

Table 1. Repeatability of the determination of HCN in Uragan D2 (research department)

Test no.	Sample weight [mg]	Consumption of AgNO ₃ (c = 0.1 M) [ml]	Calculated concentration of HCN [% w.]
1	2172.5	15.6	97.14
2	2182.3	15.8	97.95
3	2190.5	15.9	98.20
4	2142.1	15.5	97.89
5	2230.0	16.1	97.67
6	2141.2	15.5	97.93
7	2221.4	16.1	98.05
8	2215.0	15.9	97.11
9	2193.8	15.8	97.43
10	2149.4	15.6	98.19
		Mean	97.76
		S	0.4020
		v_x	0.41 %

Analysed on: 23 Feb 2007

Required condition of repeatability for the content determination $v_x < 0.5$ % HCN was met.

Table 2. Repeatability of the determination of HCN in Uragan D2 (quality assurance laboratory)

Test no.	Sample weight [mg]	Consumption of AgNO ₃ (c = 0.1 M) [ml]	Calculated concentration of HCN [% wt.]
1	2188.2	15.8	97.68
2	2178.8	15.8	98.10
3	2191.0	15.8	97.56
4	2222.5	16.0	97.39
5	2147.5	15.5	97.64
6	2211.5	16.0	97.88
7	2139.7	15.5	98.00
8	2210.3	15.9	97.32
9	2157.6	15.7	98.44
10	2187.0	15.8	97.74
		Mean	97.78
		s	0.3403
		v_x	0.35 %

Analysed on: 23 Feb 2007

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

Double-sampling F-test for dispersion		
	<i>Set 1</i>	<i>Set 2</i>
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		
	<i>Set 1</i>	<i>Set 2</i>
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	

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Section A4 Annex Point IIA IV.	ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION OF PRODUCT COMPONENTS	
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Section A4.1 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 ₂ S ₂ O ₃ volumetric solution	
3.3.3 Standard(s)	K ₂ Cr ₂ O 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	

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3.5	Specificity: 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, <5%	
3.8.2	Independent laboratory validation	See section 5-7	
		4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1	Materials and methods	<p>The assessment of the sulphur dioxide content is carried out through indirect iodometric titration with a Na₂S₂O₃ volumetric solution.</p> <p>Chemicals: HCl, 25% solution Potassium iodide solution (25 g in 30 ml water) Sodium thiosulphate (0.1 mol/l) Starch solution, indicator</p> <p><u>Method principle</u> 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.</p> <p><u>Test procedure:</u> 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. 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 SO₂ and I₂ is over, do titrate excessive iodine solution with a Na₂S₂O₃ (0.1 mol/l) volumetric solution, using starch solution as an indicator. Concurrently, a blank test shall be performed.</p>	
4.2	Conclusion	<p>Calculation:</p> <p>The sulphur dioxide content in % w (x) shall be calculated as follows: (a-b) . f . 3.203 . 100 x = ----- m</p> <p>Where a consumption of the sodium thiosulphate volumetric solution c = 0.1 mol/l for the blank test</p>	

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	<p>b..... consumption of the sodium thiosulphate volumetric solution c = 0.1 mol/l for the solution titration</p> <p>f..... factor of the sodium thiosulphate volumetric solution</p> <p>m..... HCN sample weight (mg)</p> <p>3.203... mg eqv. SO₂ (1 ml volumetric solution (Na₂S₂O₃) = 0.1 mol/l shall titre 5.406 mg SO₂</p>	
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 ₂ S ₂ O ₃ . 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	<p>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.</p> <p>Required value: coefficient of variation (v_x) < 5 %</p> <p>Where (v_x) is the coefficient of variation:</p> $v_x = \frac{s_x}{\bar{x}} \cdot 100 [\%]$ <p><u>Arithmetic mean of the set may be calculated as:</u></p> $\bar{x} = \frac{1}{N} \sum_{i=1}^N x_i$ <p>Sample standard deviation of the data measured is calculated as:</p> $s = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (x_i - \bar{x})^2}$ <p>Results are summarised in Table 1.</p>	
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	

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	<p>different solutions. On the basis of these results (using Student's t-test), reproducibility of the results was evaluated. Required value: coefficient of variation (v_x) < 5 % Results are summarised in Table 2.</p>	
	8 CONCLUSION	
	<p>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. Results are summarised in Table 3.</p>	

Table 1. Repeatability of the determination of SO₂ in Uragan D2 (research department)

Test no.	Sample weight [mg]	Consumption of Na ₂ S ₂ O 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 SO₂ in Uragan D2 (quality assurance laboratory)

Test no.	Sample weight [mg]	Consumption of Na ₂ S ₂ O 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

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

Double-sampling F-test for dispersion

	<i>Set 1</i>	<i>Set 2</i>
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

	<i>Set 1</i>	<i>Set 2</i>
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	

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Section A4.1 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 Specificity: interfering substances	Not present	
3.5 Recovery rates at different levels		
3.5.1 Relative standard deviation	0.002	

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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	<p>The assessment of the phosphoric acid content is carried out through titration with NaOH volumetric solution.</p> <p>Chemicals: Sodium hydroxide NaOH (0.1 mol/l)</p> <p><u>Method principle</u> The content of phosphoric acid is determined by titration.</p> <p><u>Test procedure:</u> 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.</p>	
4.2 Conclusion	<p>Calculation:</p> <p>The phosphoric acid content in % wt (x) shall be calculated as follows:</p> $x = \frac{a \cdot f \cdot 9.8 \cdot 100}{0.7 \cdot b \cdot 1000} = \frac{a \cdot f \cdot 9.8}{7 \cdot b}$ <p>Where</p> <p>a consumption of the sodium hydroxide volumetric solution c = 0.1 mol/l</p> <p>b..... HCN content (ml)</p> <p>f..... factor of the sodium hydroxide volumetric solution</p> <p>0.7.....specific gravity of HCN at 10 °C</p> <p>9.8.....mg eqv. H₃PO₄ (1 ml of volumetric solution equals 9.8 mg H₃PO₄)</p>	
4.2.1 Reliability	2	
4.2.2 Deficiencies	No	
	5 VALIDATION SUBJECT	
	Method of determination of the total free H ₃ PO ₄ content in Uragan D2 by titration with NaOH. The required quality parameter is H ₃ PO 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.	

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7 MEASURED VALUES				
<p>7.1 Method repeatability</p> <p>7.2 Interlaboratory reproducibility of the results</p> <p>7.3 Evaluation of interlaboratory reproducibility</p>	<p>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.</p> <p>Required value: coefficient of variation (v_x) < 5 %</p> <p>Where (v_x) is the coefficient of variation:</p> $v_x = \frac{s_x}{\bar{x}} \cdot 100 [\%]$ <p>Arithmetic mean of the set may be calculated as:</p> $\bar{x} = \frac{1}{N} \sum_{i=1}^N x_i$ <p>Sample standard deviation of the data measured is calculated as:</p> $s = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (x_i - \bar{x})^2}$ <p>Results are summarised in Table 1.</p> <p>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 %</p> <p>Results are summarised in Table 2.</p> <p>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.</p>			
8 CONCLUSION				
<p>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.</p>				

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 ₃ PO 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 H₃PO₄ in Uragan D2 (quality assurance laboratory)

Test no.	Consumption of NaOH (c = 0.1 M) [ml]	Calculated concentration of H ₃ PO 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

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Section A4.1 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 Specificity: interfering substances	Not present	
3.5 Recovery rates at different levels		
3.5.1 Relative standard deviation	Not applicable	

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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	<p>The procedure according to the method specified in ISO 760 standard - Determination of water Karl Fischer method - Direct electrometric titration</p> <p>Chemicals: Methanol Pyridine Sample solvent – mixture of 4 volumes of methanol (4:1) with 1 volume of pyridine (4:3). Fischer reagent Sodium Tartrate Dihydrate or water Water in methanol (water content 10 g/l a 2 g/l) Sodium Aluminium Silicate</p> <p><u>Method principle</u></p> <p>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.</p> <p>Procedure: 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).</p> <p>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.</p> <p><u>Principle of Endpoint Detection:</u></p> <p>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).</p>	

<p>4.2 Conclusion</p>	<p>Expression of results: Water equivalent of Karl Fischer reagent (T):</p> $T = \frac{m_3 \times 0.1566}{V_3} \quad \text{or} \quad T = \frac{m_4}{V_3}$ <p>Where m_3 weight of sodium tartrate if used for calibration in miligrams m_4 weight of added water if used for calibration in miligrams V_3 volume of Fischer reagent used for calibration in mililitres 0.1566... factor for calculation of water in sodium tartrate dihydrate</p> <p>Water content (in % wt) :</p> $\frac{V_4 \times T}{m_0 \times 10} \quad \text{or} \quad \frac{V_4 \times T}{V_0 \times p \times 10}$ <p>Where m_0 weight of sample in grams (solid) V_0 volume of sample in ml (liquid) p specific weight of samples in g/ml at 20 °C V_4 volume of Fischer reagent in ml T water equivalent of Fischer reagent in mg/ml</p>	
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.	

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Section A4.2 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	$R^2 > 0.999$

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3.4	Specificity: 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	<p>Sample preparation:</p> <p><u>AIR</u></p> <p>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.</p> <p>2 more possibilities</p> <p><u>BODY FLUIDS</u></p> <p>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.</p> <p><u>WATER</u></p> <p>HCN liberated from the sample by distillation from acidified media is absorbed in 0.1M NaOH solution.</p> <p>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.</p>
4.2	Conclusion	<p>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</p> <p>Rodkey, F.L. and Collison, H.A. analysed whole blood for CN and reported 100% CN recovery upon blood acidification. (DOC IV_70)</p> <p>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</p>

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	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

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.

6.1 Validation plan

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.

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6.2.2 Validated matrix:

Measur ement no.	Concentr ation	A (578 nm, l =1 cm)	Correlatio n 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:

- An artificial standard sample prepared from a certified calibration solution at 0.5 mg/l concentration level; measurement performed for both test methods specified.
- An artificial standard sample prepared from a certified calibration solution at 0.10 mg/l concentration level; measurement performed for both test methods specified
- A real sample of groundwater from the “Nová Tesla“ well, Draslovka Kolín, a.s., and a wastewater sample DX-1 Draslovka Kolín, a.s.
- 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.

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Measurement results:

Artificial samples:

Measu rement 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

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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
<i>Statistical parameters:</i>				
mean	0.100	0.500	0.100	0.497
median	0.100	0.501	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 %
<i>Distribution parameters</i>				
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

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Real samples:

Measu rement 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

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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]
<i>Statistical parameters:</i>				
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 %
<i>Distribution parameters</i>				
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:** $\leq \pm 6 \%$

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:** $\leq \pm 9 \%$

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 $\leq \pm 10 \%$

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:** $\leq \pm 20 \%$

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

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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 \approx 3x blind trial value \approx 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 \approx 0.005 mg/l CN**

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Operational range (valid for both methods specified)

This parameter is related to the following measurement conditions:

- Range of linear response of measuring the output quantity (absorbance) at concentration: here 0–0.8 mg/l.
- Sample quantity taken to the analysis and the recommended dilution rate of the distillate, based on technical possibilities and recommended values.
- Rate of sample concentration (provided it is performed acc. to the documented procedure – here 5 x).
- 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 $a = 3.102 / 1 \text{ mg/l} \approx 0.003A / 0.001 \text{ mg/l}$

Photometer resolving power: 0.001 A

Uncertainty of spectrophotometer reading (as specified by the manufacturer): $\pm 0.002 \text{ A}$

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.

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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:

Measurment 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

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Parameter	All CN	Easily liberatable CN
	IRM 0.500 mg/l	IRM 0.500 mg/l
<i>Statistical parameters:</i>		
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	--	--
<i>Distribution parameters</i>		
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: $89.0 \pm 7.6 \% (\pm \sigma)$
- easily liberatable cyanides: $77.0 \pm 14 \% (\pm \sigma)$

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:

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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.

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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 function equation: $A = 3.102 * c + 0.040$	
Repeatability (for artificial as well as real samples within the whole operating range)	water: $\pm 6 \%$ soil: $\pm 9 \%$	water: $\pm 10 \%$ soil: $\pm 20 \%$
Detection limit	0,005 mg/l	
Correctness and deviation (only for water samples)	better than $\pm 5 \%$ without deviation	better than $\pm 8 \%$ without deviation
Operating range	water: 0.005 – 4000 mg/l 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	

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Section 4.2 Annex Point IIA IV.4.2	ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION OF URAGAN D2 IN: (a) soil (b) air (c) water (d) animal and human body fluids and tissues	
Details	This section point is detailed in DOC III-A_ 4.2.1.	
	<p>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.</p> <p><u>For details and validation report see DOC III-A 4.2.1</u></p> <p>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.</p>	
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 Annex Point IIIA IV.1	<i>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</i>	
	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	

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Section A5 Annex Point IIA V.	EFFECTIVENESS AGAINST TARGET ORGANISMS AND INTENDED USES	
	Summary	Official use only
5.1	Function	
	Fumigant for the control of insect pests of all developmental stages. MG03: Pest control Product Type 18: Insecticides, acaricides and products to control other arthropods.	
5.2	Organism(s) to be controlled and products, organisms or objects to be protected	
	All stages of any storehouse pests, for example cockroach, cricket, mites, woodlouse, larder beetle, grain beetle, powder-post beetles, auger beetle, woodworm, spider beetle, mealworm, sawyer beetle, snout beetle, gelechid moth, meal moth), and human health pests (louse, bug, bed bug, scabies, fleas) in: a) Storehouses, depositories – museums, temples, containers, antiquities, and other buildings; b) Agro-food industry – disinfection of empty spaces; c) Transport facilities –railway wagons, sea and river boats. Tested were following species: Primary storage pests: <i>Sitophilus granarius</i> , <i>Tribolium castaneum</i> , <i>Tribolium confusum</i> Secondary storage pests: <i>Cryptolestes ferrugineus</i> , <i>Oxyzaepphilus surinamensis</i> , <i>Lasioderma serricorne</i> , <i>Plodia interpunctella</i> , <i>Ephestia kuehniella</i> Blatodea pest species: <i>Blatella germanica</i> , <i>Blatta orientalis</i>	
5.3	Effects on target organisms, and likely concentration at which zhe 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 org 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. Dosage: 10g/m³, i.e. 1kg per 100m³ in field conditions,	
5.4	Mode of action (including time delay)	
	Uragan D2 is used solely as required by fumigation procedures described in appendix. One single treatment. 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. 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	

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	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 insect pests of all developmental stages. MG03: Pest control Product Type 18: Insecticides, acaricides and products to control other arthropods.	
5.6	User: industrial, professional, general public (non-professional) 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 Annex Point IIA VI.6.1	ACUTE TOXICITY
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Section A6.1.1 Annex Point IIA VI.6.1.1	Acute Oral Toxicity
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Justification: Supportive data:	<p>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.</p> <p>Studies on acute oral toxicity of inorganic cyanides are used as supportive data.</p> <p>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).</p>
References:	<ol style="list-style-type: none"> 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) 2. Ferguson HC. 1962. Dilution of dose and acute oral toxicity. Toxicol Appl Pharmacol 4: 759-762. (DOC IV_13) 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 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:	<p>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.</p> <p>In the same study (4), LC50 of HCN in rats was 158 mg/m³ for 60 min inhalation exposures and 3778 mg/m³ 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.</p> <p>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.</p> <p>Other supporting findings are summarised below in the Table.</p>

	<p>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.</p> <p>In another study, LD95 values of potassium cyanide were even lower: 4.2 and 6.3 mg CN/kg for rats and mice, respectively.</p>	
Conclusions:	<ol style="list-style-type: none"> 1) Acute oral toxicity is similar for HCN and sodium or potassium cyanides. 2) Rabbits are only slightly more susceptible than mice or rats. 3) Oral lethal doses in rats are comparable to calculated total inhaled dose for a 60 min. inhalation exposure. 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). 5) The reliability of LD estimates is supported by many other studies. 6) Small differences between LD95 and LD50 values point to a steep dose-response regression. 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 19/20 died	(2)
LD oral by gavage	Ca(CN) ₂	Rat (not specified)	Single dose	39 mg/kg LD50	(1)
LD oral by gavage	NaCN	Rat (not specified)	Single dose	15 mg/kg LD50	(1)
LD oral by gavage	KCN	Mouse (Swiss-Webster)	Single dose	15.8mg/kg 19/20 died	(2)

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Section A6.1.1 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 (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	

3.2 Test Animals		
3.2.1 Species	Rat (Acute Oral Toxicity) Rabbit (Acute Oral Toxicity)	
3.2.2 Strain	Rat – Porton strain 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 Rabbits: 1900.0 – 2200.0 g	
3.2.6 Number of animals per group	10 animals/each dose (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 Unstarved rats – dose range: 4.50 – 6.30 mg/kg bw 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 (Necropsy and other exam. – not reported)	
3.5 Method of determination of LD₅₀	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		

	4 RESULTS AND DISCUSSION	
4.1 Clinical signs	Time to first signs/Time to death: starved rats: 0.2 – 2.0 minutes/ 0.5 – 75.0 minutes unstarved rats: 0.5 – 2.0 minutes/ 2.0 – 40.0 minutes unstarved rabbits: 0.5 – 12.0 minutes/ 14.0 – 80.0 minutes 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 LD₅₀	Oral starved rats: 5.1 mg/kg unstarved rats: 5.7 mg/kg 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. 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. 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 Annex Point IIA VI.6.1	ACUTE TOXICITY		
Section A6.1.2 Annex Point IIA VI.6.1.2	Acute Dermal Toxicity		
Justification: Supportive studies:	<p>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.</p> <p>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).</p>		
References:	<ol style="list-style-type: none"> 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) 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) 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) 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:	<p>Cyanides (HCN, NaCN and KCN) applied in aqueous solutions on intact skin of New Zealand rabbits had LD₅₀ 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 LD₅₀ for intact skin (1).</p> <p>Dermal LD₅₀ of acetone cyanohydrin was in the same range: 17 mg (5.2 mg cyanide) /kg bw (4).</p> <p>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).</p> <p>Findings are summarised below in the Table.</p>		
Conclusions:	<ol style="list-style-type: none"> 1) The lowest LD₅₀ 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. 2) The dermal LD₅₀ values for NaCN and KCN are only slightly higher (calculated as cyanide). 		

	<p>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.</p> <p>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);</p> <p>5) Single dose NOAEL for NaCN corresponds to 0.8 mg cyanide /kg bw.</p> <p>6) The dermal lethal doses are in the same range as oral LDs.</p> <p>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.</p> <p>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.</p>	
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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 New Zealand Female	Single dose	LD50 6.8mg/kg as cyanide	(1)
LD50 dermal	NaCN	Rabbit New Zealand Female	Single dose	LD50 7.7mg/kg as cyanide	(1)
LD50 dermal	KCN	Rabbit New Zealand Female	Single dose	LD50 8.9mg/kg as cyanide	(1)
LD50 dermal	NaCN	Rabbit New Zealand Female	Single dose	LD50 – damaged skin 4.1mg/kg as cyanide	(2)
LD50 dermal	NaCN	Rabbit New Zealand Female	Single dose	LD50 – wet skin 6.3mg/kg as cyanide	(2)
Single dose dermal	HCN	Rabbit albinotic female	Single dose	0.9mg/kg respiratory, rapid breathing, corneal opacity and keratitis	(3)
Single dose dermal	KCN	Rabbit albinotic female	Single dose	2.5mg/kg respiratory, rapid breathing, corneal opacity and keratitis	(3)
Single dose dermal	NaCN	Rabbit albinotic female	Single dose	NOAEL: 1.69mg/kg LOAEL 2.5 mg/kg respiratory, rapid breathing, corneal opacity and keratitis	(2)

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