3.3.2	Way of application	Solution (0.05 ml) of tested or control substance was added to flask containing cell culture in 5 ml of F12 medium. 0 or 1 ml of the S-9 activation preparation was added.	
3.3.3	Pre-incubation time	Cells for testing were obtained from frozen stock aliquots, and incubated for 16 – 24 hours in F12FCM5 medium.	
3.3.4	Other modifications		
3.4	**Examinations**	Cultures were incubated for 5 hours, washed and incubated in F12FCM5 for additional 19 hours, diluted to 1000cells /ml, plated for initial survival assessment and incubated for 7 days. After selection of mutant CHO cells (i.e., 6-TG resistant) the plates were incubated for further 7 days to allow for colony formation. The colonies of selected mutant CHO cells were fixed, stained and counted.	
3.4.1	Number of cells evaluated	The total number of clones on the five plates was determined.	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.1.1	Without metabolic activation	Negative for ACH concentrations 100 – 950 mg/L	
4.1.2	With metabolic activation	Negative for ACH concentrations 100 – 950 mg/L	
4.2	**Cytotoxicity**	Relative post-treatment survival decreased with ACH concentrations from 100% at 100mg/L to 50% at 700 mg/L to 0% at 1000 mg/L.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Acetone Cyanohydrin was evaluated for cytotoxicity in the CHO cell line at doses of >0.3, 1.0, 3.33, 10, 33.3, 100, 333 and 1000 ug/ml of treatment volume both without a metabolic activation (S-9) preparation and with 1, 2, 5 and 10% concentrations (of the total treatment volume) of a metabolic activation (S-9) preparation. Acetone Cyanohydrin was then evaluated in a Preliminary Mutagenicity Assay at dose levels of 100, 500 and 900 ug/ml of treatment volume both without a metabolic activation and with 1, 2, 5 and 10% concentrations of the metabolic activation (S-9) preparation. Acetone Cyanohydrin was then evaluated in the CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay at dose levels of 100, 500, 700, 850 and 950 ug/ml of treatment volume both without metabolic activation and with a 2% concentration of the metabolic activation preparation. Cells for testing were obtained from frozen stock aliquots, and incubated for 16 – 24 hours in F12FCM5 medium. Solution (0.05 ml) of tested or control substance was added to flask containing cell culture in 5 ml of F12 medium. 0 or 1 ml of the S-9 activation preparation was added. Cultures were incubated for 5 hours, washed and incubated in F12FCM5 for additional 19 hours, diluted to 1000cells /ml, plated for initial survival assessment and incubated for 7 days. After selection of mutant CHO cells (i.e., 6-TG resistant) the plates (5 plates per dose) were incubated for further 7 days to allow for colony formation. The colonies of selected mutant CHO cells were fixed, stained and counted. The total number of clones on the five plates was determined.	

		The mutant frequency was calculated by correcting the total number of mutant clones by the cloning efficiency of the cells at the time of mutant selection.	
5.2	**Results and discussion**	Relative post-treatment survival decreased with ACH concentrations: 0, 100, 500, 700, 850, 950, 1000 mg/L: 98, 99, 78, 58, 46, 37, 0 without activation 100, 98, 77, 58, 53, 49, 1 with 2% S-9 The results of the Preliminary Mutagenicity Assay, after critical review, indicated that the 2% concentration of S-9 demonstrated the most pronounced effect of all the concentrations employed, and would be used as the optimum S-9 level in the mutagenicity assay. No significant difference in the frequency of mutations between acetone cyanohydrin treated cells and solvent controls were detected CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay (**see Table**): the dose range included clearly cytotoxic concentrations. The effect of positive controls proves validity of the testing procedure used.	
5.3	**Conclusion**	The results were negative in the CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay according to the criteria of the test protocol.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table: Mammalian cell forward gene mutation assay of Acetone Cyanohydrin (number of mutants per 10^6 "expression survivors")

Treatment	Concentration mg/L	S-9 0	S-9 1%	S-9 2%	S-9 5%	S-9 10%
Solvent		**3.4**	**5.5**	**3.3**	**3.3**	**5.8**
ACH	**100**	**3.4**	**3.9**	**1.6**	**1.5**	**5.0**
	500	**4.1**	**3.3**	**8.2**	**3.4**	**2.5**
	900	**5.5**	**2.2**	**12.8**	**1.5**	**5.8**
EMS	**200**	**313**				
DMN	**100**			**299**		
Solvent		**11.1**		**4.0**		
ACH	**100**	**2.7**		**6.1**		
	500	**3.4**		**5.8**		
	700	**8.3**		**1.7**		
	850	**6.8**		**5.9**		
	950	**0.5**		**0.4**		

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

Section A6.6 Annex Point IIA VI.6.6	**GENOTOXICITY STUDIES**	

Section A6.6.4 Annex Point IIA VI.6.6.4	**In Vivo Mutagenicity Study**	
Justification:	Data on in vivo mutagenity have been found in literature. Data for cyanides have been used as surrogate information.	
Reference:	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. Yamamoto H, Mohanan PV. 2002. Melatonin attenuates brain mitochondria DNA damage induced by potassium cyanide in vivo and in vitro. Toxicology 179:29-36. **(DOC IV_51) Summary in DOC III 6.6.2a.** 2. Friedman MA, Staub J. 1976. Inhibition of mouse testicular DNA synthesis by mutagens and carcinogens as a potential simple mammalian assay for mutagenesis. Mutat Res 37: 67-76S **(DOC IV_55). Summary in DOC IIIA 6.6.4b.** 3. Yamamoto K, Yamamoto Y, Hattori H, et al. 1982. Effects of routes of administration on the cyanide concentration distribution in the various organs of cyanide-intoxicated rats. Tohoku J Exp Med 137: 73-78 **(DOC IV_24)**. 4. Mills EM, Gunasekar PG, Li L, et al. 1999. Differential susceptibility of brain areas to cyanide involves different modes of cell death. Toxicol Appl Pharmacol 156(1):6-16. 5. Osgood C, Sterling D (1991) Dichloroacetonitrile, a by-product of water chlorination, induces aneuploidy in Drosophila. Mutation Research, 261(2):85–91. 6. Monsanto Co. (1983b) In Vivo Bone Marrow Chromosome Study in Rats. St.Louis, MO, Monsanto Co. (Report HL-83-195; US EPA/OPTS Public Files No. 878216400).	
Findings:	No studies were located regarding genotoxic effects in humans after oral or inhalation exposure to cyanide. Increased DNA fragmentation was electrophoretically detectable in mitochondria isolated from the brains of male ddy mice that had received a subcutaneous injection of 2.8 mg CN (as potassium cyanide) per kg (**1**). DNA fragmentation was detected by *in situ* terminal deoxynucleotide transferase nick-end labeling (TUNEL) in the parietal and suprarhinal regions of the motor cortex in mice injected with potassium cyanide (2.4 mg CN/kg/day) for 1–12 days **(4).** A single oral dose of 1 mg CN–/kg as potassium cyanide did not inhibit testicular deoxyribonucleic acid (DNA) synthesis in mice (**2**). Sodium cyanide was a highly effective inducer of germline aneuploidy in *Drosophila* (**5**). No statistically significant increases in the frequency of chromosomal aberrations or changes in mitotic index compared with control values were found in bone marrow cells from four groups of 24 male and 24 female Sprague-Dawley rats administered a single dose of acetone cyanohydrin by oral gavage at levels of 0, 1.5, 5, or 15 mg/kg body weight with preparation intervals of 6, 12, and 24 h post-administration **(6).**	

Conclusion:	The doses reported to increase DNA fragmentation were in the range of oral LD50 values. No genotoxicity was found at lower doses. The dose of 7 mg/kg bw resulted in death of all the 10 rats within 10.3 minutes in another study **(3).** This confirms the conclusion that genotoxic changes are secondary to cellular toxicity.	

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

Section A6.6 Annex Point IIA VI.6.6		**GENOTOXICITY STUDIES**	
Section A6.6.4 Annex Point IIA VI.6.6.4		**In vivo mutagenicity study in mammalian cells**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Hiro-aki Yamamoto, P.V. Mohanan: Melatonin attenuates brain mitochondria DNA damage induced by potassium cyanide in vivo and in vitro Toxicology 179 (2002): 29 - 36 **(DOC IV_51)**	
1.1.1	Data owner	/	
1.1.2	Companies with letter of access	/	
1.1.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline reported. Procedure used is specifically designed to study primary toxic effects of cyanides.	
2.2	**GLP**	No	
2.3	**Deviations**	/	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Potassium cyanide	
3.1.1	Lot/Batch number	/	
3.1.2	Specification	/	
3.1.2.1	**Description**	/	
3.1.2.2	**Purity**	/	
3.1.2.3	**Stability**	Not reported	
3.2	**Study Type**	Brain mitochondria DNA damage in mice injected potassium cyanide.	
3.2.1	Organism/cell type	Twenty four male ddy mice (SLC, Shizuoka), 4 weeks old were administered lethal dose of KCN subcutaneously.	
3.2.2	Deficiencies / Proficiencies	Not applicable	
3.2.3	Metabolic activation system	/	
3.2.4	Positive control	/	
3.3	**Administration / Exposure;**		
3.3.1	Concentrations	Four groups (per 6 animals) received: Gr.1: saline sc; Gr.2: potassium cyanide 7mg/kg bw subcutaneously; Gr.3: potassium cyanide 7mg/kg bw subcutaneously + 20mg/kg bw melatonin intraperitoneally; Gr.4: 20mg/kg bw melatonin intraperitoneally;	

3.3.2	Observed effects	Severe seizures in all animals of group 2 but in none of group 3.	
3.3.3	Material sampling	Crude brain mitochondria fraction (1,000 g supernatant of homogenised brains) was prepared 10 minutes after injection.	
3.3.4	Isolation of brain mitochondrial DNA	100 mg of fresh brains was homogenised with ice cold buffer supplied along with DNA extractor kit; the homogenate was centrifuged at 1,000 g for 1 min at 4 °C.	
3.4	**Examinations**	Agarose gel electrophoresis of Hind II digested mitochondrial DNA.	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**	Potassium cyanide in a lethal dose inflicted damage to mitochondrial DNA in all animals of group 2 but in no animal of group 3.	
4.2	**Cytotoxicity**	Potassium cyanide had severe neurotoxic effects in all animals of group 2 but in no animal of group 3.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The effect of potassium cyanide on mitochondria DNA (mtDNA) in mouse brain was investigated in vivo and in vitro. Young mice were injected subcutaneously potassium cyanide in a dose of 7 mg/kg bw, the same dose and 20 mg/kg bw of melatonin, melatonin alone or saline. Crude mitochondria fraction was prepared from their brains, approximately 10 minutes after injection. Potassium cyanide (0, 0.1, 1.0 or 2.0 mM) was incubated with a crude mitochondria fraction prepared from mouse brain at 37 °C for 60 min.	
5.2	**Results and discussion**	A subcutaneous injection of potassium cyanide (7 mg/kg) caused both brain mtDNA damage and severe seizures and death in mice. The damage of mtDNA and seizures induced by potassium cyanide were abolished by the pre-injection of melatonin (20 mg/kg). When potassium cyanide (0, 0.1, 1.0 or 2.0 mM) was incubated with a crude mitochondria fraction prepared from mouse brain at 37 °C for 60 min, the damage of mtDNA was observed in a concentration-dependent manner. The mtDNA damage was prevented by a co-treatment with melatonin (1.5 mM), a scavenger of hydroxyl radicals (OH). Hydrogen peroxide (1.5 mM) inflicted similar damage to brain mtDNA in the presence of Fe^{2+}. The damage was also abolished by the co-treatment with melatonin. Furthermore, when cyanide (0, 0.1 or 1.0 mM) was incubated with the crude mitochondria fraction prepared from mouse brain, the lipid peroxidation was significantly increased in a concentration-dependent manner. The increased lipid peroxidation was completely inhibited by the co-treatment with melatonin (1.0 mM). The protective effect of melatonin against seizures and mitochondrial damage suggest that reactive oxygen species may play a cardinal role both for mitochondrial DNA damage and acute neurotoxic effect of high dose cyanide.	
5.3	**Conclusion**	Damage of DNA was observed in animals after acutely lethal dose of potassium cyanide. As concluded in the summary of human case study (DOC IIIA 6.12a), minimum lethal absorbed dose of 0.5mg/kg bw and minimum lethal concentration of cyanide in brain of 0.6 mg/kg correspond to molar cyanide concentrations of 0.02 mM. DNA damage in both in vitro and in vivo part of the summarised study was observed at acutely lethal concentrations in tissues.	
5.3.1	Reliability	3	

5.3.2 Deficiencies	Comparison of electrophoretic results does not allow for quantitative evaluation.	

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

Section A6.7 Annex Point IIA VI.6.7	**CARCINOGENICITY STUDY**	
Justification:	Dangerous properties of HCN are well explored, and toxicokinetics as well as biologic mechanism of toxic effects are well understood. As a respiratory poison, free cyanide has high acute toxicity due to inhibiting cytochrome oxidase. Tissue utilization of oxygen is impaired, and, with time, a state of histotoxic anoxia occurs. Cyanide can also inhibit approximately 40 enzymes, including a number of other important metallo-enzymes. The cells critically dependent on oxidative metabolism and limited anaerobic capacity are particularly vulnerable to cyanide intoxication. Cyanide also alters calcium homeostasis and cytosolic calcium ion overload has been implicated as an intracellular mediator of cellular injury during and after anoxic hypoxia. Hydroperoxide generation with subsequent peroxidation of lipids and subsequent changes in structure and function of membranes has been suggested as a possible further mechanism of cyanide toxicity.	
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. J. W. Howard, R. F. Hanzal, Chronic Toxicity for Rats of Food Treated with Hydrogen Cyanide, Hazleton Laboratories, Falls Church, Va., Agricultural and Food Chemistry, Volume 3, April 1955, No.4 **Summary in DOC III_6.7.1a (DOC_42)** 2. National toxicology program (NTP) (1996): Toxicology and carcinogenesis studies of acetonitrile in F344/N rats and B6C3F$_1$ mice (Inhalation studies). NIH publication No. 96 – 3363. **Summary in DOC III_6.7.1b (DOC IV_49)** 3. Doherty PA, Ferm V, Smith RP (1982) Congenital malformations induced by infusion of sodium cyanide in the golden hamster. *Toxicology and Applied Pharmacology,* 64:456–464.	
Findings:	Effects upon repeated or long-term exposures are described from epidemiological studies. No effects, leading to suspicion of HCN carcinogenity, have been described (**see section DOC III_ 6.12**.). US EPA classifies hydrogen cyanide by grade D – i.e., a substance without carcinogenic effects. No thorough long-term experimental investigations into the carcinogenic activity of cyanide were found in the literature. Group size and exposure durations in long-term experimental studies were not sufficient to yield reliable information with regard to carcinogenic potential of cyanides. Chronic study in rats **(1)** did not found increased incidence of tumours in animals fed food fumigated with HCN for two years, but the study was not specifically designed to detect carcinogenicity. **Summary in section DOC III 6.7.1a.** The results of NTP combined chronic toxicity – carcinogenicity study of acetonitrile in rats and mice offer an adequate substitute **(2)**. Extensive two-year inhalation chronic toxicity - carcinogenicity studies with acetonitrile in concentrations 168 – 670 mg/m^3 (rats) and 84 – 335 mg/m^3 (mice) did not identify any significant treatment-related effects on survival, general health, behaviour, body weight or organ weights in either species. Complete necropsy and histological examination gave no evidence of exposure-related non-neoplastic lesions in rats or mice. Non-significantly higher incidence of hepatocellular adenoma and carcinoma in the highest exposure group of male rats was evaluated as equivocal: the	

	incidences remained in the range of historical controls. The relevance of higher incidence of lung tumours in male mice is dubious with respect to increased survival in the highest exposure group of males, and to extremely wide range of historical controls. The incidence of hepatocellular adenoma or carcinoma in male mice was not a monotonous function of exposure concentration. **Summary in section DOC III 6.7.1b.** Acetonitrile is not classified as human carcinogen: the evidence in animals is equivocal in spite of the exceptionally extensive testing and thorough analysis. Experimental investigations of the genetic toxicity of cyanide have yielded conflicting results, but overall, cyanide does not appear to have significant mutagenic or clastogenic activity. The dose – response regressions for both the acute and chronic effects of cyanides are extremely steep. Medium- and long-term experimental studies indicate no significant organ pathology or developmental injury at doses below acutely toxic level. A study described in the section on teratogenic effects (**DOC III_6.8.1**) illustrates an extremely narrow range of effective cyanide doses administered in a continuous subcutaneous infusion for 3 days during gestation: teratogenic effects were observed only between doses of 3.28 to 3.37 mg cyanide/kg per hour (79 to 81 mg/kg per day), while a slightly higher dose of 3.5 mg cyanide/kg per hour caused maternal deaths **(3).**	
Conclusion	Extremely narrow interval between the lowest effective doses and lethal doses of cyanides renders a carcinogenity study not feasible. Even if such study would be attempted, the interpretation of the results could not eliminate the possibility that the effects are secondary to acute cell injury or destruction resulting from unavoidable cyanide concentration peaks. No observations in human subjects, performed during the long time HCN have been used, indicate it may be a cause of cancer occurrence. No new facts – from experimental toxicological, toxic kinetic or metabolic studies, structure/activity analyses - point to the necessity to perform a carcinogenity study in laboratory animals.	
Undertaking of intended data submission	No studies are planned.	

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

Section A6.7 Annex Point IIA VI.6.7		**CARCINOGENICITY STUDIES**	
Section A6.7 Annex Point IIA VI.6.7		**Carcinogenicity study (oral)**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	J. W. Howard, R. F. Hanzal, Chronic Toxicity for Rats of Food Treated with Hydrogen Cyanide, Hazleton Laboratories, Falls Church, Va., Agricultural and Food Chemistry, Volume 3, April 1955, No.4 **(DOC IV_42)**	
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**		
		3 MATERIALS AND METHODS	
3.1	**Test material**	HCN	
3.1.1	Lot/Batch number	Not reported	
3.1.2	Specification	Not reported	
3.1.2.1	**Description**	Hydrogen cyanide was generated by the action of sulphuric acid on sodium cyanide aeroids (supplied by the American Cyanamid Co.)	
3.1.2.2	**Purity**	Not reported	
3.1.2.3	**Stability**		
3.2	**Test Animals**		
3.2.1	Species	Albino rat	
3.2.2	Strain		
3.2.3	Source	Carworth Farm	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Male: 58g Female: 55g	
3.2.6	Number of animals per group	10 males and 10 females per group (one control and two experimental groups were used in the study)	

3.2.6.1	At interim sacrifice	No	
3.2.6.2	**At terminal sacrifice**	12 rats of the control group 12 rats at the 100 ppm group 15 rats at the 300 ppm group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral in food	
3.3.1	Duration of treatment	104 weeks	
3.3.2	Interim sacrifice(s)	No	
3.3.3	Final sacrifice	After 104 weeks	
3.3.4	Frequency of exposure	Daily	
3.3.5	Post exposure period	No	
3.3.6	Type	**Oral** In food – commercially available dog meal treated with HCN vapours. The food was prepared fresh every 2 days and analysed for its initial hydrogen cyanide content. The content of HCN in food markedly dropped after 48 hours (to about one third). Food ad libitum	
3.3.7	Concentration		
3.3.8	Vehicle	Food	
3.3.9	Concentration in vehicle	Target concentration in food was 100 and 300 ppm, average initial content 106 and 301 ppm.	
3.3.10	Total volume applied	Food ad libitum.	
3.3.11	Controls	Plain diet	
3.4	**Examinations**		
3.4.1	Number of animals	Initially – number of animals not reported At the end of study - all surviving animals	
3.4.2	Time points	Initially and at termination of the study	
3.4.3	Parameters	Not reported	
3.4.4	Body weight	Yes	
3.4.5	Food consumption	Yes	
3.4.6	Water consumption	No	
3.4.7	Clinical signs	Yes	
3.4.8	Macroscopic investigations	Yes	
3.4.9	Ophthalmoscopic examination	No	
3.4.10	Haematology	Yes	

		Cyanide and thiocyanate content in plasma and red blood cells	
3.4.11	Clinical Chemistry	No	
3.4.12	Urinalysis	No	
3.4.13	**Pathology**	Yes	
3.4.13.1	**Organ Weights**	Yes; all surviving animal Liver, kidneys, adrenals, spleen, brain, heart, ovaries, testes,	
3.4.14	**Histopathology**	Yes; all surviving animals Thyroid, stomach, small and large intestines, liver, kidneys, adrenals, spleen, heart, lungs, uterus, testes, ovary, cerebrum, cerebellum of the brain	
3.4.15	Other examinations	Cyanide and thiocyanate content in liver and kidneys	
3.5	**Statistics**	Not reported	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Body weight**	The growth curves were almost identical for the three groups of males throughout the 104-week feeding period. The females showed considerable variation. The control group exhibited an abnormal rise after 91 weeks of feeding. The variation at the dose 100 ppm was due to tumour development in one rat (died after 78 weeks) and general senility with weight loss of two rats. The 300 ppm level appeared to be of normal nature.	
4.2	**Food consumption**	The food consumption data indicate that the intake of the experimental rats was comparable to that of the control rats.	
4.3	**Water consumption**	Not performed	
4.4	**Clinical signs**	During the 2 years of feeding no gross signs of cyanide toxicity were observed.	
4.5	**Macroscopic investigations**	Autopsies revealed the same general abnormalities and signs of senility in the control and experimental rats: pale, granular, and thickened livers, congestion of medulla of the kidney, abnormally small spleens, enlarged adrenals, atrophies, encysted and inflamed genital organs, and enlarged, hemorrhagic pituitaries. Many nodes and tumours were found throughout the viscera. Infection of the ears was also evidenced.	
4.6	**Ophthalmoscopic examination**	Not performed	
4.7	**Haematology**	No effect	
4.8	**Clinical Chemistry**	Not performed	
4.9	**Urinalysis**	Not performed	
4.10	**Pathology**	No effect	
4.11	**Organ Weights**	No effect	
4.12	**Histopathology**	No effect	

4.13	**Other examinations**	Content of cyanide and thiocyanate in tissue (in γ per 100 grams or ml): **Control group:** Cyanide was absent in all tissue. Thiocyanate – average values: plasma – 361 red blood cells– 73 liver – 566 kidneys – 577. **100 ppm group:** Cyanide was absent in plasma, liver, kidneys. Red blood cells – 5.40 Thiocyanate – average values: plasma – 936 liver – 719 kidney – 1023 **300 ppm group:** Cyanide was absent in plasma, kidneys. Liver – only in one rat Red blood cells (50% rats) – 1.97 Thiocyanate – average values: plasma – 1123 red blood cells - 246 liver – 665 kidney – 1188	
4.14	**Time to tumours**	Not recorded	
4.15	**Other**		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Non-guideline study The study of the chronic toxicity to rats of food fumigated with hydrogen cyanide.	
5.2	**Results and discussion**	Food containing 100 and 300 ppm of HCN produced no noticeable signs of cyanide toxicity. Haematological values determined initially and at the termination of the study, were within normal limits. Neither gross nor microscopic examination of the tissues revealed evidence of pathology due to the HCN feeding. No free cyanide was found in the plasma, liver or kidneys of the rats sacrificed in the 100 ppm group. In most instances cyanide was found in the red blood cells. These four tissues showed a definite rise in thiocyanate content over those of the control. Cyanide was not found in the plasma or kidneys of rat at the 300 ppm level. It was found in the liver of one rat and in the red blood cells of less than 50% of the rats in this group. Definite rises in thiocyanate were found in all four tissues. This study showed that a diet containing 100 or 300 ppm of HCN as a result of fumigation is non-toxic to male and female albino rats over a 2-year period.	
5.3	**Conclusion**	Feeding of food containing 100 and 300 ppm of hydrogen cyanide produced no signs of cyanide toxicity during a 2-year- feeding period. At termination haematological values were within normal limits and neither gross nor microscopic examination of tissues revealed evidence of pathology due to hydrogen cyanide feeding. At mean food consumption of 15 – 18 g per day the average daily dose of HCN in 100ppm and 300ppm group animals was about 3 and 10 mg/kg bw, respectively.	
5.3.1	Reliability	3	
5.3.2	Deficiencies	Detailed findings and type and frequency of tumours not reported. No statistical evaluation.	

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

Section A6.7 Annex Point IIA VI.6.7		**CARCINOGENICITY STUDIES**	
Section A6.7 Annex Point IIA VI.6.7		**Carcinogenicity Study (Inhalation)**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	NTP (1996): Toxicology and carcinogenesis studies of acetonitrile in F344/N rats and B6C3F$_1$ mice (Inhalation studies). NIH publication No 96 – 3363. **DOC IV_49**	
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**	OCDE guideline 1996	
2.2	**GLP**	Yes (NTP, 1996)	
2.3	**Deviations**	/	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Acetonitrile vapours	
3.1.1	Lot/Batch number		
3.1.2	Specification	Not reported	
3.1.2.1	**Description**	Volatile liquid with boiling temperature of 82 °C	
3.1.2.2	**Purity**	≥ 99 %	
3.1.2.3	**Stability**		
3.2	**Test Animals**		
3.2.1	Species	Albino rat Mice	
3.2.2	Strain	Fischer 344 rats B6C3F1 mice	
3.2.3	Source		
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation		
3.2.6	Number of animals per group	12 male and 12 female rats per group 15 male and 15 female mice per group (one control and three experimental groups were used in the study)	

3.2.6.1	**At interim sacrifice**	4 male and 4 female rats and 5 male and 5 female mice from each dose group for the 15-months interim evaluation	
3.2.6.2	**At terminal sacrifice**	2x8 rats of the control group 2x8 rats of the 100 ppm group 2x8 rats of the 200 ppm group 2x8 rats of the 400 ppm group 2x10 mice of the control group 2x10 mice of the 50 ppm group 2x10 mice of the 100 ppm group 2x10 mice of the 200 ppm group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	103 weeks	
3.3.2	Interim examination and sacrifice(s)	15 months: haematology, weight of liver, lungs and kidney	
3.3.3	Final sacrifice	After 103 weeks	
3.3.4	**Inhalation**		
3.3.4.1 Concentrations		Rats: 0, 100, 200, 400 ppm = 0, 168, 335, 670 mg/m^3 Mice: 0, 50, 100, 200 ppm = 0, 84, 168, 335 mg/m^3 The doses selected for the 2-year study of acetonitrile in rats were based on reduced survival of 800 ppm males and 1,600 ppm males and females in the 13-week study. The exposure concentrations selected for the 2-year study in mice were based on reduced survival and gross and histopathologic lesions in 400, 800, and 1,600 ppm groups of male and female mice in the 13-week study.	
3.3.4.2 Type of exposure		Whole body	
3.3.4.3 Duration of exposure		6 h/day, 5 d/week, 103 weeks	
3.4	**Examinations**		
3.4.1	Body weight	Weekly for the first 13 weeks, thereafter at 4-week- intervals, at two-week- intervals for the last 13 weeks.	
3.4.2	Food consumption	Yes	
3.4.3	Water consumption	No	
3.4.4	Clinical signs	Yes, twice daily	
3.4.5	Macroscopic investigations	Yes	
3.4.6	Ophthalmoscopic examination	No	
3.4.7	Haematology	Yes: haematocrit, haemoglobin, erythrocyte count and volume	
3.4.8	Clinical Chemistry	No	
3.4.9	Urinalysis	No	

3.4.10	Gross pathology	Yes	
3.4.11	Organ Weights	Yes	
3.4.12	Histopathology	Complete histopathological examination in all animals. Organs examined: Tissue masses with regional lymph nodes, bones, skin, thyroid, adrenals, parathyroid, pituitary, thymus, bone marrow, salivary gland, oesophagus, stomach, small and large intestines, liver, kidneys, spleen, heart, nose, larynx, lungs, trachea, uterus, mammary gland, ovary, testes, prostate, seminal vesicle, urinary bladder, brain	
		4 RESULTS AND DISCUSSION	
4.1	**Body weight**	No treatment related effects	
4.2	**Clinical signs and survival**	The survival of male mice of the highest exposure group had significantly higher survival than controls. No other treatment related effects.	
4.3	**Macroscopic investigations**	No treatment related effects	
4.4	**Ophthalmoscopic examination**	Not performed	
4.5	**Haematology**	Haematological examination in rats has shown slightly lower haemoglobin levels and erythrocyte volume in the highest exposure groups.	
4.6	**Clinical Chemistry**	Not performed	
4.7	**Urinalysis**	Not performed	
4.8	**Gross Pathology**	No treatment related effects	
4.9	**Organ Weights**	No treatment related effects	
4.10	**Histopathology**	There was no evidence of significant exposure-related non-neoplastic lesions in rats or mice. In **rats**, there were non-significantly higher incidences of hepatocellular adenoma (0 - 2 - 2 - 6%, range of historical controls 0 - 8%) and hepatocellular carcinoma (2 - 0 - 0 - 6%, historical controls 0 - 4%) in the highest exposure group of males. In addition, male rats exhibited an increased incidence of basophilic foci in liver that was statistically significant in the 335 and 670 mg/m^3 groups. The foci were not atypical in appearance, as are those considered to be preneoplastic. NTP concluded that "a causal relationship between acetonitrile exposure and liver neoplasia in male rats is uncertain." The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in male **mice** of the highest exposure group (42%) was significantly higher than in the control group, and at the upper limit of the range of historical controls (10 - 42%). In contrast, in females the incidence was inversely related to concentration, and incidence in controls was at the upper limit of the range of historical controls. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly higher in male mice exposed to a concentration of 168 mg/m^3 (61%) compared to the control group, but in male mice exposed to a concentration of 335 mg/m^3 it was even lower than incidence in the control group. Focal hyperplasia of for stomach was observed in male and female mice exposed to acetonitrile, the incidence being significantly higher	

		compared to controls in males exposed to 335 mg/m^3 and females exposed to 168 or 335 mg/m^3. The severity of lesions ranged from minimal thickening of the stratum spinosum accompanied by increased number of basal cells, to marked epithelial thickening and folding with focal ulceration. The incidence of squamous cell papillomas was not significantly different compared to controls and remained in the range of historical controls.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	**2-year study in rats**: The doses selected for the 2-year study of acetonitrile were based on reduced survival of 800 ppm males and 1,600 ppm males and females in the 13-week study. Groups of up to 56 male and 56 female rats were exposed to 0, 100, 200, or 400 ppm (equivalent to 0, 168, 335, or 670 mg/m^3) acetonitrile by inhalation for 6 hours per day, 5 days per week for 2 years. Four male and four female rats from each exposure group were evaluated at 15 months for histopathology and haematology parameters. **2-year study in mice:** The exposure concentrations selected for the 2-year study were based on reduced survival and gross and histopathologic lesions in 400, 800, and 1,600 ppm groups of male and female mice in the 13-week study. Groups of 60 male and 60 female mice were exposed to 0, 50, 100, or 200 ppm (equivalent to 0, 84, 168, or 335 mg/m^3) acetonitrile by inhalation for 6 hours per day, 5 days per week for 2 years. Five male and five female mice from each exposure group were evaluated at 15 months for histopathology.	
5.2	**Results and discussion**	**2-year study in rats** Survival, Body Weights, Clinical Findings, and Haematology: Two-year survival, mean body weights, organ weights, behaviour, general health, and appearance of exposed male and female rats were similar to those of the controls. The hematologic effects observed were minor and of no biological significance: slightly lower haemoglobin levels and erythrocyte volume in the highest exposure groups. Pathology Findings: The incidences of hepatocellular adenoma (3/48), hepatocellular carcinoma (3/48), and hepatocellular adenoma or carcinoma (combined; 5/48) were greater in male rats exposed to 400 ppm than in the controls (one carcinoma). The incidences of hepatocellular adenoma and hepatocellular carcinoma were within the range of historical controls. However, the incidence of hepatocellular adenoma or carcinoma (combined) slightly exceeded the range of historical controls. In addition, the incidences of basophilic, eosinophilic, and mixed cell foci in 400 ppm males were marginally greater than in controls, suggesting hepatotoxicity of acetonitrile. There were no exposure-related liver lesions in female rats. **2-year study in mice** Survival, Body Weights, and Clinical Findings: Two-year survival of exposed male and female mice was similar to that of the controls, except that the survival of male mice in the 200 ppm group was significantly greater than that of the controls. Mean body weights and organ weights of exposed groups of male and female mice were similar to those of the controls, and no clinical observations in any group were clearly related to acetonitrile exposure. Pathology Findings: There were no increases in the incidences of neoplasms that were considered related to acetonitrile exposure in mice. The incidence of squamous hyperplasia of the epithelium of the forestomach was significantly increased at 15 months in 200 ppm females. At 2 years, the increased incidence of this lesion was dose	

		related in all exposed groups of males and females. The relevance of higher incidence of lung tumours in male mice is dubious with respect to increased survival in the highest exposure group of males, and to extremely wide range of historical controls (10 – 42%). In contrast, in females the incidence was inversely related to concentration, and incidence in controls was at the upper limit of the range of historical controls. The incidence of hepatocellular adenoma or carcinoma in male mice was not a monotonous function of exposure concentration.	
5.3	**Conclusion**	There was only equivocal evidence of carcinogenic activity of acetonitrile in male F344/N rats based on marginally increased incidences of hepatocellular adenoma and carcinoma. There was no evidence of carcinogenic activity of acetonitrile in female F344/N rats exposed to 100, 200, or 400 ppm. There was no evidence of carcinogenic activity of acetonitrile in male or female B6C3F1 mice exposed to 50, 100, or 200 ppm. Exposure to acetonitrile by inhalation resulted in increased incidences of hepatic basophilic foci in male rats and of squamous hyperplasia of the forestomach in male and female mice.	
5.3.1	Reliability	*1*	
5.3.2	Deficiencies	No relevant identified.	

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

Section A6.8 **Annex Point IIA VI.6.8**	**REPRODUCTIVE TOXICITY**	

Section A6.8.1 **Annex Point IIA VI.6.8.1**	**Teratogenicity Tests**	
Other existing data [x]	Technically not feasible [ ] Scientifically unjustified [ ]	
Limited exposure [ ]		
Justification:	Dangerous properties of HCN are well explored. Effects upon repeated or long-term exposures are described from epidemiological studies. No effects, leading to suspicion of HCN toxic effects for reproduction, have been described. As no teratogenicity studies were located for HCN, studies on cyanides or cyanide producing diets are used as replacement (**summaries see sections 6.8.1a,b,c).**	
Supporting studies **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. Tewe OO, Maner JH. 1981b. Performance and patho-physiological changes in pregnant pigs fed cassava diets containing different levels of cyanide. Res Vet Sci 30:147-151. **(DOC IV_62)** **2.** Monsanto Co. (1983) Range finding teratology study in the rat. St. Louis, MO, Monsanto Co. (Report IR-83-094; US EPA/OPTS Public Files No. 878216393). **(DOC IV_58)** 3. Monsanto Co. (1983a) Teratology study in rats. St. Louis, MO, Monsanto Co. (Report IR-83-105; US EPA/OPTS Public Files No. 878216401) **(DOC IV_59). Summary see section 6.8.1c.** 4. Benito Soto-Blanco, Silvana L. Go´rniak (2004).Prenatal toxicity of cyanide in goats—a model for teratological studies in ruminants. Theriogenology 62: 1012–1026 **(DOC IV_60)**. **Summary see section 6.8.1a.** 5. Altamir Benedito de Sousa, Paulo C´esar Maiorka, Ivair Donizete Goncalves, L´ılian Rose Marques de S´a, Silvana Lima G´orniak (2007) Evaluation of effects of prenatal exposure to the cyanide and thiocyanate in Wistar rats. Reproductive Toxicology 23: 568–577 **(DOC IV_38) Summary see section 6.8.1b.** 6. Doherty PA, Ferm V, Smith RP (1982) Congenital malformations induced by infusion of sodium cyanide in the golden hamster. Toxicology and Applied Pharmacology, 64:456–464. 7. Frakes RA, Sharma RP, Willhite CC, Gomez G (1986b) Effect of cyanogenic glycosides and protein content in cassava diets on hamster prenatal development. Fundamental and Applied Toxicology, 7:191–198.	
Findings:	Preliminary experiments with pregnant Golden Syrian hamsters showed that at a dose rate of 0.125 mmol/kg body weight per hour (subcutaneous infusion), no effects on the foetus were observed, while at a dose rate of 0.133 mmol/kg body weight per hour or more, there was a 100% resorption rate and maternal deaths. Toxicity to dams increased with increasing dose levels and included shortness of breath, incoordination, reduced body temperature, and loss of body weight. Co-administration of thiosulfate eliminated the teratogenic effect, protecting the dams and	

	foetuses from the toxic effects of cyanide. In a follow-up experiment, pregnant Golden Syrian hamsters (5–7 animals per group) were continuously exposed to sodium cyanide from day 6 to day 9 of gestation at 0, 0.126, 0.1275, or 0.1295 mmol/kg body weights per hour by using osmotic minipumps implanted subcutaneously. These doses are equivalent to 0, 3.28, 3.32, and 3.37 mg cyanide/kg body weight per hour or 0, 78.7, 79.6, and 80.9 mg/kg body weight per day. The treatment induced a remarkable increase in resorption as well as fetal malformations. These included non-closure of the neural tube, exencephaly, encephalocoele, and malformations of the heart and limbs or tail **(6).** **Comment:** Teratogenic effects were observed in an extremely narrow range of doses between 3.28 and 3.37 mg cyanide/kg per hour (79 to 81 mg/kg per day), while a slightly higher dose of 3.5 mg cyanide/kg per hour caused maternal death. Pregnant Wistar rats (10 animals per group) were fed a cassava diet liberating 21 mg hydrogen cyanide/kg diet, fortified with 500 mg potassium cyanide/kg diet, throughout gestation and lactation. This is equivalent to an estimated daily dose of 16 mg cyanide/kg body weight. No effects were observed on the number, mortality at birth or body weight of offspring or weight gain of pups during lactation (**6**). In equivalent studies with pregnant Yorkshire pigs, three groups of six animals were given potassium cyanide in the diet (30.3, 277, or 521 mg cyanide/kg diet) throughout gestation. No effects were seen on the number or weight of offspring or subsequent lactational performance. Pregnant sows treated at the highest dose level had proliferative changes in the kidney glomeruli and increased thyroid weights. Fetal spleen to body weight and head to body weight ratios in the high cyanide group were significantly reduced ($P < 0.05$) compared with the low-cyanide exposed group (**1); see tables 1 and 2 below**. Groups of pregnant hamsters were fed diets consisting of two types of cassava meal, either a "low-cyanide" (sweet cassava meal) or a "high-cyanide" (bitter cassava meal) variety. These were mixed (80:20 with laboratory chow and administered on days 3–14 of gestation. The cyanide concentration of the sweet cassava meals was 0.6–0.7 mmol/kg; that of the bitter cassava meal was 5–11 (mean 7.9) mmol/kg. Cassava fed dams gained significantly less weight than did control animals (fed diet similar in nutritional value as cassava, but without cyanogenic glycosides), and their offspring showed evidence of fetotoxicity (reduced fetal body weight and reduced ossification of sacrocaudal vertebrae, metatarsals, and sternebrae). The bitter cassava also produced a significant increase in the number of runts compared with litters from dams fed either low-protein or laboratory-stock diets. (**7**) (Low-protein diet was formulated to simulate the cassava diet with a low (4%) protein content, in contrast to the 25% protein content in the standard laboratory diet.) The only teratogenic effects noted were hydrocephalus in three animals in the low-cyanide (sweet cassava) test group and one encephalocoele found in one animal in the highcyanide (bitter cassava) test group (**7).** In a teratogenicity study, hamsters were given a single oral dose of linamarin (0, 70, 100, 120, or 140 mg/kg body weight, corresponding to 0, 7.4, 11, 13, or 15 mg cyanide/kg body weight per day) on day 8 of the pregnancy. The animals were killed on day 15 of the pregnancy, and the numbers of resorption sites, dead fetuses, and living fetuses were recorded. Living fetuses were examined for gross external malformations and for internal malformations using histopathological methods. Linamarin had no effect on fetal body weight, ossification, embryonic mortality, or litter size. At the two highest doses, which caused overt	

	maternal toxicity (dyspnoea, hyperpnoea, ataxia, tremor, and hypothermia), vertebral and rib anomalies and encephalocoeles were observed (**7**). **Comment:** If the hamsters weighed 110 g at the beginning of the experiment, this would lead to a daily dose of cyanide of 1 and 15 mg/kg body weight in the sweet and bitter cassava groups, respectively. A single oral dose of D,L-amygdalin at gestational day 8 resulted in exencephaly, encephalocoele, and skeletal malformations at doses of ≥250 mg/kg body weight (≥14 mg cyanide/kg body weight) in hamsters (these doses were also clearly toxic to the mothers). At the lowest dose tested, 200 mg/kg body weight (11 mg cyanide/kg body weight), fused ribs were observed in two offspring of one mother (maternal toxicity not reported). Encephalocoele and rib anomalies were also observed after a dose of prunasin (177 mg/kg body weight [16 mg cyanide/kg body weight]) in the absence of maternal toxicity in hamsters. No teratogenic effects were noted when hamsters received D,L-amygdalin (275 mg/kg body weight [16 mg cyanide/kg body weight]) intravenously. The teratogenic effects found were considered to be due to cyanide released by bacterial β-glucosidase in the gastrointestinal tract. Groups of 25 pregnant Sprague-Dawley rats were dosed by gavage on days 6–15 of gestation with 0, 1, 3, or 10 mg acetone cyanohydrin (ACH) /kg body weight (equivalent to 0, 0.3, 0.9, or 3 mg cyanide/kg body weight). Maternal toxicity was evidenced by slight reductions in body weight gain in the mid- and high-exposure groups, and statistically significant differences between the high-dose group and controls were found for the number of corpora lutea implantations per dam. There were no comparable differences in the number of viable fetuses per dam, post-implantation losses per dam, mean fetal body weight, or fetal sex distribution for all dose groups and the controls. The incidences of fetal malformations and developmental variations for all fetuses of treated animals and controls were also comparable. It was concluded that 10 mg ACH/kg body weight (3 mg cyanide/kg body weight) was not teratogenic in the rat in the presence of maternal toxicity (**ref.2, 3).** Twenty six pregnant goats were allocated into four groups and were administered KCN doses of 0, 1, 2 or 3 mg/kg bw per day orally – in two doses by gavage. Exposure lasted from Day 24 (period of implantation) to parturition (Day 150 in average). Clinical signs of acute toxicity were observed in dams of the top dose group. There were no treatment-related effects on maternal mortality (zero in all groups), body weight, length of gestation, and number of live kids per litter, on birth weight, weight curve in first 3 postnatal months and on plasma glucose. T3 concentrations higher compared to controls were found in the top dose dams and kids at the birth day, but not at postnatal day 7. No treatment related differences were in concentration of T4. No treatment related gross abnormalities were observed in kids. In conclusion, treatment related effects were detected only in top dose dams and offspring. Dams of this group displayed clear signs of acute neurotoxicity and thyrotoxicity and corresponding histopathological findings in thyroids and white matter of CNS **(4).** 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 or changes in the postnatal development of the offspring, no treatment related effects on body weight, number of corpora lutea, preimplantation loss, postimplantation loss, fetal and placental weight, fetal length and skeletal variations were observed in	

	KCN or KSCN treated groups. Visceral abnormalities were significantly more frequent in fetuses of KCN top dose dams compared to controls. 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 **(5).**	
Conclusions	All studies permitting precise estimates of cyanide doses administered concur in a conclusion that teratogenity is limited to cases of severe maternal toxicity. Oral exposure in drinking water or by gavage does not guarantee an even time distribution of cyanide intake: namely rats on dry diet are known to drink in bouts that may lead to short but high concentration peaks of cyanide in blood.	
Undertaking of intended data submission	No studies are planned.	

Table 1: Body and organ weights of exposed mothers and their fetuses (from: Tewe OO, Maner JH. 1981b. Performance and pathophysiological changes in pregnant pigs fed cassava diets containing different levels of cyanide. Res Vet Sci 30:147-151. (**DOC IV_41**))

TABLE 3: Body and tissue weight of slaughtered gilts and their fetuses

	Experimental variables			
	No added cyanide	250 ppm added cyanide	500 ppm added cyanide	Standard error of difference between means
Gilts				
Body-weight (kg)	177·2	183·5	170·8	145
Body-weight gain during gestation	42·4	41·0	41·7	0·48
Thyroid (g/100 kg body-weight)	5·52	7·44	7·98	1·29
Spleen (g/100 kg body-weight)	0·98	1·15	0·93	0·2
Liver (g/100 g body-weight)	0·97	0·80	1·14	0·24
Kidney (g/100 g body-weight)	0·19	0·17	0·19	0·06
Heart (g/100 g body-weight)	0·26	0·25	0·28	0·07
Fetuses				
Number of fetuses per litter	11	10	8	0·71
Weight of fetus (g)	997·5	934·0	931·5	3·5
Thyroid (g/kg body-weight)	$0·54^{a}$	$0·36^{b}$	$0·52^{a}$	0·18
Spleen (g/kg body-weight)	$0·91^{a}$	$0·84^{a}$	$0·72^{b}$	0·03
Liver (g/100g body-weight)	2·82	2·86	2·50	0·25
Kidney (g/100 g body-weight)	0·66	0·65	0·64	0·18
Heart (g/100 g body-weight)	$0·79^{a}$	$0·73^{ab}$	$0·71^{b}$	0·115

Samples were collected from slaughtered gilts on the 110th day of gestation
a, b, Means with common superscripts in horizontal rows are not significantly different (P>0·05)

Table 2: Litter size, body weight and feed intake of the offspring (from: Tewe OO, Maner JH. 1981b. Performance and pathophysiological changes in pregnant pigs fed cassava diets containing different levels of

cyanide. Res Vet Sci 30:147-151. (**DOC IV _41**))

TABLE 4: Performance during 56 days of lactating sows fed fresh cassava diets containing different levels of added cyanide during pregnancy

	Experimental variables			
	No added cyanide	250 ppm added cyanide	500 ppm added cyanide	Standard error of difference between means
Weight gain during first 35 days of lactation (kg)	11·7	12·3	11·9	±0·32
Litter size at birth	9	8	8	±0·44
Litter size at weaning	7	8	8	±0·64
Birth weight of offspring (kg)	0·89	0·91	1·02	0·22
Weaning weight of offspring (kg)	13·8	14·4	15·4	±0·52
Daily feed intake of sow (kg)	5·16	5·17	5·67	±0·31
Daily feed intake of offspring (kg)	0·15	0·209	0·20	±0·09

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

Section A6.8 **Annex Point IIA VI.6.8**		**REPRODUCTIVE TOXICITY**	
Section A6.8.1 **Annex Point IIA VI.6.8.1**		**Developmental Toxicity Study in the Pregnant Goat**	
		1 REFERENCE	**Official use only**
1.1	Reference	Benito Soto-Blanco, Silvana L. Go´rniak (2004) Prenatal toxicity of cyanide in goats—a model for teratological studies in ruminants. Theriogenology 62: 1012–1026 **(DOC IV_60)**	
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	/	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	No reported	
2.2	GLP	Not reported.	
2.3	Deviations		
		3 MATERIALS AND METHODS	
3.1	Test material	Potassium cyanide	
3.1.1	Lot/Batch number		
3.1.2	Specification	Merck	
3.1.2.1	Description	KCN dissolved in water	
3.1.2.2	Purity		
3.1.2.3	Stability		
3.2	Test Animals		
3.2.1	Species	Goat	
3.2.2	Strain	Mixed-breed female goats and Alpine bucks	
3.2.3	Source		
3.2.4	Sex	Females	
3.2.5	Age/weight at study initiation	1 – 3 years	
3.2.6	Number of animals per group	8+5+5+8 in dose groups: 0, 1, 2, 3 mg/kg per day	
3.2.7	Control animals	Yes	
3.2.8	Mating period		

3.3	Administration/ Exposure	Oral	
3.3.1	Duration of exposure	Day 24 of gestation (day after the period of implantation) to parturition (approx. Day 150 of pregnancy)	
3.3.2	Postexposure period	3 months	
3.3.3	Type	Gavage twice daily (7:30 – 8:00 and 16:30 – 17:00)	
3.3.4	Dose levels	0, 1, 2 or 3 mg/kg bw per day	
3.3.5	Vehicle	Water	
3.3.6	Concentration in vehicle	Not reported	
3.3.7	Total volume applied	Not reported	
3.3.8	Controls	Water	
3.4	Examinations	Pregnancies were confirmed with Doppler ultrasonic detector.	
3.4.1	Body weight	Yes, weakly. Throughout pregnancy and 3 months after birth.	
3.4.2	Food consumption	Not reported.	
3.4.3	Clinical signs	Yes, daily. Animals with severe ataxia or convulsions (two dams from the top dose group) were treated with intravenous sodium nitrite and sodium thiosulphate.	
3.4.4	Examination of ovaries	No	
3.4.5	Examination of uterus and uterine contents	One dam from control group and on from the top dose group were sacrificed on Day 120 of pregnancy for histopathological study of both dam and offspring.	
3.4.6	Examination of neonates	Birth weight, notation of external abnormalities, and sex	
3.4.6.1	General	Yes	
3.4.6.2	Skeletal	Yes	
3.4.6.3	Soft tissue	Yes	
3.5	Further remarks	Blood samples were collected from jugular vein of pregnant goats every other week for plasma glucose, cholesterol (before feeding and first dosing) and thiocyanate (6 h after dosing) determination. At the day of birth and 7, 45 and 90 days after birth plasma samples were collected from dams and kids for determination of glucose and cholesterol determination. Thyroxine and triiodothyronine were measured in kids at birth and at 7 postnatal days. At 3 months after birth, one dam from each group and all male goats from every litter were sacrificed for histopathological examination.	

		4 RESULTS AND DISCUSSION	
4.1	Maternal toxic effects	a) Interim histopathology results in one top dose group dam on Day 120: increased number of reabsorption vacuoles in the thyroid follicular colloid; status spongiosus suggestive of edema of the cerebral and cerebellar white matter b) Two dams from the top dose group displayed severe ataxia or convulsions. One of them aborted two fetuses, one of them prognatic. There were no treatment-related effects on maternal mortality (zero in all groups), body weight, length of gestation, number of live kids per litter. Two abortions were in one animal of the top dose group, one fetal death in the control group. Plasma thiocyanate levels increased with the duration of gravidity and as a function of dose: on Day 143 the levels were 10, 20, 30 and 85 mmol/L in dose groups 0, 1, 2 and 3 mg/kg. No significant differences between dose groups were observed in glucose levels; cholesterol concentrations were higher in dams of dose groups 2 and 3 mg/kg compared to controls at days 129 and 143.	
4.2	Teratogenic / embryotoxic effects	a) Interim histopathology results in both fetuse of one top dose group dam on Day 120: vacuolar degeneration of thyroid follicular cells and cerebellar white matter. b) Two prognatic kids were born to two dams of the top dose group. No other gross abnormalities were observed. No treatment effects on birth weight, weight curve in first 3 postnatal months and in plasma glucose were detected. T3 concentrations higher compared to controls were found in the top dose dams and kids at the birth day, but not at postnatal day 7. No treatment related differences were in concentration of T4. The histopathological examination of dams and kids killed at 3 postnatal months revealed no lesions.	
4.3	Other effects	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	Materials and methods	Twenty six pregnant goats were allocated into four groups and were administered KCN doses of 0, 1, 2 or 3 mg/kg bw per day orally – in two doses by gavage. Exposure lasted from Day 24 (period of implantation) to parturition (Day 150 in average).	
5.2	Results and discussion	Results are illustrated by **Table 1** (reproductive functions) and **Table 2** (thyroid functions). Clinical signs of acute toxicity were observed in dams of the top dose group. There were no treatment-related effects on maternal mortality (zero in all groups), body weight, length of gestation, and number of live kids per litter, on birth weight, weight curve in first 3 postnatal months and on plasma glucose. T3 concentrations higher compared to controls were found in the top dose dams and kids at the birth day, but not at postnatal day 7. No treatment related differences were in concentration of T4. Two abortions were in one animal of the top dose group, one fetal death in the control group. Two prognatic kids were born to two dams	

		of the top dose group. No other gross abnormalities were observed. Interim histopathology results in one top dose group dam at Day 120 indicated increased number of reabsorption vacuoles in the thyroid follicular colloid; status spongiosus suggestive of edema of the cerebral and cerebellar white matter. Similar findings were in two fetuses of this dam. On the other hand, the histopathological examination of dams and kids killed at 3 postnatal months revealed no lesions. **See Fig.1.**	
5.3	Conclusion	Treatment related effects were detected only in top dose dams and offspring. Dams of this group displayed clear signs of acute neurotoxicity and thyrotoxicity and corresponding histopathological findings in thyroids and white matter of CNS.	
5.3.1	LO(A)EL maternal toxic effects	3mg/kg bw per day	
5.3.2	NO(A)EL maternal toxic effects	2 mg/kg bw per day	
5.3.3	LO(A)EL embryotoxic / teratogenic effects	3mg/kg bw per day	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	2mg/kg bw per day	
5.3.5	Reliability	2	
5.3.6	Deficiencies	No	

Table 1: Reproductive characteristics

Table 1
Reproductive parameters from dams treated with KCN from Day 24 of pregnancy to term[a]

	Control	KCN (mg/kg per day)		
		1.0	2.0	3.0
Body weight gain (kg)				
Prenatal period	10.2 ± 1.7 (6)[b]	11.0 ± 1.7 (5)	11.3 ± 1.5 (5)	13.4 ± 2.2 (5)
Postnatal period	9.5 ± 1.5 (6)	11.1 ± 1.7 (5)	10.6 ± 1.6 (5)	13.0 ± 1.7 (5)
Length of gestation (days)	151.2 ± 1.2 (7)[c]	148.6 ± 1.3 (5)	148.4 ± 1.21 (5)	148.7 ± 0.8 (6)
Live kids	9	8	7	7
Males	5 (55.6%)	2 (25%)	3 (42.9%)	3 (42.9%)
Females	4 (44.4%)	6 (75%)	4 (57.1%)	4 (57.1%)
Live kids/litter	1.3	1.6	1.4	1.20
Twins	3 (33.3%)	3 (60%)	2 (40%)	2 (33.3%)
Prognata kids	0	0	0	2
Fetal death	1	0	0	0
Aborted fetuses	0	0	0	2
Weak kids	1	0	0	1

[a] Mean ± S.E.M.
[b] Number of dams.
[c] Number of deliveries.

Table 2: Characteristics of thyroid functions

Table 4
Plasma thyroxine (T4; μg/dl) and triiodothyronine (T3; ng/dl) concentrations in the offspring born from dams receiving KCN during gestation[1]

	Control	KCN (mg/kg per day)		
		1.0	2.0	3.0
Dams	$n = 7$[2]	$n = 5$	$n = 5$	$n = 6$
T4				
Delivery	3.1 ± 0.4	2.7 ± 0.5	3.0 ± 0.3	4.0 ± 0.7
7 day	3.6 ± 0.3	3.3 ± 0.6	3.7 ± 0.5	3.2 ± 0.4
T3				
Delivery	75.8 ± 7.7[a]	82.5 ± 8.2	84.2 ± 6.8	107.9 ± 8.3[a]
7 day	80.4 ± 2.7	73.8 ± 2.8	81.3 ± 7.3	78.7 ± 4.1
Kids	$n = 9$	$n = 8$	$n = 7$	$n = 7$
T4				
Birth	15.6 ± 0.8	13.8 ± 1.3	17.1 ± 1.4	15.9 ± 2.2
7 day	4.3 ± 0.1	3.9 ± 0.3[a]	3.9 ± 0.3[b]	4.8 ± 0.4[a,b]
T3				
Birth	300.3 ± 8.8[a]	301.7 ± 10.9[b]	332.6 ± 14.6	363.0 ± 15.1[a,b]
7 day	261.4 ± 11.9[a]	202.8 ± 13.1[a,b]	228.2 ± 13.8[c]	286.3 ± 10.7[b,c]

[#] $n = 6$. The values within same rows with similar superscripts (a, b, c) differ significantly ($P < 0.05$).
[1] Mean ± S.E.M.
[2] n: number of dams.

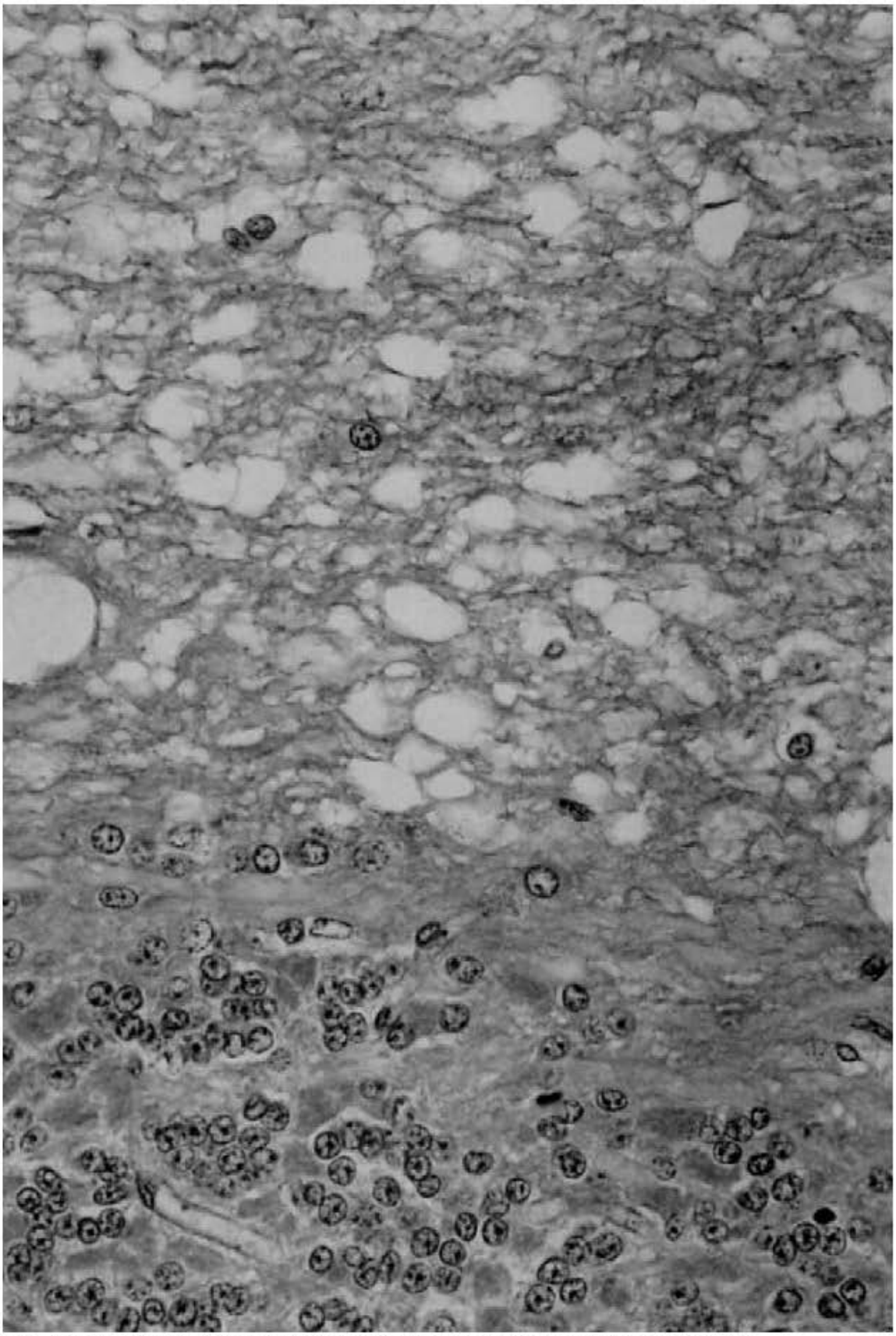

Fig. 1. Cerebellum from fetus of 3.0 mg KCN/kg group dam showing vacuolar degeneration in the white matter (H&E, 40_).

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

Section A6.8 Annex Point IIA VI.6.8		**REPRODUCTIVE TOXICITY**	
Section A6.8.1 Annex Point IIA VI.6.8.1		**Developmental Toxicity Study in Rat**	
		1 REFERENCE	**Official use only**
1.1	Reference	Sousa AB, Paulo C´esar Maiorka, Ivair Donizete Goncalves, L´ılian Rose Marques de S´a, Silvana Lima G´orniak (2007) Evaluation of effects of prenatal exposure to the cyanide and thiocyanate in Wistar rats. Reproductive Toxicology 23: 568–577 **(DOC IV_38)**	
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	/	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	No reported, but the study design and data presentation comply with most requirements of standardised one generation reproduction toxicity test, both for teratogenicity and developmental toxicity assessment.	
2.2	GLP	Not reported.	
2.3	Deviations		
		3 MATERIALS AND METHODS	
3.1	Test material	Potassium cyanide or Potassium thiocyanate	
3.1.1	Lot/Batch number		
3.1.2	Specification	KCN: Merck, KSCN: Carlo Erba	
3.1.2.1	Description	/	
3.1.2.2	Purity	/	
3.1.2.3	Stability	/	
3.2	Test Animals		
3.2.1	Species	Rats	
3.2.2	Strain	Wistar	
3.2.3	Source	School of Veterinary Medicine, Univ. of Sao Paulo	
3.2.4	Sex	Females (and males)	
3.2.5	Age/weight at study initiation	2 months, 220g	
3.2.6	Number of animals	20 per group, 10 for trial A, 10 for trial B	

	per group		
3.2.7	Control animals	Yes	
3.2.8	Mating period	Two females with one male overnight, vaginal smears following day, positive finding indicates GD1; pregnant rats housed individually.	
3.3	Administration/ Exposure	Oral	
3.3.1	Duration of exposure	GD6 to GD20	
3.3.2	Post exposure period	Trial A: No Trial B (half of each group): 21 days, litters culled to 4 males and 4 females.	
3.3.3	Type	Administration in drinking water	
3.3.4	Dose levels	Target doses: KCN: 1, 3 or 30 mg/kg bw per day KSCN: 0.8, 2.4 or 24 mg/kg bw per day	
3.3.5	Vehicle	Water	
3.3.6	Concentration in vehicle	Variable (adjusted every two days to body weight of dams)	
3.3.7	Total volume applied	Water ad libitum	
3.3.8	Controls	Water ad libitum	
3.4	Examinations	Trial A: On GD21 dams were anaesthetised after an 8 hour fast, venous blood samples were taken for biochemical analyses, uterus, ovaria and organ samples were collected after CO_2 euthanasia. The fetuses were weighed, measured and examined for macroscopic malformations. Alternate fetuses were randomly assigned to skeletal or visceral examination. Trial B: Dams were allowed to give birth naturally, litters were reduced to 4M+4F. Body weight was recorded on PND 1, 7, 14 and 21. On PND 21, the same final procedure was used as in Trial A.	
3.4.1	Body weight	Yes, on GD 0, 6, 8, 10, 12, 14, 16, 18, 20, (+GD21 in trial B)	
3.4.2	Food consumption	Individual diet consumption measured for GD 6-9, 10-12, 13-15 and 16-18.	
	Water consumption	Consumption measured and fresh solutions provided daily.	
3.4.3	Clinical signs	Yes, daily.	
3.4.4	Examination of ovaries	In trial A	
3.4.5	Examination of uterus and uterine contents	In trial A	
3.4.6	Examination of fetuses and neonates	Birth weight, external abnormalities, and sex	

3.4.6.1	General	Yes	
3.4.6.2	Skeletal	Yes	
3.4.6.3	Soft tissue	Yes	
3.5	Further remarks	See point 3.4	
		4 RESULTS AND DISCUSSION	
4.1	Maternal toxic Effects	No clinical signs of toxicity were observed. No differences in food and water consumption between groups. Serum levels of thiocyanate were higher in all treated dams in trial A but not in trial B. Serum concentration of glucose was significantly higher in dams treated with KCN dose of 30 mg/kg bw (**see Table 4**). Histopathological changes were found in dams administered the highest doses of KCN and KSCN: vacuolisation of pancreas islet cells, diffuse microvesicular vacuolisation of hepatocytes, increased number of biliary ducts, focal neuronal necrosis and focal nodular gliosis in brain, mild congestion and vacuolisation of white matter in cerebellum (**see Table 6**). A dose related increase in the number of reabsorption vacuoles in thyroid follicular colloid is reported in all KCN and KSCN treated dams and their offspring (**see Table 6**). No treatment related effects on body weight, number of corpora lutea, preimplantation loss, postimplantation loss, fetal and placental weight or fetal length were found (**see Table 1**).	
4.2	Teratogenic / embryotoxic effects	No significant inter-group differences were found in skeletal variations (**see Table 2**). Incidence of visceral abnormalities was significantly higher in fetuses of KCN top dose dams (20 of 40 examined) compared to controls (10 of 40 examined (**see Table 3**).	
4.3	Other effects – developmental toxicity	The body weight gain of the offspring was similar in all groups (**see Table 5**).	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	Materials and methods	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. Water consumption was monitored: no difference in water consumption between treated and control rats were detected. Deviations of actual doses from target values were negligible. Half of dams were sacrificed on the GD20, half lived to weaning of the offspring.	
5.2	Results and discussion	No clinical signs of toxicity or changes in the postnatal development of the offspring, no treatment related effects on body weight, number of corpora lutea, preimplantation loss, postimplantation loss, fetal and placental weight, fetal length and skeletal variations were observed in KCN or KSCN treated groups. Visceral abnormalities were significantly more frequent in fetuses of KCN top dose dams compared to controls. 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 incresed blood glucose level are reported only in KCN top dose dams, 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 diappeared 3 weeks after exposure cessation. On the other hand, histopathological changes in liver and brain were detected in dams and fetuses of the top dose groups of both KCN and KSCN. Histological alterations in 21 day pups were similar to alterations in their dams.	
5.3	Conclusion	Repeated dosing with cyanide and (with an exception of the effect on pancreatic cells) with thiocyanate induces in not-acutely toxic doses toxic injury in dams and their offspring.	
5.3.1	LO(A)EL maternal toxic effects	3 mg/kg bw (KCN) and 2.4 mg/kg bw (KSCN) for thyrotropic effects 30 mg/kg bw (KCN) and 24 mg/kg bw (KSCN) for other toxicity endpoints	
5.3.2	NO(A)EL maternal toxic effects	1 mg/kg bw (KCN) and 0.8 mg/kg bw (KSCN) for thyrotropic effects 3 mg/kg bw (KCN) and 2.4 mg/kg bw (KSCN) for other toxicity	
5.3.3	LO(A)EL embryotoxic / teratogenic effects	3 mg/kg bw (KCN) and 2.4 mg/kg bw (KSCN) for thyrotropic effects 30 mg/kg bw (KCN) and 24 mg/kg bw (KSCN) for other toxicity	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	1 mg/kg bw (KCN) and 0.8 mg/kg bw (KSCN) for thyrotropic effects 3 mg/kg bw (KCN) and 2.4 mg/kg bw (KSCN) for other toxicity	
5.3.5	Reliability	1	
5.3.6	Deficiencies		

Table 1
Reproductive performance of rats that received, in the drinking water KCN: 0, 1, 3 and 30 mg/kg/day; or KSCN: 0.8, 2.4 and 24 mg/kg

	Control	KCN (mg/kg)			KSCN (mg/kg)		
	0	1	3	30	0.8	2.4	24
Trial A							
Number of pregnant females	10	10	10	10	10	10	10
Dam body weight gain (mean ± S.E. (g))	74.89 ± 8.38	71.50 ± 7.12	70.90 ± 8.06	76.25 ± 5.84	64.90 ± 5.63	73.00 ± 6.88	69.00 ± 3.39
Gestation period: GD 1–20							
Number of corpora lutea (mean ± S.E.M.)	11.10 ± 0.64	12.67 ± 1.24	12.04 ± 1.12	11.11 ± 0.87	11.70 ± 0.91	11.67 ± 1.16	11.50 ± 0.81
Number of implantations (mean ± S.E.M.)	9.90 ± 1.04	11.33 ± 0.55	10.50 ± 1.12	10.56 ± 1.26	10.80 ± 0.59	10.78 ± 0.72	10.80 ± 0.42
Number of resorptions (mean ± S.E.M.)	0.90 ± 0.23	1.00 ± 0.53	1.40 ± 0.43	0.89 ± 0.34	1.50 ± 0.52	2.22 ± 0.94	1.10 ± 0.43
Number of live fetuses (mean ± S.E.M.)	9.10 ± 1.01	10.33 ± 0.55	8.88 ± 0.75	8.63 ± 0.59	9.70 ± 0.88	9.70 ± 0.64	9.70 ± 0.63
Males (mean ± S.E.M.)	3.90 ± 0.65	4.66 ± 0.57	5.22 ± 0.40	4.87 ± 0.44	4.10 ± 0.45	4.77 ± 0.57	4.1 ± 0.58
Females (mean ± S.E.M.)	5.20 ± 0.80	5.66 ± 0.66	3.67 ± 0.50	3.75 ± 0.88	4.60 ± 0.60	5.00 ± 0.74	5.60 ± 0.70
Dead fetuses	0	0	0	0	0	0	0
Gravid uterus weight (mean ± S.E.M. (g))	63.06 ± 6.54	74.612 ± 4.04	58.54 ± 7.26	60.81 ± 8.38	58.89 ± 5.47	69.08 ± 4.53	60.60 ± 7.13
Placental weight (mean ± S.E.M. (g))	0.57 ± 0.03	0.51 ± 0.03	0.51 ± 0.03	0.54 ± 0.03	0.52 ± 0.02	0.54 ± 0.01	0.57 ± 0.01
Fetal weight (mean ± S.E.M. (g))	4.61 ± 0.13	4.80 ± 0.10	4.88 ± ±0.10	4.74 ± 0.09	4.57 ± 0.13	4.69 ± 0.07	4.65 ± 0.09
Fetal length (mean ± S.E.M. (cm))	3.93 ± 0.08	4.00 ± 0.03	3.93 ± 0.04	4.00 ± 0.03	3.91 ± 0.04	3.96 ± 0.04	3.86 ± 0.05
Preimplantation loss (%)	10.81 ± 1.02	10.58 ± 1.00	12.79 ± 1.15	4.95 ± 0.98	7.69 ± 0.99	7.63 ± 0.55	6.09 ± 0.65
Postimplantation loss (%)	8.08 ± 0.78	8.83 ± 0.77	15.43 ± 0.99	18.28 ± 1.07	10.19 ± 0.99	10.02 ± 0.78	10.18 ± 0.77
Trial B							
Number of pregnant females	10	10	10	10	10	10	10
Dam body weight gain (mean ± S.E. (g))	84.90 ± 5.58	88.00 ± 4.20	82.50 ± 3.99	87.30 ± 2.77	84.22 ± 3.43	93.90 ± 4.84	83.80 ± 6.07
Gestation period: GD 1-21							
Number of delivered pups (mean ± S.E.M.)	8.50 ± 0.31	8.30 ± 0.59	7.30 ± 1.03	8.10 ± 0.67	8.44 ± 0.63	9.70 ± 0.47	7.50 ± 0.79
Males (mean ± S.E.M.)	4.00 ± 0.53	4.60 ± 0.60	3.70 ± 0.68	4.90 ± 0.58	4.11 ± 0.51	4.60 ± 0.58	2.50 ± 0.45
Females (mean ± S.E.M.)	4.50 ± 0.37	3.70 ± 0.45	3.80 ± 0.59	3.80 ± 0.89	4.33 ± 0.74	5.10 ± 0.53	4.90 ± 0.55
Dead fetuses (mean ± S.E.M.)	0	0.20 ± 0.13	0.30 ± 0.15	0	0.22 ± 0.14	0	0.60 ± 0.33
Number of pups alive on PND 21	8	8	8	8	8	8	8