Section A6.7 Carcinogenicity

Annex Point IIA6.7

Oral, rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	Maekawa, A., Matsushima, H., Onodera, H., Shibutani, M., Yoshida, J., Kodama, Y., Kurokawa, Y., Hayashi, Y., 1991. Long-term carcinogenicity/carcinogenicity study of calcium lactate in F344 rats. Food and Chemical Toxicology, Vol. 29, No. 9: pp 589-594	
1.2	**Data protection**	No	
1.2.1	Data owner	Published literature	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Not applicable	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not applicable	
2.2	**GLP**	Not applicable	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Calcium lactate (CAS 814-80-2),	
		In the current study calcium lactate dissolved in water was tested. As it is administered dissolved in water, the results of this study can be used for lactic acid.	X
3.1.1	Lot/Batch number	Commercial sample obtained from Musashino Chemical Inst. Ltd (Tokyo, Japan)	
3.1.2	Specification	*Deviating from specification given in section 2 as follows*	
		Calcium lactate was dissolved in distilled water	
		Clarity and colour of solution: colourless and clear, pH 6.0-8.0; heavy metals (Pb) < 20 µg/g; alkaline metals and magnesium < 1%, arsenic < 4 µg/g; volatile fatty acids: no odour of fatty acids; loss on drying 25.0 – 30.0%.	
3.1.2.1	**Description**	Odourless white powder	
3.1.2.2	**Purity**	97.0 – 101.0 %	
3.1.2.3	**Stability**	Not reported	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	SPF Fischer (F344)	
3.2.3	Source	Charles River Japan Inc., Kanagawa, Japan	
3.2.4	Sex	Male and female	

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3.2.5	Age/weight at study initiation	6 weeks / 90 to 120 grams	
3.2.6	Number of animals per group	50 males and 50 females / group	
3.2.6.1	**at interim sacrifice**	No scheduled sacrifice, autopsy was immediately performed on rats that died during the study	
3.2.6.2	**at terminal sacrifice**	Autopsy on all animals by the end of the study	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	104 weeks	
3.3.2	Interim sacrifice(s)	No scheduled sacrifices	
3.3.3	Final sacrifice	At week 113	
3.3.4	Freqency of exposure	daily	
3.3.5	Postexposure period	9 weeks recovery period	
		Oral	
3.3.6	Type	In drinking water	
3.3.7	Concentration	0, 2.5 or 5% in drinking water (distilled water)	
3.3.8	Vehicle	Drinking water	
3.3.9	Concentration in vehicle	0, 2.5 or 5% in drinking water	
3.3.10	Total volume applied	*Ad libitum*	
3.3.11	Controls	Drinking water only	
3.4	**Examinations**		
3.4.1	Body weight	Yes, once a week for the first 13 weeks, and every 4 weeks thereafter	
3.4.2	Food consumption	Not reported	
3.4.3	Water consumption	Yes, three times a week	
3.4.4	Clinical signs	Yes, daily	
3.4.5	Makroskopic investigations	Yes	
3.4.6	Ophthalmoscopic examination	Not reported	
3.4.7	Haematology	Yes/No	Yes
		Number of animals:	All animals
		Time points:	End of study

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		Parameters:	No details reported
		Other:	-
3.4.8	Clinical Chemistry		Yes
		Number of animals:	All animals
		Time points:	End of study
		Parameters:	No details reported
		Other	-
3.4.9	Urinalysis	Yes/No	Not reported
		Number of animals:	-
		Time points:	-
		Parameters:	-
		Other	-
3.4.10	Pathology	Yes	
3.4.10.1	**Organ Weights**	Yes/No	Yes
		from:	All surviving animals, at terminal sacrifice
		Organs:	Including kidney, brain
			Other
3.4.11	Histopathology	Yes/No	Yes
		from:	All dose groups
		from:	All surviving animals
		Organs:	Including pituitary gland, thyroid gland, adrenal gland, pancreas, haematopoetic organs, testis, prostate, mammary gland, uterus, vagina, ovary, lung, heart, tongue, forestomach, large instestine, liver, kidney, urinary bladder, skin/subcutis, preputial/chtoral gland, brain, thoracic cavity and., abdominal cavity
			Other
3.4.12	Other examinations	Not applicable.	
3.5	**Statistics**	Statistical analyses were performed using Fisher's exact probability test and/or the chi-square test. Also the age-adjusted statistical test recommended by Peto et al (1980) was used.	
3.6	**Further remarks**	None	

RESULTS AND DISCUSSION

3.7 Body weight

A dose-dependent inhibitory effect on the growth of rats was observed. Compared with the controls a 13% decrease in body-weight gain was observed in both male and female rats of the high-dose group (5%).

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3.8	**Food consumption**	Not reported	
3.9	**Water consumption**	Daily water consumption was almost constant in all groups of both sexes.	
3.10	**Clinical signs**	Results not reported (but daily observations were made)	
3.11	**Macroscopic investigations**	No effects reported	
3.12	**Ophthalmoscopic examination**	Not reported	
3.13	**Haematology**	No specific dose related changes were observed	
3.14	**Clinical Chemistry**	No specific dose related changes were observed	
3.15	**Urinalysis**	Not reported	
3.16	**Pathology**	No effects reported	
3.17	**Organ Weights**	Females in the high dose group exhibited slightly but significantly higher kidney weights compare with controls. However, histologically there was no difference in the severity of chronic nephropaty between different groups. No toxic lesions were observed in the kidney. A significant dose-dependent increase in relative brain weights was observed for both male and female rats, although no histological change was detected.	
3.18	**Histopathology**	Histologically, all the tumours observed in this experiment were similar to those know to occur spontaneously in F34 rats. None of the experimental groups showed a significant increase in the incidence of any specific tumour	
3.19	**Other examinations**	Not applicable	
3.20	**Time to tumours**	Not applicable, exposure by drinking water	
3.21	**Other**	Not applicable	
		4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	Published article on a long term carcinogenicity study performed by the National Institute of Hygiene Sciences in Tokyo, Japan. No reference is made to a specific test guideline (i.e. OECD), but study resembles OECD guideline 453. No intermediate examinations are reported, all reported endpoints were examined at termination of the study.	
4.2	**Results and discussion**	No clear toxic lesion was specifically caused by long-term exposure to calcium lactate. No significant dose-related increase was found in the incidences of tumours in any organ or tissue..	
4.3	**Conclusion**	The results indicated that calcium lactate had neither toxic nor carcinogenic activity in F344 rats	X
4.3.1	Reliability	2	
4.3.2	Deficiencies	Yes, study is not performed according to current guidelines. As it is a literature publication, the reporting is concise and raw data are missing. However, the study has been performed well and can be used for the purpose of this dossier. As calcium lactate was used, effects of calcium should also be taken into account.	

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/07/16
Materials and Methods	3.1 The applicant's version is acceptable with the following amendment: As calcium lactate is administered dissolved in water, the results of this study can partly be used for lactic acid considering also calcium effects.
Results and discussion	Applicant's version is acceptable.
Conclusion	Carcinogenic LO(A)EL: > 5 % calcium lactate in drinking water, Carcinogenic NO(A)EL: 5 % calcium lactate in drinking water (highest dose tested) In the article the mean total calcium lactate intake (in grams/rat) is calculated. The 5 % dose corresponds with 625.4 g/ rat for male rats and 412.1 g/rat for female rats for 104 weeks. This are per day ~880 mg/kg bw/d (male) or ~ 930 mg/kg bw/d (female).
Reliability	2
Acceptability	Acceptable with restrictions
Remarks	None
	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	