

draft

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 have published a consultation document on guidance on a strategy for testing and mutagenic hazard assessment of chemical substances (<http://www.iacom.org.uk/publications/documents/StrategyGuidanceCOMconsultation3.pdf>) The COM has advised 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.

II. Introduction

2. 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 activity¹ and hydrazine, hydrazides and hydrazones²), pesticides (e.g. malathion³ and benomyl and carbendazim⁴), food additives (e.g. saccharin⁵) and chemicals such as dyes with a wide number of uses (e.g. triphenylmethane dyes⁶ and hair dye HC Blue 1⁷). Genotoxicity tests have been used to monitor the purification of chemicals to remove genotoxic impurities^{6,8}, to investigate the potential genotoxicity of specific impurities isolated from substances⁹, and to test samples of substances for the presence of genotoxins.^{10,11} 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*^{6,7,9-15} and *invitro/invivo* genotoxicity tests.^{6,11,15} Published approaches to testing and evaluation of impurities in pharmaceuticals have suggested using QSAR and the initial step and an Ames test if appropriate.^{16,17} Negative results for QSAR and an Ames test have been suggested

as sufficient information to reach a conclusion on the genotoxicity of impurities in pharmaceuticals.¹⁷

III. Strategy for genotoxicity assessment of impurities in chemical substances

3. 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 by regulatory agencies. A case-by-case approach is recommended for the identification of impurities and quantification of levels. The use of the Threshold of Toxicological Concern (TTC) concept can be used as a pragmatic guide to selection of impurities requiring genotoxicity assessment. These are called the relevant impurities for the test substance. All impurities selected for genotoxicity assessment should be subject to a QSAR evaluation. Genotoxicity testing of impurities can be undertaken in studies using the test substance containing impurities or in studies where individual impurities have been separated and purified. As a pragmatic guide impurities present at $\geq 5\%$ can be investigated using the chemical substance containing impurities, i.e. there is no need to isolate and purify such impurities. The strategy for genotoxicity testing and assessment of impurities in chemical substances is given in Figure 1

4. 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 term relevant impurity refers to new or increased exposures to impurities which require genotoxicity evaluation. The use of the Threshold of Toxicological Concern (TTC) concept can also be used as a pragmatic guide to selection of relevant impurities which require genotoxicity assessment when comparing the impurities present in two or more chemical substances. Thus exposure to new or increased exposures to impurities but at exposures $\leq 0.15\mu\text{g/day}$ would not require further investigation. All relevant impurities 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 relevant impurities using the Ames test and in vitro micronucleus test (MNvit) as shown in Figure 1 is needed.

VI Conclusions

5. The genotoxicity assessment of impurities present in chemical substances is guided by the application of the TTC concept to select relevant 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 relevant impurities.

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 Langenbach 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. Abu-Shakra A., Johnson L., Earley K., Jameson C.W., Kari F.W., Gupta R., and Langenbach 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.
9. 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.
10. 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.

11. 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.
12. Basu A.K. and Marnett L.J. (1983) Unequivocal demonstration that malondialdehyde is a mutagen. *Carcinogenesis* 4, 331-3.
13. 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.
14. 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.
15. 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.
16. 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.
17. 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.