INTRODUCTION

Chronic inhalation of respiratory sensitisers could lead to hay fever- and occupational asthma-like symptoms such as perennial rhinitis, eczema, breathing difficulties and bronchoconstriction. In very rare cases people might experience anaphylactic responses, including death.

No validated hazard identification tests or risk analysis exist for respiratory sensitisation. Occupational limits have derived tolerable levels for some respiratory allergens, but these have generally been very low, e.g. in the range of µg/m³ for anhydrides and ng/m³ for several enzymes.

We provide a respiratory sensitisation risk assessment for a commonly used e-liquid flavour ingredient: cocoa extract.

HAZARD IDENTIFICATION

Cocoa shell extract from Theobroma cacao L. is classified as generally recognised as safe (GRAS) in the USA [1] and has no health-related classifications in Europe [2].

- **Potential to induce respiratory sensitisation?**
  - Yes, based on the presence of IgE antibodies in an occupational cohort study and a case report from the confectionery industry [3,4].

- **Potential to elicit symptoms?**
  - Yes, respiratory symptoms, including occupational asthma in two cohort studies and responses to broncho-provocation testing [3-5].

- **What is the allergenic part?**
  - Flour and other cereals have multiple allergens, and those identified have all been proteinaceous [6-12]. Recognised high-molecular-weight organic respiratory allergens are proteins from animals, plants and bacteria, and, where further classified, have generally been enzymes [2,13-15].

Conclusion

Overall weight of evidence indicates cocoa has the potential to induce and elicit respiratory sensitisation. The allergenic components are most likely proteinaceous, probably enzymes.

DERIVING A TOLERABLE CONSUMER EXPOSURE LEVEL FOR E-CIGARETTE FLAVOURING

- **Literature data not sufficiently quantitative to establish cocoa-specific no-effect or minimum-effect levels.**
  - Apply a toxicological threshold of concern approach.

- **Derived minimum-effect level (DMEL) – 15 ng/m³**
  - A default consumer DMEL of 15 ng/m³ has been derived for new and existing enzyme protein respiratory allergens [14].

Safety factors (SFs):

- **E-liquid matrix effects – 1**
  - GENERIC DMEL based on detergent experience, i.e. already takes into account irritant matrix

- **Population-specific sensitivities – 1**
  - Vaping products are for adult use only. DMEL is based on consumer and occupational data that included smokers and other population variables.

- **Product design – 10**
  - DMEL based on unintentional exposures, vaping products intended to be inhaled. Respirable fraction of cocoa dust in factory reported as 23% [4], suggesting a 4-fold SF would account for up to 100% potentially being respirable from vaping products. Apply a SF of 10 to ensure sufficient conservatism.

- **Proportion of allergens in extract – 0.2**
  - Antibody-mediated sensitisation is compound-specific. In cocoa shell extract, 79% of the total protein content comprises albumins and globulins [16], which are non-enzymatic protein types. Thus, enzymatic proteins cannot constitute more than one-fifth of the total protein content, and the levels of individual enzymes will be substantially lower.

  \[ \text{Supportable consumer exposure level for total cocoa shell extract protein from vaping is 7.5 ng/m³}. \]

  \[ \text{15 ng/m³ [DMEL]} \]

  \[ 10 \text{ [SF for efficient lung delivery]} * 0.2 \text{ [Proportion of allergen in extract]} \]

CAN PROTEIN TRANSFER INTO THE AEROSOL?

**Test case:** A 1st generation e-cigarette (a cigalike) was filled with an e-liquid containing 0.0025% total protein from cocoa extract. Aerosol was collected using puffs of 3 second duration and 80 ml puffing volume. The level of protein in aerosol using polyacrylamide gel electrophoresis/silver stain analysis was measured, using bovine serum albumin as a standard for quantification.

**Result:** Puff concentrations of total protein level (including peptides) exceeded 2000 ng/m³

**Limitation:** The level of protein break down and associated reduction in allergenicity due to the aerosolisation process is unknown.

CONCLUSIONS

- Cocoa extract has the potential to induce and elic respiratory sensitisation.
- Supportable consumer exposure level for total cocoa shell extract protein from vaping is 7.5 ng/m³.
- Proteinaceous material can transfer into the aerosol. Just 0.0025% protein in the e-liquid in a cigalike already resulted in levels more than 250 times the supportable consumer exposure level.
- Protein-containing cocoa extract is not supportable in e-liquids.

REFERENCES

1. FDA, DHHS, 2005. Section 182.20: essential oils, oleoresins (solvent free), and natural extractives (including distillates).