COM/12/S2

INTERIM GUIDANCE ON A STRATEGY FOR GENOTOXICITY TESTING AND MUTAGENIC HAZARD ASSESSMENT OF IMPURITIES IN CHEMICAL SUBSTANCES (April2012)

I. Preface

1. The Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) is an expert advisory committee whose terms of reference include advice on the principles of genotoxicity testing and assessment. The COM has published guidance on a strategy for testing and mutagenic hazard assessment of chemical substances (http://www.iacom.org.uk/guidstate/documents/COMGuidanceFINAL.pdf). The COM has been asked to advise on the need for a generic strategy to test and evaluate the genotoxicity of impurities present in chemical substances. The COM has not previously published guidance on impurities.

2. In this document the term impurity* relates to an unintended constituent present in a substance as produced. Impurities are a specific form of contaminant in that they are linked to the substance of interest because they may originate from the starting materials or be the result of secondary or incomplete reactions during the production process. Impurities may also result from degradation of the substance, for example, during storage. While it is present in the final substance the impurity(ies) was not intentionally added. [*http://echa.europa.eu/documents/10162/17235/substance_id_en.pdf ECHA Guidance for identification and naming of substances under REACH]

3. This interim guidance is intended to provide advice on identifying impurities which require a genotoxicity assessment, and the approach to be taken for such an evaluation. The Committee may choose to reconsider the subject of testing and evaluation of genotoxic impurities when the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) publishes its guidance on this subject.

II. Introduction

4. The presence and potential mutagenicity of impurities has been investigated for a wide range of chemical substances including pharmaceuticals[1,2], pesticides[3,4], food additives[5] and chemicals such as dyes with a number of uses (e.g. triphenylmethane dyes [6] and hair dye HC Blue 1 [7]). Genotoxicity tests have been used to monitor the purification of chemicals to remove genotoxic impurities[6,7], to investigate the potential genotoxicity of specific impurities isolated from substances[8], and to test samples of substances for the presence of genotoxins.[4,9] The genotoxicity testing strategy adopted to assess impurities can vary widely and needs to be designed on a case-by-case basis. Testing strategies have included both *in vitro* [6-13] and *in vitro/in vivo* genotoxicity tests.[6,9,13]

5. The approaches used for genotoxicity testing and evaluation of impurities vary between different chemical sectors (such as pharmaceuticals and pesticides). This reflects the differing risk/benefit assessments for these chemicals. Published approaches to testing and evaluation of impurities in pharmaceuticals have suggested using (Q)SAR (Quantitative Structure Activity Relationships) and the Ames bacterial mutagenicity test as the initial steps.[14,15] When assessing new or increased levels of impurities in a pesticide from a new source, the basic requirements (based on genotoxicity tests with the active ingredient manufactured to the technical specification) are: an Ames test when impurities are present between 0.1% and 1% and three *in vitro* assays (typically the Ames test, an *in vitro* chromosome aberration test and a mammalian cell gene mutation test) when impurities are present at 1% and above.

III. Strategy for genotoxicity assessment of impurities in chemical substances

Introduction

6. The genotoxicity assessment of impurities can be undertaken when the genotoxicity of the chemical is under investigation and also in situations when there is a need to compare impurities in two or more chemical substances.

An example of the latter situation is the assessment by regulatory agencies of the equivalence of a chemical substance sourced from different manufacturers. A case-by-case approach is recommended for the identification of impurities requiring genotoxicity assessment. It is assumed that, where possible, the structure of all impurities requiring genotoxicity assessment is known.

Selection of impuritiy(ies) for genotoxicity assessment.

7. The concept of a threshold of toxicological concern (TTC) was originally developed to define a common exposure level for any unstudied chemical which would not pose an unacceptable risk of carcinogenicity or other toxic effects.[16] It was extended by Kroes et al (2004) to be a robust and conservative approach for the selection of impurities requiring genotoxicity assessment if their exposures exceed 0.15 µg/person per day (0.0025 µg/kg bw/day for a 60 Kg adult).[17] The TTC does not imply that the mode of action of a genotoxic substance is thresholded. It is applicable to substances with good exposure assessment information, which have a known chemical structure which includes structural alert(s) for genotoxicity, but for which there are little or no relevant toxicity data. The Committee endorses this formulation of the TTC approach for screening and priority setting for impurities. In the context of mutagenicity testing, the Committees agrees with Kroes et al* that the TTC approach is not appropriate (and, therefore, should not be applied) for certain classes of genotoxicants that are particularly potent carcinogens, namely aflatoxin-like, *N*-nitroso compounds or azoxy compounds[17,18] . It is assumed that impurities with such structures would be potential mutagens.

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*Kroes et al,[17] full list of exceptions to the use of the TTC in general including their "cohort of concern" is:

- High potency carcinogens (e.g. aflatoxin-like, azoxy- or N-nitroso substances)
- Steroids
- Inorganic substances
- Metals, including essential metals
- Polymers, oligomers
- Proteins
- Substances known/predicted to bioaccumulate (e.g. polyhalogenated-dibenzodioxins, -dibenzofurans, -biphenyls)
- Insoluble nanomaterials
- Radioactive substances
- Substances likely to exert local effects (on GI tract, respiratory tract or skin)

8. In situations where there are multiple impurities, it may be appropriate to sum the estimated exposure for those impurities that contain the same structural alert for mutagenicity and then to compare this with the TTC for genotoxicants in order to reach a decision on which impurities require a genotoxicity evaluation. Thus, for example, it would be acceptable to sum exposures to impurities with epoxide groups. This approach implies that it would be necessary to undertake a genotoxicity assessment for all impurities included in a group containing the same structural alert and where the sum total exposure cannot be confirmed to be below the TTC.

9. In situations where it is not possible to undertake an estimation of exposure or where the structure of the impurity has not been or cannot be determined then a pragmatic cut off concentration of 0.1% can be used as a guide for priority setting for genotoxicity assessment.[19] This advice has been taken from the guidance document on the assessment of the equivalence of technical materials of pesticides regulated under regulation (EC) No 1107/2009 and represents a pragmatic approach which could be applied to all chemicals.

Approach to genotoxicity assessment

10. All impurities selected for genotoxicity assessment should, if possible, have their structures identified and be subject to a (Q)SAR evaluation. In this document (Q)SAR evaluation refers to the application of (Q)SAR models and/or knowledge-based SAR models appropriate to genotoxicity evaluation. Genotoxicity testing of isolated or synthesised impurities should be undertaken where a (Q)SAR evaluation indicates potential for mutagenicity and should include an Ames test and an *in vitro* micronucleus (MNvit) test. In situations where the structure of the impurity(ies) is unknown, then the first step for any impurity selected for genotoxicity assessment would be to undertake an Ames test and a MNvit test. The Committee considers that there are inherent limitations regarding the sensitivity of these assays to detect a dose-related genotoxic response when the impurity is tested when

present at a low level of the technical substance (or material spiked with the identified impurity).[20,21,22] Thus, the Committee recommends, where practical, that any testing should be undertaken with the isolated or synthesised impurity rather than the technical substance. The strategy for genotoxicity testing and assessment of impurities in chemical substances is given in Figure 1.

11. A case-by-case assessment of the results of the testing should be undertaken. Thus, for example, a (Q)SAR alert may not always be overruled by just a negative Ames test because there are classes of genotoxic chemicals that are poorly or not detected in the Ames test. Hence the need for both an Ames test and the MNvit test.

Genotoxicity equivalence of chemical substances

12.. An approach to the assessment of the genotoxic equivalence of chemical substances is shown in Figure 2. In this figure, the term “test substance (new)” refers to the new specification or technical material. The term “comparator substance” refers to the substance to which comparisons of the impurity profile and/or levels of impurities are being made. The use of the Threshold of Toxicological Concern (TTC) concept (as outlined in paragraph 7) and pragmatic cut off limit of 0.1% (as outlined in paragraph 9) can also be used as a guide to selection of those impurities that require genotoxicity assessment when comparing the impurities present in two or more chemical substances. All impurities which require genotoxicity assessment, identified from a comparison of two or more substances, should be subjected to a (Q)SAR evaluation and a decision made as to whether genotoxicity testing of such impurities using the Ames test and the MNvit test as shown in Figure 1 is needed. As above, genotoxicity testing should be undertaken using the isolated or synthesised impurity rather than the new test substance.

VI. Conclusion

13. The genotoxicity assessment of impurities present in chemical substances is guided by knowledge of the structure, estimated exposure and

the application of the TTC concept to select impurities which require evaluation. In situations where it is not possible to undertake an estimation of exposure or the structure of the impurity has not been or cannot be determined then a pragmatic cut off concentration of 0.1% can be used as a guide for priority setting for genotoxicity assessment The genotoxicity testing strategy needs to be derived on a case-by-case basis but should, where the structure of the impurity is known, include (Q)SAR evaluation of impurities selected for genotoxicity assessment, coupled with expert judgement and reference to genotoxicity data on similar substances. Genotoxicity testing of isolated or synthesised impurities should be undertaken where a (Q)SAR evaluation indicates potential for mutagenicity, and where exposure cannot be confirmed to be below the TTC, and should include an Ames test and an *in vitro* micronucleus (MNvit) test. In situations where the structure of the impurity has not been or cannot be determined and is unknown, and where exposure cannot be confirmed to be below the TTC, then the first step in the evaluation for impurities selected for genotoxicity assessment should be to conduct an Ames test and an MNvit test. If the available evidence suggests that an impurity should be considered to be mutagenic then levels should be controlled to as low as reasonably practical. .

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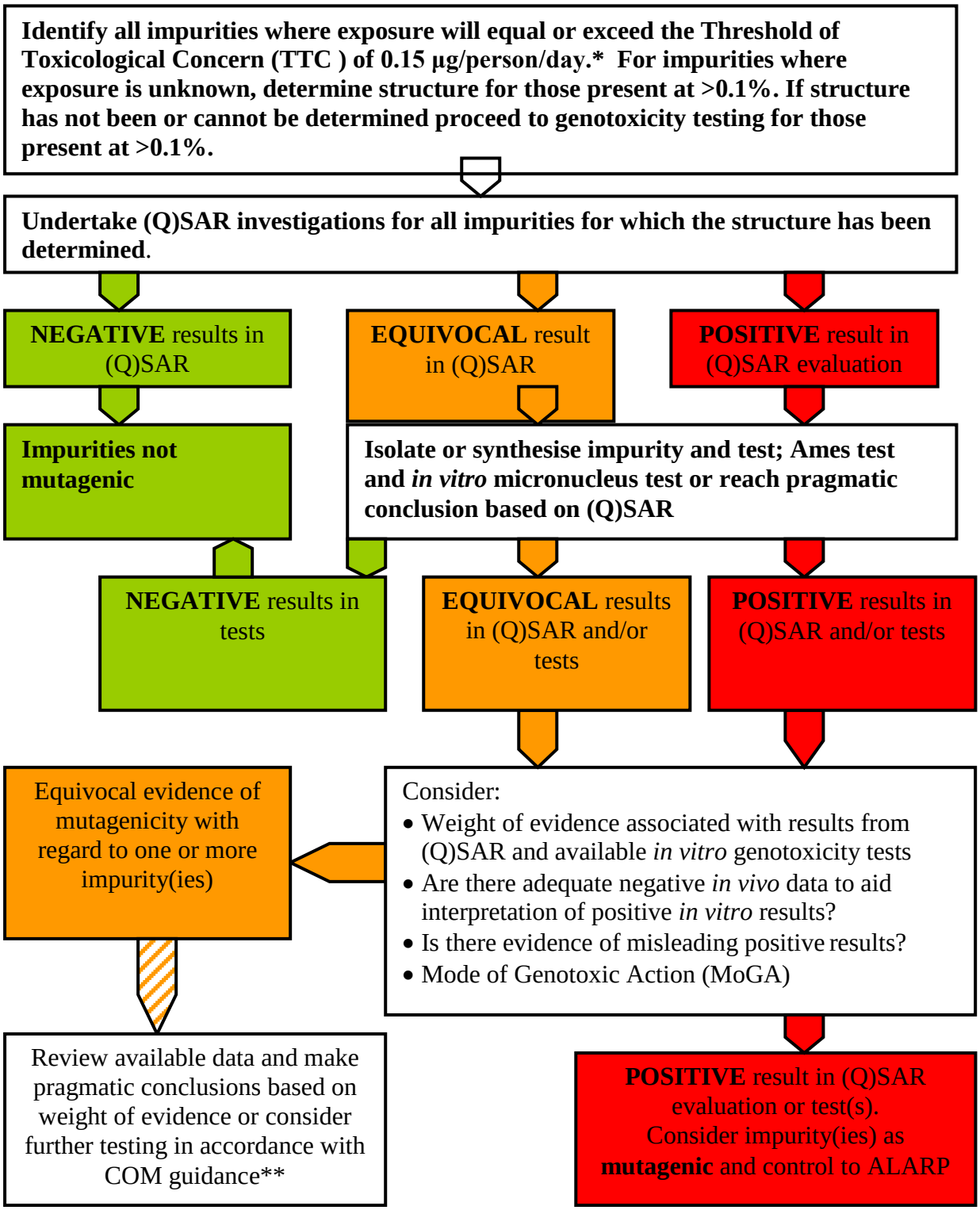
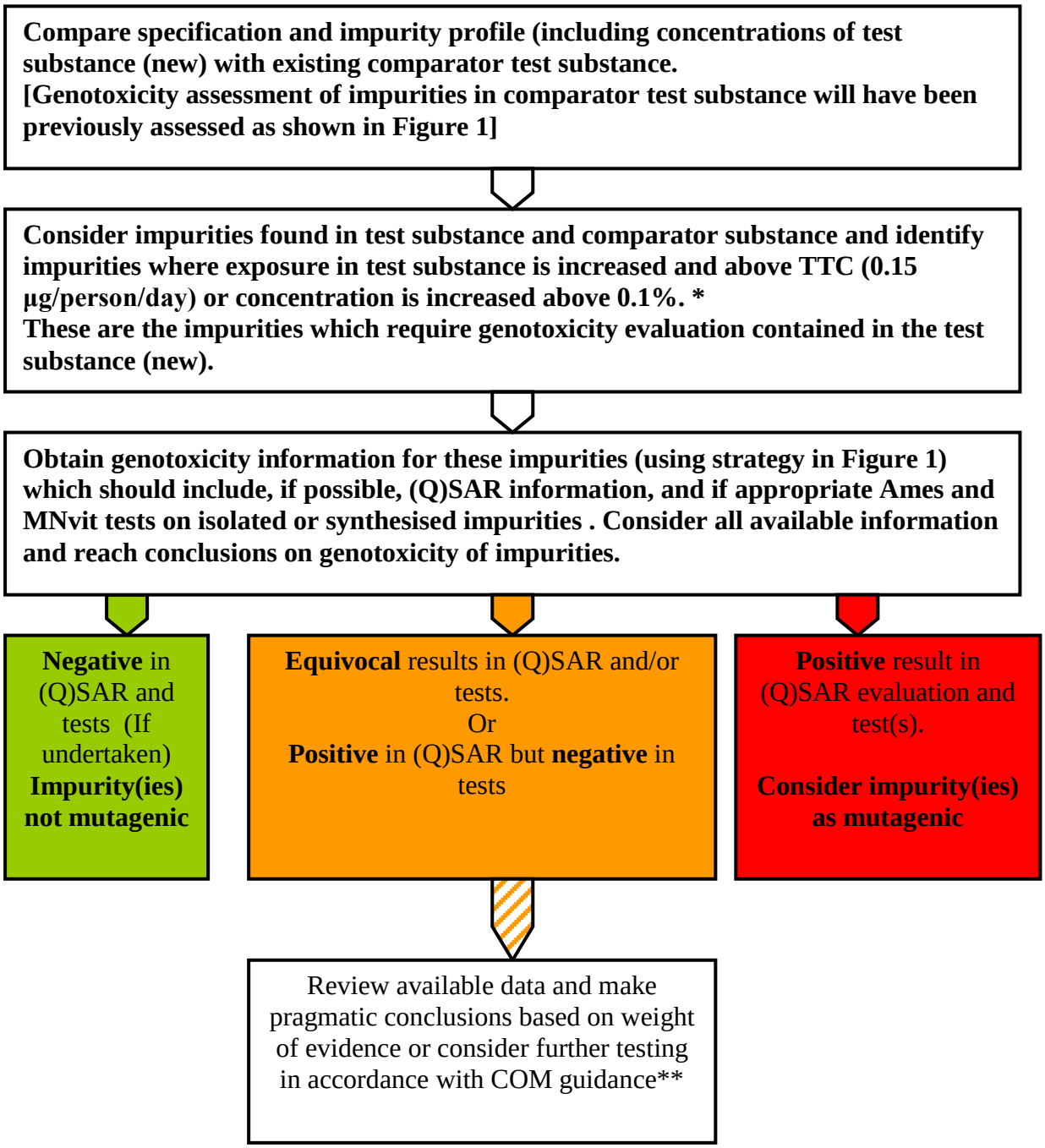
Figure 1: Strategy for the Genotoxicity Assessment of impurities in test substances



*[Impurities giving rise to exposures below TTC are considered to present negligible risk]
Impurities which are aflatoxin-like, *N*-nitroso and azoxy- compounds pose a risk at exposures below the TTC and should be considered as mutagenic. It would be appropriate to sum the exposures for impurities with the same structural alert for mutagenicity.
**http://www.iacom.org.uk/guidstate/documents/COMGuidanceFINAL.pdf

Figure 2: Strategy for the Genotoxicity Assessment of equivalence between two test substances



* * Impurities which are aflatoxin-like, *N*-nitroso and azoxy- compounds pose a risk at exposures below the TTC and should be considered as mutagenic . It would be appropriate to sum the exposures for impurities with the same structural alert for mutagenicity.

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