

Section A6.6.1/6.6.2/**6.6.3****Genotoxicity in vitro**
*Ames test***Annex Point IIA6.6.1 /
6.6.2 / 6.6.3**

		Official use only
1 REFERENCE		
1.1 Reference	Al-Ani, F.Y., Al-Lami, S.K. (1988) Absence of mutagenic activity of acidity regulators in the Ames Salmonella/microsome test Mutation Research, 206, p. 467-470 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, method comparable to OECD guidelines (Standard plate incorporation assay (Maron and Ames, 1983))	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 3 other acidity regulators (anhydrous citric acid, phosphoric acid, and malic acid)	
3.1.1 Lot/Batch number	Not reported	
3.1.2 Specification	Lactic acid obtained from BDH	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	Not reported	
3.1.2.3 Stability	Not reported	
3.2 Study Type	Standard plate incorporation assay (Maron and Ames, 1983)	
3.2.1 Organism/cell type	<i>Salmonella typhimurium</i> strains TA97, TA98, TA100, and TA104.	
3.2.2 Deficiencies / Proficiencies	Not applicable	
3.2.3 Metabolic activation system	S9 mix (rat liver homogenate S9 fraction prepared as in Ames et al. (1975))	
3.2.4 Positive control	2-aminoanthracene (2-AA)	

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3.3	Administration / Exposure; Application of test substance	
3.3.1	Concentrations	0, 0.5, 1.0, 2.0 µL/plate (all concentrations were tested in triplicate)
3.3.2	Way of application	According to Maron and Ames (1983)
3.3.3	Pre-incubation time	According to Maron and Ames (1983)
3.3.4	Other modifications	Not mentioned
3.4	Examinations	Mutagenic activity (His ⁺ revertants)
3.4.1	Number of cells evaluated	Not applicable

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)
4.2	Results and discussion	No genotoxicity detected
4.3	Conclusion	Lactic acid does not have genotoxic properties
4.3.1	Reliability	2, study conducted in compliance with generally accepted scientific principles
4.3.2	Deficiencies	No

X

Evaluation by Competent AuthoritiesUse separate "evaluation boxes" to provide transparency as to the
comments and views submitted**EVALUATION BY RAPPORTEUR MEMBER STATE****Date** 2009/01/22

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Materials and Methods	2.1 Similar to OECD test guideline 471 2.3 Deviations from OECD test guideline 471: selection of strains (TA1535 missing, TA104 instead of TA102 or E. coli WP2); 2-Aminoanthracene as sole positive control
Results and discussion	Applicant's version is acceptable. For results see CA-Table 1
Conclusion	Lactic acid revealed no genotoxic properties in the Ames test under the conditions tested.
Reliability	2
Acceptability	Acceptable
Remarks	Deviations from current OECD test guideline 471 do not affect the overall integrity of the test.
COMMENTS FROM ...	
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

CA-table 1:

Lactic acid concentration (μ l/plate)	<i>S. typhimurium</i> strain							
	TA97		TA98		TA100		TA104	
	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
0	47.0 \pm 1.73	27.0 \pm 1.73	56.3 \pm 1.15	44.0 \pm 2.64	167.6 \pm 2.51	139.3 \pm 1.52	449.3 \pm 8.14	379.3 \pm 9.29
0.5	50.3 \pm 1.52	25.6 \pm 1.15	57.6 \pm 2.51	46.6 \pm 2.08	168.0 \pm 5.29	149.3 \pm 2.51	491.6 \pm 7.63	448.3 \pm 3.51
1.00	49.0 \pm 1.00	29.3 \pm 0.57	53.0 \pm 2.64	49.3 \pm 0.57	160.0 \pm 2.00	143.3 \pm 2.88	509.3 \pm 9.01	454.3 \pm 5.13
2.00	50.3 \pm 0.57	25.6 \pm 2.08	51.3 \pm 1.15	49.3 \pm 2.51	143.0 \pm 2.64	122.0 \pm 1.00	478.3 \pm 10.40	426.0 \pm 7.63
2-AA (10 μ g/plate)	442.6 \pm 2.30	32.0 \pm 2.00	499.3 \pm 6.11	53.3 \pm 1.15	557.3 \pm 6.42	154.3 \pm 0.57	992.3 \pm 4.93	688.0 \pm 7.21

Data represent means of 3 plates.