Table 2
Incidence of skeletal alterations in litters from mothers 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
Total number of litters	10	10	10	10	10	10	10
Total fetuses examined	40	40	40	40	40	40	40
Affected litters	6	5	5	6	6	6	5
Affected fetuses	23	20	28	26	27	21	23
Skull: incomplete ossification							
Affected litters	6	5	5	6	6	6	5
Affected fetuses	23	20	28	26	27	21	23
Cranial sutures: incomplete ossification							
Affected litters	6	5	5	6	5	5	5
Affected fetuses	8	8	8	11	5	5	10
Sternebrae (bone incompletely ossified)							
Affected litters	6	5	5	6	5	5	5
Affected fetuses	10	12	12	12	11	13	13
Irregularly shaped ribs							
Affected litters	6	5	5	6	5	5	5
Affected fetuses	6	8	9	7	13	8	6
Wavy rib							
Affected litters	0	0	2	2	0	0	0
Affected fetuses	0	0	2	2	0	0	0

Table 3
Incidence of visceral alterations in litters from mothers 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
Total number of litters	10	10	10	10	10	10	10
Total fetuses examined	40	40	40	40	40	40	40
Affected litters	6	7	5	6	5	6	5
Affected fetus	10	18	6	20*	6	15	12
Dilated ureter							
Affected litters	1	7	3	5	2	3	5
Affected fetuses	1	7	3	5	2	3	5
Kinky ureter							
Affected litters	7	7	5	6	3	6	5
Affected fetuses	7	11	5	6	3	8	8
Peritoneal hemorrhage							
Affected litters	2	2	1	4	2	3	2
Affected fetuses	2	2	1	4	2	3	2
Enlarged esophagus							
Affected litters	2	0	0	6	1	4	2
Affected fetuses	2	0	0	7	1	4	2

* Significantly different from control group at $P < 0.05$ (Fischer's test).

Table 4
Serum levels of glucose, cholesterol (mg/dl) and thiocyanate (μmol/l) in control and experimental dams given, 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							
Glucose	81.38 ± 7.04	84.57 ± 9.49	84.11 ± 7.29	112.13 ± 7.56*	70.57 ± 7.10	61.71 ± 6.11	67.59 ± 6.37
Cholesterol	63.85 ± 15.98	65.94 ± 14.66	62.77 ± 14.95	60.08 ± 13.28	61.97 ± 12.47	62.42 ± 15.48	69.58 ± 11.09
Thiocyanate	32.71 ± 3.98	81.43 ± 9.20*	167.90 ± 9.37*	262.50 ± 9.61*	96.09 ± 10.02*	141.88 ± 11.00*	228.50 ± 12.99*
Trial B							
Glucose	79.20 ± 7.25	75.57 ± 10.32	73.88 ± 10.59	70.62 ± 7.34	68.71 ± 9.63	65.83 ± 11.89	68.33 ± 7.29
Cholesterol	68.34 ± 8.66	69.31 ± 7.84	69.61 ± 9.53	60.41 ± 9.39	62.50 ± 9.22	62.93 ± 8.85	60.44 ± 8.16
Thiocyanate	26.63 ± 2.77	35.11 ± 2.87	36.05 ± 2.83	36.73 ± 3.72	37.85 ± 3.25	36.95 ± 3.57	34.20 ± 4.29

* Significantly different from control group at $P < 0.05$ (Duncan's test).

Table 5
Body weight gain (g) of offspring from dams 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

Period	Control	KCN (mg/kg)			KSCN (mg/kg)		
	0	1	3	30	0.8	2.4	24
PND 01–07	51.73 ± 4.70	52.00 ± 4.80	45.93 ± 4.80	47.18 ± 4.61	43.11 ± 4.17	54.37 ± 4.15	47.34 ± 4.16
PND 08–14	76.50 ± 5.72	74.50 ± 7.07	62.67 ± 8.06	71.92 ± 6.31	72.70 ± 5.82	81.21 ± 5.57	67.79 ± 7.89
PND 15–21	83.30 ± 10.03	81.70 ± 10.11	68.40 ± 10.04	92.00 ± 7.28	84.20 ± 8.58	95.80 ± 6.43	83.20 ± 8.36
PND 01–21	211.53 ± 11.31	208.20 ± 11.22	177.33 ± 11.77	211.10 ± 8.14	225.17 ± 11.33	231.32 ± 9.00	198.24 ± 11.06

Table 6
Intensity of the lesions[a] observed in dams and their respective offspring in control and experimental groups that received, in the drinking water KCN: 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/Trial B							
Liver							
Hepatic congestion	−	+	+	++	−	+	−
Vacuolization of hepatocytes	−	−	+	+	−	−	+
Proliferation of biliary ducts	−	−	−	−	−	−	+
Brain							
CNS congestion	−	−	+	++	−	−	+
Neuronophagia	−	−	−	+	−	−	+
Hemorrhagic areas in CNS	−	−	−	+	−	−	−
Gliosis	−	−	−	++	−	+	++
Necrosis	−	−	−	+	−	−	−
Thyroid							
Increase in the number of reabsorption vacuoles in follicular colloid	−	+	++	+++	+	++	+++
Trial A							
Pancreas							
Islet cells vacuolation	−	−	−	++	−	−	−
Pups							
Liver							
Hepatic congestion	−	−	+	++	−	+	++
Vacuolization of hepatocytes	−	−	−	+	−	−	+
Proliferation of biliary ducts	−	−	−	+	−	−	+
Brain							
CNS congestion	−	−	+	++	−	+	++
Neuronophagia	−	−	−	+	−	−	+
Gliosis	−	−	−	++	−	−	++

The intensity was characterized by scores: (−) no lesion; (+) mild lesions; (++) moderated lesions; and (+++) severe lesions. It was used for four animals in each group.
[a] The identification of the lesions was considered only when all animals analyzed showed the same alterations.

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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 Rats**	
		1 REFERENCE	**Official use only**
1.1	Reference	Monsanto Co: Teratology Study In Rats With Test Article Acetone Cyanohydrin, 1984, **(DOC IV_59)**	
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	Section 83-3 of the Environmental Protection Agency Guidelines, Subdivision F. for Hazard Evaluation-Ruman and Domestic Animals, issued June, 1982. This is similar to OECD guideline for the testing of chemicals no. 414 for prenatal developmental toxicity study	
2.2	GLP	Yes, the study was conducted according to the United States Food and Drug Administration's Good Laboratory Practice Regulations of June 20, 1979.	
2.3	Deviations		
		3 MATERIALS AND METHODS	
3.1	Test material	Acetone Cyanohydrin	
3.1.1	Lot/Batch number	29T501-2	
3.1.2	Specification	Not reported	
3.1.2.1	Description	Clear amber liquid	
3.1.2.2	Purity	99.82 %	
3.1.2.3	Stability	Stable	
3.2	Test Animals		
3.2.1	Species	Rats	
3.2.2	Strain	Charles River COBS CD	
3.2.3	Source	Charllfs River Breeding Labor-atorias. Inc., Portage. Michigan on May 16, 1983	
3.2.4	Sex	Females	
3.2.5	Age/weight at study initiation	80-120 days/ 220 g (minimal weight on delivery), 218-289 g on GD0	
3.2.6	Number of animals per group	25 per group	

3.2.7	Control animals	Yes	
3.2.8	Mating period	One female with one male of the same strain and source, copulatory plugs or vaginal smears daily, positive finding indicates GD0; pregnant rats housed individually.	
3.3	Administration/ Exposure	Oral	
3.3.1	Duration of exposure	GD6 to GD15	
3.3.2	Post exposure period	Days 16 to 20of gestation	
3.3.3	Type	Oral administration via gavage in deionised water	
3.3.4	Dose levels	1) 1 mg/kg bw 2) 3 mg/kg bw 3) 10 mg/kg bw	
3.3.5	Vehicle	Deionised water	
3.3.6	Concentration in vehicle	1, 3 or 10 mg/ 5ml	
3.3.7	Total volume applied	5 ml/kg .bw	
3.3.8	Controls	Vehicle	
3.4	Examinations	Observations for mortality and signs of toxicity (such as tremors, convulsions, loss of righting, reflex, excessive blood loss and moribundity twice an each day. During treatment detailed clinical observations were performed daily. Dams were humanly killed on the GD 20 and necropsy performed.	
3.4.1	Body weight	Yes, individual body weights were recorded for all dams on gestation days 0, 6, 9, 12, 16 and 20.	
3.4.2	Food consumption	Not determined	
3.4.3	Clinical signs	Yes, daily.	
3.4.4	Examination of ovaries	Corpora lutea	
3.4.5	Examination of uterus and uterine contents	Number and locations of viable and nonviable fetuses, early and late resorption , total no. of implantations	
3.4.6	Examination of fetuses and neonates	Foetal weight, external abnormalities, sex, soft tissue, skeletal examination	
3.4.6.1	General	Yes	
3.4.6.2	Skeletal	Yes	
3.4.6.3	Soft tissue	Yes	
3.5	Further remarks	None	

		4 RESULTS AND DISCUSSION	
4.1	Maternal toxic Effects	Survival was 100% in the control group and all treated groups. Antemortem observations included hair loss (**see Table 1**), soft stool (one occurrence in 1 and 3 mg/kg/day dosage group), scabbing on the nose (control group), and single instances of red, swollen hind limb (1mg/kg/day dosage group) and a nodule on the lower lip (10 mg/kg/day dosage group). There were single instances of the following necropsy observations in the control group: hydronephrosis, intestinal worms and a subcutaneous abdominal mass. A slight treatment-related reduction of body weight gains occurred in the 3 and 10 mg/kg/day dosage groups during the treatment and gestation periods (days 6 to 15 and 0 to 20, respectively) the body weight values for the 1 mg/kg /day dosage group were comparable to those of the control group (**see Table 2**). The corpora lutea/dam and total implantations/dam for the 10 mg/kg/day dosage groups were significant1y less than the control group values. The other parameters for this group, viable fetuses/dam and total implantation losses/dam, fetal body weight, fetal sex distribution and all Cesarean section parameter values for the 1 and 3 mg/kg.day dosage groups were comparable to the control group values (**see Table 3).**	
4.2	Teratogenic / embryotoxic effects	There were two or three incidences of microphthalmia in all groups. Malformations occurring in single incidence in fetuses and/or litter were transposition of great vessels with right-sided aortic arch, interventricular septal defect, malpositioned heart, malformed lungs, diaphragmatic hernia, vestigial uterine horn and bent ribs.	
4.3	Other effects – developmental toxicity	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	Materials and methods	The conduction of the study is in accordance with OECD guideline no.414, the significant deviation being the fact that neither food nor water consumption was determined. To evaluate teratogenicity of the test compound its doses were administered to pregnant rats from GD6 to GD 15 dissolved in deionized water in n doses of 0 (control group) 1, 3 and 10 mg/kg bw. The dams were humanly sacrificed on GD 20. The doses used were determined in the range finding study which used 5 animals per dosage group. In this study dosages of 0, 1,3,5,7.5 and 10 mg/kg bw were administered daily from G.D. 6 till G.D. 15 and animals were humanly sacrificed on GD 20. No biologically meaningful differences in the uterine examination values of any of the dosage groups when compared to those of the control group were found (**DOC IV_58**).	

5.2	Results and discussion	The ante mortem observations were spurious, independent of the dose and no meaningful difference between the control and any treated group was observed. The same applies to necropsy observations. The difference between the highest dose and control group in lutea/dam and total implantations/dam concerns pretreatment parameters and is not compound related. The slight decrease in weight in the two highest dose groups is not considered as a toxic effect caused by the substance. No such difference was observed in the range finding study for any of the concentrations used **(DOC IV_58)**. There were no meaningful differences in the incidence of fetal malformations and developmental variations in the treated groups when compared to the control group. Rather, the findings were isolated showing no dependence on the dose.	
5.3	Conclusion	Treatment with acetone cyanohydrin did not produce a teratogenic response when administered orally by gavage to Charles River COBS CD rats at dosage levels of 10 mg/kg/day or less.	
5.3.1	LO(A)EL maternal toxic effects		
5.3.2	NO(A)EL maternal toxic effects	10 mg/kg.bw of acetone cyanohydrin corresponding to 3.3 mg/kg .bw of HCN	
5.3.3	LO(A)EL embryotoxic / teratogenic effects		
5.3.4	NO(A)EL embryotoxic / teratogenic effects	10 mg/kg.bw of acetone cyanohydrin corresponding to 3.3 mg/kg .bw of HCN	
5.3.5	Reliability	1	
5.3.6	Deficiencies	Neither food nor water consumption was determined	

Table 1 – Occurrence of hair loss

	Dosage of acetone cyanohydrin in (mg/kg/day)							
Hair loss location:	0 (control)		1		3		10	
	No	%	No	%	No	%	No	%
- forelimbls	4	16	6	24	4	16	1	4
- nose	1	4						
- ventral thorax and/or abdomen:			1	4	1	4	2	8
- Inguinal, left:			1	4	1	4		

Table 2 – Reduction of body weights

	Group Mean Maternal Body Weights (grams)							
	Acetone Cyanohydrin (mg/kg/day)							
	0 (Control)		1		3		10	
Day of Gestation	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
0	251	15.6	251	14.1	252	14.3	251	13.1
6	278	16.6	277	19.1	278	15.6	278	14.0
9	284	16.8	282	17.0	282	15.7	283	13.7
12	300	17.6	299	19.2	297	17.0	298	13.3
16	327	19.7	323	20.4	321	16.4	322	17.3
20	388	22.4	381	22.9	375	18.2	378	19.4
Days of Gestation	Group Mean Maternal Body Weight Change (grams)[a]							
0 to 6	27	6.3	26	11.1	26	4.3	27	6.1
6 to 9	6	4.3	5	4.9	4	4.2	6	5.8
9 to 12	16	4.4	17	6.3	15	4.4	15	5.0
12 to 16	28	6.6	24	8.0	24	5.5	24	8.1
16 to 20	61	12.4	58	12.2	53	11.8	56	8.3
6 to 15[b]	49	10.2	47	9.6	43	6.4	44	12.0
0 to 20	137	18.3	131	16.6	122	15.5	128	17.8

[a] Values represent the mean of the individual change body weight for these intervals

[b] Day 15 body weights were recorded on day 16 to reflect the 10 day treatment regimen

S.D. – standard deviation

Table 3 – Summary of Group Mean Maternal and Fetal Observations at Caesarian Section

	Acetone Cyanohydrin (mg/kg/day)											
	0 (Control)			1			3			10		
	No.	%	±S.D.	No.	%	±S.D.	No.	%	±S.D.	No.	%	±S.D.
Animals on study:	25	-	-	25	-	-	25	-	-	25	-	-
Animals that were gravid:	25	-	-	25	-	-	25	-	-	25	-	-
Animals examined at Cesarean section:	25	-	-	25	-	-	25	-	-	25	-	-
Nongravid:	0	-	-	0	-	-	0	-	-	0	-	-
Gravid:	25	-	-	25	-	-	25	-	-	25	-	-
Dams with resorptions only:	0	-	-	0	-	-	0	-	-	0	-	-
Dams with viable fetuses:	25	-	-	25	-	-	25	-	-	25	-	-
Viable fetuses/dam:	14,1	-	2,24	14,2	-	2,64	13,4	-	2,08	13,6	-	1,47
Postimplantation loss/dam:	1,4	-	1,41	1,0	-	1,17	1,0	-	0,93	0,9	-	1,01
Total implantations/dam:	15,5	-	1,42	15,1	-	2,07	14,4	-	2,55	14,4**	-	1,33
Corpora lutea/dam:	16,9	-	1,62	16,8	-	2,18	16,3	-	2,82	15,9*	-	1,54
Group mean preimplantation loss (%)[a]:	-	8,3	-	-	9,8	-	-	11,5	-	-	9,1	-
Group mean postimplantation loss (%)[b]:	-	8,8	-	-	6,3	-	-	7,2	-	-	6,1	-
Mean fetal body weight (grams):	3,2	-	0,36	3,3	-	0,24	3,2	-	0,20	3,2	-	0,24
Fetal sex distribution - male:	185	52,4	-	174	49,2	-	157	46,9	-	177	52,2	-
female:	168	47,6	-	180	50,8	-	178	53,1	-	162	47,8	-

[a] $\frac{\text{Total No. Corpora Lutea - Total No. Implantations}}{\text{Total No. Corpora Lutea}} \times 100$

[b] $\frac{\text{Total No. Implantations - Total No. Viable Fetuses}}{\text{Total No. Implantations}} \times 100$

*Significantly different from control group; $p<0.05$

**Significantly different from control group; $p<0.01$

S.D. - Standard deviation

- Not applicable

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

Section A6.8.2 Annex Point IIA VI.6.8.2	**Fertility study**	
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. No reproductive and developmental toxicity studies are available for hydrogen cyanide. Fertility studies on potassium cyanide in diet and inhalation of acetone cyanohydrin are used as replacement.	
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. 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**) **Summary see DOC III_6.8.2a.** 2. Monsanto Co. (1985b) Female fertility study of Sprague-Dawley rats exposed by the inhalation route to acetone cyanohydrin. St. Louis, MO, Monsanto Co. (Report ML-82-145; US EPA/OPTS Public Files No. 878216398) (DOCIVA / A78) (**DOC IV_61**) **Summary see DOC III_ 6.8.2b.** 3. 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) (DOCIVA / A27) **(DOC IV_40) Summary DOC III_ 6.8.2c.**	
Summaries	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. There was a dose-dependent decrease in body weight gain, blood haemoglobin (18% and 23%), and serum T4 concentration (54% and 75%). In a male fertility study **(1)**, Sprague-Dawley rats (n = 15) were exposed by inhalation to ACH (0, 35, 101, or 202 mg/m^3; 0, 11, 32, or 64 mg hydrogen cyanide/m^3), 6 h/day, 5 days/week, during a period of 69 days (i.e., 48 exposure days). After the treatment period, the males were mated with three non-exposed females each. There were no effects on the mean body weight, clinical signs of toxicity, or anatomical changes in gross necropsy. The mating efficiency, number of live implants, and pre- and post-implantation losses were not different between treated and control groups. Female Sprague-Dawley rats were exposed by inhalation (6 h/day, 7 days/week) for 21 days to ACH at at 38, 108, or 207 mg/m^3 and then mated with untreated males. Exposure of the females, which was equivalent to 12, 34, and 66 mg hydrogen cyanide/m^3, was continued until the day of mating, and the females were sacrificed at mid-gestation (gestation days 13–15). No treatment related effects on female fertility were observed in any of the exposure groups. The only frequently observed clinical sign post-exposure was red nasal discharge or encrustation. The highest exposure in the study could be considered as the no-observed-effect level (NOEL) for female reproductive effects **(2).** **Comment:** The oral and the inhalation studies in rats differ extremely in	

	doses administered: 6 hour exposure in the highest ACH concentration roughly corresponds to a daily dose of 2 mg cyanide/kg bw, while the daily dose administered in diet amounted to 1000 mg cyanide/kg bw. The first is considered as NOEL for male and female fertility, while the second suppressed female fertility completely. 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 **(3); summary see section 6.8.2c.** As part of the sub chronic toxicity study, examination of reproductive parameters incl. sperm motility and vaginal cytology examinations were accomplished on rats and mice in the 0, 30, 100, and 300 ppm groups. Sodium cyanide caused a slight reduction in cauda epididymal weight in all groups of exposed male rats (daily doses 2.7 mg/kg bw and higher) and in male mice exposed to 300 ppm (daily dose of 45.9 mg/kg bw). In male rats, the number of spermatid heads per testis in the 300ppm-group (daily dose of 23.69 mg/kg bw) was less than the number in the controls, and sperm motility in all exposed groups (daily doses 2.7 mg/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 19.1 and 54.3 mg/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. Alterations in reproductive parameters indicate that subchronic exposure to low concentrations of sodium cyanide may produce mild but significant adverse effects on rat reproductive systems, probably insufficient to decrease fertility in rats.	
Conclusions	Inhalatory exposure of rats to ACH concentrations corresponding to 63 – 66 mg hydrogen cyanide/m^3, 6 h/day, 5 days/week, for 10 weeks had no effects on male or female fertility: the exposure corresponds to a daily dose of 2 mg CN/kg bw and a total, cumulative dose of 100 mg hydrogen cyanide/kg bw. LOEL oral daily doses for reproductive parameters of 2.7 mg/kg bw per day in male rats and 45.9 mg/kg bw per day in male mice resulted from sub chronic exposure to low concentrations of sodium cyanide in drinking water.	
Undertaking of intended data submission	No studies are planned.	

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Section A6.8 Annex Point IIA VI.6.8		**REPRODUCTIVE TOXICITY**	
Section A6.8.2 Annex Point IIA VI.6.8.2		**Fertility Study in Male Rats**	
		1 REFERENCE	**Official use only**
1.1	Reference	Monsanto co: Male fertility study of Sprague-Dawley rats exposed by the inhalation route to acetone cyanohydrin. Report 1985 (**DOC IV_47**)	
1.2	Data protection	No	
1.2.1	Data owner	No	
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 fertility test.	
2.2	GLP	Yes	
2.3	Deviations	/	
		3 MATERIALS AND METHODS	
3.1	Test material	Acetone cyanhydrin	
3.1.1	Lot/Batch number	T501-3	
3.1.2	Specification		
3.1.2.1	Description	Light amber liquid	
3.1.2.2	Purity	98.5%	
3.1.2.3	Stability	Stable during the study	
3.2	Test Animals		
3.2.1	Species	Albino rats	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	Charles River	
3.2.4	Sex	Male and virgin female rats	
3.2.5	Age/weight at study initiation	Males: 27 days at receipt, 42 days at first exposure, 15 weeks at mating Females: 55 days at receipt, 12 weeks at mating	
3.2.6	Number of animals	15 males + 50 females	

	per group		
3.2.7	Control animals	Yes	
3.2.8	Mating period	Three untreated females were assigned to each male. Males were caged consecutively with each assigned female until confirmed copulation was observed or five nights occurred. Examination for copulation plugs, vaginal smears following day, positive finding indicates GD1; pregnant rats housed individually. Mated females were sacrificed at about mid-term (days 13-15).	
3.3	Administration/ Exposure	Animal (only males) were exposed in individual wire mesh cages suspended in 10 cubic meter exposure chamber.	
3.3.1	Duration of exposure	6 hours per day, 5 days per week, 10 weeks Total number of exposures varied between 48 and 58 (males were exposed until the day after the last mating opportunity).	
3.3.2	Post exposure period	3 weeks prior to sacrifice	
3.3.3	Type	Vapour inhalation	
3.3.4	Dose levels	Target levels: 0, 10, 30, 60 ppm	
3.3.5	Vehicle	Air	
3.3.6	Concentration in air	Mean daily analytical levels: 10.0, 28.5, 57.2 ppm	
3.3.7	Total volume applied	/	
3.3.8	Controls	Exposure to ambient air	
3.4	Examinations		
3.4.1	Body weight	Males were weighed once per week. Females were weighed prior to mating and on days 0 and 13 of gestation.	
3.4.2	Food consumption	No	
3.4.3	Clinical signs	Males were examined thoroughly once per week, and observed for clinical signs on exposure days.	
3.5	Paternal toxic effects	Gross examination of thoracic and abdominal organs. Male reproduction organs were preserved in formalin.	
3.6	Pregnancy status	Total nidations, number of resorption, live implantations, and corpora lutea were counted.	
3.7	Other examinations	/	
		4 RESULTS AND DISCUSSION	

4.1	Parental toxic effects	All males survived to scheduled sacrifice. No significant treatment related effects on clinical signs, body weight and necropsy findings were observed.	
4.2	Reprotoxic effects	Efficiency in mating, number of live implantations, pre- and post-implantation loss was comparable to control group values.	
4.3	Other effects	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	Materials and methods	Male Sprague-Dawley rats, in groups of fifteen, were exposed by the inhalation route (6 hrs. /day, 5 days/week) to acetone cyanohydrin at target levels of 10, 30 and 60 ppm. Mean daily analytical levels (averaged for all exposure days) were within 5% of the target levels: 10.0, 28.5 and 57.2 ppm. Males were exposed for at least 48 exposure days (69 days on study) and mated to untreated females. Exposure of the males was continued through the day after the last mating opportunity (maximum 58 exposure days).	
5.2	Results and discussion	No toxic effects were observed in males of any of the exposure groups. Mean body weights of treated male groups were not significantly different from control group body weights and no treatment-related clinical signs of toxicity were observed during or after exposure. There were no remarkable lesions detected in any of the exposed males at necropsy. No male fertility effects were observed in any of the exposure level groups. Treated males were comparable to control group males in effecting pregnancy. Females mated to exposed males were comparable to females mated to control males in numbers of live implants and pre- and post-implantation loss (**see Table1**). Therefore, this study indicates that acetone cyanohydrin does not produce male fertility effects in rats at inhalation exposure levels at least as high as 60 ppm.	
5.3	Conclusion	This study indicates that acetone cyanohydrin does not exhibit reproductive toxicity in male rats at inhalation exposure levels at least as high as 60 ppm.	
5.3.1	LO(A)EL paternal toxic effects	> 60 ppm	
5.3.2	NO(A)EL paternal toxic effects	60 ppm (the highest exposure level) Corresponds to 12.45 mg cyanide/kg body weight (calculated with a minute volume of 175 ml/min and an average body weight of 300 g and assuming 100% absorption)	
5.3.3	LO(A)EL reprotoxic effects	> 60 ppm	
5.3.4	NO(A)EL reprotoxic effects	60 ppm (the highest exposure level) Corresponds to 12.45 mg cyanide/kg body weight (calculated with a minute volume of 175 ml/min and an average body weight of 300 g and assuming 100% absorption)	
5.3.5	Reliability	1	
5.3.6	Deficiencies	No	

Table 1. Summary of mating and pregnancy data

Group ACH ppm	Mating success % of nights co-housed	Pregnant females	BW gain to d.13	Live impl. mean	Loss pre+post impl.%	Resor-ption %
0	30	34	55.0	13.4	9+7	7
10	23	34	54.4	14.7	4+3	7
30	28	36	56.4	14.2	8+5	5
60	29	36	55.3	14.0	7+5	5
Signif.	/	/	/	/	/ /	/

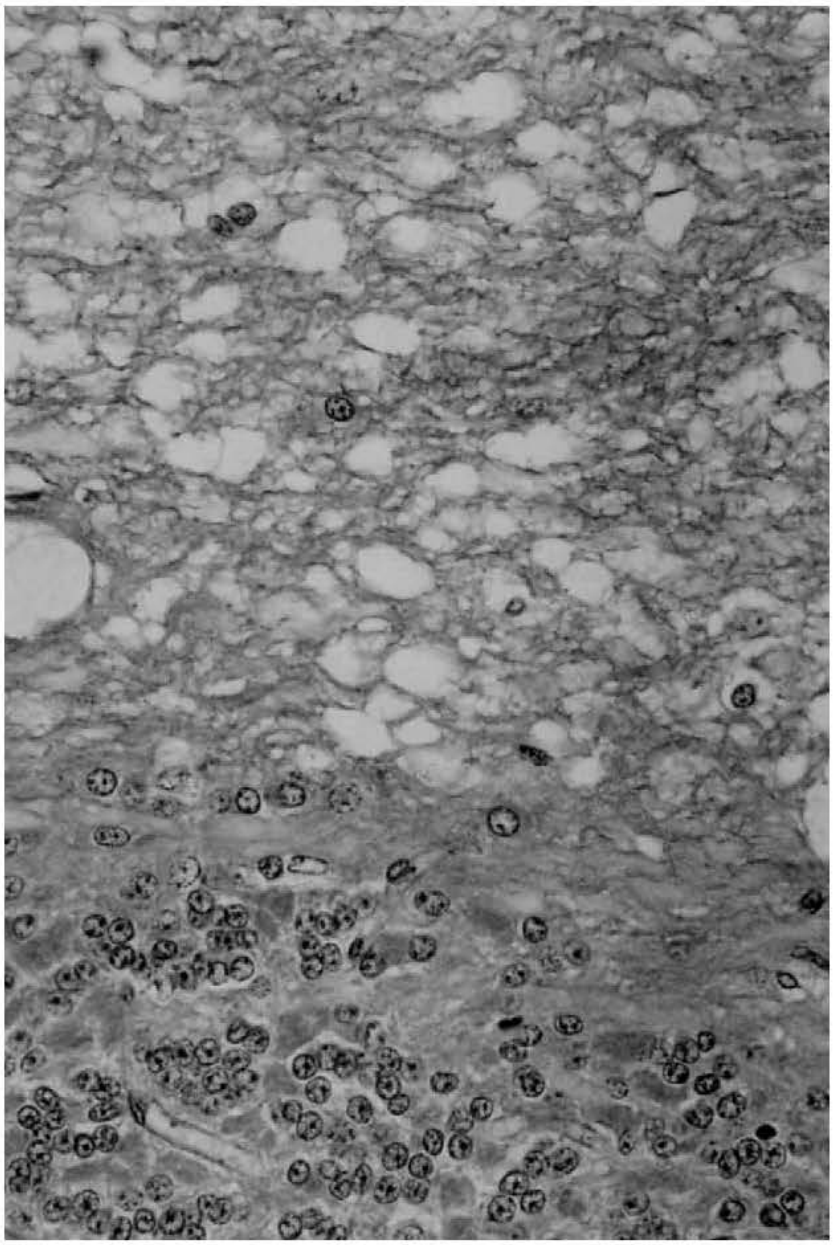

Fig. 1. Cerebellum from fetus of 3.0 mg KCN/kg group dam showing vacuolar degeneration in the white matter (H&E, 40_)

	Evaluation by Competent Authorities
Date	
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Section A6.8 **Annex Point IIA VI.6.8**		**REPRODUCTIVE TOXICITY**	
Section A6.8.2 **Annex Point IIA VI.6.8.2**		**Fertility Study in Female Rats**	
		1 REFERENCE	**Official use only**
1.1	Reference	Monsanto co: Female fertility study of Sprague-Dawley rats exposed by the inhalation route to acetone cyanohydrin. Report 1985 (**DOC IV_61**)	
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 fertility test.	
2.2	GLP	Yes	
2.3	Deviations	/	
		3 MATERIALS AND METHODS	
3.1	Test material	Acetone cyanhydrin	
3.1.1	Lot/Batch number	T501-3	
3.1.2	Specification		
3.1.2.1	Description	Light amber liquid	
3.1.2.2	Purity	98.5%	
3.1.2.3	Stability	Stable during the study	
3.2	Test Animals		
3.2.1	Species	Albino rats	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	Charles River	
3.2.4	Sex	Male and virgin female rats	
3.2.5	Age/weight at study initiation	Females: 43 days at receipt, 63 days at first exposure, 84 days at first mating Males: 41 days at receipt, 82 days at mating	
3.2.6	Number of animals	24 females + 30 males per group	

	per group		
3.2.7	Control animals	Yes	
3.2.8	Mating period	One female with one male overnight. Exposure of the females was continued until copulation was confirmed or a maximum of 5 nights co-housed with a male without signs of copulation. Examination for copulation plugs, vaginal smears following day, positive finding indicates GD1; pregnant rats housed individually. Mated females were sacrificed at about mid-term (days 13-15).	
3.3	Administration/ Exposure	Animal (only females) were exposed in individual wire mesh cages suspended in 10 cubic meter exposure chamber.	
3.3.1	Duration of exposure	6 hours per day, 7 days per week; total number of exposures varied between 21 and 26 (females were exposed until the day after the last mating opportunity).	
3.3.2	Post exposure period	Monitoring of pregnancy up to GD13	
3.3.3	Type	Vapour inhalation	
3.3.4	Dose levels	Target levels: 0, 10, 30, 60 ppm	
3.3.5	Vehicle	Air	
3.3.6	Concentration in air	Mean daily analytical levels: 10.7, 30.4, 58.6 ppm	
3.3.7	Total volume applied	/	
3.3.8	Controls	Exposure to ambient air	
3.4	Examinations		
3.4.1	Body weight	Females were weighed once per week, prior to mating and on days 0 and 13 of gestation.	
3.4.2	Food consumption	No	
	Water consumption	No	
3.4.3	Clinical signs	Females were examined thoroughly once per week, and observed for clinical signs on exposure days. Foe oestrus cycle evaluation, vaginal smears were taken on 5 consecutive days for females which did not exhibit confirmed copulation.	
3.4.4	Gross necropsy	Gross examination of thoracic and abdominal organs. Female reproduction organs were preserved in formalin.	
3.4.5	Examination of ovaries, uterus and uterine contents	Nidation sites were classified and counted and corpora lutea were counted.	
3.5	Further remarks	Males were sacrificed after mating.	

		4 RESULTS AND DISCUSSION	
4.1	Maternal toxic Effects	No clinical signs of toxicity were observed. No significant toxic effects were observed in the females at exposure levels up to 60 ppm as judged by body weights or lesions detectable at necropsy. Although there was an apparent dose response in the number of animals exhibiting red nasal discharge or encrustation in the third week of exposure, no other treatment-related clinical signs were observed and this effect was not judged to be a significant toxic response. No female fertility effects were observed at any of the exposure levels tested. Mating efficiency, pregnancy rates, numbers of live implants and pre- and post-implantation loss rates of exposed females were comparable to values for unexposed females and were consistent with values for other fertility studies in Sprague-Dawley female rats conducted in this laboratory.	
4.2	Teratogenic / embryotoxic effects	No data	
4.3	Other effects – developmental toxicity	/	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	Materials and methods	Sprague-Dawley female rats (24 rats per group) were exposed by the inhalation route (6 hrs/day, 7 days/week) to acetone cyanohydrin at target exposure levels of 0, 10, 30 and 60 ppm for 21 exposure days and mated (at the age of 84 days) to untreated males (30 rats per group, age 82 days) to assess female fertility. Exposure of the females was continued until copulation was confirmed or a maximum of 5 nights co-housed with a male without signs of copulation. Females were sacrificed at mid-gestation (gestation days 13-15).	
5.2	Results and discussion	The averages of mean daily analytical exposure levels (10.7, 30.4 and 58.6 ppm) were within 7% of the target exposure levels. The ratio of nominal/analytical values indicated that acetone cyanohydrin was primarily present as vapour at all exposure levels. No treatment-related deaths, effects on body weight or lesions detectable at necropsy were observed in any of the exposure level groups. There were no treatment related clinical signs observed pre-exposure, during exposure or at weekly physical examinations. There were no treatment-related female fertility effects observed in any of the exposure level groups. Mating efficiency, pregnancy rates, number of live implants and pre- and post- implantation loss were comparable to control values for all exposure level groups (**Table 1**).	
5.3	Conclusion	Acetone cyanohydrin did not produce female fertility effects when administered by the inhalation route to Sprague Dawley rats for 3 weeks prior to mating, at concentrations up to 60 ppm. Corresponds to a daily dose of 13.1 mg cyanide/kg body weight (calculated with a minute volume of 120 ml/min and an average body weight of 200 g and assuming 100% absorption).	

5.3.1	LO(A)EL maternal toxic effects	> 60 ppm	
5.3.2	NO(A)EL maternal toxic effects	60 ppm	
5.3.3	LO(A)EL effects on fertility	> 60 ppm	
5.3.4	NO(A)EL effects on fertility	60 ppm	
5.3.5	Reliability	1	
5.3.6	Deficiencies	/	

Table 1. Summary of mating and pregnancy data

Group ACH ppm	Mating success % of nights co-housed	Pregnancies / copulations (25 females)	BW/ gain to d.13	Live impl. mean	Loss pre+post impl.%	Resor-ption %	Corpora lutea mean
0	45	22/23	261/66	14.0	9+6	6	15.8
10	49	22/23	262/65	14.6	6+4	4	16.1
30	39	22/23	259/66	14.0	11+8	8	16.9
60	36	23/23	260/69	15.5	3+4	4	16.8
Signif.	/	/	/	/	/ /	/	/

Table 2
Incidence of skeletal alterations in litters from mothers 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
Total number of litters	10	10	10	10	10	10	10
Total fetuses examined	40	40	40	40	40	40	40
Affected litters	6	5	5	6	6	6	5
Affected fetuses	23	20	28	26	27	21	23
Skull: incomplete ossification							
Affected litters	6	5	5	6	6	6	5
Affected fetuses	23	20	28	26	27	21	23
Cranial sutures: incomplete ossification							
Affected litters	6	5	5	6	5	5	5
Affected fetuses	8	8	8	11	5	5	10
Sternebrae (bone incompletely ossified)							
Affected litters	6	5	5	6	5	5	5
Affected fetuses	10	12	12	12	11	13	13
Irregularly shaped ribs							
Affected litters	6	5	5	6	5	5	5
Affected fetuses	6	8	9	7	13	8	6
Wavy rib							
Affected litters	0	0	2	2	0	0	0
Affected fetuses	0	0	2	2	0	0	0

Table 3
Incidence of visceral alterations in litters from mothers 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
Total number of litters	10	10	10	10	10	10	10
Total fetuses examined	40	40	40	40	40	40	40
Affected litters	6	7	5	6	5	6	5
Affected fetus	10	18	6	20*	6	15	12
Dilated ureter							
Affected litters	1	7	3	5	2	3	5
Affected fetuses	1	7	3	5	2	3	5
Kinky ureter							
Affected litters	7	7	5	6	3	6	5
Affected fetuses	7	11	5	6	3	8	8
Peritoneal hemorrhage							
Affected litters	2	2	1	4	2	3	2
Affected fetuses	2	2	1	4	2	3	2
Enlarged esophagus							
Affected litters	2	0	0	6	1	4	2
Affected fetuses	2	0	0	7	1	4	2

* Significantly different from control group at $P < 0.05$ (Fischer's test).

Table 4
Serum levels of glucose, cholesterol (mg/dl) and thiocyanate (μmol/l) in control and experimental dams given, 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							
Glucose	81.38 ± 7.04	84.57 ± 9.49	84.11 ± 7.29	112.13 ± 7.56*	70.57 ± 7.10	61.71 ± 6.11	67.59 ± 6.37
Cholesterol	63.85 ± 15.98	65.94 ± 14.66	62.77 ± 14.95	60.08 ± 13.28	61.97 ± 12.47	62.42 ± 15.48	69.58 ± 11.09
Thiocyanate	32.71 ± 3.98	81.43 ± 9.20*	167.90 ± 9.37*	262.50 ± 9.61*	96.09 ± 10.02*	141.88 ± 11.00*	228.50 ± 12.99*
Trial B							
Glucose	79.20 ± 7.25	75.57 ± 10.32	73.88 ± 10.59	70.62 ± 7.34	68.71 ± 9.63	65.83 ± 11.89	68.33 ± 7.29
Cholesterol	68.34 ± 8.66	69.31 ± 7.84	69.61 ± 9.53	60.41 ± 9.39	62.50 ± 9.22	62.93 ± 8.85	60.44 ± 8.16
Thiocyanate	26.63 ± 2.77	35.11 ± 2.87	36.05 ± 2.83	36.73 ± 3.72	37.85 ± 3.25	36.95 ± 3.57	34.20 ± 4.29

* Significantly different from control group at $P < 0.05$ (Duncan's test).

Table 5
Body weight gain (g) of offspring from dams 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

Period	Control	KCN (mg/kg)			KSCN (mg/kg)		
	0	1	3	30	0.8	2.4	24
PND 01–07	51.73 ± 4.70	52.00 ± 4.80	45.93 ± 4.80	47.18 ± 4.61	43.11 ± 4.17	54.37 ± 4.15	47.34 ± 4.16
PND 08–14	76.50 ± 5.72	74.50 ± 7.07	62.67 ± 8.06	71.92 ± 6.31	72.70 ± 5.82	81.21 ± 5.57	67.79 ± 7.89
PND 15–21	83.30 ± 10.03	81.70 ± 10.11	68.40 ± 10.04	92.00 ± 7.28	84.20 ± 8.58	95.80 ± 6.43	83.20 ± 8.36
PND 01–21	211.53 ± 11.31	208.20 ± 11.22	177.33 ± 11.77	211.10 ± 8.14	225.17 ± 11.33	231.32 ± 9.00	198.24 ± 11.06

Table 6
Intensity of the lesions[a] observed in dams and their respective offspring in control and experimental groups that received, in the drinking water KCN: 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/Trial B							
Liver							
Hepatic congestion	−	+	+	++	−	+	−
Vacuolization of hepatocytes	−	−	+	+	−	−	+
Proliferation of biliary ducts	−	−	−	−	−	−	+
Brain							
CNS congestion	−	−	+	++	−	−	+
Neuronophagia	−	−	−	+	−	−	+
Hemorrhagic areas in CNS	−	−	−	+	−	−	−
Gliosis	−	−	−	++	−	+	++
Necrosis	−	−	−	+	−	−	−
Thyroid							
Increase in the number of reabsorption vacuoles in follicular colloid	−	+	++	+++	+	++	+++
Trial A							
Pancreas							
Islet cells vacuolation	−	−	−	++	−	−	−
Pups							
Liver							
Hepatic congestion	−	−	+	++	−	+	++
Vacuolization of hepatocytes	−	−	−	+	−	−	+
Proliferation of biliary ducts	−	−	−	+	−	−	+
Brain							
CNS congestion	−	−	+	++	−	+	++
Neuronophagia	−	−	−	+	−	−	+
Gliosis	−	−	−	++	−	−	++

The intensity was characterized by scores: (−) no lesion; (+) mild lesions; (++) moderated lesions; and (+++) severe lesions. It was used for four animals in each group.
[a] The identification of the lesions was considered only when all animals analyzed showed the same alterations.

	Evaluation by Competent Authorities
Date	
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Conclusion	
Remarks	

Section A6.8 Annex Point IIA VI.6.8		**REPRODUCTIVE TOXICITY**	
Section A6.8.2 Annex Point IIA VI.6.8.2		**Fertility Study**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	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 IV_40)**	
1.2	**Data protection**	No	
1.2.1	Data owner	/	
1.2.2		/	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No reported. Method and procedure essentially identical with standardised test B14.	
2.2	**GLP**	US FDA GLP regulation	
2.3	**Deviations**	Only 4 strains of S. typhimurium were used.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Sodium cyanide	
3.1.1	Lot/Batch number		
3.1.2	Specification		
3.1.2.1	**Description**		
3.1.2.2	**Purity**	98%	
3.1.2.3	**Stability**	Not known	
3.2	**Study Type**	Bacterial reverse mutation test	
3.2.1	Organism/cell type	Salmonella typhimurium strains TA100, TA1535, TA97, and TA98,	
3.2.2	Deficiencies / Proficiencies	Not applicable	
3.2.3	Metabolic activation system	S9 mix 10 or 30% rats and hamster liver, Aroclor induced	
3.2.4	Positive control	Yes	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	0.3 to 333 µg per plate	
3.3.2	Way of application		

3.3.3	Pre-incubation time	Sodium cyanide was incubated with the S. typhimurium tester strains either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37^{0}C	
3.3.4	Other modifications	Top agar supplemented with l-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates	
3.4	**Examinations**	Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37dgC. Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of sodium cyanide.	
3.4.1	Number of cells evaluated		
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.1.1	Without metabolic activation	Not genotoxic	
4.1.2	With metabolic activation	Not genotoxic	
4.2	**Cytotoxicity**	At doses ≥ 100µg per plate.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Sodium cyanide (0.3 to 333 micrograms per plate) was tested for mutagenicity in Salmonella typhimurium strains TA100, TA1535, TA97, and TA98, with and without Aroclor-induced rat and hamster S9 at concentrations of 10% and 30%. Sodium cyanide was incubated with the S. typhimurium tester strains either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37^{0}C. Top agar supplemented with l-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37^{0}C. Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of sodium cyanide.	
5.2	**Results and discussion**	**See table below.**	
5.3	**Conclusion**	Sodium cyanide in doses 0.3 to 333 micrograms per plate was not mutagenic in any of several strains of Salmonella typhimurium with or without S9 activation. The testing with the strain T100 was completely negative for doses per plate up to the level toxic for bacteria (100 or 333 micrograms per plate); positive controls indicated full sensitivity of the tes*t*	
5.3.1	Reliability	*2*	
5.3.2	Deficiencies	Only 4 strains of S. typhimurium were used.	

Table: Mutagenicity of Sodium Cyanide in Salmonella typhimurium 1

		Revertants/plate[2]						
Strain / Dose		**S9**			**+ hamster S9**		**+rat S9**	
(µg/ plate)		**Trial 1**	**Trial 2**	**Trial 3**	**10%**	**30%**	**10%**	**30%**
TA100	0.0	103± 1.5	89± 6.2	92± 3.5	107± 9.3	108± 1.2	86± 6.9	116± 3.5
	1.0	89± 9.8		104± 4.5		108± 5.5		121± 4.5
	3.3	108± 1.2	83± 9.4	89± 4.6	104± 7.0	111± 3.8	94± 1.7	120± 6.0
	10.0	101± 1.5	82± 9.0	108± 3.7	114± 6.1	117± 10.0	116± 4.6	133± 10.3
	33.0	103± 6.6	91± 8.6	85± 1.2	99± 11.0	108± 4.3	107± 12.6	121± 6.0
	100.0	101± 7.8	3± 2.5	96± 2.9	79± 9.5	115± 6.0	80± 6.6	122± 12.4
	333.0		0± 0.0		1± 0.7		12± 4.4	
Trial summary		**Negative**	**Negative**	**Negative**	**Negative**	**Negative**	**Negative**	**Negative**
Positive control[3]		415± 31.5	348± 15.5	644± 59.0	719± 25.7	448± 41.5	1,125± 21.5	706± 45.9
TA1535	0.0	14± 2.3	10± 1.5		13± 0.3	16± 2.1	11± 0.7	13± 2.8
	1.0	14± 0.3				21± 2.7		14± 2.5
	3.3	14± 0.6	9± 0.7		9± 2.1	16± 3.0	10± 2.1	14± 1.2
	10.0	9± 1.2	7± 1.8		13± 2.6	13± 1.8	8± 1.5	14± 2.4
	33.0	12± 0.9	4± 0.7		12± 2.7	19± 3.8	7± 2.6	16± 1.0
	100.0	12± 2.3	3± 0.7		11± 2.8	21± 1.2	8± 1.2	18± 1.2
	333.0		0± 0.0		4± 1.7		3± 1.5	
Trial summary		**Negative**	**Negative**		**Negative**	**Negative**	**Negative**	**Negative**
Positive control		516± 8.2	200±6.5		80± 0. 9	92± 6.4	141± 6.1	178± 6.6

Strain / Dose		**S9**			**+ hamster S9**		**+rat S9**	
(µg/ plate)		**Trial 1**	**Trial 2**	**Trial 3**	**10%**	**30%**	**10%**	**30%**
TA97	0.0	143± 3.2	115± 2.3		142± 4.7	191± 11.9	134± 8.5	206± 9.2
	1.0	119± 4.5				167± 12.7		226± 9.5
	3.3	133± 3.5	123± 5.9		154± 6.0	167± 9.6	144± 4.4	217± 8.0
	10.0	119± 6.2	100± 4.5		149± 9.4	179± 9.4	134± 4.3	192± 3.8
	33.0	130± 4.6	106± 5.9		156± 7.5	173± 9.1	143± 9.1	219± 3.9
	100.0	163± 3.0	109± 4.9		163± 6.6	179± 5.0	128± 8-9	229± 7.8
	333.0		76± 15.6		130± 21.5		116± 7.7	
Trial summary		**Negative**	**Negative**		**Negative**	**Negative**	**Negative**	**Negative**
Positive control		721± 103.8	355± 32.1		1,222± 40.0	1,900±122.0	1,533±62.5	1,097± 43.9

Table:Mutagenicity of Sodium Cyanide in Salmonella typhimurium (continued)

		Revertants/plate[2]					
Strain / Dose			**S9**			**+ hamster S9**	
(µg/ plate)		**Trial 1**	**Trial 2**	**Trial 3**	**10%**	**10%**	**30%**
TA98	0.0	19± 1.5	10± 2.3	15± 2.1	18±2.4	23± 2.5	18± 2.4
	0.3			14± 1.5		16± 2.2	
	1.0	12± 1.5		13±.3.6		20± 2.0	20± 2.0
	3.3	14± 1.5	5± 2.0	16± 3.3	13± 2.6	16± 3.8	21± 3.2
	10.0	14± 1.8	0± 0.0	13± 1.7	14± 2.9	22± 3.3	24± 1.2
	33.0	11± 1.0	1±1.0	12± 0.7	8± 0.6	16± 1.5	13± 3.2
	100.0	0± 0.0	0± 0.0		3± 0.6		7± 2.2
	333.0		0± 0.0		0± 0.3		
Trial summary		**Negative**	**Negative**	**Negative**	**Negative**	**Negative**	**Negative**
Positive control		321± 10.5	253± 67.0	423±9.7	706± 24.1	406± 26.5	387±44.5

		+rat S9	
TA98 (continued)	10%	10%	30%
0.0	18± 3.0	19± 2.3	22±0.0
0.3		19± 3.2	
1.0		23± 2.2	24±2.2
3.3	21± 2.6	17± 1.7	24±2.6
10.0	12± 2.4	20± 1.5	23±1.8
33.0	9± 0.7	17± 1.5	22±2.1
100.0	3± 0.0		7±1.5
333.0	0± 0.0		
Trial summary	**Negative**	**Negative**	**Negative**
Positive control	433±24.2	199± 8.0	407± 46.6

[1] Study was performed at Microbiological Associates, Inc. The detailed protocol is presented in Zeiger et al. (1992) – see original study; 0 µg/plate is the solvent control.

[2] Revertants are presented as mean ± standard error from three plates.

[3] The positive controls in the absence of metabolic activation were 4-nitro-o-phenylenediamine (TA98), sodium azide (TAl 00 and Al535), and 9-aminoacridine (TA97). The positive control for metabolic activation with all strains was 2-aminoanthracene.

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Section A6.9 Annex Point IIIA VI.1	**NEUROTOXICITY STUDY**	
Justification:	Due to its high dependency on oxidative metabolism the central nervous system is particularly vulnerable to cyanide intoxication. The central nervous system symptoms form a substantial part of the pattern of both acute and chronic cyanide toxicity. Many observations in humans have been described, and experimental studies carried out on laboratory animals. Data on the effects of sub chronic and chronic cyanide exposure indicate that the same pattern of effects occur in humans and experimental animals.	
Supportive data: **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**),Hazardous Substance Data Bank (HSDB), National Library of Medicine´s TOXNET system: Hydrogen cyanide *Peer reviewed* (**DOC IV_2**) and 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 IV_40).** 1) **Summary of a key study** 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) is in DOC III_ 6.11.1a.** 2) Tewe OO, Maner JH: Performance and pathophysiological changes in pregnant pigs fed cassava diets containing different levels of cyanide; Research in veterinary Science, 1981,30,147-151 **(DOC IV_62).** 3) Fechter LD, Chen G, Johnson DL. 2002. Potentiation of noise-induced hearing loss by low concentrations of hydrogen cyanide in rats. Toxicol Sci 66 (1):131-138. **(DOC IV_63)** 4) 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**) 5) Jackson LC, Bloch EF, Jackson RT, Chandler JP, Kim YL, Malveaux F (1985) Influence of dietary cyanide on immunoglobulin and thiocyanate levels in the serum of Liberian adults. Journal of the National Medical Association, 77:777 782. 6) Jackson LC (1988) Behavioural effects of chronic sublethal dietary cyanide in an animal model: Implications for humans consuming cassava (Manihot esculenta). Human Biology, 60:597–614. 7) Hertting GO, Kraupp E, Schnetz E, Wuketich ST (1960) Investigation about the consequences of a chronic administration of acutely toxic doses of sodium cyanide to dogs. Acta Pharmacologica et Toxicologica, 17:27–43 (in German). 8) Lewis TR, Anger WK, Te Vault RK (1984) Toxicity evaluation of sub-chronic exposures to cyanogen in monkeys and rats. Journal of Environmental Pathology, Toxicology and Oncology, 5:151–163. 9) Mathangi DC, Namasivayam A. 2000. Effect of chronic cyanide intoxication on memory in albino rats. Food Chem Toxicol 38:51-55. 10)Lessell S. 1971. Experimental cyanide optic neuropathy. Arch Ophthalmol 86:194-204. 11)Lessell, S., and Kuwabara, T. (1974). Fine structure of experimental cyanide optic neuropathy. Invest. Ophthamol. 13, 748-756 12)Ferraro, A. (1933) Experimental toxic encephalomyelopathy (Diffuse sclerosis following subcutaneous injections of potassium	

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Results and discussion :	Neurotoxicity findings in acute exposure to cyanides in laboratory animals are discussed in the acute toxicity section. The effects of cyanide on behaviour were studied in fasted 25-week-old miniature pigs (12 litter mates: 5 females and 7 castrated males) randomized in four groups. The animals were dosed daily for 24 weeks with a single bolus of cyanide as aqueous potassium cyanide just prior to the daily feeding. The doses were 0, 0.4, 0.7, or 1.2 mg cyanide/kg body weight, chosen to be equivalent to those consumed by West Africans in their diet **(5).** Every 6 weeks, thyroid function (T3 and T4) and fasting blood glucose were measured, but not thyroid-stimulating hormone (TSH). Daily observations were made of clinical signs and various behavioural measurements, including social, antagonistic, exploratory, learning, feeding, and excretory behaviour. In all treatment groups, dose-related decreases were evident from week 6 in blood levels of T3 and T4, and an increase in fasting blood glucose was noted, particularly in top-dose animals. Statistical analysis was not provided for each dose group versus control, but changes in top-dose animals appeared significant by week 18; by week 24, decreases of 35% for T3 and 15% for T4 and an increase of 60% in fasting blood glucose were observed. Behavioural observations revealed a picture of decreased high energy-demanding behaviour, such as exploration and aggression, slower eating, more frequent drinking, and shivering consistent with the decreased thyroid activity. A LOAEL of 1.2 mg/kg body weight per day could be suggested from this study **(6).** Dogs administered sodium cyanide in capsules at levels of 0, 0.5, 2, or 4 mg/kg body weight (one dog at each dose level) daily for 13–15 months showed severe signs of acute cyanide poisoning right after the daily dosing (the dog at the lowest dose died). In the autopsy, the only significant findings were degenerative changes in ganglia cells of the central nervous system, interpreted to be caused by multiple episodes of acute cerebral hypoxia **(7).** Transient behavioural changes in monkeys but not in rats exposed for 6 months to 54 mg cyanogen/m^3 have been reported (**8**). The 6 month (6 hr/day, 5 days/week) inhalation toxicity study was conducted with	

	cyanogen gas using male rhesus monkeys (Macacca mulatta) and male albino rats (Charles River Strain) as experimental animals. Fifteen monkeys and 90 rats were divided into three groups of 5 monkeys and 30 rats. One group, the Controls, was not exposed to the test material; the other two groups were exposed to either 11 ppm (corresponding to 27 mg cyanide/$m^{3)}$ or 25 ppm cyanogen (corresponding to 54 mg cyanide/$m^{3)}$. At the outset of exposures, 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 receiving 11 ppm exposures; the increases were transitory as the rate returned to control levels before exposures were terminated. At the end of the 6 month- exposure, there were no effects in hematologic or clinical chemistry parameters attributable to the inhalation exposure to cyanogen. The electrocardiograms and gross pathologic and histopathologic examinations of 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 cyanogen than in Control animals. Body weights were significantly lower in rats exposed to 25 ppm than in Controls. The results suggest that sub chronic 25 ppm cyanogen exposures are marginally toxic, but the evidence on 11 ppm does not support a similar conclusion **(1).** Potentiation to noise-induced hearing loss by exposure of rats to low concentrations of hydrogen cyanide was reported. It was suggested that hydrogen cyanide (34 mg/$m^3$3 for 3.5 h) plus noise produced impaired auditory function by producing significant oxidative stress in the cochlea. Hydrogen cyanide alone did not cause significant hearing loss or hair cell loss **(3).** A single intraperitoneal dose and 25 repeated intraperitoneal doses of sodium cyanide (2 mg/kg body weight [1 mg cyanide/kg body weight]), stated to represent 25% of the LD50, administered to Wistar strain albino rats resulted in similar reductions of memory (Tmaze test), along with reductions in the levels of dopamine and 5-hydroxytryptamine and increases in norepinephrine and epinephrine levels in the hippocampus, measured after a month of treatment **(9).** In experiments with rats **(4, 10, 11)**, cats, and monkeys **(12, 13)** selective destruction of white matter in the brain was a striking and consistent feature of poisoning from prolonged exposure to cyanide. In most of these experiments, animals were injected with increasing doses of sodium or potassium cyanide for up to 132 days, and the doses used were high enough to cause significant death rates from acute toxicity. However, in the study **(4)**, weanling rats exposed to low concentrations of potassium cyanide in feed had a marked decrease in weight gain, but no deaths or clinical signs of toxicity. Early necrosis of grey and white matter was a common occurrence in rats and monkeys, but repeated exposure appeared to selectively favour destruction of white matter. The histopathologic lesions observed in all species consisted of demyelination, especially of the optic nerve tracts and the corpus callosum. Swelling of astrocytes and myelin damage were apparent within 2days in rats injected with sodium cyanide at doses sufficient to keep the rats comatose for 225 to 260 minutes (**11**). Axonal damage, with vacuolation and loss of microtubules, also occurred. Blindness was common in cyanide-treated animals and was considered to be a result of persistent anoxia in the brain.	
Mechanism of neurotoxicity and observations in humans	As a respiratory poison, free cyanide (hydrogen cyanide or cyanide ion) has high acute toxicity due to inhibiting cytochrome oxidase. Tissue utilization of oxygen is impaired, and histotoxic anoxia occurs. Due to	

	its high dependency on oxidative metabolism and limited anaerobic capacity, the central nervous system is particularly vulnerable to cyanide intoxication (**14**). The central nervous system symptoms observed in cyanide toxicity parallel those observed following accumulation of calcium in the brain and cytosolic calcium ion overload has been implicated as an intracellular mediator of cellular injury during and after anoxic hypoxia (**15**). Hydro peroxide generation with subsequent peroxidation of lipids and subsequent changes in structure and function of certain membranes have been suggested as a possible further mechanism of cyanide toxicity (**16**); (see section on mechanism of action). Neurologic lesions attributed to sub chronic cyanide poisoning in humans are similar to those described for experimental animals. In rats, however, the corpus callosum appears to be more sensitive than the optic nerves, whereas in humans, optic nerve damage is frequently the only central nervous system lesion **(17)**. Numerous studies have implicated cyanide as the etiologic agent in human neuropathies, including Nigerian nutritional neuropathy, tobacco amblyopia, and Leber's optical atrophy. The syndrome of tropical ataxic neuropathy includes bilateral optic atrophy, nerve deafness, sensory spinal ataxia, weakness of legs, and numbness of feet (**18**). This condition is believed to be due to cyanide-induced demyelination in the brain and spinal cord and is attributed primarily to consumption of the plant cassava, which contains high levels of cyanogenic glycosides (**17**). Elevated plasma and urinary thiocyanate levels and demyelination of peripheral nerves, with decreased conduction velocity, were observed in patients from Nigeria with tropical ataxic neuropathy (**18, 19**). Cyanide poisoning from tobacco smoke has also been implicated in the occurrence of tobacco amblyopia, an optic disorder that is common in people who smoke tobacco. Tobacco smoke is known to contain cyanide, and in (**20**) there is reported that smokers have elevated levels of plasma and urinary thiocyanate. Hydroxocobalamin and cyanocobalamin, which are capable of complexing cyanide in the bloodstream, have been shown to be effective in treating tobacco amblyopia, suggesting that cyanide itself is the etiologic agent in this disorder. Finally, an inborn error in cyanide metabolism is thought to be the cause of Leber's hereditary optic atrophy, a condition in which bilateral vision failure occurs. Low levels of plasma thiocyanate in smokers with this condition suggest a hereditary deficiency in the ability to metabolize cyanide to thiocyanate (**20**). The neurologic lesions seen with all of these neuropathies are thought to be the result of cyanide-induced histotoxic anoxia.	
Conclusions	1) The central nervous system is the primary target of acute cyanide toxicity. Inhalation of hydrogen cyanide causes first short-time stimulation followed by depression, convulsions, unconsciousness and suppression of primary reflexes, dilatation of pupils, paralysis and even death. 2) Neurologic lesions attributed to subchronic cyanide poisoning in humans are similar to those described for experimental animals. 3) Neurological disorders (tremor, ataxia, cerebral cells injury) are observed in medium-term studies on laboratory animals with HCN concentration of 50mg/m^3 HCN and higher for several hours per day.	

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Section A6.12 **Annex Point IIA VI.6.9**	**MEDICAL DATA IN ANONYMOUS FORM**
Justification:	Hydrogen cyanide has been used for many years and its effects on humans in occupational settings are well known. Data for cyanides (in diet) are used as supporting information for oral exposure.
References:	Summaries and evaluations in this section are based mostly on exhaustive and reliably peer reviewed documents: ATSDR (2004, Toxicological profile of cyanide) **(DOC IV_1)** and IPCS (2004, WHO, CICAD 61: Hydrogen cyanide and cyanides: human health aspects) **(DOC IV_5)** and Hazardous Substance Data Bank (HSDB), National Library of Medicine´s TOXNET system (state in February 2006): Hydrogen cyanide *Peer reviewed* **(DOC IV_2).** **Summaries of two case studies are in DOC III 6.12.1a and b** (ref. 69 and 72). References from ATSDR, 2004, IPCS and HSDB: 1. American Conference of Governmental Industrial Hygienists (ACGIH); Documentation of the Threshold Limit Values and Biological Exposure Indices. 5th ed. Cincinnati, OH: American Conference of Governmental Industrial Hygienists, 1986., p. 314 2. Banerjee KK, Bishayee B, Marimuthu P (1997) Evaluation of cyanide exposure and its effect on thyroid function of workers in a cable industry. Journal of Occupational Medicine, 39:255–260. 3. Drinker P. 1932. Hydrocyanic acid gas poisoning by absorption through the skin. J Ind Hyg 14:1-2. 4. Dudley HC, Sweeney TR, Miller JW. 1942. Toxicology of acrylonitrile (vinyl cyanide). II: Studies of effects of daily inhalation. J Ind Hyg Toxicol 24:255-258 5. Chandra H, Gupta BN, Mathur N. 1988. Threshold limit value of cyanide: A reappraisal in Indian context. Indian J Environ Protection 8:170-174. 6. VanderLaan WP, Bissell A. 1946. Effects of propylthiouracil and of potassium thiocyanate on the uptake of iodine by the thyroid gland of the rat. Endocrinology 39:157-160. 7. ATSDR (1993) Toxicological profile for cyanide. Prepared by Syracuse Research Corporation under subcontract to Clement International Corporation (Contract No. 205-88-0608). 8. ATSDR (1991) Case studies in environmental medicine. Atlanta, GA, US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry 9. Hardy HL, Jeffries WM, Wasserman MM, Waddell WR (1950) Thiocyanate effect following industrial cyanide exposure. New England Journal of Medicine, 242:968–972. 10. Leeser JE, Tomenson JA, Bryson DD (1990) A cross-sectional study of the health of cyanide salt production workers. Macclesfield, ICI Central Toxicology Laboratory. 11. Okafor PN, Okorowko CO, Maduagwu EN (2002) Occupational and dietary exposures of humans to cyanide poisoning from large-scale cassava processing and ingestion of cassava foods. Food and Chemical Toxicology, 49:1001–1005. 12. Linden CH, Lovejoy Jr. FH. 1998. Poisoning and drug overdose. In: Fauci AS, Braunwald E, Isselbacher KJ, eds. Harrison's principles of internal medicine. New York: McGraw-Hill Health; Professions Division. 13. Berlin C. 1977. Cyanide poisoning--A challenge. Arch Intern Med 137:993-994. 14. Williams, 1959 – Williams RT (1959) Detoxification mechanisms, 2nd

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Findings:	Findings are summarised below in Table

Table - Summarised findings

Acute toxicity – oral (for cyanides)

Study		**Subjects**	**Dose, concentration/ exposure time**	**Effect, ED**	**Reference**
Single dose by ingestion	KNC	Human - male	Single dose	15mg/kg Respiratory effect - hyperventilation	(65)
Single dose by ingestion	KCN	Human - male	Single dose	Minimally 15mg/kg gastrointestinal vomiting and nausea	(65)
			NOAEL: 15mg/kg/day	15mg/kg blood effect	(65)