub> values for cell viability, EC<sub>1.5</sub> values below 1000 µM, and luciferase induction I<sub>max</sub> values greater than 1.5. Four of the chemicals (d-limonene, xylene, methyl isobutyl ketone, propionaldehyde) were negative in the assay. Naphthalene gave an inconclusive result because one trial was negative (I<sub>max</sub>=1.30), one trial was positive (I<sub>max</sub>=1.73), and the final trial fell within the borderline range of 1.35-1.67 (I<sub>max</sub>=1.49). The deviation in dilution ranges for p-tert-butylphenol, d-limonene, naphthalene, xylene, and 1-naphthylamine had no impact on the results because the maximum dose required for the assay was achieved. For bisphenol A, where the top molar concentration tested was greater than 2000 µM, the deviation had no impact on the study outcome because a positive prediction was achieved at doses lower than the maximum dose.

**Table 9. KeratinoSens™ luciferase induction and cell viability**

Results of the KeratinoSens™ assay are provided as the concentration for induction of luciferase activity 1.5-fold (EC<sub>1.5</sub>), 30% and 50% reduction in viability (IC<sub>30</sub> and IC<sub>50</sub>, respectively), and maximal fold-induction of luciferase activity (I<sub>max</sub>) compared to solvent controls. When values were not obtained, the highest tested dose or ND (not determined) was listed. Positivity was defined as a greater than 1.5-fold induction in luciferase activity at a concentration below the IC<sub>30</sub> concentration and the EC<sub>1.5</sub> value being below 1000 µM in at least two trials. Positive control results for cinnamic aldehyde were valid for all trials associated with assays of batch A and B chemicals. Borderline <sup>a</sup>positive and <sup>b</sup>negative results fell within the borderline range of 1.35 to 1.67-fold induction.

| CAS#      | Chemical Name            | Trial | EC <sub>1.5</sub><br>(µM) | IC <sub>30</sub><br>(µM) | IC <sub>50</sub><br>(µM) | I <sub>max</sub>  | Positive?    |
|-----------|--------------------------|-------|---------------------------|--------------------------|--------------------------|-------------------|--------------|
| 5989-27-5 | d-limonene               | A1    | ND                        | 102.98                   | 125.59                   | 1.43              | No           |
|           |                          | A3    | ND                        | 58.61                    | 74.89                    | 1.10              |              |
| 1330-20-7 | xylene                   | A1    | ND                        | 2016.20                  | 2841.71                  | 0.96              | No           |
|           |                          | A3    | ND                        | 1120.74                  | 1348.11                  | 1.03              |              |
| 100-52-7  | benzaldehyde             | A1    | 598.44                    | 2968.54                  | >3763                    | 6.89              | Yes          |
|           |                          | A3    | 716.20                    | 2831.45                  | >3763                    | 7.13              |              |
| 78-98-8   | methylglyoxal            | B10   | 253.80                    | 632.94                   | 740.61                   | 13.31             | Yes          |
|           |                          | B11   | 163.89                    | 624.52                   | 736.65                   | 20.88             |              |
| 80-05-7   | bisphenol A              | A1    | 16.25                     | 116.74                   | 146.01                   | 54.07             | Yes          |
|           |                          | A3    | 1.67                      | 138.23                   | 161.37                   | 33.40             |              |
| 98-54-4   | p-tert-butylphenol       | A1    | 156.23                    | 310.05                   | 416.98                   | 2.92              | Yes          |
|           |                          | A3    | 158.26                    | 201.42                   | 239.28                   | 1.55 <sup>a</sup> |              |
| 90-30-2   | n-phenyl-1-naphthylamine | A7    | 5.17                      | 11.96                    | 35.73                    | 6.53              | Yes          |
|           |                          | A8    | 4.80                      | 24.29                    | 39.68                    | 5.66              |              |
| 91-20-3   | naphthalene              | A1    | ND                        | 288.49                   | 430.00                   | 1.30              | Inconclusive |
|           |                          | A3    | 337.90                    | 428.53                   | 530.08                   | 1.73              |              |
|           |                          | A10   | ND                        | 571.18                   | 696.10                   | 1.49 <sup>b</sup> |              |
| 134-32-7  | 1-naphthylamine          | A1    | 7.99                      | 737.74                   | 1034.69                  | 12.36             | Yes          |
|           |                          | A3    | 13.14                     | 875.17                   | 1117.09                  | 9.64              |              |
| 4170-30-3 | crotonaldehyde           | A4    | 6.93                      | 23.19                    | 35.66                    | 80.30             | Yes          |
|           |                          | A5    | 6.76                      | 74.62                    | 90.85                    | 121.63            |              |
|           |                          | A6    | 5.75                      | 34.56                    | 43.50                    | 52.11             |              |
|           |                          | A7    | 3.95                      | 48.02                    | 65.28                    | 82.11             |              |
|           |                          | A8    | 5.32                      | 41.08                    | 47.70                    | 33.94             |              |
| 107-13-1  | acrylonitrile            | A4    | 41.02                     | 277.05                   | 553.62                   | 90.32             | Yes          |
|           |                          | A5    | 37.37                     | 545.00                   | 1026.03                  | 222.80            |              |
|           |                          | A6    | 64.40                     | 461.27                   | 638.38                   | 106.69            |              |
|           |                          | A7    | 74.13                     | >2000                    | >2000                    | 19.53             |              |
|           |                          | A8    | 61.72                     | >2000                    | >2000                    | 74.54             |              |
| 106-89-8  | epichlorohydrin          | B7    | 23.83                     | 335.74                   | 382.68                   | 33.17             | Yes          |
|           |                          | B8    | 29.47                     | 368.39                   | 406.35                   | 66.81             |              |
|           |                          | B9    | 34.93                     | 356.78                   | 397.51                   | 81.05             |              |

| CAS#                           | Chemical Name          | Trial | EC <sub>1.5</sub><br>(μM) | IC <sub>30</sub><br>(μM) | IC <sub>50</sub><br>(μM) | I <sub>max</sub> | Positive? |
|--------------------------------|------------------------|-------|---------------------------|--------------------------|--------------------------|------------------|-----------|
| 107-02-8                       | acrolein               | B10   | 15.16                     | 79.70                    | 92.85                    | 43.18            | Yes       |
|                                |                        | B11   | 23.46                     | 97.19                    | 111.81                   | 28.91            |           |
| 78-85-3                        | methacrolein           | B1    | 14.25                     | 65.75                    | 83.73                    | 16.88            | Yes       |
|                                |                        | B3    | 10.11                     | 42.61                    | 52.37                    | 21.07            |           |
| 108-10-1                       | methyl isobutyl ketone | B1    | ND                        | >2000                    | >2000                    | 1.15             | No        |
|                                |                        | B3    | ND                        | >2000                    | >2000                    | 1.19             |           |
| 620-23-5                       | m-tolualdehyde         | B1    | 128.56                    | 502.74                   | 741.04                   | 51.13            | Yes       |
|                                |                        | B3    | 82.30                     | 534.66                   | 673.36                   | 56.01            |           |
| 123-38-6                       | propionaldehyde        | B4    | 1579.17                   | >2000                    | >2000                    | 1.68             | No        |
|                                |                        | B13   | 1119.06                   | >2000                    | >2000                    | 1.75             |           |
| 110-62-3                       | valeraldehyde          | B4    | 243.81                    | 940.67                   | 1804.63                  | 7.54             | Yes       |
|                                |                        | B13   | 237.68                    | >2000                    | >2000                    | 51.28            |           |
| 529-20-4                       | o-tolualdehyde         | B1    | 122.99                    | 299.20                   | 408.26                   | 15.19            | Yes       |
|                                |                        | B3    | 83.00                     | 120.69                   | 326.32                   | 18.14            |           |
|                                |                        | B10   | 162.55                    | 379.18                   | 604.45                   | 26.86            |           |
| Batch A<br>Positive<br>Control | Cinnamic<br>Aldehyde   | A1    | 9.06                      | >64                      | >64                      | 28.46            | Yes       |
|                                |                        | A3    | 11.67                     | >64                      | >64                      | 6.87             |           |
|                                |                        | A4    | 4.60                      | 39.13                    | 58.71                    | 27.33            |           |
|                                |                        | A5    | 6.65                      | >64                      | >64                      | 5.24             |           |
|                                |                        | A6    | <4.0                      | >64                      | >64                      | 34.13            |           |
|                                |                        | A7    | 6.79                      | >64                      | >64                      | 16.99            |           |
|                                |                        | A8    | <4.0                      | >64                      | >64                      | 12.54            |           |
|                                |                        | A10   | 8.66                      | >64                      | >64                      | 6.50             |           |
| Batch B<br>Positive<br>Control | Cinnamic<br>Aldehyde   | B1    | 8.51                      | >64                      | >64                      | 22.71            | Yes       |
|                                |                        | B3    | 4.66                      | 49.27                    | >64                      | 26.35            |           |
|                                |                        | B4    | 20.78                     | >64                      | >64                      | 2.74             |           |
|                                |                        | B7    | 8.66                      | >64                      | >64                      | 6.50             |           |
|                                |                        | B8    | 14.86                     | >64                      | >64                      | 4.02             |           |
|                                |                        | B9    | 9.48                      | >64                      | >64                      | 9.74             |           |
|                                |                        | B10   | 6.15                      | >64                      | >64                      | 5.77             |           |
|                                |                        | B11   | 9.19                      | >64                      | >64                      | 5.02             |           |
|                                |                        | B13   | 6.12                      | >64                      | >64                      | 9.89             |           |

#### 4.5. EC3 Calculation and Sensitizer Potency Predictions

EC3 values were predicted from NAM assay data using the methods of Natsch and Gerberick (2022a). The EC3 values for the 19 chemicals tested using the NAM assays are presented in Table 10, along with EC3 predictions from the *in silico* models (Derek Nexus<sup>®</sup> and OECD-T). For the four chemicals with existing mouse LLNA data (d-limonene, xylene, benzaldehyde,

methylglyoxal), the derived *in vivo* EC3 values were 26.77%, 80.75%, 51.88%, and 3.57%, respectively.

Although the dataset was too limited to draw any conclusions from a formal statistical analysis, the assay-predicted EC3 values generally agreed with literature values when assigning skin sensitization potency based on EC3 values. In Table 10, the literature EC3 values, NAM assay-predicted EC3 values, and *in silico*-predicted EC3 values were categorized according to ECETOC and GHS Subcategory potencies. NAM assay-predicted EC3 values for d-limonene, xylene, and benzaldehyde were all categorized as ECETOC weak sensitizers and fell in the range of GHS Subcategory 1B (“other sensitizer”). These categorizations agreed with the literature EC3 values for these three chemicals. The agreement did not hold for methylglyoxal, however. Methylglyoxal was predicted to be a moderate and Subcategory 1B sensitizer by the NAM assays, whereas the *in vivo* data supported a designation as a Subcategory 1A (“strong sensitizer”). However, the 3.6% assay-predicted EC3 value for methylglyoxal was near the threshold for strong sensitizer (<1%) and Subcategory 1A (<2%) categorization.

Derek Nexus<sup>®</sup>-predicted EC3 values were categorized in agreement with literature GHS Subcategory for six of eleven chemicals, and ECETOC potency categories for four of eleven chemicals (Table 10). Xylene’s literature EC3 value was weak enough to essentially be called a non-sensitizer as concluded by Derek Nexus<sup>®</sup>, so this could be considered an additional accurate prediction. The most substantial difference was for benzaldehyde, for which Derek Nexus<sup>®</sup> predicted an EC3 value of 0.04% (Extreme sensitizer/Subcategory 1A) compared to the literature value of >25% (Weak sensitizer/Subcategory 1B). The predicted EC3 values for OECD-T compared favorably with the literature GHS Subcategory for five of seven chemicals and ECETOC potency categories for three of seven chemicals. The largest difference was a predicted EC3 value of 26% for formaldehyde that was substantially higher than the 0.40% value in the literature. This difference predicted formaldehyde to be a weak, Subcategory 1B sensitizer rather than a strong, Subcategory 1A sensitizer as defined by the literature EC3 value.

**Table 10. Comparison of EC3 values obtained from the literature, NAM assays, and *in silico* predictions**

EC3 values are listed for chemicals with existing LLNA EC3 values (see Table 6) and/or those that underwent testing in NAM assays. NAM assay-predicted EC3 values were derived using the method of Natsch and Gerberick (2022a). *In silico* EC3 predictions from Derek Nexus<sup>®</sup> and OECD Toolbox (OECD-T) were calculated by the software for chemicals categorically defined as sensitizers by each program. Cell colors represent ECETOC skin sensitizer potency categories (green: non-sensitizer, yellow: weak, pink: moderate, red: strong, dark red: extreme). GHS Subcategory is indicated by 1A (“strong sensitizer”) or 1B (“other sensitizer”). “ND” indicates that no result could be determined because of insufficient data available to make a prediction for that chemical.

| CAS#      | Chemical Name | Literature        | NAM Assay Prediction | Derek Nexus <sup>®</sup> Prediction | OECD-T Prediction |
|-----------|---------------|-------------------|----------------------|-------------------------------------|-------------------|
| 5989-27-5 | d-limonene    | 30%<br>(Weak/1B)  | 27%<br>(Weak/1B)     | 8.7%<br>(Moderate/1B)               | 22%<br>(Weak/1B)  |
| 1330-20-7 | xylene        | 96%<br>(Weak/1B)  | 81%<br>(Weak/1B)     | Non-sensitizer                      | 96%<br>(Weak/1B)  |
| 100-52-7  | benzaldehyde  | >25%<br>(Weak/1B) | 52%<br>(Weak/1B)     | 0.04%<br>(Extreme/1A)               | 32%<br>(Weak/1B)  |

| CAS#       | Chemical Name            | Literature             | NAM Assay Prediction   | Derek Nexus® Prediction | OECD-T Prediction     |
|------------|--------------------------|------------------------|------------------------|-------------------------|-----------------------|
| 78-98-8    | methylglyoxal            | 0.42%<br>(Strong/1A)   | 3.6%<br>(Moderate/1B)  | 4.8%<br>(Moderate/1B)   | 26%<br>(Weak/1B)      |
| 107-22-2   | glyoxal                  | 1.10%<br>(Moderate/1A) | N/A                    | 1.4%<br>(Moderate/1A)   | 0.04%<br>(Extreme/1A) |
| 50-00-0    | formaldehyde             | 0.40%<br>(Strong/1A)   | N/A                    | 0.11%<br>(Strong/1A)    | 26%<br>(Weak/1B)      |
| 7440-41-7  | beryllium                | 8.60%<br>(Moderate/1B) | N/A                    | 0.08%<br>(Extreme/1A)   | ND                    |
| 7440-02-0  | nickel                   | 3.50%<br>(Moderate/1B) | N/A                    | 0.48%<br>(Strong/1A)    | ND                    |
| 822-06-0   | HMDI                     | 0.017%<br>(Extreme/1A) | N/A                    | 0.033%<br>(Extreme/1A)  | 0.97%<br>(Strong/1A)  |
| 18540-29-9 | hexavalent chromium      | 0.06%<br>(Extreme/1A)  | N/A                    | 0.42%<br>(Strong/1A)    | ND                    |
| 7440-48-4  | cobalt                   | 0.6%<br>(Strong/1A)    | N/A                    | 0.49%<br>(Strong/1A)    | ND                    |
| 80-05-7    | bisphenol A              | N/A                    | 10%<br>(Weak/1B)       | 5.6%<br>(Moderate/1B)   | 5.7%<br>(Moderate/1B) |
| 98-54-4    | p-tert-butylphenol       | N/A                    | 14%<br>(Weak/1B)       | 3.4%<br>(Moderate/1B)   | 5.7%<br>(Moderate/1B) |
| 90-30-2    | n-phenyl-1-naphthylamine | N/A                    | 5.79%<br>(Moderate/1B) | Non-sensitizer          | ND                    |
| 91-20-3    | naphthalene              | N/A                    | 23%<br>(Weak/1B)       | Non-sensitizer          | 2.1%<br>(Moderate/1B) |
| 134-32-7   | 1-naphthylamine          | N/A                    | 1.53%<br>(Moderate/1A) | 35%<br>(Weak/1B)        | ND                    |
| 4170-30-3  | crotonaldehyde           | N/A                    | 0.37%<br>(Strong/1A)   | 2.4%<br>(Moderate/1B)   | 4.0%<br>(Moderate/1B) |
| 107-13-1   | acrylonitrile            | N/A                    | 7.69%<br>(Moderate/1B) | ND                      | 44%<br>(Weak/1B)      |
| 106-89-8   | epichlorohydrin          | N/A                    | 7.68%<br>(Moderate/1B) | 1.1%<br>(Moderate/1A)   | 22%<br>(Weak/1B)      |
| 107-02-8   | acrolein                 | N/A                    | 0.29%<br>(Strong/1A)   | 1.9%<br>(Moderate/1A)   | 7.9%<br>(Moderate/1B) |
| 78-85-3    | methacrolein             | N/A                    | 0.47%<br>(Strong/1A)   | 2.7%<br>(Moderate/1B)   | 7.9%<br>(Moderate/1B) |
| 108-10-1   | methyl isobutyl ketone   | N/A                    | >100% (Non-sensitizer) | Non-sensitizer          | 35%<br>(Weak/1B)      |
| 620-23-5   | m-tolualdehyde           | N/A                    | 19%<br>(Weak/1B)       | 0.05%<br>(Extreme/1A)   | 18%<br>(Weak/1B)      |
| 123-38-6   | propionaldehyde          | N/A                    | >100% (Non-sensitizer) | 0.19%<br>(Strong/1A)    | 26%<br>(Weak/1B)      |
| 110-62-3   | valeraldehyde            | N/A                    | 69%<br>(Weak/1B)       | 0.26%<br>(Strong/1A)    | 30%<br>(Weak/1B)      |
| 529-20-4   | o-tolualdehyde           | N/A                    | 16%<br>(Weak/1B)       | 0.05%<br>(Extreme/1A)   | ND                    |

Derek Nexus<sup>®</sup>-predicted EC3 values were categorized in agreement with assay-derived EC3 values for GHS Subcategory for 5 of 18 chemicals, and ECETOC potency categories for 3 of 18 chemicals (Table 10). The predicted EC3 values for OECD-T compared favorably with the assay-derived EC3 GHS Subcategory for 11 of 16 chemicals and ECETOC potency categories for 5 of 16 chemicals (Table 10). The biggest discrepancies between EC3 values predicted by Derek Nexus<sup>®</sup> and the NAM assays appeared to be for a subset of chemicals with aldehyde functional groups (e.g., tolualdehyde, propionaldehyde, etc.). Derek Nexus<sup>®</sup> predicted these chemicals to be strong or extreme sensitizers while the assay-derived EC3 values determined them to be weak or non-sensitizers (Table 10).

#### 4.6 Assignment of Sensitizer Status Based on the Weight of Evidence

Literature EC3 values categorized six chemicals as GHS Subcategory 1A sensitizers: glyoxal, methylglyoxal, formaldehyde, cobalt, HMDI, and hexavalent chromium. Assay-predicted EC3 values for 1-naphthylamine, crotonaldehyde, acrolein, and methacrolein categorized all four chemicals as GHS Subcategory 1A. The weight of evidence assessment also clearly indicated that all ten chemicals were sensitizers (Table 11).

In addition to the chemicals identified as strong sensitizers, the initial literature search found 14 other AF-relevant chemicals that were preliminarily identified as sensitizers, and five other AF-relevant chemicals were predicted to be sensitizers by *in silico* models and were selected for additional testing using NAM assays. The weight of evidence was relatively clear that five of these chemicals were sensitizers (acrylonitrile, epichlorohydrin, benzaldehyde, beryllium, nickel), with NAM-predicted EC3 values categorizing each as GHS Subcategory 1B (Table 11). However, there was some non-concordance among individual factors about the potency of some of these chemicals. Acrylonitrile had k-DPRA assay results categorizing it as a GHS Subcategory 1A sensitizer, although that is not far removed from its NAM-predicted EC3 value (7.69%) that designates it a higher potency GHS Subcategory 1B sensitizer (Table 11). Human data indicated that benzaldehyde, beryllium, and nickel appeared to be stronger sensitizers than were suggested by their LLNA EC3 values, with human NOELs/LOELs that were approximately ten times lower than their LLNA EC3 values (Table 6).

Bisphenol A, p-tert-butylphenol, n-phenyl-1-naphthylamine, and naphthalene, were all categorized as GHS Subcategory 1B sensitizers by NAM-predicted EC3 values but had non-concordant results in the individual NAM assays (Table 11). Each had very low peptide depletion in the k-DPRA but were positive in the KeratinoSens<sup>™</sup> assay. A follow-up review of the literature on bisphenol A found references to positive human patch-test studies and a mouse ear-swelling test (NIOSH, 2011) that supported its assignment as a sensitizer. The assignment of p-tert-butylphenol as a sensitizer was also supported by the literature. Although there was no evidence of sensitization found in the GPMT, there are some data on positive reactions to p-tert-butylphenol in human diagnostic patch testing (ECHA, 2011). The follow-up literature review also supported the assignment of n-phenyl-1-naphthylamine as a sensitizer due to the strong positive response in the GPMT (Boman *et al.*, 1980). No specific data was found in the follow-up literature review for naphthalene, but a conservative assignment of “sensitizer” was still made based on the results of the initial literature review and the GHS Subcategory 1B classification by the NAM-predicted EC3 value.

**Table 11. Weight of evidence assessment of sensitizer status**

Assessment of each chemical as a sensitizer/non-sensitizer was made based on weight of evidence from: Human or animal data in the literature, NAM-predicted EC3 values, k-DPRA-predicted GHS Subcategory, peptide depletion and KeratinoSens™ results, and *in silico* model results. TBD: pending results of h-CLAT testing to provide more confidence in the determination, can conservatively be assigned as a sensitizer. ND: No prediction could be made due to lack of data

| CAS#      | Chemical Name            | Lit ID as Sensitizer? | Literature LLNA EC3 | NAM-predicted EC3      | Derek® (Reasoning Level) | OECD-Toolbox (Status) | Peptide Depletion (%) | k-DPRA (GHS SubCat.) | KeratinoSens™ Results | Current Weight of Evidence |
|-----------|--------------------------|-----------------------|---------------------|------------------------|--------------------------|-----------------------|-----------------------|----------------------|-----------------------|----------------------------|
| 5989-27-5 | d-limonene               | Yes                   | 30% (Weak/1B)       | 27% (Weak/1B)          | Probable                 | Sensitizer            | 1.0                   | Non-GHS 1A           | Negative              | Non-Sensitizer             |
| 1330-20-7 | xylene                   | No                    | 96% (Weak/1B)       | 81% (Weak/1B)          | Non-Sensitizer           | Non-Sensitizer        | 1.0                   | Non-GHS 1A           | Negative              | Non-Sensitizer             |
| 108-10-1  | methyl isobutyl ketone   | Insufficient Data     | N/A                 | >100% (Non-sensitizer) | Non-Sensitizer           | Sensitizer            | 1.4                   | Non-GHS 1A           | Negative              | Non-Sensitizer             |
| 123-38-6  | propionaldehyde          | Insufficient Data     | N/A                 | >100% (Non-sensitizer) | Plausible                | Sensitizer            | 1.0                   | Non-GHS 1A           | Negative              | Non-Sensitizer             |
| 100-52-7  | benzaldehyde             | Yes                   | >25% (Weak/1B)      | 52% (Weak/1B)          | Equivocal                | Non-Sensitizer        | 4.6                   | Non-GHS 1A           | Positive              | Sensitizer                 |
| 78-98-8   | methylglyoxal            | Yes                   | 0.42% (Strong/1A)   | 3.6% (Moderate/1B)     | Plausible                | Non-Sensitizer        | 39.2                  | GHS 1A               | Positive              | Sensitizer                 |
| 80-05-7   | bisphenol A              | Yes                   | N/A                 | 10% (Weak/1B)          | Equivocal                | Non-Sensitizer        | 2.2                   | Non-GHS 1A           | Positive              | Sensitizer                 |
| 98-54-4   | p-tert-butylphenol       | Yes                   | N/A                 | 14% (Weak/1B)          | Equivocal                | Non-Sensitizer        | 1.0                   | Non-GHS 1A           | Positive              | Sensitizer                 |
| 90-30-2   | n-phenyl-1-naphthylamine | Yes                   | N/A                 | 5.79% (Moderate/1B)    | Non-Sensitizer           | Non-Sensitizer        | 4.0                   | Non-GHS 1A           | Positive              | Sensitizer                 |
| 91-20-3   | naphthalene              | Yes                   | N/A                 | 23% (Weak/1B)          | Non-Sensitizer           | Sensitizer            | 7.3                   | Non-GHS 1A           | Inconclusive          | Sensitizer                 |
| 134-32-7  | 1-naphthylamine          | Yes                   | N/A                 | 1.53% (Moderate/1A)    | Equivocal                | ND                    | 21.0                  | GHS 1A               | Positive              | Sensitizer                 |
| 4170-30-3 | crotonaldehyde           | Yes                   | N/A                 | 0.37% (Strong/1A)      | Plausible                | Sensitizer            | 91.9                  | GHS 1A               | Positive              | Sensitizer                 |
| 107-13-1  | acrylonitrile            | Yes                   | N/A                 | 7.69% (Moderate/1B)    | Probable                 | Sensitizer            | 99.0                  | GHS 1A               | Positive              | Sensitizer                 |

| CAS#     | Chemical Name   | Lit ID as Sensitizer? | Literature LLNA EC3 | NAM-predicted EC3   | Derek® (Reasoning Level) | OECD-Toolbox (Status) | Peptide Depletion (%) | k-DPRA (GHS SubCat.) | Keratino-Sens™ Results | Current Weight of Evidence |
|----------|-----------------|-----------------------|---------------------|---------------------|--------------------------|-----------------------|-----------------------|----------------------|------------------------|----------------------------|
| 106-89-8 | epichlorohydrin | Yes                   | N/A                 | 7.68% (Moderate/1B) | Plausible                | Sensitizer            | 91.8                  | Non-GHS 1A           | Positive               | Sensitizer                 |
| 107-02-8 | acrolein        | Insufficient Data     | N/A                 | 0.29% (Strong/1A)   | Plausible                | Sensitizer            | 99.0                  | GHS 1A               | Positive               | Sensitizer                 |
| 78-85-3  | methacrolein    | Insufficient Data     | N/A                 | 0.47% (Strong/1A)   | Plausible                | Sensitizer            | 96.0                  | GHS 1A               | Positive               | Sensitizer                 |
| 620-23-5 | m-tolualdehyde  | Insufficient Data     | N/A                 | 19% (Weak/1B)       | Equivocal                | Sensitizer            | 1.0                   | Non-GHS 1A           | Positive               | TBD                        |
| 110-62-3 | valeraldehyde   | Insufficient Data     | N/A                 | 69% (Weak/1B)       | Plausible                | Sensitizer            | 1.0                   | Non-GHS 1A           | Positive               | TBD                        |
| 529-20-4 | o-tolualdehyde  | Insufficient Data     | N/A                 | 16% (Weak/1B)       | Equivocal                | ND                    | 1.0                   | Non-GHS 1A           | Positive               | TBD                        |

Valeraldehyde, m-tolualdehyde, and o-tolualdehyde were all designated as GHS Subcategory 1B sensitizers by the NAM-predicted EC3 value (Table 11), however, there was no additional literature evidence of their sensitization potential and each of their two NAM assays were non-concordant. Each had very low peptide depletion in the k-DPRA but were positive in the KeratinoSens<sup>TM</sup> assay. Valeraldehyde had three close structural analogues (Tanimoto score 0.7-0.8) identified by Derek Nexus<sup>®</sup> that support its assignment as a sensitizer, but they were all also very weak sensitizers with an average LLNA EC3 value of 61%. Therefore, all three chemicals were not assigned a status of “sensitizer”, but could conservatively be considered sensitizers pending additional testing to more definitively assign their status. Based on the “2 out of 3” DA, additional testing in the hCLAT assay would be recommended to resolve the disparate results in the k-DPRA and KeratinoSens assays.

Xylene, d-limonene, methyl isobutyl ketone, and propionaldehyde were all determined to be non-sensitizers based on the weight of evidence assessment. Xylene was preliminarily identified by the initial literature review as a non-sensitizer, but it had a very weak LLNA EC3 value. The NAM assays also predicted a very weak EC3 value, and data from neither NAM assay indicated xylene was a sensitizer. Therefore, the weight of evidence determined it to not be a sensitizer. There was insufficient information in the literature to support an assignment of sensitizer status to methyl isobutyl ketone and propionaldehyde, and NAM-predicted EC3 values suggested that neither was a sensitizer. D-limonene was preliminarily identified as a sensitizer in the literature and had a weak median LLNA EC3 value of 30%. However, when considering the weight of evidence that included the results of the individual NAM assays and human data, d-limonene was not considered to be a sensitizer, which agreed with a recent published weight of evidence analysis of d-limonene (Na *et al.*, 2022).

#### **4.7 Development of Preliminary Candidate Surface Guidelines**

PCSGs in  $\mu\text{g}/\text{cm}^2$  were calculated from human NOEL/LOEL data or LLNA EC3 values for the nine chemicals with values available in the literature and from assay-derived EC3 values for the 13 chemicals tested in the NAM assays (Table 12). A PCSG was not calculated for d-limonene or xylene, which were determined to be non-sensitizers by the weight of evidence assessment. A PCSG was not calculated for methyl isobutyl ketone and propionaldehyde because they were also determined to be non-sensitizers by the NAM assays.

The specific parameters used in the calculation of PCSGs for each chemical are provided in Appendix C. A composite adjustment factor was applied during each calculation to account for uncertainty in translating experimental data into human responses and actual exposure conditions. The composite adjustment factors of 30 to 300 used in this study fell within the wide range of composite adjustment factors used for skin sensitization quantitative risks assessments (3-10,000, although up to 300 is typically used in practice) (Fukushima *et al.*, 2022). The composite adjustment factor range for LLNA EC3 values used during this study (90-180) was comparable to composite adjustment factors of 50-180 that have been recommended specifically for surface guidelines for occupational exposures from LLNA EC3 values (Naumann and Arnold, 2019; Fukushima *et al.*, 2022).

**Table 12. Preliminary candidate surface guidelines**

PCSGs were calculated from PoD based on human NOELs/LOELs or LLNA- or assay-derived EC3 values. A composite adjustment factor was applied to each value to account for potential uncertainty and variability in the calculation of the PCSGs. Additional details of the PCSG calculations are provided in Appendix C.

| CAS#       | Chemical Name            | Point of Departure |       | Composite Adjustment Factor | PCSG (mg/100 cm <sup>2</sup> ) |
|------------|--------------------------|--------------------|-------|-----------------------------|--------------------------------|
|            |                          | Source             | %     |                             |                                |
| 5989-27-5  | d-limonene               | Non-sensitizer     | N/A   | N/A                         | N/A                            |
| 1330-20-7  | xylene                   | Non-sensitizer     | N/A   | N/A                         | N/A                            |
| 100-52-7   | benzaldehyde             | Human NOEL         | 590   | 30                          | 2                              |
| 78-98-8    | methylglyoxal            | LLNA               | 105   | 90                          | 0.1                            |
| 107-22-2   | glyoxal                  | Human LOEL         | 345   | 90                          | 0.4                            |
| 50-00-0    | formaldehyde             | Human NOEL         | 37    | 30                          | 0.1                            |
| 7440-41-7  | beryllium                | Human LOEL         | 11    | 90                          | 0.01                           |
| 7440-02-0  | nickel                   | Human LOEL         | 28    | 90                          | 0.03                           |
| 822-06-0   | HMDI                     | LLNA               | 4     | 180                         | 0.002                          |
| 18540-29-9 | hexavalent chromium      | LLNA               | 15    | 180                         | 0.003                          |
| 7440-48-4  | cobalt                   | Human LOEL         | 313   | 90                          | 0.3                            |
| 80-05-7    | bisphenol A              | NAMs               | 2500  | 300                         | 0.8                            |
| 98-54-4    | p-tert-butylphenol       | NAMs               | 3500  | 300                         | 1                              |
| 90-30-2    | n-phenyl-1-naphthylamine | NAMs               | 1440  | 300                         | 0.5                            |
| 91-20-3    | naphthalene              | NAMs               | 5750  | 300                         | 2                              |
| 134-32-7   | 1-naphthylamine          | NAMs               | 383   | 300                         | 0.1                            |
| 4170-30-3  | crotonaldehyde           | NAMs               | 93    | 300                         | 0.03                           |
| 107-13-1   | acrylonitrile            | NAMs               | 1920  | 300                         | 0.6                            |
| 106-89-8   | epichlorohydrin          | NAMs               | 1920  | 300                         | 0.6                            |
| 107-02-8   | acrolein                 | NAMs               | 73    | 300                         | 0.02                           |
| 78-85-3    | methacrolein             | NAMs               | 118   | 300                         | 0.04                           |
| 108-10-1   | methyl isobutyl ketone   | NAMs               | N/A   | N/A                         | N/A                            |
| 620-23-5   | m-tolualdehyde           | NAMs               | 4650  | 300                         | 2                              |
| 123-38-6   | propionaldehyde          | NAMs               | N/A   | N/A                         | N/A                            |
| 110-62-3   | valeraldehyde            | NAMs               | 17300 | 180                         | 10                             |
| 529-20-4   | o-tolualdehyde           | NAMs               | 3960  | 300                         | 1                              |

## 5.0 DISCUSSION/CONCLUSIONS

### Applications of PCSGs Developed for AF-Relevant Chemicals

The PCSGs that were derived for 22 chemicals in the present study can be used to compare to the presence of these chemicals on work surfaces to better understand the risk of Airmen developing ACD from occupational exposures. Wipe samples of a known area (e.g., 100 cm<sup>2</sup>) can be collected from high-contact surfaces in occupational settings for processes that are known to use a given chemical, and the total amount of that chemical present on the wipe can be determined using analytical methods. The mass of chemical collected on the wipe can then be divided by the area wiped to give the surface concentration of the chemical in µg/cm<sup>2</sup> or mg/100 cm<sup>2</sup>. If the surface concentration of that chemical is higher than the PCSG, then there would be elevated risk for induction of ACD. However, these PCSGs are only recommendations, and would need further scientific and policy review prior to implementation, which would likely occur on a chemical-by-chemical basis. In addition to providing PCSG data to support determination of these guidelines, this study also identified specific AF-relevant chemicals as potential sensitizers, which is hazard information that can be provided for decisions on future potential uses of these chemicals.

Even as recommendations, PCSGs could be used to help identify exposures to potential sensitizers as an initial screen for targets for future in-depth studies on exposure risks. As an example of this approach, hexavalent chromium wipe samples were collected at AF aircraft structural maintenance shops after removal and re-application of surface coatings on aircraft components. Surface concentrations of hexavalent chromium were elevated on specific surfaces within the active work areas of the shops, with 4.4 µg/cm<sup>2</sup> being the highest concentration measured (Keebaugh *et al.*, 2022a; Keebaugh *et al.*, 2022b). While this concentration is much higher than the PCSG of 0.03 µg/cm<sup>2</sup> calculated for hexavalent chromium, the active work areas typically required PPE that includes gloves, a full Tyvek suit, and full-face respirator that would limit dermal exposure. Surface concentrations in transition areas associated with PPE removal and storage, where PPE was not required and there would be increased potential for dermal exposure, ranged from levels of 0.001 to 0.03 µg/cm<sup>2</sup> (Keebaugh *et al.*, 2022a; Keebaugh *et al.*, 2022b). While these levels were below the 0.03 µg/cm<sup>2</sup> PCSG for hexavalent chromium, the higher end of the range provides only a limited margin of safety.

### Limitations

There were several limitations identified when developing PCSGs that are related to this relatively new approach. The derivation of Equation 11 by Natsch and Gerberick (2022a), was performed using existing LLNA and NAM data from an OECD database. Much of the research on dermal sensitization data has been generated for cosmetic and other applications where dermal exposure is highly likely and the chemicals are potentially different from chemicals used by the AF in occupational scenarios. Therefore, the applicability of NAM-predicted EC3 values that were developed for consumer products to AF-relevant chemicals is yet to be fully validated. The lack of overlap in that chemical space is evidenced by the limited LLNA EC3 values available for comparison in this study. Aldehydes are one class of chemicals studied that would benefit from additional LLNA EC3 comparisons to NAM-derived EC3 values. A previous validation of the NAM-derived EC3 value found them to be least predictive of aldehyde EC3 values. This limited predictivity is potentially a result of poor reactivity of aldehydes in the k-DPRA assay due to the conditions of the assay not permitting Schiff base formation (Natsch *et al.*, 2015).

The EC3 prediction regression equation that was used in this study is also limited by a tendency of the equation to mildly underpredict the potency of stronger sensitizers due to a limited dynamic range (Nastch and Gerberick 2022b). This underprediction tendency could explain why methylglyoxal was predicted to be a GHS Subcategory 1B sensitizer by the NAM-derived EC3 despite being a GHS Subcategory 1A sensitizer according to its LLNA EC3.

Confidence was limited in assignment of sensitizer status for some chemicals because of the non-concordant results in the KeratinoSens<sup>TM</sup> and k-DPRA assays, especially for chemicals with little to no literature evidence of sensitizer potential. Addition of h-CLAT testing should increase confidence in the weight of evidence for certain chemicals with non-concordant peptide depletion and KeratinoSens<sup>TM</sup> results by allowing for a full pseudo-“2 of 3” DA to assign sensitizer status. It will also allow for the use of the four other EC3 prediction regression equations that incorporate h-CLAT parameters to allow for additional refinement of EC3 predictions.

Aldehydes were a chemical class for which Derek Nexus<sup>®</sup> appeared to potentially overpredict potency in the chemicals that were studied. Benzaldehyde was predicted to be a much stronger sensitizer than was indicated in the literature, and several aldehydes were predicted to be substantially stronger than were predicted by NAM-derived EC3 values. One potential explanation for the discrepancy was that Derek Nexus<sup>®</sup> included several dialdehydes as close structural matches for the aldehydes of interest. These dialdehydes were listed as very strong sensitizers according in the Derek Nexus<sup>®</sup> database, which may have weighted the overall prediction in that direction. The increased protein reactivity of dialdehydes compared to monoaldehydes has been demonstrated experimentally with glutardialdehyde being 170 times more reactive compared to corresponding monoaldehyde pentanal (Bohme, *et al.*, 2021). This substantial difference in reactivity would potentially justify the exclusion of dialdehydes from aldehyde predictions. Excluding dialdehydes for the chemicals of interest in the present study brings the predictions from the strong/extreme sensitizer level to the weak/moderate sensitizer level that more closely agreed with assay predictions.

Another limitation of the study was the minimal existence of available surface guidelines to compare to the PCSGs derived in this study and support the adequacy of the approach. Other published scientific studies have derived surface guidelines that can be used for comparison, but these values have not been adopted or reviewed by any policy-setting organizations, so they should not necessarily be considered a gold-standard. One study used a similar approach as the present study for calculating occupational surface guidelines from LLNA EC3 values, with the only difference being that they were derived from the LLNA: BrdU-ELISA rather than the traditional radioactivity-based LLNA (Fukushima *et al.*, 2022). Therefore, differences between the PCSGs and the values published in the Fukushima *et al.*, (2022) study may be more of a function of the variability in assay results than the overall approach used to calculate PCSGs. Another study performed a hexavalent chromium quantitative risk assessment using the traditional LLNA EC3 value (Basketter *et al.*, 2003), but comparison to that study is complicated because their approach was intended to be applied to consumer products use cases with intentional repeated dermal application and may be less relevant to PCSGs that represent occupational exposures with more incidental dermal contact.

Although the LLNA test was used as comparison as a benchmark for the NAM assays in deriving the EC3 prediction equation, results from animal testing are not always representative of effects in humans and that consideration needs to be factored in when making those comparisons. Consequently, human data and the weight of evidence analysis was factored into the PCSG calculations to account for this consideration. For example, a recent weight of evidence analysis of d-limonene as a human skin sensitizer that incorporated animal, human, and *in vitro* data, determined that the weight of evidence did not support it being considered a human sensitizer despite positive LLNA results (Na *et al.*, 2022). Their rationale was that it was a very weak sensitizer in human tests, and that the stronger results of animal testing may have been the result of irritancy and not sensitization. Also, there is evidence that d-limonene itself isn't sensitizing in guinea pig testing, but that the oxidation product is a sensitizer. It is significant to note that the d-limonene NAM assay results in the publication were consistent with those performed at the laboratory used in the present study. D-limonene was negative in KeratinoSens<sup>TM</sup> and had mixed results in DPRA in the publication (Na *et al.*, 2022). D-limonene was negative in the KeratinoSens<sup>TM</sup> assay and DPRA during the present study, and was negative in the KeratinoSens<sup>TM</sup> assay and positive in DPRA in a previous study performed at the same laboratory (Lamm *et al.*, 2016). The consistency of these results is meaningful because intra- and inter-laboratory reproducibility is a key factor in the supporting validity and acceptability of NAMs for risk assessment without relying on comparisons to animal data (van der Zalm *et al.*, 2022).

#### *Conclusions/Recommendations*

PCSGs were successfully derived for 22 chemicals and sensitizer status was determined for the majority of 125 AF-relevant chemicals. For chemicals that had inconclusive sensitizer status at the completion of this study, only one additional *in vitro* assay is likely necessary to definitively categorize their sensitizer status. This project will provide information to the appropriate MAJCOMs on what chemicals and concentrations are likely to induce sensitization among workers and will recommend surface guideline limits for AF relevant chemicals with potential for ACD. Long term, the data collected and PCSGs derived in this study will support improved characterization of risk of ACD in AF personnel. Moreover, this study provides a practical example of the utility of non-animal toxicological methods in increasing the efficiency of risk assessment compared to traditional animal testing to support force health protection in the AF.

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## APPENDIX A: PRELIMINARY LITERATURE IDENTIFICATION OF SENSITIZER STATUS

### Appendix A1. Chemicals identified as sensitizers in the literature (21 compounds)

| CAS #      | Chemical Name                         |
|------------|---------------------------------------|
| 134-32-7   | 1-naphthylamine                       |
| 90-30-2    | 1-naphthylamine, n-phenyl             |
| 101-77-9   | 4-4'-methylene dianiline              |
| 75-07-0    | acetaldehyde                          |
| 107-13-1   | acrylonitrile                         |
| 100-52-7   | benzaldehyde                          |
| 7440-41-7  | beryllium                             |
| 25068-38-6 | bisphenol A epichlorohydrin polymer   |
| 18540-29-9 | chromium (VI)                         |
| 7440-48-4  | cobalt                                |
| 4170-30-3  | crotonaldehyde                        |
| 5989-27-5  | d-limonene                            |
| 50-00-0    | formaldehyde                          |
| 107-22-2   | glyoxal, inhalable/vapor/aerosol      |
| 1313-13-9  | manganese dioxide                     |
| 78-98-8    | methylglyoxal                         |
| 91-20-3    | naphthalene                           |
| 7440-02-0  | nickel                                |
| 28182-81-2 | polymeric hexamethylene diisocyanate  |
| 90598-46-2 | epoxy resins                          |
| 25085-50-1 | p-tert-butylphenol formaldehyde resin |

### Appendix A2. Chemicals identified as non-sensitizers in the literature (38 compounds)

| CAS       | Chemical Name        |
|-----------|----------------------|
| 1330-20-7 | xylene (all isomers) |
| 592-41-6  | 1-hexene             |
| 109-67-1  | 1-pentene            |
| 513-35-9  | 2-methyl-2-butene    |
| 67-64-1   | acetone              |
| 71-43-2   | benzene              |
| 111-76-2  | butyl cellosolve     |
| 123-72-8  | butyraldehyde        |
| 629-62-9  | C15 as n-Pentadecane |
| 544-76-3  | C16 as n-Hexadecane  |
| 124-38-9  | carbon dioxide       |
| 630-08-0  | carbon monoxide      |

| <b>CAS</b>  | <b>Chemical Name</b>                                    |
|-------------|---------------------------------------------------------|
| 98-82-8     | cumene                                                  |
| 111-46-6    | diethylene glycol                                       |
| 112-34-5    | diethylene glycol monobutyl ether                       |
| 64-17-5     | ethanol                                                 |
| 141-78-6    | ethyl acetate                                           |
| 100-41-4    | ethylbenzene                                            |
| 64742-94-5  | heavy aromatic solvent naptha (petroleum)               |
| 110-43-0    | 2-heptanone                                             |
| 67-63-0     | isopropanol                                             |
| 590-86-3    | isovaleraldehyde                                        |
| 67-56-1     | methanol                                                |
| 79-20-9     | methyl acetate                                          |
| 78-93-3     | methyl ethyl ketone (MEK)                               |
| 123-86-4    | n-butyl acetate                                         |
| 629-78-7    | n-heptadecane                                           |
| 109-66-0    | pentane                                                 |
| 108-95-2    | phenol                                                  |
| 127087-87-0 | polyethylene glycol mono (branched p-nonylphenyl) ether |
| 104-87-0    | p-tolualdehyde                                          |
| 1310-73-2   | sodium hydroxide                                        |
| 8052-41-3   | Stoddard solvent                                        |
| 7664-93-9   | sulfuric acid                                           |
| 629-59-4    | tetradecane                                             |
| 13463-67-7  | titanium dioxide                                        |
| 108-88-3    | toluene                                                 |
| 629-50-5    | tridecane                                               |

**Appendix A3. Chemicals for which insufficient data exists in the literature to determine sensitizer potential (58 compounds)**

| <b>CAS #</b> | <b>Chemical Name</b>                 |
|--------------|--------------------------------------|
| 646-04-8     | trans-2-pentene                      |
| 526-73-8     | 1,2,3-trimethylbenzene               |
| 95-63-6      | 1,2,4-trimethylbenzene; pseudocumene |
| 106-99-0     | 1,3-butadiene                        |
| 106-98-9     | 1-butene                             |
| 872-05-9     | 1-decene                             |
| 68649-11-6   | 1-decene, dimer, hydrogenated        |
| 25339-56-4   | 1-heptene                            |
| 90-12-0      | 1-methyl naphthalene                 |
| 124-11-8     | 1-nonene                             |
| 25377-83-7   | 1-octene                             |

| <b>CAS #</b> | <b>Chemical Name</b>                               |
|--------------|----------------------------------------------------|
| 563-46-2     | 2-methyl-1-butene                                  |
| 763-29-1     | 2-methyl-1-pentene                                 |
| 107-83-5     | 2-methylpentane                                    |
| 620-14-4     | 3-ethyltoluene                                     |
| 563-45-1     | 3-methyl-1-butene                                  |
| 622-96-8     | 4-ethyltoluene                                     |
| 691-37-2     | 4-methyl-1-pentene                                 |
| 107-02-8     | acrolein                                           |
| #N/A         | asphalt                                            |
| 7727-43-7    | barium sulfate                                     |
| 124-18-5     | C10 as n-decane                                    |
| 1120-21-4    | C11 as n-undecane                                  |
| 7440-43-9    | cadmium                                            |
| 590-18-1     | cis-2-butene                                       |
| 627-20-3     | cis-2-pentene                                      |
| 115-10-6     | dimethyl ether                                     |
| 28804-88-8   | dimethylnaphthalene isomer                         |
| 112-40-3     | dodecane                                           |
| 1333-86-4    | elemental carbon/carbon black                      |
| 60-29-7      | ethyl ether                                        |
| 611-14-3     | ethyl toluene                                      |
| 107-21-1     | ethylene glycol                                    |
| 142-82-5     | heptane                                            |
| 7439-89-6    | iron                                               |
| 7439-92-1    | lead                                               |
| 1309-60-0    | lead dioxide                                       |
| 8032-32-4    | ligroine                                           |
| 108-67-8     | mesitylene, 1,3,5-trimethylbenzene                 |
| 78-85-3      | methacrolein                                       |
| 108-10-1     | methyl isobutyl ketone (MIBK)                      |
| 75-09-2      | methylene chloride                                 |
| 620-23-5     | m-tolualdehyde                                     |
| 91-57-6      | naphthalene, 2-methyl-                             |
| 111-84-2     | nonane                                             |
| 103-65-1     | n-propylbenzene                                    |
| 111-65-9     | octane                                             |
| 529-20-4     | o-tolualdehyde                                     |
| 68937-41-7   | phenol, isopropylated, phosphate 3:1 (PIP 3.1 oil) |
| 123-38-6     | propionaldehyde                                    |
| 115-07-1     | propylene                                          |

| <b>CAS #</b> | <b>Chemical Name</b>                |
|--------------|-------------------------------------|
| 7632-00-0    | sodium nitrite                      |
| 100-42-5     | styrene                             |
| 7446-09-5    | sulfur dioxide (7446-09-5)          |
| 4050-45-7    | trans-2-hexene                      |
| 1330-78-5    | tricresyl phosphate (mixed isomers) |
| 1066-40-6    | trimethylsilanol                    |
| 110-62-3     | valeraldehyde                       |

**Appendix A4. Chemicals removed from further investigation (8 chemicals)**

| <b>CAS</b> | <b>Chemical name</b> | <b>Reason for removal</b>                                                                   |
|------------|----------------------|---------------------------------------------------------------------------------------------|
| 74-86-2    | acetylene            | These light gases are commonly encountered and show no evidence of sensitization potential. |
| 7440-37-1  | argon                |                                                                                             |
| 106-97-8   | butane               |                                                                                             |
| 74-98-6    | propane              |                                                                                             |
| 74-84-0    | ethane               |                                                                                             |
| 74-85-1    | ethylene             |                                                                                             |
| 10102-43-9 | nitric oxide         |                                                                                             |
| 10102-44-0 | nitrogen dioxide     |                                                                                             |

## APPENDIX B: IN SILICO PREDICTION OF SENSITIZER STATUS

*In silico* predictions of sensitizer status for chemicals without literature EC3 values and not tested with *in chemico/in vitro* methods, ND=not determined because of lack of information

| CAS #      | Chemical Name                 | Literature ID as Sensitizer? | Sensitizer?  |                 |                  |
|------------|-------------------------------|------------------------------|--------------|-----------------|------------------|
|            |                               |                              | Derek Nexus  | OECD-T Measured | OECD-T Predicted |
| 101-77-9   | 4-4'-methylene dianiline      | Yes                          | Equivocal    | Yes             | Yes              |
| 75-07-0    | acetaldehyde                  | Yes                          | Plausible    | ND              | Yes              |
| 1313-13-9  | manganese dioxide             | Yes                          | No           | ND              | ND               |
| 646-04-8   | trans-2-pentene               | Insufficient Data            | No           | ND              | No               |
| 526-73-8   | 1,2,3-trimethylbenzene        | Insufficient Data            | No           | ND              | No               |
| 95-63-6    | 1,2,4-trimethylbenzene        | Insufficient Data            | No           | ND              | No               |
| 106-99-0   | 1,3-butadiene                 | Insufficient Data            | No           | ND              | ND               |
| 106-98-9   | 1-butene                      | Insufficient Data            | No           | ND              | No               |
| 872-05-9   | 1-decene                      | Insufficient Data            | No           | No              | No               |
| 68649-11-6 | 1-decene, dimer, hydrogenated | Insufficient Data            | No           | ND              | ND               |
| 25339-56-4 | 1-heptene                     | Insufficient Data            | No           | ND              | No               |
| 90-12-0    | 1-methyl naphthalene          | Insufficient Data            | No           | ND              | No               |
| 124-11-8   | 1-nonene                      | Insufficient Data            | No           | ND              | No               |
| 25377-83-7 | 1-octene                      | Insufficient Data            | No           | ND              | ND               |
| 563-46-2   | 2-methyl-1-butene             | Insufficient Data            | No           | ND              | No               |
| 763-29-1   | 2-methyl-1-pentene            | Insufficient Data            | No           | ND              | No               |
| 107-83-5   | 2-methylpentane               | Insufficient Data            | No           | ND              | ND               |
| 620-14-4   | 3-ethyltoluene                | Insufficient Data            | No           | ND              | No               |
| 563-45-1   | 3-methyl-1-butene             | Insufficient Data            | Equivocal    | ND              | ND               |
| 622-96-8   | 4-ethyltoluene                | Insufficient Data            | No           | ND              | No               |
| 691-37-2   | 4-methyl-1-pentene            | Insufficient Data            | No           | ND              | ND               |
| #N/A       | asphalt                       | Insufficient Data            | ND           | ND              | ND               |
| 7727-43-7  | barium sulfate                | Insufficient Data            | Inconclusive | ND              | ND               |
| 124-18-5   | C10 as n-decane               | Insufficient Data            | No           | No              | No               |
| 1120-21-4  | C11 as n-undecane             | Insufficient Data            | No           | No              | No               |
| 7440-43-9  | cadmium                       | Insufficient Data            | No           | ND              | ND               |
| 590-18-1   | cis-2-butene                  | Insufficient Data            | No           | ND              | ND               |
| 627-20-3   | cis-2-pentene                 | Insufficient Data            | No           | ND              | No               |
| 115-10-6   | dimethyl ether                | Insufficient Data            | No           | ND              | ND               |
| 28804-88-8 | dimethylnaphthalene isomer    | Insufficient Data            | No           | ND              | No               |
| 112-40-3   | dodecane                      | Insufficient Data            | No           | No              | No               |
| 1333-86-4  | elemental carbon/carbon black | Insufficient Data            | No           | ND              | ND               |

| CAS #      | Chemical Name                        | Literature ID as Sensitizer? | Sensitizer?  |                 |                  |
|------------|--------------------------------------|------------------------------|--------------|-----------------|------------------|
|            |                                      |                              | Derek Nexus  | OECD-T Measured | OECD-T Predicted |
| 60-29-7    | ethyl ether                          | Insufficient Data            | No           | No              | ND               |
| 611-14-3   | ethyl toluene                        | Insufficient Data            | No           | ND              | No               |
| 107-21-1   | ethylene glycol                      | Insufficient Data            | No           | No              | No               |
| 142-82-5   | heptane                              | Insufficient Data            | No           | ND              | No               |
| 7439-89-6  | iron                                 | Insufficient Data            | No           | ND              | ND               |
| 7439-92-1  | lead                                 | Insufficient Data            | No           | ND              | ND               |
| 1309-60-0  | lead dioxide                         | Insufficient Data            | Inconclusive | ND              | ND               |
| 8032-32-4  | ligroine                             | Insufficient Data            | ND           | ND              | ND               |
| 108-67-8   | mesitylene, 1,3,5-trimethylbenzene   | Insufficient Data            | No           | ND              | No               |
| 75-09-2    | methylene chloride                   | Insufficient Data            | No           | No              | ND               |
| 91-57-6    | naphthalene, 2-methyl-               | Insufficient Data            | No           | ND              | No               |
| 111-84-2   | nonane                               | Insufficient Data            | No           | ND              | No               |
| 103-65-1   | n-propylbenzene                      | Insufficient Data            | No           | ND              | No               |
| 111-65-9   | octane                               | Insufficient Data            | No           | ND              | No               |
| 68937-41-7 | phenol, isopropylated, phosphate 3:1 | Insufficient Data            | No           | ND              | No               |
| 115-07-1   | propylene                            | Insufficient Data            | No           | ND              | ND               |
| 7632-00-0  | sodium nitrite                       | Insufficient Data            | Inconclusive | ND              | ND               |
| 100-42-5   | styrene                              | Insufficient Data            | No           | No              | No               |
| 7446-09-5  | sulfur dioxide                       | Insufficient Data            | No           | ND              | ND               |
| 4050-45-7  | trans-2-hexene                       | Insufficient Data            | No           | ND              | No               |
| 1330-78-5  | tricresyl phosphate (mixed isomers)  | Insufficient Data            | No           | ND              | No               |
| 1066-40-6  | trimethylsilanol                     | Insufficient Data            | No           | ND              | ND               |

## APPENDIX C: PRELIMINARY CANDIDATE SURFACE GUIDELINES DERIVATION

### C1. Calculation of Preliminary Candidate Surface Guidelines

| CAS#       | Chemical Name            | Point of Departure |                       | Compound Used in LLNA                         | Mass fraction of compound | Adjustment Factors |                  |                 |           | Preliminary Candidate Surface Guideline |
|------------|--------------------------|--------------------|-----------------------|-----------------------------------------------|---------------------------|--------------------|------------------|-----------------|-----------|-----------------------------------------|
|            |                          | Source             | (µg/cm <sup>2</sup> ) |                                               |                           | AF <sub>WOE</sub>  | AF <sub>IV</sub> | AF <sub>E</sub> | Composite | (mg/100 cm <sup>2</sup> )               |
| 5989-27-5  | d-limonene               | Non-sensitizer     | N/A                   | N/A                                           | N/A                       | N/A                | N/A              | N/A             | N/A       | N/A                                     |
| 1330-20-7  | xylene                   | Non-sensitizer     | N/A                   | N/A                                           | N/A                       | N/A                | N/A              | N/A             | N/A       | N/A                                     |
| 100-52-7   | benzaldehyde             | Human NOEL         | 590                   | N/A                                           | N/A                       | 1                  | 10               | 3               | 30        | 2                                       |
| 78-98-8    | methylglyoxal            | LLNA               | 105                   | N/A                                           | 1                         | 3                  | 10               | 3               | 90        | 0.1                                     |
| 107-22-2   | glyoxal                  | Human LOEL         | 345                   | N/A                                           | N/A                       | 3                  | 10               | 3               | 90        | 0.4                                     |
| 50-00-0    | formaldehyde             | Human NOEL         | 37                    | N/A                                           | N/A                       | 1                  | 10               | 3               | 30        | 0.1                                     |
| 7440-41-7  | beryllium                | Human LOEL         | 11                    | N/A                                           | N/A                       | 3                  | 10               | 3               | 90        | 0.01                                    |
| 7440-02-0  | nickel                   | Human LOEL         | 28                    | N/A                                           | N/A                       | 3                  | 10               | 3               | 90        | 0.03                                    |
| 822-06-0   | HMDI                     | LLNA               | 4                     | N/A                                           | 1                         | 6                  | 10               | 3               | 180       | 0.002                                   |
| 18540-29-9 | hexavalent chromium      | LLNA               | 15                    | K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> | 0.35                      | 6                  | 10               | 3               | 180       | 0.003                                   |
| 7440-48-4  | cobalt                   | Human LOEL         | 313                   | N/A                                           | N/A                       | 3                  | 10               | 3               | 90        | 0.3                                     |
| 80-05-7    | bisphenol A              | NAMs               | 2500                  | N/A                                           | N/A                       | 10                 | 10               | 3               | 300       | 0.8                                     |
| 98-54-4    | p-tert-butylphenol       | NAMs               | 3500                  | N/A                                           | N/A                       | 10                 | 10               | 3               | 300       | 1                                       |
| 90-30-2    | n-phenyl-1-naphthylamine | NAMs               | 1440                  | N/A                                           | N/A                       | 10                 | 10               | 3               | 300       | 0.5                                     |
| 91-20-3    | naphthalene              | NAMs               | 5750                  | N/A                                           | N/A                       | 10                 | 10               | 3               | 300       | 2                                       |
| 134-32-7   | 1-naphthylamine          | NAMs               | 383                   | N/A                                           | N/A                       | 10                 | 10               | 3               | 300       | 0.1                                     |
| 4170-30-3  | crotonaldehyde           | NAMs               | 93                    | N/A                                           | N/A                       | 10                 | 10               | 3               | 300       | 0.03                                    |
| 107-13-1   | acrylonitrile            | NAMs               | 1920                  | N/A                                           | N/A                       | 10                 | 10               | 3               | 300       | 0.6                                     |
| 106-89-8   | epichlorohydrin          | NAMs               | 1920                  | N/A                                           | N/A                       | 10                 | 10               | 3               | 300       | 0.6                                     |
| 107-02-8   | acrolein                 | NAMs               | 73                    | N/A                                           | N/A                       | 10                 | 10               | 3               | 300       | 0.02                                    |
| 78-85-3    | methacrolein             | NAMs               | 118                   | N/A                                           | N/A                       | 10                 | 10               | 3               | 300       | 0.04                                    |
| 108-10-1   | methyl isobutyl ketone   | NAMs               | N/A                   | N/A                                           | N/A                       | N/A                | N/A              | N/A             | N/A       | N/A                                     |
| 620-23-5   | m-tolualdehyde           | NAMs               | 4650                  | N/A                                           | N/A                       | 10                 | 10               | 3               | 300       | 2                                       |
| 123-38-6   | propionaldehyde          | NAMs               | N/A                   | N/A                                           | N/A                       | N/A                | N/A              | N/A             | N/A       | N/A                                     |
| 110-62-3   | valeraldehyde            | NAMs               | 17300                 | N/A                                           | N/A                       | 6                  | 10               | 3               | 180       | 10                                      |
| 529-20-4   | o-tolualdehyde           | NAMs               | 3960                  | N/A                                           | N/A                       | 10                 | 10               | 3               | 300       | 1                                       |

## Appendix C2. Rationale for Adjustment Factor Based on Weight of Evidence

| CAS#       | Chemical Name            | Adjustment Factor Based on Weight of Evidence |                                                                                                                                                   |
|------------|--------------------------|-----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
|            |                          | Adjustment Factor                             | Rationale                                                                                                                                         |
| 5989-27-5  | d-limonene               | N/A                                           | Non-sensitizer in literature                                                                                                                      |
| 1330-20-7  | xylene                   | N/A                                           | Non-sensitizer in literature                                                                                                                      |
| 100-52-7   | benzaldehyde             | 1                                             | Human NOEL, no adjustment                                                                                                                         |
| 78-98-8    | methylglyoxal            | 3                                             | Close structural analogue (glyoxal) with human data, use same adjustment                                                                          |
| 107-22-2   | glyoxal                  | 3                                             | Adjustment for human LOEL to NOEL                                                                                                                 |
| 50-00-0    | formaldehyde             | 1                                             | Human NOEL, no adjustment                                                                                                                         |
| 7440-41-7  | beryllium                | 3                                             | Adjustment for human LOEL to NOEL                                                                                                                 |
| 7440-02-0  | nickel                   | 3                                             | Adjustment for human LOEL to NOEL                                                                                                                 |
| 822-06-0   | HMDI                     | 6                                             | Default interspecies adjustment with no human data or structural analogues                                                                        |
| 18540-29-9 | hexavalent chromium      | 6                                             | Default interspecies adjustment with no human data or structural analogues                                                                        |
| 7440-48-4  | cobalt                   | 3                                             | Adjustment for human LOEL to NOEL                                                                                                                 |
| 80-05-7    | bisphenol A              | 10                                            | Default NAM adjustment with no close structural analogues                                                                                         |
| 98-54-4    | p-tert-butylphenol       | 10                                            | Default NAM adjustment with no close structural analogues                                                                                         |
| 90-30-2    | n-phenyl-1-naphthylamine | 10                                            | Default NAM adjustment with no close structural analogues                                                                                         |
| 91-20-3    | naphthalene              | 10                                            | Default NAM adjustment with no close structural analogues                                                                                         |
| 134-32-7   | 1-naphthylamine          | 10                                            | Default NAM adjustment with no close structural analogues                                                                                         |
| 4170-30-3  | crotonaldehyde           | 10                                            | Default NAM adjustment with no close structural analogues                                                                                         |
| 107-13-1   | acrylonitrile            | 10                                            | Default NAM adjustment with no close structural analogues                                                                                         |
| 106-89-8   | epichlorohydrin          | 10                                            | Default NAM adjustment with no close structural analogues                                                                                         |
| 107-02-8   | acrolein                 | 10                                            | Default NAM adjustment with no close structural analogues                                                                                         |
| 78-85-3    | methacrolein             | 10                                            | Default NAM adjustment with no close structural analogues                                                                                         |
| 108-10-1   | methyl isobutyl ketone   | N/A                                           | Non-sensitizer based on NAM results                                                                                                               |
| 620-23-5   | m-tolualdehyde           | 10                                            | Default NAM adjustment with no close structural analogues                                                                                         |
| 123-38-6   | propionaldehyde          | N/A                                           | Non-sensitizer based on NAM results                                                                                                               |
| 110-62-3   | valeraldehyde            | 6                                             | Three structural analogues with Tanimoto score ~0.7-0.8, average LLNA EC3 of 61% (15300 µg/cm <sup>2</sup> ), use default interspecies adjustment |
| 529-20-4   | o-tolualdehyde           | 10                                            | Default NAM adjustment with no close structural analogues                                                                                         |

## LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

|                   |                                                                                                                                                    |
|-------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| ACD               | allergic contact dermatitis                                                                                                                        |
| AF                | Air Force                                                                                                                                          |
| AFB               | Air Force Base                                                                                                                                     |
| AF <sub>E</sub>   | adjustment factor for exposure conditions                                                                                                          |
| AF <sub>IV</sub>  | adjustment factor for interindividual variability of effects                                                                                       |
| AFMC              | Air Force Materiel Command                                                                                                                         |
| AFRL              | Air Force Research Lab                                                                                                                             |
| AFSC              | Air Force Specialty Code                                                                                                                           |
| AF <sub>WOE</sub> | Adjustment factor for translation to human effects                                                                                                 |
| AOP               | adverse outcome pathway                                                                                                                            |
| ARE               | antioxidant response element                                                                                                                       |
| BC                | buffer control                                                                                                                                     |
| BrdU              | 5-bromo-2-deoxyuridine                                                                                                                             |
| CAS               | Chemical Abstract Service                                                                                                                          |
| CD                | contact dermatitis                                                                                                                                 |
| CHET              | Chemical Hazard Evaluation Tool                                                                                                                    |
| CNIH              | Confirmation of No Induction in Humans                                                                                                             |
| CI                | confidence interval                                                                                                                                |
| CO <sub>2</sub>   | carbon dioxide                                                                                                                                     |
| DA                | defined approach                                                                                                                                   |
| DC                | dendritic cells                                                                                                                                    |
| DMEM              | Dulbecco's Modified Eagle Medium                                                                                                                   |
| DMSO              | dimethyl sulfoxide                                                                                                                                 |
| dp                | depletion                                                                                                                                          |
| DPRA              | Direct Peptide Reactivity Assay                                                                                                                    |
| DSA <sub>05</sub> | dose per unit skin area leading to a sensitization incidence of 5%                                                                                 |
| EC <sub>1.5</sub> | Concentration resulting in a 1.5-fold induction in gene activity in KeratinoSens assay                                                             |
| EC3               | concentration of a chemical needed to induce at least a 3-fold increase in lymph node cell proliferation when compared to vehicle treated controls |
| ECETOC            | European Center for Ecotoxicology and Toxicology of Chemicals                                                                                      |
| EESOH-MIS         | Enterprise Environmental, Safety, and Occupational Health Management Information System                                                            |
| ELISA             | Enzyme-linked immunosorbent assay                                                                                                                  |
| FCM               | flow cytometry                                                                                                                                     |
| GHS               | Globally Harmonized System                                                                                                                         |
| GPMT              | guinea pig maximization test                                                                                                                       |
| HAS               | hardened aircraft shelter                                                                                                                          |
| HaCaT             | human keratinocytes                                                                                                                                |
| h-CLAT            | human Cell Line Activation Test                                                                                                                    |
| HMDI              | hexamethylene diisocyanate                                                                                                                         |
| HMT               | Human Maximization Test                                                                                                                            |
| HRIPT             | Human Repeat Insult Patch Test                                                                                                                     |

|                  |                                                                           |
|------------------|---------------------------------------------------------------------------|
| IC <sub>30</sub> | concentration resulting in a 30% reduction in cell viability in MTT assay |
| IC <sub>50</sub> | concentration resulting in a 50% reduction in cell viability in MTT assay |
| ICD              | irritant contact dermatitis                                               |
| ICD-9/10         | International Classification of Disease code 9/10                         |
| I <sub>max</sub> | maximum induction of gene activity in KeratinoSens assay                  |
| IRR              | incidence rate ratio                                                      |
| IATA             | Integrated Approach to Testing and Assessment                             |
| ITS              | integrated testing strategy                                               |
| k-DPRA           | kinetic Direct Peptide Reactivity Assay                                   |
| KB               | knowledge base                                                            |
| KE               | key event                                                                 |
| Keap1            | Kelch-like ECH-associated protein 1                                       |
| k <sub>max</sub> | maximum reaction rate constant                                            |
| LC               | Langerhans cells                                                          |
| LCMC             | Life Cycle Management Center                                              |
| LLNA             | local lymph node assay                                                    |
| LMW              | low molecular weight                                                      |
| log              | logarithm                                                                 |
| MAJCOM           | Major Command                                                             |
| MTT              | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide          |
| mBrB             | monobromobimane                                                           |
| NAD(P)H          | nicotinamide adenine dinucleotide phosphate                               |
| NAM              | new approach methodology                                                  |
| NAMs             | new approach methodologies                                                |
| NIOSH            | National Institute for Occupational Safety and Health                     |
| NC               | negative control                                                          |
| NESIL            | no expected sensitization induction level                                 |
| NOEL             | no observed effect level                                                  |
| Nrf2             | Nuclear Factor-Erythroid Factor 2-Related Factor 2                        |
| OCD              | occupational contact dermatitis                                           |
| OD               | optical density                                                           |
| OEC              | Air Force bioenvironmental engineering career field consulting branch     |
| OECD             | Organisation for Economic Cooperation and Development                     |
| OECD-T           | OECD QSAR Toolbox                                                         |
| OHE              | occupational hand eczema                                                  |
| OSHA             | Occupational Safety and Health Administration                             |
| PAO              | polyalphaolefin                                                           |
| PC               | positive control                                                          |
| PCSG             | preliminary candidate surface guideline                                   |
| pGL4             | plasmid GL4                                                               |
| pH               | potential of hydrogen                                                     |
| PIP 3:1          | phenol, isopropylated phosphate (3:1)                                     |
| PoD              | points of departure                                                       |
| PPE              | personal protective equipment                                             |
| PCSGs            | preliminary candidate surface guidelines                                  |

|                 |                                                      |
|-----------------|------------------------------------------------------|
| QSAR            | quantitative structure-activity relationship         |
| RLU             | relative light units                                 |
| SC              | solvent control                                      |
| SD              | standard deviation                                   |
| SDS             | Safety Data Sheet                                    |
| SV40            | simian virus 40                                      |
| UN              | United Nations                                       |
| USAFSAM         | United States Air Force School of Aerospace Medicine |
| VP              | vapor pressure                                       |
| w/v             | weight per volume                                    |
| %               | percent                                              |
| ≥               | greater than or equal to                             |
| cm <sup>2</sup> | square centimeter                                    |
| mg              | milligram                                            |
| mL              | milliliter                                           |
| mM              | millimeter                                           |
| nm              | nanometer                                            |
| μg              | microgram                                            |
| μM              | micromolar                                           |