Regulatory Toxicology and Pharmacology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

An approach to ingredient screening and toxicological risk assessment of flavours in e-liquids

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ARTICLE INFO

Article history:
Received 15 January 2015
Available online xxxx

Keywords:
Electronic cigarettes
E-cigarettes
Vaping
Flavour ingredients
Toxicological risk assessment
Ingredient screening
E-liquid
Toxicological thresholds of concern
TTCs
Contaminants

ABSTRACT

Flavour ingredients are an essential part of e-liquids. Their responsible selection and inclusion levels in e-liquids must be guided by toxicological principles. We propose an approach to the screening and toxicological risk assessment of flavour ingredients for e-liquids. The screening involves purity requirements and avoiding ingredients that are carcinogenic, mutagenic or toxic to reproduction. Additionally, owing to the uncertainties involved in potency determination and the derivation of a tolerable level for respiratory sensitisation, we propose excluding respiratory sensitisers. After screening, toxicological data on the ingredients should be reviewed. Inhalation-specific toxicological issues, for which no reliable safe levels can currently be derived, can lead to further ingredient exclusions. We discuss the use of toxicological thresholds of concern for flavours that lack inhalation data suitable for quantitative risk assessment. Higher toxicological thresholds of concern are suggested for flavour ingredients (170 or 980 μg/day) than for contaminant assessment (1.5 μg/day). Analytical detection limits for measurements of potential reaction and thermal breakdown products in vaping aerosol, should be informed by the contaminant threshold. This principle leads us to recommend 5 ng/puff as an appropriate limit of detection for untargeted aerosol measurements.

1. Introduction

The main users of e-cigarettes are current smokers, especially those who have expressed an interest in quitting or cutting down cigarette consumption. In the same way that the taste of tobacco is important to cigarette smokers, flavour is an important part of the e-cigarette experience, including for regular adoption or conversion. Farsalinos et al. (2013) undertook a survey of 4618 Greek e-cigarette users to assess flavour preferences. The median duration of smoking cigarettes was 22 years and of e-cigarettes was 12 months. Respondents reported that having a variety of flavours available was very important to efforts to quit smoking, and almost half felt that restriction of variety would increase cravings for cigarettes. The authors concluded that liquid flavourings in e-cigarettes contribute substantially to the overall experience of persistent users. Similarly, when adults in the US were surveyed about their tobacco use and motivations for starting and stopping e-cigarette use, the study found the most important reason for stopping vaping was the taste of the product (Biener and Hargraves, 2014). This feature was particularly important to those who had tried e-cigarettes only once or twice, whereas taste played a notably lesser role in stopping vaping for intensive and intermittent users. These findings imply that taste, and hence flavourings, are likely to play a major role in the difference between people only trying e-cigarettes versus actually adopting them for longer term use. Indeed, flavourings might be essential to smoking cessation in e-cigarettes users, because the US study concluded that daily use of e-cigarettes for at least 1 month was strongly associated with quitting smoking after a 2-year follow-up period, compared with intermittent or no use.

The market for e-cigarettes has expanded extremely quickly worldwide. Long-term research findings on the health effects of vaping are not yet available, and methods for various assessments, such as toxicology, flavours, respiratory effects and so on, are still to be agreed upon. However, as vaping products are widely available, publication, debate and agreement on risk assessment approaches are becoming increasingly important. Regulations are still developing and are not yet up to date with vaping reality. Therefore, industry can help to develop appropriate product standards and implement robust quality management systems. Much of the focus of studies reported thus far has been related to the risk assessment of the solvents and nicotine in e-liquid. Additionally, screening and risk assessment considerations are generally

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http://dx.doi.org/10.1016/j.yrtph.2015.05.018
0273-2300/

performed on the in-going ingredients of e-liquids. However, the main consumer exposure to the e-liquid during normal use is to the aerosol. In this paper we focus on responsible product stewardship for the flavours that are essential to create consumer-relevant e-liquids. We suggest an approach to toxicological risk assessment of flavours that takes into account the in-going flavour ingredients and constituents and the identification, measurement and risk assessment of any potential thermal breakdown and reaction products.

2. Screening and risk assessments

2.1. Aerosol versus e-liquid

The aerosolisation process involves a brief heating period during every puff of an e-cigarette. Published data around heater operating temperatures are scarce, but estimates have ranged from 40–65 °C (Bertholon et al., 2013; Westenberger, 2009) to 170–180 °C (Talih et al., 2015), and even up to 350 °C or higher in the absence of e-liquid (Schripp et al., 2013). Regardless of the exact operating temperatures of individual vaping products under specific conditions, a heating period introduces the potential for pyrolysis of compounds and endothermic reactions between them. Additionally, the compounds can respond in varying degrees to the different processes involved in aerosolisation, such as evaporation and condensation. Together these factors might result in changes to the composition of the aerosol versus that of the e-liquid. Appropriately sensitive measurement of the aerosol, therefore, is required for the risk assessment to take into account potential thermal breakdown and reaction products of flavouring ingredients (Fig. 1).

2.2. Screening of in-going ingredients

The first screening step for in-going ingredients relates to the purity of the compounds (Fig. 1). As a practical way of minimising risks from potential contaminants in ingredients, we suggest that only food-grade flavouring ingredients are used to provide some reassurance on purity and systemic toxicity. Food flavours, however, are not normally assessed for inhalation exposures and further safety assurance is required.

A toxicological risk assessment also requires knowledge through full quantitative disclosure of the individual ingredients and constituents in e-liquid. This requirement sounds obvious, but besides the commercial sensitivity of flavour recipes and sub-flavours, challenges surround consistency and identification of constituents, especially for ingredients of natural origin. The compositions of naturals vary dependent on biological and geographical origins and weather and other environmental factors affecting growth and harvest, and can change over time. Thus, using only naturals that are approved food flavourings ensures that specific limits have been placed on constituents of known toxicological concern. An example of such restrictions can be found in article 6 of the European food flavouring regulations (European Parliament and the Council, 2008b).

2.2.1. Ingredients classed as carcinogenic, mutagenic or toxic to reproduction

Exclusion of ingredients from use if they have properties known to be carcinogenic, mutagenic or toxic to reproduction (CMR) is considered a basic safety precaution. In general, use of only food-grade flavourings should already have ensured they are not CMR, however, because classification criteria can differ per region and several food flavourings have been grandfathered on to approved lists on the basis of historic use, exceptions may exist. Therefore our proposed screening criteria also explicitly exclude any ingredients classed as group 1, 2A or 2B carcinogens in the International Agency for Research in Cancer classification, as well as any classified as CMR by the US Food and Drug Administration (FDA) or if a harmonised European classification exists. Additionally, ingredients that appear on the REACh list for substances of very high concern (ECHA (European Chemicals Agency), 2014) for human toxicity reasons should also be avoided, as should all compounds that have been identified by the FDA as "harmful and potentially harmful compounds" or HPHC in a tobacco smoke context (Food and Drug Administration (FDA), 2012). For ingredients that are not evaluated or classified or where only a manufacturer's self-notified classification exists, a weight-of-evidence approach is recommended that applies criteria to the data as described by the Globally Harmonized System of Classification.

2.2.2. Respiratory sensitisers

Some discussion has taken place about restricting the inclusion of contact allergens in e-liquids. An evaluation process has been proposed that includes a tolerable no effect level of 1000 ppm in e-liquids, below which the chance of induction of contact sensitisation and eliciting effects in pre-sensitised people is considered tolerable (Costigan, 2014). However, the situation is different for respiratory sensitisation. If e-liquids were to contain respiratory sensitisers (i.e. type I allergens and causative agents of immediate hypersensitivity), inhalation exposure over time could lead to IgE-mediated responses, such as are experienced with hay fever and occupational asthma (e.g. perennial rhinitis, eczema, breathing difficulties and bronchoconstriction). Although extremely rare, in the very worst case, people might experience anaphylactic responses, including death. The potential severity of symptoms related to respiratory allergens, therefore, sets these substances apart from those causing the more common contact sensitisation. Additionally, although contact sensitisation is a well-understood process with recognised, robust hazard identification tests and quantitative risk assessment processes (Api et al., 2008; OECD, 2010; United Nations, 2013), no validated hazard identification tests and quantitative risk assessment processes exist for respiratory sensitisation and the recommended approach relies on a weight of evidence evaluation (ECETOC, 1999; ILSI HESI, 2014; United Nations, 2013). Some tests are in use for hazard identification, such as the measurement of immunoglobulin E (IgE) in mice and specific guinea pig pulmonary responses (Briatico-Vangosa et al., 1994; Kimber et al., 1996; Pauluhn, 1996), but their applicability is restricted to certain chemical classes of compounds. Hazard identification and the derivation of tolerable doses are therefore based on a weight-of-evidence approach, where occupational experience especially can form an important hazard alert function. On top of the identification uncertainties, several respiratory sensitisers have very low derived no-effect levels, leading to occupational exposure guidelines being measured in g/m^3 for anhydrides (WHO, 2009), and even 5–60 ng/m^3 for several enzymes (AISE Enzymes Occupational Exposure Working Group, 2013).

3. Review of existing toxicological evidence

If flavouring ingredients pass the screening stage, a review of the existing toxicological data should follow. This approach will contribute to identifying any evidence of inhalation-specific issues that might make a compound unsuitable for use in an inhalation product. An example is the potential for diacetyl and

Please cite this article in press as: Costigan, S., Meredith, C. An approach to ingredient screening and toxicological risk assessment of flavours in e-liquids. Regul. Toxicol. Pharmacol. (2015), http://dx.doi.org/10.1016/j.yrtph.2015.05.018