	and *Thiobacillus* (**12).** Some of these species, for example *Pseudomonas*, are capable of using the cyanide ion and thiocyanate as the sole source of carbon and nitrogen and therefore, are particularly effective at cyanide degradation. In fact, *Pseudomonas* is the basis of commercial applications for degrading the cyanide ion to ammonia and carbonate in waste waters generated in mining operations that use the cyanide ion to leach gold and other precious metals for low-grade ores (**12**). Recently reviewed the role of microbes in cyanide degradation (**14**) has categorized the microbial enzymes that use the cyanide ion as a substrate according to the following types of reactions: substitution/addition, hydrolysis, oxidation, and reduction. Sulfur transferases such as rhodanese are involved in substitution reactions that result in the conversion of the cyanide ion to the less toxic thiocyanate, whereas pyridoxal phosphate enzymes are involved in substitution/addition reactions that result in production of nitrile derivatives of amino acids. These organic nitriles may then be ultimately degraded via enzyme catalyzed hydrolysis to either the corresponding amino acid and ammonia (without formation of the free amide) or the carboxylic acid and ammonia (via formation of the free amide). The cyanide hydratase and cyanidase enzymes catalyze the hydrolysis of the cyanide ion to formamide or formic acid and ammonia, respectively. A strain of *Alcaligenes xylosoxidans subsp. denitrificans* has been found to effectively hydrolyze the cyanide ion concentrations up to 300 $mg.l^{-1}$ down to very low levels (0.01–0.02 $mg.l^{-1}$) and to be resistant to inactivation by chloride, sulfate, iodide, Fe^{+2}, Zn^{+2}, or Ni^{+2} at concentrations of 70 $mg.l^{-1}$ **(17).** Thus, these hydrolytic systems are some of the most promising for detoxification of cyanide-containing waste waters (**14**). A number of microbial systems have been identified that are capable of direct oxidation or reduction of the cyanide ion. *Bacillus pumulus*, *Pseudomonas fluorescens*, and *Pseudomonas paucimobili* have all been found to oxidize the cyanide ion to ammonia and carbon dioxide (**18**). In an aerobic batch bioreactor experiment, *Pseudomonas putida* was found to significantly degrade 4 mM sodium cyanide (cyanide concentration approximately 100 mg/L) to ammonia and carbon dioxide (**19**). Other evidence indicates that formamide and formate are additional transformation products in microbial oxidation of the cyanide ion by this species, inferring that there may be more than one pathway of cyanide biotransformation involved (**20, 21**). Several bacterial species have been identified that are capable of oxidative degradation of metallocyanides (**22**). The cyanide oxygenase system involved in this process offers a new technology for the treatment of metal cyanide wastes (**14**).	
Conclusions:	Conditions of degradation: pH = 7.5 – 8.2 Temperature: 10 – 20 °C Biodegradation is an important transformation process for cyanide in natural surface waters, and is dependent on such factors as cyanide concentrations, pH, temperature, availability of nutrients, and acclimation of microbes. Although the cyanide ion is toxic to microorganisms at concentrations as low as 5–10 mg/L, acclimation increases tolerance to this compound. A number of pure cultures of microorganisms degrade low concentrations of cyanide under both aerobic and anaerobic conditions. Mixed microorganisms in sewage sludge or activated sludge acclimated to cyanide also significantly biodegrade concentrations of 100 mg/L of most simple and complex cyanides. A number of microorganisms have been identified that are capable of utilizing the cyanide ion, cyanate, and thiocyanate, including species of the genera, *Actinomyces, Alcaligenes, Arthrobacter, Bacillus, Micrococcus, Neisseria, Paracoccus, Pseudomonas*, and *Thiobacillus*. Some of these species, e. g.	

	Pseudomonas, are capable of using the cyanide and thiocyanate as the sole source of carbon and nitrogen and therefore are particularly effective at cyanide degradation. Sulfur transferases, such as rhodanese, convert cyanide ion to the less toxic thiocyanate, whereas pyridoxal phosphate enzymes are involved in reactions that result in production of nitrile derivatives of amino acids. A strain of *Alcaligenes xylosoxidans subsp. denitrificans* has been found to effectively hydrolyze the cyanide ion concentrations up to 300 $mg.l^{-1}$ down to very low levels (0.01–0.02 $mg.l^{-1}$) and to be resistant to inactivation by chloride, sulphate, iodide, Fe^{+2}, Zn^{+2}, or Ni^{+2} at concentrations of 70 $mg.l^{-1}$. *Bacillus pumulus*, *Pseudomonas fluorescens*, and *Pseudomonas paucimobili* have all been found to oxidize the cyanide ion to ammonia and carbon dioxide. In an aerobic batch bioreactor experiment, *Pseudomonas putida* was found to significantly degrade 4 mM sodium cyanide (cyanide concentration approximately 100 mg/L) to ammonia and carbon dioxide.	
Undertaking of intended data submission	No studies are planned.	

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

Section A7.1.3 **Annex Point IIA VII.7.7**	**ADSORPTION/ DESORPTION SCREENING TEST**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
References:	**1.** JACC No 53, Cyanides of Hydrogen, Sodium and Potassium, 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)** 2. JACC No 53, Cyanides of Hydrogen, Sodium and Potassium, 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 II (**DOC IV_4**)	
Detailed justification:	The biocidal product URAGAN D2 is the same as the technical grade active substance. HCN is produced as liquid which is sorbed on surface of inert material. Boiling temperature of HCN in liquid state is 25.7 °C (78.3 °F). Due to the large surface of sorbed inert material, the evaporation is very fast. Therefore the active substance is gas only. No studies have been conducted to investigate adsorption and desorption to water/sediment systems of hydrogen cyanide. Hydrogen cyanide is not expected to be present in water/sediment environments because of its physical properties and use patterns (fumigant in enclosed spaces). Hydrogen cyanide is expected to have high mobility in soils. It is not expected to be adsorbed to suspended solids and sediment in water It is not strongly partitioned into the sediments or suspended adsorbents, primarily due to its high solubility in water.	
Undertaking of intended data submission [ ]	No studies are planned.	

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

Section A7.1.4.1 **Annex Point IIIA XII.2.1**	**Field study in accumulation in the sediment**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Reference:	1. JACC No 53, Cyanides of Hydrogen, Sodium and Potassium, 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)** 2. JACC No 53, Cyanides of Hydrogen, Sodium and Potassium, 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 II **(DOC IV_4)** 3. ATSDR (2004, Toxicological profile of cyanide) **(DOC IV_1)**	
Detailed justification:	Based on its physic-chemical properties log Kow = 0.66 (at 0 °C) (**3**), log Kow = -0.25 (at 20 °C) (**1**), solubility in water, HCN is not expected to bioaccumulate in aquatic organisms. Hydrogen cyanide is not expected to be present in water/sediment environments because of its physical properties and use patterns (fumigant in enclosed spaces). Hydrogen cyanide is a gas under all environmental conditions. Since hydrogen cyanide will not reside in the sediment, a field study is not applicable and consequently has not been performed.	
Undertaking of intended data submission	No studies are planned	

	Evaluation by Competent Authorities
Date	
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Conclusion	
Remarks	

Section A7.2 **Annex Point IIIA XII.1**	**Fate and Behaviour in Soil**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
Reference:	1. JACC No 53, Cyanides of Hydrogen, Sodium and Potassium, 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)** 2. JACC No 53, Cyanides of Hydrogen, Sodium and Potassium, 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 II **(DOC IV_4)**	
Detailed justification:	Analogous to the fate of cyanides in water, it is predicted that the fate of cyanides in soil would be dependent on cyanide concentrations, pH, temperature, metal content, concentration of microorganisms, availability of nutrients, and acclimation of microbes. Cyanide may occur as hydrogen cyanide, alkali metal salts, or as immobile metallocyanide complexes. In soil, cyanide present at low concentrations would biodegrade under aerobic conditions with the initial formation of ammonia, which would be converted to nitrite and nitrate in the presence of nitrifying bacteria. With respect to the intended use, the substance is not expected to come into contact with soil.	
Undertaking of intended data submission	No studies are planned.	

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

Section A7.3.1 **Annex Point IIIA VII.5**	**Phototransformation in Air**	
References:	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 (state in February 2006): Hydrogen cyanide *Peer reviewed***(DOC IV_2).** 1. JACC No 53, Cyanides of Hydrogen, Sodium and Potassium, 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)** 2. JACC No 53, Cyanides of Hydrogen, Sodium and Potassium, 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 II **(DOC IV_4)** Supplement literature from ATDRS,2004: 3. EPA. 1984a. Health effects assessment for cyanide. Washington, DC: U.S. Environmental Protection Agency. EPA540186011. 4. EPA. 1979. Cyanides. In: Water-related environmental fate of 129 priority pollutants. Vol. 1. Washington, DC: U.S. Environmental Protection Agency, Office of Water Planning and Standards, Office of Water and Waste Management. EPA440479029a. PB80204373. 12-1-12-12. 5. Cicerone, R.J., Zellner R. 1983. The atmospheric chemistry of hydrogen cyanide (HCN). J Geophys Res 88:10689-10696. 6. Fritz B, Lorenz K, Steinert W, et al. 1982. Laboratory kinetic investigation of the tropospheric oxidation of selected industrial emissions. EUR 7624:192-202 7. Lyman, W. 1982. Atmospheric residence time. In: Lyman, WJ, Reehl, WF, Rosenblatt DH, eds. Handbook of chemical property estimation methods: Environmental behavior of organic compounds. New York, NY: McGraw Hill Book Company, 10-2-10-33. 8. Frank SN, Bard AJ. 1977. Heterogeneous photocatalyst oxidation of cyanide ion in aqueous solutions at titanium dioxide powder. J Amer Chem Soc 99(1):303-304.	
Detailed justification:	Most cyanide in the atmosphere exists almost entirely as hydrogen cyanide gas, although small amounts of metal cyanides may be present as particulate matter in the air **(3)**. Hydrogen cyanide is very resistant to photolysis at wavelengths of normal sunlight **(4)**. The most important reaction of hydrogen cyanide in air is the reaction with photochemically generated hydroxyl radicals and subsequent rapid oxidation to carbon monoxide (CO) and nitric oxide (NO); photolysis and reaction with ozone are not important transformation processes, and reaction with singlet oxygen (O 1D) is not a significant transformation process except at stratospheric altitudes where singlet oxygen is present in significant concentrations (**5**). The rate of hydroxyl radical reaction with hydrogen cyanide in the atmosphere depends on the altitude, and the rate of the reaction is at least an order of magnitude faster at lower tropospheric altitudes (0–8 km) than at upper tropospheric altitudes (10–12 km) **(5).**	

	Based on a reaction rate constant of $3x10^{-14}$ cm^3 .(molecule-sec)$^{-1}$ at 25 °C) (**6**) and assuming an average hydroxyl radical concentration of $5x10^5$ molecules.cm^{-3}, the residence time for the reaction of hydrogen cyanide vapour with hydroxyl radicals in the atmosphere is 2 years. This value compares well with the atmospheric residence time derived by **(5)** of approximately 2.5 years, with a range of 1.3–5.0 years, depending on the hydroxyl radical concentrations assumed. Using the equation t½ = 0.693. for converting residence time to half-life (t½) (**7**) and an estimated atmospheric residence time for hydrogen cyanide of 2–3 years, and assuming first-order kinetics for the reaction of hydrogen cyanide with hydroxyl radicals, an atmospheric half-life of 1.4–2.9 years can be calculated for hydrogen cyanide. The relatively slow rate of degradation of hydrogen cyanide suggests that this compound has the potential to be transported over long distances before being removed by physical or chemical processes. Since hydrogen cyanide is miscible in water, it appears that wet deposition may be an important fate process. In the presence of titanium dioxide powder, photo catalytic oxidation of cyanide proceeds at significant rates in both high intensity artificial sunlight and unfocused sunlight. With titanium dioxide powder present, more than 99% of a 1 mM (26 mg/l) solution of cyanide ion was oxidized by exposure to sunlight for two days. In the absence of titanium dioxide powder, little or no oxidation occurred (**8**). **Conclusion**: The most important reaction of hydrogen cyanide in air is the reaction with photochemically generated hydroxyl radicals and subsequent rapid oxidation to carbon monoxide (CO) and nitric oxide (NO); photolysis and reaction with ozone are not important transformation processes, and reaction with singlet oxygen (O 1D) is not a significant transformation process except at stratospheric altitudes where singlet oxygen is present in significant concentrations. Assuming an average hydroxyl radical concentration of $5x10^5$ molecules.cm^{-3}, the residence time for the reaction of hydrogen cyanide vapour with hydroxyl radicals in the atmosphere is 2 years. This compound has the potential to be transported over long distances before being removed by physical or chemical processes.	
Undertaking of intended data submission	No studies are planned.	

	Evaluation by Competent Authorities
Date	
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Conclusion	
Remarks	

Section A7.4.1.1 **Annex Point IIA VII.7.1**		**ACUTE TOXICITY TO FISH**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Ambient Water Quality Criteria for Cyanides; US EPA (1980). 440/5-80-037 (published). (**DOC IV_69**)	
1.2	**Data protection**	No	
1.2.1	Data owner	Unrestricted data	
1.2.2	Companies with letter of access	No companies with letter of access	
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 presented	
2.2	**GLP**	No	
2.3	**Deviations**	Not available	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Hydrogen cyanide	
3.1.1	Lot/Batch number	Not relevant.	
3.1.2	Purity		
3.1.3	Further relevant properties	Water solubility, vapour pressure, chemical stability, dissociation constant, and biodegradability: see: Physical and chemical properties	
3.1.4	Method of analysis	Various methods	
3.2	**Preparation of TS solution**		
3.3	**Ref.substance**	No	
3.4	**Testing procedure**		
3.4.1	Test organisms	Goldfish, *Carassius auratus* Fathead minnow, *Pimephales promelas* Largemoufh bass, *Micropterus salmonides* Black crapple, *Pomoxis nigromaculatus* Bluegill, *Lepomios macrochirus* Yellow perch, *Perca flavescens* Brook trout, *Salvelinus fontinalis* Oncorhynchus mykiss, *Salmo gairdner* Atlantic salmon, *Salmo salar*	
3.4.2	Test system		
3.4.3	Test conditions		
3.4.4	Duration of the test	96 hours	
3.4.5	Test parameter	Mortality	
3.4.6	Statistics		

		4 RESULTS	
4.1	**Results test substance**		
4.1.1	Effect data	See 5.2.1	
4.1.2	Other effects	No	
4.2	**Results of controls**		
4.2.1	Number / % of animals showing adverse effects		
4.3	**Test with ref. substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**		
5.2	**Results and discussion**	Free cyanide concentrations from about 0.050 to 0.200 mg/L eventually were fatal to juveniles of most of the more sensitive fish species, with concentrations much above 0.200 mg/L being rapidly fatal to most juvenile fish. Thus, there is a relatively narrow range of species sensitivity for fish. Certain life stages and species of fish appear to be more sensitive to cyanide than others. Embryos, sac fry, and warm water species tended to be the most resistant.	
5.2.1	LC_{50}	Goldfish, *Carassius auratus* 0.318 mg/l Fathead minnow, *Pimephales promelas* 0.1251 mg/l Largemoufh bass, *Micropterus salmonides* 0.102 mg/l Black crapple, *Pomoxis nigromaculatus* 0.102 mg/l Bluegill, *Lepomios macrochirus* 0.0993 mg/l Yellow perch, *Perca flavescens* 0.0926 mg/l Brook trout, *Salvelinus fontinalis* 0.0858 mg/l Oncorhynchus mykiss, *Salmo gairdner* 0.0447 mg/l Atlantic salmon, *Salmo salar* 0.090 mg/l	
5.3	**Conclusion**	The toxicity of cyanide increases with reduction in dissolved oxygen below the saturation and the resistance of fishes to cyanide solutions that are rapidly lethal decreases with increase in temperature.	
5.3.1	Reliability	3	
5.3.2	Deficiencies	None	

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	
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Remarks	

Section A7.4.1.1 **Annex Point IIA VII.7.1**		**ACUTE TOXICITY TO FISH**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Kovacs T. G., and G. Leduc. 1982. Acute toxicity of cyanide to rainbow trout (Salmo gairdneri) acclimated at different temperatures. Can. J. Fish. Aquat. Sci. 39: 1426-1429. (**DOC IV_70**)	
1.2	**Data protection**	No	
1.2.1	Data owner	Unrestricted data	
1.2.2	Companies with letter of access	n/a	
1.2.3	Criteria for data protection	/	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No presented	
2.2	**GLP**	Not reported, but performed (1971) by the prestigious US leading public associations using standardized methods.	
2.3	**Deviations**	Not available	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Hydrogen cyanide	
3.1.1	Lot/Batch number	Not relevant.	
3.1.2	Purity		
3.1.3	Further relevant properties	Water solubility, vapour pressure, chemica stability, dissociation constant, and biodegradability: see Physical and chemical properties	
3.1.4	Method of analysis	Temperature effects on cyanide toxicity.	
3.2	**Preparation of TS solution**		
3.3	**Reference substance**	No	
3.4	**Testing procedure**	Rainbow trout purchased form La Pisciculture, Mt. Sutton, Sutton (Qué.), were held in 200-L fiberglass oval-shaped tanks at a temperature of 12 ± 1°C. Chemical characteristics of the dechlorinated Montreal city water used during the experiments were as follows; alkalinity 87 $mg.L^{-1}$ as $CaCO_3$; hardness 127 $mg.L^{-1}$ as $CaCO_3$; CO_2 0.47 $mg.L^{-1}$. See Table A7.4.1.1-1 for oxygen and pH levels. After at least 2-week acclimation in the 200-L holding tanks at 12 °C, the fish to be tested at 6 and 18 °C were segregated and acclimated to the desired water temperatures by gradual adjustments of about 1 $°C.d^{-1}$. The fish were then held for 7 d at these temperatures, prior to transfer into the white polyethylene (68 x 57.5 x 42 cm) test tanks, where they were held for further 2 week at the test temperatures (i.e. acclimated for 3 week at test temperatures). The tanks were connected to a flow-through system provided with thermally controlled water, at a flow of 1 $L.min.^{-1}$ in each tank, giving 99% replacement in 6 h. The entire 24-tank assembly was illuminated evenly by fluorescent light, controlled by a time switch to	

		provide a 12 h photoperiod (8:30-20:30). During this period the fish were fed daily but no food was given for 48 h before the tests.	
3.4.1	Dilution water	Dechlorinated Montreal city water	
3.4.2	Test organisms	Oncorhynchus mykiss, *Salmo gairdner*	
3.4.3	Test system	Flow-through	
3.4.4	Test conditions	The acute toxicity of HCN to rainbow trout at 6, 12, and 18°C. At hour zero a calculated amount of cyanide stock solution was mixed into each tank to produce immediately the predicted concentrations which were then maintained by Mariotte bottles. Cyanide was monitored in the test tanks twice daily. The measured concentrations never differed by more than 1% of the predicted values. The concentrations tested (mg $HCN.L^{-1}$) ranged from 0.018 to 0.056 at 6°C, 0.032 to 0.087 at 12°C, and 0.042 to 0.087 at 18°C. These ranges were selected from preliminary tests and included concentrations causing zero and 100% mortality. Control groups were maintained at each temperature under identical conditions.	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality	
3.4.7	Monitoring of TS concentration	Yes 2/day	
3.4.8	Statistics		
		4 RESULTS	
4.1	**Results test substance**		
4.1.1	Effect data	See **Table 1**	
4.1.2	Other effects	No	
4.2	**Results of controls**		
4.2.1	Number/ percentage of animals showing adverse effects		
4.3	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The acute toxicity tests were carried out to determine the 96 hr LC_{50} of HCN to rainbow trout at three temperatures, 6, 12 and 18 °C. The bioassays were carried out following standard methods. The fish weighed 9-18 g with a mean weight of 12 g, they were randomly chosen, acclimated as described above, and segregated for each test concentration and control group. On the day the bioassays began, the tanks were cleaned and a calculated amount of cyanide stock solution was mixed into each tank to immediately produce the predicted concentration. This was considered to be zero hour, and the desired concentrations were then maintained by metering cyanide stock solutions from Mariotte bottles.	

		Cyanide was monitored in the test tanks twice daily and the cyanide flows were adjusted when necessary. The desired concentrations never varied by more then one percent of the predicted values. The concentrations tested (mg $HCN.l^{-1}$) ranged from 0.018 to 0.056 at 6 °C, 0.032 to 0.087 at 12 °C, and 0.042 to 0.087 at 18 °C. These ranges were selected empirically such that the approximate 96 hr LC_{50} values, determined by preliminary screening bioassays would be close to the median of the eight concentrations tested and included concentrations causing zero and 100% mortality. Control groups were maintained at each temperature under identical conditions. Observations on mortality were taken from hour on up to 96 hours, at logarithmically spaced intervals. Hower, additional incidental observations were made after 48 hours to obtain additional information on mortality. The criterion of death was absence of respiratory movements and lack of response to probing with a glass rod upon which the fish were removed, weighed and their fork length measured.	
5.2	**Results and discussion**	The resistance of juvenile rainbow trout to cyanide was markedly affected by temperature (**Table 1**) and each 96-h LC_{50} value differed significantly from those obtained at other temperatures. The cyanide level required to kill 50 % of the trout in 96 h was 2.4 times lower at 6 than at 18°C. The median survival times (MST) for each test concentration was estimated. Eye–fitted toxicity curves were constructed over cyanide concentrations ranging from 0.028 to 1.0 $mg.L^{-1}$, using data from this study and comparable data form the literature. At concentrations below 0.10 mg $HCN.L^{-1}$, the MST decreased at a lower temperature (**Fig. 1**). The lethal thresold was reached in 96 h at 18 °C but not at 12 and 6 °C. As the concentrations tested increased, the difference in the MST at each temperature became smaller (**Fig.1**). The curves intersect near 0.1 mg $HCN.L^{-1}$ above which the temperature effect on the lethal action of cyanide to trout is reversed. Thus at more rapidly lethal concentrations cyanide becomes more toxic at 18 °C than at 12 and 6 °C.	
5.2.1	LC_{50}	Oncorhynchus mykiss, *Salmo gairdner* LC50 = 0.028 ± 0.004 $mg.l^{-1}$ at 6 °C LC50 = 0.042 ± 0.004 $mg.l^{-1}$ at 12 °C LC50 = 0.068 ± 0.004 $mg.l^{-1}$ at 18 °C	
5.3	**Conclusion**	Temperature and HCN concentration effect on acute toxicity is documented. LC50 values from the study conclusions: LC50 = 0.028 ± 0.004 $mg.l^{-1}$ at 6 °C LC50 = 0.042 ± 0.004 $mg.l^{-1}$ at 12 °C LC50 = 0.068 ± 0.004 $mg.l^{-1}$ at 18 °C Warm acclimated rainbow trout survived longer in lethal concentrations of cyanide. Toxicity curves clearly showed the temperature effect on the acute toxicity of cyanide is concentration dependent. At slowly lethal concentrations, cyanide is more toxic at lower temperatures, whereas at rapidly lethal levels the reverse occurs; the reversal takes place at 0.10 mg $HCN.L^{-1}$.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	None	

Table 1: Acute toxicity of hydrogen cyanide to rainbow trouth acclimated and tested at different temperatures in flow-through system for 96 h

	6 °C	**12 °C**	**18 °C**
96-h LC50 mg.L^{-1} HCN (95% confidence interval) Slope function	0.028 (0.024-0.035) 1.30	0.042 (0.038-0.046) 1.30	0.068 (0.064-0.072) 1.12
Highest cyanide conc. with no mortality in 96 h. mg HCN.L^{-1}	0.018	0.032	0.060
Lowest cyanide conc. with 100% mortality in 96 h. mg HCN.L^{-1}	0.037	0.053	0.087
Average weight of fish (range). g.	11.79 (9.05-15.98)	12.41 (8.29-16.02)	13.19 (9.63-17.21)
Average fork length of fish (range). mm	102 (95-110)	102 (90-111)	106 (95-119)
Mean pH (range)	8.06 (7.9-8.1)	8.10 (8.06-8.11)	7.82 (7.78-7.90)
Mean oxygen saturation (range) %	89.2 (88.2-90.1)	97.1 (95.2-98.5)	86.8 (85.7-88.3)

Fig. 1: Toxicity of HCN to rainbow trout at three temperatures

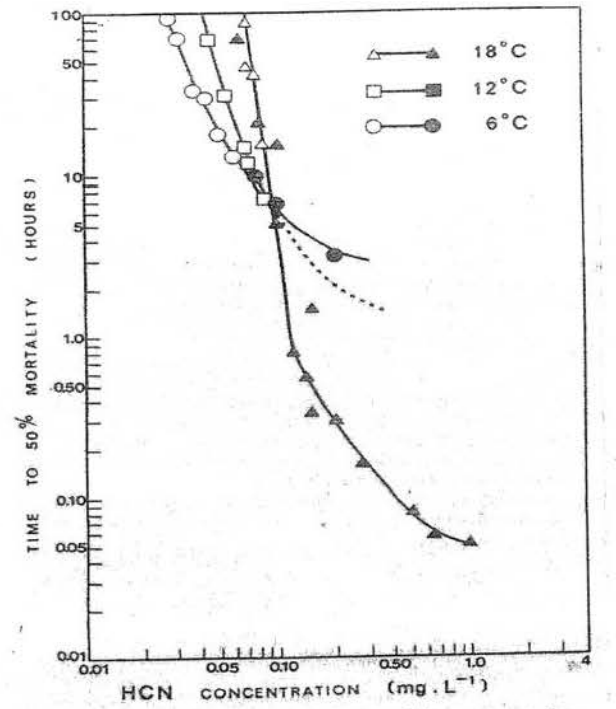

FIG. 1. Toxicity of hydrogen cyanide to rainbow trout at three temperatures: this study (open symbols); Herbert and Merkens (1952) (closed triangles); Ministry of Technology (1968) (closed circles).

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

Section A7.4.1.1 **Annex Point IIA VII.7.1**		**ACUTE TOXICITY TO FISH**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Smith L.L, Broderius S.J., Osied D.M., Kimbal G.L., Koenst W.M., Acute Toxicity of Hydrogen Cyanide to Freshwater Fishes, Paper No. 9954, under Grant No. R802914 (**DOC IV_71**)	
1.2	**Data protection**	No	
1.2.1	Data owner	Unrestricted data	
1.2.2	Companies with letter of access	No companies with letter of access	
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. Spraque, J.B.: Measurement of pollutant toxicity to fish. 1. Bioassay method for acute toxicity,	
2.2	**GLP**	Not reported, but performed (1971) by the prestigious US leading public associations using standardized methods.	
2.3	**Deviations**	Not available	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Hydrogen cyanide	
3.1.1	Lot/Batch number	Not relevant. Instead of initial identity of tested material parallel analyses during tests.	
3.1.2	Purity		
3.1.3	Further relevant properties	Water solubility, vapour pressure, chemical stability, dissociation constant, and biodegradability: see: Physical and chemical properties	
3.1.4	Method of analysis	Free cyanide concentration in each chamber was determined daily by the spectrophotometric method according to Epstein with calculated HCN concentrations based on corresponding pH and temperature measurements and using dissociation constants of molecular HCN. This method has been used as standard method for examination of water by the renowned US leading public associations.	
3.2	**Preparation of TS solution**	Sodium cyanide stock solutions were adjusted to pH 11 with NaOH. Test water adjusted to appropriate temperature and oxygen was coveyed by gravity from elevated head tanks to the diluter apparatus. The test substance was delivered to test chambers from intermitten – flow diluters modified.	
3.3	**Reference substance**	No	
3.4	**Testing procedure**		
3.4.1	Dilution water	Water from a deep well - see **Table 1**	
3.4.2	Test organisms	See **Table 2** Fathead minnow, *Pimephales promelas* (Rafinesque) Bluegill, *Lepomis macrochirus* (Rafinesque)	

		Oncorhynchus mykiss, *Salmo gairdneri* (Richardson).	
3.4.3	Test system	Flow-through - see **Table 3**	
3.4.4	Test conditions	See **Table 4**	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality	
3.4.7	Monitoring of TS concentration	All reported concentrations of test substance were based on on analyses made on water from the test chamber.	
3.4.8	Statistics	The concentration – percent mortality data were analysed with a logarithmic – probability (log-probit) program. LC50 and medial lethal threshold concentrations (TLC) were calculated.	
		4 RESULTS	
4.1	**Results test substance**		
4.1.1	Effect data (Mortality)	See **Tables 5-10**	
4.1.2	Other effects	No	
4.2	**Results of controls**		
4.2.1	Number/ percentage of animals showing adverse effects		
4.3	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Different species used for acute tests were eggs, fry and juveniles. Eggs and fry were randomly placed in test chambers of 20 l of test solution; eggs and fry were tested on screen bottomed acrylic cylinders covered with bakelite lid and held in 20 l chamber. Sodium cyanide from the stock solution was delivered to test chambers from the interminent-flow diluters. NaCN hydrolyzes to form free cyanide – CN^- ion and molecular HCN. At pH 6.0 – 8.0 in most natural water predominantes the molecular (un-ionized) HCN, with less than 6 % free cyanide occuring in the ionic form below pH 8 at 25 °C. Eggs and fry were tested immediately on introduction to the test chamebrs; juveniles were held in test chambers for three days prior to HCN expose. Free cyanide concentrations in each chamber were determined daily. Observations on mortality were made daily. Acute toxicity of hydrogen cyanide was determined at temperatures from 4 °C to 30 °C and oxygen concentrations of 3.36 to 9.26 mg. l^{-1} on different species life history stages, with single water source and using uniform test procedures. 337 acute toxicity tests were designed to determine the 96-hr median lethal HCN concentration (LC_{50}) and the median lethal threshold concentration (LTC).	
5.2	**Results and discussion**	Acute toxicity varied from 0.057 mg. l^{-1} for juvenile rainbow trout to 0.191 mg. l^{-1} for field stocks of juvenile fathead minnows. Juvenile fish	

		were more sensitive at lower temperatures and at oxygen levels below 5 mg. l^{-1}. The difference in median lethal concentration between field stock fathead minnows, the most resistant species tested, and rainbow trout juveniles was approximately threefold. Eggs of all species tested were the most resistant life history stage. Slope of the log-probit toxicity curves is smallest for egg tests and increases to that for juveniles one.	
5.2.1	LC_{50}	Fathead minnow eggs : from 0.121 to 0.352 $mg.l^{-1}$ Fathead minnow swim-up fry: from 0.081 to 0.122 $mg.l^{-1}$ Fathead minnow juveniles: from 0.082 to 0.137 $mg.l^{-1}$ Bluegill eggs: from 0.580 to 0.935 $mg.l^{-1}$ (expresed as LTC) Bluegill swim-up fry: from 0.232 to 0.371 $mg.l^{-1}$ Bluegill juveniles: from 0.075 to 0.125 $mg.l^{-1}$ Oncorhynchus mykiss: **0.0572 $mg.l^{-1}$** (95% confidence limit was 0.0557 – 0.0587 $mg.l^{-1}$).	
5.3	**Conclusion**	For most species juveniles were most sensitive and eggs more resistant to HCN. Temperature has a marked effect on acute toxicity of HCN with juvenile fish in general becoming more sensitive at lower temperatures Oxygen below 5 $mg.l^{-1}$ result in increased sensitivity for all juveniles tested.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	None	

Table 1: Dilution water

Criteria	Details
Source	Water from a deep well was transmited to experiments through polyvinyl chloride pipe.
Alkalinity	approximately 235 $mg.l^{-1}$ as $CaCO_3$
Hardness	approximately 220 $mg.l^{-1}$ as $CaCO_3$
pH	6 to 8
Conductance	
Holding water different from dilution water	No

Table 2: Test organisms

Criteria	Details
Species/strain	1. Fathead minnow, *Pimephales promelas* (Rafinesque) 2. Bluegill, *Lepomis macrochirus* (Rafinesque) 3. Oncorhynchus mykiss, *Salmo gairdneri* (Richardson).
Source	1. Fathead minnow were cultured in test laboratory from brood stock originally obtained from the U.S. EPA´s ERL, Duluth. Juvenile wild-stock fatheads were collected from Como Lake in St. Paul. 2. Bluegill was obtained from wild stock with eggs spawned and fry hatched in the laboratory. Juvenile bluegills were collected from local waters. 3. Oncorhynchus mykiss were obtained as newly hardened eggs or as 24-hr fry from state hatcheries.
Age/size	1. Sac fry, length 5-6 mm; Swim-up, length 5-6 mm, Juveniles, length 26-45 mm 2. Sac fry, length 4 mm; Swim-up, length 4 mm, Juveniles, length 13-28 mm 3. Juveniles, length 40-68 mm
Pretreatment	Juveniles held at test conditions for seven days before being placed in test chambers. Juveniles from the field were given prophylactic treatment with neomycin and tetracycline at 20 mg. l^{-1} for 4-hr periods on three consecutive days.
Feeding of animals during test	No

Table 3: Test system

Criteria	Details
Test type	Flow-through
Volume of test vessels	Test chambers for juveniles and trout swim-up were glass aquaria 50x24x20 cc high filled to 20 l of test solution. Eggs and fry were tested in screen bottomed acrylic cylinders each covered with a bakelite lid and held in a 20l chamber - the portion of water from each cycle flowed upward through the screen to the outlet.
Volume/animal	
Number of animals/vessel	1. 25-50 eggs; 25 sac fry; 25 swim-up fry; 10 juveniles 2. 25-50 eggs; 10-50 sac fry; 10-50 swim-up fry; 10-20 juveniles 3. 10 juveniles
Number of vessels/ concentration	1

Table 4: Test conditions

Criteria	Details
Test temperature	15 – 25°C
Dissolved oxygen	3 – 7 $mg.l^{-1}$ See respective tests.
pH	about 7, see respective tests
Photoperiod	All tests were conducted under two fluorescent lamps (Luxor)

Table 5: Acute toxicity of HCN to fathead minnow eggs expressed as 96-hr LC_{50} and median lethal concentrations at hatching

°C	DO $mg.l^{-1}$	pH	96-hr LC_{50} $mg.\ l^{-1}$	95% Confidence limits	Hatch LC_{50} $mg.\ l^{-1}$	99% Confidence limits
15.2	6.36	7.86	0.352	274-453	0.126	90.9-174
20.0	6.13	7.88	0.273	162-463	0.118	97.3-142
24.9	3.51	7.72	0.202	130-314	0.116	86.6-157
24.8	4.46	7.95	0.121	77.3-190	0.113	83.0-154
25.0	5.52	7.90	0.184	115-293	0.180	122-266
25.0	6.34	8.00	0.196	140-274	0.162	135-193
24.9	7.25	7.99	0.202	-	0.187	-

Table 6: Acute toxicity of HCN to fathead minnow swim-up fry expressed as 96-hr LC_{50} and median lethal threshold concentrations

°C	DO $mg.l^{-1}$	pH	96-hr LC_{50} $mg.\ l^{-1}$	95% Confidence limits	LTC $mg.\ l^{-1}$	95% Confidence limits
15.0	6.38	7.86	0.122	104-143	0.102	92.8-113
20.0	6.14	7.89	0.0991	88.9-111	0.0961	83.6-110
24.6	3.77	7.84	0.0816	71.2-93.6	0.0816	71.2-93.6
24.7	5.14	7.96	0.108	90.3-130	0.108	90.3-130
24.9	6.17	8.02	0.113	96.5-133	0.113	96.5-133

Table 7: Acute toxicity of HCN to fathead minnow juveniles expressed as 96-hr LC_{50} and median lethal threshold concentrations

°C	DO $mg.l^{-1}$	pH	96-hr LC_{50} $mg.\ l^{-1}$	95% Confidence limits	LTC $mg.\ l^{-1}$	95% Confidence limits
15.0	6.07	7.86	0.121	116-125	0.119	115-123
20.0	3.58	7.70	0.128	109-149	0.123	105-143
19.8	4.68	7.80	0.0824	76.4-88.9	0.0824	76.4-88.9
20.0	5.20	7.78	0.125	117-133	0.123	116-132
20.0	6.07	7.91	0.137	122-153	0.137	122-153
20.0	7.13	7.90	0.131	124-138	0.131	124-138
24.8	3.58	7.75	0.106	87.9-129	0.106	87.9-129
25.0	5.08	7.83	0.119	111-129	0.119	111-129
25.1	6.13	7.98	0.129	124-133	0.129	124-133
25.2	7.04	7.96	0.120	113-128	0.120	113-138

Table 8: Acute toxicity of HCN to bluegill eggs and swim-up fry expressed as 96-hr LC_{50} and median lethal threshold of hatching concentrations

°C	DO $mg.l^{-1}$	pH	96-hr LC_{50} $mg.\ l^{-1}$	95% Confidence limits	LTC $mg.\ l^{-1}$	95% Confidence limits
Eggs						
25.2	3.39	7.70	-	-	0.690	461-1033
25.0	4.99	7.79	-	-	0.535	240-1192
25.1	6.09	7.92	-	-	0.693	572-841
25.0	6.90	7.90	-	-	0.580	343-980
Fry						
20.0	5.99	7.89	0.365	188-709	0.205	156-270
24.9	3.59	7.72	0.232	147-366	0.109	99.9-120
24.9	5.08	7.80	0.232	147-366	0.149	117-189
24.9	6.01	7.93	0.276	241-316	0.218	193-247
24.8	6.81	7.90	0.271	200-368	0.194	110-340

Table 9: Acute toxicity of HCN to bluegill juveniles expressed as 96-hr LC_{50} and median lethal threshold of hatching concentrations

°C	DO $mg.l^{-1}$	pH	96-hr LC_{50} $mg.\ l^{-1}$	95% Confidence limits	LTC $mg.\ l^{-1}$	95% Confidence limits
8.4	6.08	7.80	0.083	-	0.0617	59.3-64.2
9.7	8.35	7.94	<0.092	-	-	-
15.0	6.07	7.83	0.087	81.0-93.8	0.0871	81.0-93.8
15.1	7.03	7.92	0.075	66.0-85.2		
17.8	7.97	8.12	0.099	-		
20.0	6.06	7.86	0.108	103-112	0.108	103-112
25.1	3.48	7.71	0.0997	86.0-116	0.0997	86.0-116
25.0	5.05	7.78	0.113	55.8-227	0.113	55.8-227
24.9	6.17	7.92	0.120	109-133	0.120	109-133
24.9	6.90	7.86	0.125	115-135	0.125	115-135

Table 10: Acute toxicity of HCN to Oncorhynchus mykiss juveniles expressed as 96-hr LC_{50} and median lethal threshold concentrations

°C	DO $mg.l^{-1}$	pH	96-hr LC_{50} $mg.\ l^{-1}$	95% Confidence limits	LTC $mg.\ l^{-1}$	95% Confidence limits
10	8.80	7.80	0.0572	55.7-58.7	-	-

Table 11: Effect data

Parameter	96 h
LC_{50} $[mg.l^{-1}]$[1]	Fathead minnow eggs : from 0.121 to 0.352 mg. l^{-1} Fathead minnow swim-up fry: from 0.081 to 0.122 mg. l^{-1} Fathead minnow juveniles: from 0.082 to 0.137 mg. l^{-1} Bluegill eggs: from 0.580 to 0.935 mg. l^{-1} (expresed as LTC) Bluegill swim-up fry: from 0.232 to 0.371 mg. l^{-1} Bluegill juveniles: from 0.075 to 0.125 mg. l^{-1} Oncorhynchus mykiss: 57.2 µg. l^{-1} (95% confidence limit was 0.0557 – 0.0587 mg. l^{-1})
95 % c.l.	**See Tables: 5-10**

[1] Based on mean measured concentrations.

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

Section A7.4.1.2 **Annex Point IIA VII.7.2**		**ACUTE TOXICITY TO DAPHNIA MAGNA**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Hydrogen cyanide: An acute toxicity study with the daphnid *Daphnia magna Strauss*, Research Institute of Organic syntheses, Centre for ekotoxicology, toxicology an analytics, Pardubice – Rybitví, Czech Republic,Report No. 1514/L (unpublished), 2002-02-18 (**DOC IV_72**)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lučební závody Draslovka, a. s. Kolín	
1.2.2	Companies with letter of access	No companies with letter of acces.	
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**	Decree of Ministry of Environment No. 299 /1998 Coll., Patr 103, Method II – Acute toxicity to daphnia; OECD Test Guideline No. 202, 1984, EC Method C.2, Directive 67/548/EEC	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Hydrogen cyanide liquid stabilized	
3.1.1	Lot/Batch number	No. 15/02/22	
3.1.2	Purity	98.0 %	
3.1.3	Further relevant properties		
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Solubility in water: very good	
3.3	**Reference substance**	Yes, an acute toxicity test was carried out (Report No. 1514/L) using the reference substance potassium dichromate ($K_2Cr_2O_7$), Batch number K29239062, Certificate of analysis: 1.04862.1000 Potassium dichromate cryst. extra pure, Merck KgaA, 64271 Darmstadt.	
3.4	**Testing procedure**		
3.4.1	Dilution water	Reconstituted distilled water, see **Table 1**	
3.4.2	Test organisms	*Daphnia magna Strauss*, less than 24 hours old, see **Table 2**	
3.4.3	Test system	Static, see **Table 3**. In order to obtain information about the range of concentrations to be used in the main test a range-finding test was proceeded with the definitive test. For each test one control test (with the dilution water) was carried out. Base ("primary") solution of test substance: 15 $mg.l^{-1}$ of disstilled water. Test media of the required concentration for both the range finding test and the definitive test were prepared from the base solution by serial dilution with dilution water. 7 and 8 concentrations were studied in the range-finding test and the	

		defitive test, respectively. For the definitive test 20 animals at each test concentration divided into two batches of 10 animals each were studied.	
3.4.4	Test conditions	see **Table 4**	
3.4.5	Duration of the test	24 and 48 hours	
3.4.6	Test parameter	Immobility of animals	
3.4.7	Monitoring of TS concentration	Yes. At the beginning of the definitive test (before introduction of animals), and at the end of exposure period (after withdrawal of animals), the solutions of minimum and maximum substance concentrations were analysed by argentometric titration to confirm stability of the concentrations see **Table 5**. Test (1st run) and its duplicate (2nd run) has been performed.	
3.4.8	Statistics	EC_{50} values were calculated by the use of the computational program TOXICITA (VÚV, Ostrava).	
		4 RESULTS	
4.1	**Results test substance**		
4.1.1	Initial concentrations of test substance	1. Range – finding test concentrations in $mg.l^{-1}$: 2.1, 1.8, 1.5, 1.2, 0.9, 0.6, 0.3 2. Definitive test concentrations in $mg.l^{-1}$: 1.8, 1.65, 1.50, 1.35, 1.20, 1.05, 0.90, 0.75	
4.1.2	Effect data (Immobilization)	1. Range – finding test, see **Table 6** 2. Definitive test, see **Table 6**	
4.1.3	Other effects	No abnormalities were observed at any of the test concentrations	
4.2	**Results of controls**	No immobility occured in any control	
4.3	**Test with reference substance**	Sensitivity of the test organism and the accuracy of the test were regularly in a three month interval verified by test with reference substance, potassium dichromate. According to the results of ASLAB (2001) interlaboratory tests 48h-EC_{50} = 0.41 – 1.17 $mg.l^{-1}$: The results of the valid verified test for potassium dichromate carried-out on 12-21 Dec, 2001 are as follows: 24h-EC_{50} = 1.21 $mg.l^{-1}$ and 48h-EC_{50} = 0.93 $mg.l^{-1}$	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Hydrogen cyanide liquid stabilized was tested for acute toxicity to water fleas (*Daphnia magna)* according to OECD guideline No. 202 (1984). For a range-finding test a total of 140 young daphnid (less than 24 hours old) were exposed to seven concentrations and control. For a definitive test a total 320 young daphnid (less than 24 hours old) were exposed to eight concentrations, controls and replicates. Duration of exposure was 24 and 48 hours under static conditions. Test concentrations were verified by argentometric titration.	
5.2	**Results and discussion**	Test results have been obtained by the use of computation program TOXICITA, VÚV, Ostrava. Based on mean measured concentrations, EC_{50} values of 1.23 mg. l^{-1} and 1.07 $mg.l^{-1}$ at 24 and 48 hours respectively, were determined. See **Table 7.**	

		The NOEC was not determined.	
5.2.1	EC_{50}	24 h -EC_{50} = 1.23 mg.l^{-1}, 48 h - EC_{50} = 1.07 mg.l^{-1}	
5.3	**Conclusion**	Hydrogen cyanide liquid stabilized is classified as toxic to *Daphnia magna, Strauss*	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No remarks	

Table 1: Dilution water

Criteria	Details
Source	Reconstituted dilution water was prepared at the strart of test by mix of 2.5 ml of each of four A, B, C, D stock solutions and make up to 1,000 ml with distilled water: A) $CaCl_2.2H_2O$ (calcium chloride dihydrate): 117.6 g; dissolved in, and make up 1,000 ml with distilled water B) $MgSO_4.7H_2O$ (magnesium sulphate heptahydrate): 49.3 g; dissolved in, and make up 1,000 ml with distilled water C) $NaHCO_3$ (sodium hydrogen carbonate): 25.9 g; dissolved in, and make up 1,000 ml with distilled water D) KCl (potassium chloride): 2.3 g; dissolved in, and make up 1,000 ml with distilled water
Alkalinity	-
Hardness	Concentration of Ca + Mg: 2.57 $mmol.l^{-1}$
pH	7.64
Oxygen content	8.8 $mg.l^{-1}$(at the start of the test)
Conductance	0.81 $\mu S.cm^{-1}$
Holding water different from dilution water	No

Table 2: Test organisms

Criteria	Details
Source	*Daphnia magna Straus*, from the own laboratory bred
Age	Less than 24 hours old, at the beginning of the test
Breeding method	Pregnant adult female daphnia are transferred into the vessels containing aerated reconstituted dilution water and the 24 hour´s offsprings by means of screen (mesh 0.1 mm) eliminated and washed down into the handlindg vessel (care should be paid, no air enters under the shall). Test animals are obtained by acyclic parthenogesis from the own laboratory bred, at least third genera.
Kind of food	Mixture of the algae.
Feeding frequency	-
Pretreatment	Eliminated 24 hours´s offsprings were washed down into the handling vessel and numberred to use in the test. Sensitivity of the test animals is regularly monitoring and tested with the reference substance, potassium dichromate.
Feeding of animals during test	No

Table 3: Test system

Criteria	**Details**
Renewal of test solution	No – static conditions
Volume of test vessels	50 ml test solution in a 100 ml beaker
Volume/animal	5 ml of test solution for each animal
Number of animals/vessel	10
Number of vessels/ concentration	3 beakers

Table 4: Test conditions

Criteria	**Details**
Test temperature	20 ± 2 °C; temperature constant within ± 1 °C at each single test
Dissolved oxygen	8.5 - 8.8 $mg.l^{-1}$
pH	7.63 – 7.92
Adjustment of pH	No, just measurement pH and concentration of dissolved oxygen at the beginning and the end of the definitive test for each tested concentration.
Quality/Intensity of irradiation	Not relevant
Photoperiod	A light – dark cycle

Table 5: Test conditions (monitoring of concentrations)

Test run	**Beginning of test calculated concentration HCN [$mg.l^{/1}$]**	**Beginning of test measured concentration HCN [$mg.l^{/1}$]**	**End of test measured concentration HCN [$mg.l^{/1}$]**	**Difference of measured concentration HCN [%]**
1. run	1.80	1.79	1.60	10.6
1. run	0.75	0.77	0.70	9.1
2. run	1.80	1.84	1.61	12.5
2. run	0.75	0.76	0.72	5.3

Table 6: Immobilisation data

Test substance concentration (measured) [mg/l]	Immobile Daphnia									
	Number		Percentage		Oxygen [mg/l]		pH		T [*C]	
	24 h	48 h	24 h	48 h	0 h	24 h	0 h	24 h	0 h	24 h
Range-finding test										
2.1	10	10	100	100						
1.8	10	10	100	100						
1.5	7	9	70	90						
1.2	6	7	60	70						
0.9	3	5	30	50						
0.6	0	0	0	0						
0.3	1	1	10	10						
0	0	0	0	0						
Definitive test – 1. run										
1.80	20	20	100	100	8.6	8.5	7.70	7.92	20.5	21.0
1.65	16	19	80	95	8.7	8.6	7.70	7.90	20.5	21.0
1.50	13	17	65	85	8.7	8.6	7.69	7.90	20.5	21.0
1.35	12	14	60	70	8.8	8.6	7.65	7.91	20.5	21.0
1.20	11	13	55	65	8.8	8.6	7.67	7.90	20.5	21.0
1.05	10	11	50	55	8.8	8.6	7.67	7.88	20.5	21.0
0.90	5	8	25	40	8.8	8.6	7.66	7.87	20.5	21.0
0.75	3	3	15	15	8.8	8.6	7.64	7.84	20.5	21.0
0.00	0	0	0	0	8.8	8.6	7.64	7.84	20.5	21.0
Definitive test – 2. run										
1.80	20	20	100	100	8.7	8.6	7.70	7.90	19.7	21.3
1.65	14	17	70	85	8.8	8.6	7.68	7.89	19.7	21.3
1.50	12	15	60	75	8.8	8.7	7.68	7.89	19.7	21.3
1.35	11	13	55	65	8.8	8.6	7.67	7.88	19.7	21.3
1.20	10	12	50	60	8.8	8.6	7.66	7.86	19.7	21.3
1.05	8	10	40	50	8.8	8.6	7.66	7.86	19.7	21.3
0.90	4	6	20	30	8.8	8.7	7.65	7.83	19.7	21.3
0.75	2	3	10	15	8.8	8.6	7.63	7.82	19.7	21.3
0.00	0	0	0	0	8.8	8.6	7.64	7.82	19.7	21.3

Table 7(1.run): Effect data

	EC_{50} [mg.l^{-1}]	**95 % c.l.**	**Approximation function**
24 h	1.18	0.88 – 1.47	log linear
48 h	1.03	0.90 – 1.16	log linear

Table 7(2.run): Effect data

	EC_{50} [mg.l^{-1}]	**95 % c.l.**	**Approximation function**
24 h	1.27	0.92 – 1.62	log linear
48 h	1.11	0.95 – 1.26	log linear

Table 8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	Fulfilled	**Not fullfilled**
Immobilisation of control animals <10%	**x**	
Control animals not staying at the surface	**x**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**x**	
Concentration of test substance ≥80% of initial concentration during test	**x***	

* see Table 5

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

Section A7.4.1.3 **Annex Point IIA VII.7.3**		**GROWTH INHIBITION TEST ON ALGAE**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Growth Inhibition of Green Algae (*Scenedesmus subspicatus Brinkmann 1953/SAG 86.81)* by hydrogen cyanide liquid stabilized, Research Institute of Organic syntheses, Centre for ekotoxicology, toxicology an analytics, Pardubice – Rybitví, Czech Republic,Report No. 1522/L (unpublished), 2002-03-20 (**DOC IV_73**)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lučební závody Draslovka, a. s. Kolín	
1.2.2	Companies with letter of access	No companies with letter of access.	
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**	Annex to Decree of Ministry of Environment No. 299 /1998 Coll., Part 103, Method III, Growth Inhibition of Alga ; Test Guideline No. 201, Alga, Growth Inhibition Test, OECD, 1984; Method C.3, Alga Inhibition Test, EEC Directive 67/548/EEC, Annex V	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Hydrogen cyanide liqud stabilized	
3.1.1	Lot/Batch number	15/02	
3.1.2	Purity	98%	
3.1.3	Further relevant properties	Water solubility, vapour pressure, chemical stability, dissociation constant, biodegradability, partition coefficient n-octanol/water; see: Physical and chemical properties	
3.1.4	Method of analysis	Argentometric titration	
3.2	**Preparation of TS**	Solubility in water: very good	
3.3	**Reference substance**	Yes, an acute toxicity test was carried out (Report No. 1514/L) using the reference substance potassium dichromate ($K_2Cr_2O_7$), Batch number K29239062, Certificate of analysis: 1.04862.1000 Potassium dichromate cryst. extra pure, Merck KgaA, 64271 Darmstadt.	
3.4	**Testing procedure**		
3.4.1	Culture medium	Before the use it was equilibrated with filtered air aeration for 30 minutes. pH of the medium was 7.85 ± 0.1. All chemicals were of p.a grade. Distilled water of the conductivity of 5 µS. cm^{-1} was used for the preparation of the solutions. Stock solutions were diluted to achieve the final nutrient concentrations in the test solution. Following four stock solutions were prepared:	

		Nutrient A) macronutrients: NH_4Cl $MgCl_2$. $6H_2O$ $CaCl_2$. $2H_2O$ $MgSO_4$. $7H_2O$ KH_2PO_4 B) Fe-EDTA: $FeCl_3$. $6H_2O$ Na_2EDTA. $2H_2O$ C) trace elements: H_3BO_3 $MnCl_2$. $4H_2O$ $ZnCl_2$ $CoCl_2$. $6H_2O$ $CuCl_2$. $2H_2O$ Na_2MoO_4. $2H_2O$ D) $NaHCO_3$	Concentration in the stock solution 1.5 g/l 1.2 g/l 1.8 g/l 1.5 g/l 0.16 g/l 80 mg/l 100 mg/l 185 mg/l 415 mg/l 3 mg/l 1.5 mg/l 0.01 mg/l 7 mg/l 50 g/l	
3.4.2	Test organisms	Freshwater green algae (*Scenedesmus subspicatus Brinkmann)*, see **Table 1.**	
3.4.3	Test system	Static; see **Table 2**	
3.4.4	Test conditions	See **Table 3**	
3.4.5	Duration of the test	72 hours	
3.4.6	Test parameter	Inhibition of growth of biomass Inhibition of growth rate	
3.4.7	Sampling	After 24, 48 and 72 hours, the cells counts were determined in the test concentrations and in the control. pH values were controlled at the beginning of the test and after 72 hours in the control and the test concentrations. Temperature was determined in the course of the test concentrations.	
3.4.8	Monitoring of TS concentration	Yes, see **Table 4** Determination of the test substance concentration was performed at the beginning and the end of the test by argentometric titration. The highest concentrations tested were analysed. The lowest concentrations tested were not analysed due to limit of determination of the applied analytical method which was 0.1 mg. l^{-1}. Instead of that the nearest concentration responding to the limit of determination has been analysed. Test media were filtered through the inert membrane filter of 0.2 µm porosity and the algal free analysed for the test substance concentration.	

3.4.9	Statistics	The mean value of the cell density for each three replicate test substance concentration was considered. To determine the concentration / effect relationship the following approach was used: a) comparision of area under the growth curves b) comparision of the growth rates. The percentage inhibiton of the cell growth at each test substance concentration was calculated. For the estimation of the E_bC_{50} and ErC_{50} for the interval of 0 – 72 hours the computation TOXICITA program of VÚV Ostrava was used.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Range – finding test concentrations (nominal): 2.55, 1.20, 0.75, 0.60, 0.30, 0.15,0.045 and 0.015 Definitive test concentrations (nominal): 1st and 2nd Run: 0.30, 0.15, 0.12, 0.09, 0.06, 0.03 and 0.015 Test substance concentrations at the beginnig of the test are identical with those calculated ones. See **Table 5(01).**	
4.2.2	Actual concentrations of test substance	Range – finding test concentrations (nominal): 2.55, 1.20, 0.75, 0.60, 0.30, 0.15,0.045 and 0.015 Definitive test concentrations (nominal): 1st and 2nd Run: 0.30, 0.15, 0.12, 0.09, 0.06, 0.03 and 0.015 Test substance concentrations at the beginnig of the test are identical with those calculated ones. See **Table 5(01)**.	x
4.2.3	Cell concentration data	Cell density measurements by using a microscope with counting chambers are presented in **Tables 5**.	
4.2.4	Effect data (cell multiplication inhibition)	The growth of biomass (E_bC_{50}) was found to be 0.04 mg. l^{-1} after 72 h. The algae growth rate (E_rC_{50}) was found to be 0.12 mg. l^{-1} after 72 h. See **Table 6**	
4.3	**Results of controls**	Cell counts of the control in the range – finding test and defitive test are presented in **Tables 5.**	
4.4	**Test with reference substance**	Performed. Based on the results of an ASLAB (2001) inter-laboratory tests (presentation of 14 laboratories) following results were obtained: E_bC_{50} (0 – 72h) = 0.24 – 0.89 mg.l^{-1} E_rC_{50} (0 – 72h) = 0.59 – 1.70 mg.l^{-1} The results of the valid verified test with $K_2Cr_2O_7$ performed on 17 – 20 Dec 2001 are: E_bC_{50} (0 – 72h) = 0.76 mg.l^{-1} E_rC_{50} (0 – 72h) = 1.39 mg.l^{-1} Test culture algae growth inhibition for the media with the reference substance expresed as EC_{50} showed the sensitivity of the test species has not changed significantly.	

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Hydrogen cyanide liquid stabilized (purity of 98%) was tested for its inhibition potential effect to the growth of green algae (*Scenedesmus subspicatus Brinkmann)* in accordance with Annex to Decree of Ministry of Environment No. 299 /1998 Coll., Part 103, Method III, Growth Inhibition of Alga; Test Guideline No. 201, Alga, Growth Inhibition Test, OECD, 1984 and Method C.3, Alga Inhibition Test, EEC Directive 67/548/EEC, Annex V. Algal culture were exposed under static conditions to a negative control and eight test substance concentrations in a range – finding test and to a negative control (8 replicates) and seven test substance concentrations (3 replicates each). Whole test was completly repeated once more. The cell density in each flask was determined at 0, 24, 48 and 72 hours after the start of the test and the cell density measured by microscope. Based on the algae growth rate and the growth of biomass for each time interval were calculated. Test shows no significant deviations from the given above guidelines.	
5.2	**Results and discussion**	Based on nominal test substance concentratios, the inhibition of growth of biomass E_bC_{50} (0 – 72h) for hydrogen cyanide liquid stabilised (purity 98 %) was found to be 0.04 $mg.l^{-1}$. The algae growth rate E_rC_{50} (0 – 72 h) was found to be 0.12 $mg.l^{-1}$. According to Recommendation No. 25/1999 Coll. of the Goverment of Czech Republic test substance, hydrogen cyanide liquid stabilized is classified as very toxic to aqueous organisms – algae growth. No abnormalities were observed. Decrease in test substance concentrations at the end of test was less than 20 % of that one at the beginning of test. The lowest concentration tested has no observed effect on the growth of algae. The highest concentration tested inhibited growth more than 50 relative to the control. Deviation in pH of solutions at the beginning and end of the test was not more than 1.5 units.	
5.2.1	NOEC	Not determined	
5.2.2	E_rC_{50} (0 – 72h)	0.12 $mg.l^{-1}$	
5.2.3	E_bC_{50} (0 – 72h)	0.04 $mg.l^{-1}$	
5.3	**Conclusion**	Based on nominal concentrations and under the static conditions of the test, the inhibition of growth of biomass E_bC_{50} (0 – 72h) for hydrogen cyanide liquid stabilized (purity of 98%) was found to be 0.04 $mg.l^{-1}$. The algae growth rate E_rC_{50} (0 – 72h) was found to be 0.12 $mg.l^{-1}$. The NOEC was not determined.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No deviations from the guidelines were recorded. Test substance was in a liquid state and stabilized.	

Table 1: Test organisms

Criteria	Details
Species	Frashwater green algae *Scenedesmus subspicatus Brinkmann 1953*
Strain	SAG 86/81
Source	Autotroph organisms collection of the Botanic Institute of Academy of Sciences, Czech Republic, Třeboň, 15 Oct 2001
Laboratory culture	Yes
Method of cultivation	The stock culture of the green algal species was transferred from the sloped agar tubes and grown in conical flasks containing 100 ml of the nutrient medium, incubated at laboratoty temperature with indirect dailylight and once a weak transfered to fresh medium. All nutrient solutions are prepared using filter – sterilized distilled water of the conductivity less than 5 µS. cm^{-1}. The inoculation and transfer of algae suspensions is done under sterile conditions.
Pretreatment	The pre – cultures are inoculated with 1x 10^4 cells. ml^{-1}. For the test, the algae from the exponentially growing pre – culture was used, incubated under the conditions of the test for tree days and then exposed to the various test concentrations. The density of the pre – culture cells was measured immediately before the beginning of the test and the required volume of the inoculum was calculated.
Initial cell concentration	Each test culture started with a cell concentration of 1x 10^4 cells. ml^{-1}.

Table 2: Test system

Criteria	Details
Volume of culture flasks	Conical Erlenmayer flasks provided with the air-transmition stopper filled with each 50 ml of the test suspension (containing chosen volume of the test substance stock solution, 5 ml of nutrient stock solution, calculated volume of algal culture and distilled water to make-up the volume).
Culturing apparatus	The Erlenmayer flasks were placed illuminated platform of the shaking apparatus, exposed in an incubator at 23 ± 2 °C under continuous illumination and shaking for 72 hours.
Light quality	Illumination was provided by fluorescent lamps of white type, min 6,000 lux, max 10,000 lux, continuous light.
Procedure for suspending algae	By mixing on the shaking apparatus (40 mm swing length, 4 degree frequency), without the aeration.
Number of vessels/ concentration	3 vessels/ concentration; 3 controls without test substance; in each case 6 vessels. Whole test constituted of two runs.

Table 3: Test conditions

Criteria	Details
Test temperature	Definitive test: 1st run: 22.5 °C; 2nd run: 22.0 – 22.5 °C
pH	Measured at the beginning of the test and at 72 hours
Aeration of dilution water	No aeration during the test.
Light intensity	Definitive test: 1st run: 6,200 – 6,300 lux 2nd run: 6,300 – 6,400 lux
Photoperiod	Continuous light

Table 4: Test conditions (monitoring of concentrations)

Test run	Beginning of test calculated concentration HCN [mg.l^{-1}]	Beginning of test measured concentration HCN [mg.l^{-1}]	End of test measured concentration HCN [mg.l^{-1}]	Difference of measured concentration HCN [%]
1st run	0.30	0.32	0.29	9.4
1st run	0.12	0.13	0.11	15.4
2nd run	0.30	0.30	0.26	13.3
2nd run	0.12	0.13	0.11	15.4

Table 5(1): Algal density data *Time of measurement: 25 Feb 02*

Nominal Test-Substance Concentration [mg/l]	Inhibition of algae growth (× 10^{-4} cells/mL)			pH measured		Mean growth inhibition	
	24 h	48 h	72 h	pH 0 h	pH 72 h	I_r (%)	I_A (%)
Range – finding test							
Control	3.7500	37.5000	175.0000	-	-	-	-
2.55	0.6250	0	0			100	100
1.20	0.6250	0.6250	0.6250			100	100
0.75	1.8750	0.6250	0.6250			100	99.8
0.60	1.2500	0.6250	1.2500			95.9	100
0.30	1.2500	1.2500	2.5000			82.0	99.0
0.15	2.5000	5.6250	10.0000			55.2	91.6
0.045	3.7500	13.1250	104.3750			9.9	47.3
0.015	4.3750	20.6250	131.8750			5.2	30.0

Table 5(2): Algal density data *Time of measurement: 4March 02*

Nominal Test-Substance Concentration [mg/l]	Algal cell density (× 10^{-4} cells/mL)			pH measured		Mean growth	
	24 h	48 h	72 h	pH 0 h	pH 72 h	I_r (%)	I_A (%)
1st run							
Definitive test							
Control	7.0833	45.6250	180.2083	7.78	8.87	-	-
0.30	0.8333	3.1250	4.1667	7.81	7.94	72.3	97.5
0.15	1.2500	5.0000	7.5000	7.80	7.95	61.3	94.7
0.12	2.0833	8.9583	12.2917	7.80	8.02	51.4	89.5
0.09	2.9167	10.4167	26.6667	7.80	8.19	37.0	82.8
0.06	3.7500	18.9583	37.7500	7.80	8.63	17.3	59.3