

## SCIENTIFIC OPINION

### **Genotoxicity Test Strategy for Substances belonging to Subgroups of FGE.19<sup>1</sup>**

#### **Statement of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)**

(Question No EFSA-Q-2008-710)

**Adopted on 31 October 2008 by written procedure**

#### **PANEL MEMBERS**

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#### **BACKGROUND**

Regulation (EC) No 2232/96 of the European Parliament and the Council lays down a procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all others in the EU. In application of that Regulation, a register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC, as last amended by Commission Decision 2008/478/EC.

EFSA is requested to carry out a risk assessment on groups of flavouring substances listed in the Register prior to their authorisation and inclusion in a positive list according to

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Commission Regulation (EC) No 1565/2000 and a new regulation expected to enter in force in December 2008 and amending Council Regulations (EEC) 1570/89 and (EEC) 1601/91, Regulations (EC) 2232/96 and Directive 2000/13/EC. Deadlines for completion of the evaluations have been established by Commission Regulation (EC) No 622/2002 and have recently been extended to end 2009.

After the completion of the evaluation programme, the positive list of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96).

As a part of the evaluation programme, the genotoxic potential of these substances is assessed by EFSA prior to any further evaluation. It is therefore considered necessary to develop in EFSA a Genotoxicity Test Strategy for the group evaluation of flavouring substances.

## **TERMS OF REFERENCE**

In accordance with Commission Regulation (EC) No 1565/2000, the European Food Safety Authority asks its Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) to develop a Genotoxicity Test Strategy for the Evaluation of Flavouring Substances and to identify specific requirements for genotoxicity testing for groups of substances for which additional data are required.

## **ACKNOWLEDGEMENTS**

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## **STATEMENT ON GENOTOXICITY TEST STRATEGY FOR SUBSTANCES BELONGING TO SUBGROUPS OF FGE.19**

The Panel noted that the substances in the subgroups from FGE.19 for which additional data are required ([http://www.efsa.europa.eu/EFSA/Event\\_Meeting/afc\\_minutes\\_26thplen\\_en.pdf](http://www.efsa.europa.eu/EFSA/Event_Meeting/afc_minutes_26thplen_en.pdf)) are structurally related and that some genotoxicity data are available for these subgroups. Therefore, the Panel considers that a bacterial gene mutation test (OECD guideline 471) and an *in vitro* mammalian cell genotoxicity test should be available as a minimum data set for representative substances from subgroups of FGE.19. The Panel considers the mouse lymphoma assay with colony sizing (OECD guideline 476) as the most appropriate mammalian cell genotoxicity test for substances belonging to subgroups of FGE.19 since it is well known to be a sensitive test able to detect both gene mutations and substances capable of inducing chromosomal aberrations in mammalian cells. However, the *in vitro*

micronucleus assay (OECD guideline 487 is in preparation) or the *in vitro* chromosomal aberration test (OECD guideline 473) would also be acceptable as alternative to the mouse lymphoma assay.

Positive results in any of the above *in vitro* tests will require further assessment of genotoxicity *in vivo*.

Guidance for the follow-up of *in vitro* positive results was taken from section 3.10.5.6 of the Technical Guidance Document on Risk Assessment of Chemical Substances and Biocides (TGD) (EC 2003). Since *alpha,beta*-unsaturated substances must be regarded as potential direct acting genotoxic agents and since there is some evidence that these substances can react with tissues at initial sites of contact with the body (Dittberner *et al.*, 1997) the Panel recommends an *in vivo* Comet assay in addition to a mouse micronucleus assay. The Comet assay has the advantage that it enables the investigation of tissues at initial sites of contact and that several tissues could be analysed in parallel. Furthermore, it has recently been shown that the Comet assay is able to detect several carcinogens which were negative in the *in vivo* micronucleus assay and that the sensitivity (ability to detect carcinogens as positive) of the Comet assay is clearly higher than the sensitivities of the *in vivo* UDS assay and the transgenic animals assay while the specificity (ability to give negative results with non-carcinogens) of the Comet assay is slightly higher than that of the transgenic animals assay (Kirkland and Speit, 2008). A combination of the *in vivo* micronucleus assay and the Comet assay in a single study as suggested by Pfuhler *et al.* (2007) would also be acceptable.

Studies should be conducted using internationally agreed protocols. Test methods described by OECD or in European Commission Directives are recommended. The most up-to-date edition of any test guideline should be followed. Studies should be carried out according to the principles of Good Laboratory Practice (GLP) described in Council Directives 87/18/EEC and 88/320/EEC and accompanied by a statement of GLP compliance. Use of any methods differing from internationally agreed protocols should be justified. An OECD guideline is not yet available for the Comet assay. However, recommendations for an appropriate performance of the assay using OECD guidelines for other *in vivo* tests have been published and a standard protocol and acceptance criteria for the assay have been developed through the International Workshop on Genotoxicity Working Parties and international Comet assay workshops (Tice *et al.*, 2000; Hartmann *et al.*, 2003; Burlinson *et al.*, 2007). Additional information could be taken from a website on the Comet assay (<http://cometassay.com>). There are also links to various contract laboratories which offer the Comet assay on a commercial basis.

## REFERENCES

Burlinson, B., Tice, R.R., Speit, G., Agurell, E., Brendler-Schwaab, S.Y., Collins, A.R., Escobar, P., Honma, M., Kumaravel, T.S., Nakajima, M., Sasaki, Y.F., Thybaud, V., Uno, Y., Vasquez, M., Hartmann, A. (2007): *In Vivo* Comet Assay Workgroup, part of the Fourth International Workgroup on Genotoxicity Testing. Fourth international Workgroup on genotoxicity testing: results of the *in vivo* Comet assay workgroup. *Mutation Research* 627: 31-35.

Dittberner, U., Schmetzer, B., Gölzer, P., Eisenbrand, G., Zankl, H. (1997): Genotoxic effects of 2-trans-hexenal in human buccal mucosa cells *in vivo*. *Mutation Research* 390(1-2): 161-165.

European Chemicals Bureau (2003): Technical Guidance Document (TGD) on Risk Assessment of New and existing Chemical Substances and biocides following European Regulations and Directives (abbreviated title) (second edition) [http://ecb.jrc.it/Documents/TECHNICAL\\_GUIDANCE\\_DOCUMENT/EDITION\\_2/tgdpart1\\_2ed.pdf](http://ecb.jrc.it/Documents/TECHNICAL_GUIDANCE_DOCUMENT/EDITION_2/tgdpart1_2ed.pdf)

European Council (1986): Council Directive 87/18/EEC of 18 December 1986 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances. *Official Journal of the European Communities* No L 15, 17.1.1987, p29.

European Council (1988): Council Directive 88/320/EEC of 9 June 1988 on the inspection and verification of Good Laboratory Practice (GLP). *Official Journal of the European Communities* No L 145, 11.6.1988, p35.

Hartmann, A., Agurell, E., Beevers, C., Brendler-Schwaab, S., Burlinson, B., Clay, P., Collins, A., Smith, A., Speit, G., Thybaud, V., Tice, R.R. 4th International Comet Assay Workshop (2003): Recommendations for conducting the *in vivo* alkaline comet assay. *Mutagenesis* 18: 45-51.

Kirkland, D., Speit, G. (2008): Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens III. Appropriate follow-up testing *in vivo*. *Mutation Research* 654: 114-132.

Pfuhler, S., Albertini, S., Fautz, R., Herbold, B., Madle, S., Utesch, D., Poth, A.; Gesellschaft fuer Umwelt-Mutationsforschung (2007): Genetic toxicity assessment: employing the best science for human safety evaluation part IV: Recommendation of a working group of the Gesellschaft fuer Umwelt-Mutationsforschung (GUM) for a simple and straightforward approach to genotoxicity testing. *Toxicological Sciences* 97: 237-240.

Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C., Sasaki, Y.F. (2000): The single cell gel/comet assay: Guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environmental Molecular Mutagenesis 35: 206-221.