Section A6.5/01 Repeated dose toxicity

Annex Point IIA6.5 Inhalation study with rats with an exposure over 104 weeks

3.3.3	Postexposure period	No		
3.3.4	**Inhalation**			
3.3.4.1	Concentrations	Nominal concentration	0, 500, 2500 or 5000 ppm (ca. 0, 1250, 6250 or 12500 mg/m^3)	X
		Analytical concentration	0, 504 ± 14, 2509 ± 58 or 5037 ± 115 ppm	
3.3.4.2	Particle size	Not applicable		
3.3.4.3	Type or preparation of particles	Not applicable		
3.3.4.4	Type of exposure	Whole body		
3.3.4.5	Vehicle	None		
3.3.4.6	Concentration in vehicle	Not applicable		
3.3.4.7	Duration of exposure	6 hrs/day		
3.3.4.8	Controls	Yes (0 ppm; filtered air)		
3.4	**Examinations**			
3.4.1	Observations			
3.4.1.1	Clinical signs	Yes (daily)		
3.4.1.2	Mortality	Yes (daily)		
3.4.2	Body weight	Yes prior to first exposure, weekly in the first two weeks, and every other week thereafter		
3.4.3	Food consumption	No data		X
3.4.4	Water consumption	No data		X
3.4.5	Ophthalmoscopic examination	Yes prior to 1st exposure, at 17 and 19 months and at terminal sacrifice		
3.4.6	Haematology	Yes Number of animals: All surviving animals from core groups Time points: At approximately 13 and 19 months and at terminal sacrifice Parameters: At terminal sacrifice: Total leukocyte count, differential leukocyte count, erythrocyte count, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and platelet count Parameters at additional time points: During study: Differential leukocyte counts from control and high concentration		

Section A6.5/01 **Repeated dose toxicity**

Annex Point IIA6.5 Inhalation study with rats with an exposure over 104 weeks

		groups	
3.4.7	Clinical Chemisty	No data	X
3.4.8	Urinalysis	Yes Number of animals: 10 per sex and group Time points: week 57: group with access to water and food week 58: group with access to food but not to water weeks 74 / 104: group with access to water and food Parameters: week 57: total protein, total glucose, and urine volume week 58: osmolality weeks 74 / 104: osmolality, total protein, total glucose, and urine volume	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes From all surviving animals at interim and terminal sacrifice: liver, kidneys, testes, spleen, brain, heart, lungs	
3.5.2	Gross and histopathology	Yes A complete necropsy was performed on each animal (including animals found dead or euthanized as moribund) and tissues were fixed in 10 % neutral buffered formalin. Tissue sections were prepared and stained with haematoxylin and eosin. Sections of the kidneys were also stained with Mallory Heidenhain stain. Lungs were inflated with formalin via the trachea: sectioning of the lung included two coronal cuts through all lobes and mainstem bronchi. Four standard sections of the nasal cavity at different levels were prepared. Microscopically examined tissues of control and high concentration groups included adrenals, larynx, spleen, brain, liver, testes, eyes, lungs, thymus, gross lesions, heart, trachea, kidneys, ovaries, pancreas, nasal turbinates, stomach, uterus, pituitary, thyroid/parathyroid, aorta, sternum with bone marrow, salivary glands, duodenum, skin (flank), gall bladder, jejunum, oesophagus, urinary bladder, ileum, lymph node (submandibular), mammary gland, caecum, peripheral nerve (sciatic), thigh muscle, colon, Zymbal's glands, exorbital lacrimal glands, rectum, seminal vesicles, epididymis, prostate, femur (including articular surface), and the spinal cord. In addition, microscopic evaluations of the kidneys, testes, and gross lesions from the low and intermediate groups were performed.	
3.5.3	Other examinations	No	
3.5.4	Statistics	The data for continuous, parametric variables were intercompared for the exposure and control groups by use of Levene's test for homogeneity of variances, by analysis of variance, and by t tests. The t tests were used, if the analysis of variance was significant, to delineate which groups differed from the control group. If Levene's test indicated homogeneous variances, the groups were compared by an analysis of variance for equal variances followed, when appropriate, by pooled variance t tests. If Levene's test indicated heterogeneous variances, the groups were compared by an analysis of variance for unequal variance	

		followed, when appropriate, by separate variance t tests. Frequency data, such as microscopic diagnoses, were compared using Fisher's Exact Test. Nonparametric data were statistically evaluated using the Kruskal-Wallis test and, if necessary, by the Wilcoxon rank sum test as modified by Mann-Whitney. Mortality data were analyzed by life-table analysis. All statistical tests, except the frequency comparisons, were performed using BMDP Statistical Software. The probability value of $p < 0.05$ (two-tailed) was used as the critical level of significance for all tests.	
3.6	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Exposure periods: ≥ 2500 ppm: hypoactivity and lack of a startle reflex 5000 ppm: narcosis These clinical signs were transient in nature since these signs were absent immediately following exposure. Non-exposure periods: ≥ 2500 ppm: urine stains 5000 ppm: emaciation and dehydration in males; swollen periocular tissue in females	
4.1.2	Mortality	increased in males at 5000 ppm (100 % [last death during week 100] vs. 82 % in controls) The main cause of death appeared to be chronic renal disease which was also considered to account for much of the mortality observed for animals exposed to 2500 ppm. The main cause of death for females died or euthanized moribund due was chronic renal disease in the 5000 ppm group. The main cause of death for the male and female controls was mononuclear cell leukemia. Mean survival time: decreased in males at 5000 ppm (577 vs. 631 days in controls)	X
4.2	**Body weight gain**	≥ 2500 ppm: increased body weight and body weight gain in males (these increases were typically observed throughout the remainder of the study, although statistical significance was rarely achieved following week 72). At week 52, mean body weight and body weight gain were increased 4 and 6 %, respectively, for males at 2500 ppm and 5 and 7 %, respectively, for males at 5000 ppm. Concentration-related increases in body weight and body weight gain were typically observed for females following week 5; however, the increases in body weight and body weight gain observed at 5000 ppm were very slight (≤ 1 %). Mean body weight and body weight gain were increased 4 and 7 %, respectively, for females at 2500 ppm and 6 and 10 %, respectively, for females at 5000 ppm at week 52. 5000 ppm: decreased body weight and/or body weight gain in males and females at the end of the first and second weeks of exposure. Following this time point, the body weight of these rats increased, and, by the end of week 6, increased body weight and body weight gain were noted for both males and females.	X

Section A6.5/01	Repeated dose toxicity	
Annex Point IIA6.5	Inhalation study with rats with an exposure over 104 weeks	
4.3 **Food consumption and compound intake**	No data	
4.4 **Ophtalmoscopic examination**	No adverse effects	
4.5 **Blood analysis**		
4.5.1 Haematology	No adverse effects	
4.5.2 Clinical chemistry	No data	
4.5.3 Urinalysis	At 13 months: 5000 ppm: decrease in osmolality, increase in total protein (m) and increase in total volume and glucose (f) At 17 months: ≥ 2500 ppm: decreased osmolality, increase in total protein, total volume, and total glucose excreted for males at ≥ 2500 ppm and for females at 5000 ppm At terminal euthanasia: ≥ 2500 ppm: decrease in osmolality (f) and increases in total protein (m), total volume, and total glucose for males at 2500 ppm (no survivors at 5000 ppm) and for females at 5000 ppm The individual results are summarised in Table A6.5/01_01	X
4.6 **Sacrifice and pathology**		
4.6.1 Organ weights	Interim euthanasia: ≥ 500 ppm: concentration-related increases in absolute and relative testes weights 2500 ppm: increased relative liver weights in males 5000 ppm: increase in absolute and relative lung weights in females; increased absolute and/or relative liver and kidney weights in males Terminal euthanasia: 2500 ppm: increased absolute and/or relative liver and kidney weights in males 5000 ppm: increased absolute and/or relative liver and kidney weights in females The individual results are summarised in Table A6.5/01_02	X
4.6.2 Gross and histopathology	Interim euthanasia: ≥ 2500 ppm: increased grades for some lesions associated with chronic renal disease in males 5000 ppm: increased frequency of testicular seminiferous tubule atrophy Terminal euthanasia: ≥ 2500 ppm: increase in severity of certain renal lesions in all males (including rats found dead or euthanized moribund) such as mineralization, tubular dilation, glomerulosclerosis, interstitial nephritis, interstitial fibrosis, hydronephrosis, and transitional cell hyperplasia with an increase in the frequencies of these lesions in died males or euthanized moribund. Increased severity of some of the key components for chronic renal disease such as tubular proteinosis, glomerulosclerosis, interstitial	

nephritis, and interstitial fibrosis) in all females.

Typically both the severity and incidence of the lesions which characterize chronic renal disease were greater for males than for females.

The individual results are summarised in Table A6.5/01_03.

Rats found dead or euthanized moribund:
≥ 2500 ppm: increased incidence of mineralization in a number of organs (not further specified)
5000 ppm: increased frequency of myocardial degeneration/fibrosis, fibrous osteodystrophy, glandular ectasia within the gastric mucosa (females only), cellular hyperplasia of the parathyroid glands (females only), basophilic cell foci within the liver (males only), splenic haemosiderosis, rhinitis and squamous metaplasia of the respiratory epithelium within the nasal cavity, iridocyclitis (males only), atrial thrombosis (females only), ocular keratitis (females only), and dacryosolenitis (females only).

4.7 Other

None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In this study groups of 75 F344 rats were exposed to concentrations of 0, 500, 2500 or 5000 ppm (ca. 0, 1250, 6250 or 12500 mg/m^3) on 6 hrs/day on 5 days/week over 104 weeks. An interim sacrifice with 10 rats per sex and group was performed at week 73.
The study design is comparable to OECD Guideline 451, but some experimental data such as clinical chemistry are missing.

5.2 Results and discussion

Transient signs of narcosis were observed during exposure to ≥ 2500 ppm. In male rats exposed to 5000 ppm the mortality rate was increased (100 vs. 82 % for controls) and there was also a decrease in mean survival time (577 vs. 631 days for controls). Increases in body weight and/or body weight gain were typically observed for both sexes at ≥ 2500 ppm. Urinalysis and urine chemistry changes indicative of impaired kidney function were noted for males at ≥ 2500 ppm and for females at 5000 ppm. At interim euthanasia, there was a concentration-related increase in testes weights and an increase in absolute and/or relative liver and kidney weights in males and/or females at ≥ 2500 ppm.
A number of non-neoplastic lesions was seen in males and females at ≥ 2500 ppm, with the most significant lesions being observed in the kidneys and associated with chronic renal disease such mineralization, tubular dilation, glomerulosclerosis, interstitial nephritis, interstitial fibrosis, hydronephrosis, and transitional cell hyperplasia.

5.3 Conclusion

5.3.1 LO(A)EL [redacted]

5.3.2 NO(A)EL [redacted]

5.3.3 Other [redacted]

5.3.4 Reliability [redacted]

5.3.5 Deficiencies [redacted]

Section A6.5/01 Repeated dose toxicity

Annex Point IIA6.5 Inhalation study with rats with an exposure over 104 weeks

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/11/14
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*

Section A6.5/01	**Repeated dose toxicity**
Annex Point IIA6.5	Inhalation study with rats with an exposure over 104 weeks

Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.5/01_01 Urinalysis in rats

Affected sex	parameter	Unit	Controls	500 ppm	2500 ppm	5000 ppm
13 months after start of treatment						
m	osmolality	mOsm/kg	2332	2113	2157	1574**
m	total protein	g/L	11426	11534	12768	15296*
f	total volume	mL	4.9	5.7	6.4	7.3**
f	glucose	g/L	0.71	0.70	0.64	0.54**
17 months after start of treatment						
m	osmolality	mOsm/kg	1225	1491	942	605**
f			1973	1954	1841	1254**
m	total protein	g/L	11821	13243	17306	19382**
f			8333	6795	12652	16561**
m	total volume	mL	8.7	5.9	11.9	16.5**
f			6.3	5.0	7.3	11.6*
m	glucose	g/L	0.43	0.47	0.29	0.21*
f			0.54	0.54	0.52	0.41
24 months after start of treatment						
f	osmolality	mOsm/kg	1108	1054	934	537*
f	total volume	mL	11.0	12.1	14.8	23.3**
f	glucose	g/L	0.51	0.52	0.47	0.33*

* $p < 0.05$; ** $p < 0.01$

Table A6.5/01_02 Changes in relative organ weights

	Concentration (ppm)			
Organ (g)	**0**	**500**	**2500**	**5000**
	Interim euthanasia			
Liver (m)	3.455	3.279	3.693	4.283**
Testes	0.646	0.702	0.817	0.993**
	Terminal euthanasia			
Kidney (f)	1.056	0.886*	0.875*	1.214
Liver (m)	4.693	4.603	5.855*	No survivors
Liver (f)	4.363	4.202	4.342	5.394**
Brain (f)	0.701	0.638**	0.604**	0.647*

* $p < 0.05$; ** $p < 0.01$

A6.5/01_03: Findings in kidneys of all rats (no historical data stated)

			Control		500 ppm		2500 ppm		5000 ppm	
75 animals per group, sex:			♂	♀	♂	♀	♂	♀	♂	♀
	Mineralization		13	14	11	12	24	21	46	20
		minimal	4	7	1	8	2	9	2	1
		mild	1	2	2	0	3	1	5	2
		moderate	4	1	5	2	8	4	21	10
		marked	4	4	3	2	11	7	18	7
	Glomeruloscerosis		70	65	68	66	73	64	73	70
		minimal	1	8	8	14	6	8	0	3
		mild	38	34	30	36	22	28	17	21
		moderate	18	13	18	12	19	17	10	22
		marked	12	10	12	4	26	11	43	24
		severe	1	0	0	0	0	0	3	0
	Interstitial nephritis		57	44	66	50	60	59	70	58
		minimal	4	11	9	8	5	15	0	2
		mild	44	28	41	35	22	40	36	54
		moderate	9	5	16	7	33	4	33	2
		marked	0	0	0	0	0	0	1	0
	Interstitial fibrosis		48	42	60	40	65	51	67	53
		minimal	2	8	10	11	3	10	2	3
		mild	31	22	33	19	30	26	21	20
		moderate	15	12	17	10	27	15	42	30
		marked	0	0	0	0	5	0	2	0
	Hydronephrosis		22	10	23	11	28	14	50	21
		minimal	0	0	0	0	1	0	0	0
		mild	22	9	23	11	27	13	46	19
		moderate	0	1	0	0	0	1	4	2
	Transitional cell hyperplasia		12	4	14	2	30	2	39	8
		minimal	4	0	4	1	6	0	6	6
		mild	7	4	9	1	21	2	31	2
		moderate	1	0	1	0	2	0	2	0
		marked	0	0	0	0	1	0	0	0
	Tubular proteinosis		75	73	73	73	75	74	74	75
		minimal	1	8	0	2	1	6	0	4
		mild	24	26	25	31	18	18	10	14
		moderate	28	25	25	28	20	27	13	23
		marked	16	12	16	9	19	15	16	23
		severe	6	2	7	3	17	8	35	11
	Tubular dilatation		14	5	5	7	27	6	31	24
		mild	13	2	3	5	13	5	20	16
		moderate	0	3	2	2	14	1	11	8
		marked	1	0	0	0	0	0	0	0
No statistical figures stated										

Section A6.5/02 Repeated dose toxicity

Annex Point IIA6.5 Inhalation study with mice with an exposure over 78 weeks

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1997) Isopropanol vapor inhalation oncogenicity study in Fischer 344 rats and CD-1 mice. [redacted]	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No [redacted]	X
2.2	**GLP**	[redacted]	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Propan-2-ol	
3.1.1	Lot/Batch number	No data	
3.1.2	Specification	Anhydrous isopropanol	
3.1.2.1	Description	No data	
3.1.2.2	Purity	≥ 99.9 %	
3.1.2.3	Stability	At approximate 6-month intervals throughout the study, the purity was checked by GC and determined always to be ≥ 99.9 %	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	CD-1	
3.2.3	Source	Charles River Breeding Labs. (USA)	
3.2.4	Sex	Male / female	
3.2.5	Age/weight at study initiation	ca. 7 weeks / 19 – 35 g	X
3.2.6	Number of animals per group	75 per sex	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	78 weeks interim sacrifice: 10 per sex and group at week 54	
3.3.2	Frequency of exposure	6 hrs/day on 5 days/week	

Section A6.5/02 Repeated dose toxicity

Annex Point IIA6.5 Inhalation study with mice with an exposure over 78 weeks

3.3.3	Postexposure period	Yes (recovery group with no exposure during weeks 54 – 78)		
3.3.4	**Inhalation**			
3.3.4.1	Concentrations	Nominal concentration	0, 500, 2500 or 5000 ppm (ca. 0, 1250, 6250 or 12500 mg/m^3)	X
		Analytical concentration	0, 504 ± 14, 2509 ± 58 or 5037 ± 115 ppm	
3.3.4.2	Particle size	Not applicable		
3.3.4.3	Type or preparation of particles	Not applicable		
3.3.4.4	Type of exposure	Whole body		
3.3.4.5	Vehicle	None		
3.3.4.6	Concentration in vehicle	Not applicable		
3.3.4.7	Duration of exposure	6 hrs/day		
3.3.4.8	Controls	Yes (0 ppm; filtered air)		
3.4	**Examinations**			
3.4.1	Observations			
3.4.1.1	Clinical signs	Yes (daily)		
3.4.1.2	Mortality	Yes (daily)		
3.4.2	Body weight	Yes prior to first exposure, weekly in the first two weeks, and every other week thereafter		
3.4.3	Food consumption	No data		X
3.4.4	Water consumption	No data		X
3.4.5	Ophthalmoscopic examination	No		X
3.4.6	Haematology	Yes Number of animals: All surviving animals from core groups Time points: At approximately 12 months and at terminal sacrifice Parameters: At terminal sacrifice: Total leukocyte count, differential leukocyte count, erythrocyte count, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and platelet count Parameters at additional time points: During study: Differential leukocyte counts from control and high concentration		

Section A6.5/02 **Repeated dose toxicity**

Annex Point IIA6.5 Inhalation study with mice with an exposure over 78 weeks

		groups	
3.4.7	Clinical Chemisty	No data	X
3.4.8	Urinalysis	No	X
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes From all surviving animals at interim and terminal sacrifice: liver, kidneys, testes, spleen, brain, heart, lungs	
3.5.2	Gross and histopathology	Yes A complete necropsy was performed on each animal (including animals found dead or euthanized as moribund) and tissues were fixed in 10 % neutral buffered formalin. Tissue sections were prepared and stained with haematoxylin and eosin. Sections of the kidneys were also stained with Mallory Heidenhain stain. Lungs were inflated with formalin via the trachea: sectioning of the lung included two coronal cuts through all lobes and mainstem bronchi. Four standard sections of the nasal cavity at different levels were prepared. Microscopically examined tissues of control and high concentration groups included adrenals, larynx, spleen, brain, liver, testes, eyes, lungs, thymus, gross lesions, heart, trachea, kidneys, ovaries, pancreas, nasal turbinates, stomach, uterus, pituitary, thyroid/parathyroid, aorta, sternum with bone marrow, salivary glands, duodenum, skin (flank), gall bladder, jejunum, oesophagus, urinary bladder, ileum, lymph node (submandibular), mammary gland, caecum, peripheral nerve (sciatic), thigh muscle, colon, Zymbal's glands, exorbital lacrimal glands, rectum, seminal vesicles, epididymis, prostate, femur (including articular surface), and the spinal cord. In addition, microscopic evaluations of the kidneys, testes, and gross lesions from the low and intermediate groups were performed. Also the livers from the low and intermediate groups at interim sacrifice were examined.	
3.5.3	Other examinations	No	
3.5.4	Statistics	The data for continuous, parametric variables were intercompared for the exposure and control groups by use of Levene's test for homogeneity of variances, by analysis of variance, and by t tests. The t tests were used, if the analysis of variance was significant, to delineate which groups differed from the control group. If Levene's test indicated homogeneous variances, the groups were compared by an analysis of variance for equal variances followed, when appropriate, by pooled variance t tests. If Levene's test indicated heterogeneous variances, the groups were compared by an analysis of variance for unequal variance followed, when appropriate, by separate variance t tests. Frequency data, such as microscopic diagnoses, were compared using Fisher's Exact Test. Nonparametric data were statistically evaluated using the Kruskal-Wallis test and, if necessary, by the Wilcoxon rank sum test as modified by Mann-Whitney. Mortality data were analyzed by life-table analysis. All statistical tests, except the frequency comparisons, were performed using BMDP Statistical Software. The probability value of $p < 0.05$ (two-tailed) was used as the critical level of significance for all tests.	
3.6	**Further remarks**	None	

Section A6.5/02 **Repeated dose toxicity**

Annex Point IIA6.5 Inhalation study with mice with an exposure over 78 weeks

		4 RESULTS AND DISCUSSION		
4.1	**Observations**			
4.1.1	Clinical signs	Exposure periods: ≥ 2500 ppm: 5000 ppm: These clinical signs were transient in nature since these signs (other than the ataxia) were absent immediately following exposure. Non-exposure periods: 5000 ppm:	hypoactivity, lack of a startle reflex and narcosis ataxia and prostration ataxia immediately following exposure but not at the following morning	
4.1.2	Mortality	No significant differences within the groups.		
4.2	**Body weight gain**	500 ppm: ≥ 500 ppm: 2500 ppm: ≥ 2500 ppm: 5000 ppm:	occasional small increases in body weight gain for core females concentration-related increase in body weight and body weight gain for core males (2, 5 and 7 or 6, 23 and 30 %, respectively) increase in body weight gain in core females (ca. 15 %); increased body weight gain (ca. 20 %) for recovery males throughout exposure and recovery phases occasionally increased body weight gain (ca. 10 – 20 %) for recovery females throughout exposure and recovery phases increase in body weight and body weight gain for core females (5 and 30 %, respectively); increased body weight and body weight gain for recovery males (ca. 6 and 30 %, respectively) throughout exposure and recovery phases	X
4.3	**Food consumption and compound intake**	No data		
4.4	**Ophtalmoscopic examination**	No data		
4.5	**Blood analysis**			
4.5.1	Haematology	No adverse effects		
4.5.2	Clinical chemistry	No data		
4.5.3	Urinalysis	No data		
4.6	**Sacrifice and pathology**			
4.6.1	Organ weights	≥ 500 ppm: decreased relative testes weight for core males at terminal euthanasia (not concentration-related); concentration-related increase in absolute and relative liver weights for core females and recovery males at terminal euthanasia ≥ 2500 ppm: slightly increased absolute and/or relative liver weights in males at terminal euthanasia 5000 ppm: increased absolute and/or relative liver weights in males at interim euthanasia; decreased absolute and relative brain weights for core females		X

Section A6.5/02 **Repeated dose toxicity**

Annex Point IIA6.5 Inhalation study with mice with an exposure over 78 weeks

		The individual results are summarised in Table A6.5/02_01
4.6.2	Gross and histopathology	≥ 500 ppm: increased incidence of minimal to mild renal tubular proteinosis (intraluminal protein) for core males and females at terminal euthanasia ≥ 2500 ppm: increased incidence of ectasia (dilation) of the seminal vesicles for core males found dead or euthanized moribund 5000 ppm: increased incidence of ectasia (dilation) of the seminal vesicles for core males at terminal euthanasia; increase in minimal to mild tubular dilation for core females; additional microscopic lesions noted only for females at terminal euthanasia: mucosal cell hyperplasia within the glandular portion of the stomach, congestion of the adrenal gland, and extramedullary haematopoiesis and haemosiderosis of the spleen. There were no exposure-related differences in microscopic frequencies of non-neoplastic lesions males and females of the recovery group. The individual results are summarised in Table A6.5/02_02.
4.7	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In this study groups of 75 CD-1 mice were exposed to concentrations of 0, 500, 2500 or 5000 ppm (ca. 0, 1250, 6250 or 12500 mg/m^3). Core groups (55 mice/sex/group) were exposed on 6 hrs/day on 5 days/week over 78 weeks. 10 mice/sex/group were assigned to an interim euthanasia group and were terminated during weeks 54, and 10 mice/sex/group were assigned to a recovery group and did not receive any further exposure following week 53 but were retained until the core group of animals was euthanized. The study design is comparable to OECD Guideline 451, but some experimental data such as clinical chemistry are missing.
5.2	**Results and discussion**	Transient signs of narcosis and increases in body weight and/or body weight gain were seen in both sexes at ≥ 2500 ppm. At interim sacrifice, a concentration-related increase in absolute and relative liver weights was seen in males and females. At necropsy there was an increased incidence of seminal vesicle enlargement in males exposed to ≥ 2500 ppm. Microscopically, some of the non-neoplastic lesions included an increased incidence of ectasia of the seminal vesicles for males exposed to ≥ 2500 ppm, minimal renal tubular proteinosis for males and females from all groups, and renal tubular dilation for females exposed to 5000 ppm.
5.3	**Conclusion**	
5.3.1	LO(A)EL	[redacted]
5.3.2	NO(A)EL	[redacted]
5.3.3	Other	[redacted]
5.3.4	Reliability	[redacted]
5.3.5	Deficiencies	[redacted]

Section A6.5/02 Repeated dose toxicity

Annex Point IIA6.5 Inhalation study with mice with an exposure over 78 weeks

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/11/14
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	-
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.5/02_01 Changes in relative organ weights

	Concentration (ppm)			
Organ (g)	**0**	**500**	**2500**	**5000**
	Interim euthanasia			
Liver (m)	5.732	5.708	5.788	6.547**
Brain (f)	1.491	1.591	1.490	1.360*
	Terminal euthanasia			
Liver (f)	5.822	5.903	6.139	6.642**
Testes	0.566	0.479**	0.495*	0.496**
Brain (m)	1.387	1.366	1.323	1.240**
Brain (f)	1.575	1.540	1.518	1.438**
	Recovery euthanasia			
Liver (m)	4.828	5.333*	5.611*	6.319*

* $p < 0.05$; ** $p < 0.01$

Table A6.5/02_02 Number (%) of euthanized mice with findings in selected organs

	0 ppm	500 ppm	2500 ppm	5000 ppm
Sex: male **No. of animals**	**35**	**32**	**29**	**31**
seminal vesicle: ectasia	8 (23)	6 (19)	7 (24)	20 (65)**
kidney: tubular proteinosis	8 (23)	16 (50)*	14 (48)*	14 (45)
kidney: tubular dilation	0 (0)	5 (16)*	0 (0)	1 (3)
Sex: female **No. of animals**	**42**	**35**	**43**	**37**
kidney: tubular proteinosis	7 (17)	16 (46)**	15 (35)	16 (43)*
kidney: tubular dilation	1 (2)	0 (0)	3 (7)	6 (16)*
adrenals: congestions	1 (2)	0 (0)	0 (0)	8 (22)*
stomach: mucosal cell hyperplasia	1 (2)	0 (0)	0 (0)	9 (24)**
spleen: extramedullary haematopoiesis	13 (31)	0 (0)	2 (5)	23 (62)**
spleen: extramedullary haemosiderosis	7 (17)	0 (0)	1 (2)	14 (38)*

* $p < 0.05$; ** $p < 0.01$

Section A6.6.1/01 Genotoxicity in vitro

Annex Point IIA6.6.1 Ames test with Salmonella typhimurium TA 97, TA 98, TA 100, TA 1535, TA 1537

Official use only

	1 REFERENCE	
1.1	**Reference**	Zeiger E, Anderson B, Haworth S, Lawlor T & Mortelmans K (1992) Salmonella Mutagenicity Tests: V. Results from the testing of 311 chemicals. Environ Mol Mutagen 19 (Suppl 21), 2 – 141
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Criteria for data protection	No data protection claimed
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No Method according to Haworth et al. (1983) Environ Mol Mutagen 5 (Suppl 1), 3 – 142 Study design comparable to OECD guideline 471
2.2	**GLP**	[redacted]
2.3	**Deviations**	Not applicable
	3 MATERIALS AND METHODS	
3.1	**Test material**	Propan-2-ol
3.1.1	Lot/Batch number	No data
3.1.2	Specification	Isopropanol
3.1.2.1	Description	No data
3.1.2.2	Purity	> 99 %
3.1.2.3	Stability	No data
3.2	**Study Type**	Bacterial reverse mutation test
3.2.1	Organism/cell type	Salmonella typhimurium TA 97, TA 98, TA 100, TA 1535, TA 1537
3.2.2	Deficiencies / Proficiencies	Histidine deficiency
3.2.3	Metabolic activation system	S9 mix S9 fraction was prepared from Aroclor 1254 pretreated male Sprague-Dawley rats and male Syrian hamsters according to Haworth et al. (1983) Environ Mol Mutagen 5 (Suppl 1), 3 – 142
3.2.4	Positive control	-S9 mix: TA 100 / TA 1535: sodium azide TA 97 / TA 1537: 9-aminoacridine TA 98: 4-nitro-o-phenylenediamine +S9 mix: all strains: 2-aminoanthracene
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	0, 100, 333, 1000, 3333 or 10000 µg/plate

Section A6.6.1/01		Genotoxicity in vitro
Annex Point IIA6.6.1		Ames test with Salmonella typhimurium TA 97, TA 98, TA 100, TA 1535, TA 1537
3.3.2	Way of application	Bacteria added to test substance dilutions in buffer with or without S9-mix
3.3.3	Pre-incubation time	20 min
3.3.4	Other modifications	No
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Revertant colonies per plate scored
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No
4.1.2	with metabolic activation	No
4.2	**Cytotoxicity**	> 10000 µg/plate
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Study comparable to OECD guideline 471
5.2	**Results and discussion**	2-propanol did no induce gene mutations in Salmonella typhimurium TA 97, TA 98, TA 100, TA 1535, TA 1537
5.3	**Conclusion**	
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/02/19
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A6.6.1/01 Genotoxicity in vitro

Annex Point IIA6.6.1 Ames test with Salmonella typhimurium TA 97, TA 98, TA 100, TA 1535, TA 1537

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.6.1/01: Results of the Ames test with 2-propanol

Strain TA 97					
Activation	None	10 % HL	30 % HL	10 % RL	30 % RL
Concentration (µg/plate)	Revertants (mean)				
0	179	178	230	194	202
100	183	190	225	191	180
333	180	171	220	192	163
1000	167	171	202	194	209
3333	162	162	221	215	216
10000	164	160	220	195	198
Positive control	417	603	330	410	429
Strain TA 98					
Activation	None	10 % HL	30 % HL	10 % RL	30 % RL
Concentration (µg/plate)	Revertants (mean)				
0	17	50	41	44	37
100	15	50	33	39	33
333	13	47	37	34	27
1000	14	46	41	33	26
3333	15	43	31	31	24

10000	19	44	33	39	28
Positive control	591	597	283	357	134
Strain TA 100					
Activation	None	10 % HL	30 % HL	10 % RL	30 % RL
Concentration (µg/plate)	Revertants (mean)				
0	104	171	163	156	170
100	120	162	166	163	178
333	124	156	168	147	172
1000	119	173	174	164	150
3333	134	147	148	147	159
10000	132	153	143	152	149
Positive control	415	780	667	582	576
Strain TA 1535					
Activation	None	10 % HL	30 % HL	10 % RL	30 % RL
Concentration (µg/plate)	Revertants (mean)				
0	24	19	11	19	16
100	19	18	16	16	16
333	17	16	13	15	13
1000	18	16	11	17	14
3333	17	16	10	15	11
10000	20	19	8	14	13
Positive control	418	201	403	110	96
Strain TA 1537					
Activation	None	10 % HL	30 % RL		
Concentration (µg/plate)	Revertants (mean)				
0	10	10	10		
100	8	7	10		
333	7	7	9		
1000	6	9	12		

3333	10	9	9		
10000	9	7	10		
Positive control	330	26	37		

Section A6.6.1/02 Genotoxicity in vitro

Annex Point IIA6.6.1 SOS chromotest with Escherichia coli PQ37

			Official use only
		1 REFERENCE	
1.1	**Reference**	von der Hude W, Behm C, Guertler R & Basler A (1988) Evaluation of the SOS chromotest. Mutat Res 203, 81 – 94	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No Method (with slight modifications) according to Quillardet et al. (1985) Mutat Res 147, 65 – 78	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Propan-2-ol	
3.1.1	Lot/Batch number	No data	
3.1.2	Specification	2-propanol	
3.1.2.1	Description	No data	
3.1.2.2	Purity	No data	
3.1.2.3	Stability	No data	
3.2	**Study Type**	SOS chromotest	
3.2.1	Organism/cell type	Escherichia coli PQ37	
3.2.2	Deficiencies / Proficiencies		
3.2.3	Metabolic activation system	S9 mix S9 fraction was prepared from Aroclor 1254 pretreated male Wistar rats and male Syrian hamsters according to Ames et al. (1975) Mutat Res 31, 347 – 364	
3.2.4	Positive control	1 µM 4-NQO without S9 mix and 30 µM B(a)P with S9 mix	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	3 – 5 different concentrations at half-log intervals (maximum: 100 mM)	X
3.3.2	Way of application	Dissolved in medium	
3.3.3	Pre-incubation time	No data	
3.3.4	Other modifications	No	

Section A6.6.1/02 Genotoxicity in vitro

Annex Point IIA6.6.1 SOS chromotest with Escherichia coli PQ37

3.4	**Examinations**		
3.4.1	Number of cells evaluated	No data	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.1.1	without metabolic activation	No	
4.1.2	with metabolic activation	No	
4.2	**Cytotoxicity**	No data	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In this strain of E. coli, the structural gene for ß-galactosidase lacZ is placed under control of the SOS gene sfiA. The expression of this gene, induced by DNA damage, is measured indirectly by determination of the ß-galactosidase activity in a colorimetric assay. Testing was done in 2 series of glass tubes containing 10 µl dissolved test substance and 250 µl bacterial suspension. The incubation time was 2 hrs. Thereafter, ß-galactosidase (ß-gal) was measured in one series of tubes and alkaline phosphatase (AP) in the other. The ß-gal assay was terminated 30 min after the addition of o-nitrophenyl-ß-galacto-pyranoside (ONPG) and the AP assay was terminated 10 min after the addition of p-nitrophenyl phosphate (PNPP). 3 different methods were used to determine the SOS induction by coloured chemicals with an absorption maximum near 420 nm: -centrifugation method: After incubation for 2 hrs the bacterial suspension was diluted with 5 ml Lamp medium and centrifuged for 15 min at 5000 rpm. The supernatant was discarded and the bacterial pellet was dissolved in the appropriate buffer. Subsequently ß-gal and AP were measured as above. -subtraction method: ß-gal and AP activities were determined as usual and in parallel series the same solutions were prepared without bacterial suspension. The absorption caused by the colour of the test substances itself was measured at 420 nm and this value was subtracted from the values of ß-gal and AP measured in parallel experiments with bacteria. These differences were used to calculate the SOS inducing factor. -X-gal method: To measure the ß-gal activity, ONPG was replaced by 5-bromo-4-chloro-3-indolyl-ß-D-galactoside (X-gal). In the series in which the AP activity was measured, PNPP was substituted by 5-bromo-4-chloro-3-indolyl phosphate (X-phos) with analysis at 615 nm. A result is positive if the SOS induction factor exceeds the solvent control by ≥ 0.5 combined with an increased ß-gal activity.	
5.2	**Results and discussion**	Although this study was not performed according to an actual guideline, this study gave no indication that 2-propanol will cause genotoxic effects in vitro.	
5.3	**Conclusion**		
5.3.1	Reliability	[redacted]	X
5.3.2	Deficiencies	[redacted]	

Section A6.6.1/02 Genotoxicity in vitro

Annex Point IIA6.6.1 SOS chromotest with Escherichia coli PQ37

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/02/21
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.3/01 Genotoxicity in vitro

Annex Point IIA6.6.3 CHO cell /HGPRT gene mutation assay

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1993) In vitro and in vivo assays of isopropanol for mutagenicity. [redacted]	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes [redacted]	X
2.2	**GLP**	[redacted]	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Propan-2-ol	
3.1.1	Lot/Batch number	No data	
3.1.2	Specification	2-propanol	
3.1.2.1	Description	No data	
3.1.2.2	Purity	99.98 %	
3.1.2.3	Stability	Purity remained unchanged during test period	
3.2	**Study Type**	In vitro mammalian cell gene mutation test (HGPRT)	
3.2.1	Organism/cell type	Mammalian cell lines: Chinese hamster Ovary (CHO)	X
3.2.2	Deficiencies / Proficiencies		
3.2.3	Metabolic activation system (MA)	S9 mix of liver from rats inducted 5 days prior to sacrifice with 500 mg/kg of Aroclor 1254, supplemented with CORE (nicotinamide adenine dinucleotide phosphate, glucose-6-phosphate and an ion-mix)	
3.2.4	Positive control	Without S9: BrdU with S9: 3-MCA	X
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	0, 0.5, 1, 1.5, 2, 2.5, 3, 4 , 4.5 or 5 mg/mL (- S9) 0, 0.5, 1, 2, 3, 3.5, 4, 4.5 or 5 mg/mL (+ S9)	

Section A6.6.3/01 Genotoxicity in vitro

Annex Point IIA6.6.3 CHO cell /HGPRT gene mutation assay

3.3.2	Way of application	Test substance was applied to culture dissolved in sterile deionised water
3.3.3	Pre-incubation time	3 days cleansing medium, 1 day recovery medium, normal culture medium and subculturation twice at least, used after 5-9 days
3.3.4	Other modifications	
3.3.5	Examinations	Mutant expression and cytotoxicity evaluation (see table A6.3.3./01 in appendix for examinations and results)
3.3.6	Number of cells evaluated	Mutant expression: $1.5 * 10^6$ cells per dish cytotoxicity evaluation: 200 cells per dish
3.3.7	Further remarks	- S9: 3 trials performed + S9: 2 trials performed
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No, mutant frequency in all dose groups within normal assay variation. Clear positive reaction in positive controls. See also table A6.6.3/01
4.1.2	with metabolic activation	No, mutant frequency in all dose groups within normal assay variation. Clear positive reaction in positive controls. See also table A6.6.3/01
4.2	**Cytotoxicity**	No, in all dose groups within normal assay variation.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	2-propanol was tested in an in vitro mammalian cell gene mutation test with Chinese hamster Ovary cells according to USEPA TSCA Health Effects Testing Standards: "Detection of gene mutations in somatic cells in culture" (40CFR 798.5300)
5.2	**Results and discussion**	There was no increase in mutant frequencies both in the presence and absence of S9 mix, while a clear positive reaction was seen in positive controls
5.3	**Conclusion**	[redacted]
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Section A6.6.3/01 Genotoxicity in vitro

Annex Point IIA6.6.3 CHO cell /HGPRT gene mutation assay

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/02/20
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.3/01 Genotoxicity in vitro

Annex Point IIA6.6.3 CHO cell /HGPRT gene mutation assay

Table A6.6.3/01: Results

Concentration [mg/ml]	Number of mutant cells (total number of mutants from 12 mutant selection dishes/dose), independent trials										Comments
	without S9						with S9				
Vehicle control (*: only 8 dishes)	8	3	21	38	14	6	10	3*	16	—	Duplicate control cultures were used in the mutation assays
Positive control	336		273		310	310	741		638		with S9: 50µg BrdU/ml without S9: 5µg 3-MCA/ml
0.5	3		20		—		10		10		—: no data
1.0	6		22		4		4		26		
1.5	—		10		—		—		—		
2.0	18		8		4		8		22		
2.5	—		23		—		—		—		
3.0	4		20		7		8		19		
3.5	—		—		—		—		20		
4.0	5		38		10		25		25		
4.5	—		—		12		—		24		
5.0	8		13		2		7		20		

Section A6.6.3/02 Genotoxicity in vitro

Annex Point IIA6.6.3 In vitro sister chromatid exchange assay in mammalian cells (V79)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1987) Genotoxicity of three-carbon compounds evaluated in the SCE test in vitro. [redacted]	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No [redacted]	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Propan-2-ol	
3.1.1	Lot/Batch number	No data	
3.1.2	Specification	2-propanol	
3.1.2.1	Description	No data	
3.1.2.2	Purity	99.7 %	
3.1.2.3	Stability	No data	
3.2	**Study Type**	In vitro sister chromatid exchange assay in mammalian cells.	
3.2.1	Organism/cell type	Chinese hamster lung fibroblasts (V79)	
3.2.2	Deficiencies / Proficiencies		
3.2.3	Metabolic activation system	S9 mix S9 fraction was prepared from Aroclor 1254 pre-treated male Wistar rats according to Ames et al. (1975) Mutat Res 31, 347 – 364	
3.2.4	Positive control	Epichlorohydrin (+ S9 mix: 0.3 – 10.0 mMol; - S9 mix: 0.03 – 0.3 mMol)	X
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	+/- S9 mix: 0, 3.3, 10, 33.3 or 100 mMol (0, 200, 600, 2000 or 6000 µg/mL)	X
3.3.2	Way of application	The test substance was dissolved in DMSO	

Section A6.6.3/02		**Genotoxicity in vitro**	
Annex Point IIA6.6.3		In vitro sister chromatid exchange assay in mammalian cells (V79)	
3.3.3	Pre-incubation time	The V79 cells were grown in minimal essential medium with Earle's salts (MEM) and seeded (5 * 10^5 cells) into 25 cm^2 flasks. The medium was sucked off 18 hrs later, replaced by fresh medium, after which the test substance and 10^{-5} M BrdU were added. Mitotic cells were harvested by shaking them off after 28 hrs on incubation, with 2 * 10^{-7} M Colcemid for the last 4 hrs. The cells were fixed on slides and stained according to standard protocols (Latt et al. [1981] Mutat Res 87, 17 – 62). In experiments with short-term exposure the cells were exposed to the test substance in supplemented MEM 18 hrs after seeding. After 3 hrs the treatment medium was removed, the cells were rinsed with MEM, and then placed on medium with BrdU for 28 hrs (see above). In tests with S9 mix the medium was removed 18 hrs after seeding the cells. The V79 monolayer in the 25 cm^2 flasks was incubated with the test substance and 0.5 ml of a standard S9 mix with 10 % S9 fraction.	
3.3.4	Other modifications	No	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	The distribution of 1st (M1), 2nd (M2), and 3rd (M3) mitosis was determined by counting 100 metaphases per experimental point. Statistical evaluation: 25 metaphases with harlequin-stained chromosomes were scored for SCE per experimental point. All results were confirmed in independent experiments and mean values of 2 experiments with a total of 50 metaphases per point were analysed by pair-wise comparison to the solvent control, using Student's t test.	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.1.1	without metabolic activation	No increase in the incidence of SCE even at high dose levels of 6 mg/ml	
4.1.2	with metabolic activation	No increase in the incidence of SCE even at high dose levels of 6 mg/ml	
4.2	**Cytotoxicity**	> 100 mMol	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Used method comparable to OECD guideline 479. Cells tested up to dose levels of 6 mg/ml with and without metabolic activation.	
5.2	**Results and discussion**	Although this study was not performed according to an actual guideline and data for GLP are missing, this study gave no indication that 2-propanol will cause genotoxic effects in vitro. The test substance had no cytotoxic or genotoxic activity even at the maximum concentration of 6 mg/ml.	
5.3	**Conclusion**	[redacted]	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	[redacted]	

Section A6.6.3/02 Genotoxicity in vitro

Annex Point IIA6.6.3 In vitro sister chromatid exchange assay in mammalian cells (V79)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/02/20
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.3/02 Genotoxicity in vitro

Annex Point IIA6.6.3 In vitro sister chromatid exchange assay in mammalian cells (V79)

Table A6.6.3/02 Results of the in vitro sister chromatid exchange assay with V79 cells

Concentration (mM)	SCE (mean per cell + SD)		M1		M2		M3	
	— S9	+ S9	— S9 / + S9		— S9 / + S9		— S9 / + S9	
2-propanol								
DMSO	6.3 ± 2.1	8.1 ± 2.8	1	7	90	92	9	1
3.3	5.4 ± 2.6	8.1 ± 3.1	0	10	98	90	2	0
10.0	5.8 ± 2.1	8.9 ± 2.9	0	13	93	87	7	0
33.3	5.7 ± 2.0	8.7 ± 2.9	2	9	94	91	4	0
100.0	5.8 ± 2.1	8.9 ± 2.9	0	3	100	92	0	0
Epichlorohydrin								
DMSO	6.1 ± 2.3	8.2 ± 2.9	0	1	99	99	1	0
0.03	8.5 ± 2.8	n.d.	1		98		1	
0.1	16.4 ± 4.6*	n.d.	4		96		0	
0.3	31.0 ± 6.2*	8.6 ± 3.7	0	6	100	94	0	0
1.0	n.d.	9.3 ± 3.1		2		98		0
3.3	n.d.	12.7 ± 4.6*		5		95		0
10.0	n.d.	toxic						

n.d. = not done; * = $p < 0.005$

Section A6.6.4/01 Genotoxicity in vivo

Annex Point IIA6.6.4 Micronucleus Test with mice

			Official use only
		REFERENCE	
1.1	**Reference**	[redacted] (1993) In vitro and in vivo assays of isopropanol for mutagenicity. [redacted]	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	No data protection claimed	
		GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes [redacted]	X
2.2	**GLP**	[redacted]	
2.3	**Deviations**	No	
		MATERIALS AND METHODS	
3.1	**Test material**	Propan-2-ol	
3.1.1	Lot/Batch number	No data	
3.1.2	Specification	2-propanol	
3.1.2.1	Description	No data	
3.1.2.2	Purity	99.98 %	
3.1.2.3	Stability	Purity remained unchanged during test period	
3.1.2.4	Maximum tolerable dose	Based on a range finding study with 6 m/f mice	X
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	Random bred ICR	
3.2.3	Source	Harlan Sprague-Dawley, Inc. (USA)	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Adult / no data	
3.2.6	Number of animals per group	Range finding test: 3 males and 3 females Main study: 5 males and 5 females per dose and sampling time (i.e. 15 males and 15 females per group)	
3.2.7	Control animals	Vehicle and positive control	

Section A6.6.4/01 Genotoxicity in vivo

Annex Point IIA6.6.4 Micronucleus Test with mice

3.3	**Administration/ Exposure**	Intraperitoneal		
3.3.1	Number of applications	1		
3.3.2	Interval between applications	Not applicable		
3.3.3	Postexposure period	Positive control group: vehicle control group: test groups:	24 h after treatment 24h – 48 – 72h after treatment 24h – 48 – 72h after treatment	
		Intraperitoneal		
3.3.4	Vehicle	0.9 % saline solution		
3.3.5	Concentration in vehicle	100 %		X
3.3.6	Total volume applied	10 ml/kg		
3.3.7	dose applied	Range-finding test: 500, 1625, 2750, 3875 or 5000 mg/kg bw Main study: initially 350, 1173 or 3500 mg/kg bw; a second trial with 2500 mg/kg bw was initiated due to excessive toxicity at 3500 mg/kg bw		
3.3.8	Substance used as Positive Control	Oral dosing with 80 mg cyclophosphamide/kg		
3.4	**Examinations**			
3.4.1	Clinical signs	Not further specified		
3.4.2	Tissue	Bone marrow		
		Number of animals:	all animals	
		Number of cells:	1000 polychromatic erythrocytes (PCE)	
		Time points:	24, 48 and 72 hrs after dosing	
		Type of cells	Polychromatic erythrocytes (PCE) and normo-chromatic erythrocytes (NCE) in bone marrow	
		Parameters:	PCE/NCE ratio	X
3.5	**Further remarks**	Criteria of identification of micronuclei according to Schmid W (1976) The micronucleus test for cytogenetic analysis. In: Hollaender A (ed): 'Chemical mutagens: Principles and methods for their detection.' Vol 4, NY, Plenum press, 31-53		
3.6	**Statistics**	Data were evaluated by an analysis of variance on the square root arscine transformation performed on the proportion of micronucleated cells per mouse followed by a Tukey's studentized range test with adjustment for multiple comparisons to determine statistical significance		

Section A6.6.4/01 Genotoxicity in vivo

Annex Point IIA6.6.4 Micronucleus Test with mice

4 **RESULTS AND DISCUSSION**

4.1 Clinical signs No data

4.2 Haematology / Tissue examination No data

4.3 Genotoxicity No, the frequency of micronuclei was not significantly increased and there was no effect on the PCE/NCE ratio.

4.4 Mortality In preliminary range finding test, the LD_{50} (i.p.) was determined with 4384 mg/kg bw:

[mg/kg bw]	500	1625	2750	3875	5000
mortality [72 h]	0/6	0/6	1/6	2/6	4/6

Main study:

[mg/kg bw]	350	1173	3500
Mortality	0/0	0/0	35/40 within 22 hrs, others euthanized moribund

In additional tests with an additional dose of 2500 mg/kg bw all dosed animals became prostrate. Within 72 hours 6 animals had died. Necropsy findings of died animals: fluid-filled thoracic cavities and distended stomachs or colons

APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods Mice were injected once with 2-propanol in four concentrations from 350 to 3500 mg/kg. As the top dose killed almost the whole group within 22 hrs, an intermediate dose was tested in addition (trial 2). This study was performed in accordance with applicable USEPA TSCA Health Effects Testing Standards: "In Vivo Mammalian Bone Marrow Cytogenetics Tests Micronucleus Assay" (40CFR 798.5395) under GLP conditions.

5.2 Results and discussion There was no increased incidence of micronuclei in bone marrow PCEs harvested at 24 - 72 hrs after dosing.

5.3 Conclusion [redacted]

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Section A6.6.4/01 Genotoxicity in vivo

Annex Point IIA6.6.4 Micronucleus Test with mice

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/02/21
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.6.4/01: Results of the Micronucleus Test In Vivo

				control				inter-	
				negative	positive	low	mid	mediate	high
		dose [mg/kg bw]		**0**	**CPA**	**350**	**1173**	**2500**	**3500**
24h	trial 1	animals evaluated		30	30	30	30	-	40
		mortality		0	0	0	0	-	40
		PCE/NCE ratio	male	0.58	0.84	1.05	0.65	-	-
		PCE/NCE ratio	female	0.95	1.08	0.86	0.73	-	-
		% micronucleated PCEs	male	0.12	3.24*	0.02	0.73	-	-
		% micronucleated PCEs	female	0.08	1.22*	0.04	0.10	-	-
		% micronucleated PCEs	overall	0.10	2.23*	0.03	0.08	-	-
	trial 2	animals evaluated		30	30	-	-	40	-
		mortality		0	0	-	-	0	-
		PCE/NCE ratio	male	0.41	0.52	-	-	0.43	-
		PCE/NCE ratio	female	0.62	0.81	-	-	0.57	-
		% micronucleated PCEs	male	0.02	1.56*	-	-	0.10	-
		% micronucleated PCEs	female	0.08	1.66*	-	-	0.20	-
		% micronucleated PCEs	overall	0.05	1.61*	-	-	0.15	-
48h	trial 1	animals evaluated		20	-	20	20	-	-
		mortality		0	-	0	0	-	-
		PCE/NCE ratio	male	0.63	-	0.45	0.55	-	-
		PCE/NCE ratio	female	0.86	-	0.67	0.76	-	-
		% micronucleated PCEs	male	0.04	-	0.12	0.04	-	-
		% micronucleated PCEs	female	0.10	-	0.22	0.06	-	-
		% micronucleated PCEs	overall	0.07	-	0.17	0.05	-	-
	trial 2	animals evaluated		20	-	-	-	30	-
		mortality		0	-	-	-	3	-
		PCE/NCE ratio	male	0.66	-	-	-	0.47	-
		PCE/NCE ratio	female	0.74	-	-	-	0.51	-
		% micronucleated PCEs	male	0.04	-	-	-	0.06	-
		% micronucleated PCEs	female	0.10	-	-	-	0.24	-
		% micronucleated PCEs	overall	0.07	-	-	-	0.15	-
72h	trial 1	animals evaluated		10	-	10	10	-	-
		mortality		0	-	0	0	-	-
		PCE/NCE ratio	male	0.70	-	0.88	0.79	-	-
		PCE/NCE ratio	female	0.57	-	0.53	0.70	-	-
		% micronucleated PCEs	male	0.04	-	0.10	0.02	-	-
		% micronucleated PCEs	female	0.04	-	0.14	0.02	-	-
		% micronucleated PCEs	overall	0.04	-	0.12	0.02	-	-
	trial 2	animals evaluated		10	-	-	-	17	-
		Mortality		0	-	-	-	3	-
		PCE/NCE ratio	male	0.52	-	-	-	0.42	-
		PCE/NCE ratio	female	0.88	-	-	-	0.54	-
		% micronucleated PCEs	male	0.06	-	-	-	0.04	-
		% micronucleated PCEs	female	0.12	-	-	-	0.10	-
		% micronucleated PCEs	overall	0.09	-	-	-	0.07	-

***: $p \leq 0.05$**

Section A6.7/01 Carcinogenicity

Annex Point IIA6.7 Inhalation study with rats with an exposure over 104 weeks

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1997) Isopropanol vapor inhalation oncogenicity study in Fischer 344 rats and CD-1 mice. [redacted]	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Companies with letter of access	Not applicable	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No [redacted]	X
2.2	**GLP**	[redacted]	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Propan-2-ol	
3.1.1	Lot/Batch number	No data	
3.1.2	Specification	Anhydrous isopropanol	
3.1.2.1	Description	No data	
3.1.2.2	Purity	≥ 99.9 %	
3.1.2.3	Stability	At approximate 6-month intervals throughout the study, the purity was checked by GC and determined always to be ≥ 99.9 %	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	F344	
3.2.3	Source	Harlan Sprague Dawley, Inc. (USA)	
3.2.4	Sex	Male / female	
3.2.5	Age/weight at study initiation	Ca. 7 weeks / 93 – 165 g	X
3.2.6	Number of animals per group	75 per sex	
3.2.6.1	at interim sacrifice	10 per sex and group (at week 73)	
3.2.6.2	at terminal sacrifice	65 per sex and group (at week 104)	
3.2.6.3	recovery group	No	
3.2.7	Control animals	Yes	

Section A6.7/01 Carcinogenicity

Annex Point IIA6.7 Inhalation study with rats with an exposure over 104 weeks

3.3	**Administration/ Exposure**	Inhalation		
3.3.1	Duration of treatment	104 weeks		
3.3.2	Interim sacrifice(s)	At week 73		
3.3.3	Final sacrifice	At week 104		
3.3.4	Frequency of exposure	6 hrs/day on 5 days/week		
3.3.5	Postexposure period	No		
		Inhalation		
3.3.6	Concentrations	Nominal concentration:	0, 500, 2500 or 5000 ppm (ca. 0, 1250, 6250 or 12500 mg/m^3)	X
		Analytical concentration:	0, 504 ± 14, 2509 ± 58 or 5037 ± 115 ppm	
3.3.7	Type of exposure	Whole body		
3.3.8	Vehicle	None		
3.3.9	Concentration in vehicle	Not applicable		
3.3.10	Duration of exposure/day	6 hrs		
3.3.11	Controls	Yes (0 ppm; filtered air)		
3.4	**Examinations**			
3.4.1	Body weight	Yes prior to first exposure, weekly in the first two weeks, and every other week thereafter		
3.4.2	Food consumption	No data		X
3.4.3	Water consumption	No data		X
3.4.4	Clinical signs	Yes (daily)		
3.4.5	Macroscopic investigations	Yes		
3.4.6	Ophthalmoscopic examination	Yes prior to 1st exposure, at 17 and 19 months and at terminal sacrifice		
3.4.7	Haematology	Yes		
	Number of animals:	All surviving animals from core groups		
	Time points:	At approximately 13 and 19 months and at terminal sacrifice		
	Parameters:	At terminal sacrifice: Total leukocyte count, differential leukocyte count, erythrocyte count, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and platelet count		

Section A6.7/01 Carcinogenicity

Annex Point IIA6.7 Inhalation study with rats with an exposure over 104 weeks

	Parameters at additional time points:	During study: Differential leukocyte counts from control and high concentration group	
3.4.8	Clinical Chemistry	No data	X
3.4.9	Urinalysis	Yes	
	Number of animals:	10 per sex and group	
	Time points:	week 57: group with access to water and food week 58: group with access to food but not to water weeks 74 / 104: group with access to water and food	
	Parameters:	week 57: total protein, total glucose, and urine volume week 58: osmolality weeks 74 / 104: osmolality, total protein, total glucose, and urine volume	
3.4.10	Pathology	Yes	
3.4.10.1	Organ Weights	Yes From all surviving animals at interim and terminal sacrifice: liver, kidneys, testes, spleen, brain, heart, lungs	
3.4.11	Histopathology	Yes A complete necropsy was performed on each animal (including animals found dead or euthanized as moribund) and tissues were fixed in 10 % neutral buffered formalin. Tissue sections were prepared and stained with haematoxylin and eosin. Sections of the kidneys were also stained with Mallory Heidenhain stain. Lungs were inflated with formalin via the trachea: sectioning of the lung included two coronal cuts through all lobes and mainstem bronchi. Four standard sections of the nasal cavity at different levels were prepared.	
	Organs:	Microscopically examined tissues of control and high concentration groups included adrenals, larynx, spleen, brain, liver, testes, eyes, lungs, thymus, gross lesions, heart, trachea, kidneys, ovaries, pancreas, nasal turbinates, stomach, uterus, pituitary, thyroid/parathyroid, aorta, sternum with bone marrow, salivary glands, duodenum, skin (flank), gall bladder, jejunum, oesophagus, urinary bladder, ileum, lymph node (submandibular), mammary gland, caecum, peripheral nerve (sciatic), thigh muscle, colon, Zymbal's glands, exorbital lacrimal glands, rectum, seminal vesicles, epididymis, prostate, femur (including articular surface), and the spinal cord. In addition, microscopic evaluations of the kidneys, testes, and gross lesions from the low and intermediate groups were performed.	
	Additional evaluations:	No	
3.4.12	Other examinations	No	

Section A6.7/01 Carcinogenicity

Annex Point IIA6.7 Inhalation study with rats with an exposure over 104 weeks

3.5	**Statistics**	The data for continuous, parametric variables were intercompared for the exposure and control groups by use of Levene's test for homogeneity of variances, by analysis of variance, and by t tests. The t tests were used, if the analysis of variance was significant, to delineate which groups differed from the control group. If Levene's test indicated homogeneous variances, the groups were compared by an analysis of variance for equal variances followed, when appropriate, by pooled variance t tests. If Levene's test indicated heterogeneous variances, the groups were compared by an analysis of variance for unequal variance followed, when appropriate, by separate variance t tests. Frequency data, such as microscopic diagnoses, were compared using Fisher's Exact Test. Nonparametric data were statistically evaluated using the Kruskal-Wallis test and, if necessary, by the Wilcoxon rank sum test as modified by Mann-Whitney. Mortality data were analyzed by life-table analysis. All statistical tests, except the frequency comparisons, were performed using BMDP Statistical Software. The probability value of $p < 0.05$ (two-tailed) was used as the critical level of significance for all tests.	
3.6	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Body weight**	≥ 2500 ppm: increased body weight and body weight gain in males (these increases were typically observed throughout the remainder of the study, although statistical significance was rarely achieved following week 72). At week 52, mean body weight and body weight gain were increased 4 and 6 %, respectively, for males at 2500 ppm and 5 and 7 %, respectively, for males at 5000 ppm. Concentration-related increases in body weight and body weight gain were typically observed for females following week 5; however, the increases in body weight and body weight gain observed at 5000 ppm were very slight (≤ 1 %). Mean body weight and body weight gain were increased 4 and 7 %, respectively, for females at 2500 ppm and 6 and 10 %, respectively, for females at 5000 ppm at week 52. 5000 ppm: decreased body weight and/or body weight gain in males and females at the end of the first and second weeks of exposure. Following this time point, the body weight of these rats increased, and, by the end of week 6, increased body weight and body weight gain were noted for both males and females.	X
4.2	**Food consumption**	No data	
4.3	**Water consumption**	No data	
4.4	**Clinical signs**	Exposure periods: ≥ 2500 ppm: hypoactivity and lack of a startle reflex 5000 ppm: narcosis	X

Section A6.7/01 **Carcinogenicity**

Annex Point IIA6.7 Inhalation study with rats with an exposure over 104 weeks

		These clinical signs were transient in nature since these signs were absent immediately following exposure. Non-exposure periods: ≥ 2500 ppm: urine stains 5000 ppm: emaciation and dehydration in males; swollen periocular tissue in females Mortality: increased in males at 5000 ppm (100 % [last death during week 100] vs. 82 % in controls) The main cause of death appeared to be chronic renal disease which was also considered to account for much of the mortality observed for animals exposed to 2500 ppm. The main cause of death for females died or euthanized moribund due was chronic renal disease in the 5000 ppm group. The main cause of death for the male and female controls was mononuclear cell leukemia. Mean survival time: decreased in males at 5000 ppm (577 vs. 631 days in controls)	
4.5	**Macroscopic investigations**	Interim euthanasia: ≥ 2500 ppm: increase in kidneys with a granular surface in males Terminal euthanasia: 2500 ppm: increase in kidneys with a granular surface in males ≥ 2500 ppm: in males and females which died or were euthanized due to morbidity, an increased incidence of thickened stomachs, kidneys with a granular surface, and colour change of the kidney (males only) was noted	
4.6	**Ophthalmoscopic examination**	No adverse effects	
4.7	**Haematology**	No adverse effects	
4.8	**Clinical Chemistry**	No data	
4.9	**Urinalysis**	At 13 months: 5000 ppm: decrease in osmolality, increase in total protein (m) and increase in total volume and glucose (f) At 17 months: ≥ 2500 ppm: decreased osmolality, increase in total protein, total volume, and total glucose excreted for males at ≥ 2500 ppm and for females at 5000 ppm At terminal euthanasia: ≥ 2500 ppm: decrease in osmolality (f) and increases in total protein (m), total volume, and total glucose for males at 2500 ppm (no survivors at 5000 ppm) and for females at 5000 ppm The individual results are summarised in Table A6.7/01_01	X
4.10	**Pathology**	No increased tumour incidence except of interstitial cell adenomas of the testes in male rats. However, these were attributed to an unusually low incidence in control group by the authors.	X

Section A6.7/01	Carcinogenicity	
Annex Point IIA6.7	Inhalation study with rats with an exposure over 104 weeks	
4.11 Relative Organ Weights	Interim euthanasia: ≥ 500 ppm: concentration-related increases in absolute and relative testes weights 2500 ppm: increased relative liver weights in males 5000 ppm: increase in absolute and relative lung weights in females; increased absolute and/or relative liver and kidney weights in males Terminal euthanasia: 2500 ppm: increased absolute and/or relative liver and kidney weights in males 5000 ppm: increased absolute and/or relative liver and kidney weights in females The individual results are summarised in Table A6.7/01_02	X

Section A6.7/01 Carcinogenicity

Annex Point IIA6.7 Inhalation study with rats with an exposure over 104 weeks

4.12	**Histopathology**	Interim euthanasia: ≥ 2500 ppm: increased grades for some lesions associated with chronic renal disease in males 5000 ppm: increased frequency of testicular seminiferous tubule atrophy Terminal euthanasia: ≥ 2500 ppm: increase in severity of certain renal lesions in all males (including rats found dead or euthanized moribund) such as mineralization, tubular dilation, glomerulosclerosis, interstitial nephritis, interstitial fibrosis, hydronephrosis, and transitional cell hyperplasia with an increase in the frequencies of these lesions in died males or euthanized moribund. Increased severity of some of the key components for chronic renal disease such as tubular proteinosis, glomerulosclerosis, interstitial nephritis, and interstitial fibrosis) in all females. Typically both the severity and incidence of the lesions which characterize chronic renal disease were greater for males than for females. The individual results are summarised in Table A6.7/01_03. Rats found dead or euthanized moribund: ≥ 2500 ppm: increased incidence of mineralization in a number of organs (not further specified) 5000 ppm: increased frequency of myocardial degeneration/fibrosis, fibrous osteodystrophy, glandular ectasia within the gastric mucosa (females only), cellular hyperplasia of the parathyroid glands (females only), basophilic cell foci within the liver (males only), splenic haemosiderosis, rhinitis and squamous metaplasia of the respiratory epithelium within the nasal cavity, iridocyclitis (males only), atrial thrombosis (females only), ocular keratitis (females only), and dacryosolenitis (females only). There were no increased frequencies of neoplastic lesions for females. However, an exposure-related decrease in the frequency of large granular lymphocyte leukaemia was observed for exposed females. A decrease in pituitary adenomas and mononuclear leukemia was observed for males which died or were euthanized due to morbidity at 5000 ppm. The only neoplastic lesion observed to be increased in exposed males was interstitial (Leydig) cell adenoma of the testis. At interim euthanasia, an increase in testicular interstitial cell adenomas was seen in males exposed to 5000 ppm. Concentration-related increases in interstitial cell adenomas of the testes were observed for males found dead or euthanized moribund as well as for all animals on the study. For males found dead or euthanized moribund, the frequencies of interstitial cell adenomas of the testis were 57.7, 72.2, 84.7 or 93.8 %, while the frequencies of this lesion for all males examined were 64.9, 77.3, 86.7 or 94.7 %.	X
4.13	**Other examinations**	None	

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Annex Point IIA6.7 Inhalation study with rats with an exposure over 104 weeks

4.14	**Time to tumours**	Not applicable
4.15	**Other**	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In this study groups of 75 F344 rats were exposed to concentrations of 0, 500, 2500 or 5000 ppm (ca. 0, 1250, 6250 or 12500 mg/m^3) on 6 hrs/day on 5 days/week over 104 weeks. An interim sacrifice with 10 rats per sex and group was performed at week 73. The study design is comparable to OECD Guideline 451, but some experimental data such as clinical chemistry are missing.
5.2	**Results and discussion**	Transient signs of narcosis were observed during exposure to ≥ 2500 ppm. In male rats exposed to 5000 ppm the mortality rate was increased (100 vs. 82 % for controls) and there was also a decrease in mean survival time (577 vs. 631 days for controls). Increases in body weight and/or body weight gain were typically observed for both sexes at ≥ 2500 ppm. Urinalysis and urine chemistry changes indicative of impaired kidney function were noted for males at ≥ 2500 ppm and for females at 5000 ppm. At interim euthanasia, there was a concentration-related increase in testes weights and an increase in absolute and/or relative liver and kidney weights in males and/or females at ≥ 2500 ppm. A number of non-neoplastic lesions was seen in males and females at ≥ 2500 ppm, with the most significant lesions being observed in the kidneys and associated with chronic renal disease such mineralization, tubular dilation, glomerulosclerosis, interstitial nephritis, interstitial fibrosis, hydronephrosis, and transitional cell hyperplasia. The only tumour type increased in incidence during the study was interstitial cell adenomas of the testes in male rats. However, this finding is questionable due to an unusually low incidence observed for the control group.
5.3	**Conclusion**	[redacted]
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Section A6.7/01 Carcinogenicity

Annex Point IIA6.7 Inhalation study with rats with an exposure over 104 weeks

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/10/08
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]

Section A6.7/01 Carcinogenicity

Annex Point IIA6.7 Inhalation study with rats with an exposure over 104 weeks

Reliability	[redacted]
Acceptability	[redacted]
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.7/01_01 Urinalysis in rats

Affected sex	parameter	Unit	Controls	500 ppm	2500 ppm	5000 ppm
13 months after start of treatment						
m	osmolality	mOsm/kg	2332	2113	2157	1574**
m	total protein	g/L	11426	11534	12768	15296*
f	total volume	mL	4.9	5.7	6.4	7.3**
f	glucose	g/L	0.71	0.70	0.64	0.54**
17 months after start of treatment						
m	osmolality	mOsm/kg	1225	1491	942	605**
f			1973	1954	1841	1254**
m	total protein	g/L	11821	13243	17306	19382**
f			8333	6795	12652	16561**
m	total volume	mL	8.7	5.9	11.9	16.5**
f			6.3	5.0	7.3	11.6*
m	glucose	g/L	0.43	0.47	0.29	0.21*
f			0.54	0.54	0.52	0.41
24 months after start of treatment						
f	osmolality	mOsm/kg	1108	1054	934	537*
f	total volume	mL	11.0	12.1	14.8	23.3**
f	glucose	g/L	0.51	0.52	0.47	0.33*

* $p < 0.05$; ** $p < 0.01$

Table A6.7/01_02 Changes in relative organ weights

	Concentration (ppm)			
Organ (g)	**0**	**500**	**2500**	**5000**
	Interim euthanasia			
Liver (m)	3.455	3.279	3.693	4.283**
Testes	0.646	0.702	0.817	0.993**
	Terminal euthanasia			
Kidney (f)	1.056	0.886*	0.875*	1.214
Liver (m)	4.693	4.603	5.855*	No survivors
Liver (f)	4.363	4.202	4.342	5.394**
Brain (f)	0.701	0.638**	0.604**	0.647*

* $p < 0.05$; ** $p < 0.01$

A6.7/01_03: Findings in kidneys of all rats (no historical data stated)

			Control		500 ppm		2500 ppm		5000 ppm	
75 animals per group, sex:			♂	♀	♂	♀	♂	♀	♂	♀
	Mineralization		13	14	11	12	24	21	46	20
		minimal	4	7	1	8	2	9	2	1
		mild	1	2	2	0	3	1	5	2
		moderate	4	1	5	2	8	4	21	10
		marked	4	4	3	2	11	7	18	7
	Glomeruloscerosis		70	65	68	66	73	64	73	70
		minimal	1	8	8	14	6	8	0	3
		mild	38	34	30	36	22	28	17	21
		moderate	18	13	18	12	19	17	10	22
		marked	12	10	12	4	26	11	43	24
		severe	1	0	0	0	0	0	3	0
	Interstitial nephritis		57	44	66	50	60	59	70	58
		minimal	4	11	9	8	5	15	0	2
		mild	44	28	41	35	22	40	36	54
		moderate	9	5	16	7	33	4	33	2
		marked	0	0	0	0	0	0	1	0
	Interstitial fibrosis		48	42	60	40	65	51	67	53
		minimal	2	8	10	11	3	10	2	3
		mild	31	22	33	19	30	26	21	20
		moderate	15	12	17	10	27	15	42	30
		marked	0	0	0	0	5	0	2	0
	Hydronephrosis		22	10	23	11	28	14	50	21
		minimal	0	0	0	0	1	0	0	0
		mild	22	9	23	11	27	13	46	19
		moderate	0	1	0	0	0	1	4	2
	Transitional cell hyperplasia		12	4	14	2	30	2	39	8
		minimal	4	0	4	1	6	0	6	6
		mild	7	4	9	1	21	2	31	2
		moderate	1	0	1	0	2	0	2	0
		marked	0	0	0	0	1	0	0	0
	Tubular proteinosis		75	73	73	73	75	74	74	75
		minimal	1	8	0	2	1	6	0	4
		mild	24	26	25	31	18	18	10	14
		moderate	28	25	25	28	20	27	13	23
		marked	16	12	16	9	19	15	16	23
		severe	6	2	7	3	17	8	35	11
	Tubular dilatation		14	5	5	7	27	6	31	24
		mild	13	2	3	5	13	5	20	16
		moderate	0	3	2	2	14	1	11	8
		marked	1	0	0	0	0	0	0	0
No statistical figures stated										

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Annex Point IIA6.7 Inhalation study with mice with an exposure over 78 weeks

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1997) Isopropanol vapor inhalation oncogenicity study in Fischer 344 rats and CD-1 mice. [redacted]	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Companies with letter of access	Not applicable	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No [redacted]	X
2.2	**GLP**	[redacted]	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Propan-2-ol	
3.1.1	Lot/Batch number	No data	
3.1.2	Specification	Anhydrous isopropanol	
3.1.2.1	Description	No data	
3.1.2.2	Purity	≥ 99.9 %	
3.1.2.3	Stability	At approximate 6-month intervals throughout the study, the purity was checked by GC and determined always to be ≥ 99.9 %	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	CD-1	
3.2.3	Source	Charles River Breeding Labs. (USA)	
3.2.4	Sex	Male / female	
3.2.5	Age/weight at study initiation	ca. 7 weeks / 19 – 35 g	X
3.2.6	Number of animals per group	75 per sex	
3.2.6.1	at interim sacrifice	10 per sex and group (at week 54)	
3.2.6.2	at terminal sacrifice	65 per sex and group (at week 78)	
3.2.6.3	recovery group	Yes (10 per sex and group; no exposure during weeks 54 – 78)	
3.2.7	Control animals	Yes	

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3.3	**Administration/ Exposure**	Inhalation		
3.3.1	Duration of treatment	78 weeks		
3.3.2	Interim sacrifice(s)	At week 54		
3.3.3	Final sacrifice	At week 78		
3.3.4	Frequency of exposure	6 hrs/day on 5 days/week		
3.3.5	Postexposure period	Yes (recovery group with no exposure during weeks 54 – 78)		
		Inhalation		
3.3.6	Concentrations	Nominal concentration:	0, 500, 2500 or 5000 ppm (ca. 0, 1250, 6250 or 12500 mg/m^3)	X
		Analytical concentration:	0, 504 ± 14, 2509 ± 58 or 5037 ± 115 ppm	
3.3.7	Type of exposure	Whole body		
3.3.8	Vehicle	None		
3.3.9	Concentration in vehicle	Not applicable		
3.3.10	Duration of exposure/day	6 hrs		
3.3.11	Controls	Yes (0 ppm; filtered air)		
3.4	**Examinations**			
3.4.1	Body weight	Yes prior to first exposure, weekly in the first two weeks, and every other week thereafter		
3.4.2	Food consumption	No data		X
3.4.3	Water consumption	No data		X
3.4.4	Clinical signs	Yes (daily)		
3.4.5	Macroscopic investigations	Yes		
3.4.6	Ophthalmoscopic examination	No		X
3.4.7	Haematology	Yes		
	Number of animals:	All surviving animals from core groups		
	Time points:	At approximately 12 months and at terminal sacrifice		
	Parameters:	At terminal sacrifice: Total leukocyte count, differential leukocyte count, erythrocyte count, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and platelet count		

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	Parameters at additional time points:	During study: Differential leukocyte counts from control and high concentration group	
3.4.8	Clinical Chemistry	No data	X
3.4.9	Urinalysis	No	X
	Number of animals:		
	Time points:		
	Parameters:		
3.4.10	Pathology	Yes	
3.4.10.1	Organ Weights	Yes From all surviving animals at interim and terminal sacrifice: liver, kidneys, testes, spleen, brain, heart, lungs	
3.4.11	Histopathology	Yes A complete necropsy was performed on each animal (including animals found dead or euthanized as moribund) and tissues were fixed in 10 % neutral buffered formalin. Tissue sections were prepared and stained with haematoxylin and eosin. Sections of the kidneys were also stained with Mallory Heidenhain stain. Lungs were inflated with formalin via the trachea: sectioning of the lung included two coronal cuts through all lobes and mainstem bronchi. Four standard sections of the nasal cavity at different levels were prepared.	
	Organs:	Microscopically examined tissues of control and high concentration groups included adrenals, larynx, spleen, brain, liver, testes, eyes, lungs, thymus, gross lesions, heart, trachea, kidneys, ovaries, pancreas, nasal turbinates, stomach, uterus, pituitary, thyroid/parathyroid, aorta, sternum with bone marrow, salivary glands, duodenum, skin (flank), gall bladder, jejunum, oesophagus, urinary bladder, ileum, lymph node (submandibular), mammary gland, caecum, peripheral nerve (sciatic), thigh muscle, colon, Zymbal's glands, exorbital lacrimal glands, rectum, seminal vesicles, epididymis, prostate, femur (including articular surface), and the spinal cord. In addition, microscopic evaluations of the kidneys, testes, and gross lesions from the low and intermediate groups were performed. Also the livers from the low and intermediate groups at interim sacrifice were examined.	
	Additional evaluations:	No	
3.4.12	Other examinations	No	

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3.5	**Statistics**	The data for continuous, parametric variables were intercompared for the exposure and control groups by use of Levene's test for homogeneity of variances, by analysis of variance, and by t tests. The t tests were used, if the analysis of variance was significant, to delineate which groups differed from the control group. If Levene's test indicated homogeneous variances, the groups were compared by an analysis of variance for equal variances followed, when appropriate, by pooled variance t tests. If Levene's test indicated heterogeneous variances, the groups were compared by an analysis of variance for unequal variance followed, when appropriate, by separate variance t tests. Frequency data, such as microscopic diagnoses, were compared using Fisher's Exact Test. Nonparametric data were statistically evaluated using the Kruskal-Wallis test and, if necessary, by the Wilcoxon rank sum test as modified by Mann-Whitney. Mortality data were analyzed by life-table analysis. All statistical tests, except the frequency comparisons, were performed using BMDP Statistical Software. The probability value of $p < 0.05$ (two-tailed) was used as the critical level of significance for all tests.		
3.6	**Further remarks**	None		
		4 RESULTS AND DISCUSSION		
4.1	**Body weight**	500 ppm:	occasional small increases in body weight gain for core females	X
		≥ 500 ppm:	concentration-related increase in body weight and body weight gain for core males (2, 5 and 7 or 6, 23 and 30 %, respectively)	
		2500 ppm:	increase in body weight gain in core females (ca. 15 %); increased body weight gain (ca. 20 %) for recovery males throughout exposure and recovery phases	
		≥ 2500 ppm:	occasionally increased body weight gain (ca. 10 – 20 %) for recovery females throughout exposure and recovery phases	
		5000 ppm:	increase in body weight and body weight gain for core females (5 and 30 %, respectively); increased body weight and body weight gain for recovery males (ca. 6 and 30 %, respectively) throughout exposure and recovery phases	
4.2	**Food consumption**	No data		
4.3	**Water consumption**	No data		
4.4	**Clinical signs**	Exposure periods: ≥ 2500 ppm: hypoactivity, lack of a startle reflex and narcosis 5000 ppm: ataxia and prostration These clinical signs were transient in nature since these signs (other than the ataxia) were absent immediately following exposure. Non-exposure periods: 5000 ppm: ataxia immediately following exposure but not at the following morning Mortality / mean survival time: No significant differences within the groups.		