Flavor ingredients in e-vapor products: A structure-based grouping approach for predicting their biological activity

D. Sciuisci1, T. Langston1, A. Kumar2, D. Smith1, K. M. Loel1, D. Marseschi1, F. Martin1, J. Hoeng1, R. Vanschewick1,3
1 PMI R&D, Philip Morris Products S.A., Neuchatel, Switzerland.
2 Altria Client Services LLC, Richmond, VA, U.S.A.

Introduction and Objective

Electronic nicotine delivery systems (ENDS) contain a wide variety of flavor ingredients. While most flavor ingredients used in today’s ENDS are “generally recognized as safe” (GRAS) for oral consumption, there are insufficient data on their safety via inhalation. Considering the range of different flavors used in ENDS and the resulting flavor mixtures, it is highly improbable to test all possible flavor combinations for inhalation toxicity. In this study, we developed a structure-based approach, where flavor compounds used in ENDS were clustered in groups of structurally related compounds, and a total of 38 flavor group representatives (FGRs) were selected. We propose that a representative FGR mixture could be tested in nonclinical (in vitro and in vivo) studies, and the data could be used for toxicological assessment of all structurally related ingredients. This is based on the “read-across” concept that structurally related compounds exhibit common metabolic and biological activities. In this study, we outline the development of this Flavor Toolbox Approach step-by-step, using an example FGR.

The Flavor Toolbox Approach

The first step is to generate a list of candidate flavor ingredients on the basis of known toxicity profiles, desirable sensorial properties, their use in e-vapor products, and historical use in tobacco products. The example list included 245 flavors; for each flavor, a “toxicological fingerprint” was generated by applying data from literature4,5 and computational toxicity predictions performed by using toxicity software (TOPKAT™). In addition, all 245 individual flavor ingredients were tested in vitro cytotoxicity by real-time cellular analysis (RTCA).

Figure 1 summarizes the Tox score (ratio of EC50 base solution/EC50 flavored solution) versus the p values for all individual flavors tested. As described previously6, a statistically significant Tox score value of 1.5 was applied to identify the most cytotoxic flavor ingredients in the list and to select a total of 47 flavor ingredients for additional characterization of their mode of action by high-resolution screening (HCS) (Fig. 2).

The second step is to assign individual flavor ingredients to flavor groups on the basis of their structural, toxicological, and metabolic properties. The 245 flavor ingredients selected in this case study were initially allocated to one of the 34 structural groups defined in the European Commission (EC) Regulation No 1907/2006. Some groups (e.g., EC groups 1 and 2) contained many flavor ingredients (primary aliphatic alcohols/esters/aldehydes, acids, and ethers). Therefore, these broader, heterogeneous structural groups were further subdivided to better represent the range of structural differences. By following this approach, a total of 38 groups were defined, encompassing 37 of the original 34 EC groups (Table 1).

The third step is to identify the flavor groups that can represent each flavor group. This can be achieved by ranking each flavor within each flavor group on the basis of available experimental and predicted toxicological data and subsequently applying an objective scoring and a computational procedure to rank and select the FGR. The FGR is the flavor ingredient with the predicted highest toxicity potential within its flavor group. A numerical score (code) for available toxicological attributes (if available) was assigned as follows:

- rating: Climax class, coded 0, 1, or 2 for Climax class 1, 2, and 3, respectively
- p-properties: pCarcinogenicity and pToxPiToxicity: logistic TOPKAT predictions, scored 1 (true) or 0 (false).
- p-lincom: p-genotoxicity: chemical descriptors defined as the sum of mutagenicity and genotoxicity, which were individually ranked “Negative” (0), “Equivocal” (0.5), or “Positive” (0.5) on the basis of available experimental data.
- p-Geometric: Relative ratio of the EC50 of the base matrix and flavor ingredient (Tox score) on the basis of BCA and HS in vitro findings. This continuous score was transformed into ranks across all flavor ingredients.
- p-LOSO: Predicted acute inhalation toxicity by TOPKAT. This continuous score was transformed into ranks across all flavor ingredients.
- p-LOSO: Predicted chronic inhalation (LOSO). This continuous score was transformed into ranks across all flavor ingredients.
- p-multiple: Multiple additivity NMF, LOSO, and UASG selected from literature.
- Two available features were summarized as followings: (e.g., 0 to 15 from DHCV LOSO and 0-LOSOL LOSO in rodents). If it did not fit; if missing, use 0; if 0 or > ”-“ assume the worst-case scenario (moved LOSO).

The contribution of the extracted “feature” across all flavor ingredients was subsequently used.

The p-LOSO predicted ToxP index. ToxP index is a numerical index developed by EC, it can be used for ranking by using multiple domains of information—in our case, HCS assay endpoints. It is defined as the weighted sum of the minimum effective concentration (MEC) ratio (MEC of the matrix divided by the MEC of the flavor ingredient in the matrix). MEC ratios were obtained from HCS experiments with 35 flavor ingredients at the time of this analysis.

In order to complement this attribute for all flavor ingredients, a prediction model was developed. After feature selection feature, pCramen, pCorrelation, pCLassification, and pToxicity were retained as predictors. Figure 3 shows the accuracy of the prediction model: For a subset of flavor ingredients, both the predicted and experimental ToxP values were correlated, with a correlation R of 0.67. By applying the final model, the ToxP values for all flavor ingredients were then predicted and ranked.

In order to predict objectively with FGR selection, flavor ingredients within each group were ranked on the basis of pLOSO, pToxicity, PredictedToxP, pChemicalDOS, and pPrirality scores. For each flavor ingredient, the average rank was then computed and used to generate the final ranking. The resulting FGRs are listed in Table 2.

Discussion and Conclusion

In this work, we have proposed a pragmatic approach in which a variety of flavor ingredients are assigned to groups of structurally related compounds (flavor groups) and FGRs are selected. SDS could be tested individually and as mixtures through in vitro and in vivo toxicity studies that could support evaluation of appropriate use levels for product development. This approach has some limitations. For example, we assume that structurally related compounds would have comparable metabolic and biological activity and that the toxicity data generated through SDS or TOPKAT predictions could be used with other in vitro and in vivo assays. This approach is based on the scientific principie of “read-across” and, once validated with experimental data and comparable case studies, it has the potential to significantly reduce the time and resources required for filing the data gap with regard to a large number of individual flavor ingredients, while minimizing the number of laboratory animals used for toxicity assessment.

The FGRs were tested in a 5-week range-finding inhalation study in AI mice. The results are presented in Wright et al. (2020).

Acknowledgements

We would like to acknowledge the technical assistance and support of the BioResearch, Biosafety and Aerosol Teams at PMI Singapore and the Statistics Team at PMI Neuchatel.

References