

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

**Draft interim guidance on impurities**

1. Members considered a first draft guidance document on approaches to the genotoxicity testing and evaluation of impurities at the June 2011 COM meetings. It was noted that finalisation of any guidance should await publication of advice from other international groups (in this instance ICH). Members agreed that some interim advice could be given to Government Departments. Essentially with respect to the role that QSAR approaches and the use of TTC for priority setting could be applied to impurities
2. A revised document is appended as Annex 1 (which contains 2 figures).
3. Members are asked to comment on the appended papers. .

**Secretariat September 2011**

## **Annex 1 to MUT/2011/15**

### **Second draft**

# **INTERIM GUIDANCE ON A STRATEGY FOR GENOTOXICITY TESTING AND MUTAGENIC HAZARD ASSESSMENT OF IMPURITIES IN CHEMICAL SUBSTANCES**

## **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 have not previously published guidance on impurities.

2. In this document the term impurity\* relates to an unintended constituent present in a substance as produced. It may originate from the starting materials or be the result of secondary or incomplete reactions during the production process or may result from degradation of the substance, for example, during storage. While it is present in the final substance it was not intentionally added.

[\*[http://guidance.echa.europa.eu/docs/guidance\\_document/substance\\_id\\_en.pdf](http://guidance.echa.europa.eu/docs/guidance_document/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.

## II. Introduction

4. The presence of mutagenic impurities has been investigated for a wide range of chemical substances including pharmaceuticals (e.g. alkyl halides and esters with alkylating activity<sup>1</sup> and hydrazine, hydrazides and hydrazones<sup>2</sup>), pesticides (e.g. malathion<sup>3</sup> and benomyl and carbendazim<sup>4</sup>), food additives (e.g. saccharin<sup>5</sup>) and chemicals such as dyes with a wide number of uses (e.g. triphenylmethane dyes<sup>6</sup> and hair dye HC Blue 1<sup>7</sup>). Genotoxicity tests have been used to monitor the purification of chemicals to remove genotoxic impurities<sup>6,7</sup>, to investigate the potential genotoxicity of specific impurities isolated from substances<sup>8</sup>, and to test samples of substances for the presence of genotoxins.<sup>4,9</sup> 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*<sup>6,13</sup> and *invitro/invivo* genotoxicity tests.<sup>6,9,13</sup> Published approaches to testing and evaluation of impurities in pharmaceuticals have suggested using QSAR and Ames test as the initial steps.<sup>14,15</sup> Negative results for QSAR are sufficient to eliminate concern that an impurity of a pharmaceutical substance is genotoxic. Negative results for the Ames test would eliminate concerns of genotoxicity for an impurity that was positive for QSAR. Thus in most cases, QSAR and Ames test are sufficient to reach a conclusion on the genotoxicity of impurities in pharmaceuticals.<sup>15</sup> However, if the Ames test is positive, either further testing is needed of the impurity has to be removed or controlled to pre-defined limits.

## III. Strategy for genotoxicity assessment of impurities in chemical substances

5. 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 and quantification of levels. The Threshold of

Toxicological Concern (TTC) concept for genotoxicants (0.15 µg/person per day, 0.0025 µg/kg bw/day for a 60 Kg adult for non-pharmaceuticals) can be used as a pragmatic guide to selection of impurities requiring genotoxicity assessment.<sup>16,17</sup> thus it would be prudent to assume genotoxic potential for these particular groups of compounds. The TTC level given above is not appropriate and therefore should not be applied to certain classes of genotoxicants that are particularly potent carcinogens, namely aflatoxin-like, *N*-nitroso compounds or azoxy compounds.<sup>18,19</sup> All impurities selected for genotoxicity assessment should be subject to a QSAR evaluation. The chemicals undergoing QSAR must be within the applicability domain of the model used. Genotoxicity testing of isolated impurities should be undertaken where a QSAR evaluation indicates potential for mutagenicity and should include an Ames test and an *in vitro* micronucleus test (MNvit). The Committee recommends that any testing should be undertaken with the isolated impurity rather than the technical substance. The strategy for genotoxicity testing and assessment of impurities in chemical substances is given in Figure 1.

6. An approach to assessment of genotoxicity equivalence of chemical substances is provided 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 impurity profile and/or levels of impurities are being made. The use of the Threshold of Toxicological Concern (TTC) concept (as outlined in paragraph 3) can also be used as a pragmatic guide to selection of impurities which require genotoxicity assessment when comparing the impurities present in two or more chemical substances. Thus new or increased exposures to impurities at intakes  $\geq 0.15\mu\text{g/day}$  would require further investigation. The exceptions are genotoxicants known to be potent carcinogens, namely aflatoxin-like, *N*-nitroso compounds or azoxy compounds.<sup>16,17</sup> All impurities which require genotoxicity assessment, identified from a comparison of two or more substances, should be subjected to a QSAR evaluation and a decision made as to whether genotoxicity testing of such impurities using the Ames

test and MNvit as shown in Figure 1 is needed. Genotoxicity testing should be undertaken using the isolated impurity rather than the new test substance.

## VI Conclusion

7. The genotoxicity assessment of impurities present in chemical substances is guided by the application of the TTC concept to select impurities which require evaluation. The testing strategy needs to be derived on a case-by-case basis but should as a minimum include QSAR evaluation of impurities selected for genotoxicity assessment.

**September 2011**

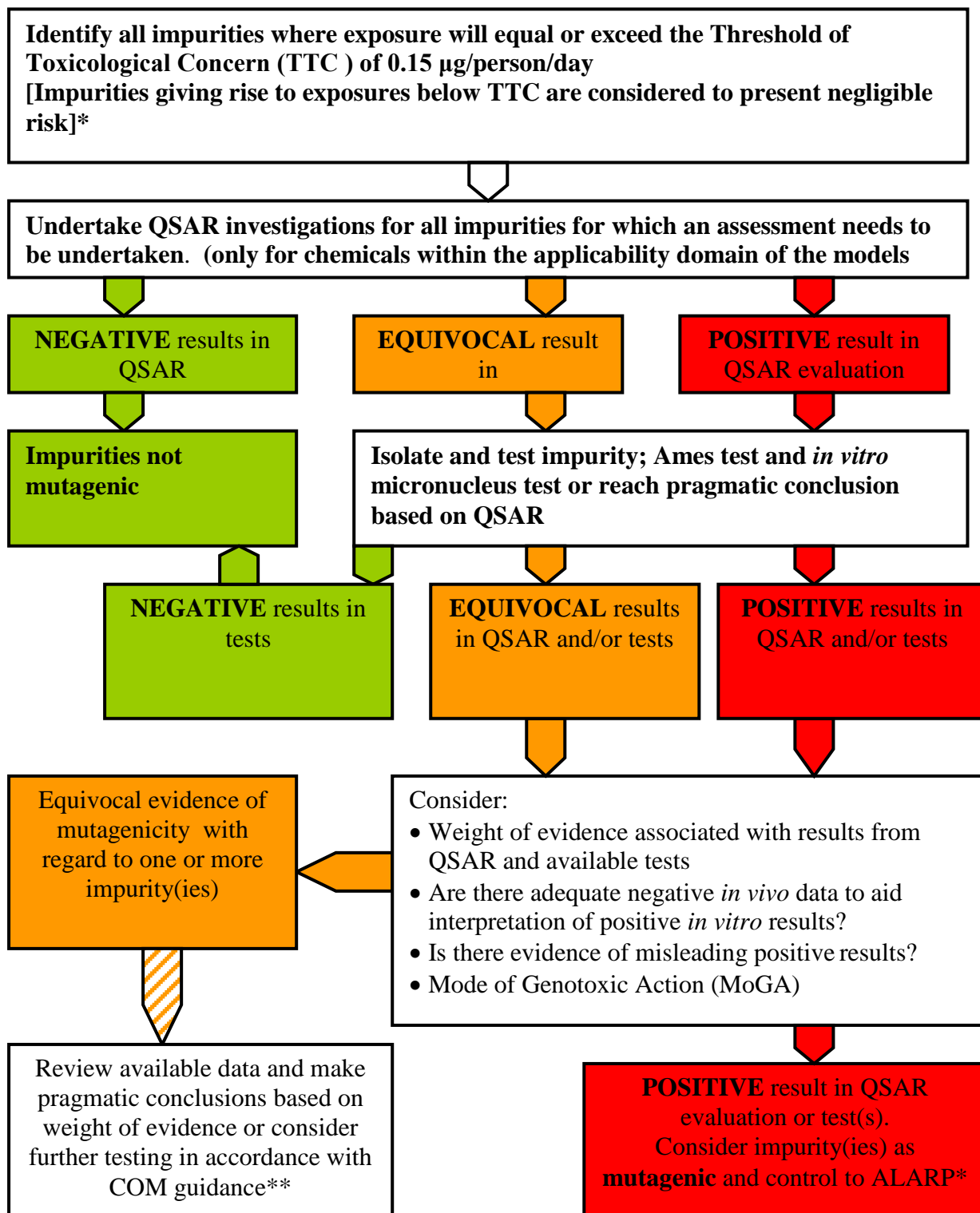
### Reference List

1. Sobol Z., Engel M.E., Rubitski E., Ku W.W., Aubrecht J., and Schiestl R.H. (2007) Genotoxicity profiles of common alkyl halides and esters with alkylating activity. *Mutat Res* 633, 80-94.
2. Elder D.P., Snodin D., and Teasdale A. (2010) Control and analysis of hydrazine, hydrazides and hydrazones-Genotoxic impurities in active pharmaceutical ingredients (APIs) and drug products. *J Pharm Biomed Anal.*
3. Blasiak J., Jalszynski P., Trzeciak A., and Szyfter K. (1999) In vitro studies on the genotoxicity of the organophosphorus insecticide malathion and its two analogues. *Mutat Res* 445, 275-83.
4. Sarrif A.M., Arce G.T., Krahn D.F., O'Neil R.M., and Reynolds V.L. (1994) Evaluation of carbendazim for gene mutations in the Salmonella/Ames plate-incorporation assay: the role of aminophenazine impurities. *Mutat Res* 321, 43-56.
5. Herbold B.A. (1981) Studies to evaluate artificial sweeteners, especially Remsen--Fahlberg saccharin, and their possible impurities, for potential mutagenicity by the Salmonella/mammalian liver microsome test. *Mutat Res* 90, 365-72.
6. Lin G.H. and Brusick D.J. (1992) Mutagenicity studies on two triphenylmethane dyes, bromophenol blue and tetrabromophenol blue. *J Appl Toxicol* 12, 267-74.
7. Abu-Shakra A., Johnson L., Earley K., Jameson C.W., Kari F.W., Gupta R., and Langanbach R. (1991) Isolation of the mutagenic and DNA adduct-inducing components from a commercial preparation of HC blue 1 using Salmonella (TA98) bioassay-directed HPLC fractionation. *Mutat Res* 260, 377-85.
8. Agarwal S.K., Bhatnagar U., and Rajesh N. (2004) Acute and genotoxic profile

of a dimeric impurity of cefotaxime. *Int J Toxicol* 23, 41-5.

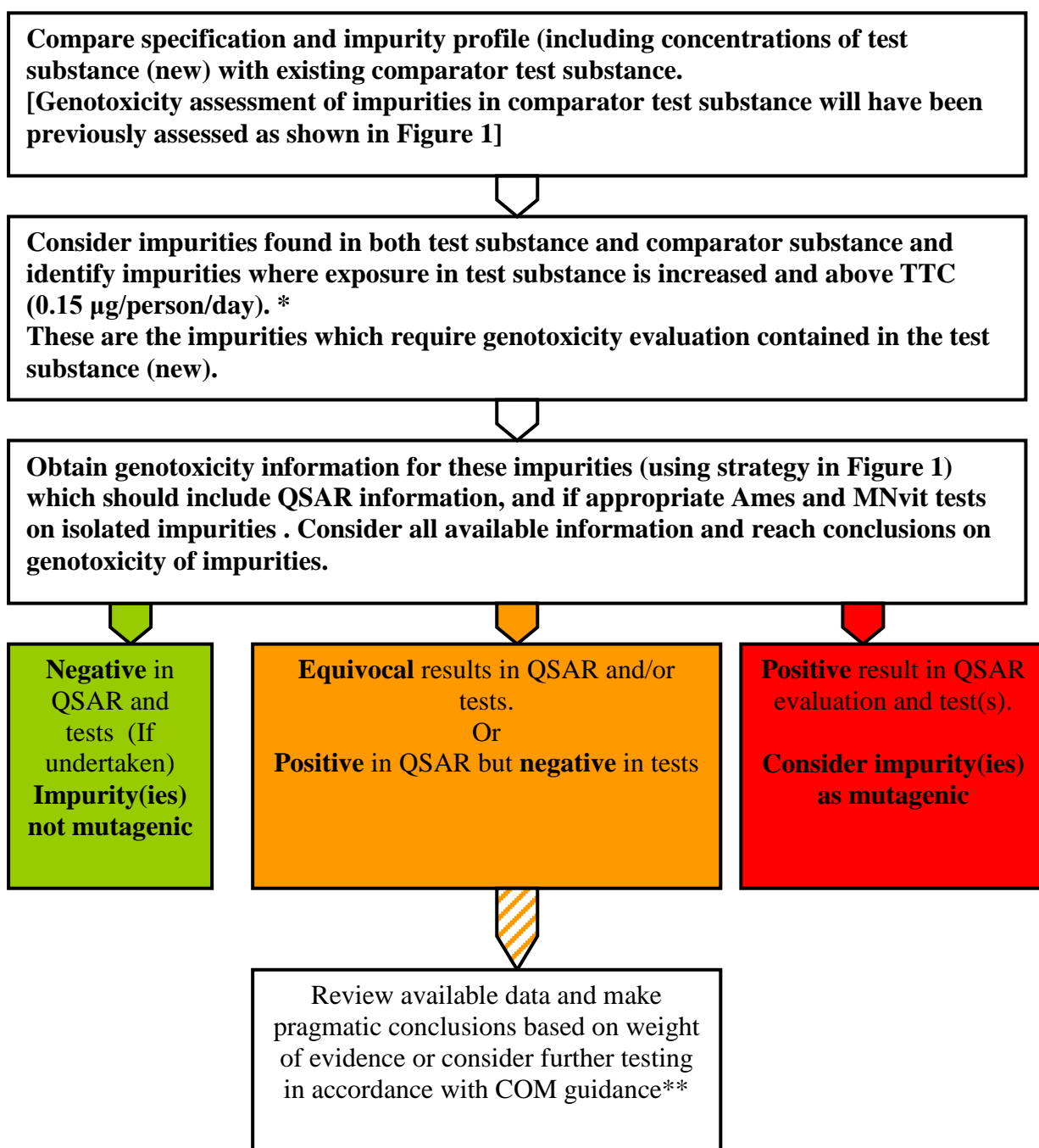
9. Fox A.W., Yang X., Murli H., Lawlor T.E., Cifone M.A., and Reno F.E. (1996) Absence of mutagenic effects of sodium dichloroacetate. *Fundam Appl Toxicol* 32, 87-95.
10. Basu A.K. and Marnett L.J. (1983) Unequivocal demonstration that malondialdehyde is a mutagen. *Carcinogenesis* 4, 331-3.
11. Eder E., Espinosa-Gonzalez J., Mayer A., Reichenberger K., and Boerth D. (2006) Autoxidative activation of the nematocide 1,3-dichloropropene to highly genotoxic and mutagenic derivatives: consideration of genotoxic/carcinogenic mechanisms. *Chem Res Toxicol* 19, 952-9.
12. Quinto I., Staiano N., Martire G., Friscia G.O., Signorini M., and de Lorenzo F. (1980) Mutagenic epoxide impurities discovered in two new beta-adrenergic blocking agents. *Toxicol Lett* 5, 109-14.
13. Proudlock R., Thompson C., and Longstaff E. (2004) Examination of the potential genotoxicity of pure capsaicin in bacterial mutation, chromosome aberration, and rodent micronucleus tests. *Environ Mol Mutagen* 44, 441-7.
14. Bercu J.P., Dobo K.L., Gocke E., and McGovern T.J. (2009) Overview of genotoxic impurities in pharmaceutical development. *Int J Toxicol* 28, 468-78.
15. Muller L., Mauthe R.J., Riley C.M., Andino M.M., Antonis D.D., Beels C., DeGeorge J., De Knaep A.G., Ellison D., Fagerland J.A., Frank R., Fritschel B., Galloway S., Harpur E., Humfrey C.D., Jacks A.S., Jagota N., Mackinnon J., Mohan G., Ness D.K., O'Donovan M.R., Smith M.D., Vudathala G., and Yotti L. (2006) A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. *Regul Toxicol Pharmacol* 44, 198-211.
16. Kroes R., Renwick AG., Cheeseman M., Kleiner R., Mangelsdorf I., Piersma A., Schilter B., Schlatter J., van Schothorst F., Vos JG., and Würtzen G. (2004) Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. *Food Chem Toxicol* 42, 65-83.
17. Munro IC., Renwick AG., and Danielewska-Nikiel B. (2008) The Threshold of Toxicological Concern (TTC) in risk assessment. *Toxicol Letters* 180, 151-156.

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



\* Exclusions from TTC for genotoxic chemicals are aflatoxin-like, *N*-nitroso compounds and azoxy-compounds. \*\* <http://www.iacom.org.uk/guidstate/documents/COMGuidanceFINAL.pdf>

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



\* Exclusions from TTC for genotoxic chemicals are aflatoxin-like, *N*-nitroso compounds and azoxy-compounds. \*\*<http://www.iacom.org.uk/guidstate/documents/COMGuidanceFINAL.pdf>