Committee on MUTAGENICITY

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COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

Revised GUIDANCE ON A STRATEGY FOR GENOTOXICITY TESTING AND MUTAGENIC HAZARD ASSESSMENT OF IMPURITIES IN CHEMICAL SUBSTANCES (November 2020)

Consideration and comments of the updated interim COM document 'Guidance on a strategy for genotoxicity testing and mutagenic hazard assessment of impurities in chemical substances'.
Members are asked to complete review of this revised interim draft as attached and consider the following questions:

1. Do members agree with the strategy presented?
2. Can members advise whether the statement in paragraph 19 (a pragmatic cut off concentration of 0.1% can be used as a guide or priority setting for genotoxicity assessment) is still correct. When comparing two or more chemicals should this be stated as 0.1% for each.
3. Can members provide a definition for 'genotoxic equivalence' (paragraph 22) that can be added to the text.
4. Members are asked to consider whether Figures 1 and 2 are still appropriate/correct.
5. Are there any other aspects which should be included within the updated guideline document?
6. Can this be released as COM guidance?

Secretariat
November 2020

Committee on MUTAGENICITY

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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 (https://www.gov.uk/government/publications/a-strategy-for-testing-of-chemicals-for-genotoxicity). (~~http://www.iacom.org.uk/guidstate/documents/COMGuidanceFINAL.pdf~~

2. As part of this overall strategy, an interim guidance specifically detailing a generic strategy to test and evaluate the genotoxicity of impurities present in chemical substances was published in 2012 (COM, 2012). The term 'chemical substance' is used to mean the test substance in which the genotoxicity of the impurities is in question. A number of initiatives in this area have been reported since 2012 and are documented here.

3. In this document the term impurity relates to that defined by ECHA as an 'unintended constituent present in a substance as manufactured. It may originate from the starting materials or be the result of secondary or incomplete reactions during the manufacture process. While it is present in the final substance it was not intentionally added' (ECHA, 2017).

4. This document provides guidance on identifying impurities for which an assessment of genotoxicity is required, and the approach to be taken in doing so.

II. Introduction

5. The presence and potential mutagenicity of impurities has been investigated for a wide range of chemical substances including pharmaceuticals (Sobol et al., 2007; Elder et al., 2010), pesticides (Blasiak et al., 1999; Sarrif et al., 1994) , food additives (Herbold, 1981) and chemicals such as dyes with a number of uses (e.g. triphenylmethane dyes (Lin and Brusick, 1992) and hair dye HC Blue (Sobol et al., 2007; Abu-Shakra et al., 1991). Genotoxicity tests have been used to monitor the purification of chemicals to remove genotoxic impurities (Lin and Brusick, 1992; Abu-Shakra et al., 1991), to investigate the potential genotoxicity of specific impurities isolated from substances (Agarwal et al., 2004) , and to test samples of substances for the presence of genotoxins (Sarrif et al., 1994; Fox et al., 1996). The genotoxicity testing strategy adopted to assess impurities can vary widely and needs to be designed on a case-by-case basis.

6. Testing strategies have included both *in vitro* (Lin and Brusick, 1992; Abu-Shakra et al., 1991; Agarwal et al., 2004; Fox et al., 1996; Basu and Marnett, 1983; Eder et al., 2006; Quinto et al., 1980; Proudlock et al., 2004) and in vitro/in vivo genotoxicity tests (Lin and Brusick, 1992; Fox et al., 1996; Proudlock et al., 2004).

7. The approaches used for the genotoxicity testing and evaluation of impurities vary between different chemical sectors (for example, pharmaceuticals and pesticides). This reflects the differing risk/benefit assessment for these types of chemicals.

Impurities in Pharmaceuticals

8. With regards to the genotoxicity testing of new pharmaceuticals, guidance has been issued by The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (ICH, 2015). The purpose of the ICH guideline is to provide a practical framework that is applicable to the identification, categorisation, qualification, and control of mutagenic[1] impurities to limit potential carcinogenic risk.

9. The process of hazard assessment recommended by ICH for actual and potential impurities starts with classification as to mutagenic and carcinogenic potential (Classes 1 – 5). This is achieved through conducting database and literature searches, with known mutagenic carcinogens considered as Class 1. If data is unavailable, an assessment of (Quantitative) Structure-Activity Relationships ((Q)SAR) with a focus on bacterial mutagenicity predictions is recommended (Brams et al.,1987). Two types of (Q)SAR prediction methodologies are advised, one using expert rule-based methodology and the second statistical-based methodology. If no structural alerts are found from the two complementary (Q)SAR methodologies, ICH consider that this is sufficient to conclude that the impurity is of no mutagenic concern, and no further testing is recommended (Classes 3, 4 or 5).

10. Where a relevant structural alert is obtained using (Q)SAR prediction, control measures can be applied, or a bacterial mutagenicity assay carried out to a fully adequate protocol (ICH S2(R1) and OECD 471), with the impurity only, is recommended. A negative assay carried out to adequate protocols is considered by ICH as sufficient to override (Q)SAR predictions and the impurity is classed as non-mutagenic (Class 5) with no further assessment needed.

11. A positive bacterial mutagenicity result from an assay carried out to a fully adequate protocol is considered to require further assessment and/or control

[1] Defined as DNA reactive substances that have a potential to directly cause DNA damage when present at low levels leading to mutations and therefore, potentially causing cancer.

measures (Class 2). If control measures cannot be applied the impurity is required to be tested in an in vivo gene mutation assay, i.e. Transgenic mutation assay; Pig-a assay (blood); Micronucleus test (blood or bone marrow); Rat liver Unscheduled DNA Synthesis (UDS) test; or Comet assay. Guidance of the choice of in vivo assay is given based on scientifically justified knowledge of the mechanism of action of the impurity and expected target tissue exposure. All in vivo studies are to be conducted under ICH genotoxicity Guidelines and results may be used to support the setting of compound specific impurity limits.

Impurities in Pesticides

12. Wherever practicable, genotoxicity studies conducted for the registration of a technical grade active ingredient (TGAI) should be performed using material conforming to registered / to be registered specifications (including any impurities) (EU, 2013). However, if it becomes necessary to retest material due to changes in manufacturing or in the source of material, which may differ in the level/types of impurities present, a tiered approach is recommended for genotoxicity testing (APVMA, 2015). This is outlined in paragraphs 13 – 15below.

13. A suitable process is suggested as (Q)SAR followed by the use of **two** well-validated in vitro genotoxicity assays. The first to detect point mutations (base-pair substitution and frameshift) in a microbial assay (for example, salmonella reverse mutation test), with and without the use of appropriate metabolic activation systems. The second to demonstrate chromosome damage in an in vitro mammalian cell assay (for example, Chinese hamster ovary/HGPRT assay), with and without the use of appropriate metabolic activation systems.

14. A positive result in either in vitro test triggers further evaluation using **two** *in vivo* tests (in rats or mice) to characterise the genotoxic potential in somatic cells. Three assays are recommended that demonstrate: production of cytogenetic damage (for example, micronuclei) in the bone marrow or other proliferative cells of intact animals; genotoxic damage, involving other than cytogenetic endpoints (for example, unscheduled DNA synthesis or P32-post-labelling adduct formation) and preferably in a suspect or known target tissue for the substance; and mutations in transgenic rats or mice that have transgenes containing reported genes for the detection of various types of mutations in somatic tissues.

15. A positive result in **one** in vivo test in somatic cells triggers further evaluation using **one** in vivo test (in rats, mice or hamsters) to assess genotoxic potential *in vivo* in germ cells. Three assays are recommended that demonstrate: a dominant lethal event in a germ cell that does not cause dysfunction of the gamete, but which is lethal to the fertilised egg or developing embryo; the production of

chromosome aberrations in spermatogonial cells; mutations in transgenic rats or mice that have transgenes containing reporter genes for the detection of various types of mutations through the germ line.

III. COM strategy for genotoxicity assessment of impurities in chemical substances

Introduction

15. 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. Where possible, the structure of all impurities requiring genotoxicity assessment should be known.

Selection of impurity(ies) for genotoxicity assessment.

16. The concept of a threshold of toxicological concern (TTC) was originally developed to define a common exposure level via the diet for any unstudied chemical which would not pose an unacceptable risk of carcinogenicity or other toxic effects (Munro et al., 1999). 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). The TTC does not infer that the mode of action of a genotoxic substance is thresholded. It is applicable to substances present at low levels in the diet (i.e. there is 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 COM endorses this formulation of the TTC approach for screening and priority setting for impurities. In the context of mutagenicity testing, the COM agrees with EFSA (2019a) that the TTC approach is not appropriate (and, therefore, should not be applied) for certain classes of genotoxins and genotoxicants that are particularly potent carcinogens, namely aflatoxin-like, azoxy or-*N*-nitroso compounds and benzidines. It is assumed that impurities with such structures would be potential mutagens.

17. In situations where there are multiple impurities, for example in a mixture, application of the TTC approach is dependent on the type and level of characterisation and needs to be considered on a case-by-case basis.

18. For mixtures that have a fully defined chemical composition and have a similar mode of action, dose addition is recommended (EFSA, 2019b). This can then be compared with the TTC for genotoxicants 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.

19. 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 (EC, 2011). 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.

COM Approach to genotoxicity assessment

20. 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 statistical and/or knowledge-based 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 COM 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 in the technical substance (or material spiked with the identified impurity) (Cyr et al., 2005; Kenyon et al., 2007; Glowienke et al. 2004). Thus, the COM 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.

21. 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

22. 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 TTC concept (as outlined in paragraph 16) and pragmatic cut off limit of 0.1% (as outlined in paragraph 19) 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

23. 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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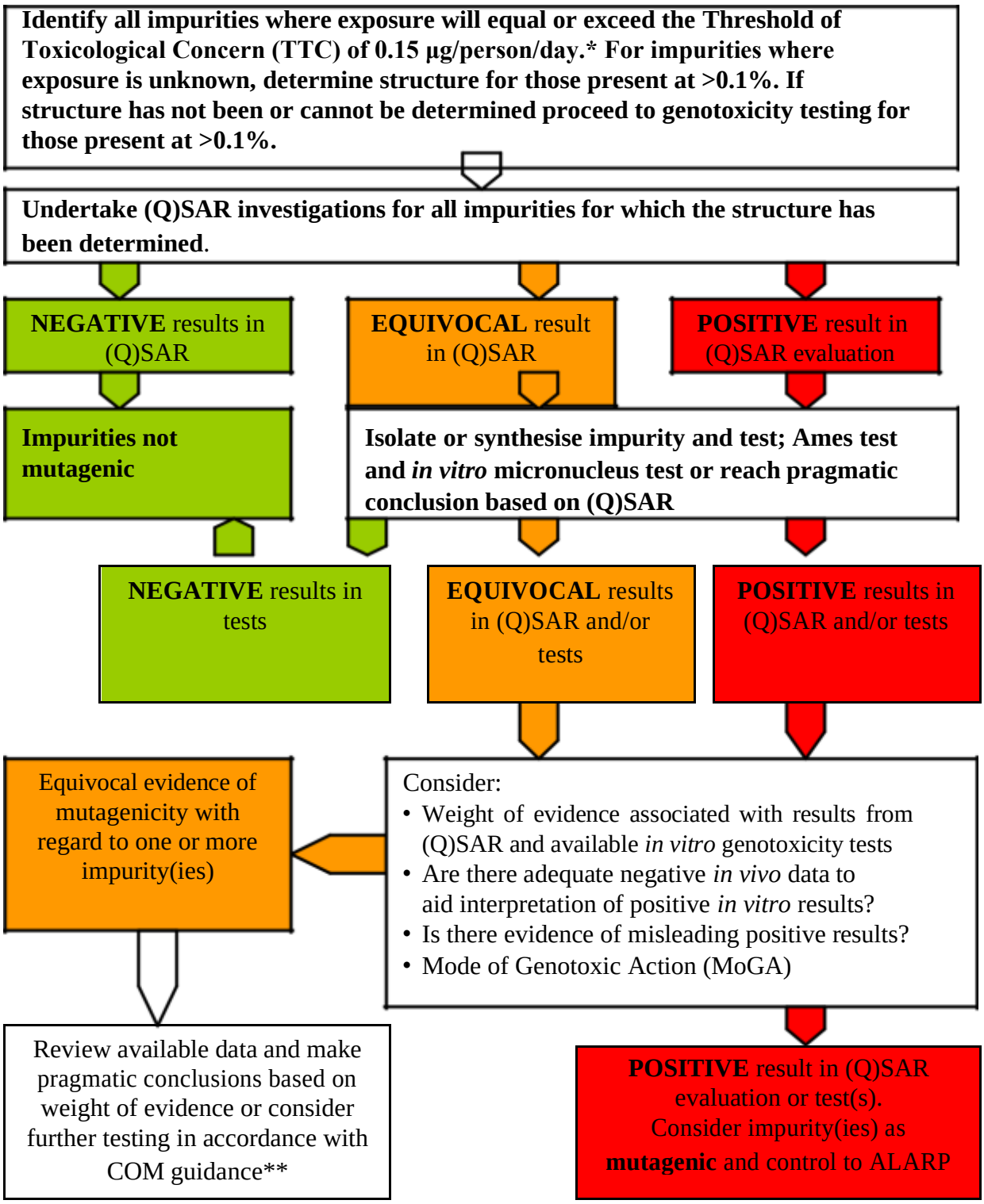
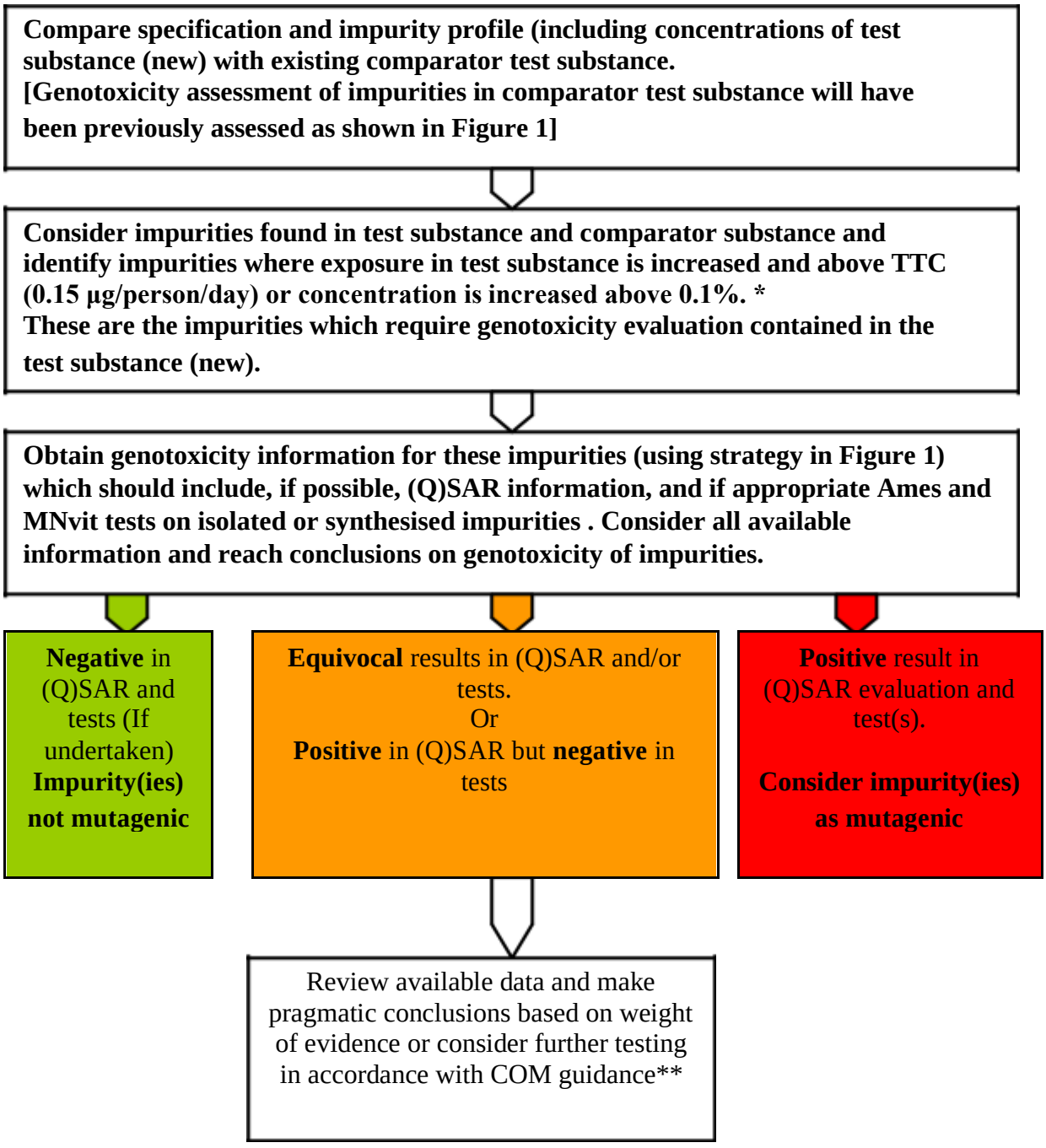
Figure 1: Strategy for the Genotoxicity Assessment of impurities in test substances



*[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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