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A Quantitative Risk Assessment for skin sensitizing plant protection products: linking Derived No-Effect Levels (DNELs) with agricultural exposure models

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Abstract

Chemical skin sensitizers produce allergic contact dermatitis, which is one of the most frequent occupational diseases associated with chemical exposures. Skin exposure is the major route of exposure when using plant protection products (PPPs). Therefore, skin sensitization is an important factor to be addressed during the regulatory risk assessment of PPPs. The main regulatory decision criterion considered when performing risk assessment for skin sensitizers is the dose applied. The equally important criteria “potency of the substance” is insufficiently considered by two potency categories as potency may vary up to five orders of magnitude. “Frequency of exposure” to the skin sensitizer is not considered at all. Consequently, an improved risk assessment methodology is essential to adequately assess health risks from skin sensitizers, especially for agricultural operators using PPPs. A quantitative risk assessment (QRA) approach for addressing PPPs sensitizing potential is proposed here. This QRA combines a methodology to derive a substance-specific threshold for skin sensitizers, a Derived No-Effect Level (DNEL), and an agricultural exposure model used for assessing chronic health risks of PPPs. The proposed QRA for skin sensitizing PPPs is a clear improvement over current risk assessment to ensure the safe use of skin sensitizers in an occupational context.

Key words

Chemical skin sensitizers, allergic contact dermatitis, plant protection products, quantitative risk assessment, Derived No-Effect Level (DNEL), agricultural operator exposure model

1 Introduction

Chemical skin sensitizers are known to produce allergic contact dermatitis, which is one of the most frequent occupational diseases associated with exposure to chemicals (Diepgen and Coenraads, 1999; McDonald et al., 2006). Allergic contact dermatitis progresses in two stages as commonly observed with other forms of allergy. After a single exposure to a skin sensitizer during an initial induction phase, sensitization to the allergen is acquired. Subsequent exposures to the same skin sensitizer elicit the actual allergic reaction (elicitation phase) (see Appendix A1 for further information on the scientific background of skin sensitization). Allergic responses to skin sensitizers are driven by the amount of substance applied per area of exposed skin (expressed in $\mu g/cm^2$) and referred to as external dose, by the potency of the skin sensitizer (i.e. its electrophilic reactivity), and by the frequency of exposure to the skin sensitizer (Friedmann, 2007; Kimber et al., 2008; Paramasivan et al., 2010). Yet, the main regulatory decision criterion currently considered in the EU when performing a risk assessment for skin sensitizing chemicals is the classification of a substance or mixture as skin sensitizer. The actual amount of substance reaching the skin is not considered for current risk assessment. According to the EU Regulation for Classification, Labelling and Packaging (CLP), a mixture containing a skin sensitizer is not considered having skin sensitizing properties if the concentration of the skin sensitizer in the mixture is below defined concentration limits of 0.1% or 1% (see Appendix A2 for further information on the classification criteria used in the EU CLP Regulation). However, this concentration-based approach does not sufficiently address potency, especially for strong sensitizers, since sensitization after exposure to strong sensitizers can occur at far lower concentrations than set forth in the CLP Regulation (Liden, 2008). Similarly, frequency of exposure is completely disregarded. Consequently, an improved risk assessment methodology for skin sensitizers is needed to adequately consider these three factors: dose, potency, and frequency of exposure to the skin sensitizers. All three are important in determining occurrence of sensitization. Ideally, a quantitative risk assessment (QRA) methodology would combine a quantitative model comparing predicted exposures to the specific skin sensitizer with an endpoint that has been derived considering these three influencing factors.

A number of authors have proposed QRA approaches for skin sensitizing chemicals, primarily focusing on cosmetic and household products and on the risk for consumers of such products (Api et al., 2006; Felter et al., 2002; Griem et al., 2003; ter Burg et al., 2010). Since the EU banned animal testing of cosmetic ingredients in 2013 (including tests for skin sensitization), considerable efforts have been and are being made in the cosmetic and fragrance industry to update skin sensitization QRA (Basketter and Safford, 2016; SCCS, 2017). Common to all these approaches is the aim to derive a quantitative endpoint to protect non-allergic individuals against skin sensitization. This endpoint is either called “No Expected Sensitizing Induction Level (NESIL)” (Api et al., 2006) or “Acceptable Non-Sensitizing Area Dose (ANSAD)” (Griem et al., 2003). Apart from focusing on risks for consumers, publications so far have concentrated on the scientific basis of skin sensitization; and appropriate use of uncertainty factors or sensitization assessment factors (SAFs) for deriving an endpoint, below which no sensitization occurs. Derived quantitative endpoints have so far not been combined with an exposure assessment, thus estimates for the likelihood of exposure to skin sensitizers have not been provided. The exposure assessment is a pre-requisite in order to perform a risk assessment where both the hazard of the substance is characterized as well as the exposure to the substance are considered.

The present study aims at developing a QRA methodology for plant protection products (PPPs) which is an important group of skin sensitizing chemicals since skin exposure is the most significant route of entry when using PPPs (Anderson and Meade, 2014; Baldi et al., 2006; Macfarlane et al., 2013). Quantitative methodologies are available for chemicals (ECHA, 2012), biocides (ECHA, 2017) and cosmetics (Api et al., 2006; Basketter and Safford, 2016; Felter et al., 2002; Griem et al., 2003; SCCS, 2017; ter Burg, 2006). For PPPs, currently only a qualitative or hazard-based approach is implemented, which consists of wearing personal protective equipment (PPE) while using sensitizing products or dilutions. The QRA approach presented here uses a methodology to derive a substance-specific threshold for skin sensitizers, a Derived No-Effect Level (DNEL) (ECHA, 2012). The DNEL explicitly includes

potency and frequency of exposure being two important determinants of skin sensitization. Subsequently, the third determinant being the actual amount of substance reaching the skin is considered by using the DNEL in an agricultural exposure model used for assessing the chronic risks of PPPs to agricultural operators[1] during the approval process of PPPs. The advantage of such an agricultural exposure model is that the estimated systemic PPP exposure is compared to a systemic endpoint. By doing this the maximum amount of PPP to which an operator may be exposed per day without any adverse health effects to be expected (Acceptable Operator Exposure Level, AOEL) can be defined. While the AOEL covers subacute and partially subchronic effects, it does not cover local skin effects such as irritation and sensitization. Hence, an endpoint reflecting skin sensitizing risk such as a DNEL is needed.

The approach presented here may help to improve the risk assessment for skin sensitizing chemicals. In addition, it addresses appropriate exposure scenarios in the risk assessment. This will eventually lead to a better protection of operators using PPPs regularly. The proposed approach will be discussed considering both the toxicological as well as the cumulative and occupational exposure assessment perspective.

2. Skin sensitizing plant protection products

Plant protection products (PPPs) aim at protecting plants from damaging influences such as weeds, fungi or insects. They are primarily used in the agricultural sector but also in forestry, horticulture, amenity areas, and private gardens to protect crops or desirable or useful plants. Given that PPPs are biologically active, they do not only have the desired plant protecting effects but also drawbacks, such as potential toxicity to humans and other non-target species in the environment. PPPs therefore undergo an authorization process in most countries where the manufacturer is required to assess the risks to human health and the environment prior to putting a product on the market (EC, 2009; PSMV, 2010). The risk assessment data have to be

[1]The term “operator” is used here according to EFSA (2014) to denominate persons who are involved in activities relating to the application of a plant protection product.

submitted by the manufacturer to governmental agencies. The appropriate authorities assess the data and eventually decide whether the health risks associated with the PPP use are acceptable and market approval can be granted. Assessing the PPP's potential to induce skin sensitization is a data requirement for placing on the market in the EU and in Switzerland (EC, 2013). Among the 1134 PPPs authorized by April 2018 in Switzerland, 323 products (i.e. 28.5%) were classified as being skin sensitizers (FOAG). They contained chemical active substances or adjuvants and co-formulants possibly being skin sensitizers.

PPP applications on agricultural crops are typically divided into four clearly separated tasks: (1) mixing and loading the PPP into a tank; (2) applying diluted PPP with spray equipment; (3) rinsing and cleaning the spray equipment; and (4) re-entering previously treated crops. The level of PPP exposure varies between these four tasks. Mixing and loading are usually tasks associated with the highest exposure because agricultural operators are handling the concentrated product. In addition, accidental spills of the concentrated product may lead to direct local skin exposure. Exposure during spraying of the diluted PPP greatly vary depending on the spray equipment used. Field crops such as cereals, potatoes, and sugar beets are predominantly sprayed with tractor-mounted boom sprayers or self-propelled sprayers. The operator often sits in a closed cabin, which significantly reduces exposure to the diluted PPP. Closed cabins may not, however, be available in all cases. Other crops such as grapes, stone or pome fruits are sprayed with tractor-mounted broadcast air-assisted sprayers. The tractors used in orchards and vineyards are usually smaller than those used in field crops and may not always include a closed cabin. Where the terrain is too steep to use machinery, operators use knapsack sprayers or backpack mist blowers. Hand held equipment is likewise used in greenhouses to spray certain vegetables such as tomatoes and cucumbers, as well as for spraying ornamental plants. Especially with hand-held equipment, exposure to the diluted product can be higher than when using a tractor-mounted spraying equipment (Baldi et al., 2006; Baldi et al., 2012). In addition, agricultural operators are exposed to contaminated surfaces on the spraying equipment during rinsing and cleaning operations. They may also come in contact with sprayed plant material following application of PPPs during pruning and

harvesting activities. Apart from the factor determined by the characteristics of the equipment described above, a number of other factor determine the level of exposure during the three tasks described. An obvious factor is exposure time, which depends on the length of the tasks performed. Spraying operations usually last several hours while mixing, loading, rinsing, and cleaning are usually shorter; about 15 – 20 minutes (Baldi et al., 2006). In addition to intensity (exposure level) and duration (exposure time), another factor is the number of tasks performed over one working day (daily frequency) such as mixing and loading tasks needed to refill empty tanks. A further crucial factor is the type of protective clothing or equipment worn by the agricultural operators. Especially for skin sensitizing PPPs, exposure is clearly influenced by the area of unprotected skin as the concentrated or diluted PPP can deposit on bare arms or legs. Finally, an additional important factor is the frequency of exposure to PPPs over a growing season (seasonal frequency). In agriculture, the seasonal frequency is related to the crops grown on the farm. Although the number of treatments may vary depending on weather conditions or pest pressure, every crop usually has a more or less defined number of treatments over a growing season. In summary, five crucial exposure determinants govern PPP exposures among agricultural workers: spills and accidents, intensity, exposure time, daily task frequency, and seasonal treatment frequency.

3 Rationale for a new quantitative risk assessment approach

The following section outlines the specific rationale behind the quantitative risk assessment (QRA) approach proposed herein. In a first paragraph, the three most important factors contributing to skin sensitization are described. Secondly, reasons for why these three factors are currently insufficiently considered in the actual risk assessment methodology of PPPs are given. Finally, the new approach for QRA to address the mentioned limitations of current risk assessment of skin sensitizing PPPs is introduced.

3.1 Dose-response relationships, potency of chemical skin sensitizers and frequency of exposure are the most important factors contributing to skin sensitization

Three principal factors determine whether a chemical induces sensitization[2]: (1) the dose that ultimately reaches the immune system; (2) the sensitizing potency of the chemical; and (3) the exposure pattern including the daily and seasonal exposure frequency. Inevitably, the three factors are linked as exposure is a function of intensity (i.e. dose), duration, and frequency. Hence, it is difficult to clearly denominate the contribution of each of the three factors. In the following, explanations of the impact of each factor is presented.

Dose influences the type and vigor of T-lymphocyte responses. The chemical dose (i.e. the amount of substance or ultimately the number of molecules) reaching the viable layers of the epidermis and finally encountering the immune system depends on a number of elements. Some elements are linked to the properties of the chemical, for example, to its molecular weight (MW) (it is assumed that compounds must have a MW less than 500 Da for efficient penetration[3]) and its solubility characteristics (lipid-soluble molecules penetrate much better than water-soluble ones having a log $K_{ow} \leq 1$[3]) (Friedmann, 2007). Other elements are exposure-related such as the concentration (i.e. the amount of the substance on a given area or in a given volume) applied to the skin surface and the duration of contact with the skin. Kimber and colleagues were able to show that the immune response to a sensitizer primarily depends on the intensity of the sensitization stimulus, in other words, on the sensitizer dose per exposed skin area (Kimber et al., 2002; Kimber et al., 2008). Similarly, Friedmann (2007) demonstrated

[2] An important additional factor for the development of an allergic reaction to a chemical sensitizer is individual susceptibility, which is determined by age, gender and genetic factors such as the human leukocyte antigen (HLA) haplotype (Menné and Wilkinson, 1992; Schnuch and Carlsen, 2011). This factor will not be discussed further here.

[3] A recent study has shown that substances with a molecular weight (MW) larger than 500 Da may be skin sensitizers too. Among a data set of 2904 substances tested for skin sensitization, 33 substances (of a total of 197 substances with a MW > 500 Da) showed to be skin sensitizers (Fitzpatrick et al., 2017b). Similarly, of 525 substances having a log $K_{ow} \leq 1$, 100 substances were found to be skin sensitizers (Fitzpatrick et al., 2017a).

that the concentration in a given volume in percent (w/v) of material applied is not per se critical, but rather the concentration applied per area of exposed skin is the crucial determinant. Hence, the relevant dose metric in skin sensitization is the amount of chemical allergen that reaches a defined unit area of skin (usually expressed as μg substance per cm^2 skin).

The **potency of chemical skin sensitizers** can be defined as the relative ability of a chemical to induce sensitization (Ezendam et al., 2012). It is thought that the more protein-reactive a chemical is, the more potent it is as a sensitizer (Friedmann, 2007). Potency is thereby primarily driven by the ability of a chemical to form a hapten-protein complex, thus the higher the hapten-protein complex concentration, the higher the potency of the chemical. Chemicals may potentially react with many different skin proteins at different amino acid sites, but in general, protein molecules are rich in nucleophiles and sensitizing chemicals are reactive electrophiles (Divkovic et al., 2005; Kaplan et al., 2012; Karlberg et al., 2008). For protein haptenation to occur (and hence lead to skin sensitization), a chemical must be electron deficient and have a high electrophilic reactivity. Chemicals with a higher electrophilic reactivity are thus more potent skin sensitizers. Chemical skin sensitizers vary by up to five orders of magnitude in skin sensitizing potency (Kimber et al., 2012). Some chemicals such as 2,4-Dinitrochlorobenzene (DNCB) (CAS 97-00-7), Diphenylcyclopropenone (CAS 886-38-4) and oxazolone (CAS 15646-46-5), are very potent immunogens (i.e. substances producing an immune response) inducing contact sensitization in 100% of humans. The stronger the potency of the sensitizer, the lower the challenge dose needed to elicit a reaction in an already sensitized individual (Ezendam et al., 2012). Thus, dose and potency are inversely related. More potent sensitizers induce reactions at much lower doses than less potent ones (and similarly higher doses are needed with less potent ones for elicitation of an allergic reaction).

Frequency of exposure is a third important determinant of the severity of the allergic reaction. Dose-response relationships are normally deducted from experiments with either a single dose or a low number of repeated doses (e.g. three exposures in the LLNA test). Yet, in occupational settings, the workers are normally repeatedly exposed weekly or even on a daily basis to

chemicals. Frequency of exposure plays a role in both induction and elicitation of skin sensitization. Basketter et al. (2006) showed that infrequent exposure at longer duration and with a higher concentration was significantly less likely to induce sensitization compared to more frequent, short duration, and lower concentration exposure. Similarly, the same degree of sensitization was induced by three exposures to 10 μg DNCB per cm^2 skin as by one exposure to 60 μg of DNCB per cm^2 skin (Paramasivan et al., 2010). Friedmann (2007) was able to show that during the early weeks and possibly months following initiation of sensitivity, repeated challenges with the sensitizer can increase the strength of sensitization and hence the reactivity. One possible explanation could be that repeated exposures result in chemical accumulation in the *stratum corneum*, acting as an epidermal reservoir as argued by Friedmann (2007). In conclusion, frequent exposure to several lower doses may induce a reaction that would otherwise only be induced by one exposure to a much higher dose. Frequency of exposure is hence equally important as dose and potency when assessing the risks of chemical skin sensitizers.

3.2 Limitations of the risk assessment for skin sensitizers according to EU CLP Regulation

It is important to understand that substances are to be classified as being hazardous according to the EU CLP Regulation's criteria (Appendix A2). The hazard-based approach put forward in the Regulation is first to determine whether a chemical substance or a mixture containing the substance are either sensitizing or not based on the defined concentration limits. The approach put forward in the EU CLP Regulation suffers a main disadvantage for assessing occupational risk to skin sensitizers. As explained above, three main factors determine whether skin sensitization occurs or not: dose, potency and frequency. The EU CLP approach, however, primarily considers dose and to a lesser extent potency, whereas frequency of exposure is completely disregarded. Hence, both potency and frequency are either not sufficiently or not at all considered. Potency is not sufficiently considered since the EU CLP approach defines only two thresholds for skin sensitizers (0.1% and 1%). Liden (2008) argues that the concentration limit of 1% for category 1 (for substances where no data is available) is far too high to prevent

sensitization and allergic contact dermatitis from many potent skin sensitizers. With strong sensitizers, a single exposure is often sufficient for the induction of an immunological response. Since more than 4'000 chemicals have the potential to cause allergic contact dermatitis (de Groot, 2008), it is likely that this category of sensitizers contains a number of substances that should potentially have lower concentration limits. The concentration limit for this category will thus likely underestimate the risk for many substances present in this category. Given that the potency of skin sensitizers may vary up to five or six orders of magnitude, concentration limits only covering two orders of magnitude are not sufficient to assess the risk of the more potent sensitizers.

Frequency of exposure to skin sensitizers, although equally important as potency, is completely disregarded by the EU CLP approach. Nevertheless, frequency of exposure is an important factor, given that PPPs are frequently applied over a growing season to protect the various crops grown on a farm against pests and diseases. Knowing that frequent exposure to several lower doses of a skin sensitizer may induce a reaction that would otherwise only be induced by one exposure to a much higher dose, frequency should be included in the risk assessment.

3.3 Quantitative risk assessment for skin sensitizers

Frequency should be considered in the quantitative risk assessment (QRA) methodology for substances or mixtures classified as sensitizers. The advantage of a QRA over a purely hazard-based assessment is that the QRA allows to derive substance-specific thresholds. These can be used in an exposure assessment to determine whether the predicted exposure exceeds the thresholds. Ideally, the threshold to be used in such a QRA would consider – beside the dose - various potency levels as well as the frequency of exposure. The ECHA proposes an approach to derive a substance-specific threshold for skin sensitizers, a Derived No-Effect Level (DNEL) (ECHA, 2012). The DNEL considers the three relevant factors: dose, potency, and frequency of exposures (see section 7.1). This approach offers the opportunity to develop a QRA for skin sensitizers as the derived threshold can be used in an agricultural exposure model. Such a

model is used prior to the regulatory market approval of PPPs to determine the risks for agricultural operators.

The aim of the present study is to propose an approach combining substance-specific DNELs for skin sensitizers with an agricultural exposure model. The DNEL is used as an endpoint to determine whether predicted skin exposure among agricultural operators exceeds the derived substance-specific DNEL. If so, specific risk mitigation measures need to be defined to control the risk.

In the following, the procedure to calculate DNELs for skin sensitizers is described for a set of existing PPPs being classified as skin sensitizers. To address actual frequencies of exposure to skin sensitizers in agriculture, work practices of operators applying PPPs in horticultural production were investigated via interviews with greenhouse managers working in horticultural production. Subsequently, the procedure of using the DNELs in an agricultural exposure model to perform a QRA for skin sensitizing PPPs is explained.

4. Material and Methods

4.1 Derived No-Effect Level calculations for skin sensitizing plant protection products

4.1.1 European Chemicals Agency's guidance for a quantitative risk assessment for skin sensitizers

The DNEL endpoint described in the QRA methodology for skin sensitizers proposed by ECHA (2012) reflects a maximum dose of skin sensitizer where no sensitization should occur. The DNEL (expressed as µg substance per cm^2 area of skin) is derived from the EC3 value (in percent) obtained from the LLNA dose-response data (see Appendix A3 for further information on OECD test methods for identifying skin sensitizers). The EC3 value is thereby interpreted to be a Lowest Observed Adverse Effect Level (LOAEL) for induction within the process of deriving a DNEL (Basketter et al., 2003). To determine the correct starting point for the induction-specific DNEL, the EC3 value (converted in $\mu g/cm^2$) is divided by (default) sensitization assessment

factors (SAFs) to account for inter- and intra-species variation, for dose response uncertainties and for uncertainties in the extrapolation of the LOAEL to the NOAEL (ECHA, 2012).

4.1.2 Data used for the calculation of Derived No-Effect Levels

The calculation was based on actual LLNA test data of PPPs submitted by manufacturers for the authorization in Switzerland. Access to these confidential test reports was possible given that some of the authors are involved in the official PPP authorization process in Switzerland. LLNA test data for some PPPs was obtained from documents in the authorization process in October 2014. The first selection criterion when screening the available dossiers was the PPP classification as a skin sensitizer according to the EU CLP Regulation. The second criterion was the availability of a positive LLNA test. Based on these two criteria, six PPPs were selected. Specific EC3 values and DNELs for these six PPPs were derived from their respective LLNA test data. The information related to product names and product characteristics is presented in an anonymized format (Product A – Product F) since the data submitted for PPP registration is confidential.

4.1.3 Derived No-Effect Level calculation procedures

Where the dose-response curve in the LLNA showed at least one SI value being above and below the threshold value of 3 (as for products E and F; see Supplemental Material), the EC3 value was calculated using Equation 1 (Table 1). The data points immediately above and below the SI value of 3 on the LLNA dose-response plot are denoted with the coordinates (a; b) and (c; d), respectively (Figure 1) (Basketter et al., 2001).

	Conc. [%]	**SI**	
	25	0.8	
c	50	1	**d**
a	100	3.6	**b**

$$\mathrm{EC3}\ [\%] = \mathrm{c} + \left[\frac{3-\mathrm{d}}{\mathrm{b}-\mathrm{d}} * (\mathrm{a}-\mathrm{c})\right] = 88.5\ \%$$

Equation 1

Table 1: Example of EC3 calculation based on a fictitious LLNA dose-response plot

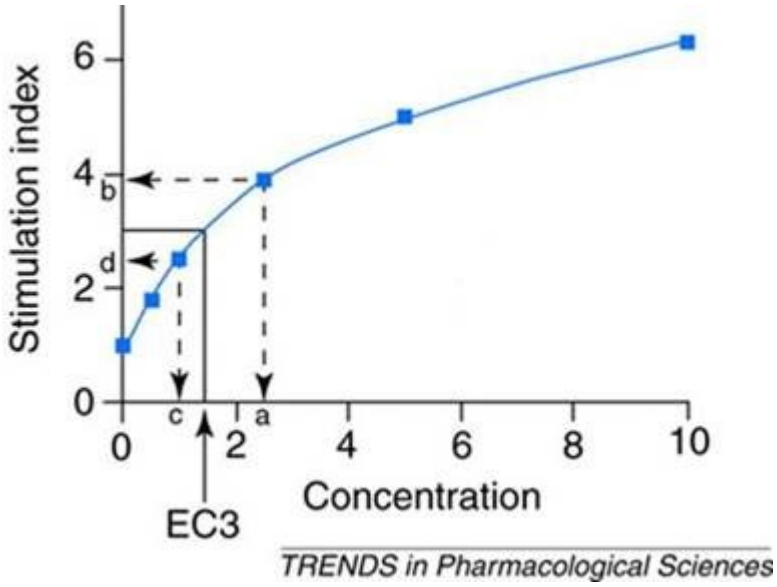

Figure 1: Graphical illustration how the EC3 value for estimation of allergenic potency is determined based on local lymph node assay (LLNA) test data. The graph shows a dose–response curve using the LLNA. The concentration of the test chemical required to produce a stimulation index (SI) of 3 (the EC3 value) is calculated using the formula EC3 =c + [(3−d)/(b−d)] ×(a−c). The data points lying immediately above and below the SI value of 3 on the LLNA dose–response plot have the coordinates (a,b) and (c,d), respectively. (adapted and reprinted from TRENDS in Pharmacological Sciences (22), Basketter, D., et al., “Measurement of allergenic potency using the local lymph node assay”. pp. 264-265, Copyright (2001) with permission from Elsevier).

In the regular LLNA protocol, EC3 is derived from plots of SI vs. concentration by linear interpolation between the two points immediately above and below SI = 3 (Roberts, 2015). Where both SI values are above the threshold value of 3, Equation 1 fails giving a correct EC3 value (as for products A, B and D; see Supplemental Material). In such a case the EC3 value has to be calculated with a log-linear extrapolation using the two SI values greater than 3 with the lowest of the SI values having the lowest test concentrations from the dose-response curve (Ryan et al., 2007). In Equation 2, the point with the higher SI was denoted (a, b) and the point with the lower SI was denoted (c, d).

Similarly, the log-linear extrapolation method outlined in Equation 2 fails where the dose-response curve is bell-shaped (i.e. the SI value at the lower dose is higher than the SI at the higher dose as for Product C where the SI at 25% = 7.9 and the SI at 50% = 7.2; see Supplemental Material). Calculating EC3 with the log-linear extrapolation from Equation 2 would give an unrealistic EC3 value of 3200%. A more realistic EC3 value can be estimated by applying the rLLNA formula, where an EC3 can be calculated from a single dose LLNA (Roberts, 2015). Using the SI value of 7.9 at the dose 25% in Equation 3 results in an EC3 value of 5%.

Equation 2

$$\text{EC3 extrapolated } [\%] = 10^{[\log c + \frac{3-d}{b-d} * (\log a - \log c)]}$$

Equation 3

$$logEC3 = \frac{\left(Probit_{78.5}\,(2) - Probit_{78.5}\,(SI-1)\right) + \,0.87 * \log Dose}{0.87}$$

Equation 4

$$\text{EC3 } [\%] * 250\ [\mu g/cm^2/\%] = \text{EC3 } [\mu g/cm^2]$$

Since the EC3 is usually expressed as a concentration (%), the value has to be converted to dose per skin area (µg/cm^2) using the formula presented in Equation 4 (ECHA, 2012). The EC3 in (µg/cm^2) can be calculated by considering the dose volume of 25 µl (according to the standard LLNA protocol) and an estimated application area of 1 cm^2 for the mouse ear. Assuming the density of the liquid is 1, the conversion factor to be applied to the EC3 (%) of 250 is calculated by converting 25 µl/cm^2 into µg/cm^2. The converted EC3 values were subsequently used as a starting point for deriving substance-specific DNELs by applying sensitization assessment factors (SAFs).

4.1.4 Application of sensitization assessment factors (SAFs).

Sensitization assessment factors (SAFs) are numerical values that address the differences between the experimental data and the human situation by taking into account uncertainties in the extrapolation procedure and in the available data set. Five different SAFs were considered:

SAF for interspecies variation (mouse => human)

Mouse EC3 data generally correlate well with human skin sensitization thresholds derived from historical predictive testing (Basketter et al., 2005). There are, however, cases where this correlation is poor and the two values may differ by a 10-fold or more. In view of this variation, ECHA proposes to use a default SAF of 10 for interspecies variation unless there is evidence of good correlation between EC3 and human NOAEL/LOAEL (ECHA, 2012). The Dutch National Institute for Public Health and the Environment (RIVM) suggests to use a factor of 3 instead of the classical factor of 10 (ter Burg, 2006; ter Burg et al., 2010). Yet, a recent publication of RIVM showed that an interspecies factor is required to ensure that the sensitization threshold determined in the LLNA does not underestimate the human threshold (Bil et al., 2017). While the geometric means of the probability distributions of murine and human sensitization threshold ratios were close to one, the 95th of these distributions resulted in an interspecies SAF of 15. In the present case, a SAF of 10 was applied to account for interspecies variation in accordance with the proposal by ECHA representing a compromise between the two proposals by RIVM.

SAF for inter-individual difference among humans

Humans differ in sensitivity to intoxications due to a multitude of biological factors such as genetic polymorphism (affecting e.g. toxicokinetics/metabolism), age, gender, health status and nutritional status. These differences can be the result of genetic and/or environmental influences. Inter-individual variation is greater in humans than in the more inbred experimental animal population. ECHA (2012) suggests to use a SAF of 5 for workers, based on the fact that this sub population does not cover the very young, the very old, and the very ill. In the present case a SAF of 10 was chosen for inter-individual differences as suggested by a number of authors (Basketter and Safford, 2016; ECHA, 2017; SCCS, 2017).

SAF for dose-response relationship

When the starting point for the DNEL calculation is a LOAEL, ECHA suggests to use an assessment factor between 3 (as minimum/majority of cases) and 10 (as maximum/exceptional

cases) (ECHA, 2012). As, in the present case, the EC3 value is considered to be a LOAEL, a SAF of 3 was applied.

SAF for vehicle or matrix effect

Skin sensitization studies are usually performed using a test solution in a simple matrix as recommended by the OECD testing guideline (OECD, 2010). ECHA (2012) recommends the use of a SAF of 3 if human exposure is expected in a matrix with no penetration enhancers. Yet, actual human exposure to PPPs might involve exposure to sensitizers in a different or more complex matrix which might increase the potential for induction of sensitization (e.g. matrix with irritant or/and penetration enhancing properties). PPPs typically contain solvents, surfactants and other chemical agents that aim at enhancing spray retention to the leaf surface. Such agents are known to affect dermal absorption, often increasing the absorption potential (EFSA, 2012). In these cases, ECHA suggests considering the application of an additional SAF of 1-10-fold, depending on the information available on the vehicle or matrix relevant for human exposure (Felter et al., 2002). Since skin sensitization studies are performed with the formulated product, the increased dermal absorption potential is already considered in the LLNA data. However, PPPs may be used in the field in tank mixes with additional substances with penetration enhancing properties. Thus a SAF of 5 was applied in the present case.

SAF for different exposure scenarios

According to ECHA, an additional SAF should be considered to account for differences in experimental exposure condition from actual human exposure scenarios (ECHA, 2012). One of the differences relates to the exposure frequency between the animal study and actual human exposure situations (Felter et al., 2002). In the LLNA, the mouse ear receives topical application of the test chemical once a day for three consecutive days. Skin sensitizers may nevertheless induce allergic responses at dosages, which do not induce a response in the LLNA. This occurs especially after prolonged and repeated exposure of the skin to low doses (de Jong et al., 2005; Paramasivan et al., 2010). Since it is unclear whether this occurs with most or only some sensitizing chemicals, ECHA suggests the application of a SAF of 1 – 10 fold to account for the

uncertainty related with repeated exposure. An interview-based study among greenhouse managers working in horticultural production was performed to understand the frequency of exposure to skin sensitizing PPPs (see Appendix A4 for further information). The interviews showed that a single operator might perform up to 60 applications of various PPPs in horticulture over a growing season from March to October. Horticulture was used as a worst-case scenario since a multitude of different PPPs are frequently used in this production. Other agricultural production areas generally use PPPs less frequently than horticulture. In the present case, a SAF of 5 was applied to account for repeated exposures during application of PPPs in different agricultural production areas.

Multiplication of the different SAFs resulted in a final SAF of 7'500 (Table 2). In conclusion, the DNEL representing the maximum dose of skin sensitizer where no sensitization should occur was calculated using Equation 5.

Sensitization assessment factor	**Accounting for**	**Default value used**
Interspecies variation	Extrapolation mouse => human	10
Inter-individual differences	Differences among humans (here worker)	10
Dose-response relationship	Extrapolation LOAEL => NOAEL	3
Vehicle or matrix effect	Exposure via test solution => human exposure via PPP	5
Exposure conditions	Frequency of exposure during test => frequency of exposure during real-use	5
Total		**7'500**

Table 2: Overview of the different sensitization assessment factors assigned for the calculation of DNELs for skin sensitizers.

Equation 5

$$\mathrm{DNEL} = \frac{\mathrm{LOAEL}}{\mathrm{SAFs}} = \frac{\mathrm{EC3}\ [\mu g/cm^2]}{2250}$$

4.2 Linking the Derived No-Effect Levels with an agricultural exposure model

4.2.1 Agricultural operator exposure models

Exposure models provide estimates for exposures to chemical substances during a particular working task such as the PPP application. In the approval process of PPPs, potential exposure is determined by using deterministic mathematical exposure models that are based on a number of parameters. Simulations of PPP exposure scenarios are calculated with the Agricultural Operator Exposure Model (AOEM) developed by the European Food Safety Authority (EFSA) (AOEM, 2013; EFSA, 2014). The AOEM estimates potential exposure absorbed via the skin and via inhalation when spraying PPPs using different types of spraying machinery (e.g. tractor-mounted boom and air-assisted sprayer as well as knapsack sprayer). The exposure is estimated by taking into account parameters that influence exposure such as application rate per hectare, treated area per day, percentage of dermal and inhalation absorption and body weight. Potential exposure can then be converted into systemic exposure using respective dermal absorption values. In the risk assessment during the approval process of PPPs, resulting exposure is compared to an endpoint defining the maximum amount of active substance to which an operator may be exposed per day without any adverse health effects to be expected over a life time (AOEL = Acceptable Operator Exposure Level (EC, 2006)). Taking into account typical PPP exposure patterns, the AOEL is based on the NOAEL from an oral short-term toxicity study (typically a 90-day study) provided that the critical endpoint(s) of the substance (e.g. reproductive/developmental toxicity) are covered and no irreversible effects occur at lower dose levels after chronic exposure. If a more sensitive, relevant end-point has been determined in a study investigating specific end-points (e.g. neurotoxicity, reproductive toxicity or developmental toxicity) the respective NOAEL is considered for AOEL setting. The AOEL does however not cover local skin effects such as irritation and sensitization. Thus, the AOEL is not appropriate to assess the risks to skin sensitizing PPPs.

When performing risk assessment with an agricultural exposure model, a first step is to estimate exposure based on a “no PPE scenario”. In this step, it is assumed that the operator does not wear any personal protective equipment (PPE) such as gloves, coverall or face shield that

would reduce exposure (1st tier approach). In cases where the estimated exposures in the "no PPE scenario" exceed the exposure limit (i.e. the AOEL), various PPE can be incorporated into the model to assess whether the selected PPEs would allow a reduction in exposure below the AOEL (2nd tier approach). Each type of PPE has a specific protection factor. The AOEM uses measured values for hand and body protection using gloves or work wear depending on the application scenario chosen. Gloves reduce hand exposures by a factor of 0.01 to 0.11 (= 89-99% protection for liquids). A coverall (i.e. certified work wear) reduces body exposure by a factor of 0.02 to 0.15 (= 85-98% protection), while a face shield reduces head exposure by a factor of 0.05 (= 95% protection) (AOEM, 2013).

4.2.2 Use of agricultural exposure models to estimate exposure to skin sensitizers

The AOEM allows to determine the amount of PPPs that is present on the outer skin surface. It can therefore be used to perform a risk assessment for skin sensitizing PPPs by comparing the amount of PPP on the skin surface to the DNEL for skin sensitizing PPPs. In the present case, exposure scenarios were calculated with the AOEM assuming that six skin sensitizer products would be applied in horticultural production using a knapsack sprayer at the actual dosage prescribed. The scenario "ornamentals, outdoor, upward spraying, manual knapsack" was chosen in the AOEM. An outdoor scenario had to be chosen as the current version of the AOEM provided by EFSA (version of March 2015) (EFSA, 2014) does not include a module for calculating indoor applications in greenhouses. The exposure scenario was selected as it is the pertinent exposure scenario for spraying horticultural plants. Moreover, the scenario "Hand-held upward spraying" is one of the scenarios leading to highest exposure among the scenarios present in the AOEM. Consequently, this can be considered a worst-case scenario.

The model determines the amount of substance deposited on the skin or "external dermal exposure" [in μg substance per person]. To determine the potential exposure on bare skin during application of the six PPPs, a "no PPE scenario" was calculated in a first step. The resulting external dermal exposure values for hands, body and head were then added to the exposure occurring during mixing and loading of the product. As PPE has to be worn by default

during mixing and loading of skin sensitizing PPPs, the corresponding exposure values for the protected body parts were also considered. The sum of the external dermal exposure during mixing and loading with PPE for the three body parts plus their exposure during application without PPE was then divided by the respective default body surface area [in cm^2] for hands, body, and head (Table 3). The amount obtained per cm^2 of skin was then compared to the DNELs defining the maximum amount of substance of skin sensitizer on bare skin where no sensitization should occur. Skin sensitization can occur on any body parts wherever the DNEL is exceeded. Where the estimated exposure in the no PPE scenario exceeded the DNEL, exposure was calculated again with appropriate PPEs to each of the three body parts. Here, the measured values from the AOEM were used to determine potential exposure values for PPP application with PPE. Again, the external dermal exposure during mixing and loading with PPE was added to the obtained values and the sum of both exposures were compared to the DNELs (see Supplemental Material for Excel screenshots of the six products analyzed).

Body part	Body surface area [in cm^2]
Hands (palms and backs of both hands)	820
Arms (both)	2'270
Trunk (bosom, neck, shoulders, abdomen, back, genitals, buttocks)	5'710
Legs (both legs and thighs)	5'330
Head	1'110

Table 3: Default surface areas [in cm^2] of different parts of the body of an adult (EFSA, 2014). The surface area of the body (= 13'310 cm^2) is composed of arms, trunk, and legs assuming that an agricultural operator would at least wear a T-shirt and short trousers in a no PPE scenario.

	Product A	Product B	Product C	Product D	Product E	Product F
Type of PPP	Fungicide	Herbicide	Fungicide	Herbicide	Fungicide	Fungicide
Application rate (kg active substance per hectare)	2.448	0.75	0.249	0.06	1.325	0.072
EC3 [%]	3.1	0.036	5.1	14.4	88.5	96.9
EC3 [µg/cm^2]	780	9	1284	3602	22115	24231
DNEL [µg/cm^2]	0.35	0.004	0.171	1.60	9.83	10.77
EU CLP classification	Skin Sens 1	Skin Sens 1	Skin Sens 1	Skin Sens 1B	Skin Sens 1	Skin Sens 1
Concentration limit	1%	1%	1%	1%	1%	1%
Conc. of product in spray solution	0.46%	0.22%	0.15%	0.15%	0.28%	0.17%
Spray solution classified according to EU CLP	no	no	no	no	no	no
Exposure calculation AOEM model						
Exposure hands > DNEL necessary PPE	yes (6'780%) no reasonable PPE	yes (218'195%) no reasonable PPE	yes (619%) gloves	no (70%)	yes (143%) gloves	no (12%)
Exposure body > DNEL necessary PPE	yes (5'112%) coverall	yes (366'326%) no reasonable PPE	yes (2'171%) coverall	yes (619%) coverall	yes (164%) coverall	no (95%)
Exposure head > DNEL necessary PPE	yes (183%) visor	yes (10'761%) no reasonable PPE	no (54%)	no (13%)	no (5%)	no (2%)

Table 4: Risk assessment for six skin sensitizing PPPs by comparing DNELs with estimated exposure of the skin calculated with the agricultural operator exposure model (AOEM). Where estimated exposure of the skin sensitizer on the skin exceeds the DNELs (shown in red), the necessary

personal protective equipment (PPE) is indicated to avoid induction of skin sensitization. For products A (hand exposure) and B (hand, body and head exposure) no reasonable PPE can be recommended since the DNEL is exceeded even when wearing PPE during application..

5 Results

5.1 Risk assessment using Derived No-Effect Levels for skin sensitizing plant protection products

DNELs were derived for the six PPPs analyzed and they varied from 0.004 to 10.77 µg/cm^2 (Table 4). Hence the difference in potency between the strongest and the weakest sensitizer varied by a factor of 2'693. Comparing the estimated exposure during spraying of the products with the respective DNELs showed that five out of six products would need protective measures to cover bare skin to avoid sensitization. Product B was the strongest sensitizer among the six products analyzed. The DNEL would be exceeded for product B even if PPE would be worn during application (see Supplemental Material). Only product F of the five remaining products would not require PPE in order for the value to remain below the DNEL. PPE would be required for products A, C, D and E. Similar to product B, the DNEL for hand exposure is also exceeded for product A even if gloves are worn during application. It is noteworthy that none of the five products (A, B, C, D, E) would have required PPE during spraying if the protective measures had been derived based on the classification criteria set forth in the EU CLP Regulation. Being classified as Skin Sens 1 or 1B according to the EU CLP Regulation, the general concentration limit for the spray solution for these five products would be 1%. However, the actual spray solution concentration ranged from 0.15 to 0.46%. Since the concentration remained below the concentration limit of 1%, the spray solutions would not have to be classified as skin sensitizing. Hence, the purely qualitative hazard assessment according to the EU CLP Regulation would have underestimated the risk. Deriving product-specific DNELs with the suggested quantitative approach clearly showed that protective measures are needed for three products to avoid sensitization.

6 Discussion

6.1 Limitations of the risk assessment for skin sensitizers according to EU CLP Regulation

The considerations and analyses made in the present publication show that the current risk assessment for skin sensitizers based on the EU CLP Regulation approach bears two important limitations:

1) **A toxicological limitation:** the actual risk is only insufficiently covered by the current approach as the two concentration limits defined by the EU CLP Regulation cover a rather narrow potency range of one order of magnitude, while the potency of skin sensitizers varies up to five orders of magnitude (Kimber et al., 2012). As shown in section 5, sensitization can occur at far lower concentrations than 0.1% since the EC3 (i.e. the concentration leading to the classification as skin sensitizer) of one of the assessed PPPs (product B) was as low as 0.036%. Where the concentration-based EU CLP Regulation approach would have indicated no risk, the DNEL-approach showed that protective measures are needed to avoid sensitization.

2) **An exposure assessment limitation:** the current risk assessment is based on the assumption of a single exposure scenario, that is, as long as the concentration limit is not exceeded during a single application of a PPP, no sensitization occurs. However, frequency of exposure plays an important role in developing an allergic contact dermatitis. Especially after prolonged and repeated skin exposure to low doses, the skin sensitizers may induce allergic responses at dosages, which do not induce responses in commonly used standard test assays such as the LLNA (de Jong et al., 2005; Paramasivan et al., 2010). As shown in section 4, frequency of exposure is a crucial component of the exposure assessment given that most companies interviewed estimated that a single worker applies PPPs 1-2 times per week over a growing period of eight months.

6.2 A toxicological approach to address the insufficient consideration of potency

The current approach set forth in the EU CLP Regulation is unable to consider the full potency range of skin sensitizers. Consequently, the inability to identify strong allergens inevitably reduces the protection of operators regularly exposed to skin sensitizers. One aim of the present study was to address this potency issue by combining the DNEL approach proposed by ECHA with existing agricultural exposure models to develop a QRA for skin sensitizing PPPs. The results described in section 5 show that DNELs can be derived for PPPs if LLNA data is available. A critical part in the DNEL derivation process is the assignment of the different SAFs, which are often selected based on a weight of evidence approach (WoE) that involves expert judgements. In a WoE approach, different pieces of the available information are analyzed for their strengths and weaknesses based on the quality and consistency of the data and the relevance of the given information. In the present case, one could argue that the assigned total SAF of 7'500 is rather high. Yet, each of the five SAFs is substantiated both by scientific data and by recommendations made in the literature (see section 4.1.4). The number of SAFs to be assigned could be debated as some authors suggest to only define three different SAFs to account for inter-individual variability (SAF = 10), for vehicle/product matrix effects (SAF from 1 to 10) and for use considerations (SAF from 1 to 10) (Api et al., 2006; Felter et al., 2002; ter Burg et al., 2010). Assigning the highest value of the three SAFs would result in a total SAF of 1'000. It is important to note that the question of which SAF is to be assigned, is primarily relevant for moderate skin sensitizers (i.e. for products D, E and F where the resulting exposure was slightly below or above the DNEL, see section 5). In the case of moderate sensitizers, assigning an SAF of 1'000 or 2'000 can change the decision whether the DNEL is exceeded or not. However, these considerations have no influence on the QRA performed for strong sensitizers (e.g. products A and B for which the DNEL is exceeded by 3 or 4 orders of magnitude).

A limitation of the proposed approach is that product-specific DNELs can currently only be derived if LLNA data is available, while test data from a guinea pig assay (Maximisation or Buehler) cannot be used to derive DNELs. Since 2013, the data requirements for the

authorization of new PPPs in the EU and Switzerland, regard the LLNA as the method of choice to assess skin sensitization[4] (EC, 2013). New PPP authorizations in Switzerland often contain LLNA data tested on the formulated product (personal communication, Christoph Geiser, Federal Food Safety and Veterinary Office). However, the EU data requirements for PPPs also foresee that where a guinea pig assay is available for the substance to be authorized, further testing with a LLNA shall not be carried out for animal welfare reasons. It may thus be difficult to derive DNELs for many older PPPs that were authorized based on data from a guinea pig assay. It might be possible to derive LLNA potency based on the read-across from structurally related chemical substances for which experimental data are available or from QSAR for highly reactive electrophilic compounds (Roberts et al., 2016; Roberts and Aptula, 2008). The same sensitization assessments factors (SAFs) as when using LLNA data would need to be applied; however, on a case-by-case basis an additional SAF to account for the uncertainty related to the use of the read-across should be considered (ECHA 2012).

The novel non-animal test methods for skin sensitization still have a restricted AOP mechanistic coverage and they can therefore not yet fully substitute the presently used animal tests (Appendix A3). At present, they primarily support the discrimination between skin sensitizers and non-sensitizers for the purpose of hazard classification and labelling. They currently do not predict potency as the LLNA would allow, nor can a precise EC3 value be determined, which is needed to derive a specific DNEL for skin sensitizers. It is nevertheless possible to determine potency classes or a rough EC3 value that could be used by adding an extra safety factor (Jaworska et al., 2015; Natsch et al., 2015). Since considerable research effort is currently ongoing to further develop the integrated testing strategy for skin sensitizers (Hoffmann et al., 2018; Kleinstreuer et al., 2018), we are confident that the novel non-animal test methods could

[4] The ECHA announced in June 2016 that the REACH requirements for skin sensitization were changing, making non-animal testing the default requirement for the registration of chemicals (http://echa.europa.eu/view-article/-/journal_content/title/registrants-to-use-alternative-test-methods-for-skin-sensitisation). It might be possible that these requirements might soon also apply to PPPs.

soon be integrated into the approach of linking DNELs with agricultural exposure models proposed herein.

6.3 Exposure: approach to address the single exposure scenario issue

Another aim of this publication was to suggest a QRA approach that would better take into account actual exposure to skin sensitizers when applying PPPs. Linking DNELs with agricultural exposure models may be an elegant approach to properly address varying exposure patterns to skin sensitizers since application frequency can be taken into account by including SAFs for different exposure conditions. The interviews with the greenhouse managers showed that operators in horticultural industry are significantly exposed to PPPs (Appendix A4). A single worker performs on average 1-2 PPP applications per week for about 1-4 h per day. The same person may thus perform more than 60 applications of various PPPs over a whole growing season from March to October. The interviews also showed that between 30-40% of the PPPs used are classified as skin sensitizers. Consequently, assuming that approximately every third application (= 33%) is performed with a skin sensitizing PPP, a single worker in horticultural production may be exposed up to 20 times to a skin sensitizer over a period of eight months. Exposure patterns similar to the horticultural production are likely to occur, especially in areas such as viticulture or arboriculture in which spraying is performed every 1 to 2 weeks over a growing season. The SAFs needed to derive the DNELs could be adapted to various agricultural production areas by defining production area-specific SAFs based on PPP application frequencies.

6.4 Risk characterization combining toxicology and exposure

From an occupational health and safety perspective, it is important to consider that the concentration limits stipulated in the EU CLP Regulation are classification criteria. As such they are used to decide whether a mixture is to be classified as being a skin sensitizer if substance A is mixed with substance B. However, classification criteria are of limited value for an occupational risk assessment as they are much too generic to reflect a specific work place setting. Occupational exposure limits (OELs) define a maximum concentration of a substance A

in an occupational setting. Use is considered safe as long as the concentration of substance A in the air or deposition of airborne substance onto the body remains below the OEL. Where a generic concentration limit may indicate a safe use of substance A, a better assessment of the occupational setting may reveal that the OEL is exceeded. This may be because substance A is applied with a specific method that cannot be covered by a generic concentration limit. The DNELs can be considered as specific OELs that assess the risk in a specific occupational setting as, for example, the spraying of PPPs. The proposed QRA approach thus addresses both the toxicological and the exposure-related limitation of the current risk assessment for skin sensitizers. Since DNELs are derived from substance-specific EC3 values, they account for the complete potency range of skin sensitizers given that the EC3 value is a continuous variable whereas the potency range of the EU CLP Regulation is an ordinal variable. Furthermore, the DNELs consider actual frequency of PPPs use as repeated exposure can be included as an additional assessment factor. The DNEL – in combination with the existing agricultural exposure models – can be used to develop a risk assessment that accounts for both the toxicology and the exposure during use of the product. This can be used to determine PPEs needed to protect exposed body parts where exposure is likely to exceed the DNEL and where a risk for the induction of an allergic skin reaction exists.

The underlying paradigm of the here presented approach is that there is no induction as long as there is no contact between a skin sensitizer and bare skin. Thus, risk only exists if operators do not or insufficiently protect themselves during spraying. The interviews performed showed that the horticultural companies questioned were aware of the need for PPEs during spraying to protect operators from PPPs' acute and chronic risks. The horticultural industry may however be an exception when compared to other areas with a high use of PPPs, such as agricultural

production[5]. Most horticultural companies are Swiss GAP certified[6], where adherence to occupational safety measures is part of the certification scheme. Since the Swiss GAP certification is almost a prerequisite for market access, approximately 80% of the companies working in flower production (but only 6% working in nursery garden and shrub production) are certified (personal communication, Jardin Suisse). The positive outcome obtained in the interviews with regard to PPE use may partly be due to that 8 out of 9 companies within the interviewed sample were Swiss GAP certified. Furthermore, most people working in horticultural industry are employees and the employer has a legal obligation to take all necessary measures to ensure that the health of its employees is protected. In contrast, in agricultural production, approximately 81% of the people (= 128'000) working on farms are family members, while only about 19% (= 31'000) are employees (FSO, 2013; FSO, 2014). The legal obligations to ensure occupational health and safety of family members is less controlled by the Swiss cantonal authorities than for employees. Since protective measures are primarily relying on the individual responsibility of the farmers, some farmers might adhere less to PPEs than people working in horticultural industry. As there is currently no public national health register recording chronic effects from PPP use in Switzerland, it is difficult to conclude whether allergic contact dermatitis is frequent among Swiss PPP users. In the absence of conclusive data showing that allergic contact dermatitis is not frequently occurring among operators using PPPs, it is reasonable to adhere to a precautionary approach and to call for the necessity for adequate protection among PPP users.

7 Conclusions and recommendations

This study showed that the proposed QRA for skin sensitizing PPPs is a clear improvement over the current risk assessment approach based on the EU CLP Regulation to ensure the safe

[5] However, small horticultural companies (≤ 5 FTE) were underrepresented among the interviewed companies. These might invest less in occupational safety measures than larger companies do.

[6] Swiss GAP (Good Agricultural Practice) is a certification program ensuring a common standard for agricultural management practice bringing conformity to different retailers' supplier standards.

use of skin sensitizing chemicals in an occupational context. The QRA considers both the broad potency range of skin sensitizing chemicals and the frequency of exposure to PPPs. Both are crucial determinants for the development of an allergic reaction. From a practical point of view, the new QRA could be immediately implemented. However, to be accepted as a new risk assessment methodology for skin sensitizing chemicals, it is necessary to formally suggest this new approach to the respective regulatory channels such as the ECHA and EFSA. Nevertheless, a few points might need to be elucidated in the new QRA to ensure the safe use of skin sensitizing chemicals.

The LLNA is an endpoint to avoid induction in non-sensitized persons. It does not allow the determination of a threshold of elicitation. DNELs will not protect already sensitized individuals. Methods and approaches to derive specific elicitation thresholds should ideally be developed, for example, by complementing the LLNA with a test with lower doses on already sensitized mice. The trend for determining the chemical classification is towards promoting non-animal test methods. Since the novel non-animal test methods for skin sensitization cannot currently be used to assign specific and precise potency to chemicals, there is a need to further develop the potency perspective in the non-animal test methods. Similarly, the novel non-animal test methods should also be expanded to derive elicitation thresholds. Considering the significant efforts that are being made by the cosmetics and fragrance industry to improve non-animal test methods, there is hope that such approaches will be available in the near future. In fact, multiple *in vitro*, *in chemico*, and *in silico* skin sensitization assays evaluated by the Cosmetics Europe Skin Tolerance Task Force (STTF) performed as well as or better than the LLNA in predicting human skin sensitization endpoints for both hazard and potency (Hoffmann et al., 2018; Kleinstreuer et al., 2018).

Improving the risk assessment methodology for chemicals is an important step in assuring the health protection of people regularly using sensitizing products in an occupational setting. However, the proposed QRA approach only helps to improve operators' health if people can be motivated to adhere to the necessary protective measures. Apart from performing a sound risk

assessment, there will always be the need for educating and training PPP users on how to adequately protect themselves.

Conflict of interest statement

The authors declare that there are no conflicts of interests.

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Appendix A

A1 Scientific background of skin sensitization

Epidemiology of allergic contact dermatitis

Chemical skin sensitizers are known to produce allergic contact dermatitis, and can be regarded as the most frequent manifestation of immunotoxicity in humans (Kaplan et al., 2012; Kimber et al., 2002). More than 4'000 chemicals that have been tested with patch testing showed having the potential to cause allergic contact dermatitis (de Groot, 2008). Allergic contact dermatitis is one of the most frequent diseases associated with chemical exposures both in the general population as well as in occupational settings. About 20% of the general population in Europe suffers from contact allergy to at least one contact allergen (Peiser et al., 2012; Thyssen et al., 2007). Most common are allergies to nickel, fragrances and preservatives. Contact dermatitis ranks first among all occupational diseases in many countries (Diepgen and Coenraads, 1999; McDonald et al., 2006). The prognosis of occupational contact dermatitis is generally poor. A review of studies on the prognosis of contact dermatitis showed that less than half of the patients had healed after several years of follow-up (Diepgen, 2003).

Immunological mechanism of skin sensitization

The clinical picture of allergic contact dermatitis is a polymorphic pattern of skin inflammation characterized by a wide range of clinical features such as itching, redness, erythema, and clustered papulovesicles. The allergic reaction is typically elicited by much lower concentrations than those needed for inducing the immune response (Ezendam et al., 2012; Friedmann, 2007). Chemical skin sensitizers are haptens (i.e. low molecular weight molecules) that as such are unable themselves to directly stimulate an adaptive immune response. In order to elicit an immune response, they need to be attached to a larger carrier such as a protein (Kimber et al., 2002). Consequently, immunogenicity must be acquired by stable association with protein and the formation of hapten-protein conjugates. A chemical must be inherently protein-reactive or must be metabolized to protein-reactive species for sensitization to proceed. A cascade of sequential and parallel steps has to occur in order for an allergic reaction to develop (Kaplan et

al., 2012; Kimber et al., 2002). The OECD has developed an adverse outcome pathway (AOP) for skin sensitization by chemical agents (OECD, 2012). The OECD AOP summarizes skin sensitization elicited by covalent binding of substances to proteins as eleven steps, which include four key events (KE):

Key Event 1: The chemical sensitizer is converted to a reactive metabolite that covalently binds to nucleophilic sites in skin proteins (e.g. cysteine and lysine residues) forming hapten-protein complexes by covalent binding of the hapten to cell surface proteins.

Key Event 2: Hapten binding to cell-surface proteins activates mitogen-activated protein kinase (MAPK) signaling pathways. This leads to the release of inflammatory cytokines and to the induction of cyto-protective pathways in keratinocytes.

Key Event 3: Recognition and internalization of haptens lead to activation and maturation of skin-resident dendritic cells (DCs) (so-called Langerhans cells).

Key Event 4: Dendritic cells migrate to lymph nodes to present major histocompatibility complex (MHC) molecules to naïve T-lymphocytes (T-cells), T-cell differentiation and proliferation as allergen-specific memory T-cells.

A2 Classification criteria for skin sensitizers according to EU CLP Regulation

Classification of substances

The EU CLP Regulation defines classification criteria for various hazards to human health and the environment (EC, 2008). Within the EU CLP Regulation, skin sensitization is one of the health hazards to be assessed when placing a substance on the European market. If the test results of the OECD test methods for skin sensitization show that the test substance exceeds the thresholds for classification as skin sensitizer (Table 1), the substance is classified as being a skin sensitizer and labelled with the hazard statement “H317: May cause an allergic skin reaction”. In addition to the yes/no classification, the EU CLP Regulation also allows for a

potency assessment, with allocation of skin sensitizers into sub-category 1A (strong sensitizers), or sub-category 1B (weak to moderate skin sensitizers).

OECD test guidelines	Threshold for classification of a substance as skin sensitizer
Mouse local lymph node assay (LLNA)	Stimulation Index ≥ 3
Guinea pig maximization test (GPMT)	Response in ≥ 30% of the test animals
Buehler occluded patch test	Response in ≥ 15% of the test animals

Table 1: Thresholds for classification of a substance as a skin sensitizer according to the EU CLP Regulation (EC, 2008).

The GPMT and the Buehler test allow to categorize skin sensitizers into weak, moderate and strong sensitizers, where potency is assigned based on test substance concentration and animal incidence of sensitization. The LLNA gives a more accurate picture of potency since potency is measured as a function of derived EC3-values. An inverse relationship exists between EC3-value and potency meaning that extremely potent sensitizers have extremely low EC3-values. Skin sensitizers vary by up to four or five orders of magnitude with respect to the minimum concentration required inducing skin sensitization. Potency is graded based on these minimum concentrations, each grade reflecting a concentration range of approximately one order of magnitude. The potency profile to skin sensitizers is broadly equivalent in mice and human. LLNA data obtained in mice can thus be used in risk assessment as No Observed Adverse Effect Level (NOAEL) for humans (Basketter et al., 2005).

Classification of mixtures

Many chemicals are supplied on the market as preparations of mixtures and often no data is available on the mixture as such but only on its individual components or substances. PPPs are usually tested for skin sensitization as a whole; however, they are frequently diluted in water before being sprayed on agricultural crops or horticultural plants. When assessing the risks of skin sensitizing PPPs, it is therefore important to know whether the diluted spray solution is still

classified as being sensitizing. According to the European Chemicals Agency (ECHA) Guidance on the application of the EU CLP criteria (ECHA, 2015), the spray solution can be regarded as a mixture of two components: the PPP is a skin sensitizing component A, which is diluted with a second component B (i.e. water). The spraying solution is often classified based on generic concentration limits for classifying a mixture as skin sensitizing. The mixture can be classified as a skin sensitizer when at least one of the skin sensitizing ingredients (e.g. the concentrated PPP) is present in the spray solution at or above the appropriate generic concentration limit for sub-category 1A or 1B, respectively (Table 2) (EC, 2008). In addition, specific concentration limits (below 0.1%) are defined for a limited number (ca. 90 substances) of very potent skin sensitizing chemicals (cf. Annex VI of the EU CLP Regulation).

Component classified as	**Generic concentration limits for classification as skin sensitizer**	**Concentration limits for elicitation of an allergic reaction**	**Potency**
Skin sensitizer category 1	≥ 1,0%	≥ 0,1%	no data available
Skin sensitizer sub-category 1A	≥ 0,1%	≥ 0,01%	EC3 ≤ 2%
Skin sensitizer sub-category 1B	≥ 1,0%	≥ 0,1%	EC3 > 2%
	=> classification H317	**=> hazard information EUH208**	

Table 2: Generic concentration limits of components of a mixture for the classification as skin sensitizers according to tables 3.4.5 and 3.4.6 of the EU CLP Regulation. Concentration limits for elicitation are used to protect already sensitized individuals (EC, 2008).

A3 OECD test methods for identifying skin sensitizers

Current test methods for skin sensitization

Three OECD test methods can be used in identifying potential skin sensitizers according to the data requirements for placing of PPPs on the EU market (EC, 2013). The method of choice in the EU is currently the mouse local lymph node assay (LLNA) (OECD, 2010). In case the LLNA cannot be conducted, a guinea pig assay (Guinea Pig Maximisation Test (GPMT) or Buehler

occluded patch test) (OECD, 1992) can be performed instead. The LLNA is used both to determine skin sensitizing potential (hazard identification) and relative skin sensitization potency (hazard characterization). In both instances, the metric is cellular proliferation induced in draining lymph nodes following topical exposure to various dilutions of a chemical. The test is based on the fact that lymph node cell proliferation is causally and quantitatively correlated with the acquisition of skin sensitization (Basketter et al., 2002; Kimber et al., 2012). Three concentrations of the active substance are selected from a concentration series of 100%, 50%, 25%, 10%, 5%, 2.5%, 1%, 0.5%, etc. These dilutions of the test substance are applied daily for three consecutive days at a volume of 25 µL to the upper surface of the mouse ear. Three days after the last exposure, the number of proliferating cells in the draining auricular lymph nodes is quantified using *in vivo* radioactive thymidine labeling as a measure of lymphocyte proliferation (i.e. memory T-cell clonal expansion). The test concentration causing a threefold increase of lymph node cell proliferation compared to the vehicle control is called the EC3 value (i.e. the effect concentration at which the stimulation index SI ≥ 3). Relative potency in the LLNA is based on the EC3 value, which is expressed as % concentration, molar value or dose per unit area (Kimber et al., 2003). The threshold for classification of a substance as skin sensitizer is a SI ≥ 3. The GPMT and the Buehler test both measure whether dermal application of a substance elicits skin reactions after previous induction by intradermal injection of the substance with adjuvant (GPMT) or by topical application of the substance without adjuvant (Buehler). These tests use a single test substance at a single concentration for induction, assumed to cause mild-to-moderate skin irritation. The severity of elicited skin responses compared to controls is recorded, yet classification as skin sensitizer is only based on the number of guinea pigs with positive skin test reactions (i.e. ≥ 30% positive animals in the GPMT and ≥ 15% in the Buehler test). Both the GPMT and the Buehler assay use only single test concentrations, so dose-effect relationships cannot be derived, whereas the mouse LLNA allows a dose-response assessment.

Novel non-animal test methods for skin sensitization

Substantial effort has in recent years focused on reducing and ultimately replacing current animal test methods for skin sensitization. This is due to the widespread agreement that alternative test methods must be developed to replace, reduce and refine (3R strategy) the number of animals used for toxicology testing. The 3R strategy aims at encouraging alternatives to animal testing, but also to improve animal welfare and scientific quality where the use of animals cannot be avoided. Moreover, the EU banned animal testing of cosmetic ingredients in 2013 which includes test for skin sensitization. The cosmetic industry is thus a major driver in developing non-animal test methods and has an urgent need for predictive and robust *in vitro* tests (Mehling et al., 2012). Within this remit, novel *in silico*, *in chemico* and *in vitro* test methods for skin sensitization have been developed both by industry consortia as well as by universities. Three alternative methods have recently been adopted as official OECD test methods, that is, the Direct Peptide Reactivity Assay (DPRA) (OECD., 2015a), the ARE-Nrf2 Luciferase Test Method (OECD., 2015b), and the Human Cell Line Activation Test (h-CLAT) (OECD, 2017).

Direct Peptide Reactivity Assay (DPRA) provides an *in chemico* procedure to support the discrimination between skin sensitizers and non-sensitizers. The DPRA addresses the molecular initiating event leading to skin sensitization (i.e. protein reactivity) by quantifying the reactivity of test chemicals towards model synthetic peptides containing either lysine or cysteine. Cysteine and lysine percent peptide depletion values are calculated and used in a prediction model to categorize a substance in one of four classes of reactivity for supporting the discrimination between skin sensitizers and non-sensitizers.

ARE-Nrf2 Luciferase Test Method provides an *in vitro* procedure to support the discrimination between skin sensitizers and non-sensitizers. It addresses the second KE on the AOP leading to skin sensitization in the keratinocytes. It includes inflammatory responses as well as gene expression associated with specific cell signaling pathways such as the antioxidant/electrophile response element (ARE)-dependent pathways. The cell line contains the luciferase gene under the transcriptional control of a constitutive promoter fused with an ARE element from a gene that is known to be up-regulated by contact sensitizers. The luciferase signal reflects the

activation by sensitizers of endogenous Nrf2 dependent genes. This allows quantitative measurement (by luminescence detection) of luciferase gene induction, using light producing luciferase substrates, as an indicator of the activity of the Nrf2 transcription factor in cells following exposure to electrophilic test substances. Currently, the only *in vitro* ARE-Nrf2 luciferase test method covered is the KeratinoSensTM test method.

Human Cell Line Activation Test (h-CLAT) provides an *in vitro* procedure used for supporting the discrimination between skin sensitizers and non-sensitizers. The h-CLAT method is proposed to address the third key event of the skin sensitization AOP by quantifying changes in the expression of cell surface markers associated with the process of activation of monocytes and DCs (i.e. CD86 and CD54), in the human monocytic leukaemia cell line THP-1, following exposure to sensitizing test chemicals. These surface molecules are typical markers of monocytic THP-1 activation and may mimic DC activation, which plays a critical role in T-cell priming. The changes of surface marker expression are measured by flow cytometry following cell staining with fluorochrome-tagged antibodies. The relative fluorescence intensity of surface markers compared to solvent/vehicle control are calculated and used in the prediction model, to support the discrimination between sensitizers and non-sensitizers.

A4 Interviews with greenhouse managers to identify actual plant protection products use

Face-to-face interviews of about an hour were performed with greenhouse managers working in horticultural companies in January and February 2016 to understand the frequency of exposure to skin sensitizing PPPs. The greenhouse managers were selected based on a list provided by the Swiss horticultural association. This list contained 12 horticultural companies in the German speaking part of Switzerland and one company in the French speaking part. These companies were considered by the Swiss horticultural association as likely willing to participate in such an interview. Nine companies (69%) accepted to participate in the interviews after having been contacted by mail and by phone. The interviews aimed at obtaining a better picture of actual PPP use and estimating the type and amount of PPP used. Horticultural production was chosen as a case study as a multitude of different PPPs are used. Since most horticultural plants

usually have a short growth period of a few weeks, the PPPs are applied on a regular basis resulting in a considerable cumulative PPP exposure for the operators over the year. An occupational hygiene survey questionnaire was developed by the authors and used in the interviews which were tape-recorded for later proofreading. Prior to the first interview, the questionnaire was tested with a Swiss horticultural association representative to check the questions for suitability, comprehension, and ambiguity. Questions included company characteristics and PPP use patterns such as type of products used, the dosage applied, the frequency and duration of use, and the method of application. Workers use of personal protection such as chemical protective suits, chemical resistant gloves, respirators, and technical equipment during application of the PPPs were also included.

All participating companies stated that they generally treated all plant types with PPPs. The frequency of treatments depended primarily on the plant type and on the pest or disease damage. Among the different types of plants produced, the frequencies of treatment varied; flowers were treated twice per week to once per month; and nursery gardens and shrubs once per week to three times per year. Most companies emphasized that - especially in flower production - customers expect to buy “flawless” products and that treatments against pests and diseases attacking either leaves or blossoms were crucial to be able to sell the products. All companies used a wide spectrum of products or active substances. A considerable number of the products were classified as skin sensitizers: 25 fungicides (40% skin sensitizers), 20 insecticides (40% skin sensitizers), 9 herbicides (33% skin sensitizers), and 4 growth regulators (no skin sensitizers). While fungicides and insecticides were both used in the greenhouse and open-air on a variety of plants, herbicides were primarily used outdoor in nursery gardens and shrubs to clean the surroundings of the cultivated plants from weeds. Growth regulator products were only used in the greenhouse, mostly on seasonal and balcony plants. Pests and diseases most frequently treated were fungal diseases (e.g. Mildews, Botrytis, Rusts) producing damages to leaves as well as insect pests such as spider mites, aphids, white flies, caterpillars and thrips producing damages to leaves and blossoms.

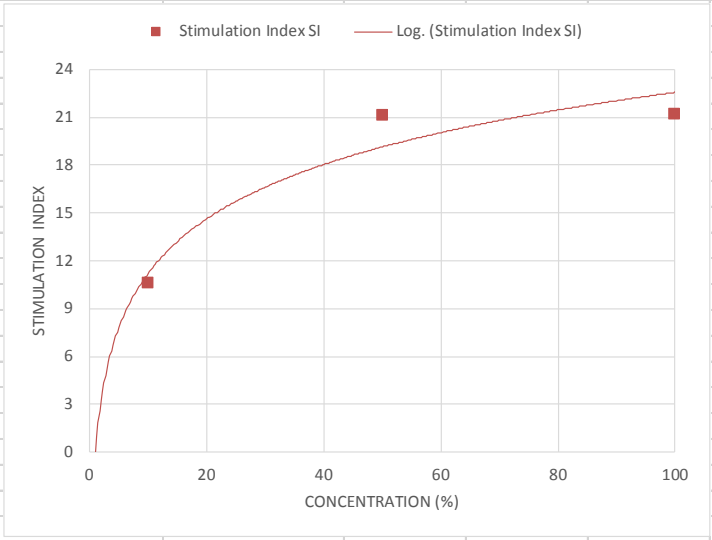
To spray small amounts of products on small areas, the machinery used were either hand sprayers or motorized knapsack sprayer. Mobile motorized sprayers were used for different types of spray operations in the greenhouse and open-air. All companies determined the need for spraying based on optical checks of plant damage or on damage threshold. All companies confirmed that two or more products for the same application were mixed. This was primarily done to reduce work load by avoiding additional spraying or to enlarge the spectrum of efficacy. Product types that were most often used in a mixture were fungicides and insecticides, and less often in combinations with the same type (e.g. a mixture of two herbicides). The frequency of spray operations performed by a single operator within the same week or month (i.e. the cumulative exposure) varied among the nine companies, mainly due to different types of plants cultivated and different necessities to treat. Most of the companies estimated that a single operator would perform 1-2 treatments per week, while two companies indicated 3-5 treatments per week. One of the companies producing nursery gardens and shrubs estimated that a single operator would apply PPPs only 1 – 2 times per month. The duration of treatments was estimated to range from 1 – 2 hours per day to 3 – 4 hours per day, depending on the area to be treated. Only exceptionally did the treatments last up to 5 – 8 hours per day. In conclusion, the same person may perform up to 60 applications of various PPPs in horticulture over a whole growing season from March to October.

Supplemental Material

Screenshots of the Excel files used for the comparison of estimated exposures of hands, body and head resulting from the Agricultural Operator Exposure model (AOEM) with Derived No-Effect Levels (DNELs) for products A - F in Table 4. The precise methodology is described in section 4 Materials and Methods.

Product A – Fungicide

Concentration (%)	Stimulation Index SI								
10	10.6		a	50	Dose				
50	21.1		b	21.1	SI				
100	21.2		c	10	Dose				
			d	10.6	SI				
$EC3\ extrapolated\ [\%] = 10^{[\log c + \frac{3-d}{b-d} * (\log a - \log c)]}$			EC3ex	3.11945593					
Using the two SI values greater than 3 with the lowest of the SI values having the lowest % concentration.									



LOAEL (ug/cm2)	779.863982		DNEL	0.10398186	(AF=7500)			
External dermal exposure mixing&loading (ML) (ug/person)			External dermal exposure application (ug/person)					
with PPE			no PPE					
hands	29		hands	5752				
body	41		body	70708				
head	8		head	203				
Sum external dermal exposure ML with PPE+application no PPE (ug/person)								
		body surface	ug/cm2	ug/cm2	% of LOAEL	% of DNEL		
hands	5781	820	7.05	7.05	0.9040038	6780		
body	70749	13310	5.31547708	5.31547708	0.68159028	5112		
head	211	1110	0.19009009	0.19009009	0.02437477	183		
External dermal exposure mixing&loading (ug/person)			External dermal exposure application (ug/person)					
with PPE			with PPE					
hands	29		Gloves	hands	59			
body	41		Coverall	body	1033			
head	8		Visor	head	10.15			
Sum external dermal exposure ML with PPE+application with PPE (ug/person)								
			body surface	ug/cm2	ug/cm2	% of LOAEL	% of DNEL	
Gloves	hands	88	820	0.10731707	0.10731707	0.013761	103	
Coverall	body	1074	13310	0.08069121	0.08069121	0.01034683	78	
Visor	head	18.15	1110	0.01635135	0.01635135	0.00209669	16	

Product B – Herbicide

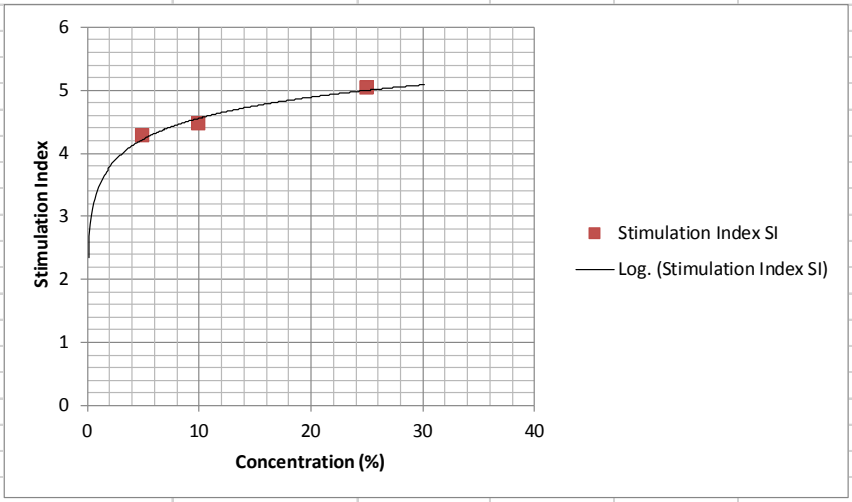
Concentration (%)	Stimulation Index SI
5	4.28
10	4.46
25	5.04

a	10	Dose
b	4.46	SI
c	5	Dose
d	4.28	SI

$$EC3\ extrapolated\ [\%] = 10^{[\log c + \frac{3-d}{b-d} * (\log a - \log c)]}$$

EC3ex	0.03616698

Using the two SI values greater than 3 with the lowest of the SI values having the lowest % concentration.



LOAEL (ug/cm2)	9.041745237	DNEL	0.001205566	(AF=7500)

External dermal exposure mixing&loading (ML) (ug/person)			External dermal exposure application (ug/person)	
with PPE			no PPE	
hands	18		hands	2139
body	25		body	58756
head	5		head	139

Sum external dermal exposure ML with PPE+application no PPE (ug/person)

			body surface	ug/cm2	ug/cm2	% of LOAEL	% of DNEL
hands	2157		820	2.6304878	2.630487805	29.0926999	218195
body	58781		13310	4.41630353	4.416303531	48.8434856	366326
head	144		1110	0.12972973	0.12972973	1.43478639	10761

External dermal exposure mixing&loading (ug/person)			External dermal exposure application (ug/person)		
with PPE			with PPE		
hands	18		Gloves	hands	18
body	25		Coverall	body	1033
head	5		Visor	head	6.95

Sum external dermal exposure ML with PPE+application with PPE (ug/person)

				body surface	ug/cm2	ug/cm2	% of LOAEL	% of DNEL
Gloves	hands	36		820	0.043902439	0.04390244	0.48555271	3642
Coverall	body	1058		13310	0.079489106	0.07948911	0.87913455	6594
Visor	head	11.95		1110	0.010765766	0.01076577	0.11906734	893

Product C – Fungicide

Concentration (%)	Stimulation Index SI		a	25	Dose
25	7.9		b	7.9	SI
50	7.2		c	50	Dose
100	12.3		d	7.2	SI

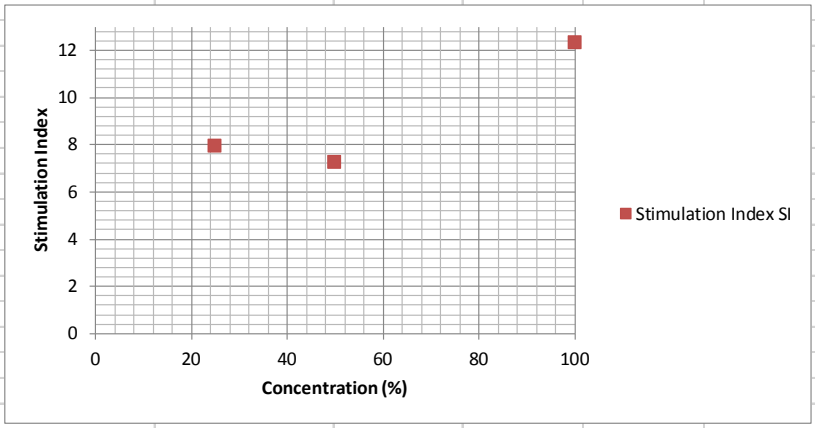
$$logEC3 = \frac{\left(Probit_{78.5}\,(2) - Probit_{78.5}\,(SI-1)\right) + 0.87 * \log Dose}{0.87}$$

logEC3 0.71053485

EC3 5.13493383

according to Roberts 2015

SI max	SI	SI-1	(SI-1)/SI max	Prob78.5(SI-1)
78.5	3	2	0.02547771	-1.95185507
	7.9	6.9	0.08789809	-1.35381258



LOAEL (ug/cm2)	1283.733458	DNEL	0.17116446	(AF=7500)

External dermal exposure mixing&loading (ML)(ug/person)		External dermal exposure application (ug/person)	
with PPE		no PPE	
hands	18	hands	851
body	25	body	49443
head	5	head	97

Sum external dermal exposure ML with PPE+application no PPE (ug/person)

		body surface	ug/cm2	ug/cm2	% of LOAEL	% of DNEL
hands	869	820	1.0597561	1.0597561	0.08255266	619.1
body	49468	13310	3.71660406	3.71660406	0.28951524	2171.4
head	102	1110	0.09189189	0.09189189	0.00715818	53.7

External dermal exposure mixing&loading (ug/person)		External dermal exposure application (ug/person)		
with PPE		with PPE		
hands	18	Gloves	hands	6
body	25	Coverall	body	1033
head	5	Visor	head	4.85

Sum external dermal exposure ML with PPE+application with PPE (ug/person)

			body surface	ug/cm2	ug/cm2	% of LOAEL	% of DNEL
Gloves	hands	24	820	0.02926829	0.02926829	0.002279935	17.099515
Coverall	body	1058	13310	0.07948911	0.07948911	0.006192026	46.4401929
Visor	head	9.85	1110	0.00887387	0.00887387	0.000691255	5.18441376

Product D – Herbicide

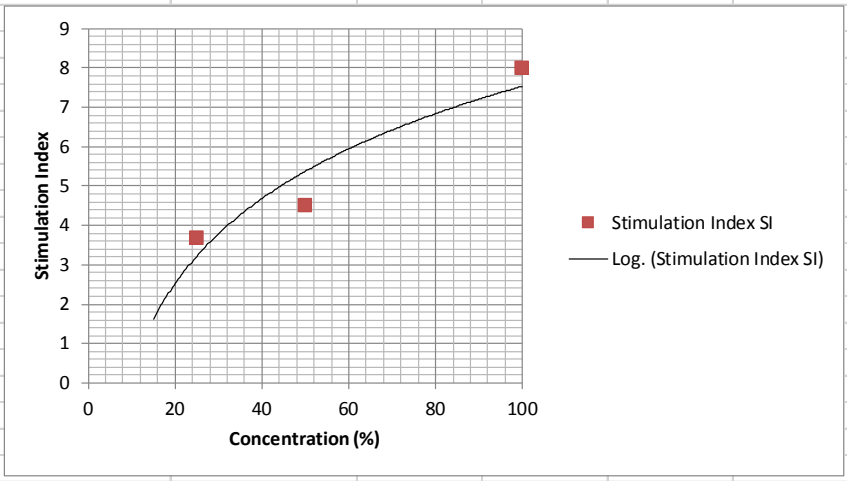
Concentration (%)	Stimulation Index SI
25	3.66
50	4.49
100	7.98

a	50	Dose	
b	4.49	SI	
c	25	Dose	
d	3.66	SI	

$$\text{EC3 extrapolated } [\%] = 10^{[\log c + \frac{3-d}{b-d} * (\log a - \log c)]}$$

EC3ex 14.4067745

Using the two SI values greater than 3 with the lowest of the SI values having the lowest % concentration.



LOAEL (ug/cm2) 3601.69363

DNEL 0.48022582 (AF=7500)

External dermal exposure mixing&loading (ug/person)	
with PPE	
hands	18
body	25
head	5

External dermal exposure application (ug/person)	
no PPE	
hands	259
body	39570
head	62

Sum external dermal exposure ML with PPE+application no PPE (ug/person)

		body surface	ug/cm2	ug/cm2	% of LOAEL	% of DNEL
hands	277	820	0.33780488	0.33780488	0.00937906	70
body	39595	13310	2.97483095	2.97483095	0.08259534	619
head	67	1110	0.06036036	0.06036036	0.00167589	13

External dermal exposure mixing&loading (ug/person)	
with PPE	
hands	18
body	25
head	5

External dermal exposure application (ug/person)		
with PPE		
Gloves	hands	1
Coverall	body	1033
Visor	head	3.1

Sum external dermal exposure ML with PPE+application with PPE (ug/person)

			body surface	ug/cm2	ug/cm2	% of LOAEL	% of DNEL
Gloves	hands	19	820	0.02317073	0.02317073	0.00064333	4.82496585
Coverall	body	1058	13310	0.07948911	0.07948911	0.00220699	16.5524433
Visor	head	8.1	1110	0.0072973	0.0072973	0.00020261	1.51955539

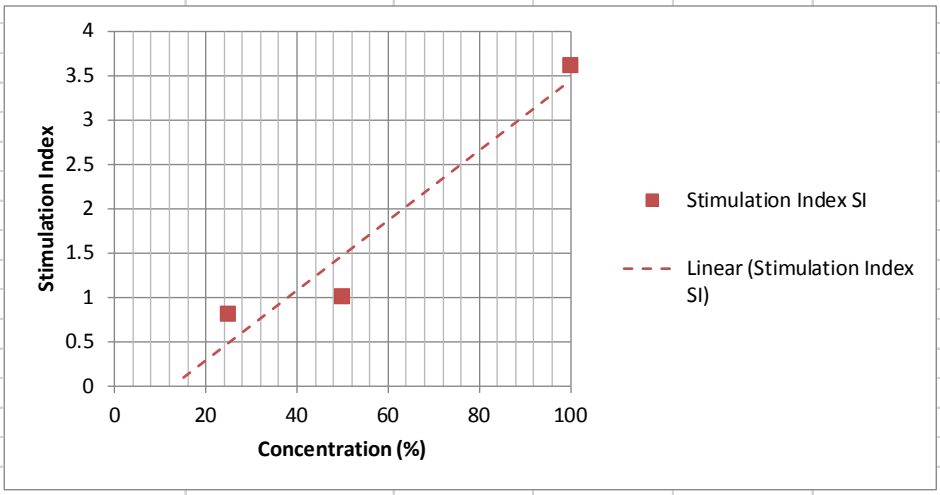
Product E – Fungicide

Concentration (%)	Stimulation Index SI
25	0.8
50	1
100	3.6

a	100	Dose (above SI=3)
b	3.6	SI
c	50	Dose (below SI =3)
d	1	SI

$$EC3\ [\%] = c + \left[\frac{3-d}{b-d} * (a-c)\right]$$

EC3 [%] 88.4615385



LOAEL (ug/cm2) 22115.3846

DNEL 2.94871795 (AF=7500)

External dermal exposure mixing&loading (ML) (ug/person)	
with PPE	
hands	18
body	25
head	5

External dermal exposure application (ug/person)	
no PPE	
hands	3443
body	64230
head	167

Sum external dermal exposure ML with PPE+application no PPE (ug/person)

		body surface	ug/cm2	ug/cm2	% of LOAEL	% of DNEL
hands	3461	820	4.22	4.22	0.0191	143
body	64255	13310	4.83	4.83	0.0218	164
head	172	1110	0.15	0.15	0.0007	5

External dermal exposure mixing&loading (ug/person)	
with PPE	
hands	18
body	25
head	5

External dermal exposure application (ug/person)		
with PPE		
Gloves	hands	32
Coverall	body	1033
Visor	head	8.35

Sum external dermal exposure ML with PPE+application with PPE (ug/person)

			body surface	ug/cm2	% of LOAEL	% of DNEL
Gloves	hands	50	820	0.06	0.0003	2.1
Coverall	body	1058	13310	0.08	0.0004	2.7
Face shield	head	13.35	1110	0.01	0.0001	0.4

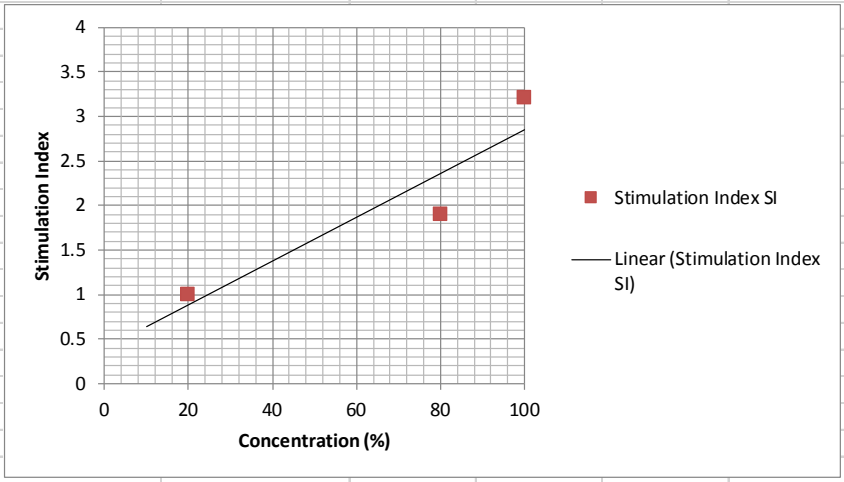
Product F – Fungicide

Concentration (%)	Stimulation Index SI
20	1
80	1.9
100	3.2

a	100	Dose (above SI=3)	
b	3.2	SI	
c	80	Dose (below SI =3)	
d	1.9	SI	

$$EC3\ [\%] = c + \left[\frac{3-d}{b-d} * (a-c)\right]$$

EC3 [%] 96.9230769



LOAEL (ug/cm2) 24230.7692

DNEL 3.23076923 (AF=7500)

External dermal exposure mixing&loading (ML) (ug/person)	
with PPE	
hands	18
body	25
head	5

External dermal exposure application (ug/person)	
no PPE	
hands	301
body	40715
head	65

Sum external dermal exposure ML with PPE+application no PPE (ug/person)

		body surface	ug/cm2	ug/cm2	% of LOAEL	% of DNEL
hands	319	820	0.38902439	0.38902439	0.0016055	12
body	40740	13310	3.0608565	3.0608565	0.01263211	95
head	70	1110	0.06306306	0.06306306	0.00026026	2

External dermal exposure mixing&loading (ug/person)	
with PPE	
hands	18
body	25
head	5

External dermal exposure application (ug/person)		
with PPE		
Gloves	hands	2
Coverall	body	1033
Visor	head	3.25

Sum external dermal exposure ML with PPE+application with PPE (ug/person)

			body surface	ug/cm2	ug/cm2	% of LOAEL	% of DNEL
Gloves	hands	20	820	0.02439024	0.02439024	0.00010066	0.8
Coverall	body	1058	7600	0.13921053	0.13921053	0.00057452	4.3
Visor	head	8.25	1110	0.00743243	0.00743243	3.0674E-05	0.2