

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)**Guidance Statement : Strategy for genotoxicity testing and mutagenic hazard assessment of impurities****Introduction**

1. The COM guidance published for consultation <http://www.iacom.org.uk/publications/index.htm> provides a strategy for testing all chemical substances including those which have existing (and often limited or inadequate) genotoxicity data. Test substances may also contain impurities at varying levels which may also exhibit genotoxic activity. Separate guidance on the genotoxicity assessment of impurities was identified as a priority project during the COM horizon scanning exercise in 2010 (see minutes of COM meeting of October 2010 <http://www.iacom.org.uk/meetings/index.htm>)
2. The COM considered a number of aspects associated with genotoxicity testing of impurities at its June 2008 meeting (MUT/08/10). A copy of the paper is appended as Annex 1. In brief, a survey of 454 mutagens which had been tested in the Ames test showed 87% were identified at <250 µg/plate with 11% between 250-2500 µg/plate and 2% between 2500-5000 µg/plate. Overall the authors estimated that 85% of mutagenic impurities should be detected if present at ≥ 5% assuming the chemical is tested up to 5000 µg/plate.¹
3. The COM also considered a proposed rationale for determining, testing, controlling specific impurities in pharmaceuticals that are or maybe genotoxic.² Muller and colleagues identified five categories of compound with either positive carcinogenicity and/or genotoxicity data and/or alerting structures. The authors proposed restricting exposure to the Threshold of Toxicological Concern (TTC) as the most appropriate risk management option.
4. The general advice of the COM when considering the risk assessment of chemicals which are mutagenic *in vivo* has been that it is prudent to assume a non threshold dose response. <http://www.iacom.org.uk/guidstate/documents/Thresholdsforinternetfinal.pdf>
The draft COM guidance on testing and evaluation suggests that TTC can aid in ranking priorities for testing for chemicals where there is little or no genotoxicity data available. The current draft guidance statement on impurities also utilises the TTC concept. ICH guidance for impurities in pharmaceuticals also makes use of the TTC exposure level for genotoxins as a pragmatic approach to making risk management decisions particularly with regard to acceptable levels of impurities. This aspect of risk management has not been included in the draft COM guidance statement for impurities since COM guidance is generic for all categories of chemicals (with differing

risk/benefit assessments) and conclusions regarding the application of ALARP are for risk managers to consider.

5. Published regulatory guidance on impurities may also stipulate limits for identification or quantification of impurities. Thus ICHQ3A(R) stipulates 0.1% for pharmaceuticals with a maximum daily dose of $\leq 2\text{g/kg}^3$ and SANCO/10597/2003-rev 8.1 stipulates a similar level of $\geq 0.1\%$ for the determination of 'significant' impurities in pesticides under Regulation EC 91/414/EEC. The approach suggested for generic guidance on selection of impurities for genotoxicity testing is based on the TTC concept (see para 7 below).

http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3A_R2/Step4/Q3A_R2_Guideline.pdf

http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkd23_en.pdf

Proposed approach to testing and evaluation of impurities

6. A draft guidance Statement is appended as Annex 2 to this discussion paper. Figure 1 presents a strategy for genotoxicity assessment of impurities in test substances. Some brief comments on the TTC and its application to genotoxicants are given below.

Threshold of Toxicological Concern (TTC).

7. The TTC approach is based on the concept that reasonable assurance of safety can be given, even in the absence of chemical-specific toxicity data, provided that the intake is sufficiently low.^{4,5} Kroes derived a decision tree approach for genotoxins for identifying genotoxins where the life-time cancer risk exceeded 10^{-6} at an intake of $1.5 \mu\text{g/day}$. It was noted that this cancer risk was exceeded at an intake of $0.15 \mu\text{g/day}$ by 5% of aromatic amines, aromatic nitrates, azo compounds, benzidine derivatives, heavy metal containing compounds, highly chlorinated compounds (excluding dioxins), compounds with miscellaneous Ashby alerts, nitrofuryl-compounds, compounds with strained rings and vinyl containing compounds. The estimated risks for the majority of aflatoxin-like, azoxy- and nitroso-compounds exceeded 10^{-6} even at an intake of $0.15 \mu\text{g/day}$ and therefore the proposed TTC value was not appropriate for such compounds.^{4,5} A copy of the review by Munro and colleagues is appended as Annex 4⁶

8. The secretariat sought comments on the proposed approach from Professor Andy Renwick (Emeritus Professor, Faculty of Medicine, University of Southampton) (see Annex 3) The risk characterisation estimates in the TTC approach are likely to be highly conservative. They assume there is no threshold in the dose response relationship and are derived by linear extrapolation from the TD50 rodent bioassay. The animal data for all compounds were treated as if all compounds acted via a direct genotoxic and mutagenic mode of action and is applicable considered to be applicable to in vivo mutagens for which there are no cancer data.

9. Thus the current proposal uses the TTC intake level of 0.15 µg/day and is applied to all potential impurities. Is this correct or should there be some structural classes of impurity which should automatically be considered for genotoxicity testing?

10. Müller and colleagues have suggested an acceptable life-time intake value of 1.5 µg/day for genotoxic impurities in pharmaceuticals and scaled higher intakes for shorter duration treatments of 120 µg/day for ≤ 1 month.² An exposure of 1.5 µg/day for 12 months or more would give a theoretical risk 10⁻⁵; this was considered to be acceptable because of the controlled exposure and direct benefit to the exposed individual. A copy of this paper is appended as Annex 4.

11. These reviews suggest that applying a TTC value of 0.15 µg/day to the selection of impurities for genotoxicity evaluation should result in a suitable and conservative assessment approach.

Overview of testing approach

12. The genotoxicity assessment of impurities can be undertaken when a test substance is considered for genotoxicity and when making a comparison between one test substance and other test substance(s). An example of the latter situation is the assessment of the equivalence of a chemical substance sourced from different manufacturers by regulatory agencies. Thus two flow diagrams for genotoxicity testing and assessment of impurities have been developed.

13. A review of the literature on genotoxicity testing approaches which have been used for impurities was undertaken for the COM review in 2008, and a number of additional references have been identified.⁷⁻⁹ Testing strategies have predominantly been limited to Ames testing or additional *in vitro* tests in mammalian cells of either the isolated impurity or marketed chemical substance containing the impurity. For specific impurities, additional *in vivo* testing has been used in some instances to resolve which risk management approach should be used.⁷

14. Approaches to genotoxicity of impurities developed within the pharmaceutical industry have proposed that QSAR and an Ames test are sufficient to resolve the genotoxicity of impurities.^{1,10,11} In the proposed approach given in Annex 2 figure 1 a combination of QSAR, Ames and *in vitro* micronucleus tests are suggested as adequate. Is this a reasonable approach?

15. The approach set out in Annex 2 using a combination of TTC to screen for chemicals requiring evaluation and concentration limit to aid in decisions as to whether impurities should be tested in isolation. If it is not practical to isolate an impurity then pragmatic assessment could be based on QSAR and test with the test substance including the impurity under evaluation.

16. Guidance on whether to test the chemical substance containing impurities or to isolate impurities prior to testing can in part be related to the

sensitivity of the genotoxicity assays to detect genotoxicants. There are data available regarding the sensitivity of the Ames test¹ but no corresponding data for the *in vitro* micronucleus test have been retrieved. Although it may be preferential to isolate individual impurities for testing, there may be significant practical and resource implications in trying to undertake such an approach to all impurities. Thus if there were only limited amount of available test substance containing impurities available, then any isolation procedure might be impractical.

17.. One proposed approach could be based on the review by Kenyon et al 2007 which reviewed a wide range of genotoxic structures, utilising a value of <50 µg/mg (i.e. 5%)(which equates to 250 µg/plate if a dose of 5000 µg/plate is tested in an Ames test) to aid in the selection of impurities which need to be isolated before genotoxicity testing. Kenyon's overall conclusion was that a dose of 250 µg/plate would be sufficient to detect about 85% of Ames positive mutagens.¹²

18. However, it is envisaged that many impurities in test substances will be present at levels lower than 5%. Would suitable reassurance be available with regard to such impurities if the test substance gave negative results in both Ames and MNvit tests?

Proposed approach to evaluation of equivalence of test substances

19. The assessment of equivalence of test substances can form quite a significant work load for regulatory agencies. This can involve assessing technical equivalence of generic sources of the same chemical(s) (e.g. a pesticide). Regulatory guidance for the assessment of equivalence of technical materials (pesticides) has been published http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc23_en.pdf and is also currently being updated. Another requirement for any approach to assessing equivalence is to consider the potential role of impurities when the regulatory toxicology package for a particular substance contains genotoxicity tests undertaken with different batches of the same test substance and consequently levels of impurities may vary between batches and the need for genotoxicity testing should be reconsidered.

20. An approach to assessment of genotoxicity equivalence of test substances is provided in figure 2 in Annex 2. The term test substance (new) refers to the new specification or technical material. The term comparator test substance refers to the test substance to which comparisons of impurity profile and/or levels of impurities are being made. The term relevant impurity refers to new or increased exposures to impurities which require genotoxicity evaluation. Once a relevant impurity has been identified then the approach to genotoxicity testing and hazard assessment would be as described in figure 1

COM Discussion

21. The COM is asked to consider the questions posed in paras 9, 14, and 18 and the appended guidance statement and advice on a suitable generic approach to the genotoxicity testing and evaluation of impurities.

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Annex 1 MUT/08/10 Previous consideration of impurities.

Annex 2 Draft guidance statement on impurities.

**Annex 3 Comments from Professor Andy Renwick, Emeritus Professor
Faculty of Medicine, University of Southampton.**

**Annex 4 Munro I.C., Renwick A.G., and Danielewska-Nikiel B. (2008) The
Threshold of Toxicological Concern (TTC) in risk assessment. *Toxicol
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Notes: CORPORATE NAME: European branch of the International Life Sciences Institute
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