Section A6.6.1/6.6.2/ 6.6.3

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3

Genotoxicity in vitro

Ames test and chromosomal aberration test

			Official use only
		1 REFERENCE	
1.1	**Reference**	Ishidate, M. Jr., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., Matsuoka, A. (1984) Primary mutagenicity screening of food additivies currently used in Japan Fd Chem. Toxic., Vol. 22, No. 8, pp. 623-636 Not GLP, published	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Companies with letter of access	Not applicable	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	X
2.2	**GLP**	No, not common to report in literature	
2.3	**Deviations**	Not applicable	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Lactic acid and 241 other food additives	
3.1.1	Lot/Batch number	Not reported	
3.1.2	Specification	Not reported	
3.1.2.1	**Description**	Not reported	
3.1.2.2	**Purity**	90.5%	
3.1.2.3	**Stability**	Not reported	
3.2	**Study Type**	Standard plate incorporation assay (Maron and Ames, 1983) and chromosomal aberration tests in vitro	
3.2.1	Organism/cell type	Ames test: *Salmonella typhimurium* strains TA92, TA1535, TA100, and TA1537, TA94, and TA98. Chromosomal aberration test: Chinese hamster fibroblast cell line	
3.2.2	Deficiencies / Proficiencies	Not applicable	
3.2.3	Metabolic activation system	Ames test:: S9 mix (rat liver microsome fraction) Chromosomal aberration test: no metabolic activation system applied	
3.2.4	Positive control	No	

Section A6.6.1/6.6.2/ 6.6.3

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3

Genotoxicity in vitro

Ames test and chromosomal aberration test

3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	Ames test: maximum dose: 10 mg/plate	
		Chromosomal aberration test: maximum dose: 1.0 mg/mL	
3.3.2	Way of application	Ames test: dissolved in phosphate buffer	
		Chromosomal aberration test: dissolved in physiological saline	
3.3.3	Pre-incubation time	Ames test: pre-incubation with both the test sample and the S-9 mix for 20 min at 37°C before plating	
		Chromosomal aberration test: no pre-incubation	
3.3.4	Other modifications	Not applicable	
3.4	**Examinations**	Ames test: mutagenic activity (His^+ revertants)	
		Chromosomal aberration test: incidence of polyploidy cells and cells with structural chromosomal aberrations (chromatid or chromosome gaps, breaks, exchanges, ring formations, fragmentations and others)	
3.4.1	Number of cells evaluated	Ames test: not applicable	
		Chromosomal aberration test: a hundred well-spread metaphases	
		RESULTS AND DISCUSSION	
3.5	**Genotoxicity**		
3.5.1	without metabolic activation	No	
3.5.2	with metabolic activation	No	
3.6	**Cytotoxicity**	No	
		4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	Standard plate incorporation assay (Maron and Ames, 1983) and chromosomal aberration tests in vitro	
4.2	**Results and discussion**	No genotoxicity detected	
4.3	**Conclusion**	Lactic acid does not have genotoxic properties	X
4.3.1	Reliability	2, study conducted in compliance with generally accepted scientific principles	
4.3.2	Deficiencies	No	

Section A6.6.1/6.6.2/ 6.6.3

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3

Genotoxicity in vitro

Ames test and chromosomal aberration test

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20089/~~1~~012/~~18~~22
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Lactic acid revealed no genotoxic properties in the Ames test and a chromosomal aberration assay under the conditions tested.
Reliability	2
Acceptability	Acceptable
Remarks	No detailed data (e.g. no. of revertants) are ~~None~~ reported.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	