**Table 3 Evaluation of interlaboratory reproducibility of the results using Student's t-test (α = 0.05).**

| Double-sampling F-test for dispersion | | |
|---|---|---|
| | | |
| | *Set 1* | *Set 2* |
| Mean value | 97.77596119 | 97.75687361 |
| Dispersion | 0.115819783 | 0.161626268 |
| No. of observations | 10 | 10 |
| Difference | 9 | 9 |
| F | 0.716590098 | |
| P(F<=f) (1) | 0.313793987 | |
| F crit (1) | 3.178893105 | |

| Double-sampling t-test with disparity of dispersions | | |
|---|---|---|
| | | |
| | *Set 1* | *Set 2* |
| Mean value | 97.77596119 | 97.75687361 |
| Dispersion | 0.115819783 | 0.161626268 |
| No. of observations | 10 | 10 |
| Hyp. difference of mean values | 0 | |
| Difference | 18 | |
| t stat | 0.114593951 | |
| P(T<=t) (1) | 0.455017787 | |
| t crit (1) | 1.734063592 | |
| P(T<=t) (2) | 0.910035575 | |
| t ckrit (2) | 2.100922037 | |

| | **Evaluation by Competent Authorities** |
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| **Date** | |
| **Evaluation of applicant's justification** | |
| **Conclusion** | |
| **Remarks** | |

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| **Section A4**<br>**Annex Point IIA IV.** | **ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION OF PRODUCT COMPONENTS** | |

| **Section A4.1**<br>**Annex Point IIA IV.4.1** | **Determination of Sulphur Dioxide Content** | |
|---|---|---|
| | **1 REFERENCE** | **Official use only** |
| **1.1 Reference** | Company standard PND 47-189-03 | |
| **1.2 Data protection** | No | |
| 1.2.1 Data owner | Lučební závody Draslovka a.s. Kolín | |
| 1.2.2 | | |
| 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 Preliminary treatment** | | |
| **3.1.1 Enrichment** | No enrichment involved in the method | |
| **3.1.2 Clean-up** | No clean-up involved in the method | |
| **3.2 Preliminary treatment** | | |
| 3.2.1 Enrichment | No enrichment involved in the method | |
| 3.2.2 Cleanup | No clean-up involved in the method | |
| **3.3 Detection** | | |
| 3.3.1 Separation method | Determination of sulphur dioxide iodometrically | |
| 3.3.2 Detector | Indirect iodometric titration with an $Na_2S_2O_3$ volumetric solution | |
| 3.3.3 Standard(s) | $K_2Cr_2O_7$ | |
| 3.3.4 Interfering substance(s) | Not present | |
| **3.4 Linearity** | | |
| 3.4.1 Calibration range | Not applicable | |
| 3.4.2 Number of measurements | Not applicable | |
| 3.4.3 Linearity | Not applicable | |

| | | |
|---|---|---|
| **3.5 Specifity: interfering substances** | Not present | |
| **3.6 Recovery rates at different levels** | | |
| 3.6.1 Relative standard deviation | Max 0.05 | |
| **3.7 Limit of determination** | 1000 ppm | |
| **3.8 Precision** | | |
| 3.8.1 Repeatability | See section 7.1,<br><5% | |
| 3.8.2 Independent laboratory validation | See section 5-7 | |
| | **4 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **4.1 Materials and methods** | The assessment of the sulphur dioxide content is carried out through indirect iodometric titration with a $Na_2S_2O_3$ volumetric solution.<br>Chemicals:<br>HCl, 25% solution<br>Potassium iodide solution (25 g in 30 ml water)<br>Sodium thiosulphate (0.1 mol/l)<br>Starch solution, indicator<br><br>Method principle<br>The content of sulphur dioxide is determined iodometrically. A sample of hydrogen cyanide is charged to iodide solution excess. After the reaction, the excess of iodide is determined by titration with a solution of sodium thiosulphate to a starch solution as an indicator.<br><br>Test procedure:<br>Put 20 ml of distilled water to a 250ml ground-joint Erlenmeyer flask, add 20 ml of iodide volumetric solution (0.1 mol/l), weigh the Erlenmeyer flask with the prepared solution on pre-scales.<br>Measure approx. 2 g HCN (2-3 ml HCN) from a syringe to the solution. Close the flask immediately, mix the content, and weigh the flask on pres-scales. After the reaction of $SO_2$ and $I_2$ is over, do titrate excessive iodine solution with a $Na_2S_2O_3$ (0.1 mol/l) volumetric solution, using starch solution as an indicator.<br>Concurrently, a blank test shall be performed. | |
| **4.2 Conclusion** | Calculation:<br>The sulphur dioxide content in % w (x) shall be calculated as follows:<br>$\mathbf{x = \frac{(a-b) . f . 3.203 . 100}{m}}$<br>Where<br>a ……. consumption of the sodium thiosulphate volumetric solution<br>c = 0.1 mol/l for the blank test | |

| | | |
|---|---|---|
| | b……… consumption of the sodium thiosulphate volumetric solution<br>c = 0.1 mol/l for the solution titration<br>f……… factor of the sodium thiosulphate volumetric solution<br>m………. HCN sample weight (mg)<br>3.203….mg eqv. $SO_2$ (1 ml volumetric solution ($Na_2S_2O_3$) = 0.1 mol/l<br>shall titre 5.406 mg $SO_2$ | |
| 4.2.1 Reliability | *2* | |
| 4.2.2 Deficiencies | *No* | |
| | **5 VALIDATION SUBJECT** | |
| | Method of determination of sulphur dioxide ($SO_2$) content in Uragan D2 by iodometric back-titration, using a volumetric solution of $Na_2S_2O_3$.<br>The required quality parameter is $SO_2$ content 0.9-1.1 % wt. | |
| | **6 METHOD DESCRIPTION** | |
| | Iodometric back-titration. The method is described in detail in a company standard PND 47-189-03. | |
| | **7 MEASURED VALUES** | |
| **7.1 Method repeatability** | The repeatability determination is performed on production samples from ten independent analyses in research department laboratories, using identical solutions and the same method described in the company standard PND 47-189-03. The analyses are performed by the same analytical chemist on the same day.<br>Required value: coefficient of variation ***($v_x$) < 5 %***<br>Where ***($v_x$)*** is the coefficient of variation:<br>$v_x = \frac{s_x}{\overline{x}} \cdot 100\,[\%]$<br><u>Arithmetic mean of the set may be calculated as</u>:<br>$\overline{x} = \frac{1}{N}\sum_{i=1}^{N} x_i$<br>Sample standard deviation of the data measured is calculated as:<br>$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2}.$<br>**Results are summarised in Table 1.** | |
| **7.2 Interlaboratory reproducibility of the results** | The determination is performed on an identical sample on the same day in the quality assurance laboratory. The analysis according to company PND 47-189-03 is performed by a different analytical chemist using | |

| | | |
|---|---|---|
| | different solutions. On the basis of these results (using Student’s t-test), reproducibility of the results was evaluated.<br>Required value: coefficient of variation ***$(v_x) < 5$ %***<br>**Results are summarised in Table 2.** | |
| | **8 CONCLUSION** | |
| | Both sets of measured values meet the condition or repeatability ($v_x < 5$ %). Processed results also meet the interlaboratory reproducibility condition (result of Student’s test $t < t_{crit}$ ). The method is suitable for a standard chemical analytical laboratory. The procedure does not require any special equipment or chemicals.<br>**Results are summarised in Table 3.** | |

**Table 1. Repeatability of the determination of $SO_2$ in Uragan D2 (research department)**

| **Test no.** | **Sample weight [mg]** | **Consumption of $Na_2S_2O_3$ (c = 0.1 M) [ml]** | **Calculated concentration of $SO_2$ [% wt.]** |
|---|---|---|---|
| 1 | 1790.5 | 13.5 | 1.02 |
| 2 | 1830.3 | 13.5 | 0.99 |
| 3 | 1870.4 | 13.6 | 0.96 |
| 4 | 1837.1 | 13.5 | 0.99 |
| 5 | 1751.2 | 13.4 | 1.06 |
| 6 | 1817.6 | 13.5 | 1.00 |
| 7 | 1853.3 | 13.5 | 0.98 |
| 8 | 1749.6 | 13.4 | 1.06 |
| 9 | 1892.0 | 13.6 | 0.95 |
| 10 | 1795.0 | 13.5 | 1.01 |
| | | **Mean** | **1.00** |
| | | **s** | **0.0374** |
| | | **$v_x$** | **3.73 %** |

Analysed on: 23 Feb 2007

Required condition of repeatability for the content determination $v_x < 5$ % HCN was met.

**Table 2. Repeatability of the determination of $SO_2$ in Uragan D2 (quality assurance laboratory)**

| **Test no.** | **Sample weight [mg]** | **Consumption of $Na_2S_2O_3$ (c = 0.1 M) [ml]** | **Calculated concentration of $SO_2$ [% wt.]** |
|---|---|---|---|
| 1 | 1722.5 | 13.4 | 1.08 |
| 2 | 1802.3 | 13.5 | 1.01 |
| 3 | 1870.8 | 13.5 | 0.97 |
| 4 | 1775.4 | 13.5 | 1.03 |
| 5 | 1736.2 | 13.4 | 1.07 |
| 6 | 1739.7 | 13.4 | 1.06 |
| 7 | 1845.1 | 13.6 | 0.97 |
| 8 | 1764.8 | 13.4 | 1.05 |
| 9 | 1796.2 | 13.6 | 1.00 |
| 10 | 1769.3 | 13.5 | 1.03 |
| | | **Mean** | **1.03** |
| | | **s** | **0.0385** |
| | | **$v_x$** | **3.76 %** |

Analysed on: 23 Feb 2007

**Table 3. Evaluation of interlaboratory reproducibility of the results using Student's t-test (α = 0.05).**

Double-sampling F-test for dispersion

| | *Set 1* | *Set 2* |
|---|---|---|
| Mean value | 1.025800016 | 1.001716138 |
| Dispersion | 0.001484981 | 0.001396742 |
| No. of observations | 10 | 10 |
| Degrees of freedom | 9 | 9 |
| F | 1.063174551 | |
| P(F<=f) (1) | 0.464393994 | |
| F crit (1) | 3.178893105 | |

Double-sampling t-test with disparity of dispersions

| | *Set 1* | *Set 2* |
|---|---|---|
| Mean value | 1.025800016 | 1.001716138 |
| Dispersion | 0.001484981 | 0.001396742 |
| No. of observations | 10 | 10 |
| Hyp. difference of mean values | 0 | |
| Degrees of freedom | 18 | |
| t stat | 1.418731736 | |
| P(T<=t) (1) | 0.086530372 | |
| t crit (1) | 1.734063592 | |
| P(T<=t) (2) | 0.173060743 | |
| t crit (2) | 2.100922037 | |

| | **Evaluation by Competent Authorities** |
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| **Date** | |
| **Evaluation of applicant's justification** | |
| **Conclusion** | |
| **Remarks** | |

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|---|---|---|
| **Section A4**<br>**Annex Point IIA IV.** | **ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION OF PRODUCT COMPONENTS** | |

| **Section A4.1**<br>**Annex Point IIA IV.4.1** | **Determination of Free Phosphoric Acid Content** | **Official use only** |
|---|---|---|
| | **1 REFERENCE** | |
| **1.1 Reference** | Company standard 47-189-03 | |
| **1.2 Data protection** | No | |
| 1.2.1 Data owner | Lučební závody Draslovka a.s. Kolín | |
| 1.2.2 | | |
| 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 Preliminary treatment** | | |
| **3.1.1 Enrichment** | No enrichment involved in the method | |
| **3.1.2 Clean-up** | No clean-up involved in the method | |
| **3.2 Detection** | | |
| 3.2.1 Separation method | Determination of phosphoric acid by acidometric titration | |
| 3.2.2 Detector | pH-meter, glass electrode | |
| 3.2.3 Standard(s) | pH Buffer standards | |
| 3.2.4 Interfering substance(s) | Not present | |
| **3.3 Linearity** | | |
| 3.3.1 Calibration range | Not applicable | |
| 3.3.2 Number of measurements | Not applicable | |
| 3.3.3 Linearity | Not applicable | |
| **3.4 Specifity: interfering substances** | Not present | |
| **3.5 Recovery rates at different levels** | | |
| 3.5.1 Relative standard deviation | 0.002 | |

| | | |
|---|---|---|
| **3.6 Limit of determination** | 100 ppm | |
| **3.7 Precision** | | |
| 3.7.1 Repeatability | <5% | |
| 3.7.2 Independent laboratory validation | See section 5-7 | |
| | **4 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **4.1 Materials and methods** | The assessment of the phosphoric acid content is carried out through titration with NaOH volumetric solution.<br>Chemicals:<br>Sodium hydroxide NaOH (0.1 mol/l)<br>Method principle<br>The content of phosphoric acid is determined by titration.<br>Test procedure:<br>In a well-venting fume cupboard, pour 100 ml HCN to a 400ml beaker. The beaker content then slowly vaporizes at 50 °C (water bath). Add 10 ml of distilled water, mix, and pour the content to a 50ml beaker and do titrate with a NaOH solution (0.1 mol/l) to pH 4.5, while using a pH-meter with a glass electrode. | |
| **4.2 Conclusion** | Calculation:<br>The phosphoric acid content in % wt (x) shall be calculated as follows:<br>$x = \frac{a \cdot f \cdot 9.8 \cdot 100}{0.7 \cdot b \cdot 1000} = \frac{a \cdot f \cdot 9.8}{7 \cdot b}$<br>Where<br>a ……… consumption of the sodium hydroxide volumetric solution c = 0.1 mol/l<br>b……… HCN content (ml)<br>f……… factor of the sodium hydroxide volumetric solution<br>0.7……..specific gravity of HCN at 10 °C<br>9.8……..mg eqv. $H_3PO_4$ (1 ml of volumetric solution equals 9.8 mg $H_3PO_4$) | |
| 4.2.1 Reliability | *2* | |
| 4.2.2 Deficiencies | *No* | |
| | **5 VALIDATION SUBJECT** | |
| | Method of determination of the total free $H_3PO_4$ content in Uragan D2 by titration with NaOH. The required quality parameter is $H_3PO_4$ content 0.08-0.12 % wt. | |
| | **6 METHOD DESCRIPTION** | |
| | Titration with a volumetric solution of NaOH up to pH 4.5. The method is described in detail in a company standard PND 47-189-03. | |

## 7 MEASURED VALUES

**7.1 Method repeatability**

The repeatability determination is performed on production samples from ten independent analyses in research department laboratories, using identical solutions and the same method described in the company standard PND 47-189-03. The analyses are performed by the same analytical chemist on the same day.

Required value: coefficient of variation ***($v_x$)*** ***< 5 %***

Where ***($v_x$)*** is the coefficient of variation:

$$v_x = \frac{s_x}{\overline{x}} \cdot 100\,[\%]$$

<u>Arithmetic mean of the set may be calculated as</u>:

$$\overline{x} = \frac{1}{N}\sum_{i=1}^{N} x_i$$

Sample standard deviation of the data measured is calculated as:

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2}.$$

**Results are summarised in Table 1.**

**7.2 Interlaboratory reproducibility of the results**

The determination is performed on an identical sample on the same day in quality assurance laboratory. The analysis according to company standard PND 47-189-03 is performed by a different analytical chemist using different solutions. Required value: coefficient of variation ***($v_x$)*** ***< 5 %***

**Results are summarised in Table 2.**

**7.3 Evaluation of interlaboratory reproducibility**

The method brought the same results in both laboratories, including dispersion of values, which represents unique confirmation of the reproducibility (mean 1 = mean 2, $s_1$=$s_2$). The identity of results is to some degree due to the determination principle itself, since a unified sample volume 100 ml is used – thus the results differ only in the volumetric solution consumption, within the error of measurement.

## 8 CONCLUSION

Both sets of measured values meet the condition or repeatability (***$v_x$***< 5 %). Processed results also meet the interlaboratory reproducibility condition. The method is suitable for a standard chemical analytical laboratory. The procedure does not require any special equipment or chemicals.

**Table 1. Repeatability of the determination of $H_3PO_4$ in Uragan D2 (research department)**

| **Test no.** | **Consumption of NaOH (c = 0.1 M) [ml]** | **Calculated concentration of $H_3PO_4$ [% wt.]** |
|---|---|---|
| 1 | 7.1 | 0.099 |
| 2 | 7.1 | 0.099 |
| 3 | 7.2 | 0.100 |
| 4 | 7.2 | 0.100 |
| 5 | 7.1 | 0.099 |
| 6 | 7.1 | 0.099 |
| 7 | 7.1 | 0.099 |
| 8 | 7.0 | 0.098 |
| 9 | 7.0 | 0.098 |
| 10 | 7.0 | 0.098 |
| | **Mean** | **0.10** |
| | **s** | **0.0010** |
| | **$v_x$** | **1.04 %** |

Analysed on: 21 Feb 2007

Required condition of repeatability for the content determination $v_x < 5$ % HCN was not met.

**Table 2. Repeatability of the determination of $H_3PO_4$ in Uragan D2 (quality assurance laboratory)**

| **Test no.** | **Consumption of NaOH (c = 0.1 M) [ml]** | **Calculated concentration of $H_3PO_4$ [% wt.]** |
|---|---|---|
| 1 | 7.2 | 0.100 |
| 2 | 7.1 | 0.099 |
| 3 | 7.0 | 0.098 |
| 4 | 7.1 | 0.099 |
| 5 | 7.0 | 0.098 |
| 6 | 7.1 | 0.099 |
| 7 | 7.2 | 0.100 |
| 8 | 7.1 | 0.099 |
| 9 | 7.1 | 0.099 |
| 10 | 7.0 | 0.098 |
| | **Mean** | **0.10** |
| | **s** | **0.0010** |
| | **$v_x$** | **1.04 %** |

Analysed on: 21 Feb 2007

| | **Evaluation by Competent Authorities** |
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| **Date** | |
| **Evaluation of applicant's justification** | |
| **Conclusion** | |
| **Remarks** | |

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|---|---|---|
| **Section A4**<br>**Annex Point IIA IV.** | **ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION OF PRODUCT COMPONENTS** | |

| | | |
|---|---|---|
| **Section A4.1**<br>**Annex Point IIA IV.4.1** | **Determination of Water Content** | |
| | **1 REFERENCE** | **Official use only** |
| **1.1 Reference** | ISO 760 Determination of water Karl Fischer method | |
| **1.2 Data protection** | No | |
| 1.2.1 Data owner | / | |
| 1.2.2 | | |
| 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 Preliminary treatment** | | |
| **3.1.1 Enrichment** | No enrichment involved in the method | |
| **3.1.2 Clean-up** | No clean-up involved in the method | |
| **3.2 Detection** | | |
| 3.2.1 Separation method | Water determination by Karl Fischer method | |
| 3.2.2 Detector | Direct electrometric titration | |
| 3.2.3 Standard(s) | Fischer reagent | |
| 3.2.4 Interfering substance(s) | Not present | |
| **3.3 Linearity** | | |
| 3.3.1 Calibration range | Not applicable | |
| 3.3.2 Number of measurements | Not applicable | |
| 3.3.3 Linearity | Not applicable | |
| **3.4 Specifity: interfering substances** | Not present | |
| **3.5 Recovery rates at different levels** | | |
| 3.5.1 Relative standard deviation | Not applicable | |

| | | |
|---|---|---|
| **3.6 Limit of determination** | The volume of Karl Fischer reagent consumed for titration is recorded | |
| **3.7 Precision** | | |
| 3.7.1 Repeatability | The determination is made strictly according to ISO 760 standard | |
| 3.7.2 Independent laboratory validation | The determination is made strictly according to ISO 760 standard | |
| | **4 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **4.1 Materials and methods** | The procedure according to the method specified in ISO 760 standard - Determination of water Karl Fischer method - Direct electrometric titration<br>Chemicals:<br>Methanol<br>Pyridine<br>Sample solvent – mixture of 4 volumes of methanol (4:1) with 1 volume of pyridine (4:3).<br>Fischer reagent<br>Sodium Tartrate Dihydrate or water<br>Water in methanol (water content 10 g/l a 2 g/l)<br>Sodium Aluminium Silicate<br><br>Method principle<br><br>Water determination by Karl Fischer method. The principle of the test consists in reaction of any water present in a test portion with a solution of iodine and sulphur dioxide in a pyridine/methanol mixture (Karl Fischer reagent), previously standardized by titration with an exactly known mass of water.<br><br>**Procedure:**<br>Cyanide sample is collected in liquid state before final filling (racking). Pre-cooled air-tight containers are used for transporting and measuring the sample. For sampling it is required to use approved protective equipment - face mask corresponding to EN 136 (e.g. type CM4 equipped with a filter type B2, corresponding to EN 141), rubber gloves (EN 374-1), rubber boots (EN 346).<br><br>In laboratory, open samples are manipulated with in the hood using a protective face mask with filter B2. The sample weight is usually between 1 and 2 grams. Sample weight in this range is adjusted according to the estimated amount of water. The sample is fed through the septum directly into the solvent submitted for Karl Fischer titration.<br><br>Principle of Endpoint Detection:<br><br>Two platinum electrodes with embedded potential difference are immersed into the solution; if there is water in the solution, the polarisation of the cathode prevents the passage of current. The end of titration is indicated during depolarization of the cathode by a sudden increase in current (recorded by a suitable electrical device). | |

| | | |
|---|---|---|
| 4.2 **Conclusion** | Expression of results:<br>Water equivalent of Karl Fischer reagent (T):<br>$T = \frac{m_3 \times 0.1566}{V_3}$ **or** $T = \frac{m_4}{V_3}$<br>Where<br>$m_3$ ……. weight of sodium tartrate if used for calibration in miligrams<br>$m_4$……… weight of added water if used for calibration in miligrams<br>$V_3$............ volume of Fischer reagent used for calibration in mililitres<br>0.1566… factor for calculation of water in sodium tartrate dihydrate<br>Water content (in % wt) :<br>$\frac{V_4 \times T}{m_0 \times 10}$ **or** $\frac{V_4 \times T}{V_0 \times p \times 10}$<br>Where<br>$m_0$……….weight of sample in grams (solid)<br>$V_0$……….volume of sample in ml (liquid)<br>p ………… specific weight of samples in g/ml at 20 °C<br>$V_4$………..volume of Fischer reagent in ml<br>T………… water equivalent of Fischer reagent in mg/ml | |
| 4.2.1 Reliability | *2* | |
| 4.2.2 Deficiencies | *No* | |
| | **5 VALIDATION SUBJECT** | |
| | The method validation was not performed, it is performed strictly according ISO 760 standard. | |

| | **Evaluation by Competent Authorities** |
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| **Date** | |
| **Evaluation of applicant's justification** | |
| **Conclusion** | |
| **Remarks** | |

| **Section A4.1**<br>**Annex Point IIA IV.4.1** | **ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION OF PRODUCT COMPONENTS** | |
|---|---|---|
| **Details:** | This section point is detailed in DOC III-A_4.1.1, 4.1.2, 4.1.3 a 4.1.4. | |
| **Method of active substance determination** | **Hydrogen cyanide**<br>Densimetry with a densimeter with a measuring range 0.690–0.740 and 0.001 division, fitted with a thermometer with a range –30 to +30 °C and 2 °C division.<br>The assessment of the hydrogen cyanide content is carried out through indirect argentometric titration with a silver nitrate volumetric solution.<br>**For details and validation protocol, see DOC III-A_4.1.1** | |
| **Methods of additives determination** | **Sulphur dioxide**<br>The assessment of the sulphur dioxide content is carried out through indirect iodometric titration with a $Na_2S_2O_3$ volumetric solution.<br>**For details and validation protocol, see DOC III-A_4.1.2** | |
| | **Phosphoric acid**<br>The assessment of the phosphoric acid content is carried out through titration with NaOH volumetric solution.<br>**For details and validation protocol, see DOC III-A_4.1.3** | |
| **Method of impurity determination** | **Water**<br>Water determination by Karl Fischer method. The principle of the test consists in reaction of any water present in a test portion with a solution of iodine and sulphur dioxide in a pyridine/methanol mixture (Karl Fischer reagent), previously standardized by titration with an exactly known mass of water.<br>**For details see DOC III-A_4.1.4** | |
| **Undertaking of intended data submission** | No further studies are planned. Described methods are sufficiently robust and accurate for their intended uses. | |

| | **Evaluation by Competent Authorities** |
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| **Date** | |
| **Evaluation of applicant's justification** | |
| **Conclusion** | |
| **Remarks** | |

| **Section A4.2**<br>**Annex Point IIA IV.4.2** | **Determination of Hydrogen Cyanide Content in Soil, Air, Water, Body Fluids** | |
|---|---|---|
| | **1 REFERENCE** | |
| **1.1 Reference** | Modification of “Standard Methods for the Examination of Water and Wastewater No. 413A-D”, American Public Health Association, American Water Works Association, Water Pollution Control Federation **(DOC IV_6)** | |
| **1.2 Data protection** | Yes | |
| 1.2.1 Data owner | GEOtest Brno, a.s., Laboratory | |
| 1.2.2 Companies with letter of access | Lučební závody Draslovka, a. s. Kolín | |
| 1.2.3 Criteria for data protection | Data submitted to the CA MS after 13 may 2000 for the purpose of entry existing active substance into Annex I/IA | |
| | **2 GUIDELINES AND QUALITY ASSURANCE** | |
| **2.1 Guideline study** | No | |
| **2.2 GLP** | No | |
| **2.3 Deviations** | No | |
| | **3 MATERIALS AND METHODS** | |
| **3.1 Preliminary treatment** | | |
| 3.1.1 Enrichment | Enrichment factor 5-10: distillation of HCN from acidified sample to alkaline solution | |
| 3.1.2 Cleanup | No | |
| **3.2 Detection** | | |
| 3.2.1 Separation method | distillation from strongly acidic media | |
| 3.2.2 Detector | Spectrophotometer HELIOS GAMMA (Unicam), 578 nm, l=10 mm | |
| 3.2.3 Standard(s) | External standard “Cyanide Standard Solution Merck Ord.qwNo. 1.19533.0500, 1000 mg/l” | |
| 3.2.4 Interfering substance(s) | Sulphides, free chlorine | |
| **3.3 Linearity** | | |
| 3.3.1 Calibration range | 0.025-0.700 mg/l directly | |
| 3.3.2 Number of measurements | 8 points for calibration relation | |
| 3.3.3 Linearity | $R^2 > 0.999$ | |

| | | | |
|---|---|---|---|
| **3.4** | **Specifity: interfering substances** | Sulphides, free chlorine | |
| **3.5** | **Recovery rates at different levels** | 85-90% for whole measuring range | |
| 3.5.1 | Relative standard deviation | ± 6 % for water samples | |
| **3.6** | **Limit of determination** | 0,005 mg/l for enrichment factor 5 | |
| **3.7** | **Precision** | better than 5 % for water samples | |
| 3.7.1 | Repeatability | ± 10 % for water samples | |
| 3.7.2 | Independent laboratory validation | ± 20 % (Z-score ≤ ± 2) | |
| | | **4 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **4.1** | **Materials and methods** | **Sample preparation:**<br>**AIR**<br>Sample preparation: air containing HCN is driven to a receiver containing 0.1 M sodium hydroxide solution where HCN is absorbed. Then the same procedure as described for water can be used.<br>2 more possibilities<br>**BODY FLUIDS**<br>Liberation of HCN from blood: sample preparation as described in **DOC IV_32**: venous blood (10 ml) is drawn off, heparinized and centrifuged at 2000 G. for 10 minutes, the sediment is washed with 0.9% saline solution. The erythrocytes can be either analysed immediately or stored in airtight containers at 4 °C. The erythrocytes (500 µl) are placed in 4N sulphuric acid in a micro distillation apparatus. The HCN given off is driven over to a receiver containing sodium hydroxide solution and then the same procedure as described for water can follow.<br>**WATER**<br>HCN liberated from the sample by distillation from acidified media is absorbed in 0.1M NaOH solution.<br>The emerging cyanide ion is then converted by chloramine T chlorination to cyanogen chloride (CAS no. 506-77-4) which by addition to pyridine will form a cyanogen-pyridine cation. During hydrolysis a pyridic core gets open with the formation of glutacone aldehyde. Glutacone aldehyde reacts with barbituric acid, getting colouration with intensity proportional to the hydrogen cyanide concentration and is measured spectrophotometrically. | |
| **4.2** | **Conclusion** | Determination of CN in erythrocytes: The limit of determination of CN in blood is reported to be around 0.5 nmol CN/ml in **DOC IV_32**<br>Rodkey, F.L. and Collison, H.A. analysed whole blood for CN and reported 100% CN recovery upon blood acidification. (**DOC IV_70)**<br>It should be noted that the method described for determining HCN content in air in section 4.1 allows for lowering HCN limit of quantification as needed | |

| | | |
|---|---|---|
| | simply by increasing the amount of air pumped through the receiver. | |
| 4.2.1 Reliability | 2 | |
| 4.2.2 Deficiencies | No | |

## 5 VALIDATION

**SOP AA-10:** Determination of all cyanides

**SOP AA-11:** Determination of easily liberatable cyanides

**5.1 Test methods:**

## 6 METHOD VALIDATION

**6.1 Validation plan**

**Objective:**
To verify, or prove the quality of test methods by validating and documenting that the methods comply with the given purpose by meeting applicable criteria.

**Procedure of validation:**
internal verification of the method fitness by measuring validation parameters.

*Note: Validation of some parameters is performed jointly for both methods because it is the same principle and nature of the methods (calibration, measurement), they differ only in the analyte isolation.*

**Validation performance date: January 2001**

**6.2 Validated parameters:**

1. Calibration dependence and linearity through testing the record of calibration functions subjectively and through a correlation coefficient.
2. Accuracy characterised by repeatability for an artificial sample and a real sample.
3. Correctness and deviation through measuring an independent standard with a known declared value.
4. Operating range upon a concentration range for which a linear response is proved.
5. Detection limit and determination limit through a statistical evaluation of the calibration dependence, or a relative standard deviation of the repeatability of calibration standard with a very low concentration.
6. Sensitivity as a slope of the calibration line in the discovered operating range in appropriate units.
7. Method yield through measuring the certified reference sample with a known value of analyte concentration.
8. Robustness on selected parameters of test conditions.

**6.2.1 Validated substances:**

All cyanides, easily liberatable cyanides
Water, soil, solution

Parameters validated jointly for both methods:1, 4, 5, 6, 8
Parameters validated separately for individual methods:2, 3, 7

The validation is performed in such a way so that conditions of all measurements comply with documented procedures!

Evaluation is performed with the ADSTAT 1.25 statistical program.

**6.2.2 Validated matrix:**

| Measurement no. | Concentration | A (578 nm, l =1 cm) | Correlation coefficient* |
|---|---|---|---|
| 1 | 0 | 0.023 | -- |
| 2 | 0.1 | 0.334 | -- |
| 3 | 0.2 | 0.670 | -- |
| 4 | 0.3 | 0.977 | -- |
| 5 | 0.4 | 1.294 | -- |
| 6 | 0.5 | 1.614 | 0.9999 |
| 7 | 0.6 | 1.925 | 0.9999 |
| 8 | 0.7 | 2.213 | 0.9998 |
| 9 | 0.8 | 2.480 | 0.9994 |
| 10 | 0.9 | 2.725 | 0.9985 |
| 11 | 1.0 | 2.776 | 0.9911 |

**Calibration dependence** (valid for both methods specified)

Direct determination through a set of calibration standards prepared from CRM in the concentration range 0–1.0 mg $CN^-$/l.

* Tested by linear regression for the validity range from C = 0 to C = adequate concentration

*Note: Graphic recordings of the dependence are given in the Appendix.*

**Evaluation:**

As it is apparent from both the visual survey of the graphic recording and monitoring of the correlation coefficient development for linear regression, calibration dependence is linear within the 0–0.8 mg/l concentration range with $k_f = 0.9994$.

**Calibration function:**

Straight line in the shape $A = 3.102 * c + 0.040$

**Determination of repeatability**

SAMPLES USED:

a) An artificial standard sample prepared from a certified calibration solution at 0.5 mg/l concentration level; measurement performed for both test methods specified.

b) An artificial standard sample prepared from a certified calibration solution at 0.10 mg/l concentration level; measurement performed for both test methods specified

c) A real sample of groundwater from the "Nová Tesla" well, Draslovka Kolín, a.s., and a wastewater sample DX-1 Draslovka Kolín, a.s.

d) A real sample of contaminated soil from the Draslovka Kolín, a.s., plant premises.

A utility standard sample prepared from a certified calibration solution of a different batch was used for calibration. Twenty repeated determinations were performed acc. to documented test procedures, evaluation performed acc. to real calibration specified.

**Measurement results:**

**Artificial samples:**

| **Measurement no.** | **All cyanides [mg/l]** | | **Easily liberatable cyanides [mg/l]** | |
|---|---|---|---|---|
| | **IRM 0.100 mg/l** | **IRM 0.500 mg/l** | **IRM 0.100 mg/l** | **IRM 0.500 mg/l** |
| 1 | 0.097 | 0.532 | 0.095 | 0.504 |
| 2 | 0.097 | 0.484 | 0.101 | 0.449 |
| 3 | 0.096 | 0.530 | 0.109 | 0.540 |
| 4 | 0.101 | 0.508 | 0.102 | 0.540 |
| 5 | 0.093 | 0.490 | 0.095 | 0.491 |
| 6 | 0.093 | 0.449 | 0.105 | 0.480 |
| 7 | 0.105 | 0.509 | 0.093 | 0.525 |
| 8 | 0.110 | 0.469 | 0.097 | 0.477 |
| 9 | 0.101 | 0.494 | 0.100 | 0.499 |
| 10 | 0.102 | 0.512 | 0.099 | 0.510 |
| 11 | 0.105 | 0.505 | 0.096 | 0.520 |
| 12 | 0.109 | 0.476 | 0.108 | 0.495 |
| 13 | 0.098 | 0.520 | 0.100 | 0.516 |
| 14 | 0.099 | 0.522 | 0.101 | 0.511 |
| 15 | 0.100 | 0.505 | 0.093 | 0.480 |
| 16 | 0.095 | 0.499 | 0.105 | 0.492 |
| 17 | 0.100 | 0.497 | 0.098 | 0.471 |
| 18 | 0.101 | 0.500 | 0.102 | 0.480 |
| 19 | 0.102 | 0.501 | 0.110 | 0.510 |
| 20 | 0.098 | 0.495 | 0.090 | 0.444 |

**Statistical evaluation of data using ADSTAT program**

Parameters estimations:

| **Parameter** | **All CN** | | **Easily liberatable CN** | |
|---|---|---|---|---|
| | IRM 0.100 mg/l | IRM 0.500 mg/l | IRM 0.100 mg/l | IRM 0.500 mg/l |
| *Statistical parameters:* | | | | |
| mean | 0.100 | 0.500 | 0.100 | 0.497 |
| median | 0.100 | 0501 | 0.100 | 0.497 |
| standard deviation | 0.005 | 0.020 | 0.008 | 0.032 |
| reliability: lower limit 95 % | 0.097 | 0.492 | 0.097 | 0.479 |
| reliability: upper limit 95 % | 0.103 | 0.509 | 0.103 | 0.514 |
| **RSD** | **5 %** | **4 %** | **8 %** | **6 %** |
| *Distribution parameters* | | | | |
| obliquity | -0.32 | - 0.64 | 0.18 | - 0.24 |
| pointedness | 2.09 | 3.47 | 2.26 | 2.56 |
| distribution shape | normal to uniform | normal to Laplace | normal | normal |
| data homogeneity | homogeneous, independent | homogeneous, independent | homogeneous, independent | homogeneous, independent |
| outlying points | no | point no. 6 | no | no |

**Real samples:**

| Measurement no. | All cyanides [mg/l] | | Easily liberatable cyanides [mg/l] | |
|---|---|---|---|---|
| | **Water** | **Soil** | **Water** | **Soil** |
| 1 | 0.213 | 75.1 | 0.079 | 1.08 |
| 2 | 0.204 | 73.5 | 0.084 | 0.80 |
| 3 | 0.204 | 74.9 | 0.077 | 1.12 |
| 4 | 0.190 | 78.1 | 0.072 | 0.95 |
| 5 | 0.210 | 66.5 | 0.060 | 0.84 |
| 6 | 0.199 | 78.3 | 0.080 | 1.10 |
| 7 | 0.199 | 76.2 | 0.067 | 1.00 |
| 8 | 0.199 | 65.8 | 0.075 | 1.05 |
| 9 | 0.207 | 68.6 | 0.080 | 0.94 |
| 10 | 0.195 | 67.7 | 0.078 | 0.93 |
| 11 | 0.210 | 68.3 | 0.085 | 0.74 |
| 12 | 0.205 | 68.0 | 0.081 | 0.89 |
| 13 | 0.193 | 55.3 | 0.080 | 1.05 |
| 14 | 0.206 | 65.2 | 0.090 | 0.90 |
| 15 | 0.192 | 66.9 | 0.079 | 1.09 |
| 16 | 0.196 | 69.8 | 0.071 | 0.77 |
| 17 | 0.196 | 77.2 | 0.084 | 0.94 |
| 18 | 0.185 | 70.3 | 0.070 | 1.10 |
| 19 | 0.187 | 68.5 | 0.084 | 0.70 |
| 20 | 0.199 | 57.8 | 0.088 | 0.96 |

**Statistical evaluation of data using ADSTAT program**

Parameters estimations:

| **Parameter** | **All CN** | | **Easily liberatable CN** | |
|---|---|---|---|---|
| | **Water [mg/l]** | **Soil [mg/l]** | **Water [mg/l]** | **Soil [mg/l]** |
| *Statistical parameters:* | | | | |
| mean | 0.199 | 69.6 | 0.078 | 0.95 |
| median | 0.199 | 68.6 | 0.080 | 0.95 |
| standard deviation | 0.011 | 6.19 | 0.008 | 0.19 |
| **RSD** | **6 %** | **9%** | **11 %** | **20 %** |
| *Distribution parameters* | | | | |
| obliquity | -0.08 | - 0.54 | - 0.69 | - 0.38 |
| pointedness | 2.13 | 3.02 | 3.19 | 2.07 |
| distribution shape | normal | normal | normal to Laplace | normal |
| data homogeneity | homogeneous, independent | homogeneous, independent | homogeneous, independent | homogeneous, independent |
| outlying points | no | no | no | no |

CONCLUSION:

**RSD repeatability:**

All cyanides

- ***Water***: ≤ ± 6 %

Repeatability for both artificial and real samples in various concentrations shows close values, thus the value specified may be accepted for water matrixes within the whole operating range of the method.

- ***Soil***: ≤ ± 9 %

Repeatability is expressed for the real sample; comparison with CRM may not be performed due to the absence of this material on the market.

Easily liberatable cyanides

- ***Water***: average around ≤ ± 10 %

Repeatability for artificial samples v in various concentrations as well as for real samples show very slight differences, thus for the whole operating range of the method an adjusted pessimistic estimation is considered as the repeatability resultant value.

- ***Soil:*** ≤ ± 20 %

Repeatability is expressed for the real sample; comparison with CRM may not be performed due to the absence of this material on the market.

## Method correctness

The correctness of both methods for water matrixes is confirmed by the statistical evaluation of the mean and median of previous measurements of artificial samples under specified conditions. Both the mean and median values correspond to the expected declared value of the analyte concentration, while various certified solutions were used for the calibration a measurement.

The method correctness for solid samples may not be tested due to the absence of CRM on the market.

## Detection limit and determination limit (valid for both methods specified)

These parameters are derived from statistical processing of calibration dependence measured values (Chapter 1) regarding factors of sample concentration (at conditions of the method specified, the concentration factor is 5) and regarding the blank trial background.

a) Blind trial testing

| Measurement no. | Blind trial value (mg/l)* |
|---|---|
| 1 | 0.003 |
| 2 | 0.001 |
| 3 | 0.001 |
| 4 | 0.001 |
| 5 | 0.002 |
| 6 | 0.003 |
| 7 | 0.002 |
| 8 | 0.001 |
| 9 | 0.002 |
| 10 | 0.001 |
| average | 0.0017 |

* With inclusion of concentration factor 5.

DL ≈ 3x blind trial value ≈ 0.005

b) Parameters derived from calibration by statistical processing of measurements:

| Parameter | Absorbance | Corresponding concentration mg/l | Resultant value after correction to conc. factor |
|---|---|---|---|
| Critical level | 0.074 | 0.011 | 0.002 |
| Detection limit | 0.105 | 0.021 | 0.004 |
| Determinability level | 0.269 | 0.074 | 0.015 |

**Conclusion:**
As the resultant value of the detection limit after utilising the concentration factor 5 of the methods, the following value may be accepted: ***DL ≈ 0.005 mg/l $CN^-$***

**Operational range** (valid for both methods specified)

This parameter is related to the following measurement conditions:

a) Range of linear response of measuring the output quantity (absorbance) at concentration: here 0–0.8 mg/l.

b) Sample quantity taken to the analysis and the recommended dilution rate of the distillate, based on technical possibilities and recommended values.

c) Rate of sample concentration (provided it is performed acc. to the documented procedure – here 5 x).

d) Detection limit size (here 0.005 mg/l)

| Matrix | Minimal suitable sample quantity for distillation | Recommended max. dilution of the distillate | Resultant range [mg/l], or [mg/kg] |
|---|---|---|---|
| waters | 1 ml | 1: 200 | 0.005 – 4000 |
| solid samples* | 1 g | 1 : 200 | 0.1 – 4000 |

* Without correction to solid matter.

*The method has not been tested for higher dilution!*

**Method sensitivity** (valid for both methods specified)

It is given by the slope of a calibration line within the linear response range and by the instrument sensitivity (resolving power):

Slope $a = 3.102 / 1\ \text{mg/l} \approx 0.003\text{A} / 0.001\ \text{mg/l}$
Photometer resolving power: 0.001 A
Uncertainty of spectophotometer reading (as specified by the manufacturer): ± 0.002 A

**Conclusion**:
The method sensitivity regarding the instrument resolving power and declared uncertainty of the method reading, and regarding the concentration factor and recommended results rounding, is sufficient within the ranges above DL considering the intended usage.

**Method yield**

Tested with regard to the necessity to isolate the analyte from the distillation samples. Due to the absence of master CRM for solid samples the method is tested only for water and solution samples.

The testing was performed by observation of measured values of the resultant concentration of an artificially prepared internal laboratory sample (sample spiked with CRM) after distillation according to a documented procedure with a declared concentration value.

Sample concentration 0.500 mg/l (i.e. within the range approx. 1/2 - 2/3 of the calibration solution) was selected for the testing. Results are given after conversion to % yield:

| Measurement no. | All cyanides [%] | Easily liberatable cyanides [%] |
|---|---|---|
| 1 | 88.0 | 81.6 |
| 2 | 88.8 | 84.2 |
| 3 | 91.2 | 85.4 |
| 4 | 87.6 | 74.1 |
| 5 | 75.2 | 79.4 |
| 6 | 82.0 | 81.0 |
| 7 | 95.4 | 75.0 |
| 8 | 85.4 | 64.0 |
| 9 | 86.4 | 80.3 |
| 10 | 88.8 | 72.2 |
| 11 | 84.7 | 84.4 |
| 12 | 86.4 | 81.6 |
| 13 | 87.4 | 65.6 |
| 14 | 87.6 | 63.4 |
| 15 | 94.4 | 75.8 |
| 16 | 87.0 | 78.0 |
| 17 | 88.0 | 69.6 |
| 18 | 88.1 | 81.4 |
| 19 | 93.2 | 88.1 |
| 20 | 90.8 | 74.5 |

| **Parameter** | **All CN** | **Easily liberatable CN** |
|---|---|---|
| | IRM 0.500 mg/l | IRM 0.500 mg/l |
| *Statistical parameters:* | | |
| mean | 89.0* | 77.0 |
| median | 88.0* | 78.7 |
| standard deviation | 3.8* | 7.2 |
| reliability: lower limit 95 % | 86.4* | 73.6 |
| reliability: upper limit 95 % | 90.9* | 80.3 |
| **RSD** | -- | -- |
| *Distribution parameters* | | |
| obliquity | 0.50* | - 0.52 |
| pointedness | 2.73* | 2.31 |
| distribution shape | normal to Laplace | normal |
| data homogeneity | homogeneous, independent* | homogeneous, independent |
| outlying points | point no. 5 | no |

* After omitting the outlying point!

**Conclusion:**

The yield values measured for water samples:
- all cyanides: 89.0 ± 7.6 % (± $\sigma$)
- easily liberatable cyanides: 77.0 ± 14 % (± $\sigma$)

The method yields are reproducible regarding accepted values of the determination uncertainty.
For solid samples the method yield as not determined – the laboratory shall state this fact in test reports.

**Robustness**

For robustness testing, the following factors were selected upon the possibility of results being influenced by small changes:

- distillation time
- air flow through the distillation assembly
- sample heating during distillation

Tests were performed through an internal reference standard with concentration 0.500 mg/l.

Results are given in the following table:

| **Tested parameter** | **Conditions** | **Results [mg/l]*** | |
|---|---|---|---|
| | | **All cyanides** | **Easily liberatable cyanides** |
| Distillation time | specified: 60 min | 0.498 | 0.495 |
| | changed: 50 min | 0.496 | 0.490 |
| | changed: 70 min | 0.500 | 0.496 |
| Air flow | specified: 3 bubbles / sec | 0.498 | 0.495 |
| | changed: 2 bubbles / sec | 0.497 | 0.498 |
| | changed: 4 bubbles / sec | 0.502 | 0.500 |
| Heating intensity | specified: mild boil | 0.498 | 0.495 |
| | changed: boil not reached | 0.450 | 0.412 |
| | changed: violent boil | 0.500 | 0.499 |

* Average from 5 measurements, corrected to the yield.

**Conclusion:**

The methods are robust against small changes of the distillation time and bearing medium flow, however they are not completely robust against boil reaching and intensity. IN order to achieve correct measurement results it is absolutely necessary to reach intensive boil of the sample during distillation.

# Validation report

The evaluation was performed upon data measured according to SOP AA-10 and SOP AA-11 test methods in January 2001 and upon statistical processing performed, under the following preconditions:

- Output validation parameters are evaluated for all cyanides and easily liberatable cyanides according to their character either jointly or separately, and are given in the following table.
- Values expressed as a relative standard deviation in % are for the validation purposes rounded to 1 %.
- Declared values – if not specified otherwise – apply within the whole operating range.

## Validation results for individual parameters

| Validation parameter | All cyanides | Easily liberatable cyanides |
|---|---|---|
| Calibration function | linear within the concentration range 0.025 – 0.80 mg/l<br>function equation: A = 3.102 * c + 0.040 | |
| Repeatability (for artificial as well as real samples within the whole operating range) | water: ± 6 %<br>soil: ± 9 % | water: ± 10 %<br>soil: ± 20 % |
| Detection limit | 0,005 mg/l | |
| Correctness and deviation (only for water samples) | better than ± 5 % without deviation | better than ± 8 % without deviation |
| Operating range | water: 0.005 – 4000 mg/l<br>soil: 0.1 – 4000 mg/kg | |
| Sensitivity | 0.001 mg/l or 0.01 mg/kg with respect to recommended rounding of results | |
| Yield (only for water samples) | 88 % | 77 % |
| Robustness | The method is sufficiently robust with the exception of heating samples during distillation. | |

| | **Evaluation by Competent Authorities** |
|---|---|
| | |
| **Date** | |
| **Evaluation of applicant's justification** | |
| **Conclusion** | |
| **Remarks** | |

| | | |
|---|---|---|
| **Section 4.2**<br>**Annex Point IIA IV.4.2** | **ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION OF URAGAN D2 IN:**<br>**(a) soil**<br>**(b) air**<br>**(c) water**<br>**(d) animal and human body fluids and tissues** | |
| **Details** | This section point is detailed in DOC III-A_4.2.1. | |
| | Method for cyanide determination in air, water and body fluid is the same: spectrophotometric measurement of color intensity of sample. They differ only in sample preparation/liberation of cyanides from raw sample.<br>**For details and validation report see DOC III-A_4.2.1**<br>Methods for soil samples and treated food/feeding stuff are not described as use pattern of product excludes food/feeding stuff from being fumigated and physical properties of product rule out soil exposure. | |
| **Undertaking of intended data submission** | No studies are planned. Data from literature show that described methods are appropriate. | |

| | **Evaluation by Competent Authorities** |
|---|---|
| | |
| **Date** | |
| **Evaluation of applicant's justification** | |
| **Conclusion** | |
| **Remarks** | |

| | | |
|---|---|---|
| **Section A4.3**<br>**Annex Point IIIA IV.1** | ***Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, in/on food or feedstuffs and other products where relevant*** | |
| | **JUSTIFICATION FOR NON-SUBMISSION OF DATA** | Official use only |
| **Other existing data [ ]** | **Technically not feasible [ ]** **Scientifically unjustified [ ]** | |
| **Limited exposure [x ]** | **Other justification [ x ]** | |
| **Justification:** | If hydrogen cyanide is used as specified and all conditions are met (emptying places to be fumigated as specified), it may not contaminate foodstuffs, water or surfaces coming to touch with foodstuffs. | |
| **References** | | |
| **Undertaking of intended data submission** | No studies are planned. | |

| | |
|---|---|
| | **Evaluation by Competent Authorities** |
| | |
| **Date** | |
| **Evaluation of applicant's justification** | |
| **Conclusion** | |
| **Remarks** | |

| **Section A5**<br>**Annex Point IIA V.** | **EFFECTIVENESS AGAINST TARGET ORGANISMS AND INTENDED USES** | |
|---|---|---|
| | **Summary** | Official use only |
| **5.1** | **Function** | |
| | Fumigant for the control of rodents.<br>MG03: Pest control<br>**Product Type 14: Rodenticides** | |
| **5.2** | **Organism)s) to be controlled and products, organisms or objects to be protected** | |
| | Mice, rats and other rodent population in:<br>a) Storehouses, depositories – museums, temples, containers, antiquities and other buildings;<br>b) Agro-food industry – disinfection of empty spaces;<br>c) Transport facilities –railway wagons, sea and river boats. | |
| **5.3** | **Effects on target organisms, and likely concentration at which the active substance will be used** | |
| | The substance functions as a respiratory poison, killing pests by damaging their metabolism. It is absorbed mainly through airways, digestive tract, unbroken skin and mucous membranes. It causes asphyxiation directly in tissues. Experience shows that target organisms do not develop resistance.<br>The impact efficiency is immediate after reaching a lethal concentration of hydrogen cyanide in air inhaled by target organisms. Exposure time shall be set depending on the pest species, level of attack, conditions of hydrogen cyanide spreading within the treated building and weather conditions.<br>**Dosage: $10g/m^3$, i.e. 1kg per $100m^3$ in field conditions,** | |
| **5.4** | **Mode of action (including time delay)** | |
| | Uragan D2 is used solely as required by fumigation procedures described in appendix.<br>One single treatment.<br>For fumigation air temperature inside the treated building must be higher than 12 °C for the whole time of the process. Exposure time is 24 hours at temperature above 18 °C and 48 hours at temperature below 18 °C.<br>After fumigation, fumigated spaces shall be ventilated until the air concentration is below 0.6 $mg/m^3$ in order to protect operators shall they have to re-enter the fumigated spaces without PPE, and must in any case be below $3mg/m^3$ for the re-entry of bystanders. In practice the ventilation time of an empty building is usually 24-48 hours, depending on weather conditions or increased humidity. | |
| **5.5** | **Field of use envisaged** | |
| | Fumigant for the control of rodents.<br>MG03: Pest control<br>**Product Type 14: Rodenticides** | |
| **5.6** | **User: industrial, professional, general public (non-professional)**<br>**Efficacy Data** | |

| | | |
|---|---|---|
| Industrial | No industrial use | |
| Professional | Uragan D2 is used by the professional user only | |
| General public | Uragan D2 is not used by the general public | |
| **5.7** | **Information on the occurrence or possible occurrence of development of resistance and appropriate management strategies** | |
| | As the substance is used in single treatment biocidal product no occurrence of resistance is expected. | |
| **5.8** | **Likely tonnage to be placed on the market per year** | |
| | 30 – 50 tonnes/ year | |

| | **Evaluation by Competent Authorities** |
|---|---|
| | |
| **Date** | |
| **Evaluation of applicant's justification** | |
| **Conclusion** | |
| **Remarks** | |

| | | |
|---|---|---|
| **Section A6.1**<br>**Annex Point IIA VI.6.1** | **ACUTE TOXICITY** | |

| **Section A6.1.1**<br>**Annex Point IIA VI.6.1.1** | **Acute Oral Toxicity** | |
|---|---|---|
| **Justification:**<br>**Supportive data:** | The active substance hydrogen cyanide is a gas at body temperature. Oral exposure to HCN is due to its physical and chemical properties practically out of question.<br>Studies on acute oral toxicity of inorganic cyanides are used as supportive data.<br>Summaries and evaluations in this section are based mostly on exhaustive and reliably peer reviewed documents: ATSDR (2004, Toxicological profile of cyanide) (**DOC IV_1**) and IPCS (2004, WHO, CICAD 61: Hydrogen cyanide and cyanides: human health aspects) (**DOC IV_5**) and Hazardous Substance Data Bank (HSDB), National Library of Medicine´s TOXNET system: Hydrogen cyanide *Peer reviewed* (**DOC IV_2**). | |
| **References:** | 1. Smyth HF, Carpenter CP, Weil CS, et. Al. 1969. Range-finding toxicity data: List VII. Am Ind Hyg Assoc J 30: 470-476 **(DOC IV_12)**<br>2. Ferguson HC. 1962. Dilution of dose and acute oral toxicity. Toxicol Appl Pharmacol 4: 759-762. **(DOC IV_13)**<br>3. Ballantyne B. 1988 Toxicology and hazard evaluation of cyanide fumigation powders. Clin Toxicol 26: 325 – 335. **(DOC IV_14)**; **Summary in DOC III_6.1.1a**<br>4. Ballantyne B. 1983a. The influence of exposure route and species on the acute lethal toxicity and tissue concentrations of cyanide. In: Hayes AW, Schnell RC, Miya TS, eds. Developments in the science and practice of toxicology. New York, NY: Elsevier Science Publishers, 583-586 (**DOC IV_15);** | |
| **Guidelines**: | Not presented. | |
| **GLP:** | No. All studies before GLP required. | |
| **Material and methods**: | Various methods of oral lethal dose determination in rats, mice and rabbits. Administration of HCN and various cyanides in water solution by gavage. | |
| **Results and discussion**: | Oral LD50 molar values for HCN, NaCN and KCN were similar both in rats (0.156, 0.117 and 0.115 mmol/kg body weight, respectively) and in rabbits (0.092, 0.104 and 0.090 mmol/kg, respectively) corresponding to 3 - 4 mg/kg cyanide per kg bw in rats and 2.34 – 2.70 mg cyanide/kg bw in rabbits.<br>In the same study **(4)**, LC50 of HCN in rats was 158 $mg/m^3$ for 60 min inhalation exposures and 3778 $mg/m^3$ for a 10 second exposure; the calculated dose resorbed during the 60 minute exposure (at complete desorption) is similar to the oral LD50 (1 mg cyanide per animal, i.e., 2 - 3 mg/kg bw). These LD50 values are twofold compared to an average fatal dose of 1.52 mg/kg cyanide for humans calculated from case report studies.<br>These values are at least by two orders of magnitude lower than toxic doses of cyanides administered chronically in diet, as discussed in the section on sub chronic oral toxicity. The difference is ascribed to rapid metabolic disposal of cyanide ions.<br>Other supporting findings are summarised below **in the Table**. | |

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| | Expressed as CN mg/kg bw, the LD50 of calcium cyanide (11 mg/kg) is higher than LD50 of sodium cyanide (8 mg/kg), but the confidence intervals overlap. These values are the highest reported estimates of LD50.<br>In another study, LD95 values of potassium cyanide were even lower: 4.2 and 6.3 mg CN/kg for rats and mice, respectively. | |
| **Conclusions**: | 1) Acute oral toxicity is similar for HCN and sodium or potassium cyanides.<br>2) Rabbits are only slightly more susceptible than mice or rats.<br>3) Oral lethal doses in rats are comparable to calculated total inhaled dose for a 60 min. inhalation exposure.<br>4) The lowest LD50 value for cyanides for rats was determined at 3 mg/kg. (as cyanide, i.e. 7.5 mg/kg bw of KCN) and for rabbits 2.34 mg/kg bw (as cyanide, i.e. 5.8 mg/kg bw of KCN).<br>5) The reliability of LD estimates is supported by many other studies.<br>6) Small differences between LD95 and LD50 values point to a steep dose-response regression.<br>7) Acute oral LD values are at least by two orders of magnitude lower than toxic doses of cyanides administered repeatedly in diet. The rate of supply seems to be a decisive factor of acute toxicity. | |

**Table: Acute toxicity – oral** (for cyanides)

| **Study type, application technique, substance** | | **Test organism** | **Dose Nr /** | **Oral LD values** | **Reference** |
|---|---|---|---|---|---|
| LD oral by gavage | KCN | Rat (Sprague-Dawley) | Single dose | 10.5 mg/kg<br>19/20 died | **(2)** |
| LD oral by gavage | $Ca(CN)_2$ | Rat (not specified) | Single dose | 39 mg/kg<br>LD50 | **(1)** |
| LD oral by gavage | NaCN | Rat (not specified) | Single dose | 15 mg/kg<br>LD50 | **(1)** |
| LD oral by gavage | KCN | Mouse (Swiss-Webster) | Single dose | 15.8mg/kg<br>19/20 died | **(2)** |

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| **Section A6.1**<br>**Annex Point IIA VI.6.1** | **ACUTE TOXICITY** | |
| **Section A6.1.1**<br>**Annex Point IIA VI.6.1.1** | **Acute Oral Toxicity** | |
| | **1 REFERENCE** | **Official use only** |
| **1.1 Reference** | Bryan Ballantyne, 1988, Toxicology and Hazard Evaluation of Cyanide Fumigation Powders, Applied Toxicology Department, Union Carbide Corporation, Danbury, Connecticut 06817, Clinical Toxicology, 26 (5&6), 325-335; Copyright © 1988 by Marcel Dekker, Inc. **(DOC IV_14)** | |
| **1.2 Data protection** | No | |
| 1.2.1 Data owner | | |
| 1.2.2 Companies with letter of access | | |
| 1.2.3 Criteria for data protection | No data protection claimed | |
| | **2 GUIDELINES AND QUALITY ASSURANCE** | |
| **2.1 Guideline study** | No<br>(methods used comparable to guideline of Acute Oral Toxicity,) | |
| **2.2 GLP** | Not reported | |
| **2.3 Deviations** | No | |
| | **3 MATERIALS AND METHODS** | |
| | | |
| **3.1 Test material** | **NaCN powder** | |
| 3.1.1 Lot/Batch number | Not reported | |
| 3.1.2 Specification | Pure NaCN | |
| **3.1.2.1 Description** | Powder | |
| **3.1.2.2 Purity** | Pure | |
| **3.1.2.3 Stability** | Not reported | |

| | | |
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| **3.2 Test Animals** | | |
| 3.2.1 Species | Rat (Acute Oral Toxicity)<br>Rabbit (Acute Oral Toxicity) | |
| 3.2.2 Strain | Rat – Porton strain<br>Rabbit – New Zealand white | |
| 3.2.3 Source | Not reported | |
| 3.2.4 Sex | Females only | |
| 3.2.5 Age/weight at study initiation | Rats: 161.6 – 204.9 g<br>Rabbits: 1900.0 – 2200.0 g | |
| 3.2.6 Number of animals per group | 10 animals/each dose<br>(3 groups of animals: 1.starved rats – deprived of food for 24 hours before dosing, 2.unstarved rats and 3.unstarved rabbits) | |
| 3.2.7 Control animals | Not reported | |
| **3.3 Administration/ Exposure** | Starved or unstarved animals | |
| 3.3.1 Post exposure period | 7 days | |
| 3.3.2 Type | Gavage | |
| 3.3.3 Concentration | Starved rats – dose range: 3.95 – 7.90 mg/kg bw<br>Unstarved rats – dose range: 4.50 – 6.30 mg/kg bw<br>Unstarved rabbits – dose range: 4.00 – 6.35 mg/kg bw | |
| 3.3.4 Vehicle | Aqueous solution | |
| 3.3.5 Concentration in vehicle | Not reported | |
| 3.3.6 Total volume applied | 1 ml/kg | |
| 3.3.7 Controls | Not reported | |
| **3.4 Examinations** | Clinical observations (signs of toxic effects, the time of onset of signs, time of death), examination of eyes<br>(Necropsy and other exam. – not reported) | |
| **3.5 Method of determination of LD$_{50}$** | LD$_{50}$ was computed from the dose-mortality data by probit analysis using a Fortran computer program (LD$_{50}$ with 95% confidence limits and slopes of regression lines). | |
| **3.6 Further remarks** | | |

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| | **4 RESULTS AND DISCUSSION** | |
| **4.1 Clinical signs** | Time to first signs/Time to death:<br>starved rats: 0.2 – 2.0 minutes/ 0.5 – 75.0 minutes<br>unstarved rats: 0.5 – 2.0 minutes/ 2.0 – 40.0 minutes<br>unstarved rabbits: 0.5 – 12.0 minutes/ 14.0 – 80.0 minutes<br>Clinical signs: rapid breathing, weak movements, tremors, respiratory distress, severe spasms, convulsions, irregular shallow breathing, coma. | |
| **4.2 Pathology** | Not reported | |
| **4.3 Other** | | |
| **4.4 $LD_{50}$** | Oral<br>starved rats: 5.1 mg/kg<br>unstarved rats: 5.7 mg/kg<br>unstarved rabbits: 5.1 mg/kg | |
| | **5 APPLICANT'S SUMMARY AND CONCLUSION** | |
| **5.1 Materials and methods** | Non-guideline study; the test substance (NaCN powder) was administered in a single dose by gavage using a stomach tube to starved or unstarved rats and to unstarved rabbits - several groups of animals with various dose levels.<br>Following exposure animals were observed for signs of toxic effects and the times of onset of signs and times to death were noted. Survivors were kept only for 7 days (according to the Guidelines observation period after exposure is 14 days). Body weights of animals were recorded only at the beginning of the study. | |
| **5.2 Results and discussion** | The time of onset of first signs of toxicity was quicker in rats compared to rabbits. NaCN was slightly more toxic in the starved than unstarved animals. Comparison of the species (unstarved) showed that NaCN was more toxic to the rabbit than the rat.<br>Study was conducted to assess potential handling hazards from pesticidal use of powdered NaCN. On coming into contact with water NaCN powder liberates HCN vapour - it can evolve 20% (by weight) of HCN. | |
| **5.3 Conclusion** | | |
| 5.3.1 Reliability | 2 | |
| 5.3.2 Deficiencies | The study comes from 1988, it is not in the GLP system, but the method used is comparable to a method used according to GLP; a control group of animals is not described. | |

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| **Section A6.1**<br>**Annex Point IIA VI.6.1** | **ACUTE TOXICITY** | |

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| **Section A6.1.2**<br>**Annex Point IIA VI.6.1.2** | **Acute Dermal Toxicity** | |
| **Justification:**<br>**Supportive studies:** | Acute dermal toxicity values for HCN have been found in literature in one LD50 study for rabbits. Studies for cyanide salts have been used as further supporting information.<br>Summaries and evaluations in this section are based mostly on exhaustive and reliably peer reviewed documents: ATSDR (2004, Toxicological profile of cyanide) (**DOC IV_1**) and IPCS (2004, WHO, CICAD 61: Hydrogen cyanide and cyanides: human health aspects) (**DOC IV_5**) and Hazardous Substance Data Bank (HSDB), National Library of Medicine´s TOXNET system: Hydrogen cyanide *Peer reviewed* (**DOC IV_2**). | |
| **References:** | **1)** Ballantyne B. 1983a. The influence of exposure route and species on the acute lethal toxicity and tissue concentrations of cyanide. In: Hayes AW, Schnell RC, Miya TS, eds. Developments in the science and practice of toxicology. New York, NY: Elsevier Science Publishers, 583-586 (**DOC IV_15)**<br>**2)** Ballantyne B. 1988. Toxicology and hazard evaluation of cyanide fumigation powders. Clin Toxicol 26:325-335. **Summary in DOC III_ 6.1.2a, b (DOC IV_14)**<br>**3)** Ballantyne B. 1983b. Acute systemic toxicity of cyanides by topical application to the eye. J Toxicol, Cutan, Ocular Toxicol 2: 119-129 **Summary in DOC III_ 6.1.2c (DOC IV_16)**<br>**4)** Smyth HF, Carpenter CP, Weil CS, et. Al. 1969. Range-finding toxicity data: List VII. Am Ind Hyg Assoc J 30: 470-476 **(DOC IV_12)** | |
| **GLP:** | No. All studies before GLP requested. | |
| **Material and methods**: | Various methods of LD and ED determination. Water solutions applied on intact or abraded skin of rabbits. | |
| **Results and discussion**: | Cyanides (HCN, NaCN and KCN) applied in aqueous solutions on intact skin of New Zealand rabbits had $LD_{50}$ values 0.260, 0.298 and 0.343 mmol/kg body weight, respectively, corresponding to 7 – 9 mg/kg bw of cyanide, 16 mg/kg of NaCN and 22 mg/kg KCN; for abraded skin the respective values were 0.087, 0.220 and 0.300 mmol/kg; the value for HCN is significantly lower than $LD_{50}$ for intact skin **(1).**<br>Dermal $LD_{50}$ of acetone cyanohydrin was in the same range: 17 mg (5.2 mg cyanide) /kg bw **(4).**<br>Non-lethal systemic effects after dermal application of HCN in female rabbits were observed after a dose of 0.9 mg.kg$^{-1}$ - rapid breathing, cornea opacity and inflammation. For sodium and potassium cyanides, the LOAEL values were similar (2.5 mg.kg$^{-1}$, 1.25 and 1.0 mg cyanide /kg bw); single dose NOAEL for NaCN corresponds to 0.8 mg cyanide /kg bw (**3**).<br>Findings are summarised below in the **Table.** | |
| **Conclusions**: | 1) The lowest $LD_{50}$ value for dermal exposure to hydrogen cyanide was determined for female rabbits: 6.7 mg.kg$^{-1}$. The reliability of the estimate is supported by all other data available.<br>2) The dermal $LD_{50}$ values for NaCN and KCN are only slightly higher (calculated as cyanide). | |

3) For non-lethal systemic effects after dermal application of HCN, LOAEL was 0.9 mg.kg$^{-1}$, which is 1/7 of LD50. These findings have shown that the dose - response for acute systemic toxicity of HCN is very steep.
4) For sodium and potassium cyanides, the LOAEL values were similar (2.5 mg.kg$^{-1}$, i.e., 1.25 and 1.0 mg cyanide /kg bw);
5) Single dose NOAEL for NaCN corresponds to 0.8 mg cyanide /kg bw.
6) The dermal lethal doses are in the same range as oral LDs.
7) Permeability of abraded skin for HCN (in aqueous solution) is approx. 3 times higher than permeability of intact skin. Increased permeability should be assumed also for gaseous HCN.
8) No data were found on dermal toxicity of gaseous HCN, but with respect to solubility of HCN the resorption proportional to time and exposed skin area should be assumed.

**Table Acute toxicity – dermal** (hydrogen cyanide and cyanides)

| **Study** | | **Test organism** | **Dose / exposure time** | **Dermal toxicity LD and ED values** | **Reference** |
|---|---|---|---|---|---|
| LD50 dermal | HCN | Rabbit<br>New Zealand<br>Female | Single dose | LD50<br>6.8mg/kg as cyanide | **(1)** |
| LD50 dermal | NaCN | Rabbit<br>New Zealand<br>Female | Single dose | LD50<br>7.7mg/kg as cyanide | **(1)** |
| LD50 dermal | KCN | Rabbit<br>New Zealand<br>Female | Single dose | LD50<br>8.9mg/kg as cyanide | **(1)** |
| LD50 dermal | NaCN | Rabbit<br>New Zealand<br>Female | Single dose | LD50 – damaged skin<br>4.1mg/kg as cyanide | **(2)** |
| LD50 dermal | NaCN | Rabbit<br>New Zealand<br>Female | Single dose | LD50 – wet skin<br>6.3mg/kg as cyanide | **(2)** |
| Single dose dermal | HCN | Rabbit<br>albinotic<br>female | Single dose | 0.9mg/kg<br>respiratory, rapid breathing, corneal opacity and keratitis | **(3)** |
| Single dose dermal | KCN | Rabbit<br>albinotic<br>female | Single dose | 2.5mg/kg<br>respiratory, rapid breathing, corneal opacity and keratitis | **(3)** |
| Single dose dermal | NaCN | Rabbit<br>albinotic<br>female | Single dose | NOAEL: 1.69mg/kg<br>LOAEL 2.5 mg/kg<br>respiratory, rapid breathing, corneal opacity and keratitis | **(2)** |

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