Supplementary file to:

Review of industry reports on EU priority tobacco additives part B: Methodological limitations

This supplementary file presents the assessment framework applied by the review panel in the evaluation of the chemistry and toxicity parts of the assessment of each additive in the review panel report (i.e. Chapter 5.1 to 5.15 in our report [1]).

1. Exposure and chemistry

Two types of experiments are commonly reported in the literature to assess altered exposure due to inclusion of an additive in cigarettes. These are chemical analysis of i) mainstream smoke (MSS) i.e. comparative testing and ii) emissions from pyrolysis experiments, as illustrated in

Figure ***S1***. In comparative testing experiments, the smoke chemistry of test cigarettes containing additives are compared with an additive-free control cigarette. In pyrolysis experiments, pure additives are combusted in well controlled experimental laboratory conditions (temperature, temperature increase rate, duration time, etc.), so in the absence of the tobacco matrix.

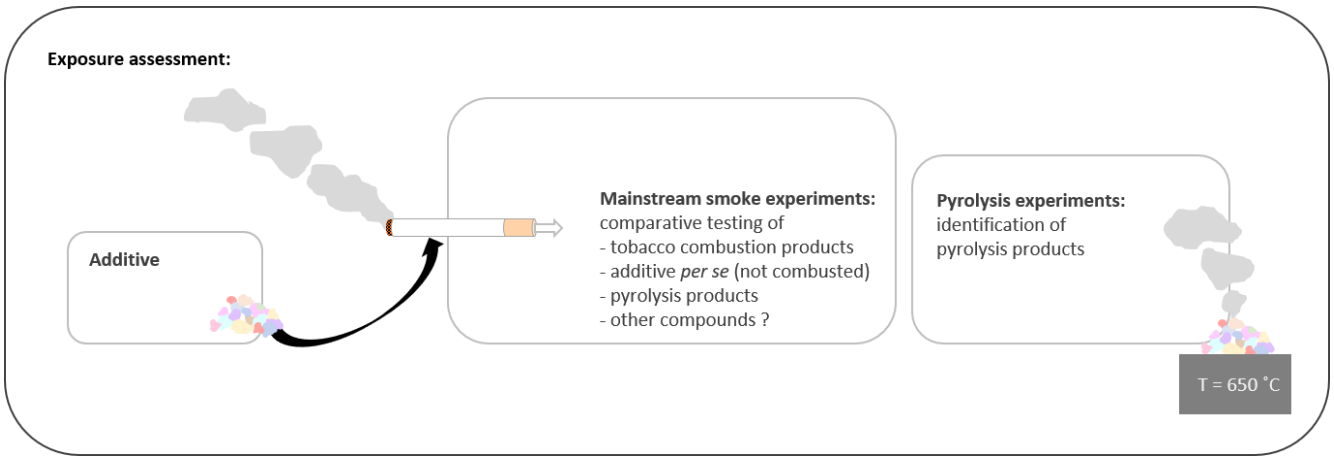

Figure S1: *Schematic illustration of the two types of experiments commonly performed to assess altered exposure due to inclusion of an additive in cigarettes.*

The information required from these experiments to evaluate the risk resulting from application of the additive in the tobacco includes:

- What is the transfer rate of the *additive itself* to the smoke? (using comparative testing)
- What are the *pyrolysis products* of the additive? (using pyrolysis experiments)
- Are these *pyrolysis products* present in mainstream or sidestream smoke, and in which amounts? (using comparative testing)

- Does inclusion of the additive alter the levels of *tobacco combustion products or the formation of new ones*? (using comparative testing)
- Are the levels of *other compounds* (than the additives specific pyrolysis products and tobacco combustion products) increased or modified by inclusion of the additive? (using comparative testing)

2. Toxicity

As illustrated in Figure S2, a selection of approaches may be applied in a toxicological evaluation of the consequences of inclusion of the additive in the tobacco. These include, but are not limited to:

A. Evaluation of the hazard properties of the **additive itself** based on existing information, in particular the oral route (**ingestion**) based on its application as a food additive. This should include evaluation of the general toxicity of the additive based on reports from regulatory bodies. Information regarding Acceptable Daily Intake (ADI) and the general exposure level of the population should be reported. This route of exposure is not appropriate for the evaluation of the toxicity of a compound as a tobacco additive, but this information may provide some clues about a possible target organ, toxic effect and/or dose-response curves.

B. Evaluation of the **additive itself via inhalation** due to transfer of additive (non-combusted) in the smoke. Experiments and evaluations specific for inhalation should be included. In addition, toxicokinetic assessment following inhalation compared to the oral route is highly relevant. Evaluation of the toxicity of the metabolites formed following inhalation as compared to those formed following ingestion is required. Finally, evaluation of the health risk, should consider the total exposure to the additive and its metabolites resulting from its use in both food and tobacco products.

C. Evaluation of **pyrolysis products** of the additive (inhalation exposure route and CMR properties). First, pyrolysis products have to be identified through pyrolysis experiments. Then, their presence in mainstream tobacco smoke must be verified in comparative chemical testing. Finally, assessment of their toxicity is required. Experiments and evaluations for toxicity specific for inhalation and CMR properties should be included.

D. Evaluation of **MSS** (inhalation exposure route). Comparative experiments to assess whether the additive contributes to increased toxicity of the generated smoke. This can be assessed either i) directly in terms of comparative toxicity testing or ii) indirectly by comparative chemistry testing followed by toxicological evaluation of the significant changes in MSS chemistry. However, for the characterization of the additive's toxicity, the comparative testing approach is hampered by its limited sensitivity and lack of discriminative power.

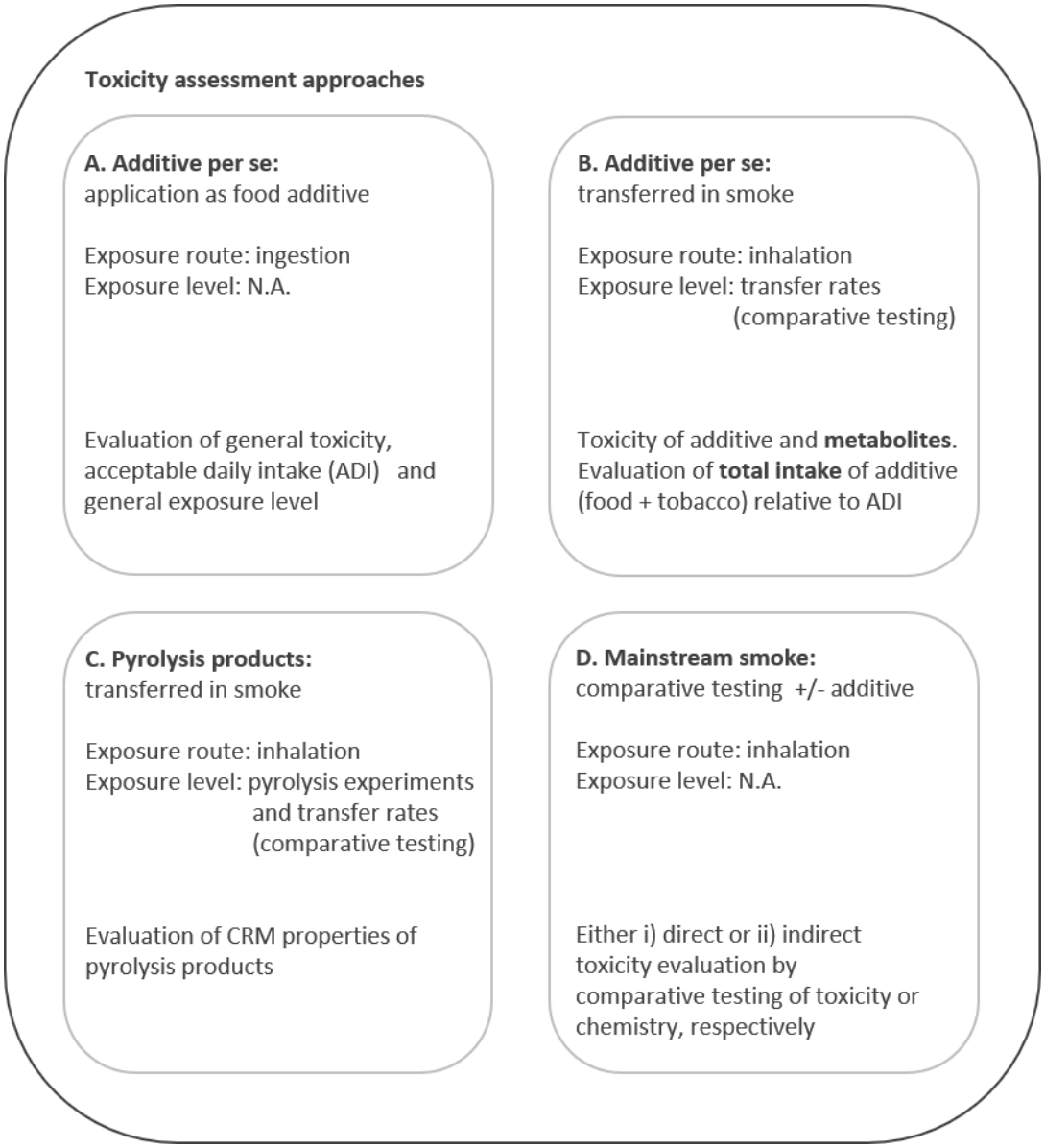

Figure S2: *Schematic illustration of toxicity assessment strategies. The figure summarizes four approaches that may be applied in a toxicological evaluation of the consequences of inclusion of the additive in the tobacco. See text for explanation. N.A. = not applicable*

3. References

1. National Institute for Public Health and the Environment (RIVM), Environment and Occupational Health & Safety (ANSES), Norwegian Institute of Public Health (NIPH), Italian National Institute of Health (ISS), and the WP 9 Independent Review Panel *D9.3 Report on the peer review of the enhanced reporting information on priority additives.* 2020.