## Section A6.3.2 Repeated dose toxicity

**Annex Point IIA6.3** 6.3.2 Subacute dermal toxicity study in rats

Official use only

### 1 REFERENCE

| | | |
|---|---|---|
| **1.1** | **Reference** | [redacted], 2003, Dichlofluanid – Study for suacute dermal toxicity in rats, [redacted], Report No. [redacted], 2003-04-02 (unpublished) |
| **1.2** | **Data protection** | Yes |
| 1.2.1 | Data owner | Bayer Chemicals AG |
| 1.2.2 | Companies with letter of access | — |
| 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

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| **2.1** | **Guideline study** | Yes<br>The methods used in this study are in accordance with:<br>- the OECD-Guideline 410,<br>- the EEC-Annex V, Part B.9 and<br>- the US-EPA, OPPTS 870.3200, EPA 712-C-98-201 |
| **2.2** | **GLP** | Yes |
| **2.3** | **Deviations** | Yes<br>- Analytical determination (stability) is missing,<br>- No use of satellite groups for the detection of reversibility, persistence or delayed occurrence of toxic effects. |

### 3 MATERIALS AND METHODS

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| **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] |
| 3.1.2.3 | Stability | Stable within the timeframe of the study. |
| **3.2** | **Test Animals** | |
| 3.2.1 | Species | Wistar rat |
| 3.2.2 | Strain | HsdCpb:WU (SPF-bred) |
| 3.2.3 | Source | [redacted] |
| 3.2.4 | Sex | Males and females |

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| 3.2.5 | Age/weight at study initiation | Males:<br>Age: 9 – 10 weeks<br>Weight: mean 259 g (244 – 285 g)<br>Females:<br>Age: 12 – 13 weeks<br>Weight: mean 221 g (206 – 230 g) |
| 3.2.6 | Number of animals per group | 5 animals per sex per dose group |
| 3.2.7 | Control animals | Yes |
| **3.3** | **Administration/ Exposure** | Dermal |
| 3.3.1 | Duration of treatment | 28 days |
| 3.3.2 | Frequency of exposure | 5 days per week for the first three weeks, daily in the fourth week. |
| 3.3.3 | Post-exposure period | None |
| **3.3.4** | **Dermal** | |
| 3.3.4.1 | Area covered | Greater than 10 % of body surface: 30.0 $cm^2$ |
| 3.3.4.2 | Occlusion | Occlusive |
| 3.3.4.3 | Vehicle | Phosphate buffer solution (pH 6); only used for moistening the gauze pads. |
| 3.3.4.4 | Concentration | 0, 100, 300 or 1000 mg/kg bw |
| 3.3.4.5 | Doses applied | See Table A6_3-1 in the appendix.<br>Depending on the body weight and the surface area of the gauze covered by the test substance layer (100 mg/kg: 1.5 – 2.25 $cm^2$; 300 mg/kg: 4.0 – 6.25 $cm^2$; 1000 mg/kg: 9.0 – 14.0 $cm^2$), the amounts of test substance which were applied per $cm^2$ skin were calculated for each treatment week. |
| 3.3.4.6 | Duration of exposure | 6 h |
| 3.3.4.7 | Removal of test substance | Soap and water. |
| 3.3.4.8 | Controls | Treated with phosphate buffer solution (pH 6). |
| **3.4** | **Examinations** | |
| 3.4.1 | Observations | |
| 3.4.1.1 | Clinical signs | Yes, at least once a day. |
| 3.4.1.2 | Mortality | Yes, at least once a day. |
| 3.4.2 | Body weight | Yes, before study begin and than weekly. |
| 3.4.3 | Food consumption | Yes, once weekly. |
| 3.4.4 | Water consumption | Yes, once weekly. |
| 3.4.5 | Ophthalmoscopic examination | No. |

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| 3.4.6 | Haematology | Yes,<br>Number of animals: all animals<br>Time points: end of study<br>Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, morphology of erythrocytes, total and differential leukocyte count, platelet count, reticulocyte count, mean corpuscular haemoglobin concentration, mean corpuscular volume, clotting time (hepatoquick) |
| 3.4.7 | Clinical Chemistry | Yes,<br>Number of animals: all animals<br>Time points: end of study<br>Parameters: sodium, potassium, inorganic phosphate, calcium, chloride, glucose (non-fastened), total cholesterol, urea, total bilirubin, creatinine, total protein, albumen, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase. |
| 3.4.8 | Urinalysis | No |
| **3.5** | **Sacrifice and pathology** | |
| 3.5.1 | Organ Weights | Yes<br>Organs: liver, kidneys, adrenals glands, testes. |
| 3.5.2 | Gross and histopathology | Yes<br>Necropsy: all animals<br>Systematic gross examination of each animal's general physical condition, body orifices, external and internal organs and tissues.<br><br>Histopathology:<br>all animals: skin (treated and untreated), organs and tissues with macroscopic findings<br>control and high dose group: liver and kidneys |
| 3.5.3 | Other examinations | Open field observations: before study begin and than once a week. The inspection was done in the morning before the starting the treatment. Parameters: any clinical findings, abnormalities, body surfaces and orifices, posture, general behaviour, breathing and excretory products, irritation at the dose site.<br><br>Test for local tolerability: the shaved skin areas were examined before study begin and than daily during the treatment. The assessment of reddening was done according the guidelines published by the US Department of Agriculture (US-Federal Register, 38, 187 27019, 1973) and according to Draize (The Appraisal of Chemicals in Food, Drugs and Cosmetics, 26 – 30. Association of Food and Drug Officials of the United States, Austin, Texas, 1959) as follows:<br><br>No reddening = 0<br><br>Very little reddening = 1<br><br>Moderate reddening = 2<br><br>Distinct reddening = 3<br><br>Intensive reddening (dark red) = 4<br><br>The evaluation of swellings (in males and in females on study days 1, 4, 8, 11, 15, 18, 22, 25, 29) was done by measuring the skinfold thickness on the back in the center of the application area using a cutimeter of skinfold caliper by Kroeplin. Measurements were taken at two different locations within the treatment area. From these a mean value was |

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| | | calculated. |
| 3.5.4 | Statistics | The quantitative results for individuals were used to calculate arithmetic group means and standard deviations. The results for the groups that received the test substance were compared with those for the control group at significance level $\alpha = 5\%$ and $\alpha = 1\%$. |
| **3.6** | **Further remarks** | — |
| | | **4 RESULTS AND DISCUSSION** |
| **4.1** | **Observations** | See also tables in the appendix. |
| 4.1.1 | Clinical signs | No systemic treatment-related effects. |
| 4.1.2 | Mortality | No mortalities at any dose level. |
| **4.2** | **Body weight gain** | No effects. |
| **4.3** | **Food consumption** | No effects. |
| **4.4** | **Water consumption** | No effects. |
| **4.5** | **Ophthalmoscopic examination** | — |
| **4.6** | **Blood analysis** | |
| 4.6.1 | Haematology | No effects. |
| 4.6.2 | Clinical chemistry | The concentrations of creatinine and albumin were decreased at 1000 mg/kg in males. Chloride concentrations were found not dose-related decreased in females at 100 mg/kg and above. All individual values were in the historical range. Therefore, these differences were considered as not toxicologically relevant. |
| 4.6.3 | Urinalysis | — |
| **4.7** | **Sacrifice and pathology** | |
| 4.7.1 | Organ weights | No effects. |
| 4.7.2 | Gross and histopathology | The only gross pathology findings made at necropsy were discoloured (pale) kidneys in one or two males of each treatment group and in two females dosed with 100 mg/kg bw. Histopathological examinations of the kidneys showed no treatment-related effects. This was also true for the investigation of the liver. Microscopic analysis of the skin displayed inflammatory infiltrates in all groups including controls. In the treated skin area of the females was a slightly elevated incidence of inflammatory infiltrates and an increased grading observable. However, these skin effects were not dose-dependent. In males only the grading was slightly increased. An epidermal thickening was observed at the treated of almost all animals of the dose groups. It also occurred in the untreated skin areas of some rats, probably due to an extension of these induced effects in this untreated location. |
| **4.8** | **Other** | Open field observation: no effects.<br><br>Test for local tolerability: skin reddening was observed at 100 mg/kg and above in both sexes. The intensity was increased up to marked reddening. In most animals of both sexes formation of scale was observed in all dose groups. Due to formation of rhagades, one male and one female of the high dose groups were treated only up to day 15 and |

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| | | one female of the 300 mg/kg dose group only up to day 16 of the study.<br><br>The mean skinfold thickness was slightly increased in animals of both sexes at 100 mg/kg and above in week two. | |
| | | **5 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **5.1** | **Materials and methods** | A subacute dermal study in rats was performed with the active substance dichlofluanid to determine toxic effects and establish a dose-response relationship. Particular emphasis was given to detecting potential effects requiring a lengthy latent period and/or cumulating under the test conditions.<br><br>The methods used in this study are in accordance with the OECD-Guideline 410, the EEC-Annex V, Part B.9 and the US-EPA, OPPTS 870.3200, EPA 712-C-98-201. | |
| **5.2** | **Results and discussion** | Dermal treatment of rats with dichlofluanid caused no systemic substance-related effects. Examination of the skin showed skin reddening and formation of scale (100 mg/kg and above, both sexes), rhagades (300 mg/kg and 100 mg/kg one female each, 1000 mg/kg: one male) were observed. Skinfold thickness was increased (100 mg/kg and above, both sexes, week 2). Microscopically, epidermal thickening occurred ( 100 mg/kg and above, both sexes). A no-effect level for the local skin effects could not be determined. | |
| **5.3** | **Conclusion** | | |
| 5.3.1 | LO(A)EL | $LOEL_{local\ skin\ effects}$: 1000 mg/kg bw | X |
| 5.3.2 | NO(A)EL | $NOEL_{systemic}$: 1000 mg/kg bw<br><br>$NOEL_{local\ skin\ effects}$: not applicable due to results of study. | |
| 5.3.3 | Other | — | |
| 5.3.4 | Reliability | 2 | |
| 5.3.5 | Deficiencies | Analytical determinations (stability, homogeneity) were not performed, because the substance was applied neat and was only moistened with phosphate buffer solution (pH 6) immediately before application. | |

| Evaluation by Competent Authorities | |
|---|---|
| | Use separate "evaluation boxes" to provide transparency as to the comments and views submitted |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 1/11/04 |
| **Materials and Methods** | As described above [IUCLID 5.1 11/11] |
| **Results and discussion** | As described above |
| **Conclusion** | $NOAEL_{systemic\ effects}$: 1000 mg/kg/day<br>$LOAEL_{skin\ effects}$: 100 mg/kg/day |
| **Reliability** | 2 |
| **Acceptability** | Acceptable |
| **Remarks** | The UK CA generally agrees with the applicant's summary and conclusions. However the LOAEL for local skin effects is considered to be 100 mg/kg/day. |
| | **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** | |

## Table A6_3-1. Doses applied in mg/cm$^2$ skin

Depending on the body weight and the surface area of the gauze covered by the test substance layer (100 mg/kg: 1.5 – 2.25 cm$^2$; 300 mg/kg: 4.0 – 6.25 cm$^2$; 1000 mg/kg: 9.0 – 14.0 cm$^2$), the following amount of test substance was applied per cm$^2$ skin:

| Applied doses in mg/cm$^2$ skin | | | | |
|---|---|---|---|---|
| **Dose (mg/kg bw)** | **Week 1 of treatment** | **Week 2 of treatment** | **Week 3 of treatment** | **Week 4 of treatment** |
| **Males** | | | | |
| **100** | **17.4** | **12.6** | **13.8** | **14.7** |
| **300** | **15.8** | **17.2** | **14.9** | **15.8** |
| **1000** | **24.4** | **26.5** | **24.7*** | **22.6*** |
| **Females** | | | | |
| **100** | **14.8** | **9.9** | **10.0** | **10.2** |
| **300** | **16.7** | **13.5** | **11.1*** | **11.2*** |
| **1000** | **24.2** | **21.1** | **21.4*** | **18.5*** |

*** only four animals, without animals showing rhagades**

## Table A6_3-2. Results of clinical chemistry haematology and urinalysis

**Not needed due to results of the study.**

## Table A6_3-3. Results of subacute dermal toxicity study in rats

| | Control 0 mg/kg | | Low dose 100 mg/kg | | Medium dose 300 mg/kg | | High dose 1000 mg/kg | | Dose-response +/- | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **m[a]** | **f[a]** | **m[a]** | **f[a]** | **m[a]** | **f[a]** | **m[a]** | **f[a]** | **m** | **f** |
| **Number of animals examined** | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | |
| **Organ: skin** | | | | | | | | | | |
| **Local skin findings:** | | | Formation of scale, | | Formation of scale, | rhagades | Formation of scale, rhagades | rhagades | + | + |
| Reddening* | 0 | 0 | ↑ | ↑↑ | ↑↑ | ↑↑ | ↑↑↑ | ↑↑ | + | + |
| Skinfold thickness | | | slightly increased in week 2 | | slightly increased in week 2 | | slightly increased in week 2 | | | |
| **Microscopic pathology** | | | Epidermal thickening | | Epidermal thickening | | Epidermal thickening | | + | + |

*Reddening in week 4 of study:

No reddening = 0

Mainly very little reddening to moderate reddening: ↑

Mainly moderate to distinct reddening: ↑↑

Mainly distinct reddening: ↑ ↑ ↑