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**	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)**	
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 vapour	
3.1.1	Lot/Batch number	Not reported	
3.1.2	Specification	Not reported	
3.1.2.1	**Description**	Vapours of liquid HCN	
3.1.2.2	**Purity**	Not reported	
3.1.2.3	**Stability**	Not reported	
3.1.2.4	**Radiolabelling**	No labelling	
3.2	**Test Animals**		
3.2.1	Species	Guinea pig Rabbit	
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	Guinea pigs: 567 – 680 g Rabbits: 1588 – 1814 g	
3.2.6	Number of animals per group	Guinea pigs: 6 (total number) Rabbits: 20 (total number)	
3.2.7	Control animals	No	

3.3	**Administration/ Exposure**	Dermal	
3.3.1	Preparation of test site	Guinea pigs: clipping of hair on the abdomen (approx. 1/60 of body surface)	
3.3.2	Concentration of test substance	Guinea pigs: saturated vapours (30 – 50%) Rabbits: 4,000 ppm – 33,000 ppm; after a single introduction the concentration of HCN fell (in one hour: from 1:175 to 1:425) so HCN was introduced repeatedly to maintain a level concentration	
3.3.3	Specific activity of test substance		
3.3.4	Volume applied	Not relevant	
3.3.5	Size of test site	Guinea pigs: 7.93 cm^2 Rabbits: entire body surface, head excluded	
3.3.6	Exposure period	Guinea pigs: single exp. 15 - 97 minutes; repeated exp. - 1st day 15 minutes, 2nd day 10′, 5th day 10′, 6th day 10′, 7th day 15′, 8th day 10′, 9th day 10′, 10th day 10′, 12th day 10′, 14th day 15′ and 15th day 78′ Rabbits: 12 – 90 minutes	
3.3.7	Sampling time	Not reported	
3.3.8	Samples		
		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	**Guinea pigs** after a single exposure (5 animals): clinical symptoms – shallow, laboured or gasping breathing, convulsions or collapse and coma; death or euthanasia - all (euth. – in 2 animals); autopsy results – dark blood (in 4 anim.), submucous haemorrhages in stomach (3 anim.), stomach distended with air (3 anim.) microscopic examination of exposed skin – slight vascular congestion (3 anim.), swelling of connective tissue (1 anim.) Guinea pig after repeated exposure (1 animal): clinical symptoms – breathing distressed, loss of equilibrium (1st , 7th and 15th day); convulsions and coma – 15th day death autopsy results – dark blood **Rabbits** without $Na_2S_2O_3$ (12 animals): clinical symptoms – an appearance of alarm, lateral swaying movements of head, quick breathing, slight grunt (10 anim.), tremor (1 anim.), convulsions (9 anim.), collapse (9 anim.) death – 6 animals autopsy results – dark blood **Rabbits** protected by $Na_2S_2O_3$ (8 animals): clinical symptoms – an appearance of alarm, lateral swaying movements of head, quick breathing, slight grunt (3 anim.), convulsions (4 anim.), collapse (3 anim.) death – 3 animals autopsy results – dark blood	
4.2	**Dermal irritation**	No effects	

4.3	**Recovery of labelled compound**	Not reported	
4.4	**Percutaneous absorption**	Not reported	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Non-guideline study; Small area of abdominal skin of 6 guinea pigs was exposed to HCN. Entire body surface (with head excluded) of 20 rabbits were exposed to HCN.	
5.2	**Results and discussion**	An atmosphere saturated with HCN is readily absorbed by a skin surface in the guinea pig and will produce death if the exposure be prolonged. Atmospheres containing HCN (in different concentrations) readily passes through the skin of rabbit. Rabbits can tolerate a concentration 1:210 (average) for 90 minutes. Sodium thiosulphate approximately doubled the time of exposure without symptoms, except in high concentration (1:30 – 1:50). Acute poisoning with HCN does not appear to produce any post-mortem macroscopic or microscopic characteristic appearances, from which a diagnosis could reasonably be made.	
5.3	**Conclusion**	Hydrocyanic acid vapour can pass through the skin of guinea pig and rabbit and can produce serious or lethal toxicity in these animals.	
5.3.1	Reliability	3	
5.3.2	Deficiencies	No	

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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**	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 Dermal Toxicity)	
2.2	**GLP**	Not reported	
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	Rabbits	
3.2.2	Strain	Rabbit – not reported (albino)	
3.2.3	Source	Not reported	
3.2.4	Sex	Males only	
3.2.5	Age/weight at study initiation	Rabbit – 2040 g (average)	
3.2.6	Number of animals per group	Skin Absorption Toxicity 4 rabbits (total number)	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Post exposure period	Not reported	

3.3.2	Area covered	Entire body surface (their fur was closely clipped), head excluded	
3.3.3	Occlusion	Not possible	
3.3.4	Vehicle	Air	
3.3.5	Concentration in vehicle	10 000 ppm	
3.3.6	Total volume applied	Not relevant	
3.3.7	Duration of exposure	8 hours	
3.3.8	Removal of test substance	Not relevant	
3.3.9	Controls	No	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	No clinical signs.	
4.2	**Pathology**	No gross lesions on autopsy.	
4.3	**Other**	None	
4.4	**LD_{50}**	>>10 000 ppm	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Non-guideline studies Entire body surface (with head excluded) of 4 rabbits were exposed to a cyanogen concentration of 10 000 ppm for a period of 8 hours.	
5.2	**Results and discussion**	It was determined that male albino rabbits could endure an exposure to their skin of 10 000 ppm for eight hours without any apparent detrimental effects. Cyanogen hydrolyses to yield one molecule of hydrogen cyanide and one of cyanate. This is the basis for the supposition that cyanogen is comparable in toxicological effect to hydrogen cyanide.	
5.3	**Conclusion**	Based on results of this study, cyanogen has a very low dermal toxicity (LD50 >>10 000 ppm for 8 hour exposure): comparison with high acute toxicity of inhaled cyanogen (LD50 <350 ppm for 1 hour exposure) indicates slow dermal absorption.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	The study comes from 1960, it is not in the GLP system, but the method used is comparable to a standard method for acute dermal toxicity testing.	

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Section A6.3 Annex Point IIA VI.6.3	**SHORT-TERM REPEATED DOSE TOXICITY (28 DAYS)**	

Section A6.3.1 Annex Point IIA VI.6.3	**Repeated dose toxicity (oral)**	
Detailed justification:	Hydrogen cyanide is used as a gas so exposure by ingestion cannot happen in practice. HCN evaporates easily from water and diet, sub-acute oral toxicity tests are, therefore, not technically feasible, because repeated administration of water solution of HCN by gavage cannot be interpreted as a model of sub-acute exposure but of repeated acute poisoning. Relevant qualitative and quantitative information can be extracted from three studies on the effects of short-term exposure to potassium cyanide in drinking water. 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.** Sousa AB, Soto-Blanco B, Guerra JL, Kimura ET, Górniak S (2002) Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity? *Toxicology*, 174:87–95. **(DOC IV_37)** **2.** Sousa AB, Paulo C´esar Maiorka, Ivair Donizete Goncalves, L´ılian Rose Marques de Sá, Silvana Lima Górniak (2007) Evaluation of effects of prenatal exposure to the cyanide and thiocyanate in Wistar rats. Reproductive Toxicology 23: 568–577 **Summary in section 6.8.1b. (DOC IV_38).** 3. Pritsos CA. 1996. Mitochondrial dysfunction and energy depletion from subchronic peroral exposure to cyanide using the Wistar rat as a mammalian model. Toxic Subst Mech 15(3):219-229. 4. Olusi SO, Oke OL, Odusote A (1979) Effects of cyanogenic agents on reproduction and neonatal development in rats. *Biology of the Neonate*, 36:233–234.	
Results and discussion	Forty-six male adult inbred Wistar rats were used in four experimental groups and one control group and treated with 0, 0.3, 0.9, 3.0, or 9.0 mg potassium cyanide/kg body weight per day in the drinking-water for 15 days. This was equivalent to 0, 0.12, 0.36, 1.2, and 3.6 mg cyanide/kg body weight per day. The high-dose group exhibited a 70% lower body weight gain than the control animals. In qualitative histological analysis, without statistical treatment or morphometric analysis, changes were observed in the kidney, liver, and thyroid. Cytoplasmic vacuolation, considered to reflect hydropic degeneration of proximal tubular epithelial cells, was noted in animals treated at doses of 3.0–9.0 mg potassium cyanide/kg body weight per day and in hepatocytes of those animals treated at a dose of 9.0 mg potassium cyanide/kg body weight per day. A dose-dependent increase in the number of reabsorption vacuoles on follicular colloid in the thyroid gland was noted in all animals of the experimental groups. No changes were observed in serum triiiodothyronine (T3), thyroxine (T4), creatinine, or urea levels; a decrease was observed in serum alanine aminotransferase (ALAT) activity at the two lowest exposure levels. Serum aspartate aminotransferase (ASAT) was elevated by 30% at the two lowest dose levels and by 21% at the 3.0 mg potassium cyanide/kg body weight per day dose; it was decreased by 29% at the highest dose	

	level **(1).** To verify the toxic effects of prenatal exposure to cyanides, pregnant rats received daily in drinking water potassium cyanide (KCN) in doses of 1, 3 or 30 mg/kg bw or potassium thiocyanate (KSCN) in doses of 0.8, 2.4 or 24 mg/kg bw. No clinical signs of toxicity and no treatment related effects on body weight were observed in KCN or KSCN treated groups. Histology revealed the effect of cyanide and thiocyanate on thyroid (increased number of vacuoles in the folicular colloid) in all KCN or KSCN treated groups. The changes were dose related and indicate probably a compensatory increase in hormone synthesis, as no differences in plasma cholesterol were found. Vacuolisation of pancreatic islet cells and increased blood glucose level were found only in KCN top dose animals, sacrificed immediately after cessation of exposure. Diabetogenic effect seems to be due to direct effect of cyanide and not to thiocyanate metabolite. Histopathological findings in pancreas were transitory: they disappeared 3 weeks after exposure cessation. On the other hand, histopathological changes in liver and brain were detected in animals of the top dose groups of both KCN and KSCN. These results are in agreement with a previous similar experiment: potassium cyanide in a daily dose of 3.7 mg/kg bw - but not in a half dose - decreased ATP levels and mitochondrial respiration in female rats exposed for 30 days via drinking water **(3)**. After 2 weeks on a diet containing 5 or 10 g potassium cyanide/100 g diet, female rats (10 per group) were mated with untreated males. No pregnancies resulted. The dose corresponds roughly to 1000 and 2000 mg cyanide/kg body weight per day (**4**). There was a dose-dependent decrease in body weight gain, blood haemoglobin (18% and 23%), and serum T4 concentration (54% and 75%).	
Conclusions:	Short-term (15 – 30 day) oral exposure of rats to potassium cyanide at a daily dose of 1.2 mg/kg bw (as cyanide) induced minimal effects. NOAEL was 0.12 mg/kg bw.	
Undertaking of intended data submission	No studies are planned.	

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Date	
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Conclusion	
Remarks	

Section A6.3 Annex Point IIA VI.6.3	**SHORT-TERM REPEATED DOSE TOXICITY (28 DAYS)**	

Section A6.3.2 Annex Point IIA VI.6.3	**Repeated dose toxicity (dermal)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Hydrogen cyanide is a gas at body temperature. The main route of exposure is inhalation, and upon this exposure HCN is classified as highly toxic, its inhalation may be fatal.	
References:	1. Ballantyne B. 1988. Toxicology and hazard evaluation of cyanide fumigation powders. Clin Toxicol 26: 325-335. **(DOC IV_14). Summary in DOC III_ 6.1.2a, b.** 2. Ballantyne B. 1983b. Acute systemic toxicity of cyanides by topical application to the eye. J Toxicol, Cutan, Ocular Toxicol 2: 119-129 **(DOC IV_16) Summary in DOC III_ 6.1.2c.**	
Results and discussion	Since poisoning symptoms have been observed even after a single short-time contact of hydrogen cyanide with skin, it may be assumed that signs of HCN absorption through skin will occur also with repeated doses. Skin exposure to gaseous HCN is thus considered a possible route of exposure with poisoning symptoms similar to oral and inhalation exposure but with a longer latency, as indicated by experimental studies or short-term observations **(1)** and **(2)**. No 28-day dermal study for HCN or for cyanides has been found in literature. Dangerous properties of HCN have been known for a long time; upon longer-term exposures, as sub chronic exposure, observations of effects on humans and epidemiological studies are of primary significance. **See 6.12.**	
Undertaking of intended data submission	No studies are planned. To conduct a dermal repeated dose toxicity study would constitute unjustified use of animals.	

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Date	
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Section A6.3 Annex Point IIA VI.6.3	**SHORT-TERM REPEATED DOSE TOXICITY (28 DAYS)**	

Section A6.3.3 Annex Point IIA VI.6.3	**Repeated dose toxicity (inhalation)**	
Justification: Literature data:	Hydrogen cyanide is a highly toxic substance by inhalatory exposure for humans and for all species of laboratory organisms. Its effects are well known. Although literature provides a large number of data, no single study meets requirements for key studies and the data below are of supporting character. The toxic effects of repeated or protracted inhalatory exposures were studied either with hydrogen cyanide or with acetone cyanhydrin. 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. Valade MP. 1952. Central nervous system lesions in chronic experimental poisoning with gaseous hydrocyanic acid. Bull Acad Natl Med (Paris) 136: 280-285. (in French) (**DOC IV_39**) 2. 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) 3. Hugod C. 1981. Myocardial morphology in rabbits exposed to various gas-phase constituents of tobacco smoke: an ultra-structural study. Atherosclerosis 40: 181 - 190	
Guidelines:	Not presented	
GLP:	No	
Material and methods:	Various methods of inhalatory exposures to hydrogen cyanide or acetone cyanhydrin.	
Results and discussion:	The two studies **(1)** and **(3)** shortly described below represent two extremes with respect to HCN concentrations used and injury found. In a 28-day study, dogs were exposed to hydrogen cyanide in an airborne concentration of 50 mg/m^3 for 30 min every third day **(1)**. Extensive vasodilation haemorrhages and cellular lesions were described in the central nervous system. For other findings **see Table.** In an ultra-structural study of the role of individual components of tobacco smoke on atherosclerosis, no statistically significant increase in the incidence of histolopathological findings in the lungs and the myocardium were found in rabbits exposed to 0.6 mg hydrogen cyanide/m^3 continuously for 1 or 4 weeks **(3).** Rats (Sprague-Dawley, 10 males and 10 females per concentration group) were exposed for 4 weeks to acetone cyanhydrin in airborne concentrations of 33, 106 and 211 mg/m^3, 6h/day, 5 days/week **(2)**. Exposure levels correspond to HCN concentrations of 10, 24, and 67 mg/m^3. The exposed animals displayed only slight deviations in laboratory values in the two higher concentration groups, and no organ macroscopic or histologic pathology. Individual findings: decreased blood haemoglobin and increased blood urea nitrogen (high-dose females), decreased total serum protein (high- and mid-dose males), increased T_3 level (mid-dose males). The scarcity of cumulative effects contrasts with the death of 3/10 males after a single	

	exposure to a concentration only slightly higher than the highest concentration used in the sub chronic phase of the study (225 mg/m^3, equivalent to 71 mg HCN /m^3). The NOAEL (equiv. HCN concentration 10 mg/m^3) is estimated to correspond to a daily dose of 2.7 mg CN/kg bw (i.e., cumulative dose of 54 mg/kg bw).	
Conclusions:	1. The NOAEL of acetone cyanhydrin for rats (equiv. HCN concentration 10 mg/m^3 for 6 hours per day) is estimated to correspond to a daily dose of 2.7 mg CN/kg bw (i.e., cumulative dose of 54 mg/kg bw). 2. Mild effects of high cumulative doses (tens of mg/kg bw in a 4-week study in dogs) contrast with severe injury or death after intermittent short exposure (30 min) to high airborne concentrations (about 50 mg/m^3). 3. Concentration of 0.6mg HCN/ m^3 was NOAEL for continuous 4-week exposure of rabbits.	

Table - Short-time (28day) toxicity – inhalatory (hydrogen cyanide)

Study		**Test organism**	**Dose / exposure time**	**Target organ / basic effect as LOEL**	**Reference**
28-day study inhalatory	HCN	Dog not specified	2-day interval 30 minutes / day	50mg/m^3 25% died	**(1)**
28-day study inhalatory	HCN	Rat Long-Evans	4-day interval 12.5 minutes / day	224mg/m^3 systemic effects increased activity of creatine-phospho-kinase	
28-day study inhalatory	HCN	Dog not specified	2-day interval 30 minutes / day	45mg/m^3 systemic effects respiratory, breathlessness	
28-day study inhalatory	HCN	Dog not specified	2-day interval 30 minutes / day	45mg/m^3 systemic effects gastrointestinal vomiting, painful compulsion to stool, diarrhoea	
28-day study inhalatory	HCN	Dog not specified	2-day interval 30 minutes / day	45mg/m^3 neurological effects	

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Section A6.4 Annex Point IIA VI.6.4	**SUBCHRONIC TOXICITY**	

Section A6.4.1 Annex Point IIA VI.6.4	**Subchronic Oral Toxicity Test**	
Justification: **Literature data:**	Hydrogen cyanide is used as a gas so exposure by ingestion cannot happen in practice. HCN evaporates easily from water and diet, subchronic oral toxicity tests are therefore not technically feasible for hydrogen cyanide. Relevant information can be extracted from studies on medium term and long-term oral exposures to inorganic cyanides. (On the other hand, experimental evidence on subchronic toxicity of copper cyanide cannot be convincingly used as supportive information on toxicity of hydrogen cyanide.) The method used in the NTP study on sodium cyanide administered in drinking water complies best with guidelines for subchronic oral toxicity studies and serves as a key study. NTP. 1993. Sodium cyanide administered in drinking water to F344/N rats and $B6C3F_1$ mice. NTP, Toxicology Report Series No. 37. (NIH Publication 94-3386) (DOC IVA / A27) **(DOC IV_40)**; **Summary in section 6.4.1a**	
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: Hydrogen cyanide *Peer reviewed* (**DOC IV_2**). 1. Hayes WJ (1967) The 90 day LD50 and chronicity factors as a measure of toxicity. Toxicology and Applied Pharmacology, 11:327–335. 2. 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. 3. Olusi SO, Oke OL, Odusote A (1979) Effects of cyanogenic agents on reproduction and neonatal development in rats. Biology of the Neonate, 36:233–234. 4. Okolie NP, Osagie AU (1999) Liver and kidney lesions and associated enzme changes induced in rabbits by chronic cyanide exposure. Food and Chemical Toxicology, 37:745–750. 5. Soto-Blanco B, Maiorka PC, Gorniak SL (2002a) Neuropathologic study of long term cyanide administration to goats. Food and Chemical Toxicology, 40:1693–1698. 6. Kamalu BP, Agharanya JC (1991) The effect of a nutritionallybalanced cassava (Manihot esculenta Crantz) diet on endocrine function using the dog as a model. 2. Thyroid. British Journal of Nutrition, 65:373–379. 7. Mathangi DC, Namasivayam A (2000) Effect of chronic cyanide intoxication on memory in albino rats. Food and Chemical Toxicology, 38:51–55. 8. Tewe OO, Maner JH (1985) Cyanide, protein and iodine interactions in the performance and metabolism of rats. *Journal of Environmental Pathology and Toxicology*, 6:69–77. **(DOC IV_41).** 9. Howard JW, Hanzal RF. 1955. Chronic toxicity for rats of food treated with hydrogen cyanide. Agric and Food Chem 3:325-329. **(DOC IV_42).**	

	10. US EPA (1993b) *Hydrogen cyanide (CASRN 74-90-8).* US Environmental Protection Agency, Integrated Risk Information System. **(DOC IV_43).** 11. Philbrick DJ, Hopkins JB, Hill DC, et al. 1979. Effects of prolonged cyanide and thiocyanate feeding in rats. J Toxicol Environ Health 5:579-592. **(DOC IV_44).**	
Summaries	The study in **(1)** permits to compare in the same animals (Sherman rats) cumulative toxicity of potassium cyanide administered in diet with acute toxicity of the same substance administered by gavage in a single dose: a single dose of 10 mg/kg bw killed 50% of animals, while no deaths at all were observed in animals receiving 250 mg/kg bw (100 CN mg/kg) per day for 90 days (cumulative dose 9 g CN /kg bw). The results show the dependence of toxicity of cyanides on the rate of supply and an extremely low chronicity (cumulative capacity), as confirmed by many other subchronic studies. In a 13-week- study, male Sprague-Dawley rats were administered potassium cyanide in drinking-water at a dose level of 40, 80, or 160/140 mg/kg body weight per day. These doses correspond to 16, 32, and 64/56 mg cyanide/kg body weight per day (**2**). Histopathological investigation of the brain, heart, liver, testes, thyroid, and kidneys did not reveal adverse effects. Urinary protein excretion was increased in dosed animals, and dose-dependent increases were observed in organ weights; these were interpreted to have arisen from decreased food and water consumption caused by decreased palatability. All rats survived, but there was a dose-dependent loss of body weight, an increase in thyroid weight, and a decrease in blood haemoglobin and serum T4 levels in rats after 14 weeks on a diet containing 5 or 10 g potassium cyanide/100 g diet, corresponding to approximately 800 and 1600 mg cyanide/kg body weight per day(**3**). No effects were noted in Sprague-Dawley rats fed potassium cyanide at concentrations up to 187.5 mg/100 g diet (750 mg cyanide/kg diet) for 56 days. On protein-deficient diets, the lowest body weight gain was obtained at the highest dietary cyanide concentration (**8**). In a 40-week- study in rabbits, the animals were fed potassium cyanide at a level of 1.76 g/kg diet (corresponding to 24–17 mg cyanide/kg body weight per day (**4**). The weight gain of the treated animals was decreased by 33%; at the end of the experimental period, serum urea and creatinine levels were elevated, as were the activities of serum lactate dehydrogenase, sorbitol dehydrogenase, ALAT, and alkaline phosphatase. In a neuropathological study (**5**), goats, 30–45 days old at the beginning of the study, were given potassium cyanide in milk (until weaning) and in drinking-water thereafter at a dose level of 0.3, 0.6, 1.2, or 3.0 mg (0.12, 0.24, 0.48, or 1.2 mg cyanide)/kg body weight per day for 5 months. In a qualitative morphological and immunohistochemical study, presence of gliosis and spongiosis in the medulla oblongata and spinal cord and gliosis in the pons and damage to Purkinje cells in the cerebellum were observed at the highest dose, but no increase in apoptotic cells was reported. Congestion and haemorrhage in the cerebellum were observed at the 0.48 mg cyanide/kg body weight per day group. No quantification or statistical analysis of the findings was presented. In reference (**6**) there are compared the effects of cassava containing linamarin with those of a diet containing an equivalent amount of sodium cyanide in three groups of growing dogs, each comprising six animals, for 14 weeks. One group was fed cassava (gari) as the carbohydrate source, which was expected to release 10.8 mg hydrogen cyanide/kg cooked food; another group was fed on the control diet with rice as the carbohydrate source, to which enough sodium cyanide was added at feeding time to release 10.8 mg hydrogen cyanide/kg cooked food (for both, the daily dose was 1.08 mg hydrogen cyanide/kg body weight). The control group	

was fed this rice diet without added sodium cyanide. Nephrosis and changed plasma free amino acid profile were observed in the sodium cyanide-treated group, while no effect was observed in the plasma glutamyltransferase, ALAT, or isocitrate dehydrogenase activities or in the histology of the liver, kidney, or myocardium. Adrenal hyperplasia and hypertrophy and pancreatic necrosis and fibrosis were observed. (In contrast, the gari diet caused generalized congestion and haemorrhage, periportal vacuolation of the liver, swelling, vacuolation, and rupture of the epithelial cells of the proximal convoluted tubule of the kidney, myocardial degeneration, adrenal gland degeneration, and pancreatic haemorrhage, necrosis, and fibrosis.) A 36% decrease in the serum T3 concentration was noted, together with histological changes of the thyroid consistent with parenchymatous goitre. A significantly reduced frequency of testicular tubules in stage 8 of the spermatogenic cycle as well as marked testicular germ cell sloughing and degeneration were also observed.
In a 2-year- dietary study, weanling albino rats (10 per sex per group) were administered food fumigated with hydrogen cyanide (special jars were used in order to limit volatilization of hydrogen cyanide from the feed) **(9).** The average concentrations of cyanide in the feed were 0, 73, and 183 mg/kg diet, as estimated **(10)** based on the authors' data for concentrations at the beginning and end of each food preparation and by assuming a first-order rate of loss for the intervening period and on the corresponding daily doses of 4.3 and 10.8 mg cyanide/kg body weight per day. No treatment-related effects on survival or growth rate, signs of toxicity, or haematological or histopathological changes in the organs examined (heart, lung, liver, spleen, gastrointestinal tract, kidneys, adrenals, thyroid, testes, uterus, ovaries, cerebrum, cerebellum, and brain) were observed in the treated male or female animals. A NOAEL of 10.8 mg cyanide/kg body weight per day was established.

The effects of cyanide on thyroid function were investigated in groups of 10 male weanling rats fed a semipurified casein-based diet for 11.5 months either supplemented by added methionine, vitamin B_{12}12, and potassium iodide or without added vitamin B_{12}12 and potassium iodide and with the methionine addition restricted to a third. Both dietary groups were divided into three: one served as the control, the second received 1500 mg potassium cyanide/kg, and the third received 2240 mg potassium thiocyanate/kg. The rats given the potassium cyanide would have received doses of 30 mg cyanide/kg body weight per day. Cyanide, but not thiocyanate, caused a consistent reduction in weight gain in the complete and restricted diet fed animals. Both cyanide and thiocyanate caused decreased thyroid gland activity in young rats, particularly in those fed the restricted diet. Depression of both plasma T4 and the T4 secretion rate, suggestive of depressed thyroid function, was found at 4 months, but to a lesser degree after 1 year. At autopsy, the animals were found to have enlarged thyroids, which suggested a mechanism of adaptation **(11).**

In order to study the possible contribution of cyanide exposure to malnutrition-related diabetes mellitus **(4)** fed New Zealand White rabbits potassium cyanide for 10 months (702 mg cyanide/kg diet, corresponding to approximately 20 mg/kg body weight per day). No effects were observed on the serum amylase activity, blood glucose concentration, or the morphology of the pancreas, while degenerative changes were reported in the liver and kidney. Similarly, 1-year- feeding of cassava to rats induced no changes in blood glucose homeostasis or pancreatic histology **(7).**

Because of the small group size and limited exposure time in most of them, these studies are not informative with regard to the possible

	carcinogenicity of cyanides.	
Conclusions	1) Cyanides administered in water or diet caused little toxic effect in doses up to 10 mg/kg body weight per day. 2) Specific deviations in the thyroid and reproduction functions were observed after repeated administration of cyanide daily doses of 1 mg/kg body weight. 3) Summing up the results of short- term and lomg-term studies, the daily doses of 0.1 mg cyanide/kg body weight is the lowest reported NOAEL. Thorough analysis of the selected key study on subchronic oral toxicity of sodium cyanide, and a series of supporting studies justify the use of surrogate data for non feasible repeated dose oral studies with hydrogen cyanide. Exposure to rapidly metabolised and eliminated substances such as inorganic cyanides via drinking water in rodents with food and water ad libitum and regular dark - light cycles is not equivalent to a continuous exposure, but rather to several short exposures per day, with most of the total daily dose received during the dark period of the day.	
Undertaking of intended data submission	No studies are planned.	

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

Section A6.4 **Annex Point IIA VI.6.4**		**SUBCHRONIC TOXICITY**	
Section A6.4.1 **Annex Point IIA VI.6.4**		**Subchronic toxicity oral (13 weeks)**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	NTP. 1993. Sodium Cyanide Administered In Drinking Water to F344/N Rats and B6c3f$_1$ Mice. Toxicology Report Series No. 37. (NIH Publication 94-3386) **(DOC IV_40)**	
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**	The animal studies of sodium cyanide were performed in compliance with U.S. Food and Drug Administration Good Laboratory Practices regulations (21CFR, Part58)	
2.2	**GLP**	The Quality Assurance Unit of Southern Research Institute performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.	
2.3	**Deviations**	/	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Sodium cyanide	
3.1.1	Lot/Batch number	Lot01410ML from Aldrich Chemical Company	
3.1.2	Specification		
3.1.2.1	**Description**	White powder	
3.1.2.2	**Purity**	Initial 99.9%, cumulative at least 98%	
3.1.2.3	**Stability**	Water solutions stable for at least 4 days under animal room conditions	
3.2	**Test Animals**		
3.2.1	Species	Rat, mouse	
3.2.2	Strain	F344/N rats and B6C3F$_1$ mice	
3.2.3	Source	Rats: Taconic Laboratory Animals and Services (Germantown, NY); Mice: Simonsen Laboratories (Gilroy, CA).	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	6 weeks	
3.2.6	Number of animals per group	Base study: 10 per species, per sex, per dose group Supplemental clinical pathology groups: 10 male rats per dose group.	
3.2.7	Control animals	Yes	

3.3	**Administration/ Exposure**	Oral	
3.3.1	Exposure and post exposure period	Exposure for 13 weeks, no post exposure period	
3.3.2	Type	In drinking water	
3.3.3	Doses	The calculated daily doses (in mg NaCN/kg body weight) in individual dose groups were in male rats: 0, 0.2, 0.5, 1.4, 4.5, and 12.5 in female rats: 0, 0.2, 0.5, 1.7, 4.9, and 12.5 in mice: 0, 0.3, 1, 3, 9, and 26 The exposure levels selected for the 13-week studies were based on the results of 2-week drinking water studies conducted by SRI, International. In these studies, male and female rats and mice exposed to sodium cyanide at concentrations greater than 300 ppm had significantly depressed weight gains.	
3.3.4	Vehicle	Deionised, filtered water	
3.3.5	Concentrations in vehicle	0, 3, 10, 30, 100 and 300 mg/L.	
3.3.6	Total volume	Water *ad libitum*	
3.3.7	Controls	Water	
3.4	**Examinations**	Clinical observations, Haematology, Clinical Chemistry , Pathology	
3.4.1	Observations	Twice /day	
3.4.2	Body weight	Prior to the start of the study, prior to sacrifice, and weekly during exposures.	
3.4.3	Food consumption	No	
3.4.4	Ophthalmoscopic examination	No	
3.4.5	Clinical Pathology studies	Blood for haematology and clinical chemistry evaluations was collected on days 5, 25, 45, and 92 from rats in the supplemental clinical pathology study group. Base-study rats were evaluated on days 86 (males) and 93 (females). Urine samples were collected from supplemental rats overnight on days 8, 22, 43, and 88. Blood for haematology and clinical chemistry evaluations was collected from base-study mice on days 89 (males) and 93 (females).	
3.4.6	Haematology	Haematology parameters included hematocrit (Hct), hemoglobin (Hgb) concentration, erythrocyte (RBC) count, reticulocyte count, nucleated erythrocyte count, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count, and leukocyte (WBC) count and differential.	
3.4.7	Clinical Chemistry	Clinical chemistry parameters included urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase (ALT), alkaline phosphatase, creatine kinase (CK), sorbitol dehydrogenase (SDH), 5'-nucleotidase, and total bile acids.	
3.4.8	Urinalysis	Urinalysis parameters included thiocyanate, sorbitol dehydrogenase (SDH), N-acetyl-β-D-glucosaminidase (NAG), ribonuclease, volume, specific gravity, and pH.	
3.5	**Sacrifice and pathology**	Immediately after exposure cessation.	

3.5.1	Organ Weights	Yes	
3.5.2	Gross and histopathology	Gross necropsy in all animals in the base studies and microscopic examination in 0 and 300 ppm groups. The following tissues were examined: adrenal glands, brain (three sections), clitoral glands, esophagus, eyes (if grossly abnormal), femur and marrow, gallbladder (mice only), gross lesions and tissue masses, heart, kidneys, large intestine (cecum, colon, rectum), liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nasal cavity and turbinates (threesections), ovaries, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial glands, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spinal cord/sciatic nerve (if neurologic signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thigh muscle, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only). The liver (males only), spleen, and urinary bladder of rats, the spleen and mammary gland (females only) of mice, and gross lesions in rats and mice were examined in the lower exposure groups.	
3.5.3	Other examinations	Sperm Motility and Vaginal Cytology Evaluations: Sperm motility and vaginal cytology evaluations were performed on base- study animals at the end of the 13-week studies. Animals in the 0, 30, 100, and 300ppm groups were evaluated. Male rats and mice were evaluated for necropsy body and reproductive tissue weights, epididymal spermatozoal data, and spermatogenesis. Females were evaluated for necropsy body weight, estrous cycle length, and the percentage of cycle spent in the various stages.	
3.5.4	Statistics	Organ and body weight data, which are approximately normally distributed, were analysed with the parametric multiple comparisons procedures of Williams or Dunnett. Clinical chemistry, haematology, spermatid, and epididymal spermatozoal data, which typically have skewed distributions, were analysed with the nonparametric multiple comparisons methods.	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
		Results are illustrated in Tables 1, 2, 3 and 4	
4.1	**Clinical signs and mortality**	All rats survived to the end of the study. One control female mouse died during Week9, and a female in the 30ppm group died during Week13; the death of the exposed female was attributed to an ovarian tumour. All male mice survived to the end of the study. No clinical signs were observed that were considered related to exposure to sodium cyanide.	
4.2	**Body weight**	The final mean body weights and mean body weight gains of male rats in the 10 and 300 ppm groups were slightly less than those of the controls, while the final mean body weights and mean body weight gains of exposed and control female rats were similar. The final mean body weights of male and female mice in the 3ppm groups and males in the 30ppm group were slightly greater than those of the controls; the final mean body weight of females exposed to 300ppm was less than that of the control females.	
4.3	**Water consumption and**	Water consumption by males and females in the 100 and 300ppm groups was by more than 10% less than that by the controls. Actual	

	compound intake	compound intakes are illustrated in tables.	
4.3.1	**Blood analysis**	Changes in haematology and clinical chemistry parameters occurred in supplemental and base-study rats in various exposure groups at various time points. In general, these changes were minor and sporadic and were not considered to be clinically significant. Few hematologic or clinical chemistry changes occurred in mice. These changes were minimal and were not considered to be biologically significant.	
4.3.2	**Urinalysis**	Decreases in urine volume and increases in urine specific gravity occurred in supplemental rats in the 300 ppm group at all-time points and in the 100ppm group on Day 8. These changes were consistent with the observed decreases in water consumption and with subsequent decreases in urine output, suggesting a palatability problem with the dosed water. Increases in urinary thiocyanate occurred in rats at all but the 3 and 10ppm exposure levels on Days 22 and 88 and all but the 3ppm exposure level on Day43. Changes in urine pH, sorbitol dehydrogenase, and N-acetyl-β-D-glucosaminidase were minorand not exposure related; these changes were not considered to be clinically significant.	
4.4	**Sacrifice and pathology**		
4.4.1	Gross and histopathology	There were no treatment-related gross or histopathologic lesions in rats of either sex. There were no morphologic differences in the follicle size, colloid staining, or follicular epithelium of the thyroid gland of rats administered sodium cyanide compared to the controls. In histologic sections of the brain, there was no evidence of treatment-related degenerative changes in the corpus callosum. There were no treatment-related gross or histopathologic lesions in mice of either sex.	
4.5	**Other:**	**Reproductive tissues and oestrous cycle characteristics: (See Tables 1 and 2.)** Rats: The left cauda epididymal weights of all groups of exposed males were significantly lower than the control value; left epididymal and testis weights and the number of spermatid heads per testis for males in the 300ppm group were also lower than those of the controls. Sperm motility in all groups of exposed males was less than that in the controls, but these motility changes were not considered to be biologically significant. Female rats in the 100 and 300ppm groups spent more time in proestrus and diestrus and less time in estrus and metestrus than control females. Mice: The left epididymal and cauda epididymal weights of males in the 300ppm group were significantly less than those of the controls. No changes in sperm motility or spermatid head density such as those seen in male rats exposed to 300ppm sodium cyanide occurred in male mice. No significant changes in estrous cycle length occurred in females.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Groups of 10 rats and 10 mice per sex were administered sodium cyanide in drinking water at concentrations of 0, 3, 10, 30, 100, and 300ppm for 13weeks. Concentrations of 100 ppm and greater resulted in reduced water consumption (by 10 – 30%). Thus, higher concentrations of sodium cyanide could not be administered by the drinking water route of administration. Actual daily intakes of sodium cyanide (base study dose group average	

		for weeks 2 – 13 in mg/kg bw) calculated from water consumption data were 0.3, 0.9, 2.7, 8.5 and 23.6 in male rats, 0.3, 1.0, 3.2, 9.2 and 23.5 in female rats, 0.5, 1.8, 5.1, 16.2 and 45.9 in male mice, 0.6, 2.1, 6.2, 19.1 and 54.3 in female mice. The absorption of administered cyanide was confirmed by increases in urinary thiocyanate excretion. Clinical observations, haematological, clinical chemistry evaluation urine analysis, body weight and water consumption measurements were performed in the course of exposures. Organ weights, gross and microscopical findings were evaluated immediately after 13-week exposure. Reproductive parameters incl. sperm motility and vaginal cytology examinations were accomplished on rats and mice in the 0, 30, 100, and 300 ppm groups.	
5.2	**Results and discussion**	No deaths attributed to sodium cyanide administration occurred in either species. In animals exposed to 300ppm, male rats had slightly lower final mean body weights and mean bodyweight gains and female mice had slightly lower final mean body weights than the respective controls, apparently due to lower water intake. **(See Tables 1 and 2.)** No gross or microscopic changes specifically related to cyanide toxicity occurred at any site in males or females of either species. In particular, no lesions were found in the brain or thyroid gland. Differences between absolute and relative organ weights of exposed and control animals were minor and sporadic and were not exposure concentration dependent; these differences were not considered to be related to sodium cyanide administration. Hematologic, clinical chemistry, and urinalysis evaluations of rats and mice revealed minimal changes that were not considered biologically significant; decreased urine volume and increased urine specific gravity observed in male rats in the 300ppm group of the supplemental clinical pathology study were consistent with the observed decreases in water consumption. Sodium cyanide caused a slight reduction in cauda epididymal weight in all groups of exposed male rats (daily doses 1.5 mg CN/kg bw and higher) and in male mice exposed to 300-ppm (daily dose of 14 mg CN/kg bw). In male rats, the number of spermatid heads per testis in the 300ppm group (daily dose of 14 mg CN/kg bw) was less than the number in the controls, and sperm motility in all exposed groups (daily doses 2.5 mg CN/kg bw and higher) was non-significantly lower than in the controls. Sodium cyanide produced no adverse effects on estrous cyclicity in female mice, but at higher concentrations (100 and 300 ppm, daily doses 4.6 and 14 mg CN/kg bw, respectively), sodium cyanide caused an increase in the amount of time spent by female rats in proestrus and diestrus relative to estrus and metestrus. **(See Tables 3 and 4.)**	
5.3	**Conclusion**	Administration of low concentrations of sodium cyanide in drinking water to rats and mice for 13 weeks resulted in no clinically significant body weight, organ weight, histopathologic, or clinical pathology changes.	
5.3.1	LO(A)EL	N/A	
5.3.2	NO(A)EL	The results of the study are artefact caused by experimental design. Hence the proposed LOAELs of 6.9 mg/kg.bw and 14mg/kg .bw for reproductive organs of male rats and male mice respectively are not correctly assigned. Actually the doses used were 12.5 (male rat) and 26 CN (male mice) mg/kg should be considered as NOAELs. Justification why effects on rodent reproductive organs are considered	

	artifacts are explained for data on male rats, Male rats (groups used for histopathological examination) Dose groups mg/kg bw per day NaCN 0 2.7 8.8 23.6 CN 0 1.4 4.5 12.5 Water consumption g/day 24.6 23.0 22.1 20.1 Organ weights mg testis 1.58 1.56 1.52 1.46 epididymis 0.448 0.437 0.425 0.417 epididymis - cauda 0.162 0.150 0.148 0.141 Relative organ weights mg / g bw testis 4.67 4.65 4.50 4.58 epididymis 1.32 1.30 1.26 1.31 epididymis - cauda 0.48 0.45 0.44 0.44 Total number of spermatozoa in the cauda epididymidis. (x 10^6): 100 103 103 100 Number of spermatozoa in the cauda epididymidis. (10^6/g), = total number/weight of cauda epididymidis): 615 684 699 709 Lower body weight in top dose group is probably related to lower consumption of water: the top doses have been selected in preliminary tests as the highest concentration of NaCN that did not significantly inhibit drinking in rats and mice. There is no treatment related difference in relative weights of testis and epididymis. Slightly lower relative weight of left epididymal cauda (max. by 8%) in all treated groups is probably technical artifact (e.g., due to absence of randomization in performing the study) , as indicated by equal or higher number of spermatozoa stored in cauda epididymidis in treated animals compared to controls.	
5.3.3 Other		
5.3.4 Reliability	1	
5.3.5 Deficiencies		

Table 1: Summary of reproductive tissue evaluations in male rats

TABLE C1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Drinking Water Study of Sodium Cyanide[1]

Study Parameters	0 ppm	30 ppm	100 ppm	300 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	338 ± 5	335 ± 5	338 ± 4	319 ± 5*
Left epididymis	0.448 ± 0.006	0.437 ± 0.005	0.425 ± 0.007	0.417 ± 0.005**
Left cauda epididymis	0.162 ± 0.003	0.150 ± 0.004*	0.148 ± 0.004*	0.141 ± 0.003**
Left testis	1.58 ± 0.03	1.56 ± 0.02	1.52 ± 0.02	1.46 ± 0.02**
Spermatid measurements				
Spermatid heads (10^7/g testis)	11.35 ± 0.38	10.88 ± 0.53	10.92 ± 0.37	10.57 ± 0.33
Spermatid heads (10^7/testis)	17.86 ± 0.61	16.94 ± 0.81	16.58 ± 0.63	15.42 ± 0.44*
Spermatid count (10^{-4} mL suspension)	89.28 ± 3.05	84.68 ± 4.03	82.90 ± 3.16	77.10 ± 2.20*
Epididymal spermatozoal measurements				
Motility (%)	94.24 ± 0.58	90.67 ± 1.25*	92.09 ± 0.85*	90.66 ± 1.46*
Concentration (10^6/g cauda epididymal tissue)	615 ± 42	684 ± 40	699 ± 33	709 ± 45

1 Data are presented as mean ± standard error. Differences from the control group for spermatid heads/g testis and spermatozoal concentration are not significant by Dunn's test.
* Significantly different (P≤0.05) from the control group by Dunnett's (necropsy body weight only) or Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

Table 2: Summary of estrous cycle characteristics in female rats

TABLE C2 Summary of Estrous Cycle Characterization in Female F344/N Ratsin the 13-Week Drinking Water Study of Sodium Cyanide[1]

Study Parameters	0 ppm	30 ppm	100 ppm	300 ppm
n	10	10	10	10
Necropsy body weight (g)	193 ± 4	197± 3	195 ± 3	198 ± 4
Estrous cycle length (days)	4.95 ± 0.12	5.10 ± 0.16	4.75 ± 0.11	5.25 ± 0.11
Estrous stages[2] (% of cycle)				
Diestrus	33.3	41.7	38.3	41.7
Proestrus	12.5	7.5	14.2	20.0
Estrus	35.0	35.0	33.3	24.2
Metestrus	18.3	15.8	12.5	14.2
Uncertain diagnoses	0.8	0.0	1.7	0.0

1 Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for necropsy body weight are not significant by Dunnett's test. Differences from the control group for estrous cycle length are not significant by Dunn's test.
2 Evidence suggests that females in the 100 and 300ppm groups differ significantly (P=0.03, Wilk's Criterion) from the control females in the relative length of time spent in estrous stages. Females in these two groups spent more time in proestrus and diestrus and less time in estrus and metestrus than control females.

Table 3: **Survival, body weight, water consumption and compound intake data for rats**

TABLE 3Survival, Body Weight, Water Consumption, and Compound Consumption Data for F344/N Rats in the 13-Week Drinking Water Study of Sodium Cyanide

Dose		Mean Body Weight (grams)			Final Weight Relative to Controls[2] (%)	Average Water Consumption[3] (g/day)	Average Dose[3] (mg/kg/day)
(ppm)	Survival[1]	Initial	Final	Change			
MALE							
0	10/10	121	330	209		24.6	
3	10/10	127	325	199	99	23.9	0.3
10	10/10	126	315	189	96	22.6	0.9
30	10/10	123	330	207	100	23.0	2.7
100	10/10	124	327	203	99	22.1	8.5
300	10/10	121	315	193	95	20.1	23.6
FEMALE							
0	10/10	105	197	91		18.4	
3	10/10	105	195	90	99	17.3	0.3
10	10/10	106	196	90	100	17.6	1.0
30	10/10	105	198	93	101	18.3	3.2
100	10/10	101	196	96	100	15.4	9.2
300	10/10	107	198	91	101	13.5	23.5

1 Number surviving at 13 weeks/number of animals per group.

2 (Dose group mean/control group mean) × 100.

3 Average of individual consumption values for Weeks2-13 for all animals in the base study.

Table 4: Survival, body weight, water consumption and compound intake data for mice

TABLE 4 Survival, Body Weight, Water Consumption, and Compound Consumption Data for B6C3F$_1$ Mice in the 13-Week Drinking Water Study of Sodium Cyanide

Dose		Mean Body Weight (grams)			Final Weight Relative to Controls[2] (%)	Average Water Consumption[3] (g/day)	Average Dose[3] (mg/kg/day)
(ppm)	Survival[1]	Initial	Final	Change			
MALE							
0	10/10	22.5	37.1	14.6		5.7	
3	10/10	21.7	39.2	17.5	106	5.8	0.5
10	10/10	22.3	38.3	16.1	103	5.9	1.8
30	10/10	23.0	39.1	16.1	105	5.5	5.1
100	10/10	21.4	38.7	17.3	104	5.2	16.2
300	10/10	23.1	37.6	14.5	101	4.8	45.9
FEMALE							
0	9/10[4]	18.5	31.1	12.2		5.7	
3	10/10	18.5	33.1	14.6	107	5.7	0.6
10	10/10	17.5	32.0	14.4	103	5.6	2.1
30	9/10[5]	17.4	29.8	12.4	96	5.3	6.2
100	10/10	17.1	31.4	14.2	101	5.0	19.1
300	10/10	17.8	29.0	11.2	93	4.4	54.3

1 Number surviving at 13 weeks/number of animals per group.

2 (Dose group mean/control group mean) × 100.

3 Average of individual consumption values for Weeks2-13 for all animals in the base study.

4 Week of death: 9.

5 Week of death: 13.

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

Section A6.4 Annex Point IIA VI.6.4	**SUBCHRONIC TOXICITY**	

Section A6.4.2 Annex Point IIA VI.6.4	**Subchronic toxicity dermal**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Hydrogen cyanide is a gas at body temperature. The main route of exposure is inhalation, and upon this exposure HCN is classified as highly toxic, its inhalation may be fatal. Dangerous properties of HCN have been known for a long time; upon longer-term exposures, as subchronic exposure, observations of effects on humans and epidemiological studies are of primary significance. **Summary see section III_6.12.** Skin exposure to gaseous HCN is considered a possible route of exposure with poisoning symptoms similar to oral and inhalation exposure but with a longer latency, as indicated by experimental studies or short-term observation and by toxicokinetic data: In subchronic exposures, the difference between oral and dermal route may be considered to be negligible. No subchronic dermal study for HCN or for cyanides has been found in the literature and subchronic tests with laboratory animals seem not to be warranted.	
References	1. Ballantyne B. 1988. Toxicology and hazard evaluation of cyanide fumigation powders. Clin Toxicol 26: 325-335. **(DOC IV_14)** 2. Ballantyne B. 1983b. Acute systemic toxicity of cyanides by topical application to the eye. J Toxicol, Cutan, Ocular Toxicol 2: 119-129 **(DOC IV_16)**	
Undertaking of intended data submission	No studies are planned.	

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

Section A6.4 Annex Point IIA VI.6.4	**SUBCHRONIC TOXICITY**	

Section A6.4.3 Annex Point IIA VI.6.4	**Subchronic inhalation toxicity test**	
Justification:	Hydrogen cyanide is a highly toxic substance by inhalatory exposure for humans and for all species of laboratory organisms. The effects on humans and epidemiological studies are of primary significance. **Summary see section III_6.12.** Medium-term exposures of animals to hydrogen cyanide or acetone cyanhydrin are of supportive value.	
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: Hydrogen cyanide *Peer reviewed* (**DOC IV_2**). 1. Lewis TR, Anger WK, Te Vault RK. 1984. Toxicity evaluation of sub-chronic exposures to cyanogen in monkeys and rats. J Environ Pathol Toxicol Oncol 5:151-163. **Summary in section 6.4.3a (DOC IV_45).** 2. Monsanto Co. (1984a) Three-month inhalation toxicity of acetone cyanohydrin in male and female Sprague-Dawley rats. St. Louis, MO, Monsanto Co. (Report ML-82-143; US EPA/OPTS Public Files No. 878216397). **(DOC IV_46). Summary in section 6.4.3** 3. Monsanto Co. (1985a) Male fertility study of Sprague-Dawley rats exposed by the inhalation route to acetone cyanohydrin. St. Louis, MO, Monsanto Co. (Report ML-82-144; US EPA/OPTS Public Files No. 878216404). **(DOC IV_47)** 4. National toxicology program (1996): Toxicology and carcinogenesis studies of acetonitrile in F344/N rats and B6C3F$_1$ mice (Inhalation studies). NIH publication No 94 – 3363. **DOC IV_49**	
Summaries:	**Cyanogen:** Groups of 30 male albino rats (Charles River) were exposed for 6 months (6 h/day, 5 days/week) by inhalation to 0, 24, or 54 mg cyanogen/m^3 (corresponding to 0, 25, and 56 mg hydrogen cyanide/m^3). There were no effects on haematological or clinical chemistry parameters, gross pathology, or histopathology (liver, kidney, cardiovascular system) attributable to the cyanogen exposure. Body weights were significantly lower in rats exposed to 54 mg cyanogen/m^3 than in the controls **(1).** In an inhalation study, groups of five rhesus monkeys (*Macacca mulatta*) were exposed to 24 or 54 mg cyanogen/m^3 for 6 h/day, 5 days/week, for 6 months. This corresponded to 25 and 56 mg hydrogen cyanide/ m^3. There were no effects on haematological or clinical chemistry parameters attributable to the inhalation exposure. Total lung moisture content was significantly lower in both treatment groups than in control animals **(1).** **Acetone cyanohydrin:** Sprague-Dawley rats (15 per sex and dose level) were exposed to ACH at concentrations of 0, 36, 101, or 204 mg ACH/m^3, 6 h/day, 5 days/week, for 14 weeks. The exposures were equivalent to 0, 11, 32, and 65 mg hydrogen cyanide/m^3 **(2).**There were no treatment-related deaths or significant changes in body weight gain or haematology. Irritation of the nose and eyes was observed, but no more in exposed than in non-exposed animals. A decrease in blood glucose was recorded in high- and mid-	

	exposure females, and a decrease in total serum protein and globulin concentrations was noted in the mid- and low- dosed females. A comprehensive microscopic evaluation of tissues revealed no abnormalities, and no changes in serum T3 or T4 levels were observed. The NOAEL reported from was 204 mg ACH/m^3, corresponding to 65 mg hydrogen cyanide/m^3. This can be estimated to correspond to a daily dose of 15 mg cyanide/kg body weight per day. In the male fertility study, no mortality, clinical signs of toxicity, changes in body weight, or changes in gross necropsy were observed in rats after 48 exposures to up to 202 mg ACH (64 mg hydrogen cyanide)/m^3, 6 h/day, 5 days/week **(3).** Groups of 10 male and 10 female F344/N rats were exposed to 0, 100, 200, 400, 800, or 1,600 ppm (equivalent to 0, 168, 335, 670, 1,340, or 2,681 mg/m^3) acetonitrile by inhalation for 6 hours per day, 5 days per week for 13 weeks (**4**). Six male and three female rats that received 1,600 ppm and one male that received 800 ppm died during the study. At exposure concentrations up to and including 800 ppm, the final mean body weights and body weight gains were generally similar to those of the controls. At 1,600 ppm, body weight gain was lower and the final mean body weights of both males and females were significantly lower than those of the controls. Hypoactivity and ruffled fur were observed during the first week of the study in males receiving 800 ppm and males and females receiving 1,600 ppm. Additional clinical findings in 1,600 ppm males that died during week 1 were ataxia, abnormal posture, and clonic convulsions. Clinical pathology findings included nonresponsive, normocytic, normochromic anaemia in 1,600 ppm males and females and in 800 ppm females, and decreased triiodothyronine (T3) concentrations in 1,600 ppm females. Absolute and relative thymus weights were significantly lower than those of the controls in the 800 and 1,600 ppm males and females. Females exposed to 1,600 ppm had significantly greater absolute and relative heart, kidney, and liver weights than those of the controls. There were no clear exposure-related histopathologic effects, although pulmonary congestion and edema and haemorrhage in the lung and brain were seen in some rats that died early. These lesions are consistent with cyanide-induced anoxia. Groups of 10 male and 10 female B6C3F1 mice were exposed to 0, 100, 200, 400, 800, or 1,600 ppm (equivalent to 0, 168, 335, 670, 1,340, or 2,681 mg/m^3) acetonitrile by inhalation for 6 hours per day, 5 days per week for 13 weeks (**4**). All mice exposed to 1,600 ppm died during the first 3 weeks of the study. In addition, one 400 ppm female and one male and four females from the 800 ppm groups also died before the end of the study. Body weight gains were similar to those of controls for all surviving groups of mice except the 800 ppm males, for which the final mean body weight was slightly lower than that of the controls. Clinical findings observed during the first week in 800 and 1,600 ppm mice were hypoactivity and a hunched, rigid posture. In males that received 200 ppm and above, absolute liver weights were greater than that of the controls and relative liver weights were greater in all exposed groups. In 800 ppm females, the absolute liver weight was greater than that of the controls and relative liver weights of females that received 400 ppm and above were greater than that of the controls. Lesions clearly associated with acetonitrile exposure were observed in the stomach, predominantly the forestomach, of males that received 400 ppm and above and of females that received 200 ppm and above. Histologically, these focal or multifocal pale to dark raised lesions consisted of areas of focal epithelial hyperplasia and ulceration, sometimes associated with hemosiderin deposition. An increased incidence of cytoplasmic vacuolation occurred in the liver of males and females exposed to 400 or 800 ppm. A lack of fatty degenerative change was observed in the X-zone of the adrenal	

	cortex of 800 and 1,600 ppm female mice.	
Conclusion	The subchronic inhalation of HCN in a concentration of 10 mg/m^3 for 6 h/day, 5 days/week was without effect.	
Undertaking of intended data submission	No studies are planned.	

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

Section A6.4 Annex Point IIA VI.6.4		**SUBCHRONIC TOXICITY**	
Section A6.4.3 Annex Point IIA VI.6.4		**Subchronic toxicity inhalation (180 days)**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	T.R. Lewis, W.K. Anger, R.K.Te Vault: Toxicity evaluation of sub-chronic exposures to cyanogen in monkeys and rats. JEPTO 5-4/5:151 – 163, 1984 **(DOC IV_45).**	
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	
2.2	**GLP**	The study from 1984 is not in the GLP system.	
2.3	**Deviations**	/	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cyanogen $(CN)_2$	
3.1.1	Lot/Batch number		
3.1.2	Specification		
3.1.2.1	**Description**	Colourless gas	
3.1.2.2	**Purity**	99% pure	
3.1.2.3	**Stability**		
3.2	**Test Animals**		
3.2.1	Species	Rhesus monkey, albino rats	
3.2.2	Strain	Macacca mulatta, Charles River Sprague-Dawley COBS	
3.2.3	Source	Charles River	
3.2.4	Sex	Male monkeys, male rats	
3.2.5	Age/weight at study initiation		
3.2.6	Number of animals per group	5 monkey per treatment group 30 rats per treatment group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Inhalation	

3.3.1	Duration of treatment	180 days		
3.3.2	Frequency of exposure	6 hr/day, 5 days/week.		
3.3.3	Postexposure period	14 days, 4 weeks or other		
3.3.4	**Inhalation**			
3.3.4.1	**Concentrations**	Nominal concentrations	0 ppm; 11 ppm; 25 ppm	
		Analytical concentrations	≤1ppm; 11.2 ±1.5 ppm; 25.3 ± 3.3 ppm	
3.3.4.2	**Particle size**			
3.3.4.3	**Type or preparation of particles**			
3.3.4.4	**Type of exposure**	Whole body		
3.3.4.5	**Vehicle**			
3.3.4.6	**Concentration in vehicle**			
3.3.4.7	**Duration of exposure**	6hr/day		
3.3.4.8	**Controls**	Sham exposed		
3.4	**Examinations**	Behavioural testing (only in monkeys), Clinical observations, Haematology, Clinical Chemistry, Pathology (gross necropsy and microscopic examination), Moisture in lungs		
3.4.1	Observations	Daily		
3.4.1.1	**Clinical signs**			
3.4.1.2	**Behavioural testing**	1 day per week for 12 monkeys, and 5 days per week for 3 monkeys (one in each of the groups).		
3.4.1.3	**Mortality**	Yes/no		
3.4.2	Body weight	Prior to the start of the study, prior to sacrifice, and at least monthly during exposures to verify growth in rats and deprivation maintenance in monkeys.		
3.4.3	Food consumption	No		
3.4.4	Water consumption	No		
3.4.5	Ophthalmoscopic examination	No		
3.4.6	Haematology	Yes Parameters: haematocrit, haemoglobin concentration. number of animals: each monkey, 6 rats per exposure level time points: monkey – 0 day, 30 days, 90 days, 180 days of exposure rats -2 days, 5 days, 30days, 90 days, 180 days of exposure		
3.4.7	Clinical Chemistry	Yes Number of animals: each monkey, 6 rats per exposure level time points: monkey – 0 day, 30 days, 90 days, 180 days of exposure rats -2 days, 5 days, 30days, 90 days, 180 days of exposure Parameters: T3 and T4		

3.4.8	Urinalysis	No	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes Organs: lungs	
3.5.2	Gross and histopathology	Yes All dose groups (2 days of exposure and again 5 days, 1 months, 3 months, 180 days – rats; 11 monkeys were similarly sacrificed immediately after the termination of exposures, 3 monkey – 4 weeks later) Organs: thyroid, liver, kidneys, spleen, heart, lungs, bone marrow, cerebellum, cerebrum	
3.5.3	Other examinations		
3.5.4	Statistics	ANOVA, non-parametric tests	
3.6	**Further remarks**	ECG in monkeys before exposures and after the last exposure.	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs		
4.1.2	Behavioural testing	There was an increase in response rate in all three groups during the exposure period compared to the baseline period. The mean increase was 20%, 14%, 145% in T-CO, T-11 and T-25 subjects, respectively. The rate changes for each group were evaluated statistically by mean of a randomization test for matched pair. The increase in response rate in the T-25 group was marginally significant. The probability that the rate increases in the T-CO and T-11 groups could have occurred by chance was greater than 0.10. **Results see Table 1**	
4.1.3	Mortality	One (control) monkey died near the start of the exposures from causes unrelated to the experiment. 3 rats (control) died, 1 (11 ppm), 4 (25 ppm) – was not significantly different from change.	
4.2	**Body weight gain**	Mean body weights of rats exposed to cyanogen at 25 ppm was significantly depressed compared to control.	
4.3	**Food consumption and compound intake**	Not reported	
4.4	**Ophtalmoscopic examination**	Not reported	
4.5	**Blood analysis**		
4.5.1	Haematology	No consistent effects **Results see Table 2**	
4.5.2	Clinical chemistry	No effects on T3 uptake and T4 concentration **Results see Table 2**	
4.5.3	Urinalysis	Not available	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Lungs from control animals contained more moisture than lungs from	

		animals exposed to cyanogens gas; the difference was statistically significant in monkeys.	
4.6.2	Gross and histopathology	No effects	
4.7	**Other**		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Cyanogen $(CN)_2$, subchronic (180 days) inhalation toxicity study . A 6 months (6 hr/day, 5 days/week) inhalation exposure was conducted with cyanogens gas using male rhesus monkeys (Macacca mulatta) and male albino rats (Charles River Strain). Fifteen monkeys and 90 rats were divided into three groups of 5 monkeys and 30 rats. One group, the Controls, was exposed to the air; the other two groups were exposed to cyanogene concentrations of 11 or 25 ppm.	
5.2	**Results and discussion**	At the end of the 6 months exposure, there were no differences in hematologic or clinical chemistry (T3, T4) parameters attributable to the inhalation exposure to cyanogens. The electrocardiograms and gross pathologic and histopatologic examinations of the test animals were normal when compared with the control animals. Total lung moisture content was significantly lower in monkeys exposed to either 11 ppm or 25 ppm cyanogens than in control animals; the difference was not significant in rats. This effect indicates irritation of the respiratory tract by inhaled cyanogen. Body weights were lower in cyanogen exposed rats, significantly in rats exposed to 25 ppm probably as a result of respiratory irritation (cyanogen is known to irritate in air concentrations above 10 ppm). There was a doubling of the rate of responding on a variable interval 2.9 min schedule of reinforcement in monkeys exposed to 25 ppm cyanogen, and increases were also seen in the monkeys exposed to 11 and 0 ppm; the increases were transitory as the rate returned to control levels before exposures were terminated.	
5.3	**Conclusion**	Body weights were lower in 25 ppm cyanogen exposed rats. Lower body weights in cyanogen 25 ppm exposed rats result probably from respiratory irritation by inhaled cyanogen (cyanogen is known to irritate in air concentrations above 10 ppm). Behavioral conclusions disregarded as intristically unreliable No other effects were detected.	
5.3.1	LO(A)EL	LOAEL > 25 ppm;	
5.3.2	NO(A)EL	≥ 25 ppm (the highest exposure level used) corresponding approx. to 25 mg HCN/m^3 and to 4.7 mg/kg bw per day	
5.3.3	Other		
5.3.4	Reliability	2	
5.3.5	Deficiencies	The study from 1984 is not in the GLP system	