Introduction and objectives

This poster describes the non-clinical and clinical assessments performed to fulfill the regulatory requirements as per Art. 6 (2) of the EU Tobacco Products Directive 2014/40/EU, under which Member States shall require manufacturers and importers of cigarettes and roll-your-own tobacco containing an additive included in the priority list established by Commission Implementing Decision (EU) 2016/787 to carry out comprehensive studies. The Directive requires manufacturers and importers of cigarettes and roll-your-own tobacco to examine each additive for whether it contributes to and increases the toxicity or additivity of tobacco products to a significant or measurable degree; if it leads to a characterization of the product; if it facilitates inhalation or nicotine uptake; and if it results in the formation of CRM (carcinogenic, mutagenic, and reprotoxic constituents) and if these substances increase the CRM properties of the respective tobacco product to a significant or measurable degree.

This poster gives an overview on comprehensive smoke chemistry, in vitro toxicity, and human clinical studies commissioned by the members of the Priority Additives Tobacco Consortium to independent Contract Research Organizations (CROs), where the emissions of test cigarettes containing priority additives were compared to emissions emerging from an additive-free reference cigarette.

Methods

Additives tested

- The study specifies 15 additives subject to enhanced reporting obligations: cocoa, carob bean, diacetyl, fig, fenugreek, geraniol, glycerol, guar gum, licorice, maltol, menthol, propylene glycol, sorbitol, and titanium dioxide. Two of these additives are not covered by the work described in this poster: diacetyl (not used) and guar gum (other testing approach).
- All additives were food-grade additives, certified to be compliant with the requirements of European Regulations or equivalent obligations.
- In addition to certificates of analysis, which were obtained from the suppliers of the additives, further analyses were performed on the six additives that are extracts of plant material (i.e., carob bean, cocoa, fenugreek, fig, guar gum, and licorice extract). The analyses were performed by two independent contract organizations (Yorda Group, UK, and UFGF Laboratory, Switzerland) to determine those constituents of relevance listed by SCHEER (SCHIER, 2016).

Test and reference cigarettes

- Additives were added to tobacco as flavorings or casings as in the standard process of manufacturing, except for guar gum, which was added as usual in the form of cast leaf (i.e., as a cut tobacco sheet consisting of ground tobacco with guar gum as a binding agent).
- The additive levels in the test cigarettes were defined to cover the Quantity Not Exceeded (QNE) level used by the EU Directive for testing (see Table 1 for details).
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Testing approach

- The studies were performed in independent CROs under Good Laboratory Practice (GLP) guidelines. The studies were performed in accordance with the respective national legislation as per the following guidelines and best practices: reaching, REACH, JECFA, SCF, and EFSA data were identified.

Additive transfer

- Additives were added to tobacco as flavorings or casings as in the standard process of manufacturing, except for guar gum, which was added as usual in the form of cast leaf (i.e., as a cut tobacco sheet consisting of ground tobacco with guar gum as a binding agent).
- The additive levels in the test cigarettes were defined to cover the Quantity Not Exceeded (QNE) level used by the EU Directive for testing (see Table 1 for details).
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Literature review

- A systematic literature review of relevant databases was performed by an external company by following highest standards and best practices. A total of 1500 publications were identified.
- Information on prior work was integrated into the Priority Additives study database, which is detailed in Table 1.

Results – Licorice

The comprehensive results of analysis of licorice-containing test cigarette and additive-free reference cigarette are used as an example in this poster, because licorice was subject to the entire repertoire of tests.

- Neutral licorice extract
- Chemical profile
- CAS No., CRF
- Purify
- Lot specification
- Analysis of contaminants or impurities

According to the scientific literature, licorice has no carcinogenic, mutagenic/genotoxic, or reprotoxic properties (JECFA 2005; SCF 2005).

- Test cigarettes with licorice extract
- Concentration added
- Target concentrations: 0.6% (Low), 1.2% (Max), and 1.8% (Max Plus)

Cigarette

- NRU Assay
- Ames Assay
- Lactobacillus assay
- Salmonella assay
- Activation
- Positive
- Negative

Conclusions

The comprehensive results required by EU TPD (2014/40/EU) Article 6(2) were commissioned by a consortium of 12 tobacco companies to independent CROs (Simms et al., 2019). Of the 13 priority additives tested, some showed a minor increase or decrease in smoke chemistry parameters upon comparison of emissions from test cigarettes with reference cigarettes without priority additives. In some cigarettes with sorbitol, a significant increase in formaldehyde and acrolein levels was observed relative to the additive-free reference cigarette. Increased formic acid and acetaldehyde levels were observed test cigarettes with guar gum (Stabbart et al., 2019). These increases did not translate into increased in vitro toxicity, and they were also not observed when sorbitol or guar gum was tested in a mixture with other priority additives. Relative to the additive-free reference cigarette, none of the tested priority additives caused an increase in in vitro toxicity (Ames, microincrustation, and neutral red uptake assay) or changes in smoking behavior or nicotine absorption or uptake (rate or concentration) during human clinical studies (McIlvan et al., 2019).