Joint Action on Tobacco Control (JATC)
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WP9- D9.1 Assessment/Evaluation Framework for enhanced reporting of priority additives and guidelines for 'Good Experimental Practicing'

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1. The Assessment/Evaluation Framework for enhanced reporting of priority additives

Background

Manufacturers and importers of cigarettes and RYO tobacco (individual or in consortia) are obliged to submit a report containing comprehensive studies on each priority additive (if used as additive).[1,2] These studies should be submitted by 1 July 2018, 18 months after the additive concerned has been included in the priority list at 1 January 2017.

The Commission and the Member States concerned may require these reports to be peer reviewed by an independent scientific body (TPD art. 6.4). To provide guidelines on how the enhanced reporting documents on priority additives will be assessed an 'assessment and evaluation framework' was composed. The framework is meant to assist the commission and member states to identify missing information that needs to be requested from industry. In addition, it provides a structure by which to assess methodology and conclusions of submitted studies, along with an overview of most relevant risks associated with each of the priority listed additives. The framework will be used by an independent review panel, which has been established in light of the Joint Action on Tobacco Control.

Enhanced reporting requirements

Manufacturers or importers shall establish a report on the results of studies on priority additives. The report shall include an executive summary, and a comprehensive overview compiling the available scientific literature on that additive and summarizing internal data on the effects of the additive (TPD article 6.4).

Aspects that are included in the assessment framework are based on TPD article 6.4:
- Comprehensiveness
- Methodology
- Conclusions

Furthermore, the reports should evaluate priority additives in relation to criteria specified in TPD article 6:
- Contributions to toxicity and addictiveness
- Formations of additional CMR compounds (metabolites, pyrolysis products)
- Facilitation of inhalation or nicotine up-take
- Flavoring properties
- Whether this priority additive compound has the effect of increasing the toxicity or addictiveness, CMR properties of any of the products concerned to a significant or measurable degree

Those studies shall take into account the intended use of the products concerned and examine in particular the emissions resulting from the combustion process involving the additive concerned. The studies shall also examine the interaction of that additive with other ingredients contained in the products concerned. Information of the recently published SCHEER opinion II on Additives used in Tobacco Products was used as a basis for the framework that was developed and can be used for the assessment of the submitted reports.

The Commission and the Member States concerned may also request supplementary information from manufacturers or importers regarding the additive concerned. This supplementary information shall form part of the report (TPD 6.4). The information received shall assist the Commission and Member States in taking the decisions pursuant to Article 7 (Regulation of ingredients).

[1] Tobacco Product Directive 2014/40/EU Article 6

[2] Commission Implementing Decision EU 2016/787 point 6 and Annex 1

2. Advice for 'Good Experimental Practicing'

This advice is a work in progress, as industry reports have already been submitted and drafting should be continued after an initial assessment of these reports.

According to the European Tobacco Product Directive (TPD, EU 2016/787; article 6), importers and manufacturers of cigarettes and RYO tobacco (individual or in consortia) are required to submit a comprehensive report on each priority additive (if used as additive). The reports should be based on studies carried out to examine for each additive whether it contributes to or increases the toxicity and addictiveness of cigarettes or RYO tobacco to a significant degree. Moreover, studies should be carried out to examine whether the additive results in a characterizing flavour, facilitates inhalation or nicotine uptake or leads to the formation of CMR (carcinogenic, mutagenic, or reproductive) properties. Submitted studies and reports should be sufficiently comprehensive to allow a reliable assessment of whether an additive can contribute to or significantly increases the toxicity or addictiveness of cigarettes or RYO tobacco.

Priority listed additives are no novel compounds as they are widely used in food and other consumer goods. Because all listed additives are registered or pre-registered under REACH, substantial data may be available. Manufacturers need to confirm that these registrations cover the intended application as tobacco additive. However, data requirements according to REACH are not necessarily sufficient for compliance with Article 6 of the TPD or commission implementing decision (EU) 2016/787. The importer's or manufacturer's report should therefore also address existing data gaps, especially concerning inhalation toxicology and CMR effects of pyrolysis products of the additive itself and pyrolysis products of the combination of additives in the product.

The recently published SCHEER opinion II on Additives used in Tobacco Products[3] proposes a comprehensive step-wise approach to assess the toxic effects, addictive potential, inhalation facilitation properties, and characterizing flavour properties of tobacco additives. After thorough evaluation, the JATC WP 9 recommends the use of this pragmatic and efficient approach for the assessment of priority additives. That is, the complete SCHEER opinion II should be followed as guideline for good experimental practicing (GEP).

As suggested in the SCHEER opinion, validated and internationally standardized testing guidelines should be applied when conducting studies on priority additives. The methodological approach of studies should be based on the most recent existing state-of-the-art protocols and regulations where applicable. These can be used or translated from existing guidelines for study designs and reporting developed by e.g. the EQUATOR network.[4] For studies regarding pyrolysis and toxicology, OECD testing guidelines are available, and should be adopted. An overview of relevant OECD guidelines within the SCHEER step-wise approach is provided in Annex I (*decision tree for toxicological assessment of tobacco additives).*

There is currently no validated framework or set of testing guidelines for the testing of the addictiveness of tobacco additives. However, JATC recommends the use of multiple study designs in order to provide sufficient and robust information. The SCHEER report provides a list of study designs that can be adopted to test for addictiveness, these are also provided in Annex II (*decision tree for assessing addictiveness of tobacco additives).* Additional sources that provide guidance on the assessment of abuse liability are:

1. Assessment of Abuse Potential of Drugs. Draft Guidance. US Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER), 2010.
2. Carter LP, Griffiths RR. Principles of laboratory assessment of drug abuse liability and implications for clinical development. Drug Alcohol Depend. 2009 Dec 1;105 Suppl 1:S14-25.

[3] SCHEER opinion II on Additives used in Tobacco Products;
https://ec.europa.eu/health/sites/health/files/scientific_committees/scheer/docs/scheer_o_001.pdf
[4] http://www.equator-network.org/

The issue whether additives can lead to a characterizing flavor is complex and will be decided for particular products by an expert panel according to Decision (EU) 2016/786. However, submitted studies for priority-listed compounds should include data on aroma properties, as well as information on whether these compounds are used as flavor in tobacco products or in other products. Since uniform rules and procedures have been specified by the European Commission to determine whether a tobacco product has a characterizing flavor (EU 2016/779), requested studies should only report on the flavoring properties of listed compounds/additives, but not on specific products. Further, if these compounds are used in complex flavors, technical details, including typically applied levels, as well as odor detection thresholds, should be provided. Studies may also include sensory analysis in consumer or expert panel studies, and studies on consumer preferences/ marketing data. Guidance for sensory studies is provided in guidelines on the sensory evaluation of foods, such as those from The Institute of Food Science & Technology: https://www.ifst.org/resources-resource-search/ifst-guidelines-ethical-and-professional-practices-sensory-analysis-foods

Besides the (characterising) flavour features, other features can contribute to make a tobacco product more attractive. In humans, the attractiveness of individual tobacco products can be compared in panel studies, surveys and by experimental measures. The SCHEER opinion provides more detail on several attractiveness features and methods to evaluate them.

In addition to guidelines on experimental practice, aspects of scientific validity can be increased by using checklists and reporting templates. Therefore, a separate document is provided in Annex 3 that outlines minimum reporting requirements and reporting template for studies on additives subject to increased reporting obligations.

Annex I - General approach to assess the toxicological effects of tobacco additives

Step 1. Evaluation of the additive in unburned form

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Step 1.1 *In silico* testing, taking into regard mutagenicity, carcinogenicity, and reproductive toxicity.

At least: (1) Identification of the relevant structural characteristics and potential mechanisms or mode of action of a target chemical.
(2) Identification of other chemicals that have the same structural characteristics and/or mechanism or mode of action.

E.g. by using: JRC QSAR database, Toxtree, Dart, Toxmatch, Stat4tox

↓

Indication of CMR properties?

← No, conflicting results, or data unavailable. This should be reported during the evaluation. The substance still may have CMR properties. Proceed to next step.

→ Yes. This should be reported during the evaluation. The additive may not meet the requirements of TPD art. 7. Proceed to next step.

Step 1.2 *In vitro* testing, taking into regard mutagenicity, carcinogenicity, and reproductive toxicity.

Carcinogenicity
- Syrian hamster cell transformation assay
- Bhas 42 cell transformation assay
- Other assays if appropriate

Mutagenicity
Testing for both 1) point mutations in prokaryotic and mammalian cells, as well as 2) chromosomal aberrations and DNA damage and repair.
- Bacterial reverse mutation assay
- *In vitro* mammalian chromosomal aberration test
- *In vitro* mammalian cell gene mutation test
- *In vitro* SCE Assay in mammalian cells
- S. cerevisae gene mutation assay
- S. cerevisae mitotic rec. assay
- DNA damage and repair, UDS in mammalian cells *in vitro*
- *In vitro* mammalian cells micronucleus test
- *In vitro* mammalian cell gene mutation test using the thymidine kinase gene
- Other assays if appropriate

Reproductive toxicity
See OECD conceptual framework for endocrine disrupting chemicals.
- Estrogen or androgen receptor binding activity
- Estrogen receptor transactivation
- Androgen or thyroid transactivation
- Steroidogenesis *in vitro*
- Androgen transactivation assay
- Estrogen or androgen receptor binding activity
- MCF-7 cell proliferation assays (ER ant/agonist)
- Other assays if appropriate

↓

Indication of CMR properties?

← No. This should be reported during the evaluation. Data in category 1 and 2 must be present; no conflicting data should exist or this should be well explained. Proceed to next step.

→ Yes. This should be reported during the evaluation. The additive may not meet the requirements of TPD art. 7. Proceed to next step.

Step 1.3 (optional) *In vivo* **historical data**, taking into regard mutagenicity, carcinogenicity, and reproductive toxicity.

Carcinogenicity
- 2-year carcinogenicity study
- Other assays if appropriate

Mutagenicity
- Mammalian erythrocyte micronucleus test
- Mammalian bone marrow chromosome aberration test
- Rodent dominant lethal test
- Mammalian spermatogonial chromosome aberration test
- Unscheduled DNA synthesis test with mammalian liver cells
- Transgenic rodent somatic and germ cell gene mutation assays
- Alkaline single-cell gel electrophoresis assay for DNA strand breaks
- Other assays if appropriate

Reproductive toxicity
- Prenatal development toxicity study
- Two-generation reproduction toxicity
- Other assays if appropriate

↓

Indication of CMR properties?

← No, or no data available. This should be reported during the evaluation. Proceed to next step.

→ Yes. This should be reported during the evaluation. Proceed to next step.

Step 2. Evaluation of the pyrolysis products after analysis of both:
- Gradually heating the sample from 200 to 900 degrees and
- Conventional pyrolysis, in which the new sample is pyrolysed at minimally 3 different temperatures (~300, 600, and 900 degrees)

Step 2.1 Repeat step 1.1, 1.2, and 1.3 for all pyrolysis products reported.

SCHEER, 2016. Tobacco Additives II. https://ec.europa.eu/health/sites/health/files/scientific_committees/scheer/docs/scheer_o_001.pdf
ECHA, 2016. Chapter R.7a. Endpoint specific guidance. https://echa.europa.eu/documents/10162/23047722/ir-csa_r7a_r7-5_rdt_peg_draft_en.pdf/a0a7a777-4cd8-4ee9-92c0-f356b7429a81

Annex II - General approach to assess the addictiveness of tobacco additives

Step 1. Evaluation of the additive in unburned form

Step 1.1 *In silico* testing, taking into account effects on nicotine bioavailability, duration, and concentration in the blood circulation or nicotine-dependent activation of mesolimbic pathways in the brain.

For instance:

1. Nicotinic acetylcholine receptor (nAChR) computer models, integrating protein (sub-)structures, dynamics, and functional relationships
   - α4β2*nACh receptor model
2. Ligand-based monoamine oxidase (MOA) models, providing insight in enzyme selectivity, mechanisms of action and the relationship between MOA inhibitory activity and the molecular structure of several inhibitors
   - QSAR
   - CoMFA
   - 3D-pharmacophores
   - Ligand-network models

Providing information on dependence potential?

No, conflicting results, or data unavailable.This should be reported during the evaluation. Proceed to next step.

Yes. This should be reported during the evaluation.The additive may not meet the requirements of TPD art. 7. Proceed to next step.

Step 1.2 *In vitro* testing, taking into account effects on nicotine bioavailability, duration, and concentration in the blood circulation or nicotine-dependent activation of mesolimbic pathways in the brain.

For instance:

1. 3D lung-on-a-chip combined with mathematical computer models, providing predictive information on lung uptake and particle deposition. This provides insight in additive-dependent altered nicotine uptake
2. Capacity of the additive to change PH values. A higher PH will result in larger amounts of uncharged nicotine and hence better absorption of nicotine by endothelial cells
3. (Inhibition of) the enzymatic activity of MOA.
   - Peroxidase-linked spectrophotometric assay
4. Inhibition of nicotine metabolism (performed by CYP2A6 and CYP2B6 in the liver) increases the bioavailability of nicotine (CYP metabolism inhibitor ratio)
   - *In vitro* analysis using recombinant enzyme or human liver microsomal preparations

Providing information on dependence potential?

No. This should be reported during the evaluation. More data may be required. Proceed to next step.

Yes. This should be reported during the evaluation. The additive may not meet the requirements of TPD art. 7. Proceed to next step.

Step 1.3 (optional) *In vivo* **historical data**, taking into account effects on nicotine bioavailability, duration, and concentration in the blood circulation or nicotine-dependent activation of mesolimbic pathways in the brain.

For instance:

1. Biomarker analysis of nicotine
   - Biomarker analysis of nicotine in blood samples of smokers.
     E.g. alkalizing compounds in cigarretes may increase nicotine uptake
2. Dopamine release and turn over
   - *Ex vivo* or *in vivo* measurement of dopamine release and turn over via isolation of specific brain tissue or microdialysis
3. Observing neurobiological effects (nicotine dependent activation of the mesolimbic pathway) using imaging techniques
   - fMRI
   - PET
   - SPECT
4. Radiotracer for nicotine, to study the pattern of nicotine accumulation in the brain
   - PET with radiolabelled nicotine
5. Radiotracers for α4β2*nACh receptors. Habitual smoking is linked to upregulated α4β2*nACh receptors.
   - Radiolabelled A-85380
   - PET with 2-FA/6-FA
   - SPECT with 5-IA
6. Radiotracer for dopamine receptor
   - PET with [11C]raclopride or [11C]PHNO
   - SPECT with [123I]IBZM
7. Radiotracers for μ-opioid receptors
8. Behavioural responses in rodents
9. Behavioural outcome measured in humans
   - Fagerström Test for Nicotine Dependence (FTND)
   - The cigarette withdrawal scale (CWS-21)

Report study results and proceed to the next step.

Step 2. Evaluation of the pyrolysis products after analysis of both:

- Gradually heating the sample from 200 to 900 degrees and
- Conventional pyrolysis, in which the new sample is pyrolysed at minimally 3 different temperatures (~300, 600, and 900 degrees)

Step 2.1 Repeat step 1.1, 1.2, and 1.3 for all pyrolysis products reported.

SCHEER, 2016. Tobacco Additives II. https://ec.europa.eu/health/sites/health/files/scientific_committees/scheer/docs/scheer_o_001.pdf

Annex III - Guidance document for the reporting of studies on additives subject to increased reporting obligations.

1. Introduction

According to Article 2(2) of the TPD, member states (MS) shall require manufacturers and importers to carry out comprehensive studies for additives in the priority list as published on the 20th May 2016 [1] The studies that are to be carried out in the context of these increased reporting obligations are to be formulated as a report that apriori should include "an executive summary, and a comprehensive overview compiling the available scientific literature on that additive and summarising internal data on the effects of the additive" as outlined by Art. 6(4) of the TPD, which further states that these reports may be peer reviewed by an independent scientific body, in particular as regards their comprehensiveness, methodology and conclusions."

In line with the above and the consensus that checklists and reporting templates are needed to increase aspects of scientific validity the purpose of this guide is to outline the minimum reporting requirements and reporting template for the studies to be requested under Article 6 of the TPD. It is important primarily that this template should allow for the easy understanding of the data submitted, should be clear and concise, mainly though for experts and hence these templates must be structured in a way to aid the regulatory review process.

It is vital that for each of the priority additives, all information collected is included in the report. This includes internal documents and data, as well as open literature on peer-review journals and grey literature (e.g. unpublished reports of studies used for regulatory purposes), including JECFA, EFSA and FEMA documents or data coming from any other regulatory request, in case the additive is used in other contexts. For each part of missing information, verifiable justification should be offered.

The information given in this guide does not describe the requirements to pass the peer review process but should be seen as guide for preparation of the reports which would allow thorough evaluation and derivation of conclusions by the independent scientific body.

2. Background documents.

Overall there are several reporting checklists and guides that are designed to support the reporting of research studies, which were evaluated for their relevance to the TPD. These include, but are not limited to, the following documents:

- COUNCIL REGULATION (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)[2],

[1] Commission Implementing Decision (EU) 2016/787 of 18 May 2016 laying down a priority list of additives contained in cigarettes and roll-your-own tobacco subject to enhanced reporting obligations. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2016.131.01.0088.01.ENG&toc=OJ:L:2016:131:TOC

[2] COUNCIL REGULATION (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration,

- The ECHA report on “Practical Guide 1: How to report in vitro data”.[3]
- The ECHA report on “Practical Guide 3-How to report robust study summaries” [4]
- the Good Cell Culture Practice advices on in vitro experimentation and provides standards for any work involving cell and tissue cultures, including the preparation of cells and tissues derived from humans and animals, characterization and maintenance of important characteristics, quality assurance, recording and reporting, safety, education and training, and ethics. [5]
- Gold Standard Publication Checklist of animal studies[6]
- STROBE[7], which is an initiative to strengthening the reporting of observational studies in epidemiology. STROBE does not make quality assessments but provides a checklist with items that are important to include in reports of observational studies. Multiple extensions of the STROBE statement have now been developed for specific fields of study.
- PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses[8]
- The ARRIVE Guidelines Checklist (Animal Research: Reporting In Vivo Experiments)[9] ARRIVE Guidelines were designed to improve the design, analysis and reporting of research using animals.
- Other guidelines – reporting checklists developed by the EQUATOR[10] network, such as CONSORT[11], RATS, etc. have been developed to standardise the reporting requirements of research within peer reviewed journals. While not all fields are pertinent to the requirements in this specific situation, these too were evaluated for their relevance.

A comprehensive overview of reporting guidelines for various types of studies can be found on the website of the Equator Network: http://www.equator-network.org/

The templates presented on the following pages are based on the above documents, appropriately adapted to the current regulatory context.

Evaluation, Authorisation and Restriction of Chemicals (REACH) http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32008R0440

[3] ECHA Practical Guide 1: How to report in vitro data https://echa.europa.eu/documents/10162/13655/pg_report_in_vitro_data_en.pdf

[4] ECHA Practical Guide 3.How to report robust study summaries - http://echa.europa.eu/documents/10162/13643/pg_report_robust_study_summaries_en.pdf

[5] Coecke S, Balls M, Bowe G, Davis J, Gstraunthaler G, Hartung T, Hay R, Merten OW, Price A, Schechtman L, Stacey G, Stokes W; Second ECVAM Task Force on Good Cell Culture Practice. Altern Lab Anim. 2005 Jun;33(3):261-87

[6] Hooijmans C., de Vries R, Leenaars M, Ritskes-Hoitinga M. The Gold Standard Publication Checklist (GSPC) for improved design, reporting and scientific quality of animal studies GSPC versus ARRIVE guidelines. Lab Anim. 2011 Jan; 45(1): 61.

[7] www.strobe-statement.org

[8] Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med. 2009; 6(7):e1000097. PMID: 19621072

[9] Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 8(6): e1000412. doi:10.1371/journal.pbio.1000412

[10] The Equator Network. http://www.equator-network.org/

[11] Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ 340:c332.

3. Template for the reporting of literature reviews

A comprehensive overview compiling the available scientific literature on that additive and summarizing internal data on the effects of the additive as identified through a systematic review of the literature, a grey literature review and evidence that may be available to the submitter. For this purpose, modified PRISMA guidelines were used as a base.

	Domain	Explanation and/or requested parameters
1	Title	Provide as accurate and concise a description of the content report.
2	Abstract	Structured summary
3	Executive Summary	A longer detailed summary in English and the national language in the MS in which the report is to be submitted to.
4	Objective	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. Provide detail on each of the outcomes that will be explored
	Materials and Methods	The material and methods section should comprehensively cover both the published literature as also the grey literature on that specific additive. For each review design the following should be reported
5	Information sources	Describe all information sources (for systematic reviews provide information on the databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. State in detail the information sources for grey and industry research literature
6	Search criteria	For systematic reviews provide full electronic search strategy for at least one database, including any limits used, such that it could be repeated. For other sources provide criteria as suitable
7	Study Selection	For systematic reviews state the process for selecting studies (i.e., screening, eligibility etc.). A flowchart should be provided.
8	Data collection process	List and define all variables for which data were sought and any assumptions and simplifications made
	Results & Discussion	
9	Primary results	Results presented for every outcome noted in the introduction, grouped by outcome and/or type of test performed. Results should be supported with the information in tabular format.
10	Conclusion	Conclusion that stems from the results in the report
11	References	

4. Template for the reporting of pyrolysis studies

The following checklist/template is provided to aid the reporting of pyrolysis studies that will be used to evaluate if some additive leads to the formation of substances with CMR properties when in its burnt form.

	Domain	Explanation and/or requested parameters
1	Title	Provide as accurate and concise a description of the content report.
2	Abstract	Structured summary
3	Executive Summary	A longer detailed summary in English and the national language in the MS in which the report is to be submitted to.
	Introduction	
4	Background	Scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.
5	Rationale	Explanation of rationale for the study and for the choice of procedures and materials used;
6	Objective	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.
	Materials and Methods	
7	Protocols	Relevant Standard Operating procedures followed
8	Equipment	Equipment used to include all noted parameters including maintenance also analytical parameters as LOD, LOQ, accuracy, precision, etc. of the method
9	Procedure	Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.
10	Exposure parameters	Detailed information on all related exposure parameters related to the additive that is being studied
11	Statistical methods;	Provide details of the statistical methods used for each analysis (if applied)
	Results & Discussion	
12	Primary results	Results presented for the primary outcome including complete qualitative and quantitative assessment of the products created after pyrolysis. Complete diagrammes and results of the qualitative and quantitative analyses are to be provided. Tables and figures should be provided

		Also target analysis, full screening analysis, identification of unknown compounds. Characterization of compound toxicity, exposure data to each compound-inhalation, relative reference data
13	Secondary results	Special focus should be given to the potential interactions that may take place within substances produced after pyrolysis
14	Conclusion	Conclusion that stems from the results in the report
15	References	

5. Template for the reporting of in vitro/in silica studies

	Domain	Explanation and/or requested parameters
1	Title	Provide as accurate and concise a description of the content report.
2	Abstract	Structured summary
3	Executive Summary	A longer detailed summary in English and the national language in the MS in which the report is to be submitted to.
	Introduction	Overall this should be very limited in size, simply to provide a basic introduction into the following noted domains
4	Background	Scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.
5	Rationale	Explanation of rationale for the study and for the choice of procedures and materials used;
6	Objective	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.
	Materials and Methods	
7	Origin	Type of culture, Cell/tissue type, Species
8	Protocols	Relevant Standard Operating procedures (OECD or EU) followed
9	Equipment	Equipment used including all noted parameters including maintenance
10	Design	Planning and experimental design, including endpoints and measures to assess them.
11	Procedure, maintenance and handling	Culture media (including all supplements and additives, antibiotics); Culture substrate, medium change parameters, transport solution; Surface coating, subculture detachment solutions; Maintenance conditions, storage conditions; procedures for preparation or use of cells or tissues Ethical statement (if applicable)
12	Exposure parameters	Detailed information on all related exposure parameters including but not limited to time, dose, route, and justification of the exposure etc.
13	Statistical methods;	Provide details of the statistical methods used for each analysis
	Results	Tables and figures should be provided
14	Baseline data	Baseline information

15	Primary results	Results presented for the primary outcome
16	Secondary results	Secondary outcomes
17	Sensitivity analyses	Potential sensitivity or additional analyses
	Discussion	
18	Comparability	Comparison of main findings with existing literature
19	Adequacy	adequacy and suitability of the in vitro method
20	Limitations	Potential biases Deviations from predefined protocols or SOPs Dropouts or exclusion of lines or of individual outcomes
21	Conclusion	Conclusion that stems from the results in the report
22	References	

6. Template for the reporting of in vivo studies

The template for the reporting of in vivo experiments is slightly adapted from a well-received guidelines checklist.[12] New animal studies are discouraged, this template is to be used solely for reporting of historical data.

	Domain	Explanation and/or requested parameters
1	Title	Provide as accurate and concise a description of the content report.
2	Abstract	Structured summary
3	Executive Summary	A longer detailed summary in English and the national language in the MS in which the report is to be submitted to.
	Introduction	Overall this should be very limited in size, simply to provide a basic introduction into the following noted domains
4	Background	Scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.
5	Rationale	Explanation of rationale for the study and for the choice of procedures and materials used;
6	Objective	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.
	Materials and Methods	
7	Ethical statement	Indicate the nature of the ethical review permissions, relevant licenses (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research
8	Study design	a. The number of experimental and control groups. b. Any steps taken to minimize the effects of subjective bias when allocating animals to treatment (e.g. randomization procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.
9	Experimental procedures	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of

[12] Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 8(6): e1000412. doi:10.1371/journal.pbio.1000412

		administration, anesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anesthetic, route of administration, drug dose used).
10	Experimental animals	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.
11	Housing and husbandry	a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; b. Husbandry conditions (e.g. breeding program, light/dark cycle, temperature, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.
12	Sample size	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.
13	Allocating animals to experimental groups	a. Give full details of how animals were allocated to experimental groups, including randomization or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.
14	Experimental outcomes	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes), and method used to assess them (kit, supplier).
15	Statistical methods	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the

		assumptions of the statistical approach.
	Results	Tables and figures should be provided
16	Baseline data	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing (this information can often be tabulated).
17	Numbers analysed	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%). b. If any animals or data were not included in the analysis, explain why
18	Outcomes and estimation	Report the results for each analysis carried out, with a measure of precision (E.g. standard error or confidence interval).
19	Adverse events	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.
	Discussion	
20	Comparability	Comparison of main findings with existing literature
21	Adequacy	adequacy and suitability of the in vitro method
22	Limitations	Potential biases Deviations from predefined protocols or SOPs limitations of the animal model and potential imprecision
23	Conclusion	Conclusion that stems from the results in the report
24	References	

7. Other issues

In addition to the above, it is important to outline additional parameters that would facilitate the peer review process which include the following:

Language: Since the independent peer review panels are most likely to have a broad geographical variation, the reports should be prepared in English so as to facilitate international peer review. On the other hand, the executive summary and abstract should also be provided in the national language of each EU MS when submitted to the relevant competent authority of that EU MS.

Definitions of terminology within the reports: Within the reports, terms used for the first time should be provided with definitions. In all other cases definitions of terms referred to should be taken from previous European Commission documents, including REACH “Guidance on information requirements and chemical safety assessment”.

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