## Section A6.6.4 Genotoxicity in vivo

**Annex Point IIA6.6** 6.6.4 In vivo cytogenetic study of the bone marrow in Chinese hamsters (cytogenetic in-vivo-test)

Official use only

### 1 REFERENCE

| | | |
|---|---|---|
| **1.1** | **Reference** | [redacted], 1989, Chromosome aberration assay in bone marrow cells of the Chinese hamster with KUE 13032 C, [redacted], Report No. [redacted], 1989-11-09 (unpublished) |
| **1.2** | **Data protection** | Yes |
| 1.2.1 | Data owner | Bayer CropScience AG |
| 1.2.2 | Companies with letter of access | Bayer Chemicals AG |
| 1.2.3 | Criteria for data protection | Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA. |

### 2 GUIDELINES AND QUALITY ASSURANCE

| | | |
|---|---|---|
| **2.1** | **Guideline study** | No<br>The methods used in this study are comparable to the OECD-Guideline 475. |
| **2.2** | **GLP** | Yes |
| **2.3** | **Deviations** | Yes<br>Deviations of the OECD-Guideline 475:<br>- Only one sampling was performed. The schedule for sampling was 40 hours for the vehicle control and the dose groups and 24 hours for the positive control.<br>- Only 100 cells per animal were scored for the mitotic index. |

### 3 MATERIALS AND METHODS

| | | |
|---|---|---|
| **3.1** | **Test material** | As given in section 2 of dossier. |
| 3.1.1 | Lot/Batch number | [redacted] |
| 3.1.2 | Specification | As given in section 2 of dossier. |
| 3.1.2.1 | Description | White powder |
| 3.1.2.2 | Purity | [redacted] (analytical finding of November 22, 1988) |
| 3.1.2.3 | Stability | In vehicle: at pH 4 = 15.3 days; at pH 7 = 18.8 hours; at pH 9 = < 10 minutes |
| 3.1.2.4 | Maximum tolerable dose | 1000 mg/kg bw |
| **3.2** | **Test Animals** | |
| 3.2.1 | Species | Hamster |
| 3.2.2 | Strain | Chinese hamsters inbred strain |
| 3.2.3 | Source | [redacted] |
| 3.2.4 | Sex | males and females |

## Section A6.6.4 Genotoxicity in vivo

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| | | |
|---|---|---|
| 3.2.5 | Age/weight at study initiation | Age: minimum 10 weeks<br>Weight: approximately 25 g |
| 3.2.6 | Number of animals per group | 5 males + 5 females per dose and sampling time were evaluated.<br>Remaining animals of each test group were evaluated in case an animal died in its test groups. |
| 3.2.7 | Control animals | Yes |
| **3.3** | **Administration/ Exposure** | Oral |
| 3.3.1 | Number of applications | 1 |
| 3.3.2 | Interval between applications | — |
| 3.3.3 | Postexposure period | 40 hours<br>24 hours (positive control) |
| 3.3.4 | Type | Gavage |
| 3.3.5 | Concentration | 0, 1000, 2000 or 4000 mg/kg bw |
| 3.3.6 | Vehicle | 0.5 % aqueous Cremophor emulsion |
| 3.3.7 | Concentration in vehicle | 0, 25, 50 or 100 mg/ml |
| 3.3.8 | Total volume applied | Dichlofluanid groups: 40 ml/kg bw<br>Positive control: 10 ml/kg bw |
| 3.3.9 | Controls | Vehicle (negative control),<br>30 mg/kg bw cyclophosphamide (positive control) dissolved in physiological saline. |
| **3.4** | **Examinations** | |
| 3.4.1 | Clinical signs | Yes |
| 3.4.2 | Tissue | Bone marrow |
| | | Number of animals: all animals |
| | | Number of cells: 100 metaphases per animal<br>100 cells for mitotic index per animal |
| | | Time points: 40 h after treatment,<br>positive control: 24 h after treatment |
| | | Type of cells: femoral marrow cells |
| | | Parameters: numbers and types of structural aberrations<br>mitotic index |
| **3.5** | **Further remarks** | — |

## Section A6.6.4 Genotoxicity in vivo

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### 4 RESULTS AND DISCUSSION

| | | |
|---|---|---|
| **4.1** | **Clinical signs** | After administration of 2000 mg dichlofluanid /kg bw one female died. Five males and three females died after treatment with 4000 mg dichlofluanid/kg bw. |
| **4.2** | **Haematology / Tissue examination** | As determined by evaluation of mitotic indices all dose levels of the test substance induced slight cytotoxic effects. |
| **4.3** | **Genotoxicity** | No |
| **4.4** | **Other** | — |

### 5 APPLICANT'S SUMMARY AND CONCLUSION

| | | |
|---|---|---|
| **5.1** | **Materials and methods** | Dichlofluanid was tested for chromosome-damaging effects using the cytogenetic test on bone marrow, which represents a sensitive in vivo test for chromosome damage in mammals. The methods used in this study were comparable with the OECD-Guideline 475. Existing deviations were described in 2.3 (see above). |
| **5.2** | **Results and discussion** | The medium and the high dose level induced lethalities.<br>At all dose levels dichlofluanid induced a slight cytotoxicity. As compared to the negative control value, treatment with dichlofluanid did not result in a significant enhancement of the aberration frequency at any dose level tested.<br>In contrast the positive control exerted a distinct chromosome-damaging effect. |
| **5.3** | **Conclusion** | It can be stated that during the mutagenicity test described and under the experimental conditions reported dichlofluanid did not induce chromosome mutations as determined by the chromosome aberration test with bone marrow cells of the Chinese hamster.<br>Therefore dichlofluanid is considered to be non-mutagenic in this chromosome aberration assay. |
| 5.3.1 | Reliability | 2 |
| 5.3.2 | Deficiencies | No |

| Evaluation by Competent Authorities | |
|---|---|
| | Use separate "evaluation boxes" to provide transparency as to the comments and views submitted |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 29/10/04 |
| **Materials and Methods** | As described above [IUCLID 5.6 3/9] |
| **Results and discussion** | As described above |
| **Conclusion** | As described above |
| **Reliability** | 2 |
| **Acceptability** | Acceptable |
| **Remarks** | The UK CA agrees with the applicant's summary and conclusions. |
| | **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.*<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** | |

## Table A6_6_4-2. Table for cytogenetic in-vivo-test: chromosomal analysis in femoral marrow cells

| | | Negative control | 1000 mg/kg bw | 2000 mg/kg bw | 4000 mg/kg bw | Positive control |
|---|---|---|---|---|---|---|
| **Sampling time (h)** | | 40 | 40 | 40 | 40 | 24 |
| **Number of cells evaluated** | | 1000 | 1000 | 1000 | 1000 | 1000 |
| **Mitotic index (%)** | | 4.49 | 2.59 | 2.79 | 2.77 | 2.31 |
| **Metaphases with aberrations excl. Gaps (%)** | | 0.2 | 0.7 | 0.6 | 0.8 | 26.3 |
| **Metaphases with aberrations incl. gaps (%)** | | 0.8 | 1.3 | 1.4 | 1.3 | 27.1 |
| **Chromatid aberrations** | gaps | 6 | 7 | 7 | 5 | 30 |
| | breaks | — | 4 | 4 | 5 | 79 |
| | fragment | 1 | — | — | 1 | 39 |
| | deletion | — | — | 1 | — | 9 |
| | exchange | — | — | — | — | 303 |
| | multiple aberrations* with exchanges | — | — | — | — | 117 |
| | chromosomal disintegration[#] | — | — | — | — | 14 |
| **Isochromatid aberrations** | gaps | — | — | 1 | — | 1 |
| | breaks | — | 2 | — | — | 13 |
| | fragment | 1 | 1 | 1 | 1 | 12 |
| | deletion | — | — | — | — | 2 |

* multiple aberration = more than 5 events, excluding gaps, in one cell; only exchanges, but no other aberrations, were recorded in these cells.

[#] chromosomal disintegration = pulverisation