



An approach to allergy risk assessments for e-liquid ingredients



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ABSTRACT

Many flavours and fragrances are known allergens. Their selection and inclusion levels in e-liquids must therefore be guided by toxicological principles, taking into account the exposure pattern and inhalation route of exposure. For contact sensitisation, a general, agreed quantitative risk assessment approach to prevent dermal sensitisation exists. Here we propose exposure parameters and safety factors to apply this approach to e-liquid ingredients. Additionally, as a risk management approach for pre-sensitised individuals, we derive a threshold of 0.1% for indicating the presence of a contact sensitizer in e-liquid. Risk assessment for respiratory sensitisation is not well established. Occupational exposure limits that protect against respiratory allergy are generally very low. Cocoa shell extract is used as a case study to discuss the issues. A tolerable exposure level is derived and estimates of consumer exposure are presented, leading to the practical risk management approach of excluding respiratory sensitizers as e-liquid ingredients. Related to this, if natural extracts are used as flavourings in e-liquids, we recommend only protein-free versions are used. Additionally, we recommend the presence of any potential food allergens should be noted on the product information.

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1. Introduction

The use of various flavours in e-cigarettes and e-liquids can lead to concerns over the potential for allergic responses from the use of these vaping products. Different types of allergy can be relevant to vaping exposures. Skin or contact sensitisation, also known as type IV delayed cell-mediated type hypersensitivity, needs to be considered due to the use of flavours or fragrances, and sometimes other compounds, such as preservatives, many of which are known potential contact sensitizers. Dermal quantitative risk assessment for contact sensitisation is well established (Api et al., 2008), and this paper proposes the details for an analogous approach for vaping exposures. Early thinking in this area has been briefly described in a poster presentation elsewhere (Costigan, 2014).

Because the main intended exposure from vaping products is via inhalation, respiratory allergy (also referred to as type I immediate IgE-mediated hypersensitivity) also requires consideration. Although chemical respiratory allergy is much less common than contact sensitisation, the potential adverse effects are much more

severe. We have previously introduced respiratory sensitisation as an exclusion criterion for ingredient selection (Costigan and Meredith, 2015). In this Position Paper, we expand upon that by discussing cocoa shell extract as a case study to illustrate the risk assessment process and related challenges, and include some practical resources for identifying type I allergies. Additionally, we propose to extend this exclusion criterion to risk management of ingredients associated with IgE-mediated food allergies and discuss various food immunological responses.

2. Type IV delayed cell-mediated hypersensitivity

Vaping transforms e-liquid into an aerosol that enters the consumer's mouth and respiratory tract. E-liquid formulations generally contain nicotine, solvents and flavourings, and might contain other ingredients, such as preservatives. Some flavour ingredients, whether natural extracts or synthetic, can induce contact sensitisation. Thus, a hazard identification approach is needed to decide whether an intended ingredient has that potential. And if it does, whether the risk of allergy responses from the expected exposures is acceptably low.

Sensitisation has two basic phases, induction and elicitation of a reaction (OECD, 2012). Induction 'primes' an individual's immune system to a specific substance by inducing specialised

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immunological memory on exposure to an allergen. Induction might take weeks to years of exposure to develop and, after induction, further exposure can elicit the classic inflammatory reaction associated with allergic contact dermatitis. Both induction and elicitation are threshold mechanisms. The threshold for elicitation, however, is typically lower than that for induction. The network of Langerhans cells in oral tissue, means that oral exposure is also relevant. However, contact sensitisation reactions are not known to extend into the respiratory tract.

Typical types of compounds with contact sensitisation potential are flavourings and preservatives. Flavourings often contain natural flavours, which are effectively mixtures of constituents. To assess the risks associated with single ingredients and constituents of naturals, three questions should be addressed:

1. Does the ingredient/constituent have any sensitisation potential?
2. If yes, is the sensitiser present at a sufficiently low level that it is not expected to elicit reactions even in pre-sensitised individuals?
3. If present at higher levels, are the levels expected or not expected to induce sensitisation?

In this article, we derive a risk assessment approach specific to vaping products. Fig. 1 summarises the resultant practical process for assessment of contact sensitisation of e-liquid ingredients.

2.1. Contact sensitisation hazard identification

The main toxicological test for determining sensitisation potential of a substance is the local lymph node assay (OECD, 2010). This test measures the concentration needed to stimulate a three-fold increase in lymph node cell proliferation in mice. Other classic animal methods are the Buehler and guinea pig maximisation tests. The classic clinical study, which shows an absence of sensitisation induction in humans at specific levels, is the human repeat insult patch test (Basketter, 2008).

Good progress has been made with *in silico* and *in vitro* methods for contact sensitisation hazard identification. The OECD has published two new *in vitro* test guidelines (442C and 442D) that address key events in the adverse outcome pathway for contact sensitisation and, therefore, can be part of a weight of evidence approach to hazard assessment. No *in vitro* test is validated to assess the relative potency of sensitisers.

2.2. Quantitative risk assessment (QRA)

The QRA methodology for contact sensitisation is well established and has been adopted by industry (e.g. International Fragrance Association and Research Institute for Fragrance Materials) and regulators (e.g. REACH). The method is based on defining a no expected sensitisation induction level (NESIL) and applying appropriate uncertainty factors to establish a level where the risk is acceptably low—the acceptable exposure level (AEL) (Api et al., 2008). The consumer exposure level (CEL) is then estimated and compared against the AEL. If $AEL/CEL \geq 1$, the proposed use of the compound is deemed supportable. NESILs can be derived from animal and/or human data and are quantitative measures of the potency of the sensitiser. Compilations of appropriate sensitisation data for NESILs derived from flavours and fragrances have been published (Gerberick et al., 2005; Kern et al., 2010).

Although elicitation is also of some interest, the main interest of the QRA is in avoiding sensitising consumers at all. The usual approach to protecting pre-sensitised consumers is to define levels above which consumers should be informed of the presence of

known sensitisers, so they may decide whether to use the product if they know of, or suspect a sensitivity. This approach is analogous to that taken in the EU Cosmetics Directive 76/768/EEC which requires known sensitisers at concentrations greater than 0.001% in leave-on products and 0.01% in rinse-off products to be mentioned on the label (European Commission, 1976). In the REACH, CLP legislation the threshold for mentioning sensitisers is 0.1%.

How to practically risk assess multiple sensitizers with a similar chemistry in one product, that might exhibit some level of cross-reactivity, still requires further debate. Quantitative evidence for levels of cross-reactivity exists for only a limited number of compounds and is not currently available for the bulk of flavour ingredients used in e-liquids. Where such information is available, this should be considered in the risk assessment.

2.2.1. Acceptable exposure level (AEL)

The AEL is the weight of evidence NESIL divided by the product safety assessment factor (SAF). The product SAF was defined by Api et al., (2008) and actually combines three separate SAFs: inter-individual variation, product-specific matrix effects and product-specific use. A further refinement for sensitising fragrance ingredients (Basketter and Safford, 2016) suggested decreasing the product matrix SAF range from 1 to 10 to 0.3–3 and expanding the product use SAF into three different SAFs: frequency or duration of product use, potential presence of occlusion and skin condition/site. However, specifically for the risk assessment of e-liquids, we consider the SAF defined by Api et al. (2008) to be more conservative and applicable. Occlusion, such as from clothing, is not applicable to the oral and respiratory exposure from e-liquids. The proposed skin condition/site factor considers pre-existing inflammation, particularly in areas prone to increased levels of inflammation (e.g. hands, underarms, peri-anal and peri-ocular regions). The mouth, however, can normally sustain considerable abrasion forces from chewing and salivary enzymes and, hence, is not particularly prone to inflammation. The possible accumulation of a product through repeated use, which is part of the product use SAF proposed by Basketter and Safford, is incorporated in the calculation of CELs presented here.

As vapers represent a general, adult population, we recommend a default inter-individual variation SAF of 10. The matrix SAF accounts for the compound having been tested in a different vehicle than the e-liquid aerosol to which the consumer will be exposed. As the presence of irritants or certain solvents in the e-liquid aerosol might increase epithelial penetration, we recommend a matrix SAF of 3. The product-specific use SAF takes in account exposure-related matters such as site of contact, epithelial integrity and duration of exposure. During vaping, a brief peak of oral exposure occurs during the actual puff and holding of the aerosol in the mouth when the formulation is deposited in the oral mucosal cavity. Lips and parts of the mouth are highly vascular, which results in increased absorption of compounds, although rapid dispersion and salivary dilution significantly limit exposure. For such exposure, we recommend a product-specific use SAF of 3 (Api et al., 2008). The overall product SAF, therefore, is $10 \times 3 \times 3 = 90$, meaning the AEL for a compound is the “weight of evidence NESIL”/90.

2.2.2. Consumer exposure level (CEL)

For contact sensitisation, the CEL represents the quantity of compound per exposed surface area (Kimber et al., 2008). A worst case approach is to estimate exposure to an ingredient during the initial mouth-hold phase and assume that all the ingredient in the puff deposits in the mouth. This is extremely conservative as it would mean no product deposition in the lungs whatsoever.

Few reports on adult mouth surface area are available (Collins

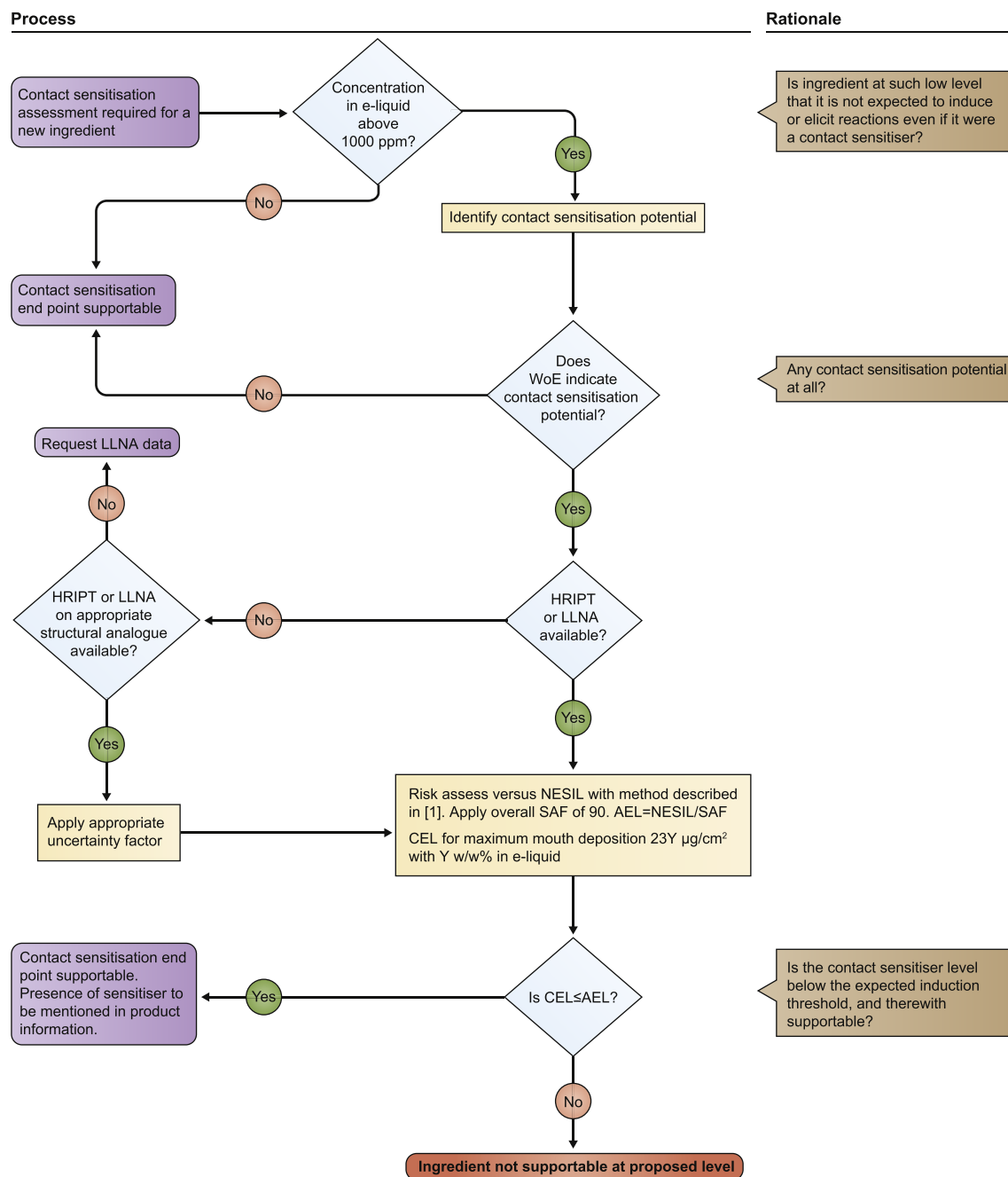


Fig. 1. Contact sensitisation risk assessment process for e-liquid ingredients.

and Dawes, 1987; Kerr et al., 1991). In one report the mean total surface area of the mouth for 20 adults was $214.7 (\pm 12.9) \text{ cm}^2$ (Collins and Dawes, 1987). Aerosol is unlikely to distribute over all surfaces equally, and the tongue and palate surfaces will receive the first exposure. In addition, the buccal areas are highly variable between people and change over time. Thus, we suggest estimating aerosol deposits over the mean area of the palate and dorsum of the tongue (45.8 cm^2 (Collins and Dawes, 1987)).

The amount of e-liquid aerosolised per puff depends on the type of vaping product used and the volume and duration of the puff. The average aerosol weight per 15 puffs, as measured on a vaping product simulator with the power range set to represent products available on the US market and with a 4 s puffing duration (chosen to represent experienced e-cigarette users), was 30–153 mg (2–10

mg/puff). When puffing conditions were set to represent extreme behaviour in experienced vapers, which could apply to an e-liquid sold separately and used in a high-delivery product, the highest value was 333 mg (i.e. 22 mg/puff) (Talih et al., 2015). Although the maximum deliveries of commercial devices may continue to increase, the high deliveries, combined with low pressure drops, lead to direct to lung vaping, i.e. where the mouth hold phase is bypassed. Most mouth deposition in the traditional puff – mouth hold – mouth to lung exposure, takes place during the mouth hold phase. By passing this significantly reduces the mouth deposition opportunity. Maximum mouth deposition was estimated based on anecdotal evidence from product developers, estimation via daily e-liquid consumption with puffs per day and deliveries of devices which are mouth to lung versus those generally used for direct to

lung. On that basis, 35mg/puff is proposed as a practical, conservative limit of maximum mouth delivery. This is above the currently reported maximum delivery value of 22 mg/puff discussed earlier. Thus, 35 mg/puff is used to cover realistic worst case consumer exposures for our generic assessment. Lower values could be used for risk assessments performed for specific products, especially where product-specific limitations allow a lower estimate of the maximum realistic achievable puff delivery.

Despite rapid dispersion and salivary dilution that occurs between puffs, there might be some accumulation of compounds over serial puffs. We have found no literature on cumulative mouth deposition from e-cigarettes, but make this assumption in line with the increase in plasma nicotine reported in experienced e-cigarette users in the course of a 1 h ad lib puffing period (Farsalinos et al., 2014; Vansickel and Eissenberg, 2013). To account for potential dose accumulation with frequent or extended use, when estimating a worst case dose over a puffing session on the basis of single-puff information, we have added a frequency/duration of product use SAF of 3, which is aligned with Basketter and Safford (2016).

Taking all the above into account, if the ingredient were present at Y %w/w, the CEL would be:

$$\text{accumulation SAF} \times \text{level of ingredient in formulation [\%w/w]} \times \text{formulation per puff [mg]} / \text{mean surface area of the palate and dorsum of the tongue [cm}^2\text{]}$$

or

$$3 \times (Y/100) \times 35/45.8 = 0.023Y \text{ mg/cm}^2 \text{ or } 23Y \text{ } \mu\text{g/cm}^2,$$

Thus, for example, if the ingredient were present at 1%, CEL would be 23 $\mu\text{g/cm}^2$.

Using AEL/CEL > 1, it can be calculated whether a specific ingredient level is supportable. However, this may also be used to calculate the maximum supportable level of an ingredient: AEL/23Y > 1, therefore 23Y < AEL and Y < AEL/23.

For example, cinnamaldehyde is a moderate contact sensitizer, for which the weight of evidence NESIL is 590 $\mu\text{g/cm}^2$ (Api et al., 2008). The AEL from vaping is thus 590/90 = 6.55 $\mu\text{g/cm}^2$, and the maximum level supportable in an e-liquid from a contact sensitisation point of view, would be 6.55/23 = 0.28%. By contrast, a weak sensitizer would be supportable at much higher levels. For example eugenol, which has a NESIL of 5900 $\mu\text{g/cm}^2$ (Api et al., 2008), would be supportable up to 5900/(90*23) = 2.85%. For weak sensitizers, therefore, contact sensitisation is unlikely to be the toxic end point that limits their concentration for use in an e-liquid.

2.3. Threshold for indicating presence of a contact sensitizer

As a practical shorthand, the proposed threshold for indicating the presence of a contact sensitizer will be referred to here as the “informing threshold”.

The EU Cosmetics Directive requires consumers are informed of the presence of contact sensitizers, with cosmetic thresholds being 10 ppm for leave-on products and 100 ppm for rinse-off products. Aerosolised e-liquid is rinsed off by salivary dilution and swallowing, and the rinse-off threshold can thus be used as a starting point for deriving an informing threshold for contact sensitizers applicable for e-liquids. Besides the rinsing off by saliva soon after the initial exposure, it should be considered that even the initial exposure is not to the e-liquid, but to a substantially diluted form of the e-liquid in air. E-liquid density varies between 1.0 (glycerol-free) and 1.26 g/ml (100% glycerol). The reported worst case of 22 mg/puff is therefore maximally 22 $\mu\text{l/puff}$, which was measured in a

puff volume of 264 ml (Talih et al., 2015). Thus, this theoretically indicates a dilution of 0.022/264 = 8×10^{-5} (i.e. >10,000 times). Applying a dilution factor of 10 to the cosmetic rinse off threshold is therefore a very conservative, precautionary approach. It results in an “informing threshold” for contact sensitisation for e-liquids of 1000 ppm (0.1 %w/w).

To put this into context, a threshold of 1000 ppm would be in line with the European labelling requirement for contact sensitizers in mixtures, including e-liquids (European Parliament and the Council, 2008a) and would be comparable to derived Dermal Sensitisation Thresholds (DST). A DST of 900 $\mu\text{g/cm}^2$ was derived for chemicals that are not directly reactive to skin proteins (which includes many fragrance contact sensitizers) and 64 $\mu\text{g/cm}^2$ for reactive compounds (Safford et al., 2011; Safford et al., 2015). Fragrance allergens are generally only weak to moderate sensitizers, as is demonstrated by the 55 that are used for benchmarking in the reactive DST derivation, where none falls in the potent category and only one is deemed a strong sensitizer (Safford et al., 2015). The probabilistic threshold represented by a DST for untested fragrances of unidentified reactivity is thus likely to be somewhere between the two DSTs reference values, closer to the one for non-reactive compounds. Using the values presented here, the DST for non-reactive compounds would be 0.7% and for reactive compounds 0.05%.

2.4. Type I immediate IgE-mediated hypersensitivity

IgE-mediated hypersensitivity is involved in respiratory and food allergies. Food allergies are included here because evidence indicates that respiratory exposure can elicit reactions, even if induction has occurred via a different route. For instance, systemic reactions resulting from the odour of cooking fish or shellfish have been reported (Casimir et al., 1997; Pascual et al., 1996). Also, dermal exposure appears to play an augmenting role or even to be the route of induction for some established respiratory allergens (de Vooght et al., 2010; Kimber and Dearman, 2002).

Deriving acceptable levels of allergens in e-liquids for respiratory sensitisation differs from the methods for contact sensitisation. No hazard identification tests and quantitative risk analysis processes have been validated for respiratory sensitisation and, therefore, these assessments rely on a weight of evidence approach (Costigan and Meredith, 2015; ECETOC, 1999; North et al., 2016; Isola et al., 2008; United Nations, 2013). Additionally, the potential severity of symptoms related to respiratory sensitisation far outstrips that of contact sensitisation. Chronic inhalation exposure to respiratory sensitizers could lead to symptoms experienced with hay fever and occupational asthma (e.g. perennial rhinitis, eczema, breathing difficulties and bronchoconstriction). In the very worst cases, although extremely rare, people might experience anaphylactic responses, including death. Not only can the possible effects upon the user include very severe effects, but for most compounds suspected of being respiratory sensitizers, there is no clear quantitative data to derive a tolerable exposure level from. And in the limited number of cases where tolerable inhalation levels have been identified for respiratory sensitizers, they are mostly very low. Occupational exposure guidelines are measured in $\mu\text{g/m}^3$ for anhydrides (WHO, 2009) and 5–60 ng/m^3 for several enzymes (International Association for Soaps, Detergents and Maintenance Products, 2015). Such low tolerable levels do not provide useful levels for use as a flavour in e-liquid, as will be demonstrated in the cocoa shell extract case study below. Overall, it is therefore recommended that known respiratory sensitizers should be avoided completely in e-liquids.

To demonstrate the challenges of respiratory sensitisation hazard assessment and the reality of the tolerable levels being too low

to be of practical use for an e-liquid flavour ingredient, we present a case study of cocoa shell extract.

2.5. Cocoa shell extract case study

2.5.1. Hazard identification for cocoa flavouring

Cocoa extracts are used quite commonly in e-liquids. The cocoa shell extract from *Theobroma cacao* L. is classified as generally recognised as safe in the USA (Food and Drug Administration, Department of Health and Human Services, 2005) and has no harmonised or self-notified health-related classifications under the European Classification Labelling and Packaging regulation (ECHA, 2013). There is a growing consensus that the potential for respiratory sensitisation could be excluded for cocoa or its extracts if a lack of potential for skin sensitisation could be shown in good-quality hazard identification tests (ECHA, 2012; Dearman et al., 2013). However, although no animal data indicate contact sensitisation for cocoa products, in four human sensitisation studies, some individuals have demonstrated a typical sensitisation response to skin challenge (Burks et al., 1998; Kivity et al., 1994; Maslansky and Wein, 1971; Rademaker and Forsyth, 1989).

Two studies of occupational cohorts and one case study from the confectionery industry have yielded data on respiratory sensitisation potential for cocoa shell extract (Perfetti et al., 1997; Zuskin et al., 1994, 1998). The presence of IgE antibodies demonstrated the potential to induce type I allergy in humans. Respiratory symptoms, especially occupational asthma, revealed by broncho-provocation testing, confirmed the potential to elicit symptoms after induction. Lung exposures from vaping might be significantly higher than these occupational exposures. In the study by Zuskin and colleagues (Zuskin et al., 1998) the mean total dust level in the cocoa processing area was 9.1 mg/m³ and the mean respirable fraction was 2.1 mg/m³. Our measurements of cocoa powder particle size and inhalation modelling suggest that cocoa powder entering the human mouth will be intercepted and deposited in the head with no bronchial and pulmonary deposition (unpublished data). By contrast, a significant proportion of e-cigarette aerosol is expected to enter the lungs, which could present a significant risk of respiratory allergy.

Zuskin et al., (1998) also investigated a cohort of 53 flour processing workers in a different part of the same confectionery factory and found that the prevalence for all respiratory symptoms was remarkably similar to that for the workers processing cocoa. Additionally, among the cocoa workers, 73% (29 of 40) had a positive skin prick test to cocoa versus 36% (19/53) of the flour workers to flour. Therefore, the overall allergenicity of cocoa dust seems similar to that of flour, which is a well-recognised occupational respiratory allergen (viz. baker's lung). Flour and other cereals have multiple allergens, and those identified have all been proteinaceous (Dannenbaum and Grieshaber, 2007; Pahr et al., 2012; Palosuo, 2003; Quirce et al., 1992; Reed et al., 1985; Sander et al., 2001; Swanson et al., 1991). Indeed, most recognised high-molecular-weight organic respiratory allergens are proteins from animals, plants and bacteria, and, where further classified, have generally been enzymes (Aberer et al., 2002; Basketter et al., 2010; Baur, 2013; ECHA, 2013). The specific allergenic component(s) of cocoa dust have not been investigated, but it seems likely they are proteinaceous and enzymatic.

The Chemical Safety Report for Cocoa Powder (ADM Cocoa BV et al., 2014), which includes cocoa shell extract in the substance identity, lists the constituents as being cocoa glycerides, cocoa protein, sucrose, starch, cellulose, pectin, theobromine, caffeine, acetic acid, lactic acid, oxalic acid, (+)-epicatechin, tannins (pro-cyanidins) and water. Aregheore (2002) reported 16.0% crude protein in cocoa shell extract and 13.8% in cocoa dust. Thus, the

amount of protein differs between individual cocoa extracts. Testing performed on a cocoa shell extract from a reputable flavour supplier, using a standard methodology measuring N-content by titration and multiplied by 6.25 (LFGB, 2014), indicated an estimated total protein content of 8.3% (unpublished data). The potential of cocoa extracts for respiratory sensitisation must, therefore, be taken into account, and the levels for safe use derived.

2.5.2. Deriving a tolerable cocoa exposure level for e-cigarette flavouring

The data on levels of factory cocoa dust and the level of cocoa shell extract used in the broncho-provocation tests are too limited to establish cocoa-specific no-effect or minimum-effect levels. Therefore, it is appropriate to apply a toxicological threshold of concern approach. Derived minimum-effect level (DMEL) values are the functional equivalents of supportable levels for toxic end points not amenable to derived no-effect levels. In the absence of more-specific data on the protein of interest, a default consumer DMEL of 15 ng/m³ has been derived for new and existing enzyme protein respiratory allergens (Basketter et al., 2010). This threshold is based on a clinical study that demonstrated no sensitisation, confirmed via skin prick testing, with a consumer spot-cleaning device that contained enzymes and was used for 30 sprays (expected exposure 12–17 ng/m³) per day for 6 months by 96 atopic subjects (Weeks et al., 2011). This limit is substantiated by occupational experience, where exposure guidelines that successfully control occupational asthma vary between 5 ng/m³ and 60 ng/m³, dependent on the specific enzyme and matrix (Basketter et al., 2010). The default consumer DMEL for e-cigarettes should take into account potential e-liquid matrix effects, any population-specific sensitivities, product design and the uncertainty in the precise identity of any potentially allergenic fraction of the extracts.

Irritancy of a product matrix might increase people's sensitivity to allergy induction and/or elicitation. Vaping studies suggest that the e-liquid matrix is likely to induce limited irritation (Farsalinos and Polosa, 2014; Hua et al., 2013). In the detergent industry, a safety factor of 4 for matrix effect has resulted in occupational exposure guidelines of 15 ng/m³ in individual factories for proteases in an irritant matrix (Sarlo, 2003; Schweigert et al., 2000). Thus, as the generic consumer DMEL of 15 ng/m³ was based on experience with a cleaning product, it already takes into account an enhancing matrix effect. Although a product intended for inhalation might be expected to induce less respiratory irritation than a cleaning product, a correlation between inhaled environmental propylene glycol exposures and an increased likelihood of IgE sensitisation in children has been reported (Choi et al., 2010). No potential mechanism has yet been identified and, therefore, whether this relation is statistical or causal is unclear. Overall, though, the assumption that the matrix has an enhancing effect roughly equivalent to that factored into the 15 ng/m³ value from a cleaning product seems appropriate.

Most new vapers start as existing smokers of traditional tobacco products, but cigarette consumption generally decreases over time, leading to smoking cessation in most long term vapers (ASH, 2016; Biener and Hargraves, 2015; Brown et al., 2014; Etter and Bullen, 2011). Although smoking is often assumed to be a risk factor in occupational asthma, reviews have found few supporting data (Siracusa et al., 2006; Vandenplas, 2011). Regardless of the exact relationship between respiratory sensitisation risk and smoking, the 15 ng/m³ consumer DMEL is based on data that included smokers as a normal part of the population. Thus, a further uncertainty factor for sensitivity in smokers is not needed.

The experience from which the supportable levels are derived is based on unintentional occupational and consumer inhalation exposures. As aerosol from vaping products is intended to be inhaled,

it is prudent to include a safety factor to account for more efficient lung delivery. Few quantitative data are available on which to base such a factor, but with factory measurements showing a respirable fraction of cocoa dust of only 23% (2.1 mg/m³ respirable versus 9.1 mg/m³ total cocoa dust (Zuskin et al., 1998)), a safety factor of 4 should allow for the worst case of total aerosol being respirable in a vaping product. As a conservative approach, however, we have applied a safety factor of 10.

Barring cross-reactivity between similar molecules, antibody-mediated sensitisation is specific to the compound against which antibodies are raised. Mixture effects from combining different allergens cannot be excluded, but are effectively taken into account because safe levels are based on, among other factors, occupational exposures, which often involve mixtures. The proteinaceous fraction in the cocoa extracts will consist of many different proteins, each with potential allergenicity varying from none to sufficiently potent to have been identified from occupational experience. In cocoa shell extract, 79% of the total protein content comprises albumins and globulins (Bonvehí and Coll, 1999), which are non-enzymatic protein types that have not currently been identified as causative agents for respiratory sensitisation. Thus, enzymatic proteins cannot constitute more than one-fifth of the total protein content, and the levels of individual enzymes will be substantially lower. In principle, a supportable respiratory CEL will apply per identified allergenic protein. A conservative estimate, therefore, is that one-fifth of the total protein measured in cocoa shell extract accounts for the maximum level of any individual allergenic enzyme.

Taking all the safety factors above into account:

$$\text{CEL} = 15 \text{ ng/m}^3 [\text{DMEL}]/(10 [\text{safety factor for efficient lung delivery}] * 0.2 [\text{maximum level of an individual allergenic enzyme in the protein mix}])$$

This yields a supportable CEL of 7.5 ng/m³ for total cocoa shell extract protein from vaping.

2.5.3. Estimating consumer cocoa protein exposure

A supportable exposure level of 7.5 ng/m³ for cocoa shell extract is extremely low and unsuitable to allow noticeable cocoa flavour levels in e-liquid if all the protein were to transfer across into the aerosol. However, it seems unlikely that a high-molecular-weight compound, such as a protein, would efficiently transfer when aerosolised. We therefore measured the level of protein in aerosol using polyacrylamide gel electrophoresis/silver stain analysis. Vype eStick devices, a first-generation “cig-a-like” e-cigarette, were filled with an e-liquid containing 25 ppm total protein. An unflavoured sample was used as a control. The devices were vaped on a smoking machine with 80 ml puff volume, 3 s puff duration and 30 s puff interval regime. Approximately 50 L of aerosol per e-liquid sample was collected on filter pads to provide sufficient aerosol condensate for testing. A sample spiked with 100 ng bovine serum albumin was run in the polyacrylamide gel electrophoresis alongside the aerosol samples for protein quantification. We found various low-molecular-weight peptides and several high-molecular-weight proteins in the flavoured e-liquid aerosol. The total protein level (including peptides) exceeded 2000 ng/m³, compared with no protein content in the unflavoured e-liquid aerosol.

The level of denaturation and break down of the original proteins due to the aerosolisation process is unknown and, therefore, no estimate of relative allergenicity of the proteinaceous fraction in the aerosol versus that in the e-liquid can be made. However, because even just 25 ppm total protein in the e-liquid already resulted in a puff concentration of more than 250 times the derived supportable air concentration, cocoa shell extract flavouring is not

considered to be supportable.

2.6. Non-IgE-mediated food allergies

Food allergies not mediated by IgE comprise a diverse set of clinical diagnoses and are scientifically not well understood. Examples are certain forms of soy and wheat allergies, which are believed to be T-cell mediated. Symptoms occur several hours after exposure and, although they can be debilitating, do not involve potentially deadly systemic reactions such as anaphylaxis. The diversity of responses and limited mechanistic understanding mean that there is not a single well-established way of identifying whether a compound has the potential to cause non-IgE-mediated food allergy. Equally, whether respiratory exposure to compounds would result in symptoms is unclear. Nevertheless, as at least part of the vaping aerosol deposit exposure is via the oral route, the potential for effects should be taken into account.

In the absence of a clear scientific approach for quantitative risk assessment, we suggest a practical approach analogous to regulatory risk management approach for foods, i.e. identify on product labels whether any identified food allergens are included so that consumers with known or self-suspected food allergies may decide whether or not to use or consume these products.

One practical source of information on compounds that have been established as potential food allergens is the European food regulations (European Parliament and the Council, 2008b). We recommend against using any that can induce IgE-mediated allergy in inhalation products. However, for listed compounds with no evidence of IgE-mediated mechanisms, we suggest informing consumers of their presence in a list of ingredients on the packaging or other product information source, or by adding a specific warning such as “contains gluten”.

2.7. Conclusions

For contact sensitisers, NESIL values should be derived to protect against induction of sensitisation. Known contact sensitisers should be mentioned in the product information if present at levels above 0.1% in the e-liquid. In contrast, the use of known respiratory sensitisers should be avoided completely in e-liquids. Identification of respiratory sensitisers is by a weight of evidence approach. Occupational experience is particularly informative in this area. Finally, we recommend using only protein-free versions of natural extracts. The presence of any potential food allergens, regardless of their use level, should be noted on the product information to help consumers already sensitised to help themselves.

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