## Section A6.5 Repeated dose toxicity

**Annex Point IIA6.3 / 6.4 / 6.5**

*Long-term toxicity, oral, rat*

| | | | Official use only |
|---|---|---|---|
| | | **1 REFERENCE** | |
| **1.1** | **Reference** | [redacted] 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 |
| 3.2.7 | Control animals | Yes |
| **3.3** | **Administration/ Exposure** | Oral |
| 3.3.1 | Duration of treatment | 104 weeks |
| 3.3.2 | Frequency of exposure | Daily |
| 3.3.3 | Postexposure period | 9 weeks recovery period |
| **3.3.4** | **Oral** | |
| **3.3.4.1** | **Type** | In drinking water |
| **3.3.4.2** | **Concentration** | 0, 2.5 or 5% in drinking water (distilled water) |
| **3.3.4.3** | **Vehicle** | Drinking water |
| **3.3.4.4** | **Concentration in vehicle** | 0, 2.5 or 5% in drinking water |
| **3.3.4.5** | **Total volume applied** | Not applicable |
| **3.3.4.6** | **Controls** | Drinking water without test substance |
| **3.4** | **Examinations** | |
| 3.4.1 | Observations | Daily |
| **3.4.1.1** | **Clinical signs** | Yes, daily |
| **3.4.1.2** | **Mortality** | Yes, daily |
| 3.4.2 | Body weight | Yes, once a week for the first 13 weeks, and every 4 weeks thereafter |
| 3.4.3 | Food consumption | Not reported |
| 3.4.4 | Water consumption | Yes, three times a week |
| 3.4.5 | Ophthalmoscopic examination | Not reported |
| 3.4.6 | Haematology | Yes on all surviving rats at week 113 |
| | | No details reported |
| 3.4.7 | Clinical Chemisty | Yes, on all surviving rats at week 113 |
| | | No details reported |

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|---|---|---|
| 3.4.8 | Urinalysis | Not reported |
| **3.5** | **Sacrifice and pathology** | |
| 3.5.1 | Organ Weights | Yes on all surviving rats at week 113<br>Including kidney, brain |
| 3.5.2 | Gross and histopathology | Yes on all surviving rats at week 113<br>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 |
| 3.5.3 | Other examinations | Carcinogenicity: see under A6.7 |
| 3.5.4 | Statistics | Statistical analyses were performed using Fisher's exact probability test and/or the chi-square test.<br>Also the age-adjusted statistical test recommended by Peto et al (1980) was used. |
| **3.6** | **Further remarks** | None |

**4 RESULTS AND DISCUSSION**

| | | |
|---|---|---|
| **4.1** | **Observations** | |
| 4.1.1 | Clinical signs | Results not reported. |
| 4.1.2 | Mortality | First mortalities occurred after 56 weeks. For females the mortality rate in the highest dose group (5%) was slightly higher than those in the other two groups; not statistically significant. |
| **4.2** | **Body weight gain** | 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%). |
| **4.3** | **Food consumption and compound intake** | Not reported |
| **4.4** | **Ophtalmoscopic examination** | Not reported |
| **4.5** | **Blood analysis** | |
| 4.5.1 | Haematology | No specific dose related changes were observed |
| 4.5.2 | Clinical chemistry | No specific dose related changes were observed |
| 4.5.3 | Urinalysis | Not reported |

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| **4.6** | **Sacrifice and pathology** | | |
| 4.6.1 | Organ weights | Females in the high dose group exhibited slightly but significantly higher kidney weights compared 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.<br><br>A significant dose-dependent increase in relative brain weights was observed for both male and female rats, although no histological change was detected. | |
| 4.6.2 | Gross and histopathology | A number of non-neoplastic lesions (e.g. myocardial fibrosis, bile-duct proliferation, hepatic microgranulomas and chronic nephropathy) were observed in all groups. | |
| **4.7** | **Other** | None | |
| | | **5 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **5.1** | **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 guidelines 453. However, no intermediate examinations are reported. It is not clear if these have been performed. All reported endpoints were examined at termination of the study. | |
| **5.2** | **Results and discussion** | No clear toxic lesion could be attributed to long-term exposure to calcium lactate. No significant dose-related increase was found in the incidences of tumours in any organ or tissue. | |
| **5.3** | **Conclusion** | The results indicate that calcium lactate had neither toxic nor carcinogenic activity in F344 rats. | |
| 5.3.1 | LO(A)EL | Not applicable | |
| 5.3.2 | NO(A)EL | No adverse effects were observed at the highest dose level. Therefore the NOAEL is > 5% (in drinking water). | X |
| | | In the article the mean total calcium lactate intake (in grams/rat) is calculated. The 5% dose corresponds with 6254 g/ rat for male rats and 4121 g/rat for female rats. (over 104 weeks? Thus per day: 8.6 g/day (male) or 5.6 g/day (female) | X |
| 5.3.3 | Other | | |
| 5.3.4 | Reliability | 2 | |
| 5.3.5 | 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. | |

| **Evaluation by Competent Authorities** |
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| Use separate "evaluation boxes" to provide transparency as to the comments and views submitted |
| **EVALUATION BY RAPPORTEUR MEMBER STATE** |

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| **Date** | 2008/07/16 |
| **Materials and Methods** | 3.1 The applicant's version is acceptable with the following amendment:<br>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** | LO(A)EL: 5 % calcium lactate in drinking water, based on 13 % decreased decrease in body weight gain<br>NO(A)EL: 2.5 % calcium lactate in drinking water<br><br>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. This is per day approx. 880 mg/kg bw/d (male) or approx. 930 mg/kg bw/d (female).<br><br>2.5 % intake is estimated to be approx. 464 mg/kg bw/d (male) or 535 mg/kg bw/d (female). |
| **Reliability** | 2 |
| **Acceptability** | Acceptable with restrictions |
| **Remarks** | The results of this study can only be used as a very rough approximation for a NOAEL for L(+) lactic acid because the effects observed (decrease in food consumption and body weight gain) might be due to high calcium intake, low water/food intake (no data presented in the publication) and/or malabsorption due to local gastrointestinal irritation (provoked by calcium or lactate). Thus, in the view of the RMS, the study seems to be inadequate to use the obtained NOAEL for derivation of reference values. |

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| | **COMMENTS FROM ...** *(specify)* |
| **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.*<br>*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** | |