	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 A6.2/03 Percutaneous absorption (in vivo test)

Annex Point IIA6.2 Human data

Official use only

		1 REFERENCE
1.1	**Reference**	Turner P, Saeed B & Kelsey MC (2004) Dermal absorption of isopropyl alcohol from a commercial hand rub: implications for its use in hand decontamination. J Hosp Infect 56, 287 – 290
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 no guidelines available
2.2	**GLP**	[redacted]
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	2-Propanol
3.1.1	Lot/Batch number	No data
3.1.2	Specification	Formulation (Sterisol hand disinfectant®) containing 52.6 % (w/w) isopropyl alcohol
3.1.2.1	Description	No data
3.1.2.2	Purity	Not applicable
3.1.2.3	Stability	No data
3.1.2.4	Radiolabelling	Not applicable
3.2	**Test Animals**	
3.2.1	Species	10 human volunteers
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	4 Male / 6 female
3.2.5	Age/weight at study initiation	48 – 105 kg / 27 – 54 years
3.2.6	Number of animals per group	
3.2.7	Control animals	
3.3	**Administration/ Exposure**	Dermal
3.3.1	Preparation of test site	
3.3.2	Concentration of test substance	52.6 % (w/w)

Section A6.2/03 Percutaneous absorption (in vivo test)

Annex Point IIA6.2 Human data

3.3.3	Specific activity of test substance		
3.3.4	Volume applied	Total of 72 ml of the formulation (ca. 30 g 2-propanol)	
3.3.5	Size of test site		
3.3.6	Exposure period	The formulation was applied to the hands every 10 minutes for 4 hours	
3.3.7	Sampling time	5 min after final application	
3.3.8	Samples	Blood	
		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	None	
4.2	**Dermal irritation**	One participant noted mild erythema and itching of her hands	
4.3	**Recovery of labelled compound**	Not applicable	
4.4	**Percutaneous absorption**	The blood levels of 2-propanol were in the range of < 0.5 – 1.8 mg/L. Assuming a mean blood volume of 5 L, the total amount absorbed was ≤ 9 mg, i.e. the estimated penetration was ≤ 0.03 %.	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In an experimental study with 10 adult volunteers, the dermal absorption of 2-propanol was studied after hand washing every 10 minutes for 4 hours with a formulation containing 52.6 % 2-propanol (total amount applied: ca. 30 g 2-propanol).	
5.2	**Results and discussion**	Mean blood levels of 2-propanol were in the range of < 0.5 – 1.8 mg/L, i.e. the estimated penetration was ≤ 0.03 %.	X
5.3	**Conclusion**	[redacted]	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	[redacted]	

Section A6.2/03 Percutaneous absorption (in vivo test)

Annex Point IIA6.2 Human data

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2010/01/16
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 A6.2/04 Percutaneous absorption (in vivo test)

Annex Point IIA6.2 Human data

			Official use only
		1 REFERENCE	
1.1	**Reference**	Peschel O, Bauer MF, Gilg T & von Meyer L (1992) Veraenderung von Begleitstoffanalysen durch perkutane Resorption propanolhaltiger Antiseptika. Blutalkohol 29, 172 – 184	
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 no guidelines available	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	2-Propanol	
3.1.1	Lot/Batch number	No data	
3.1.2	Specification	Formulation (Sterillium®) containing 45 g propan-2-ol, 30 g propan-1-ol and 0.2 g mecetronium etilsulfat per 100 g	
3.1.2.1	Description	No data	
3.1.2.2	Purity	Not applicable	
3.1.2.3	Stability	No data	
3.1.2.4	Radiolabelling	Not applicable	
3.2	**Test Animals**		
3.2.1	Species	9 human volunteers	
3.2.2	Strain		
3.2.3	Source		
3.2.4	Sex	Male / female	
3.2.5	Age/weight at study initiation		
3.2.6	Number of animals per group		
3.2.7	Control animals		
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Preparation of test site	Chirurgical disinfection of hands and forearms	
3.3.2	Concentration of test substance		

Section A6.2/04 Percutaneous absorption (in vivo test)

Annex Point IIA6.2 Human data

3.3.3	Specific activity of test substance		
3.3.4	Volume applied	30 – 50 ml of the formulation (total amount applied: 14 – 23 g 2-propanol) 100 ml (inhalation)	X
3.3.5	Size of test site		
3.3.6	Exposure period	5 min 10 min (inhalation)	
3.3.7	Sampling time	Over a period of 5 hrs after initiation of skin contact	
3.3.8	Samples	Blood	
		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	No data	
4.2	**Dermal irritation**	No data	
4.3	**Recovery of labelled compound**	Not applicable	
4.4	**Percutaneous absorption**	Blood taken from the back of the foot (7 subjects): maximum values of 0.9 – 1.9 mg/L (mean 1.2 mg/L) after 40 minutes. Blood taken from the cubital vein (2 subjects): maximum values of 14 mg/L (male) or 4.5 mg/L (female) after 60 and 40 minutes, respectively. Blood levels declined slowly. After 90 minutes the concentration in the male was 8.8 mg/l, in the female 5.2 mg/L Uptake by inhalation: maximum value shortly after the end of the 10 min exposure period: 2.82 mg/L (1 subject)	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In an experimental study with 9 adult volunteers, the dermal absorption of 2-propanol was studied after chirurgical disinfection of hands and forearms with a formulation containing 30 g propan-1-ol per 100 g. 30 – 50 ml of the formulation (total amount applied: 14 – 23 g 2-propanol) was applied over a period of 5 min. Blood analysis was done via routine GC.	
5.2	**Results and discussion**	Maximum blood levels were 1.2 mg/L for blood taken from the back of the foot and 14 and 4.5 mg/L for blood from the cubital vein. Higher levels of 1-propanol taken from the cubital vein compared to the vein at the back of the foot can be explained by absence of the first pass effect for the site near to the application of the test substance. The results indicate that dermal absorption of 2-propanol still goes on for some time after application, although 2-propanol evaporates easily. Based on the maximum value of 14 mg/L obtained after 60 minutes and assuming a blood volume of 5 l, the amount absorbed is as a minimum 70 mg. This would correspond to a dermal absorption of 0.3-0.5 % of the dose administered. Uptake by inhalation may contribute to the dermal uptake by hand disinfection.	X

Section A6.2/04	**Percutaneous absorption (in vivo test)**	
Annex Point IIA6.2	Human data	
5.3 Conclusion	[redacted]	X
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	2008/02/27
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*

Section A6.2/04	**Percutaneous absorption (in vivo test)**
Annex Point IIA6.2	Human data
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.2/05 Metabolism study in mammals

Annex Point IIA6.2 Absorption, metabolism, disposition, and excretion of isopropanol in F344 rats and B6C3F1 mice

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	1 REFERENCE	
1.1	**Reference**	[redacted] (1994) Disposition and pharmacokinetics of isopropanol in F-344 rats and B6C3F1 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
2.2	**GLP**	[redacted]
2.3	**Deviations**	Not applicable
	3 MATERIALS AND METHODS	
3.1	**Test material (unlabelled test item)**	Isopropyl alcohol
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	> 99.8 %
3.1.2.3	Stability	No data
3.1.2.4	Molecular formula	C_3-H_8-O
3.2	**Test material (labelled test item)**	[2-^{14}C] isopropyl alcohol
3.2.1	Lot/Batch number	No data
3.2.2	Specification	2-propanol
3.2.2.1	Description	No data
3.2.2.2	Radiochemical purity	> 98 %
3.2.2.3	Stability	No data
3.2.2.4	Molecular formula	C_3-H_8-O
3.2.2.5	Radiolabelling	[2-^{14}C]
3.3	**Test Animals**	
3.3.1	Species	Rat Mouse
3.3.2	Strain	Fischer F-344 B6C3F1/CrlBR

Section A6.2/05		Metabolism study in mammals	
Annex Point IIA6.2		Absorption, metabolism, disposition, and excretion of isopropanol in F344 rats and B6C3F1 mice	
3.3.3	Source	Charles River Laboratories, Inc. (Raleigh, NQ)	
3.3.4	Sex	Male / Female	
3.3.5	Age/weight at study initiation	Rat : 7 – 9 weeks / 98 – 225 g Mouse: 5 – 7 weeks / 14 – 31 g	
3.3.6	Number of animals per group	Metabolism and excretion studies: 4 rats of each sex and 4 groups of 3 mice each for each sex Pharmacokinetic studies: 4 mice of each sex per time point	
3.3.7	Control animals	Yes	X
3.4	**Administration/ Exposure**	Oral, intravenous and inhalation	
3.4.1	Target dose level	See Table 6.2/05_01	
3.4.2	Animal treatment prior to administration	No data	
3.4.3	Specific activity of test substance	No data	
3.4.4	Vehicle	Intravenous application: TS was dissolved in isotonic saline Oral dosing via gavage: TS was formulated in distilled, deionised water	
3.4.5	Volume applied	Intravenous application: 2 ml/kg Oral dosing via gavage: 5 ml/kg	
3.4.6	Time to death	No deaths were observed	
3.4.7	Observation period	≥ 72 hrs	
3.5	**Study design and investigated endpoints**	Inhalation exposures: rats: nose-only mice: whole-body Rats (but not mice) in all pharmacokinetic studies were prepared with indwelling jugular cannulae.	
3.5.1	Sampling time		
3.5.2	Samples	Radiolabeled compounds exhaled in breath Urine and faeces were collected from each animal separately In pharmacokinetics studies, blood samples were collected at selected times via jugular cannulae in rats or by serial euthanasia of groups of 4 mice per time point	
3.5.3	Determination of radioactivity	The radiochemical purity of the TS was confirmed each time that a radiolabeled dosing solution or inhalation feed stock was used. Each formulation was analyzed by HPLC on an Aminex HPX-87H ion-exclusion column. Radioactivity was detected using a Ramona LS scintillation detector equipped with a flow-through 180 µl yttrium silicate solid scintillator cell. Radiochemical purity was determined by analysis of discrete fractions of HPLC eluate by liquid scintillation spectrometry. All biological samples were assayed for total radiolabel either directly or following digestion in an organic tissue solubiliser. Large tissues (i.e. liver) were homogenized and weighed samples of the homogenate were solubilised, neutralized, and decolorized prior to analysis. The residual carcasses of rats or of groups of 3 mice were digested in 2 N	

ethanolic sodium hydroxide and weighed aliquots were analyzed for ^{14}C content.
Liquid scintillation spectrometers were calibrated for quench correction using an external standard method.

3.5.4 Other

Metabolite profiles:
Composite 0 – 24 and 24 – 48 hr urine and volatile breath trap samples were prepared for each study by combining equivalent fractions (by weight) of urine or breath trap solutions from each rat or from groups of 3 mice of one sex for the indicated intervals. The composition of radiolabeled metabolites in these composite samples was determined by HPLC. Quantitation was performed using a flow-through radioactivity detector. The identities of the TS and acetone in excreta were established by comparison of their retention times to those of authentic standards.

Chromatographic purification of metabolite 1:
In addition to the TS and acetone, a minor urinary metabolite was found to be excreted by both species, which was purified from pooled rat urine using a multistep HPLC procedure.
Spectroscopic techniques used for identification of metabolite 1:
The residue of combined, lyophilized fraction of HPLC eluate containing purified metabolite 1 obtained from the last preparative chromatography step was reconstituted in 10 ml of HPLC grade distilled, deionised water. This was shell frozen and lyophilized overnight. This process was repeated three times in order to remove all traces of volatile solvents and ammonium acetate buffer. The resulting residue was a white, crystalline powder. This residue was stored in a sealed flask in the dark at -20°C until reconstituted for NMR analysis. The purified metabolite 1 residue was reconstituted in 1 ml of D_20 and transferred to an appropriate NMR tube for spectrometric analysis.

Analysis of blood samples for the TS and acetone using HPLC:
Two ~ 0.05 g aliquots were taken for total radioactivity analysis and the remaining blood was delivered to 300 µl septum-sealed, crimped-top, silated glass tubes. After precipitation of the protein by addition of 15 µl of 60 % trichloroacetic acid each sample was centrifuged, and the resulting supernatants were removed for analysis by HPLC. The deproteinized blood samples were analyzed for the TS and acetone by HPLC. Carbon-14 eluting from the column was detected by the in-line radioactivity detector and the peak areas measured by a Maxima 820 Data System. The radiochromatographic peak area response which was directly proportional to the amount of ^{14}C present in each respective peak was used to quantitate the mass of the TS and acetone in each sample. A calibration curve was generated for each study to correct for the changing efficiency of the yttrium silicate solid scintillator in the flow-through scintillation cell caused by the extremely low pH of the mobile phase. Control samples spiked with a range of different concentrations of ^{14}C isopropyl alcohol were analyzed prior to, during, and following the analysis of all the study samples. The results of the analysis of these samples were combined to generate a single calibration curve for all samples from a single study.

Pharmacokinetic methods:
All pharmacokinetic analyses were performed using the NLIN procedure (SAS Version 5.18)

4 RESULTS AND DISCUSSION

4.1	**Toxic effects, clinical signs**	No effects reported
4.2	**Recovery of labelled compound**	
4.3	**Absorption**	2-propanol was rapidly absorbed independently of the route (oral, i.v. or inhalation).
4.4	**Tissue distribution**	2-propanol and its radiolabeled metabolites were widely distributed to the tissues in all of the studies conducted and efficient elimination from the body resulted in low residual radioactivity. The terminal body burdens were in a range of ca. 1 – 5 %. In all studies most of the residual radiolabel remained in the adipose tissue, skeletal muscle, and skin and in no case did any individual tissue retain more than 2.4 % of the dose (not further specified). There were no obvious organs in which radiolabel was found to be retained, although the liver and kidney had slightly elevated concentrations of radiolabel relative to the blood in most studies. It is expected that 2-propanol will be metabolised at these sites, so that this could contribute to a slightly elevated concentration of 2-propanol and its radiolabeled metabolites in these tissues. The peak levels in blood for 2-propanol were given as follows: Oral studies in rats: 300 mg/kg: ca. 277 µg eq/g for males and 275 µg eq/g for females 3000 mg/kg: ca. 1295 µg eq/g for males and 1387 µg eq/g for females Multiple dosing with 300 mg/kg: ca. 253 µg eq/g for males and 222 µg eq/g for females Intravenous application: rats: ca. 336 µg eq/g for males and 281 µg eq/g for females mice: ca. 237 µg eq/g for males and 168 µg eq/g for females Exposure via inhalation: rats: 500 ppm: ca. 28 µg eq/g for males and 36 µg eq/g for females 5000 ppm: ca. 800 µg eq/g for males and 951 µg eq/g for females mice: 500 ppm: ca. 69 µg eq/g for males and 60 µg eq/g for females 5000 ppm: ca. 1948 µg eq/g for males and 1822 µg eq/g for females
4.5	**Metabolism**	From other studies it is known that acetone is the primary metabolite of 2-propanol, which was also confirmed in this study. In exhaled breath acetone accounted for 75 – 100 % of the radiolabeled organic volatile compounds being exhaled (see also table 6.2/05_05). The balance of the exhaled radioactivity was accounted for by CO_2 and 2-propanol itself. In urine, three radiolabeled compounds were found. Acetone was about 15 – 50 % of the radiolabel in urine. 2-propanol was less than 10 % of the urinary excretion of radiolabel except for high oral dosing where 2-propanol was about 15 % of the total urinary radioactivity due to saturation of the normal elimination pathways. A 3rd radiolabeled compound excreted in urine has been tentatively identified as the glucuronide conjugate of 2-propanol with an average of 3.5 % of the dose.

Section A6.2/05 Metabolism study in mammals

Annex Point IIA6.2 Absorption, metabolism, disposition, and excretion of isopropanol in F344 rats and B6C3F1 mice

Pharmacokinetics (see also table 6.2/05_06):
The half-life for the disappearance of 2-propanol from blood predicted by the model was 0.6 – 2 hrs, which was observed in all cases except for the high oral dose. In all studies the rate of elimination of 2-propanol was distinctly dose-dependent and the half-life for elimination increased and the elimination rate constant decreased directly with increasing dose in both species. There were no apparent differences between males and females of either species or between the different routes of administration. After oral dosing with 3000 mg/kg, the model predicted half-lives of 6.8 and 4.0 hrs for male and female rats.

4.6 Excretion

Oral studies in rats (see also table 6.2/05_03):
After dosing with 300 mg/kg, the major route of excretion was via exhalation (ca. 56 % as volatile breath and 26 % as CO_2 breath), while urine and faeces were minor routes of excretion (< 7 %). In no tissue more than 2 % of the dose 72 hrs following dosing was found and carcasses contained an average of ca. 3.8 % of the dose (not further specified).
After dosing with 3000 mg/kg, the major route of excretion also was via exhalation (ca. 70 % as volatile breath and 15 % as CO_2 breath), while excretion via urine and faeces was < 10 %. In no tissue more than 0.5 % of the dose 72 hrs following dosing was found and carcasses contained an average of < 1.5 % of the dose (not further specified).
After multiple dosing with 300 mg/kg (nominal) of unlabeled 2-propanol for 7 days and dosing on day 8 with 300 mg/kg radiolabeled 2-propanol, the disposition was determined over the ensuing 96 hrs. About 54 % of the dose was exhaled as volatile breath and ca. 28 % as CO_2 breath, while via urine and faeces < 7 % were excreted. No tissue retained more than 2.4 % of the dose and carcasses contained ca. 4 % of the dose (not further specified).

Intravenous application (see also table 6.2/05_02):
In rats and mice the major route of excretion was via exhalation (ca. 45 – 55 % as volatile breath and 28 – 33 % as CO_2 breath) after dosing with 300 mg/kg, and only minor amounts (< 7 %) were excreted via urine and faeces.
In rats no tissue retained more than 2.2 % of the dose and carcasses contained an average of < 4 % of the dose (not further specified).
In mice the residual carcasses contained 3.3 – 4 % of the dose (not further specified).

Exposure via inhalation (see also table 6.2/05_04):
In rats exposed to 500 ppm (nose-only over 6 hrs), the major route of excretion was via exhalation (29 – 38 % as volatile breath and 45 – 53 % as CO_2 breath), while excretion via urine and faeces was < 9 %. In no tissue more than 1.6 % of the dose was found and carcasses contained an average of 5 % of the dose (not further specified).
In rats exposed to 5000 ppm (nose-only over 6 hrs), the major route of excretion also was via exhalation (ca. 65 % as volatile breath and 21 % as CO_2 breath), while excretion via urine and faeces was < 9 %. In no tissue more than 0.5 % of the dose was found and carcasses contained an average of 1.5 % of the dose (not further specified).
In mice exposed to 500 ppm (whole-body over 6 hrs), the major route of excretion was via exhalation (46 – 54 % as volatile breath and 32 – 38 % as CO_2 breath), while excretion via urine and faeces was < 10 %. In tissues about 6.5 % of ^{14}C were found (not further specified).

Section A6.2/05	**Metabolism study in mammals**
Annex Point IIA6.2	Absorption, metabolism, disposition, and excretion of isopropanol in F344 rats and B6C3F1 mice

		In mice exposed to 5000 ppm (whole-body over 6 hrs), the major route of excretion also was via exhalation (70 – 75 % as volatile breath and 18 – 22 % as CO_2 breath), while excretion via urine and faeces was < 9 %. In carcasses an average of 1.7 – 2.1 % of the dose was found (not further specified).	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The absorption, metabolism, disposition, and excretion of 2-propanol was studied in male and female rats and mice. Animals were exposed by iv (300 mg/kg) and inhalation (500 and 5000 ppm for 6 hr) routes; additionally, 2-propanol was given by gavage to rats only in single and multiple 300 and 3000 mg/kg doses.	X
5.2	**Results and discussion**	In rats ca. 81 – 89 % of the administered dose was exhaled as acetone, CO2 or non-metabolised 2-propanol. In mice ca. 76 % of the dose was exhaled after iv bolus and ca. 92 % was exhaled after exposure via inhalation. In urine ca. 3 – 8 of the applied dose was excreted as 2-propanol, acetone and as a metabolite tentatively identified as isopropyl glucuronic acid. Small amounts of radiolabel were found in faeces and in the carcass. There were no major differences in the rates or routes of excretion observed either between sexes or between routes of administration. Additionally, repeated exposure had no effect on excretion. However, both the route of administration and the exposure or dose level influenced the form in which material was exhaled. After exposure to 5000 ppm a higher percentage of non-metabolised 2-propanol was found in expired air than following exposure to 500 ppm, implying saturation of metabolism.	
5.3	**Conclusion**	[redacted]	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	[redacted]	X

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]

Section A6.2/05 Metabolism study in mammals

Annex Point IIA6.2 Absorption, metabolism, disposition, and excretion of isopropanol in F344 rats and B6C3F1 mice

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 6.2/05_01: Summary of experimental treatments

			Actual doses (mg/kg) or concentration (ppm)	
Study design	**Route**	**Target dose (mg/kg) / concentration (ppm)**	**Males**	**Females**
Rat				
ETD	Intravenous	300	307	307
PK	Intravenous	300	306	318
ETD	Gavage	300	296	292
PK	Gavage	300	330	331
ETD	Gavage	3000	3121	3116
PK	Gavage	3000	3042	3069
ETD-PK	Inhalation	500	84	89
ETD-PK	Inhalation	5000	915	1010
MD-ETD	Gavage	300	302	299
MD-PK	Gavage	300	302	300
Mouse				
EXC	Intravenous	300	305	313
PK	Intravenous	300	295	292
EXC	Inhalation	500	206	225
PK	Inhalation	500	518	
EXC	Inhalation	5000	208	213
PK	Inhalation	5000	5012	

ETD = excretion and tissue distribution; EXC = excretion; PK = pharmacokinetic; ETD-PK = excretion, tissue distribution and pharmacokinetic; MD-ETD = multiple dose excretion and tissue distribution; MD-PK = multiple dose pharmacokinetic

Table 6.2/05_02: Comparison of the cumulative excretion of radiolabel by rats and mice following intravenous bolus administration of 300 mg/kg (% administered dose)

Sample time (hrs)	Volatile breath	CO_2 breath	Urine	Faeces	Total
Rat					
Male					
72	54.7	29.2	5.1	1.5	90.5
Female					
72	55.3	30.0	4.4	1.0	90.7
Mouse					
Male					
72	44.6	32.7	3.9	1.5	82.8
Female					
72	46.1	27.7	2.5	1.4	77.8

Table 6.2/05_03: Cumulative excretion of radiolabel by groups of rats following oral administration of radiolabeled TS (% administered dose)

Sample time (hrs)	Volatile breath	CO_2 breath	Urine	Faeces	Total
300 mg/kg					
Male					
72	56.6	24.6	5.9	0.7	87.9
Female					
72	54.7	27.4	4.8	0.6	87.4
3000 mg/kg					
Male					
72	68.2	15.8	8.3	0.8	93.0
Female					
72	70.9	15.4	6.8	0.5	93.6
300 mg/kg * 8 days					
Male					
72	52.8	28.6	5.4	0.9	87.8
Female					
72	55.3	27.3	4.5	1.0	88.1

Table 6.2/05_04: Cumulative excretion of radioactivity by groups of rats or mice following 6 hr inhalation exposure to atmosphere containing radiolabeled TS (% recovered dose)

Sample time (hrs)	Volatile breath	CO_2 breath	Urine	Faeces	Total
Rat 500 ppm					
Male					
72	29.2	52.5	7.6	1.2	90.4
Female					
72	38.2	45.5	5.5	1.7	90.8
Rat 5000 ppm					
Male					
72	65.8	21.6	7.0	0.6	95.0
Female					
72	65.3	20.7	8.0	0.4	94.5
Mouse 500 ppm					
Male					
72	45.6	38.0	7.7	1.6	92.9
Female					
72	54.2	31.8	4.2	1.7	91.5
Mouse 5000 ppm					
Male					
72	75.4	17.5	7.0	1.2	96.8
Female					
72	69.9	21.6	4.6	1.2	97.0

Table 6.2/05_05: Comparison of the excretion of radiolabeled parent compound and metabolites by rats and mice as a function of dose and route

		Breath			Urine		
Route	**Sex**	**CO_2 (% of dose)**	**Acetone (% of dose)**	**Isopropanol (% of dose)**	**Glucuronide (% of dose)**	**Acetone (% of dose)**	**Isopropanol (% of dose)**
Rat 300 mg/kg							
i.v.	M	29.2	44.6	9.2	3.6	0.8	0.4
i.v.	F	30.0	43.4	11.2	3.4	0.7	ND
Mouse 300 mg/kg							
i.v.	M	32.7	36.3	7.8	3.1	ND	ND
i.v.	F	27.7	45.7	ND	1.7	ND	ND
Rat 300 mg/kg							
Oral	M	24.6	55.9	ND	4.4	1.0	0.2
Oral	F	27.4	54.1	ND	3.3	1.1	ND
Rat 3000 mg/kg							
Oral	M	15.8	40.7	11.9	3.8	0.9	0.7
Oral	F	15.4	44.4	14.9	3.2	1.0	1.0
Rat 300 mg/kg * 8 days							
Oral	M	28.6	52.2	ND	4.1	0.7	0.3
Oral	F	27.3	54.9	ND	3.2	0.9	ND
Rat 500 ppm							
Inhalation	M	52.2	28.8	ND	4.2	2.4	ND
Inhalation	F	45.5	33.9	3.8	2.6	1.7	ND
Rat 5000 ppm							
Inhalation	M	21.6	50.1	11.6	3.2	2.3	0.2
Inhalation	F	20.7	50.0	13.5	2.8	3.9	0.5
Mouse 500 ppm							
Inhalation	M	38.0	37.7	6.6	5.7	0.5	0.5
Inhalation	F	31.3	52.7	ND	3.1	1.0	1.0
Mouse 5000 ppm							
Inhalation	M	17.5	59.8	13.4	3.8	1.0	0.6
Inhalation	F	21.6	53.4	14.0	3.3	0.4	0.4

ND = no peak detectable

Table 6.2/05_06: Pharmacokinetic parameter estimates derived from one-compartment modelling of isopropanol blood concentration vs. time data

Sex	Route	Target dose (mg/kg or ppm)	Elimination rate constant (h)	Half-life (h)	AUC (µg * h/g)
Rat					
M	i.v.	300	0.535	1.3	707
F	i.v.	300	0.612	1.2	511
M	Oral	300	0.547	1.3	744
F	Oral	300	0.557	1.3	492
M	Oral	3000	0.135	6.8	19507
F	Oral	3000	0.183	4.0	11839
M	Oral	300 * 8 days	0.430	1.7	667
F	Oral	300 * 8 days	0.415	1.7	442
M	Inhalation	500	0.956	0.8	111
F	Inhalation	500	0.767	0.9	152
M	Inhalation	5000	0.338	2.1	4648
F	Inhalation	5000	0.394	1.8	5436
Mouse					
M	i.v.	300	0.907	0.8	327
F	i.v.	300	0.788	0.9	379
M	Inhalation	500	1.22	0.6	393
F	Inhalation	500	0.930	0.8	551
M	Inhalation	5000	0.388	1.8	17516
F	Inhalation	5000	0.439	1.6	15610

Section A6.2/06 Percutaneous absorption in humans

Annex Point IIA6.2

Official use only

1 REFERENCE

1.1 Reference

Bieber N (2006) Absorption of alcohol from hand disinfection (Alkoholresorption nach Händedesinfektion) Dissertation Ernst-Moritz-Arndt-Universität Greifswald, Germany

1.2 Data protection

No

1.2.1 Data owner

n.a.

1.2.2 Criteria for data protection

No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No

2.2 GLP

[redacted]

2.3 Deviations

n.a.

3 MATERIALS AND METHODS

3.1 Test material

Ethanol
Propan-1-ol
Propan-2-ol
In different disinfectants

3.1.1 Lot/Batch number

3.1.2 Specification

Content of the different disinfectants:

Sterillium®Virugard	Ethanol	95 %
Sterillium®Gel	Ethanol	85 %
Manorapid Synergy®	Ethanol	55 %
	Propan-1-ol	10 %
	Propan-1,2-diol	5.9 %,
	Buan-1,3-diol	
Poly-Alcohol Händeantisepticum®	Propan-2-ol	70 %
Sterillium®Lösung	Propan-2-ol	45 %
	Propan-1-ol	30 %
	Mecetroniumetilsulfat	0.2 %

3.1.2.1 Description

n.g.

3.1.2.2 Purity

n.g.

3.1.2.3 Stability

n.g.

3.1.2.4 Radiolabelling

No

3.2 Test Animals

Section A6.2/06 Percutaneous absorption in humans

Annex Point IIA6.2

3.2.1	Species	Human volunteers	
3.2.2	Strain	n.a.	
3.2.3	Source	n.a.	
3.2.4	Sex	m, f	
3.2.5	Age/weight at study initiation	> 18 years	
3.2.6	Number	12 (6 m, 6 f)	
3.2.7	Controls	No	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Preparation of test site		
3.3.2	Concentration of test substance	Propan-1-ol: 10, 30 %	X
3.3.3	Specific activity of test substance	n.a.	
3.3.4	Volume applied	Hygienic hand disinfection: 3-5 ml Surgical hand disinfection: 4-6 times 3-5 ml	
3.3.5	Size of test site	Hygienic disinfection: Hands Surgical hand disinfection: hands and forearms	
3.3.6	Exposure period	Hygienic hand disinfection: 30 sec Surgical hand disinfection: 3 min (skin was kept wet during application)	
3.3.7	Exposure frequency	Hygienic hand disinfection: 20 times, 1 minute break after each disinfection Surgical hand disinfection:5 times, 5 minutes break after each disinfection	X
3.3.8	Exposure duration	Hygienic hand disinfection: Hand rub 10 minutes, including breaks: 30 min Surgical hand disinfection: Hand rub 30 min, including breaks: 80 min	
3.3.9	Sampling time	Hygienic hand disinfection: 2.5,5, 10, 20, 30, 60, 90 min after last disinfection Surgical hand disinfection: 5, 10, 20, 30, 60, 90, 120 min after last disinfection	
3.3.10	Samples	Blood	
3.3.11	Determination of test substance	GC with FID-detection:	

	Detection Limit mg/L	Quantification limit mg/L
Ethanol	0.14	0.34
Propan-1-ol	0.13	0.34
Propan-2-ol	0.03	0.09

4 RESULTS AND DISCUSSION

.

4.1 Absorption

Hygienic hand disinfection:

Product	Alcohol	Concentration	AUC mg/l x min	Uptake range mg	% of dose absorbed median
Sterillium® Virugard	Ethanol	95 %	1211.3	286.1-2185.4	2
Sterillium® Gel	Ethanol	85 %	545.3	95.2-1514.1	1
Manorapid Synergy®	Ethanol	55 %	311.0	95.2-980.2	0.9
	Propan-1-ol	10 %	81.2	28.1-263.9	1.3
Poly-Alcohol Händeanti-septicum®	Propan-2-ol	70 %	335.8	98.5-1180-9	0.8
Sterillium® Lösung	Propan-2-ol	45 %	330.1	141-2112.9	1.2
	Propan-1-ol	30 %	536.8	223.4-4476.6	2.8

Surgical hand disinfection:

Product	Alcohol	Concentration	AUC mg/l x min	Uptake range mg	% of dose absorbed
Sterillium® Virugard	Ethanol	95 %	1151.4	514.1-4817.7	0.8
Sterillium® Gel	Ethanol	85 %	1487.1	543.9-18816.0	1.1
Manorapid Synergy®	Ethanol	55 %	468.0	281.4-6377.8	0.5
	Propan-1-ol	10 %	143.0	61.0-1995.3	0.9
Poly-Alcohol Händeanti-septicum®	Propan-2-ol	70 %	447.4	271.3-2406.9	0.4
Sterillium® Lösung	Propan-2-ol	45 %	585.4	164.2-4072.9	0.8
	Propan-1-ol	30 %	788.8	314-7777.3	1.6

Section A6.2/06 Percutaneous absorption in humans

Annex Point IIA6.2

4.2	**Time course**	Maximum values were obtained 30 min after the end of the disinfection, at 90 minutes (hygienic hand disinfection) and 120 min (surgical hand disinfection) the values still were above the initial values for all 3 alcohols.	
4.3	**Dermal irritation**	Not investigated	
4.4	**Recovery of labelled compound**	n.a.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	12 human volunteers were exposed to different alcohol based disinfectants either for hygienic or surgical hand disinfection under worst case conditions (20 disinfections for hygienic hand disinfection, 5 disinfections for surgical hand disinfection). Uptake of the ethanol, prpan-1-ol and propan-2-ol was determined via measurement of the alcohols in the blood.	X
5.2	**Results and discussion**	The medians for the uptake ranged from 0.4-2 % of the dose. Although by surgical hand disinfection higher blood levels were found due to the higher dose applied, given as % of the dose, the levels tend to be lower, indicating saturation of absorption at higher dose levels. There were no differences in absorption between ethanol, propan-1-ol and propan-2-ol. Uptake by inhalation was not prevented. Therefore it may have contributed to the overall uptake. However, the time course of uptake was rather slow, and progressed during about 30 minutes after the end of exposure indicating rather accumulation of the alcohols in the skin and subsequent uptake than immediate uptake via the lung.	X
5.3	**Conclusion**	[redacted]	
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	2010/01/16
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 A6.4.1/01 Repeated dose toxicity

Annex Point IIA6.4.1 Subchronic oral toxicity test with rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1993) Toxic effects in rats of twelve weeks' dosing of 2-propanol, and neurotoxicity measured by densitometric measurement of glial fibrillary acidic protein in the dorsal hippocampus. [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	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	Not applicable	X
		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	HPLC grade (Rathburn)	
3.1.2.3	Stability	No data	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	MOL:WIST	
3.2.3	Source	Møllegard breeding centre Ltd, Denmark	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	3 months / 270 g	
3.2.6	Number of animals per group	22	
3.2.7	Control animals	Yes (tap water)	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	90 days	X
3.3.2	Frequency of exposure	Continuously via drinking water	
3.3.3	Postexposure period	None	

Section A6.4.1/01 Repeated dose toxicity

Annex Point IIA6.4.1 Subchronic oral toxicity test with rats

3.3.4	**Oral**		
3.3.4.1	Type	Drinking water	
3.3.4.2	Concentration	0, 1, 2, 3 or 5 %	X
3.3.4.3	Vehicle	Water	
3.3.4.4	Concentration in vehicle	0, 1, 2, 3 or 5 %	X
3.3.4.5	Total volume applied	Not applicable	
3.3.4.6	Controls	Vehicle (tap water)	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes	X
3.4.1.2	Mortality	Yes	X
3.4.2	Body weight	Yes (once weekly)	
3.4.3	Food consumption	No data	
3.4.4	Water consumption	Yes (twice weekly)	
3.4.5	Ophthalmoscopic examination	No	
3.4.6	Haematology	No	
3.4.7	Clinical Chemistry	No	
3.4.8	Urinalysis	No	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes liver, heart, spleen, testes, kidneys, adrenals	
3.5.2	Gross and histopathology	Yes liver, heart, spleen, testes, kidneys, adrenals, brain 12 rats from each group: pathological examination 10 rats from each group dosed with 0 – 3 %: densitometry of the brain tissue 9 rats dosed with 5 %: densitometry of the brain tissue	X
3.5.3	Other examinations	The content of glial fibrillary acidic protein (GFAP) was measured semiquantitatively by a densiometric method applied to immunohistochemically stained sections from dorsal hippocampus.	
3.5.4	Statistics	Body weight, relative water consumption, relative organ weight and absorbances: one-way analysis of variance (ANOVA) followed by Dunnetts two-tailed t-test. Absorbance against section thickness and organ weights against dose: linear regression	
3.6	**Further remarks**	None	

Section A6.4.1/01 Repeated dose toxicity

Annex Point IIA6.4.1 Subchronic oral toxicity test with rats

		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	5 %: Hyperactivity in handled rats.	X
4.1.2	Mortality	5 %: one rat died within the 1st week due to dehydration.	X
4.2	**Body weight gain**	1 %: statistically significant increased 3 or 5 %: statistically significant decrease	X
4.3	**Food consumption and compound intake**	No data on food intake. In the highest dose group the content of 2-propanol was reduced to 4 % in the 2nd week (animals drank very little in the 1st week) and thereafter returned to 5 % for the rest of the study. The mean intake for 2-propanol (based on water consumption) was calculated with 0, 870, 1280, 1680 or 2520 mg/kg bw.	
4.4	**Ophtalmoscopic examination**	No data	
4.5	**Blood analysis**		
4.5.1	Haematology	No data	
4.5.2	Clinical chemistry	No data	
4.5.3	Urinalysis	No data	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Relative organs weight statistically significant increased for liver, testes, kidneys and adrenals (for testes not dose-dependent; see table A6.4.1/01_01)	X
4.6.2	Gross and histopathology	Increased formation of hyaline casts and content of hyaline droplets in the proximal tubules of the kidneys (severity of change was dose-dependent). No abnormalities recorded for liver, heart, spleen, testes, adrenals and brain.	X
4.7	**Other**	There was no indication for astrogliosis caused by dosing with 2-propanol.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In this study groups of 22 male rats were dosed with 0, 1, 2, 3 or 5 % 2-propanol via drinking water over 90 days. The mean intake for 2-propanol (based on water consumption) was calculated with 0, 870, 1280, 1680 or 2520 mg/kg bw.	
5.2	**Results and discussion**	The treatment caused changes in relative organs weights and dose-dependent adverse effects on the kidneys, while there was no indication for neurotoxic effects on the dorsal hippocampus.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	[redacted]	X
5.3.2	NO(A)EL		
5.3.3	Other	[redacted]	
5.3.4	Reliability	[redacted]	

Section A6.4.1/01 **Repeated dose toxicity**

Annex Point IIA6.4.1 Subchronic oral toxicity test with rats

5.3.5 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	2008/02/21
Materials and Methods	[redacted]
Results and discussion	[redacted]

Section A6.4.1/01 Repeated dose toxicity

Annex Point IIA6.4.1 Subchronic oral toxicity test with rats

	[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 A6.4.1/01_01 Changes in relative organ weights (g/100 g; adrenals: mg/100 g)

	liver	heart	spleen	testes	kidneys	adrenals
0 %	2.9	0.251	0.157	0.785	0.483	10.9
1 %	3.02	0.251	0.163	0.741	0.515	11.5
2 %	3.15*	0.246	0.16	0.736	0.582***	12.5
3 %	3.22**	0.257	0.169	0.788	0.601***	13.8***
5 %	3.26***	0.259	0.153	0.888**	0.654***	14.6***

* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$

Section A6.4.3/01 Repeated dose toxicity

Annex Point IIA6.4.3 13-Week Inhalation Toxicity Study with mice

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994) Isopropanol 13-week vapor inhalation study in rats and mice with neurotoxicity evaluation in rats. [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	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	No data	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	CD-1	
3.2.3	Source	Charles River Breeding Labs., MI (USA)	
3.2.4	Sex	Male / female	
3.2.5	Age/weight at study initiation	8 weeks / 19 – 37 g	
3.2.6	Number of animals per group	10 m / 10 f	
3.2.7	Control animals	Yes (10 per sex)	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	90 days	
3.3.2	Frequency of exposure	6 hrs/day on 5 days/week for 13 weeks	
3.3.3	Postexposure period	None	

Section A6.4.3/01 Repeated dose toxicity

Annex Point IIA6.4.3 13-Week Inhalation Toxicity Study with mice

3.3.4	**Inhalation**		
3.3.4.1	Concentrations	Nominal concentration	0, 100, 500, 1500 or 5000 ppm (ca. 0, 250, 1250, 3750 or 12500 mg/m^3)
		Analytical concentration	0, 100, 506, 1508 or 5008 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	Not applicable	
3.3.4.6	Concentration in vehicle	Not applicable	
3.3.4.7	Duration of exposure	6 hrs/day	
3.3.4.8	Controls	sham exposed	
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 start of exposures, weekly during study, and immediately before euthanasia)	
3.4.3	Food consumption	Yes (weekly)	
3.4.4	Water consumption	Yes (weekly)	
3.4.5	Ophthalmoscopic examination	Yes (Prior to 1st exposure and during week 12)	
3.4.6	Haematology	Yes (at termination in all animals): total leukocyte count, mean corpuscular volume (MCV), platelet count, mean corpuscular haemoglobin (MCH), erythrocyte count, differential leukocyte count, mean corpuscular haemoglobin concentration, reticulocyte count, haematocrit, and haemoglobin.	
3.4.7	Clinical Chemistry	Yes (at termination in all animals): glucose, alanine aminotransferase, calcium, urea nitrogen, aspartate aminotransferase, phosphorus, creatinine, gamma-glutamyl transferase, sodium, total protein, potassium, albumin chloride, globulin, and total, conjugated, and unconjugated bilirubin.	
3.4.8	Urinalysis	No	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes: brain, liver, lungs, kidneys, adrenals, testes, and ovaries from all surviving mice were weighed at termination	
3.5.2	Gross and histopathology	Yes (complete necropsy on all animals): Tissues were fixed in 10 % neutral buffered formalin. Tissue sections were prepared and stained with haematoxylin and eosin. Sections of the	

Section A6.4.3/01 **Repeated dose toxicity**

Annex Point IIA6.4.3 13-Week Inhalation Toxicity Study with mice

		kidneys were also stained with Mallory Heidenhain stain. The tissues which were microscopically examined in controls 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 lungs and livers from animals of the 100, 500 and 1500 ppm 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. 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	≥ 1500 ppm: narcosis, ataxia, hypoactivity 5000 ppm: lack of a startle reflex	X
4.1.2	Mortality	No mortalities at any concentration level	
4.2	**Body weight gain**	5000 ppm: statistically significant increase in body weight and body weight gain in females (13 % or 71 %, respectively)	
4.3	**Food consumption and compound intake**	Food uptake: no adverse effects Water uptake: ≥ 1500 ppm: increased uptake in males 5000 ppm: increased uptake in females	
4.4	**Ophtalmoscopic examination**	No adverse effects	
4.5	**Blood analysis**		
4.5.1	Haematology	5000 ppm: increased haematocrit, haemoglobin, MCV and MCH values in females	

Section A6.4.3/01	Repeated dose toxicity	
Annex Point IIA6.4.3	13-Week Inhalation Toxicity Study with mice	
4.5.2	Clinical chemistry	5000 ppm: increased total protein, albumin, globulin, total / direct bilirubin, and inorganic phosphorus in females; decreased serum chloride in females.
4.5.3	Urinalysis	No data
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	≥ 1500 ppm: increase in relative liver weights in females (10 % or 21 %, respectively)
4.6.2	Gross and histopathology	No adverse effects
4.7	**Other**	No data
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In this study CD-1 mice were exposed to 2-propanol concentrations of 0, 100, 500, 1500 or 5000 ppm (ca. 0, 250, 1250, 3750 or 12500 mg/m^3) on 6 hrs/day on 5 days/week for 13 weeks.
5.2	**Results and discussion**	No exposure-related mortalities occurred. Narcotic effects were noted during exposure to ≥ 1500 ppm. In females exposed to 5000 ppm an increase in body weight and body weight gain and changes in haematological parameters and clinical chemistry were seen. Concentrations of ≥ 1500 ppm caused an increase in relative liver weights also in females. There were no treatment-related effects at gross necropsy or at histopathological examination.
5.3	**Conclusion**	
5.3.1	LO(A)EL	[redacted]
5.3.2	NO(A)EL	[redacted]
5.3.3	Other	
5.3.4	Reliability	[redacted]
5.3.5	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	2008/10/08
Materials and Methods	[redacted]

Section A6.4.3/01 Repeated dose toxicity

Annex Point IIA6.4.3 13-Week Inhalation Toxicity Study with mice

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	

Section A6.4.3/02 Repeated dose toxicity

Annex Point IIA6.4.3 13-Week Inhalation Toxicity Study with rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994) Isopropanol 13-week vapor inhalation study in rats and mice with neurotoxicity evaluation in rats. [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	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	No data	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	F344	
3.2.3	Source	Harlan Sprague-Dawley, Inc., IN (USA)	
3.2.4	Sex	Male / female	
3.2.5	Age/weight at study initiation	8 weeks / 112 – 165 g	
3.2.6	Number of animals per group	a.) 10 m / 10 f b.) 15 m / 15 f (for assessment of neurobehavioral function [FOB])	
3.2.7	Control animals	Yes a.) 10 per sex b.) 15 per sex	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	90 days	
3.3.2	Frequency of exposure	6 hrs/day on 5 days/week for 13 weeks	

Section A6.4.3/02 Repeated dose toxicity

Annex Point IIA6.4.3 13-Week Inhalation Toxicity Study with rats

3.3.3	Postexposure period	None		
3.3.4	**Inhalation**			
3.3.4.1	Concentrations	Nominal concentration	a.) 0, 100, 500, 1500 or 5000 ppm (ca. 0, 250, 1250, 3750 or 12500 mg/m^3) b.) 0, 500, 1500 or 5000 ppm (ca. 0, 1250, 3750 or 12500 mg/m^3)	X
		Analytical concentration	0, 100, 506, 1508 or 5008 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	Not applicable		
3.3.4.6	Concentration in vehicle	Not applicable		
3.3.4.7	Duration of exposure	6 hrs/day		
3.3.4.8	Controls	Sham exposed		
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 start of exposures, weekly during study, and immediately before euthanasia)		
3.4.3	Food consumption	Yes (weekly)		
3.4.4	Water consumption	Yes (weekly)		
3.4.5	Ophthalmoscopic examination	Yes (Prior to 1st exposure and during week 12)		
3.4.6	Haematology	Yes (during week 6 of the study and at termination in all animals): total leukocyte count, mean corpuscular volume (MCV), platelet count, mean corpuscular haemoglobin (MCH), erythrocyte count, differential leukocyte count, mean corpuscular haemoglobin concentration, reticulocyte count, haematocrit, and haemoglobin.		
3.4.7	Clinical Chemistry	Yes (at termination in all animals): glucose, alanine aminotransferase, calcium, urea nitrogen, aspartate aminotransferase, phosphorus, creatinine, gamma-glutamyl transferase, sodium, total protein, potassium, albumin chloride, globulin, and total, conjugated, and unconjugated bilirubin.		
3.4.8	Urinalysis	No		
3.5	**Sacrifice and pathology**			

Section A6.4.3/02 Repeated dose toxicity

Annex Point IIA6.4.3 13-Week Inhalation Toxicity Study with rats

3.5.1	Organ Weights	Yes: brain, liver, lungs, kidneys, adrenals, testes, and ovaries from all surviving rats (except for rats having neuroanatomic pathology evaluation) were weighed at termination. For rats which had neuroanatomic pathology evaluation, the brain was weighed and measured (length and width)
3.5.2	Gross and histopathology	Yes (complete necropsy on all animals): 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. The tissues which were microscopically examined in controls 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 lungs, livers and kidneys from animals of the 100, 500 and 1500 ppm groups were performed. Neuroanatomic pathology evaluation was performed on 10 of the 15 rats/sex/group designated for neurobehavioural function assessments. These rats were anesthetized with sodium pentobarbital, and tissues were fixed by intracardiac perfusion, with a phosphate buffered solution of 5 % methanol-free EM grade formaldehyde followed by a phosphate solution of 5 % glutaraldehyde. The brain, spinal cord, and peripheral nerves were removed and immersion fixed in methanol-free EM grade formalin for light microscopic examination or in glutaraldehyde for possible electron microscopic examination. Light microscopic examinations were performed on the following tissues from 6 rats/sex/group: forebrain, spinal cord (cervical and lumbar), centre of the cerebrum, dorsal root ganglia, centre of the midbrain, Gasserian ganglia, cerebellum and pons, dorsal and ventral root fibres, medulla oblongata, common peroneal nerve (below the knee), tibial nerve (below the knee), sural (fibular) nerve (below the knee), and proximal sciatic nerve (mid-thigh and sciatic notch). Electron microscopic examination was not performed on the remaining 4 rats/sex/group due to the absence of significant toxicological or pathologic findings during light microscopic examination.
3.5.3	Other examinations	Ten of the 15 rats/sex designated for neurobehavioural function assessments were evaluated with the functional observational battery (FOB) prior to the first exposure and on the weekend following weeks 1, 2, 4, 9, and 13. Approximately 42 hrs elapsed between the end of the exposure and the beginning of FOB testing for male and female rats. These rats were observed for signs of convulsions, tremors, stereotypy, and piloerection. Respiration, urination, gait, arousal, rears, and startle response were also evaluated during this initial observation period. The rats were then grasped, and pupil size, pupil response to light, vocalization, salivation, mouth breathing, lacrimation, diarrhoea, visual placing, and muscle tone were evaluated. Catatonia, grip strength, surface and air righting reflexes, toe and tail withdrawal reflexes, hind leg splay, rectal temperature, and body weight were subsequently assessed.

Section A6.4.3/02 **Repeated dose toxicity**
Annex Point IIA6.4.3 13-Week Inhalation Toxicity Study with rats

		Motor activity evaluations were conducted on all 15 rats/sex prior to the first exposure and on the weekend following weeks 4, 9 and 13. The time between the end of the exposure and the beginning of motor activity testing was approximately 20 hr for female rats and 24 hrs for male rats. The rats were tested using an automated photocell recording apparatus designed to measure activity in a novel environment. Data were collected automatically for subsequent analysis. The length of the test session was 90 min.	
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. Intrasession motor activity data were analyzed using a repeated-measures analysis with concentration as grouping factor and session time as the within-subject factor. Group comparisons at each reporting epoch were made (as described above) if significant concentration effects or time-by-concentration interactions were observed. The epsilon-adjustment procedure (Greenhouse-Geisser correction) was used in repeated-measures analysis of motor activity data. Frequency data, such as microscopic diagnoses and FOB data, 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. 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	≥ 500 ppm: increased incidence of perinasal encrustation (males) ≥ 1500 ppm: hypoactivity 5000 ppm: lack of a startle reflex, ataxia, narcosis (not observed during exposure after week 2); markedly increased incidence of swollen periocular tissue (females).	X
4.1.2	Mortality	No mortalities at any concentration level	
4.2	**Body weight gain**	1500 ppm: decreased body weight and body weight gain in females at the end of week 1. ≥ 1500 ppm: absolute body weight and body weight gain were increased (usually statistically significantly) beginning at approximately week 5; at the end of week 13, the percentage increases in body weight gain were 12 and 16 % for males and females of the 5000 ppm group, respectively, and 7 and 8 % for males and females of the 1500 ppm group, respectively 5000 ppm: statistically significantly decreased body weight and/or body weight gain at the end of week 1	

Section A6.4.3/02 Repeated dose toxicity

Annex Point IIA6.4.3 13-Week Inhalation Toxicity Study with rats

4.3	**Food consumption and compound intake**	Food uptake: 5000 ppm: statistically significant decrease in females at the end of week 1; statistically significant increase in food consumption beginning at weeks 4 – 5 (% increases: 5 and 13 % at the end of week 13 for males and females, respectively). Water uptake: ≥ 1500 ppm: increased water consumption (beginning at approximately week 2)	
4.4	**Ophtalmoscopic examination**	No adverse effects	
4.5	**Blood analysis**		
4.5.1	Haematology	1500 ppm: decreased total erythrocytes in females at week 6 ≥ 1500 ppm: decreased platelet counts in males at week 6 and increased platelet counts in males at week 14. 5000 ppm: decreased total erythrocytes, haemoglobin, haematocrit, and platelet counts at week 6; increased MCV and MCH at week 6 in males; increased lymphocytes at week 6 in females; certain of these haematological effects seen in males and females (e.g. decreased total erythrocytes, haemoglobin, and haematocrit) were no longer present at week 14; at week 14, MCV and MCH were still increased in males and increased MCV was also observed in females.	X
4.5.2	Clinical chemistry	No adverse effects	
4.5.3	Urinalysis	No data	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	5000 ppm: increased relative liver weights in males and females (8 and 5 %, respectively).	
4.6.2	Gross and histopathology	≥ 100 ppm: increased numbers and sizes of hyaline droplets within the kidneys of exposed males compared with controls (not clearly concentration related, although this change was most pronounced in the high-concentration group). Due to the lack of other renal histopathological changes, the biological significance of these droplets is unclear.	X
4.7	**Other**	No changes in any of the parameters of the FOB. An increase in motor activity was observed in females of the 5000 ppm group following weeks 9 and 13 (57 and 26 %, respectively). Differences in mean activity for females of the 5000 ppm group compared to the control group were observed at several of the 10-min session intervals at week 9 but not at week 13. There were no alterations in motor activity at any time point for males.	X

Frequency and grade of hyaline droplets in the kidneys of males

	Concentration (ppm)				
Grade	**0**	**100**	**500**	**1500**	**5000**
Minimal	9 / 10	2 / 10	0 / 10	4 / 10	0 / 10
Mild	0 / 10	3 / 10	1 / 10	5 / 10	0 / 10
Moderate	1 / 10	5 / 10	9 / 10	1 / 10	1 / 10
Marked	0 / 10	0 / 10	0 / 10	0 / 10	9 / 10

Section A6.4.3/02 Repeated dose toxicity

Annex Point IIA6.4.3 13-Week Inhalation Toxicity Study with rats

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In this study Sprague-Dawley rats were exposed to 2-propanol concentrations of 0, 100, 500, 1500 or 5000 ppm (ca. 0, 250, 1250, 3750 or 12500 mg/m^3) on 6 hrs/day on 5 days/week for 13 weeks.
In addition 15 rats per sex were exposed to 0, 500, 1500 or 5000 ppm (ca. 0, 1250, 3750 or 12500 mg/m3) for assessment of neurobehavioural function (FOB).

5.2 Results and discussion

No exposure-related mortalities occurred.
Concentrations of ≥ 500 ppm caused an increased incidence of perinasal encrustation in males, ≥ 1500 ppm hypoactivity and at 5000 ppm there was a lack of a startle reflex, ataxia, and narcosis.
Changes in body weights, body weight gain and water uptake were noted at ≥ 1500 ppm, while changes in food consumption were seen only at 5000 ppm.
Haematological effects such as decreased total erythrocytes, decreased or increased platelet counts, decreased haemoglobin and haematocrit were observed at concentrations of ≥ 1500 ppm.
At 5000 ppm there was an increase in relative liver weights in males and females.
At necropsy there was increase in numbers and sizes of hyaline droplets within the kidneys of exposed males compared with controls. However, the biological significance of this finding is unclear as there was no clear concentration dependency and there were also no renal histopathological changes.

There were no changes in any of the parameters of the FOB.

5.3 Conclusion

5.3.1	LO(A)EL	[redacted]	X
5.3.2	NO(A)EL	[redacted]	X
5.3.3	Other		
5.3.4	Reliability	[redacted]	
5.3.5	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

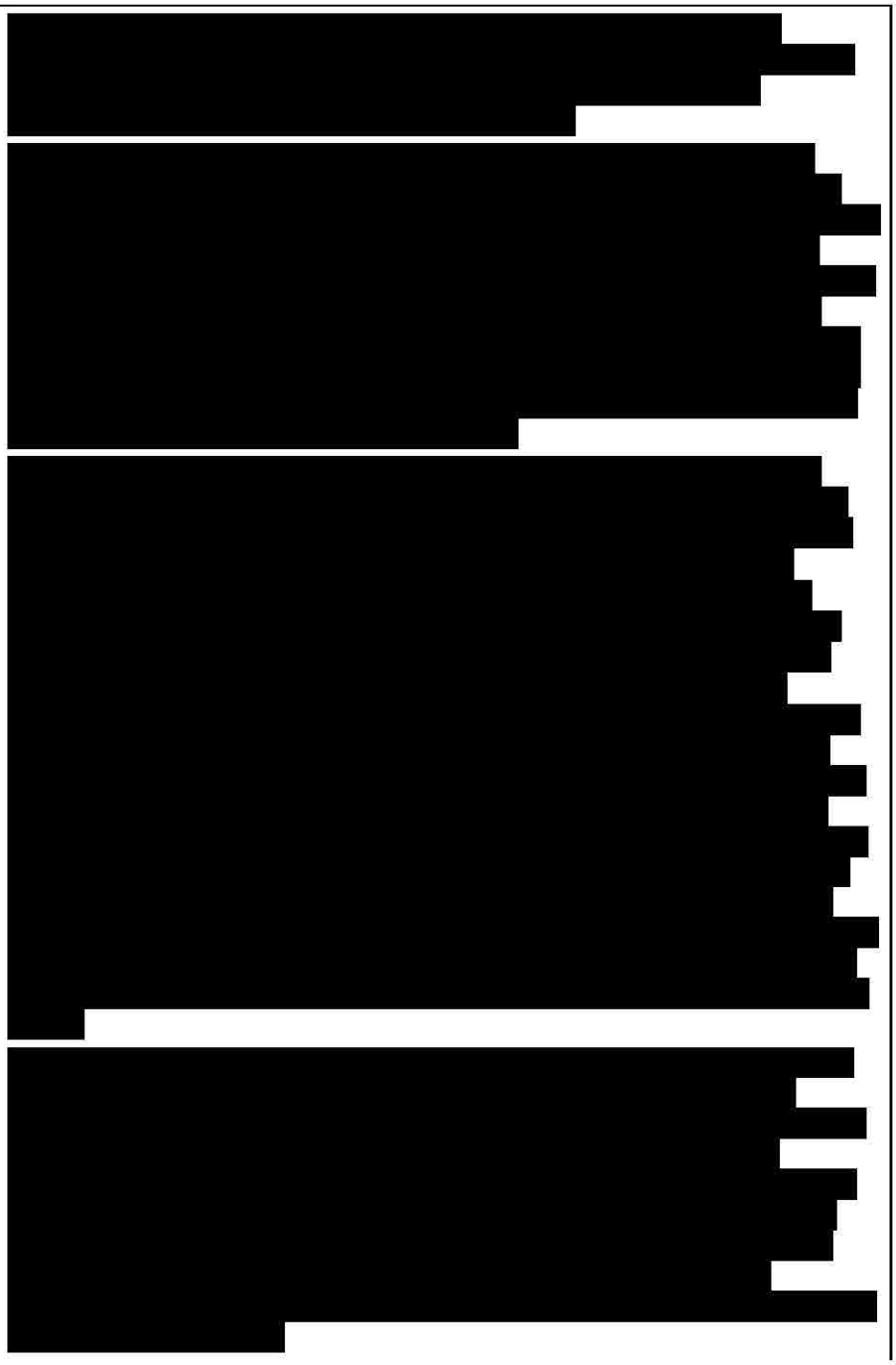
Date 2008/10/08

Materials and Methods [redacted]

Section A6.4.3/02 Repeated dose toxicity

Annex Point IIA6.4.3 13-Week Inhalation Toxicity Study with rats

Results and discussion



Section A6.4.3/02 Repeated dose toxicity

Annex Point IIA6.4.3 13-Week Inhalation Toxicity Study with rats

Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

	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	

Section A6.4.3/03 Repeated dose toxicity

Annex Point IIA6.4.3 13 Week Inhalation Toxicity Study with rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1991) Toxicity of isopropyl alcohol (IPA). Part 2. Repeated inhalation exposures in rats. [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 data	
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	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar	
3.2.3	Source	No data	
3.2.4	Sex	Male	X
3.2.5	Age/weight at study initiation	Ca. 8 weeks / no data	
3.2.6	Number of animals per group	6	X
3.2.7	Control animals	Yes (12)	X
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	90 days	X
3.3.2	Frequency of exposure	4 hrs/day on 5 days/week	X
3.3.3	Postexposure period	12 weeks	

Section A6.4.3/03 Repeated dose toxicity

Annex Point IIA6.4.3 13 Week Inhalation Toxicity Study with rats

3.3.4	**Inhalation**			
3.3.4.1	Concentrations	Nominal concentration	0, 400, 1000, 4000 or 8000 ppm over 12 weeks	
		Analytical concentration	No data	
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	No data		
3.3.4.5	Vehicle	Not applicable		X
3.3.4.6	Concentration in vehicle	Not applicable		X
3.3.4.7	Duration of exposure	4 hrs/day		X
3.3.4.8	Controls	Sham exposed		X
3.4	**Examinations**			
3.4.1	Observations			
3.4.1.1	Clinical signs	Yes		X
3.4.1.2	Mortality	Yes		X
3.4.2	Body weight	Yes		X
3.4.3	Food consumption	No data		
3.4.4	Water consumption	No data		
3.4.5	Ophthalmoscopic examination	No data		
3.4.6	Haematology	Yes		X
3.4.7	Clinical Chemistry	Yes		X
3.4.8	Urinalysis	No data		
3.5	**Sacrifice and pathology**			
3.5.1	Organ Weights	No data		
3.5.2	Gross and histopathology	No data		
3.5.3	Other examinations	No		X
3.5.4	Statistics	No data		
3.6	**Further remarks**			
		4 RESULTS AND DISCUSSION		
4.1	**Observations**			
4.1.1	Clinical signs	≥ 1000 ppm: marked local irritation (not further specified)		
4.1.2	Mortality	No		X
4.2	**Body weight gain**	≥ 1000 ppm: decreased body weight		

Section A6.4.3/03	Repeated dose toxicity
Annex Point IIA6.4.3	13 Week Inhalation Toxicity Study with rats

4.3	**Food consumption and compound intake**	No data
4.4	**Ophtalmoscopic examination**	No data
4.5	**Blood analysis**	
4.5.1	Haematology	≥ 4000 ppm: decrease in erythrocyte and haemoglobin values
4.5.2	Clinical chemistry	8000 ppm: increase in serum GOT and GPT, and total cholesterol
4.5.3	Urinalysis	No data
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No data
4.6.2	Gross and histopathology	No data
4.7	**Other**	No

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In this study male Wistar rats were exposed to 2-propanol concentrations of 0, 400, 1000, 4000 or 8000 ppm over 12 weeks.
5.2	**Results and discussion**	A concentration of ≥ 1000 ppm caused marked local irritation and a decrease in body weight. A decrease in erythrocyte and haemoglobin values was seen at ≥ 4000 ppm and at 8000 ppm an increase in serum GOT and GPT, and total cholesterol was noted.
5.3	**Conclusion**	
5.3.1	LO(A)EL	[redacted]
5.3.2	NO(A)EL	[redacted]
5.3.3	Other	
5.3.4	Reliability	[redacted]
5.3.5	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	2008/02/21
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	

Section A6.4. Annex Point IIA6.4.	Repeated dose toxicity in dogs (second species)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [x] Other justification [ ]	
References:		
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/01/30
Evaluation of applicant's justification	[redacted]
Conclusion	[redacted]
Remarks	[redacted]
	COMMENTS FROM OTHER MEMBER STATE (specify)
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.5/01 Repeated dose toxicity

Annex Point IIA6.5 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	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	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Inhalation	X
3.3.1	Duration of treatment	104 weeks interim sacrifice: 10 per sex and group at week 73	
3.3.2	Frequency of exposure	6 hrs/day on 5 days/week	

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	