Section A6.1 **Annex Point IIA VI.6.1**		**ACUTE TOXICITY**	
Section A6.1.2 **Annex Point IIA VI.6.1.2**		**Acute Percutaneous 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 Dermal 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	Rabbit	
3.2.2	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	Rabbits: 2200 - 2600 g	
3.2.6	Number of animals per group	6-12 animals/dose (3 groups of rabbits: 1.with exposure on dry skin, 2.with exposure on moist skin and 3.with exposure on abraded skin)	
3.2.7	Control animals	Not reported	

3.3	**Administration/ Exposure**	Dry, moist or abraded skin	
3.3.1	Post exposure period	Not reported	
3.3.2	Area covered	Clipped dorsal trunk skin (% of body surface – not reported)	
3.3.3	Occlusion	Occlusive contact (polyethylene sheeting held in place with bandaging tape)	
3.3.4	Vehicle	No (only powdered NaCN was applied)	
3.3.5	Concentration in vehicle	N/A	
3.3.6	Total volume applied	Dose range Dry skin: 200 mg/kg bw Moist skin: 7 – 20 mg/kg bw Abraded skin: 5 – 10 mg/kg bw	
3.3.7	Duration of exposure	6 hours	
3.3.8	Removal of test substance	Not reported	
3.3.9	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 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**		
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Time to first signs/Time to death: dry skin: no signs/ no death moist skin: 9.0 – 145.0 minutes/ 21.0 – 170. 0 minutes abraded skin: 5.0 – 110.0 minutes/ 12.0 – 180. 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	**LD_{50}**	Percutaneous dry skin: >200 mg/kg moist skin: 11.8 mg/kg abraded skin: 7.7 mg/kg	

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Non-guideline study; the test substance (NaCN powder) was applied on clipped dry, moist or abraded skin and held in occluded contact for 6 hours - several groups of unstarved rabbits 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**	Applied to dry intact skin NaCN did not produce systemic toxicity. However, on moistened intact skin or abraded skin lethal amounts of cyanide were absorbed. Time to first signs and time of death were shorter in animals with the abraded skin than moistened skin 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**	Percutaneous LD_{50} dry skin: >200 mg/kg moist skin: 11.8 mg/kg abraded skin: 7.7 mg/kg	
5.3.1	Reliability	2	
5.3.2	Deficiencies	The study from 1988 is not in the GLP system, but the method used is comparable to methods standardised by EU directive 440/2008	

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

Section A6.1 Annex Point IIA VI.6.1		**ACUTE TOXICITY**	
Section A6.1.2 Annex Point IIA VI.6.1.2		**Acute Eye 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 guidelines for this route of exposure (for systemic toxicity testing).	
2.2	**GLP**	No	
2.3	**Deviations**	No guideline available	
		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	Rabbit	
3.2.2	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	Rabbits: 1900.0 – 2200.0 g	
3.2.6	Number of animals per group	10 animals/each dose	
3.2.7	Control animals	Not reported	
3.3	**Administration/ Exposure**	ocular, dermal (dry, moist or abraded skin)	
3.3.1	Post exposure period	7 days	

3.3.2	Vehicle	No (only powdered NaCN was applied)	
3.3.3	Concentration in vehicle	Dose range 3.18 – 9.96 mg/kg bw	
3.3.4	Total volume applied	/	
3.3.5	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 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**		
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Time to first signs/ Time to death: unstarved rabbits: 2.0 – 7.0 minutes/ 2.0 – 12.0 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**	Local signs of irritation after ocular exposure: lacrimation, moderate conjunctival hyperaemia, mild chemosis; in survivors – more severe conjunctival hyperaemia, moderate corneal opacification and mild iritis after 24 hours; mild conjunctival inflammation and mild to moderate keratitis after 7 days.	
4.4	**LD_{50}**	Eye- unstarved rabbits: 4.5 mg/kg	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Non-guideline study; the test substance (NaCN powder) was applied into the inferior conjunctival sac of one eye of unstarved rabbits – several groups 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**	Lethal systemic toxicity was produced by contamination of rabbit eye with NaCN powder, which also caused a rapid onset of moderately severe conjunctivitis and keratitis 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**	Ocular LD_{50} of NaCN powder in unstarved rabbits: 4.5 mg/kg bw	
5.3.1	Reliability	2	
5.3.2	Deficiencies	The study from 1988 is not in the GLP system.	

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

Section A6.1 Annex Point IIA VI.6.1		**ACUTE TOXICITY**	
Section A6.1.2 Annex Point IIA VI.6.1.2		**Acute systemic toxicity by topical application to the eye**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	BRYAN BALLANTYNE, M.D., D.Sc., Ph.D., 1983, Acute systemic toxicity of cyanides by topical application to the eye, Applied Toxicology Department, Union Carbide Corporation, P.O. Box 8361, South Charleston, West Virginia 25303(J.Toxicol.-Cut.&Ocular Toxicol. 2(2&3),119-129) **(DOC IV_16)**	
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 guidelines available	
2.2	**GLP**	No	
2.3	**Deviations**	The study from 1983 is not in the GLP system.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Hydrogen cyanide	
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification		
3.1.2.1	**Description**		
3.1.2.2	**Purity**	Not stated	
3.1.2.3	**Stability**	Not stated	
3.2	**Test Animals**		
3.2.1	Species	Rabbits	
3.2.2	Strain	Not stated	
3.2.3	Source	Not stated	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Adult/ average weight 1.99 kg (S.D. ± 0.34 kg; range 1.3 to 2.78 kg)	
3.2.6	Number of animals per group	10 animals in each group	
3.2.7	Control animals	Not stated	

3.3	**Administration/ Exposure**	Ocular	
3.3.1	Vehicle	Water	
3.3.2	Concentration in vehicle	Concentrations (w/v) of cyanide in the solution were 3.13% - 3.97% HCN	
3.3.3	Total volume applied	Constant dose-volume of 0.03 ml/kg was used in all cases. Resulting dose = 0.94 – 1.19 mg/kg bw	
3.3.4	Controls		
3.4	**Examinations**	Clinical observations, necropsy, haematology	
3.5	**Method of determination of LD_{50}**		
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Tight blepharospasm;, rapid panting breathing; weak and ataxic movements; convulsions; tonic spasms; loss of consciousness; irregular, shallow and gasping breathing; cessation of breathing and death (average 2.5 min.). The times for these sign to appear were 30-60 and 45-90 sec. Sign of toxicity were seen at the following and higher dosage: 0.94 mg/kg. Rapid shallow breathing, the first sign of toxicity, appeared more quickly with solutions of HCN but was present in all animals by 2.5 min.	
4.2	**Pathology**	Congestion of the lung and kidneys and presence of multiple scattered subpleural and epicardial petechiae.	
4.3	**Other**	Cyanide concentrations (µg/100g of wet tissue)±S.E. for dosage of 5.25 mg CN/kg: 6 animals per group Heart.....205±28 µg/100g Lung.....224±51 µg/100g Brain.....107±15 µg/100g Spinal Cord.....29±8 µg/100g Liver.....15±8 µg/100g Kidney.....14±10 µg/100g Whole blood.....552±51 µg/dl Serum.....341±53 µg/dl S.E. = standard error of the mean Concentrations were measured in adult female albino rabbits of average weight 2.24 kg(S.D. ±0.24; range 1.9 – 2.8 kg)	
4.4	**LD_{50}**	1.04 mg/kg (0.96 – 1.13) ; 0.039 (0.36 – 0.042) mmol/kg with 95% confidence limits	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The acute toxicity of hydrogen cyanide by topical application to the eye	

5.2	**Results and discussion**	Using the rabbits, the LD_{50} values (with 95% confidence limits), in mmol/kg, with aqueous solutions instilled into the inferior conjunctival sac were determined to be 0.039 (0.036-0.042) for HCN. Sign of toxicity appeared rapidly and death occurred within 3 to 12 min of the eye being contaminated.	
5.3	**Conclusion**	Contamination of the eye with hydrogen cyanide solution could be hazardous: for this route of exposure. LD_{50} is about 1 mg/kg bw.	
5.3.1	Reliability	3	
5.3.2	Deficiencies	The study from 1983 is not in the GLP system. No serious deficiencies.	

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

Section A6.1 Annex Point IIA VI.6.1	**ACUTE TOXICITY**	

Section A6.1.3 Annex Point IIA VI.6.1.3	**Acute Inhalation Toxicity**	
Justification: **Supportive data:**	The active substance hydrogen cyanide is a gas at body temperature. Hydrogen cyanide is known to be a highly toxic substance by inhalatory exposure for humans and for all species of laboratory organisms. The mechanism of its toxic action is well known. Although literature provides a large number of data, no single study meets requirements for a key study. 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);** 2. AMRL. 1971. The acute toxicity of brief exposures to hydrogen fluoride, hydrogen chloride, nitrogen dioxide, and hydrogen cyanide singly and in combination with carbon monoxide. Wright-Patterson Air Force Base, OH: Aerospace Medical Research Laboratory. AD751442 3. Hume AS, Mozigo JR, McIntyre B, et al. 1995. Antidotal efficacy of alpha-ketoglutaric acid and sodium thiosulfate in cyanide poisoning. Clin Toxicol 33(6):721-724. 4. Matijak-Schaper M, Alarie Y. 1982. Toxicity of carbon monoxide, hydrogen cyanide and low oxygen. J Combust Toxicol 9:21-61. (**DOC IV_17);** 5. Fundamental and Applied Toxicology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1-40, 1981-97. For publisher information, see TOSCF2 v. 9, p. 236, 1987 (FAATDF) 6. Toxicology and Applied Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1959- v. 42, p. 417, 1977 (TXAPA9); 7. Arvind K. Chaturvedi, Boyd R. Endecott, Roxane M. Ritter, Donald C. Sanders Variations in Time-to-Incapacitation and Blood Cynanide Values for Rats Exposed to Two Hydrogen Cyanide Gas Concentrations, Washington, D.C. 20591 8. Monsanto Co.Report 1985. One-month inhalation toxicity of acetone cyanohydrin in male and female Sprague-Dawley rats. St Louis, Monsato Co. Report ML-81-178/810068 (US EPA/OPTS Public Files No. 878216393). 9. J.M.McNerney, M.P.H., H.H.Schrenk, PhD., 1960, The Acute Toxicity of Cyanogen, Industrial Hygiene Foundation, 4400 Fifth Avenue, Pittsburg 13, Pennsylvania, Industrial Hygiene Journal, April 1960, 121 – 124; summarised in section 6.1.3a. **(DOC IV_18)** 10. The Merck Index -An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse´Station, NJ: Merck and Co., Inc., 2006., p. 830] **PEER REVIEWED**	
Guidelines:	Not presented.	
GLP:	No. All studies before GLP requested.	
Material and methods:	Inhalation exposures to HCN or acetone cyanohydrin; time vs. concentration exposures of rats, mice, guinea pigs, rabbits, dogs, goats and monkeys. A general	

	toxicity and lethality measure, classical LC_{50} calculations, estimates of lethal doses.
Results and discussion:	Relative sensitivity to HCN vapours has been tested in various species, from mice to monkeys (mice, rats, guinea pigs, rabbits, cats, dogs, goats and monkeys); time to death of half animals exposed to a concentration of 1000 mg/m^3 varied between 1.0 and 3.5 minutes, values (i.e., resistance) increased with body mass (***Barcroft 1931, this study is not included in the list of references***). The range of sensitivity values corresponds to the range of minute respiratory volumes per kg body weight, indicating that the received LD_{50} dose (per kg bw) was similar across species. Inhalation LC_{50} values in rats ranged from 158 mg/m^3 for a 60 minute exposure to 3,778 mg/m^3 for exposure time 10 sec - see **Table 2**. These LC values correspond to total doses inhaled: 0.16mg/kg bw for 10 second exposure and 2.36 mg/kg bw for 60 min. exposure. For longer exposures, the LC50 values seem not to decrease markedly, perhaps as a result of balanced resorption and elimination of CN ions. LC_{50} values interpolated from rat data for exposure times 5 to 30 minutes are similar to fatal concentrations from case reports in humans (100 – 300 mg/m^3, exposure times 30 to 5 minutes). Exposure of rats (Sprague-Dawley) to acetone cyanohydrin for 6 hours in an airborne concentration of 225 mg/m^3(equivalent to 71 mg/m^3 of hydrogen cyanide) resulted in the death of 3/10 males but none of 10 females. Similar values were found in other animal species and in other studies, as summarised below in **Table 1.** Non- lethal effects of a single exposure. Exposure of cynomolgus monkey to HCN vapours led to incapacitation after 8 minutes in a concentration of 180 mg/m^3 and after 19 minutes in 110 mg/m^3. While 30 min exposure to HCN concentration of 110 mg/m^3 induced semi consciousness, respiratory disorders and EEG changes, concentration of 70 mg/m^3 led only to slight nervous depression. (**9**).
Conclusions:	1) For rats, LC_{50} values ranged from 158 mg/m^3 for a 60 minute exposure to 3778 mg/m^3 for 10 sec exposure. 2) The reliability of these estimates is supported by similar values found in other animal species and in other studies. 3) Human fatal concentrations from case reports fall into the same range. 4) LC_{50} values increased linearly with square root of the inverse value of exposure time between 30 minutes and 10 seconds: $Ln(LC_{50}) = 9.53 - 0.56 \ln t$, t time in seconds., R-squared = 99.17%. (Similar regression is reported in the study by McNerney et al. for cyanogen**: this study is summarised in section 6.1.3a.**) LC_{50} values increase much slower in the range of longer exposures, when the cumulation of cyanide is efficiently counterballanced by transformation to thiocyanate.

Table 1 Acute toxicity – inhalation (hydrogen cyanide)

Study		**Test organism**	**Exposure time**	**HCN concentration**	**Reference**
LC50 inhalatory	HCN	Rat Wistar Male	5 minutes	563mg/m^3 (503ppm)	(2)
LC50 inhalatory	HCN	Rat not specified	60 minutes	160mg/m^3 (143ppm)	**(1)**
LC50 inhalatory	HCN	Mouse ICR Male	5 minutes	362mg/m^3	(2)
LC50 inhalatory	HCN	Mouse ICR Male	3 minutes	448mg/m^3 (400ppm)	(3)
LC50	HCN	Mouse	30 minutes	180mg/m^3	**(4)**

inhalatory		Swiss-Webster Male		(166ppm)	
LC50 inhalatory	HCN	Rabbit Not specified	35 minutes	207mg/m^3 (188ppm)	**(1)**
LC50 inhalatory	HCN	Dog	3 minutes	336 mg/m^3 300ppm	(5)
LC50 inhalatory	HCN	Mouse	30 minutes	189 mg/m^3 169ppm	(5)
LC50 inhalatory	HCN	Rat	30 minutes	179 mg/m^3 160ppm	(6)

Table 2 Acute inhalation toxicity of hydrogen cyanide for rats in dependence on the exposure time (ref. 1)

Exposure time	LC_{50} (mg.m^{-3})
10 s	3778
1 min	1471
5 min	493
30 min	173
60 min	158

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

Section A6.1 **Annex Point IIA VI.6.1**		**ACUTE TOXICITY**	
Section A6.1.3 **Annex Point IIA VI.6.1.3**		**Acute Inhalation Toxicity**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	J.M.McNerney, M.P.H., H.H.Schrenk, PhD., 1960, The Acute Toxicity of Cyanogen, Industrial Hygiene Foundation, 4400 Fifth Avenue, Pittsburg 13, Pennsylvania, Industrial Hygiene Journal, April 1960, 121 – 124 **(DOC IV_18)**	
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 Inhalation Toxicity)	
2.2	**GLP**	No, study older than GLP	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cyanogen (NCCN)	
3.1.1	Lot/Batch number	Not reported	
3.1.2	Specification	Cyanogen gas	
3.1.2.1	**Description**	Colourless gas	
3.1.2.2	**Purity**	99.5% (0.5% - nitrogen, chlorine, cyanogen chloride)	
3.1.2.3	**Stability**	Not reported	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Albino rat – strain not reported	
3.2.3	Source	Not reported	
3.2.4	Sex	Males only	
3.2.5	Age/weight at study initiation	Rat – 135 g (average)	
3.2.6	Number of animals per group	13 groups of six rats – six different concentrations, six different time periods and control	
3.2.7	Control animals	Yes	

3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Post exposure period	14 days observation	
3.3.2	Concentrations	Nominal concentration : 0, 533, 537, 851, 851, 1054, 1066, 2115, 2111, 4207, 4223, 8508, 8571 mg/m³ (0, 250, 250, 400, 400, 500, 500, 1000, 1000, 2000, 2000, 4000 and 4000 ppm)	
		Analytical concentration – not reported	
3.3.3	Particle size	/	
3.3.4	Type or preparation of particles	/	
3.3.5	Type of exposure	Whole body	
3.3.6	Vehicle	No	
3.3.7	Concentration in vehicle	/	
3.3.8	Duration of exposure	120, 60, 45, 30, 15, 7.5 and 0 minutes.	
3.3.9	Controls	Not reported	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Acute Inhalation Toxicity: asphyxiation, lacrimation, upper respiratory tract irritation, pink coloration of the noticeable skin, blinking eyes, rubbing of forepaws over eyes and snout, huddling together with inactivity, slow gasping, tearful eyes, yellow fluid dripping from nares and mouth, restless and panic type movements, accentuated and poorly coordinated motions, bright pink coloration of the skin, laboured breathing, gasping, tremors, sluggishness, prostration, shallow breathing, death.	
4.2	**Pathology**	No effects reported	
4.3	**Other**	None	
4.4	**LC_{50}**	LC_{50} for cyanogen = 23,400 ppm / t; t= exposure duration in min See **Table II** - Effects of the Acute Inhalation Exposures of Cyanogen Upon Male Albino Rats and Inhalation toxicity of cyanogen in rats – time/concentration graph – **see Figure 1.**	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Non-guideline studies Rats were housed in wire mesh cages within the chamber and exposed to a total of six different concentrations of cyanogen and six different time periods. Survivors were observed for 14 days after exposure. Body weight of rats was measured before exposure and after 14 days.	
5.2	**Results and discussion**	The present study showed that rats withstood 250 ppm of cyanogen for 120 minutes with only partial mortality and 500 ppm for 30 minutes with no deaths. In addition, the capacity of the rats in this study to tolerate the excessive concentrations of 1000 and 2000 ppm of cyanogen for periods of approximately 15 and 7.5 minutes, respectively, points toward a lower toxicity.	

5.3 Conclusion	Assuming transformation of one molecule of cyanogen to one molecule of hydrogen cyanide, following approximate LC values may be calculated for HCN (:t= exposure duration in min): LC_0 = 15,900 mg/m^3 / t; LC_{50} = 25,850 mg/m^3 / t; LC_{100} = 41,050 mg/m^3 / t	
5.3.1 Reliability	2	
5.3.2 Deficiencies	The study from 1960 is not in the GLP system, but the method used is comparable to methods standardised by EU directive 440/2008.	

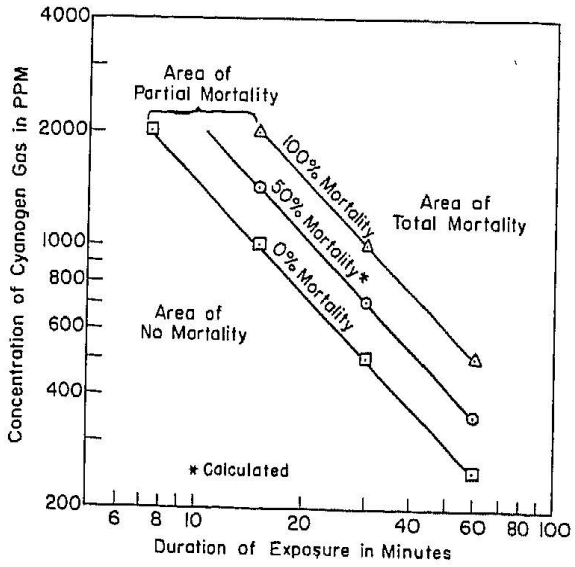


FIGURE 1. Inhalation toxicity of cyanogen in rats.

TABLE II

Effects of the Acute Inhalation Exposures of Cyanogen Upon Male Albino Rats

Concentration of Cyanogen (ppm)	Concentration of Cyanogen (mg/m³)	Average Temp. (°C)	Length of exposure (minutes)	Length of build-up period (minutes)	Mortality ratio (dead/dosed)	Initial average weight of rats (grams)	Average weight gain after 14 days (grams)
4000	8571	22.8	7.5	3.0	3/6	162	44
4000	8508	25.0	15	3.0	6/6	156	—
2000	4223	27.2	7.5	1.5	0/6	126	55
2000	4207	28.3	15	1.5	6/6	121	—
1000	2111	27.2	15	0.5	0/6	123	52
1000	2115	26.7	30	0.5	6/6	123	—
500	1066	24.4	30	0.3	0/6	134	49
500	1054	27.8	45	0.3	6/6	122	—
400	851	25.0	45	0.25	0/6	144	46
400	851	25.0	60	0.25	6/6	137	—
250	537	22.2	60	0.15	0/6	160	59
250	533	24.4	120	0.15	4/6	127	38
Control	—	—	—	—	0/6	167	53

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

Section A6.1 Annex Point IIA VI.6.1	**ACUTE TOXICITY**	

Section A6.1.4 Annex Point IIA VI.6.1.4	**Skin Irritation**	
Justification: Supportive data:	No formal study on irritating effects of cyanides on skin in humans or animals is known and no such study can be realised with regard to easy penetration of HCN through skin and extremely high acute toxicity. Data from the observation of HCN effects on human skin, resulting from the observation performed during HCN and cyanides use, are reported as surrogate information. None of the observation data meet requirements for labelling of hydrogen cyanide as a skin irritating substance, resulting from the requirements for substance classification according (ES) 1272/2008. 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**).	
Reference:	1. Blanc P, Hoan M, Mallin K, et al. 1985. Cyanide intoxication among silver-reclaiming workers. J Am Med Assoc 253: 367-371 **(DOC IV_19)** 2. El Ghawabi SH, Gaafar MA, El-Saharti AA, et al. 1975. Chronic cyanide exposure: A clinical, radioisotope, and laboratory study. Br J Med 32:215-219 **(DOC IV_20)** 3. McNerney JM, Schrenk HH. 1960. The acute toxicity of cyanogen. Am Ind Hyg Assoc J 21:121-124 **(DOC IV_18)** 4. Fairley A, Linton EC, Wild FE. 1934. The absorption of hydrocyanic acid vapours through the skin with notes on other matters relating to acute cyanide poisoning. J Hyg 34: 283-294 **(DOC IV_21)**	
Guidelines:	Not presented	
GLP:	No	
Findings:	Cyanide caused rash in 42 workers exposed to 15ppm HCN. **(1)** Brick-red burns were observed in a man exposed to 200ppm HCN for an unspecified time. No skin inflammation was observed in workers exposed to 6.4–10.4 ppm of sodium cyanide and copper cyanide. **(2)** No dermal damage was observed on rabbit skin after exposure to 10,000ppm of cyanogen for 8 hours**. (3)** Vascular congestion was observed in skin of a guinea pig after exposure to unknown doses of hydrogen cyanide for 65 minutes. **(4)**	
Conclusion:	Hydrogen cyanide does not show signs of a skin irritating substance despite the fact that skin penetration is considered to be a possible route of exposure, **see Doc 6.1.2.** Notes: - Dermal rash in silver reclaiming workers (**1**) are described on the basis of anamnestic data (questionnaire); concentrations of HCN in the hall should have been enormous: the investigation has been prompted by a case of acute fatal HCN poisoning; in	-

	addition, workers of silver reclaiming factory had been exposed to many chemical substances that may cause rash. - The skin of guinea pig was exposed to saturated vapours of HCN (i.e. approx. 915g/m^3) Cyanogen as surrogate for dermal irritation by HCN can be justified as it is likely to be hydrolysed to cyanide and cyanate during skin penetration.	

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

Section A6.1 Annex Point IIA VI 6.1	**ACUTE TOXICITY**	

Section A6.1.4 Annex Point IIA VI.6.1.4	**Eye Irritation**	
Justification: Supportive data:	No formal study on irritating effects of cyanides on eyes in humans or animals is known and no such study can be realised with regard to extremely high acute toxicity. Data from the observation of HCN effects on human eyes, resulting from the observation performed during HCN and cyanides use, are reported as surrogate information. None of the observation data meet requirements for classification of hydrogen cyanide as irritating to eyes. 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**).	
Reference:	1. McNerney JM, Schrenk HH. 1960. The acute toxicity of cyanogen. Am Ind Hyg Assoc J 21:121-124 **(DOC IV_18)** 2. Bonsall JL. 1984. Survival without sequelae following exposure to 500 mg/m^3 hydrogen cyanide. Hum Toxicol 3:57-60 **(DOC IV_22)** 3. Chandra H, Gupta BN, Ghargava SK, et al. 1980. Chronic cyanide exposure: A biochemical and industrial hygiene study. J Anal Toxicol 4:161-165. 4. Blanc P, Hogan M, Mallin K, et al. 1985. Cyanide intoxication among silver-reclaiming workers. J Am Med Assoc 253: 367-371 **(DOC IV_19)** 5. El Ghawabi SH, Gaafar MA, El-Saharti AA, et al. 1975. Chronic cyanide exposure: A clinical, radioisotope, and laboratory study. Br J Med 32:215-219 **(DOC IV_20)** 6. BRYAN BALLANTYNE, M.D., D.Sc., Ph.D., 1983, Acute systemic toxicity of cyanides by topical application to the eye, Applied Toxicology Department, Union Carbide Corporation, P.O. Box 8361, South Charleston, West Virginia 25303(J.Toxicol.-Cut.&Ocular Toxicol. 2(2&3),119-129), **summary see Section 6.1.2c) (DOC IV_16)** 7. Bryan Balantyne, 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; **summary see Section 6.1.2a and Section 6.1.2b. (DOC IV_14)**	
Guidelines:	None.	
GLP:	No	
Material and methods:	Observation in volunteers and in workers. Observation in animals tested for inhalation toxicity or for systemic toxicity of HCN applied into the conjunctival sac.	
Findings:	Cyanogen caused eye irritation in volunteers during short exposure to 16ppm **(1).** A negligible loss of peripheral vision was the only permanent effect observed in a man, whose eyes had been exposed to 452 ppm HCN for	

	13min during tank cleaning. **(2)** Eye irritation in two workers engaged in electrolytic coating was observed during chronic occupational exposure. **(3)** In other studies, cyanides caused eye irritation in 5 workers exposed to 15ppm HCN **(4)**, and lacrimation in workers exposed to 6.4 ppm of cyanide. **(5)**. Eye irritation may not be caused solely by cyanides; workers engaged in electrolytic coating may be exposed also to other chemicals irritating to eyes. Data on eye effects for animals by inhalation are available only for rats which were acutely exposed for 7.5-120 minutes to 250 ppm cyan, and 125ppm cyanide. **(1)** Tight belepharospasm after application of 3 – 4% HCN water solution indicates acute irritation. **(6)** Local signs of irritancy and inflammation were seen promptly after placing NaCN in the inferior conjunctival sac, and considered of marked lachrymation, moderate conjuctival hyperemia and mild chemosis. Conjuctivitis and lachrymation slowly resolved after 24 hours, but mild residual inflammation was still present at 7 days **(7).**	

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

Section A6.1 **Annex Point IIA VI.6.1**		**ACUTE TOXICITY**	
Section A6.1.4 **Annex Point IIA VI.6.1.4**		**Acute Eye Irritation**	
		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 **(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 (method used comparable to guideline of Acute Toxicity: Eye Irritation/Corrosion)	
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	Rabbit	
3.2.2	Strain	New Zealand white	
3.2.3	Source	Not reported	
3.2.4	Sex	Females only	
3.2.5	Age/weight at study initiation	Rabbits: 1770.0 – 2470.0 g (age – not reported)	
3.2.6	Number of animals per group	10 animals/each dose	
3.2.7	Control animals	Not reported	
3.3	**Administration/ Exposure**	Ocular – into the inferior conjunctival sac of one eye	

3.3.1	Preparation of test substance	Test substance was used as delivered.	
3.3.2	Amount of active substance instilled	3.18 – 9.96 mg/kg	
3.3.3	Exposure period	Not reported	
3.3.4	Post exposure period	7 days	
3.4	**Examinations**	Examination of eyes and examination of systemic signs of toxicity	
3.4.1	Ophthalmoscopy examination	Not reported	
3.4.1.1	**Scoring system**	Not reported	
3.4.1.2	**Examination time points**	1. immediately after application 2. 24 hours after exposure 3. 7 days after exposure	
3.4.2	Other investigations	/	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	1. immediately after application: marked lacrimation, moderate conjunctival hyperaemia, mild chemosis; 2. in survivors – 24 hours after exposure: more severe conjunctival hyperaemia, mild to moderate corneal opacification and mild iritis after 24 hours; 3. in survivors – 7 days after application: mild conjunctival inflammation and mild to moderate keratitis Systemic clinical signs: rapid breathing, weak movements, tremors, respiratory distress, severe spasms, convulsions, irregular shallow breathing, coma, death.	
4.2	**Average score**		
4.2.1	Cornea	Score – not reported	
4.2.2	Iris	Score – not reported	
4.2.3	Conjunctiva	Non-entry field	
4.2.3.1	**Redness**	Score – not reported	
4.2.3.2	**Chemosis**	Score – not reported	
4.3	**Reversibility**	Rabbits were observed only for 7 days - mild conjunctival inflammation and mild to moderate keratitis were observed.	
4.4	**Other**		
4.5	**Overall result**		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test substance (NaCN powder) was applied into the inferior conjunctival sac of one eye of rabbits – several groups of animals with various dose levels. Following exposure animals were observed for signs of toxic effects and for local signs of eye irritation. Survivors were kept only for 7 days.	

5.2	**Results and discussion**	Application of NaCN powder to rabbit eye caused a rapid onset of moderately severe conjunctivitis and keratitis. Mild conjunctival inflammation and mild to moderate keratitis were observed in survival animals 7 days after application. Lethal systemic toxicity was also produced by contamination of rabbit eye with NaCN powder.	
5.3	**Conclusion**	Application of NaCN powder conjunctival sac caused a rapid onset of moderately severe conjunctivitis and keratitis, persisting at least 7 days.	
5.3.1	Reliability	*2*	
5.3.2	Deficiencies	Scoring system is not specified, post-exposure observation is too short.	

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

Section A6.1 Annex Point IIA VI.6.1	**ACUTE TOXICITY**	

Section A6.1.5 Annex Point IIA VI.6.1.5	**Skin Sensitisation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [x] Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Justification:	It is practically difficult, if not impossible, to conduct a specific study on skin contact sensitization with hydrogen cyanide vapours; when applied on skin in a water solution hydrogen cyanide is also easily resorbed and causes acute systemic poisoning. To our knowledge, there are no confirmed cases in humans to suggest that hydrogen cyanide is a skin sensitizer. Hydrogen cyanide does not present any structural alert for skin sensibilization, standard skin sensibilization test is not feasible and sensibilization properties of cyanides have not been suggested by the experience in humans over a period of many years of production and use. This conclusion is supported by exhaustive and reliable 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		
Conclusion	There are no confirmed cases in humans to suggest that hydrogen cyanide is a skin sensitizer.	
Undertaking of intended data submission	No studies are planned.	

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

Section A6.2 Annex Point IIA VI.6.2	**METABOLISM STUDIES IN MAMMALS. BASIC TOXICOKINETICS, INCLUDING A DERMAL ABSORPTION STUDY**	
	Information On Dermal Absorption	
Justification: **Supportive data:**	HCN absorption through skin is described in literature a number of studies, however with respect to the fact that no complete studies are available, the data found and given below are used as supporting information.	
Reference:	**1.** D.C. Walton, M.G. Witherspoon 1925. Skin absorption of certain gases. J Pharmacol Exp Ther 26: 315-324 **(DOC IV_25). Summary in DOC III_ 6.1.7a.** 2. JACC No 53, Cyanides of Hydrogen, Sodium and Potasium, and acetone Cyanohydrin (CAS No. 74-90-8, 143-33-9, 151-50-8 and 75-86-5), ECETOC JACC REPORT No. 53 European Centre for Ecotoxicology and Toxicology of Chemicals Volume I **(DOC IV_3)** 3. J.M.McNerney, M.P.H., H.H.Schrenk, PhD., 1960, The Acute Toxicity of Cyanogen, Industrial Hygiene Foundation, 4400 Fifth Avenue, Pittsburg 13, Pennsylvania, Industrial Hygiene Journal, April 1960, 121 – 124 **(DOC IV_18) Summary in DOC III_ 6.1.7c.** 4. A. Fairley, E.C.Linton, F.E.Wild , The Absorption of Hydrocyanic Acid Vapour through the Skin (with notes on other matters relating to acute cyanide poisoning), Journal of Hyg., Volume 34, October 1934, No. 3: 283 - 294 **(DOC IV_21) Summary in DOC III_ 6.1.7b.**	
Guidelines:	Not presented	
GLP:	No	
Material and methods:	Peer review	
Findings:	**Skin absorption** No study dealing with quantitative absorption of gaseous cyanides or common inorganic salts after exposure of human skin has been carried out. Evidence of the ability of cyanides and hydrogen cyanide to be absorbed through skin results from toxic effects from incidental contacts of human skin with hydrogen cyanide or cyanides. Data relating to absorption of hydrogen cyanide by animals come from studies on guinea pigs and dogs (**1**): Shaved area of abdominal skin of 8 guinea pigs has been exposed to saturated vapours of HCN. All exposed animals died at 7 -8 min; clinical symptoms of toxicity and autopsy results were the same in all animals. Dogs tolerated a concentration of 5.5 mg/l for up to 180 minutes without any ill effects. Clinical signs of toxicity (muscle twitching) appeared in animals exposed to HCN concentrations 10.9 mg/L and higher. At concentrations 11.6 mg/L and higher (concentration x time product values of 11 $g.h.m^{-3}$ and higher) 6 of 7 animals died (1 of them was euthanized). Protection of skin by hair in dogs seems to slightly enhance the tolerance. Dogs (with shaved fur on their bellies) exposed to HCN vapours showed after 30-60 minutes symptoms of toxicity including rapid breathing, muscle twitching, unconsciousness and death.	
Conclusion:	According to information available, upon absorption through skin symptoms of HCN or cyanide poisoning appear. The lethal doses are in the same range as oral LDs. 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. The dermal LD_{50} values for NaCN and KCN are only slightly higher (calculated as cyanide) (**2**). 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. 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. Absorbed hydrogen cyanide is distributed within the body by blood. According to one study, up to 80% absorbed cyanides are metabolised.	

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

Section A6.2 Annex Point IIA VI.6.2	**METABOLISM STUDIES IN MAMMALS. BASIC TOXICOKINETICS, INCLUDING A DERMAL ABSORPTION STUDY**	
Justification: Literature data:	Toxic kinetics, metabolism and distribution of HCN, cyanides and other sources of cyanide ion are described in literature in a number of studies. 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. Gettler AO, Baine JO. 1938. The toxicology of cyanide. Am J Med Sci 195: 182-198 **(DOC IV_27)**. **2.** Walton DC, Witherspoon MG. 1925. Skin absorption of certain gases. J Pharmacol Exp Ther 26: 315-324 **(DOC IV_25)**. **Summary see section III_6.1.7a.** 3. Yamamoto K, Yamamoto Y, Hattori H, et al. 1982. Effects of routes of administration on the cyanide concentration distribution in the various organs of cyanide-intoxicated rats. Tohoku J Exp Med 137: 73-78 **(DOC IV_24)**. 4. BRYAN BALLANTYNE, M.D., D.Sc., Ph.D., 1983, Acute systematic toxicity of cyanides by topical application to the eye, Applied Toxicology Department, Union Carbide Corporation, P.O. Box 8361, South Charleston, West Virginia 25303 (J.Toxicol.-Cut.&Ocular Toxicol. 2(2&3),119-129) **(DOC IV_16)**. **Summary see section III_6.1.2c.** 5. J.M.McNerney, M.P.H., H.H.Schrenk, PhD., 1960, The Acute Toxicity of Cyanogen, Industrial Hygiene Foundation, 4400 Fifth Avenue, Pittsburg 13, Pennsylvania, Industrial Hygiene Journal, April 1960, 121 – 124 **(DOC IV_18)**. **Summary see section III_6.1.3a, 6.1.7c.** 6. A. Fairley, E.C.Linton, F.E.Wild , The Absorption of Hydrocyanic Acid Vapour through the Skin (with notes on other matters relating to acute cyanide poisoning), Government Experimental Establishment at Porton, Journal of Hyg., Volume 34, October 1934, No. 3 **(DOC IV_21)**. **Summary see section III_6.1.7b.** 7. Chandra H, Gupta BN, Bhargava SK, Clerk SH, Mahendre PN (1980) Chronic cyanide exposure: a biochemical and industrial hygiene study. Journal of Analytical Toxicology, 4:161–165. **(DOC IV_23)**. 8. Ansell M, Lewis FAS (1970) A review of cyanide concentrations found in human organs: A survey of literature concerning cyanide metabolism, "normal," non-fatal, and fatal body cyanide levels. Journal of Forensic Medicine, 17:148–155. **(DOC IV_28)**. 9. Schultz V, Gross R, Pasch T, Busse J, Loeschcke G (1982) Cyanide toxicity of sodium nitroprusside in therapeutic use with and without sodium thiosulfate. *Klinische Wochenschrift*, 60:1393–1400. **(DOC IV_32)**. 10. Schultz V, Bonn R, Kindler J (1979) [Kinetics of elimination of thiocyanate in 7 healthy subjects and 8 subjects with renal failure.] *Klinische Wochenschrift*, 57:243–247 (in German). **(DOC IV_34)**.	

	11. Aminlari M, Vaseghi T, Kargar MA (1994) The cyanidemetabolizing enzyme rhodanese in different parts of the respiratory systems in sheep and dog. Toxicology and Applied Pharmacology, 124:64–71. 12. Dahl AR (1989) The cyanide-metabolizing enzyme rhodanese in rat nasal respiratory and olfactory mucosa. *Toxicology Letters,* 45:199–205. 13. Sylvester DM, Holmes RK, Sander C, Way JL (1982) Interference of thiosulfate with potentiometric analysis of cyanide in blood and its elimination. *Toxicology and Applied Pharmacology*, 65:116–121. 14. Boxer GE, Rickards JC (1952) Studies on the metabolism of the carbon of cyanide and thiocyanate. *Archives of Biochemistry and Biophysics*, 36:7–26. 15. Rieders F (1971) Noxious gases and vapors. I: Carbon monoxide, cyanides, methemoglobin, and sulfhemoglobin. In: De Palma JR, ed. Drill's pharmacology in medicine, 4th ed. New York, NY, McGraw-Hill Book Company, pp. 1180–1205. 16. Tor-Agbidye J, Palmer VS, Lasarev MR, Craig AM, Blythe LL, Sabri MI, Spencer PS (1999) Bioactivation of cyanide to cyanate in sulfur amino acid deficiency: relevance to neurological disease in humans subsisting on cassava. Toxicological Sciences, 50:228–235. 17. Hartung R (1982) Cyanide and nitriles. In: Clayton GD, Clayton FE, eds. Patty's industrial hygiene and toxicology, 3rd ed. Vol. II C. New York, NY, John Wiley & Sons, pp. 4845–4906. 18. Schubert J, Brill WA (1968) Antagonism of experimental cyanide toxicity in relation to the in vivo activity of cytochrome oxidase. Journal of Pharmacology and Experimental Therapeutics, 162:352–359. 19. Lawrence WS (1947) The toxicity of sodium cyanide at slow rates of infusion. Federation Proceedings, 6(1):349. 20. Bright JE, Marrs TC (1988) Pharmacokinetics of intravenous potassium cyanide. Human Toxicology, 7:183–186. 21. Leuschner F, Neumann BW, Otto H, Möller E (1989) 13-week toxicity study of potassium cyanide administered to Sprague-Dawley rats in the drinking water. Unpublished study, Laboratory of Pharmacology and Toxicology, July [cited in JECFA, 1993]. 22. Blaschle TF, Melmon KL (1980) Antihypertensive agents and the drug therapy of hypertension. In: Goodman LS, ed. Goodman and Gilman's the pharmacological basis of therapeutics, 6th ed. New York, NY, Macmillan Publishing Co., pp. 805–806. 23. Bödigheimer K, Nowak F, Schoenborn W (1979) Pharmakokinetik und thyreotoxizität des nitroprussid-Natrium-Metaboliten Thiocyanat. Deutsche Medizinische Wochenschrift, 104:939–943.	
Absorption	**Inhalatory absorption** Cyanide as hydrogen cyanide is quickly absorbed after being inhaled (within seconds). Humans hold in their lungs 58% gaseous hydrogen cyanide at normal breathing. The following data come from exposure of dogs to a concentration of hydrogen cyanide lethal within 15 minutes and 10 minutes (1):	

1st dog ... ... absorption 16.0 mg HCN (1.55 mg/kg)
2nd dog ... ... absorption 10.1 mg HCN (1.11 mg/kg)

Oral absorption

Three dogs were administered lethal doses of hydrogen cyanide solution by gastric gavage. The quantity of absorbed cyanide was determined by the difference between supplied cyanide and cyanide that remained in stomach and intestines. The dogs were given doses of 8.4, 4.4 and 1.6mg HCN/kg, and died after 8, 21, a 155 minutes; absorption of 17, 24 and 72% of the given doses (1).

Skin absorption

No study dealing with quantitative absorption of gaseous cyanides or common inorganic salts after exposure of human skin has been carried out.
Evidence of the ability of cyanides and hydrogen cyanide to be absorbed through skin results from toxic effects from incidental contacts of human skin with hydrogen cyanide or cyanides.
In a case study, a worker carrying a new breathing apparatus was exposed to liquid hydrogen cyanide through his hand. Although inhalation of HCN was prevented, the worker fell unconscious within five minutes due to extensive absorption of liquid HCN through skin.
Absorption of gaseous HCN through dry skin is much slower: nevertheless, persons working in 20,000 ppm HCN for 8–10 minutes with protective masks are reported to experience nausea, weakness and headache.
Data relating to absorption of hydrogen cyanide by animals come from studies on guinea pigs and dogs.
Guinea pigs (with shaved fur on their bellies) exposed to saturated HCN vapours showed symptoms of toxicity including rapid breathing, muscle twitching, unconsciousness and death after 30-60 minutes. In similar tests with dogs whose bodies (shaved as well unshaved) were exposed (excluding heads) to hydrogen cyanide vapours, no symptoms of toxicity were observed for 180-minute exposure to HCN concentration of 5,572mg.m^{-3}. Exposure to HCN concentration of 15,000 mg.m^{-3} led to death after 47 minutes of dermal absorption (2).

Distribution - inhalatory exposure

Absorbed hydrogen cyanide is quickly distributed by blood into the whole body. Levels of hydrogen cyanide measured were 0.75, 0.42, 0.41, 0.33 and 0.32mg/ 100g tissue in lungs, heart, blood, kidneys and brain; the values come from a male who had died after inhalatory exposure to hydrogen cyanide. In one case of death caused by oral exposure to hydrogen cyanide, oral exposure was estimated at 30mg of CN in food approx. 3 hours before the death **(1).**

In another case, a tissue of a male who died after inhalation of hydrogen cyanide was examined with the following levels measured: 0.5mg HCN on 100ml of blood and 0.11g / 100g kidneys, 0.07mg / 100mg brain and 0.03mg/100mg liver. Cyanide level in urine was 0.2mg / 100ml and in stomach content 0.03mg/ 100g.

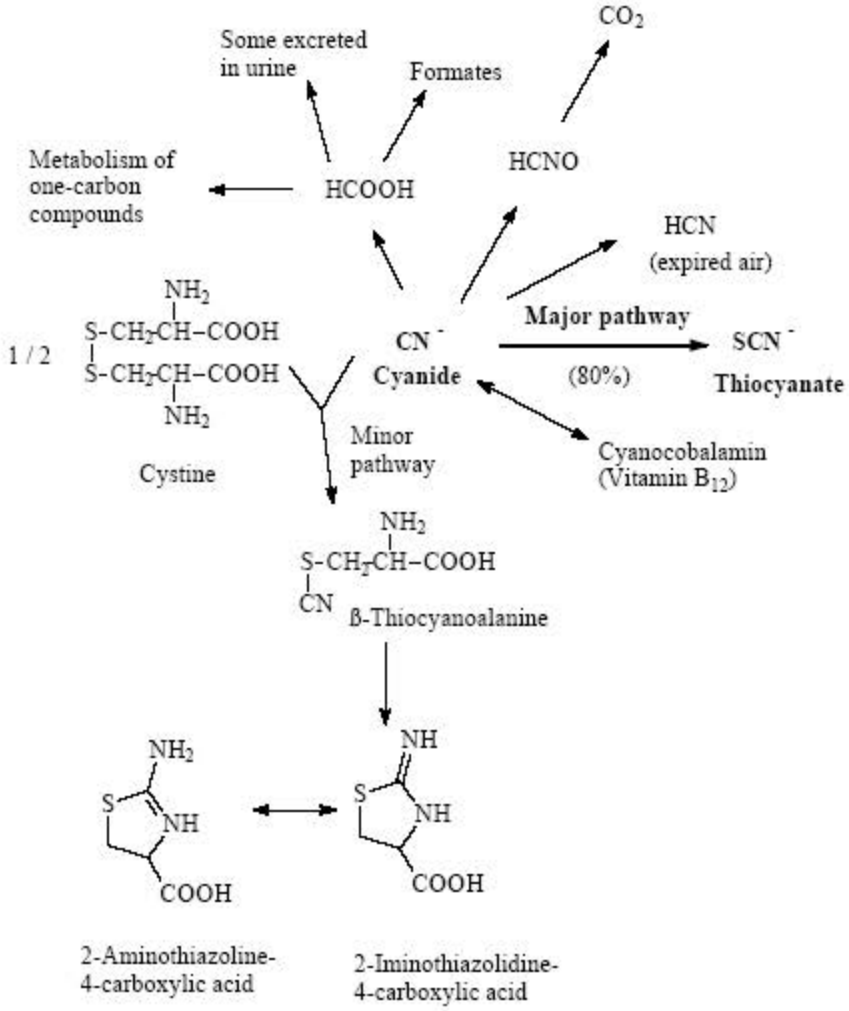
Following chronic exposure to HCN the concentration measured in the blood of smokers and non-smokers was 0.19-0.75ppm, 56.0 and 18.3µg cyanide / 100ml. Cyanide levels in control groups were 4.8µg/ml for smokers and 3.2µg/ml for non-smokers **(7).**

In rats exposed to HCN concentrations of 400 or 1,320 mg/m^3 (death after 10 or 5 minutes) no differences in cyanide concentrations in

	various body tissues, which would depend on HCN exposure concentrations, were observed. Average concentrations of cyanides in tissues in both groups of rats were 4.4µg/g of wet weight of the organ in lungs, 3.0µg/g of wet weight in blood, 2.15µg/g of wet weight in liver, 1.4µg/g of wet weight in brain, and 0.68µg/g of wet weight in spleen **(3).** In rabbits exposed to 3,040mg HCN/m^3 for 5 minutes, the following levels of cyanide content in their tissues were measured: 170µg/ 100ml blood, 48µg/ 100ml plasma, 0µg/ 100g in liver, 6µg/ 100g in kidneys, 50µg/100g in brains, 62µg/ 100g in heart, 54µg/ 100g in lungs, and 6µg/ 100g in spleen **(4).** **Distribution - oral exposure** No study of HCN distribution in a human body after oral exposure is available. In rats administered NaCN solution, CN doses 7 or 21 mg/kg bw (death after 10 or 3.3 minutes) no differences in cyanide concentrations in various body tissues, which would depend on CN dose, were observed. Average concentrations of cyanides in tissues in both groups of rats were 5.85µg/g of wet weight of the organ in lungs, 1.91 µg/ml of blood, 8.9µg/g of wet weight in liver, 1.52µg/g of wet weight in brain, and 2.1µg/g of wet weight in spleen (3). **Distribution - dermal exposure** No study of HCN distribution in a human body after dermal exposure is available. In six rabbits exposed through their skin (the skin surface is not known) to 33.75mg cyanides (in the form of HCN, approx. 5 LD50), the following levels were measured in blood and blood serum: 310 and 144µg/dl, and the following levels in tissues (in µg/100g): 26 in liver, 66 in kidneys, 97 in brain, 110 in heart, 120 in lungs, and 21 in spleen. The cyanide levels were measured immediately after the rabbits died (4).	
	Metabolism and excretion Although cyanide can interact with substances such as methaemoglobin in the bloodstream, the majority of cyanide metabolism occurs within the tissues. Cyanide is metabolized in mammalian systems by one major route and several minor routes. The major route of metabolism for hydrogen cyanide and cyanides is detoxification in the liver by the mitochondrial enzyme rhodanese, which catalyses the transfer of the sulfane sulphur of thiosulfate to the cyanide ion to form thiocyanate.(**Figure; adapted from (8)**). About 80% of cyanide is detoxified by this route. The rate-limiting step is the amount of thiosulfate. While rhodanese is present in the mitochondria of all tissues, the species and tissue distributions of rhodanese are highly variable. In general, the highest concentrations of rhodanese are found in the liver, kidney, brain, and muscle, but the supply of thiosulfate is limited **(11).** Rhodanese is present in rat nasal mucosal tissues, particularly in the olfactory region, at a 7-fold higher concentration (on a per milligram of mitochondrial protein basis) than in the liver (**12**). Dogs have a lower overall activity of rhodanese than monkeys, rats, and rabbits. A number of other sulfur transferases can also metabolize cyanide, and albumin, which carries elemental sulfur in the body in the sulfane form, can assist in the catalysis of cyanide to thiocyanate as well (**13**) . Cyanide and thiocyanate can also be metabolized by several minor routes, including the combination of cyanide with hydroxycobalamin (vitamin B12a) to yield cyanocobalamin (vitamin B12) **(14)** and the non-enzymatic combination of cyanide with cystine, forming 2-	

	iminothiazoline-4-carboxylic acid, which appears to be excreted without further change (**15) (see Figure).** In studies with rats orally administered potassium cyanide and maintained for up to 4 weeks on either a balanced diet or a diet lacking the sulfur amino acids L-cystine and L-methionine, a strongly positive linear relationship was found between blood cyanide and plasma cyanate (OCN–) concentration **(16).** It was suggested that in Africa, where there are protein-deficient populations whose levels of sulfurcontaining amino acids are low, cyanide (from prolonged use of cassava) may conceivably be converted to cyanate, which is known to cause neurodegenerative disease in humans and animals. While absorbed cyanide is principally excreted as thiocyanate in the urine, traces of free hydrogen cyanide may also be excreted unchanged in the lungs, saliva, sweat, or urine, as carbon dioxide in expired air, or as β-thiocyanoalanine in saliva and sweat **(17).** Thiocyanate was found in the urine of non-exposed people at average concentrations of 2.16 mg/litre urine for non-smokers and 3.2 mg/litre urine for smokers **(7).** Urinary excretion of thiocyanate was monitored in a man after ingestion of about 3–5 g potassium cyanide (15–25 mg cyanide/kg body weight). The results indicated that the patient excreted 237 mg of thiocyanate over a 72-h period. This quantity was substantially more than the normal average amount of thiocyanate in urine, which varies from 0.85 to 14 mg/24 h. The limiting factor in cyanide metabolism is the low concentration of the sulfur-containing substrates in the body — primarily thiosulfate, but also cystine and cysteine. The rate of spontaneous detoxification of cyanide in humans is about 1 µg/kg body weight per minute **(9),** which is considerably slower than in small rodents **(18)** or dogs (**19**). After administration of an intravenous dose of 3– 4 mg potassium cyanide to beagle dogs, blood levels decreased in a manner consistent with first-order elimination kinetics for the first 80 min. **(20).** The half-time for this phase was about 24 min, corresponding to an elimination rate constant of 0.03/min. After 80 min, the blood cyanide concentrations fell at a slower rate, with a half-time of 5.5 h. In rats, after a single oral dose, the blood elimination halftime of cyanide was 14.1 min, corresponding to a rate constant of 0.05/min. **(21)** Rats treated orally with 2 mg cyanide/kg body weight excreted 47% of the dose in the urine within 24 h. A [14C] cyanide intake study with rats (exposed to a regular intake of cyanide in the diet for 3 weeks) indicated the existence of a gastrointestinal circulation of thiocyanate, in which a substantial amount of thiocyanate, which was excreted into the stomach contents of the rat, was reabsorbed by the intestine into the body fluid, to be partly excreted in the urine and partly resecreted into the gastric contents. The relative proportion of cyanide to thiocyanate in body fluids is about 1:1000. The half-time for hydrogen cyanide elimination is about 1 h. **(8)** Half-time values of the principal metabolite thiocyanate in humans have been reported as 4 h (**22**), 2 days (**23**), and 27 days (**10**). In patients with renal insufficiency, a mean half-time of 9 days was reported. (**23**) Metabolism of cyanides includes also other sulphur transferases. Further metabolic processes of cyanides taking place in mammal organism can be seen in the following picture taken from literature **(8).**	
Distribution		
Metabolism and excretion		

Figure: Basic processes of cyanide metabolism



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

Section A6.2 Annex Point IIA VI.6.2		METABOLISM STUDIES IN MAMMALS. BASIC TOXICOKINETICS, INCLUDING A DERMAL ABSORPTION STUDY	
		Information On Dermal Absorption	
		1 REFERENCE	**Official use only**
1.1	**Reference**	D.C.Walton, M.G.Witherspoon, 1925, Skin Absorption of Certain Gases, Medical Research Division, Edgewood Arsenal, Received for Publication May 15, 1925 **(DOC IV_25)**	
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 guidelines available	
2.2	**GLP**	No (GLP was not compulsory at the time the study was performed)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	HCN vapours	
3.1.1	Lot/Batch number	Not reported	
3.1.2	Specification	Not reported	
3.1.2.1	**Description**		
3.1.2.2	**Purity**	97% of the liquid HCN (impurity – water in the liquid HCN)	
3.1.2.3	**Stability**	Not reported	
3.1.2.4	**Radiolabelling**	No	
3.2	**Test Animals**		
3.2.1	Species	Guinea Pig Dog	
3.2.2	Strain	Not reported	
3.2.3	Source	Not reported	
3.2.4	Sex	Not reported	
3.2.5	Age/weight at study initiation	Not reported	
3.2.6	Number of animals per group	8 guinea pigs (total number) 11 dogs (total number)	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Dermal Inhalation of HCN vapours excluded.	

3.3.1	Preparation of test site	Shaving of hair on the abdomen – 24 hours before the experiment Dogs were lightly morphinised 30 min before exposure	
3.3.2	Concentration of test substance	Guinea pigs: saturated vapours Dogs: 5.5 – 16.9 mg/l	
3.3.3	Specific activity of test substance		
3.3.4	Volume applied	Not relevant	
3.3.5	Size of test site	Guinea pigs: 5.06 cm^2 Dogs: whole body excl. head, abdomen shaved in all but two animals	
3.3.6	Exposure period	Guinea pigs: 7-8 minutes Dogs: 30 – 180 minutes	
3.3.7	Sampling time	Samples of air for analysis from the exposure chamber for dogs were taken in 10 min intervals.	
3.3.8	Samples		
		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	**Guinea pigs** clinical symptoms – all animals, at 6 – 7 min: rapid respiration, general twitching of muscles, convulsions; death – all, at 7 – 8 min; autopsy results - all: only pink colour of lungs **Dogs** no clinical symptoms - 3 dogs (concentration 5.5 – 6.6 mg/l, exposure 30 – 180 minutes); clinical symptoms (twitching of muscles), no death –1 dog (10.9 mg/L, exposure 60 min), 1 dog (not shaved, 15.5 mg/L, 60 min) clinical symptoms, death 5 dogs (concentration 11.6 – 16.9 mg/l, exposure 47 – 105 minutes): twitching of face and throat muscles; entire body twitching; excessive salivation; slow, laboured and irregular respiration; gasping breathing; unconsciousness; absence of corneal reflex; death euthanasia - 1 dog with persisting paralysis (15.68 mg/l, 60 minutes) autopsy results - 6 dogs: pink, dry and collapsed lungs	
4.2	**Dermal irritation**	Not reported	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Non-guideline study. Shaved area of abdominal skin of 8 guinea pigs has been exposed to saturated vapours of HCN. Whole body (except head) exposure of 11 dogs to HCN vapours in concentrations of 5.5 to 16.9 mg/L air.	

5.2	**Results and discussion**	**Guinea pigs**: all exposed animals died at 7 -8 min; clinical symptoms of toxicity and autopsy results were the same in all animals. **Dogs** tolerated a concentration of 5.5 mg/l for up to 180 minutes without any ill effects. Clinical signs of toxicity (muscle twitching) appeared in animals exposed to HCN concentrations 10.9 mg/L and higher. At concentrations 11.6 mg/L and higher (concentration x time product values of 11 $g.h.m^{-3}$ and higher) 6 of 7 animals died (1 of them was euthanized). Protection of skin by hair in dogs seems to slightly enhance the tolerance. The human skin is quite unlike that of the dog (greater number of gland openings and protection of body by hair in dogs) so no unequivocal conclusions can be drawn as to the possible resistance of man to skin absorption of HCN. On the other hand, the observation that man without special protection of skin could tolerate a concentration of 11 mg/L for three hours shows that penetration through animal and human skin is of the same order.	
5.3	**Conclusion**	HCN gas passes through the uninjured skin of guinea pig and dog and can produce death of these animals at concentration x time product values of 11 $g.h.m^{-3}$ and higher.	
5.3.1	Reliability	3	
5.3.2	Deficiencies		

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