Section A6.8.1/01 **Teratogenicity Study**

Annex Point IIA6.8.1 Developmental neurotoxicity after oral dosing in rats

3.4.5.4	Behavioural Testing	One female/male pair from each litter was assigned to each behavioural test on: motor activity (PND 13, 17, 21, 47 and 58) auditory startle (PND 22 and 60) learning and memory in an active avoidance test (daily on PND 60 to 64). *Motor activity* was assessed in a figure-eight maze for a continuous 1 hour period. Activity counts were summarized in 5 min blocks. *Auditory startle* was assessed by reaction to a short 120-dB tone and response was assessed as maximum amplitude and time latency to the maximum response (50 repetitions with 8 sec intervals in 5 blocks with ten tones). *Learning and memory* was assessed in an active avoidance test with an acoustic and optic stimulus followed by a mild electric current to a foot grid. After conditioning learning and memory was evaluated in number and times of avoidances and escapes.	
3.5	**Further remarks**	None	

4 RESULTS AND DISCUSSION

4.1 Maternal toxic Effects

One rat dosed with 1200 mg/kg bw died on PND 15. There were no other clinical observations or effects on maternal weight, food consumption or gestation length.

	Dose (mg/kg)			
	0	200	700	1200
Number mated	64	64	64	64
Number pregnant	34	35	31	35
Number pregnant that died	0	0	0	1
Number with live pups at birth	34	35	31	35
Number with usable litters	27	27	26	31

4.2 Teratogenic / embryotoxic effects

No adverse effects on pup survival, weight, sex ratio and sexual maturation. X

4.3 Other effects

There was no evidence of developmental neurotoxicity up to the highest dose tested.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In this study Sprague-Dawley rats were dosed with 0, 200, 700 or 1200 mg 2-propanol/kg bw/d on gestation day 6 through postnatal day 21. The method was according to Guidelines for developmental neurotoxicity studies (40 CFR 795.250)

5.2 Results and discussion

One of 35 pregnant animals (3 %) in the high dose group died, but there were no specific clinical signs of toxicity in the remaining animals. Neither in dams nor offspring adverse treatment related-findings were found.

5.3 Conclusion

[redacted]

Section A6.8.1/01 Teratogenicity Study

Annex Point IIA6.8.1 Developmental neurotoxicity after oral dosing in rats

5.3.1	LO(A)EL maternal toxic effects	[redacted]	
5.3.2	NO(A)EL maternal toxic effects	[redacted]	
5.3.3	LO(A)EL embryotoxic / teratogenic effects	[redacted]	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	[redacted]	X
5.3.5	Reliability	[redacted]	
5.3.6	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	2008/02/07
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*

Section A6.8.1/01	**Teratogenicity Study**
Annex Point IIA6.8.1	Developmental neurotoxicity after oral dosing in rats

Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.8.1/02 Teratogenicity Study

Annex Point IIA6.8.1 Exposure of rats via inhalation on days 1 - 19 of gestation

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1988) Teratogenicity of n-propanol and isopropanol administered at high inhalation concentrations to 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	
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	97.6 % (reagent grade)	
3.1.2.3	Stability	Samples were analysed for purity by GC- or IR-analysis.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	Charles River Breeding Laboratories, Wilmington, MA	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	No data / 200 - 300 g	
3.2.6	Number of animals per group	Control ..... 15 3500 ppm ..... 15 7000 ppm ..... 13 10000 ppm 15	X
3.2.7	Control animals	Yes	
3.2.8	Mating period	No data on time period, but mating was controlled by vaginal smear and examination for sperm plugs	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of exposure	Days 1 - 19 of gestation	
3.3.2	Postexposure period	1 day	

Section A6.8.1/02 Teratogenicity Study

Annex Point IIA6.8.1 Exposure of rats via inhalation on days 1 - 19 of gestation

		Inhalation			
3.3.3	Concentrations	Nominal concentrations: (given as	8750 mg /m³ 3500 ppm	17500mg/m³ 7000 ppm	25000 mg/m³ 10000 ppm)
		Analytical concentrations:	3510, 7042 or 10023 ppm (direct IR analysis)		
3.3.4	Type of exposure	Whole body			
3.3.5	Vehicle	None			
3.3.6	Exposure period / day	7 hrs / day			
3.3.7	Controls	Sham exposure			
3.4	**Examinations**				
3.4.1	Body weight	Yes (daily for the first week and weekly thereafter)			
3.4.2	Food consumption	Yes (weekly)			
3.4.3	Clinical signs	Yes (daily)			
3.4.4	Examination of uterine content	Number of corpora lutea Number of implantations Number of resorptions (early, middle, late)			
3.4.5	Examination of foetuses				
3.4.5.1	General	Number of live foetuses, foetal weight, sex ratio, external malformations			
3.4.5.2	Skelet	Skeletal malformations after evisceration and maceration and staining with Alizarin Red S			
3.4.5.3	Soft tissue	Visceral malformations after placement in Bouin's solution using the Wilson razor-blade cross-sectioning technique			
3.5	**Further remarks**	No water supply during inhalation period. Statistics: multivariate analysis (maternal data); non-parametric multivariate procedure (feed and water intake) followed where appropriate by a Kruskal-Wallis test with paired comparisons; Kruskal-Wallis test for group comparisons of numbers of corpora lutea; analysis of variance to compare foetal weights across groups by sex; Kruskal-Wallis test for group comparisons of litter size, percentage of liver foetuses/litter, percentage of normal foetuses/litter, and percentage of females/litter; Fisher's exact test for skeletal and visceral malformations and variations, external malformations and abnormal foetuses. The test results were adjusted for multiple comparisons using the Bonferroni technique.			
		4 **RESULTS AND DISCUSSION**			
4.1	**Maternal toxic Effects**	≥ 7000 ppm:	reduced feed intake and bodyweight gain		
		7000 ppm:	unsteady gait at the end of daily exposure (not noticeable after exposure for 19 days)		
		10000 ppm:	narcosis at the end of daily exposure (only slight effects after 19 days of exposure)		
4.2	**Teratogenic / embryotoxic effects**	≥ 3500 ppm:	significantly decreased foetal weights when compared with controls		
		≥ 7000 ppm:	significantly increased number of litters with malformations		

X

		10000 ppm: significantly increased resorptions	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In this study groups of 13 – 15 SD rats were exposed in 0.5 m^3 Hinner-type exposure chambers on gestational days 1-19 (7 hrs/day) to concentrations of 0, 3500, 7000 or 10000 ppm isopropanol. Dams were killed on gestational day 20 and offspring was examined for external and visceral malformations.	
5.2	**Results and discussion**	In dams concentrations of ≥ 7000 ppm caused a reduced feed intake and bodyweight gain. At 7000 ppm unsteady gait and at 10000 ppm narcosis were seen at the end of daily exposures. At ≥ 3500 ppm significantly decreased foetal weights were noted when compared with controls. At concentrations of ≥ 7000 ppm the number of litters with malformations was significantly increased and at 10000 ppm the resorptions also were significantly increased.	X
5.3	**Conclusion**		
5.3.1	LO(A)EL maternal toxic effects	[redacted]	X
5.3.2	NO(A)EL maternal toxic effects	[redacted]	X
5.3.3	LO(A)EL embryotoxic / teratogenic effects	[redacted]	X
5.3.4	NO(A)EL embryotoxic / teratogenic effects	[redacted]	X
5.3.5	Reliability	[redacted]	
5.3.6	Deficiencies	[redacted]	

Section A6.8.1/02	**Teratogenicity Study**
Annex Point IIA6.8.1	Exposure of rats via inhalation on days 1 - 19 of gestation

Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.8.1/02_01 Maternal effects

Parameter Historical data not given	0 ppp	3500 ppm	7000 ppm	10000 ppm
Number pregnant / number bred	15 /15	14 / 15	13 / 13	9 / 15
Clinical findings during application of test substance	None	None	Unsteady gait	Narcosis
Mortality of dams	None	None	None	None
Abortions	None	None	None	None
Body weight gain		→	↓	↓
Food consumption		→	↓	↓
Corpora lutea (mean no. per dam)	15.9	15.6	15.6	14.9
Implantations (mean no. per dam)	14.9	15.5	14.8	13.1*
Resorptions (% implants per litter)	6	4	7	59*
Mean foetal weight female	3.12	3.00*	2.63*	1.88*
male	3.27	3.13*	2.82*	1.89*

* = P ≤ 0.05

Table A6.8.1/02_02 Foetal effects

	0 ppm	3500 ppm	7000 ppm	10000 ppm
External malformations				
Number of litters (foetuses) examined	*15 (210)*	*14 (209)*	*13 (181)*	*5 (48)*
Short / missing tail	0 (0)	0 (0)	1 (1)	0 (0)
Total external malformations	0 (0)	0 (0)	1 (1)	0 (0)
Skeletal malformations				
Number of litters (foetuses) examined	*15 (103)*	*14 (104)*	*13 (89)*	*5 (24)*
Vertebral malformations (abnormal thoracic arches)	1 (1)	0 (0)	0 (0)	0 (0)
Rib malformations (rudimentary cervical)	2 (2)	3 (4)	11 (27)	5 (10)
Rib malformations (extra cervical)	0 (0)	0 (0)	2 (3)	0 (0)
Rib malformations (fused / wavy)	1 (1)	0 (0)	0 (0)	0 (0)
Rib malformations (missing)	1 (1)	1 (1)	2 (3)	1 (1)
Total skeletal malformations	3 (3)	4 (5)	11 (30)*	5 (11)*
Total skeletal variants	11 (36)	12 (39)	11 (37)	5 (20)
Visceral malformations				
Number of litters (foetuses) examined	*15 (103)*	*14 (104)*	*13 (89)*	*5 (24)*
Brain (encephalocoel)	0 (0)	0 (0)	0 (0)	1 (1)
Cardiovascular malformations (dextrocardia)	0 (0)	0 (0)	0 (0)	1 (1)
Urinary malformations (hydronephrosis)	0 (0)	0 (0)	1 (2)	0 (0)
Urinary malformations (hydroureter)	0 (0)	0 (0)	1 (1)	0 (0)
Total visceral malformations	0 (0)	0 (0)	2 (3)	2 (2)
Total visceral variants	6 (9)	6 (8)	9 (22)	4 (12)

* = P ≤ 0.05

Section A6.8.1/03 Teratogenicity Study
Annex Point IIA6.8.1 Oral dosing of rats on days 6 – 15 of gestation

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994) Developmental toxicity evaluation of isopropanol by gavage in rats and rabbits. [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	Isopropanol	
3.1.2.1	Description	Colourless liquid	
3.1.2.2	Purity	99.95 %	
3.1.2.3	Stability	The dosing solutions were determined to be homogeneous and stable for at least 49 days.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	CD (SD) outbreed albino	
3.2.3	Source	Charles River Laboratories, Inc., Raleigh (USA)	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	10 weeks / 214 – 275 g	
3.2.6	Number of animals per group	25	
3.2.7	Control animals	Yes	
3.2.8	Mating period	Bred in-house overnight (1:1) to mating colony males of the same strain from the same source	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of exposure	Days 6 – 15 post mating	
3.3.2	Postexposure period	5 days (euthanasia on gestational day 20)	

Section A6.8.1/03	Teratogenicity Study
Annex Point IIA6.8.1	Oral dosing of rats on days 6 – 15 of gestation

		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	0, 400, 800 or 1200 mg/kg bw
3.3.5	Vehicle	Distilled water
3.3.6	Concentration in vehicle	0, 80, 160 or 240 mg/mL
3.3.7	Total volume applied	5 mL/kg
3.3.8	Controls	Vehicle (distilled water)
3.4	**Examinations**	
3.4.1	Body weight	Yes On gestational days 0, 6, 9, 12, 15, 18 and 20
3.4.2	Food consumption	Yes On gestational days 0, 6, 9, 12, 15, 18 and 20
3.4.3	Clinical signs	Yes At least once daily prior to the dosing period (gestational days 0 – 5) and subsequent to the dosing period (gestational days 16 – 20). During the dosing period (gestational days 6 – 15) animals were examined twice daily, at dosing and 1 – 2 hrs post dosing.
3.4.4	Examination of uterine content	Uterine weights were recorded and any uteri with no visible implantation sites were stained with 10 % ammonium sulfide to visualize implantation sites which may have undergone very early resorptions. Ovarian corpora lutea were counted and the status of uterine implantation sites (i.e. total sites, resorptions, dead and live foetuses) was recorded.

Section A6.8.1/03 **Teratogenicity Study**
Annex Point IIA6.8.1 Oral dosing of rats on days 6 – 15 of gestation

3.4.5	Examination of foetuses	
3.4.5.1	General	Live foetuses were euthanized, weighed, sexed, and examined for external alterations including cleft palate.
3.4.5.2	Skelet	All foetuses were eviscerated, macerated, and stained with alizarin red S (for ossified skeletal districts) and Alcian blue (for cartilaginous skeletal districts). Intact foetuses (50 % per litter) were examined for skeletal alterations. The designation of foetal alterations as malformations or variations was based on the literature and on historical control data.
3.4.5.3	Soft tissue	50 % of the foetuses per litter were decapitated and examined for visceral alterations by a longitudinal dissection method. Foetal heads were decalcified in Bouin's fixative, free-hand serially sectioned, and examined for soft tissue craniofacial alterations.
3.5	**Further remarks**	In a range-finding study 12 sperm-positive dams per group were dosed with 0, 625, 1250 or 2500 mg/kg/day. All dams at 2500 mg/kg/day died or were euthanized moribund by gestational day 13. Two dams died at 1250 mg/kg/day. Also at 1250 mg/kg/day, dams exhibited reduced body weights and weight gain, reduced food consumption, and treatment-related clinical signs of toxicity. At 1250 mg/kg/day, there was a significant decrease in foetal body weight per litter unaccompanied by any external malformations or variations. There was no maternal or developmental toxicity observed at 625 mg/kg/day. Thoracic and abdominal organs and cavities were examined and maternal body, liver, and uterine weights were recorded. Statistics: Parametric statistical procedures were applied to selected measures from this toxicity study. Appropriate general linear models (GLM) procedures (SAS) for the proposed analyses of variance (ANOVA) were employed. Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data and Bartlett's test for homogeneity of variance (alpha level = 0.001) was performed on all data to be analyzed by ANOVA. GLM analysis was used to determine the significance of the dose-response relationship (test for linear trend), and to determine whether significant dose effects had occurred for selected measures (ANOVA). When a significant ($p < 0.05$) main effect for dose occurred, Williams' multiple comparison test and/or Dunnett's multiple comparison test was used to compare each chemical exposed group to the vehicle control group for that measure. A one-tailed test (i.e. Williams' test and/or Dunnett's test) was used for all pair wise comparisons except that a two-tailed test was used for maternal body and organ weight parameters, maternal food consumption, foetal body weight, and percentage males per litter. Nominal scale measures were analyzed by χ^2 test for independence for differences among treatment groups, and by a test for linear trend on proportions. When χ^2 revealed significant ($p < 0.05$) differences among groups, then a one-tailed Fisher's exact probability test was used for pair wise comparisons between each treated group and the vehicle control group.

Section A6.8.1/03 **Teratogenicity Study**
Annex Point IIA6.8.1 Oral dosing of rats on days 6 – 15 of gestation

4 **RESULTS AND DISCUSSION**

4.1 Maternal toxic Effects

Pregnancy rate was high and approximately equivalent across all groups (92 – 100 %). No dams aborted, delivered early, or were removed from the study. 4 females were non-pregnant: two at 0 mg/kg and one each at 800 and 1200 mg/kg.
Mortality:
800 mg/kg: 1 dam died on gestational day 16
1200 mg/kg: 2 dams died on gestational days 16 and 18

Maternal body weights were statistically equivalent across all groups for all time points measured. Maternal weight change was statistically significantly reduced at 1200 mg/kg only for the gestational period, gestational days 0 – 20 (158 vs. 142 g; 89.9 % of control value). This was attributed to a reduction in foetal body weights, as corrected gestational weight change (weight gain through gestation minus gravid uterine weight) was statistically equivalent across all groups. However, the mean value at this dose level was 90.8 % of the control value.

Mean maternal gravid uterine weight was also significantly reduced at 1200 mg/kg: 86 vs. 77 g (89.5 % of control value).

There were no adverse clinical signs as well as no adverse effects on food consumption, liver weights and absolute / relative body weights at any dose level. In addition no necropsy observations appeared treatment related at scheduled euthanization on gestational day 20.

4.2 Teratogenic / embryotoxic effects

All pregnant animals had one or more live foetuses at scheduled euthanization (i.e. there were no fully resorbed litters). The numbers of litters evaluated were 23, 25, 23, and 22 at 0, 400, 800, and 1200 mg/kg, respectively. There were no adverse effects on gestational parameters such as number of ovarian corpora lutea/dam, number of total, non-live (resorptions, dead foetuses, total), live implants (foetuses)/litter as well as pre- and post-implantation loss. There were no late foetal deaths. Number and sex ratio of live foetuses per litter were also equivalent, although the percentage of male foetuses per litter was statistically significantly increased at 800 mg/kg (most probably due to biologic variation with no dose-related pattern). At ≥ 800 mg/kg foetal body weights per litter were statistically significantly reduced: for all foetuses and males and females separately the values at 800 mg/kg were 97.3, 94.7 and 94.3 % of control values and at 1200 mg/kg/day the values were 92.1, 91.9 and 95.4 % of control values.

There were no treatment-related changes in incidence of external, visceral, skeletal, or total malformations or total variations when examined by number of foetuses, number of foetuses per litter, number of litters with one or more affected foetuses, or by percentage of males or females malformed per litter. The lack of any treatment-related statistically significant changes in malformation rate was observed in the unexpected absence of any control male foetuses with malformations (0 % male foetuses malformed per litter; the historical control mean value for this parameter based on 534 litters was 2.4 %). At 400 mg/kg one foetus showed all of the external malformations observed in this group (types of malformations observed are a constellation of spontaneous findings occasionally noted in term Sprague-Dawley rat foetuses) and two foetuses at 800 mg/kg showed external malformations. Visceral malformations were seen in 1, 5, 0, and 4 foetuses, while skeletal malformations were observed in 0, 2, 1, and 1 foetuses at 0, 400, 800 and 1200 mg/kg. Foetal variations (visceral and skeletal, none were observed externally) were distributed across all groups with no treatment-related

patterns of incidence or severity.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In this study groups of 25 CD rats were dosed orally with 0, 400, 800 or 1200 mg/kg/day on gestational days 6 – 15. Maternal body weights, clinical observations and food consumption were recorded throughout gestation. The dams were euthanized on gestational day 20 and foetuses were weighed, sexed, and examined for external, visceral (including craniofacial) and skeletal alterations.

5.2 Results and discussion

The pregnancy rate was high and equivalent across all groups. There were no abortions or early deliveries and no dams were removed from study. The mortality rate was 4 and 8 % of the dams at 800 or 1200 mg/kg bw, respectively. Maternal body weights and weight gain were equivalent across all groups, except for statistically significantly reduced gestational weight gain (gestational days 0 – 20; 89.9 % of control value), associated with statistically significantly reduced gravid uterine weights at 1200 mg/kg bw (89.5 % of control value). Treatment-related clinical signs or effects on maternal food consumption were not seen and all gestational parameters such as pre- and post-implantation loss, foetal sex ratios and litter size were equivalent across all groups (22 – 25 litters were examined per group). Foetal body weights per litter were statistically significantly reduced at 800 and 1200 mg/kg bw: 97.3/94.7/94.3 % of controls and 92.1/91.9/ 95.4 % of controls (all foetuses/males/females). There was no evidence of increased teratogenicity at any dose level tested.

5.3 Conclusion

5.3.1 LO(A)EL maternal toxic effects [redacted]

5.3.2 NO(A)EL maternal toxic effects [redacted]

5.3.3 LO(A)EL embryotoxic / teratogenic effects [redacted]

5.3.4 NO(A)EL embryotoxic / teratogenic effects [redacted]

5.3.5 Reliability [redacted]

5.3.6 Deficiencies [redacted]

Section A6.8.1/03 **Teratogenicity Study**

Annex Point IIA6.8.1 Oral dosing of rats on days 6 – 15 of gestation

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/02/05
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 A6.8.1/03_01 Maternal effects

	Dose (mg/kg/day)			
	0	**400**	**800**	**1200**
All litters (n)	(23)	(25)	(23)	(22)
Corpora lutea per dam	14.9	15.4	14.6	14.4
Implantation sites per litter	14.4	14.9	14.2	14.1
Percentage pre-implantation loss	4.0	4.2	4.0	2.9
Percentage resorptions per litter	1.5	1.4	1.8	4.1
No. (%) litters with resorptions	5 (21.7)	5 (20.0)	3 (13.0)	9 (40.9)
Percentage late foetal deaths per litter	0	0	0	0
No. litters with late foetal deaths	0	0	0	0
Percentage adversely affected implants per litter	1.8	3.8	2.8	5.7
No. (%) litters with adversely affected implants	6 (26.1)	9 (36.0)	6 (26.1)	13 (59.1)
Live litters (n)	(23)	(25)	(23)	(22)
Live foetuses per litter	14.0	14.7	13.9	13.5
Percentage male fetuses per litter	44.4	50.2	56.0**	46.9
Average foetal body weight per litter				
All foetuses	3.866	3.794	3.682	3.559**
Male foetuses	3.972	3.875	3.762*	3.649**
Female foetuses	3.971	3.717	3.574*	3.487**

* $p < 0.05$ (Dunnett's test) ** $p < 0.01$ (Dunnett's test)

Table A6.8.1/03_02 Foetal effects

	Dose (mg/kg/day)			
	0	**400**	**800**	**1200**
External malformations				
No. foetuses examined	326	367	320	298
No. litters examined	23	25	23	22
Percentage foetuses with external malformations per litter	0.0	0.3	0.7	0.0
No. (%) litters with external malformations	0 (0.0)	1 (4.0)	2 (8.7)	0 (0.0)
Visceral malformations				
No. foetuses examined	159	184	161	153
No. litters examined	23	25	23	22
Percentage foetuses with visceral malformations per litter	0.6	3.2	0.0	2.5
No. (%) litters with visceral malformations	1 (4.3)	5 (20.0)	0 (0.0)	4 (18.2)
Skeletal malformations				
No. foetuses examined	165	183	159	145
No. litters examined	23	25	23	22
Percentage foetuses with skeletal malformations per litter	0.0	1.1	0.7	0.6
No. (%) litters with skeletal malformations	0 (0.0)	2 (8.0)	1 (4.3)	1 (4.5)
All malformations				
No. foetuses examined				

No. litters examined	326	367	320	298
Percentage foetuses malformed per litter	23 0.3	25 2.5	23 1.0	22 1.6
No. (%) foetuses malformed				
No. (%) litters with malformations	1 (0.3)	9 (2.5)	3 (0.9)	5 (1.7)
Percentage males malformed per litter	1 (4.3) 0.0	7 (28.0) 2.3	3 (13.0) 0.5	5 (22.7) 1.4
Percentage females malformed per litter	0.4	2.6	1.7	1.7
All variations				
No. foetuses examined	326	367	320	298
No. litters examined	23	25	23	22
Percentage foetuses with variations per litter	4.9	7.5	5.4	6.5
No. (%) foetuses with variations	15 (4.6)	27 (7.4)	18 (5.6)	19 (6.4)
No. (%) litters with variations	10 (43.5)	15 (60.0)	9 (39.1)	12 (54.5)

Section A6.8.1/04 Teratogenicity Study

Annex Point IIA6.8.1 Oral dosing of rabbits on days 6 – 18 of gestation

			Official use only
		1 **REFERENCE**	
1.1	**Reference**	[redacted] (1994) Developmental toxicity evaluation of isopropanol by gavage in rats and rabbits. [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	Isopropanol	
3.1.2.1	Description	Colourless liquid	
3.1.2.2	Purity	99.95 %	
3.1.2.3	Stability	The dosing solutions were determined to be homogeneous and stable for at least 49 days.	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	NZW	
3.2.3	Source	Hazleton Research Products, Inc., Denver, PA	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	5.5 months / 2750 – 3800 g	
3.2.6	Number of animals per group	15	
3.2.7	Control animals	Yes	
3.2.8	Mating period	The animals were artificially inseminated in-house using semen from breeding colony males of the same strain from the same source	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of exposure	Gestational days 6 – 18	
3.3.2	Postexposure period	12 days (euthanasia on gestational day 30)	

Section A6.8.1/04 Teratogenicity Study

Annex Point IIA6.8.1 Oral dosing of rabbits on days 6 – 18 of gestation

		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	0, 120, 240 or 480 mg/kg bw
3.3.5	Vehicle	Distilled water
3.3.6	Concentration in vehicle	0, 60, 120 or 240 mg/mL
3.3.7	Total volume applied	2 mL/kg
3.3.8	Controls	Vehicle (distilled water)
3.4	**Examinations**	
3.4.1	Body weight	Yes On gestational days 0, 6, 9, 12, 15, 18, 21, 24 and 30
3.4.2	Food consumption	Yes On gestational days 0, 6, 9, 12, 15, 18, 21, 24 and 30
3.4.3	Clinical signs	Yes At least once daily prior to the dosing period (gestational days 0 – 5) and subsequent to the dosing period (gestational days 19 – 30). During the dosing period (gestational days 6 – 18) animals were examined twice daily, at dosing and 1 – 2 hrs post dosing.
3.4.4	Examination of uterine content	Uterine weights were recorded and any uteri with no visible implantation sites were stained with 10 % ammonium sulfide to visualize implantation sites which may have undergone very early resorptions. Ovarian corpora lutea were counted and the status of uterine implantation sites (i.e. total sites, resorptions, dead and live foetuses) was recorded.

Section A6.8.1/04 Teratogenicity Study

Annex Point IIA6.8.1 Oral dosing of rabbits on days 6 – 18 of gestation

3.4.5	Examination of foetuses	
3.4.5.1	General	Live foetuses were euthanized, weighed and examined for external alterations
3.4.5.2	Skelet	All foetuses were eviscerated, macerated, stained with alizarin red S (for ossified skeletal districts) and Alcian blue (for cartilaginous skeletal districts) and were examined for skeletal alterations. The designation of foetal alterations as malformations or variations was based on the literature and on historical control data.
3.4.5.3	Soft tissue	All foetuses per litter were sexed internally and examined for visceral alterations by longitudinal dissection and 50 % per litter were decapitated. Foetal heads were decalcified in Bouin's fixative, free-hand serially sectioned, and examined for soft tissue craniofacial alterations.
3.5	**Further remarks**	In a range-finding study 10 inseminated does per group were dosed with 0, 312.5, 625 or 1250 mg/kg/day. All does at 1250 mg/kg/day died or were euthanized moribund by gestational day 8. Seven does at 625 mg/kg/day died or were euthanized moribund by gestational day 12. There was no observable maternal or developmental toxicity at 312.5 mg/kg/day. Thoracic and abdominal organs and cavities were examined and maternal body, liver, and uterine weights were recorded. Statistics: Parametric statistical procedures were applied to selected measures from this toxicity study. Appropriate general linear models (GLM) procedures (SAS) for the proposed analyses of variance (ANOVA) were employed. Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data and Bartlett's test for homogeneity of variance (alpha level = 0.001) was performed on all data to be analyzed by ANOVA. GLM analysis was used to determine the significance of the dose-response relationship (test for linear trend), and to determine whether significant dose effects had occurred for selected measures (ANOVA). When a significant ($p < 0.05$) main effect for dose occurred, Williams' multiple comparison test and/or Dunnett's multiple comparison test was used to compare each chemical exposed group to the vehicle control group for that measure. A one-tailed test (i.e. Williams' test and/or Dunnett's test) was used for all pair wise comparisons except that a two-tailed test was used for maternal body and organ weight parameters, maternal food consumption, foetal body weight, and percentage males per litter. Nominal scale measures were analyzed by χ^2 test for independence for differences among treatment groups, and by a test for linear trend on proportions. When χ^2 revealed significant ($p < 0.05$) differences among groups, then a one-tailed Fisher's exact probability test was used for pair wise comparisons between each treated group and the vehicle control group.

Section A6.8.1/04 **Teratogenicity Study**

Annex Point IIA6.8.1 Oral dosing of rabbits on days 6 – 18 of gestation

4 **RESULTS AND DISCUSSION**

4.1 Maternal toxic Effects

Pregnancy rate was high and equivalent across all groups (86.7 – 100 %). No does aborted, delivered early, or were removed from study. Four does died at 480 mg/kg/day (4/15; 26.7 %): one on gestational day 11, one on gestational day 12 (sacrificed moribund), and two on gestational days 19. All four does were pregnant. All deaths occurred during or immediately after the dosing period and were considered treatment related. Only two females were non-pregnant (96.7 % pregnancy rate), both at 120 mg/kg/day. Maternal body weights were statistically equivalent across all groups for all time points measured, although the mean values at 480 mg/kg/day were substantially lower than those in all other groups from the onset of dosing until sacrifice. Maternal body weight change was statistically significantly reduced at 480 mg/kg/day for gestational days 6 – 18 (187 vs. 85 g; 45.4 % of control value) associated with reduced maternal food consumption during this period. Corrected maternal gestational weight change (gestational weight change minus gravid uterine weight) was substantially reduced at 480 mg/kg/day (-124.6 g) relative to the control value (-0.9 g), but the difference was not statistically significant due to the large variation typical of rabbits. Gravid uterine weights and absolute and relative liver weights were equivalent across all groups. Maternal food consumption was statistically significantly reduced at 480 mg/kg/day for gestational days 6 – 9 (86.5 and 86.3 % of controls), 9 – 12 (75.4 and 77.2 % of controls), 12 – 15 (75.5 and 77.2 % of controls) and 6 – 18 (80.4 and 81.6 % of controls). There was also a significant downward trend for gestational days 15 – 18, but the pair wise comparisons to the concurrent control group were not significantly different.
Clinical findings were observed almost exclusively at 480 mg/kg/day and included flushed and/or warm ears in one to five does early in the dosing period (gestational days 6 – 9). Apparent rupture of peripheral capillaries in the ear(s) was observed in one doe on gestational days 13 and 14 at 240 mg/kg/day. Cyanosis, lethargy, laboured respiration, and diarrhoea were also noted in one to two does at 480 mg/kg/day. Clinical weight loss (defined as > 150 g in a weigh period) was observed in two does at 480 mg/kg/day, and in one doe at 120 mg/kg/day. Perinasal wetness was observed in two does at 120 mg/kg/day, and in two does at 480 mg/kg/day; perioral wetness was observed in one doe at 480 mg/kg/day. The clinical signs were clearly treatment related only at 480 mg/kg/day, while at lower doses these signs were transient, relatively minor, and predominantly nonspecific indicators of stress. There were no treatment-related findings at scheduled necropsy on gestational day 30.

4.2 Teratogenic / embryotoxic effects

All pregnant does had one or more live foetuses at scheduled sacrifice (i.e. there were no fully resorbed litters). The numbers of litters evaluated were 15, 13, 15, and 11 at 0, 120, 240 and 480 mg/kg/day. There were no significant effects of treatment on any gestational parameters such as number of ovarian corpora lutea per doe, number of total, non-live, or live implantations per litter, or in pre- or post-implantation loss or in litter size (number of live foetuses per litter). The sex ratios were also equivalent across groups. Foetal body weights per litter were statistically equivalent across all groups, although the mean weights per litter for all foetuses, males, or females were slightly reduced at 480 mg/kg/day: 93.5, 92.7 and 86.0 % of control values. The female foetal weights decreased with increasing doses but were not significantly different from the control value in any dose group. Analyses of foetal findings indicated no treatment-related changes in incidence of external, visceral, skeletal, or

total malformations or total variations. One foetus at 480 mg/kg/day exhibited all of the external malformations observed in this group, while no external malformations were seen at ≤ 240 mg/kg/day. Visceral malformations were observed in 3, 5, 5, and 5 foetuses, and skeletal malformations were observed in 1,0, 4, and 0 foetuses at 0, 120, 240 and 480 mg/kg/day. Most foetal variations were visceral or skeletal; only one, at 240 mg/kg/day, was observed externally. These variations were distributed across all groups with no treatment-related patterns of incidence or severity.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In this study groups of 15 NZW rabbits rats were dosed orally with 0, 120, 240 or 480 mg/kg/day on gestational days 6 – 18. Maternal body weights, clinical observations and food consumption were recorded throughout gestation. The does were euthanized on gestational day 30 and foetuses were weighed, sexed, and examined for external, visceral and skeletal alterations.

5.2 Results and discussion

The pregnancy rate was high and equivalent across all groups. There were no abortions or early deliveries and no does were removed from study. At 480 mg/kg four animals (26.7 %) died and maternal body weights were statistically significantly reduced during treatment (45.4 % of control value) with a non-significant reduction in gestational weight change (gestation days 0 – 30; 77.3 % of control value). There were also profound clinical signs of toxicity and statistically significantly reduced maternal food consumption. All gestational parameters were equivalent in all dose groups. 13 – 15 litters were evaluated per group except for the 480 mg/kg (11 litters due to maternal deaths). There were no treatment-related effects on pre- or post-implantation loss, foetal sex ratio, litter size, or foetal body weight/litter and there was also no evidence of increased teratogenicity at any dose level.

5.3 Conclusion

5.3.1 LO(A)EL maternal toxic effects [redacted]

5.3.2 NO(A)EL maternal toxic effects [redacted]

5.3.3 LO(A)EL embryotoxic / teratogenic effects [redacted]

5.3.4 NO(A)EL embryotoxic / teratogenic effects [redacted]

5.3.5 Reliability [redacted]

5.3.6 Deficiencies [redacted]

Section A6.8.1/04 Teratogenicity Study

Annex Point IIA6.8.1 Oral dosing of rabbits on days 6 – 18 of gestation

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/02/05
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 A6.8.1/04_01 Maternal effects

	Dose (mg/kg/day)			
	0	**120**	**240**	**480**
All litters (n)	(15)	(13)	(15)	(11)
Corpora lutea per doe	9.5	10.5	10.7	10.7
Implantation sites per litter	7.5	7.8	8.8	8.5
Percentage pre-implantation loss	21.4	23.5	17.5	22.0
Percentage resorptions per litter	6.6	7.1	9.3	7.1
No. (%) litters with resorptions	5 (33.3)	6 (46.2)	7 (46.7)	4 (36.4)
Percentage late foetal deaths per litter	0	0	0.7	0
No. (%) litters with late foetal deaths	0	0	1 (6.7)	0
Percentage non-live implants per litter	6.6	7.1	10	7.1
No. (%) litters with non-live implants	5 (33.3)	6 (46.2)	7 (46.7)	4 (36.4)
Percentage adversely affected implants per litter	9.5	12	15.6	12.3
No. (%) litters with adversely affected implants	8 (53.3)	10 (76.9)	10 (66.7)	6 (54.6)
Live litters (n)	(15)	(13)	(15)	(11)
Live foetuses per litter	7	7.3	7.9	8.2
Percentage male foetuses per litter	50.3	47.3	45.4	53.1
Average foetal body weight per litter All foetuses Male foetuses Female foetuses	 49.71 49.68 49.75	 49.71 50.42 48.68	 47.92 48.99 46.65	 46.48 46.04 42.79

Table A6.8.1/04_02 Foetal effects

	Dose (mg/kg/day)			
	0	**120**	**240**	**480**
No. foetuses examined	105	95	119	90
No. litters examined	15	13	15	11
External malformations Percentage foetuses with external malformations per litter No. (%) litters with external malformations	 0.0 0 (0.0)	 0.0 0 (0.0)	 0.0 0 (0.0)	 0.9 1 (9.1)
Visceral malformations Percentage foetuses with visceral malformations per litter No. (%) litters with visceral malformations	 2.2 3 (20)	 5.1 5 (38.5)	 3.7 4 (26.7)	 4.5 3 (27.3)
Skeletal malformations Percentage foetuses with skeletal malformations per litter No. (%) litters with skeletal malformations	 0.8 1 (6.7)	 0.0 0 (0.0)	 2.8 2 (13.3)	 0.0 0 (0.0)
All malformations Percentage foetuses malformed per litter No. (%) foetuses malformed No. (%) litters with malformations	 3.1 4 (3.8) 4 (26.7)	 5.1 5 (5.3) 5 (38.5)	 5.8 8 (6.7) 5 (33.3)	 5.4 6 (6.7) 3 (27.3)
All variations Percentage foetuses with variations per litter No. (%) foetuses with variations No. (%) litters with variations	 65.5 65 (61.9) 15 (100)	 67.1 65 (68.4) 13 (100)	 53.4 66 (55.5) 14 (93.3)	 80.2 69 (76.7) 11 (100)

Section A6.8.2/01 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 Two-generation study with rats (oral application)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1995) Two-generation reproduction toxicity study with isopropanol 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	
		GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	Not applicable	
		MATERIALS AND METHODS	
3.1	**Test material**	Propan-2-ol	
3.1.1	Lot/Batch number	Not available	
3.1.2	Specification	Anhydrous isopropanol	
3.1.3	Description	Colourless liquid	
3.1.4	Purity	99.9 %	
3.1.5	Stability	Before starting the study, dosing solutions were analyzed for stability and analyses for concentration were conducted periodically throughout the study. Pre-study analysis of dosing solutions demonstrated stability for up to 28 days under refrigeration and pre- and post-study analyses confirmed the identity of the test substance as well as the stability of the undiluted test material.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley (Crl:CD® BR VAF/Plus®)	
3.2.3	Source	Charles River Breeding Laboratories, Kingston, NY	
3.2.4	Sex	Male / Female	
3.2.5	Age/weight at study initiation	No data / no data	
3.2.6	Number of animals per group	P_1: 30 of each sex	
3.2.7	Mating	During the mating period, one male rat was co-housed with one female selected randomly within the same treatment group for a maximum of 7 days. After 7 days, the females of those pairs that were not confirmed as having mated were placed with males of other unmated pairs within the treatment group. The same procedure was repeated for a third 7-day interval for unmated females. Mating was confirmed by observation of a copulatory plug or by the presence of sperm in a vaginal rinse.	
3.2.8	Duration of mating	1 week	X
3.2.9	Deviations from standard protocol	Not applicable	

Section A6.8.2/01 **Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2 Two-generation study with rats (oral application)

3.2.10	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Animal assignment to dosage groups	P_1: animals were allocated randomly to groups by a computer-generated program to equalize initial group mean body weights Offspring of the P_1 generation were designated as the F_1 generation. At weaning on postnatal day 21 (PND 21), two pups of each sex per litter were selected at random to become a pool of animals from which the P_2 adult generation would be chosen for each treatment group. The selected P_2 populations consisted of 30 neonates of each sex from the control, 100 and 500 mg/kg groups, while the selected P_2 population of the 1000 mg/kg group consisted of 26 neonates of each sex due to increased mortality during the early post-weaning period. Neonates began receiving treatment on PND 21. Procedures for the treatment, mating and disposition of the P_2 generation were as described for the P_1 generation. Sibling matings were avoided.
3.3.2	Duration of exposure before mating	At least 10 weeks
3.3.3	Duration of exposure in general P, F_1, F_2 males, females	P_1 females: at least 10 weeks prior to mating, continued through mating, gestation, lactation until the day before euthanasia P_1 males: at least 10 weeks prior to mating until the day before euthanasia, following delivery of the last litter they sired F_1: neonates treatment started on PND 21, continued as P_1
		Oral
3.3.4	Type	Gavage
3.3.5	Concentration	0, 100, 500 or 1000 mg/kg bw
3.3.6	Vehicle	Reverse osmosis water
3.3.7	Concentration in vehicle	0, 20, 100 or 200 mg/ml
3.3.8	Total volume applied	5 ml/kg bw
3.3.9	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Clinical signs	Parentals: At least once daily and detailed clinical examinations were performed weekly. In addition, clinical examinations of mated females were performed on GDs 0, 7, 14 and 2l and on PPDs 0, 4, 7, 14 and 21. Litters: Twice daily for general appearance of the pups and for dead offspring. On PNDs 0, 1, 4, 7, 14 and 21, the number of offspring were counted, each live animal was sexed and examined externally for anomalies

Section A6.8.2/01		Multigeneration Reproduction Toxicity Study	
Annex Point IIA6.8.2		Two-generation study with rats (oral application)	
3.4.2	Body weight	Parentals: males: weekly females: weekly before mating, on GDs 0, 7, 14 and 21 and on PPDs 0, 4, 7, 14 and 21. Litters: PNDs 0, 1, 4, 7, 14 and 21	
3.4.3	Food/water consumption	Food consumption: Measured on a similar schedule as body weight; an additional food consumption measurement was performed on PPD 10 for the P_2 generation in order to accommodate normally high maternal food consumption during the PPD 7 - 14 interval. Water consumption: no data	
3.4.4	Oestrus cycle	No data	
3.4.5	Sperm parameters	No data	
3.4.6	Offspring	Twice daily for general appearance of the pups and for dead offspring. On PNDs 0, 1, 4, 7, 14 and 21, the number of offspring were counted, each live animal was sexed and examined externally for anomalies	
3.4.7	Gross post-mortem	On all adult animals used for mating, including those that died spontaneously or were euthanized in moribund condition	
3.4.8	Organ weights	Liver and kidney weights were recorded for all mated adults that survived to scheduled termination	
3.4.9	Microscopic Examination	Pituitary, testes and epididymides, prostate and seminal vesicles, vagina, uterus, ovaries and gross lesions were examined microscopically for all parental animals in the control and 1000 mg/kg groups	
3.4.10	Histopathology P_1 and P_2 animals	Liver and kidneys	
3.4.11	Histopathology F_1 not selected for mating, F_2	No data	

Section A6.8.2/01	Multigeneration Reproduction Toxicity Study	
Annex Point IIA6.8.2	Two-generation study with rats (oral application)	
3.5 Further remarks	On PND 4, after counting, weighing and examining the offspring, the size of each litter was reduced by random selection to yield, as nearly as possible, four male and four female pups per litter. Culled pups were euthanized and given an external examination. Culled pups that appeared normal were discarded without further examination, while those that appeared abnormal were given a gross post-mortem examination. On PND 21, five male and five female pups from each treatment group were selected randomly for a gross post-mortem examination. Statistical methods: Adult body weight, food consumption, organ weight and relative organ weight means were analyzed in the following way. If the variances were homogeneous by Bartlett's test at the 1 % level, a set of parametric analyses was performed, otherwise the analyses were non-parametric. The parametric analyses were the Standard one-way ANOVA, with Dunnett's test if the ANOVA indicated that there were differences in means, followed by linear regression with a test for lack of fit. The corresponding non-parametric tests were the Kruskal-Wallis test, Dunn's Rank Sum test and Jonckheere's test for ordered response (no tests for lack of fit). Reproduction, offspring survival and histopathological parameters were analyzed by a Standard chi-squared test followed by Armitage's test for trend if the expected values were greater than five. If the chi-squared test was significant at the 5 % level (or could not be performed), each treatment group was compared to the control using the 2*2 Fisher Exact Test. Male and female offspring weights were analyzed separately by nested analyses of covariance, with pups nested within dams, dams nested with dose and total litter size as the covariate. The least significant difference test was used to determine group differences when the overall analysis was statistically significant.	
	4 **RESULTS AND DISCUSSION**	
4.1 Effects		
4.1.1 P_1 males	≥ 500 mg/kg: histopathological effects in the kidneys: increased number of hyaline droplets in the epithelial cells of the proximal convoluted tubules, increased incidence and severity of epithelial degeneration and hyperplasia, increased incidence of proteinaceous casts in the renal tubules and increased incidence of focal interstitial mononuclear cell infiltration 1000 mg/kg: increased absolute and relative liver weights	
4.1.2 P_1 females	≥ 500 mg/kg: increased relative liver weights 1000 mg/kg: two deaths increased body weight gain during the overall postpartum period (40.4 g vs. 15.1 g) increased relative kidney weights	

Section A6.8.2/01	Multigeneration Reproduction Toxicity Study	
Annex Point IIA6.8.2	Two-generation study with rats (oral application)	
4.1.3	P_2 males	100 mg/kg: two deaths ≥ 100 mg/kg: histopathological effects in the kidneys: increased number of hyaline droplets in the epithelial cells of the proximal convoluted tubules, increased incidence and severity of epithelial degeneration and hyperplasia, increased incidence of proteinaceous casts in the renal tubules and increased incidence of focal interstitial mononuclear cell infiltration 500 mg/kg: increased absolute liver weights ≥ 500 mg/kg: increased relative liver weights 1000 mg/kg: increased relative kidney weights centrilobular hepatocyte hypertrophy in 6/26 rats decrease in mating index
4.1.4	P_2 females	500 mg/kg: one death ≥ 500 mg/kg: increased body weight gain during the overall postpartum period (19.2 g and 25.3 g vs. 3.1 g) increased relative liver weights 1000 mg/kg: two deaths increased absolute liver weights increased relative kidney weights
4.1.5	F_1 males and females	100 mg/kg: one death during post-weaning period (PNDs 21 - 41) prior to selection of the P2 generation 500 mg/kg: one death during post-weaning period (PNDs 21 - 41) prior to selection of the P2 generation 1000 mg: 18 offspring deaths during post-weaning period (PNDs 21 - 41) prior to selection of the P2 generation slight decrease in survival during PND 0 - 4 interval compared with controls (statistically significantly reduced PND 1 survival index) significantly decreased male body weights on PNDs 0 and 1 (as several higher offspring body weights were observed in the treated groups compared with controls during the study, these were considered incidental and unrelated to treatment)
4.1.6	F_2 males and females	1000 mg: significantly decreased body weights in males and females on PNDs 0, 1 and 4 when compared with controls (as several higher offspring body weights were observed in the treated groups compared with controls during the study, these were considered incidental and unrelated to treatment)
4.2 Other		No

Section A6.8.2/01	Multigeneration Reproduction Toxicity Study	
Annex Point IIA6.8.2	Two-generation study with rats (oral application)	
	APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	In this two-generation reproduction toxicity study, 30 rats of each sex per group (P_1) were dosed once daily by oral gavage with 0, 100, 500 or 1000 mg isopropanol/kg bw for at least 10 weeks prior to mating. Parental animals were mated within groups for up to 3 weeks. Parental females were dosed during mating, gestation and lactation, while parental males were dosed during mating through delivery of their last litter sired. The P_2 adults were selected from the F_1 litters and were dosed for 10 - 13 weeks before mating to produce a single litter.	
5.2 Results and discussion	Findings in the parental animals included increased lactation body weight gain in females at ≥ 500 mg/kg bw, increased liver and kidney weights at ≥ 500 mg/kg bw in both sexes and centrilobular hepatocyte hypertrophy in some P_2 males. At ≥ 500 mg/kg bw there was also accumulation of hyaline droplets and other microscopic findings in the kidneys from P_1 males and from all treated groups of the P_2 males. At 1000 mg/kg bw there was an increased mortality in F_1 offspring during the early postnatal period, although no other clinical signs of toxicity were observed in the offspring of either generation. Offspring body weight was reduced at 1000 mg/kg bw during the early postnatal period in F_1 males and in F_2 pups of both sexes. At this dose level, 18/70 F_1 weanlings died or were euthanized prior to P_2 selection. No treatment-related post-mortem findings were observed in the offspring from either generation. At 1000 mg/kg bw there was a statistically significant reduction in the male mating index of P_2 males compared with controls, but there were no treatment-related microscopic changes in reproductive tissues or biologically meaningful differences in other reproductive parameters in adults of either generation. The original NOEL of 500 mg/kg bw for reproductive effects derived by Bevan et al. was based on the reduced male mating index in high-dosed P_2 males. Exposure to 1000 mg/kg/day and to a lesser extent 500 mg/kg/day did result in a reduction in postnatal survival in both F_1 and F_2 litters. Derivation of an appropriate NOAEL for offspring effects was made difficult because of conflicting interpretations of the reductions in postnatal survival for the 500 mg/kg/day treatment group. The US EPA concluded the reductions were treatment- and dose-related, a conservative interpretation that supports a NOAEL of 100 mg/kg/day. Alternatively, Bevan et al. deemed the observations not to be biologically significant and concluded the NOAEL to be 500 mg/kg/day. In order to clarify this issue a benchmark dose (BMD) assessment was conducted for the study's developmental and reproductive findings. For the offspring developmental effects, BMD dosages (BMDL5) of 449 and 418 mg/kg/day were estimated for the F_1 and F_2 generations, respectively. Based upon the decrease in male mating index observations in the P_2 males, a BMDL10 of 407 mg/kg/day was estimated for reproductive effects.	X
5.3 Conclusion		
5.3.1 LO(A)EL		X
5.3.1.1 Parent males		X
5.3.1.2 Parent females		X
5.3.1.3 F_1 males		X
5.3.1.4 F_1 females		X

Section A6.8.2/01	Multigeneration Reproduction Toxicity Study	
Annex Point IIA6.8.2	Two-generation study with rats (oral application)	
5.3.1.5 F_2		X
5.3.2 NO(A)EL		X
5.3.2.1 Parental	[redacted]	X
5.3.2.2 F_1 offspring	[redacted]	X
5.3.2.3 F_2 offspring	[redacted]	X
5.3.3 Reliability	[redacted]	
5.3.4 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]

Section A6.8.2/01	**Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2	Two-generation study with rats (oral application)
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 6.8.2/01_01: Effects on mean absolute and relative organ weights of P_1 and P_2 male SD rats

	Dose (mg/kg bw)			
	0	**100**	**500**	**1000**
P1				
Body weight (g)	604	584	619	612
Absolute organ weight (g)				
Liver	23.0	21.9	24.0	25.6**
Kidney	4.7	4.5	4.8	5.0
Relative organ weight (%)				
Liver	3.8	3.7	3.9	4.2**
Kidney	0.79	0.76	0.78	0.83
P2				
Body weight (g)	661	678	683	630
Absolute organ weight (g)				
Liver	24.1	25.2	27.2*	25.9
Kidney	4.6	4.5	4.8	4.7
Relative organ weight (%)				
Liver	3.6	3.7	4.0**	4.1**
Kidney	0.70	0.66	0.70	0.75**

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

Table 6.8.2/01_03: Summary of reproductive data

	Dose (mg/kg bw)			
	0	**100**	**500**	**1000**
P1				
Male mating index (%)	86.7	90.0	93.1	96.7
Male fertility index (%)	80.0	83.3	82.8	70.0
Female fertility index (%)	89.7	90.0	93.3	96.7
Female fecundity index (%)	88.5	88.9	85.7	72.4
Gestational index (%)	100	100	100	100
Mean gestation length (days)	22.5	22.5	22.4	22.6
Mean litter size	12.4	13.3	14.2	14.4
Mean live/litter	12.2	13.2	13.7	13.8
Mean dead/litter	0.2	0.1	0.5	0.6
P2				
Male mating index (%)	93.3	96.4	93.1	73.1*
Male fertility index (%)	80.0	82.1	72.4	61.5
Female fertility index (%)	93.3	96.7	93.1	82.6
Female fecundity index (%)	82.1	79.3	77.8	78.9
Gestational index (%)	100	95.8	100	100
Mean gestation length (days)	22.7	22.6	22.7	22.6
Mean litter size	13.2	14.0	14.1	14.4
Mean live/litter	13.0	13.7	13.7	14.0
Mean dead/litter	0.2	0.3	0.4	0.4

* $p < 0.05$

Table 6.8.2/01_04: Summary of offspring survival data

	Dose (mg/kg bw)			
	0	**100**	**500**	**1000**
F1				
Live birth index (%)	98.3	99.4	96.3	95.7*
Day 1 survival index (%)	98.6	97.6	98.0	84.5**
Day 4 survival index (%)	99.7	98.4	96.7**	91.0**
Day 7 survival index (%)	100	100	99.5	99.2
Day 14 survival index (%)	100	100	99.5	100
Day 21 survival index (%)	100	99.5	100	99.2
Lactation index (%)	99.4	99.5	99.0	92.2
F2				
Live birth index (%)	98.4	97.9	97.0	97.0
Day 1 survival index (%)	99.4	98.8	94.8**	94.2**
Day 4 survival index (%)	99.0	99.1	97.1	96.2*
Day 7 survival index (%)	100	99.4	96.3*	91.0**
Day 14 survival index (%)	100	98.9	98.1	100
Day 21 survival index (%)	100	99.4	100	100
Lactation index (%)	100	97.8	94.4**	91.0**

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

Table 6.8.2/01_05: Summary of F1 offspring body weights (g)

	Dose (mg/kg bw)			
Postnatal day	**0**	**100**	**500**	**1000**
Males				
0	6.61	6.68	6.46	6.27*
1	7.00	7.19	6.77	6.51*
4	9.47	9.94	9.30	8.94
7	15.09	15.96	14.21	14.31
14	30.71	32.65	30.46	30.21
21	48.01	52.94*	48.24	49.19
Females				
0	6.22	6.37	6.25	5.98
1	6.57	6.84	6.59	6.25
4	9.05	9.54	9.09	8.35
7	14.21	15.34	13.62	12.97
14	29.32	31.89*	29.77	28.35
21	45.56	50.79**	46.94	45.85

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

Section A6.9/01 Neurotoxicity

Annex Point IIA6.9 Acute inhalation study with rats

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1995) Isopropanol: Acute vapor inhalation neurotoxicity study in rats. [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**	Yes [redacted]
2.2	**GLP**	[redacted]
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Anhydrous isopropanol
3.1.1	Lot/Batch number	No data
3.1.2	Specification	No data
3.1.2.1	Description	No data
3.1.2.2	Purity	≥ 99.9 %
3.1.2.3	Stability	No data
3.2	**Reference Substance (positive control)**	None
3.3	**Test Animals**	
3.3.1	Species	Rat
3.3.2	Strain	F344
3.3.3	Source	Harlan Sprague Dawley, Inc. (Indianapolis, IN)
3.3.4	Sex	Male / Female
3.3.5	Rearing conditions	
3.3.6	Age/weight at study initiation	Animals designated for functional observational battery (FOB) evaluations: ca. 9 weeks / 120 – 223 g Animals designated for motor activity evaluations: ca. 10 – 11 weeks / 135 – 261 g
3.3.7	Number of animals per group	25 rats of each sex per group Behavioural observations with 10 rats of each sex Motor activity with 15 rats of each sex

Section A6.9/01 Neurotoxicity

Annex Point IIA6.9 Acute inhalation study with rats

3.3.8	Control animals	Yes
3.4	**Administration**	Inhalation
3.4.1	Exposure	Each exposure group was randomly divided into 10 rats of each sex for FOB evaluations and 15 rats of each sex for motor activity measurements. Separate animals were employed for motor activity and FOB measurements to accommodate the schedule for post-exposure evaluations. The animals were exposed once to isopropanol vapour for 6 hrs (whole body). Exposures were carried out over a 17-day period to accommodate the behavioural testing schedule.
3.4.2	Dose Levels	Nominal: 0, 500, 1500, 5000 or 10000 ppm (ca. 0, 1250, 3750, 12500 or 25000 mg/m^3) Analytical: FOB: 0, 522, 1546, 5126 or 10003 ppm Motor activity: 0, 505, 1572, 5252 or 10458 ppm for males and 0, 501, 1460, 4915 or 9892 ppm for females
3.4.3	Vehicle	Not applicable
3.4.4	Concentration in vehicle	Not applicable
3.4.5	Total volume applied	Not applicable
3.4.6	Postexposure period	
3.4.7	Anticholinergic substances used	
3.4.8	Controls	sham exposed
3.5	**Examinations**	
3.5.1	Body Weight	For all animals prior to exposure and during each behavioural testing session
3.5.2	Signs of Toxicity	Observations of mortality and overt signs of toxicity were made twice daily, beginning on the day after exposure and continuing until sacrifice
3.5.3	Observation schedule	
3.5.4	Clinical Chemistry	No
3.5.5	Pathology	No
3.5.6	Histopathology	No

Section A6.9/01 Neurotoxicity

Annex Point IIA6.9 Acute inhalation study with rats

3.6 Further remarks

Behavioural function was evaluated for 10 animals of each sex per group using a screening battery of tests (FOB) designed to detect possible alterations in central and peripheral nervous system function. The FOB was performed prior to exposure, immediately following a single 6 h exposure (designated 1-h post-exposure) and 6 and 24 hrs following exposure. The earliest post-exposure behavioural lest was initiated within 30 min of the time the animals were removed from the exposure chambers. Animals selected for the FOB were divided into five replicates each consisting of two animals of each sex per exposure group. One replicate of 20 animals was exposed in a given day. During examination, an animal was placed on a clean laboratory cart covered with a thin disposable paper board. The animal was 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 animal was 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 (landing foot splay), rectal temperature and body weight were subsequently evaluated using simple equipment. In order to avoid injury, certain tests were not performed on individual animals considered by the observer to be incapacitated as a result of sedation.

Motor activity was evaluated for 15 animals of each sex per group prior to exposure and immediately following a single 6 h exposure. Post-exposure test sessions were started within 15 min of the time the animals were removed from the exposure chambers. Males and females were each divided into three replicates consisting of five males or five females per exposure group. One replicate was exposed on a given day. Males were tested first, and then the same testing schedule was used during the next week for females. Animals were tested using an automated recording apparatus designed to measure activity in an unfamiliar environment. The test sessions lasted either 90 min (pre-exposure) or 300 min (post-exposure). The lights in the testing room were shut off approximately 180 min after the start of the post-exposure test sessions to coincide with the normal change in light cycle. The time of day for the motor activity test sessions remained constant throughout the study.

The expected pattern of post-exposure motor activity for an untreated animal includes three successive phases: an initial period of relatively high activity while the animal explores the environment of the enclosure; a secondary period of low activity once the animal habituates to the enclosure; and a final period of relatively high activity while the room lights are off. This final phase begins ca. 30 min prior to the beginning of the dark period. These conditions were chosen for the post-exposure test session in order to detect potential effects of isopropanol soon after exposure and the potential for recovery from these effects 4 – 5 hrs after exposure.

Section A6.9/01 **Neurotoxicity**

Annex Point IIA6.9 Acute inhalation study with rats

Statistical procedures:
The data for continuous, parametric variables were intercompared for the dose and control groups by use of Levene's test for homogeneity of variances, by analysis of variance and by pooled variance t-tests. The t-tests were used if the analysis of variance was significant ($P < 0.05$), to delineate which groups differed from the control group. If Levene's test indicated heterogeneous variances, the groups were compared by an analysis of variance for unequal variances followed, when appropriate, by separate variance t-tests.
Intra-session motor activity data were analyzed using a repeated measures analysis with dose as the grouping factor and session time as the within-subject factor. Group comparisons at hourly test session epochs were made if significant dose effects or time by dose interactions were observed. The epsilon adjustment procedure (Greenhouse-Geisser correction) was used in repeated measures analysis of motor activity data.
Frequency data from FOB tests were evaluated using Fisher's exact probability test. All statistical tests were performed using BMDP Statistical Software. A P value of 0.05 was used as the critical level of significance for all tests.

4 RESULTS AND DISCUSSION

4.1	**Body Weight**	10000 ppm: mean body weights tended to be lower at the 6 and 24 hrs post-exposure FOB evaluations compared to controls (3 - 8 %; not statistically different)
4.2	**Clinical signs of toxicity**	FOB: The findings were similar for male and female rats and exposure concentration-related findings were observed at ≥ 5000 ppm. At 5000 ppm exposure-related behavioural alterations 1 h after evaluation included altered gait, decreased toe and tail withdrawal reflexes, decreased mean rearing events, decreased mean rectal temperature and grip strength and increased mean hind-leg splay. Complete recovery, with the exception of decreased mean rectal temperature of males, was observed by the 6 h post-exposure evaluation. At 10000 ppm all of the animals were prostrate 1 h after exposure. Some FOB measurements could not be made for these animals and other tests for nervous system function were omitted to prevent injury. Partial recovery was apparent by the 6 h post-exposure evaluation, i.e. most of the animals were able to walk, albeit with severe ataxia, and effects on arousal, gait and surface righting tended to be less severe at 6 h post-exposure than at the 1 h evaluation. Except for increased mean hind-leg splay, complete recovery was observed by the 24 h post-exposure evaluation. Motor activity: Exposure-related changes in motor activity were generally consistent with FOB results. Decreased mean motor activity was observed throughout the 5 h test session for animals at 10000 ppm and until just before the end of the test session for animals at 5000 ppm. Total test session activity for the 10000 and 5000 ppm exposure groups was decreased ca. 90 % and 40 %, respectively, compared to the mean for the control groups. A slight, but statistically significant, decrease in total test session activity (15 % decrease) was observed for males at 1500 ppm. This decrease reflected a tendency toward decreased activity for that group during the first 4 hrs of the test session. A slight but statistically significant increase in total test session activity (10 % increase) was observed for females at 1500 ppm. This slight increase from the control was not attributed to exposure with isopropanol, because motor activity for females in this group was also increased compared to the control during the pre-exposure evaluation.
4.3	**Clinical Chemistry**	Not done
4.4	**Pathology**	Not done
4.5	**Histopathology**	Not done
4.6	**Other**	

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.9/01 Neurotoxicity

Annex Point IIA6.9 Acute inhalation study with rats

5.1	**Materials and methods**	In this study male and female F344 rats were exposed once over 6 hrs to concentrations of 0, 500, 1500, 5000 or 10000 ppm isopropanol (ca. 0, 1250, 3750, 12500 or 25000 mg/m^3). Behavioural observations for 10 rats of each sex were made prior to and 1, 6, and 24 hrs after exposure, while motor activity was evaluated for 15 rats of each sex prior to and immediately following exposure.	
5.2	**Results and discussion**	The exposure caused a spectrum of transient effects indicative of narcosis at 10000 ppm and sedation at 5000 ppm. At 10000 ppm prostration or severe ataxia, decreased arousal, slowed or laboured respiration, decreased neuromuscular function, hypothermia and loss of reflex function were observed 1 and 6 hrs after exposure. Similar – but less severe – effects were observed at 5000 ppm 1 h after exposure. At ≥ 5000 ppm concentration-related decreases in motor activity were observed in males and females, while 1500 ppm caused slight decreases in motor activity in males. Rats exposed to 1500 and 5000 ppm recovered from these motor activity effects within 5 hrs.	
5.3	**Conclusion**		
5.3.1	LOAEL	[redacted]	X
5.3.2	NOAEL	[redacted]	X
5.3.3	Reliability	[redacted]	
5.3.4	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	2008/02/28
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A6.9/01 Neurotoxicity

Annex Point IIA6.9 Acute inhalation study with rats

	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 A6.9/02 Neurotoxicity

Annex Point IIA6.9 Inhalation study with rats

3.4.2	Dose Levels	Nominal: 0 or 5000 ppm (ca. 0 or 12500 mg/m^3) Analytical: 0 or 5011 ppm (ca. 0 or 12528 mg/m^3)
3.4.3	Vehicle	Not applicable
3.4.4	Concentration in vehicle	Not applicable
3.4.5	Total volume applied	Not applicable
3.4.6	Postexposure period	Exposure over 9 weeks: 1 week Exposure over 13 weeks: 6 weeks
3.4.7	Anticholinergic substances used	
3.4.8	Controls	sham exposed
3.5	**Examinations**	
3.5.1	Body Weight	All rats were weighed prior to the start of the exposures, weekly during the study, on the days of motor activity testing and immediately preceding sacrifice
3.5.2	Signs of Toxicity	Rats were observed individually on a daily basis for clinical signs of toxicity, with special attention paid to effects such as ataxia and hypoactivity. Clinical signs were also recorded on a group basis during exposures.
3.5.3	Observation schedule	
3.5.4	Clinical Chemistry	No
3.5.5	Pathology	No
3.5.6	Histopathology	No

Section A6.9/02 Neurotoxicity

Annex Point IIA6.9 Inhalation study with rats

3.6 Further remarks

Biological procedures:
Motor activity evaluations were performed prior to the initiation of exposures (ca. 1 week) and on the Saturday following 4, 7 and 9, 11 and 13 weeks of exposure. To assess reversibility, animals exposed over 9 weeks were further evaluated 2, 4 and 7 days following their final exposure. Animals exposed over 13 weeks were further evaluated 2, 4, 7, 14, 21, 28, 35 and 42 days following their final exposure. Approximately 18 and 20 hrs elapsed between the end of the exposure and the beginning of the 1 day post-exposure motor activity test sessions for the 13 and 9 week groups, respectively. Motor activity measurements were conducted in an isolated room modified to control sound levels, light levels and environmental odours. Animals were tested individually using an automated photocell-recording apparatus designed to measure activity in a novel environment. The length of the test session was 90 min and data for ambulatory activity, fine motor activity, rearing activity and the sum of these individual types of activity (sum of all counters or total activity) were collected automatically in nine consecutive 10 min intervals for subsequent analysis.

Statistical procedures:
The data for continuous, parametric variables were intercompared for the exposure and control groups by use of Levene's test for homogeneity of variances and by t-tests. If Levene's test indicated homogeneous variances, the groups were compared by pooled variance t-tests. If Levene's test indicated heterogeneous variances, the groups were compared by separate variance t-tests.
The shape of the motor activity versus test session time curves (hereafter referred to as the motor activity habituation curves) were analyzed for possible exposure-related changes using repeated-measures analyses with exposure concentration as the grouping factor and test session time as the within-subjects factor. These analyses used the epsilon-adjustment procedure (Greenhouse–Geisser correction). Repeated measures analyses were performed for ambulatory activity, fine movements, rearing activity and total activity. Numerical differences in motor activity between exposed and control groups at within-session intervals were not analyzed statistically.
Cumulative test session motor activity data were analyzed for possible exposure-related changes if the results of the repeated measures analyses indicated an effect of treatment. These analyses were performed using the methods described above for continuous parametric variables. All statistical tests 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.

4 RESULTS AND DISCUSSION

4.1	**Body Weight**	Body weight and body weight gain were decreased for the exposed animals after 1 week of exposure. Following 3 weeks of exposure, statistically significant increases in body weight and body weight gain were observed. Statistically significant increases in body weight were noted in exposed rats throughout the remainder of the study. In rats exposed over 9 week, the final mean body weight and body weight gain was increased by ca. 6 and 17 %, respectively. In rats exposed over 13 weeks, the final mean body weight and body weight gain was increased by ca. 5 and 13 %, respectively. During the recovery period, increases in body weight and body weight gain remained for exposed rats as compared to controls, although smaller increases in body weight variables were observed during the recovery period than during the exposure regimen. At week 19, the mean body weight and body weight gain was increased by 3 and 9 %, respectively.
4.2	**Clinical signs of toxicity**	No exposure-related mortality. During exposure an apparent decrease in movement within the animal enclosures and a diminished startle response to tapping on the wall of the inhalation chamber was noted. During the non-exposure periods swollen periocular tissue was observed.
4.3	**Clinical Chemistry**	Not done
4.4	**Pathology**	Not done
4.5	**Histopathology**	Not done

Section A6.9/02 **Neurotoxicity**

Annex Point IIA6.9 Inhalation study with rats

4.6	**Other**	In exposed rats increases in mean cumulative motor activity (the sum of total activity across the 90 min test session) were observed at all of the evaluation time points during the exposure regimen. The increase in total activity reflected increases in ambulation, fine motor activity and rearing activity. There was no evidence for a preferential increase in any of these individual types of activity. In rats exposed over 9 weeks, increases in mean cumulative motor activity (the sum of total activity across the 90 min test session) were noted following the completion of 4, 7 and 9 weeks of exposure. Mean cumulative test session activity for these animals was increased by 41, 79 and 76 % at weeks 4, 7 and 9. During the recovery period, mean cumulative test session activity was not different from control values. In rats exposed over 13 weeks, mean cumulative test session activity was increased following the completion of 4, 7, 9, 11 and 13 weeks of exposure (35, 53, 144, 103 and 116 %, respectively). During the recovery period, increases in mean cumulative test session activity for these animals were also observed 2, 4, 7 and 28 days following their last exposure (79, 69, 38 and 50 %, respectively). Mean cumulative test session activity was not different from controls at days 14, 21, 35 and 42 days after exposure. Repeated measures analysis of motor activity habituation curves indicated statistically significant differences between exposed rats and controls at some study weeks. For rats exposed over 9 weeks, a change in the shape of the motor activity habituation curve was observed at week 6. For rats exposed over 13 weeks, changes in the shape of the motor activity habituation curve that were coincident with increased cumulative test session activity were observed at weeks 4, 9 and 11 as well as 4 days following the last exposure. At these time points, the mean activity was increased at most of the 10 min intra-session intervals. There were also statistically significant differences in the shape of the motor activity habituation curves for animals in both groups at time points where mean cumulative activity was not increased. These findings were attributed to an increase activity during the initial 10 – 30 min of the test session and were noted at day 7 or days 14, 21 and 35 following the last exposure over 9 or 13 weeks, respectively. No differences in the shape of the motor activity habituation curves were apparent between controls and the exposed group on day 42 following the last exposure.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In this study two groups of 30 female Fischer 344 rats were exposed to concentrations of 0 or 5000 ppm (ca. 0 or 12500 mg/m^3) on 6 hrs day and 5 days per week. 15 rats in each group were exposed over 9 or 13 weeks, respectively. Motor activity was assessed for both subgroups prior to exposure and following 4, 7, 9, 11 and 13 weeks of exposure. These motor activity measurements were made 18 – 20 hrs following the end of the last exposure for that week. In addition, to evaluate the reversibility of motor activity effects, measurements were made on three occasions during the week following the final exposure for rats in both the 9 and 13 week subgroups and weekly thereafter for five additional weeks for rats in the 13 week subgroup.

Section A6.9/02 Neurotoxicity

Annex Point IIA6.9 Inhalation study with rats

5.2	**Results and discussion**	Increases in cumulative test session motor activity counts were observed following 4, 7 and 9 weeks of exposure for rats in the 9 week subgroup. Increases in cumulative test session motor activity counts were also observed following 4, 7, 9, 11 and 13 weeks of exposure for rats in the 13 week subgroup. Reversibility of this effect was observed for rats in the 9 week subgroup within 2 days following the last exposure. Reversibility was also noted for rats in the 13 week subgroup but not until study week 15. Minor changes were observed in the shape of the motor activity habituation curves for exposed rats in the 9 and 13 week subgroups at ca. 50 % of the measurement intervals beginning at week 4. Most of these statistical changes were observed in conjunction with increases in cumulative test session motor activity and some were observed following time points where recovery of the cumulative test session motor activity counts had occurred. No change in the shape of the motor activity habituation curve was observed 6 weeks following the last exposure, i.e. there was a complete recovery of motor activity effects.	
5.3	**Conclusion**		
5.3.1	LOAEL	[redacted]	X
5.3.2	NOAEL	[redacted]	X
5.3.3	Reliability	[redacted]	
5.3.4	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	2008/02/28
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A6.12/01 Medical data

Annex Point IIA 6.12

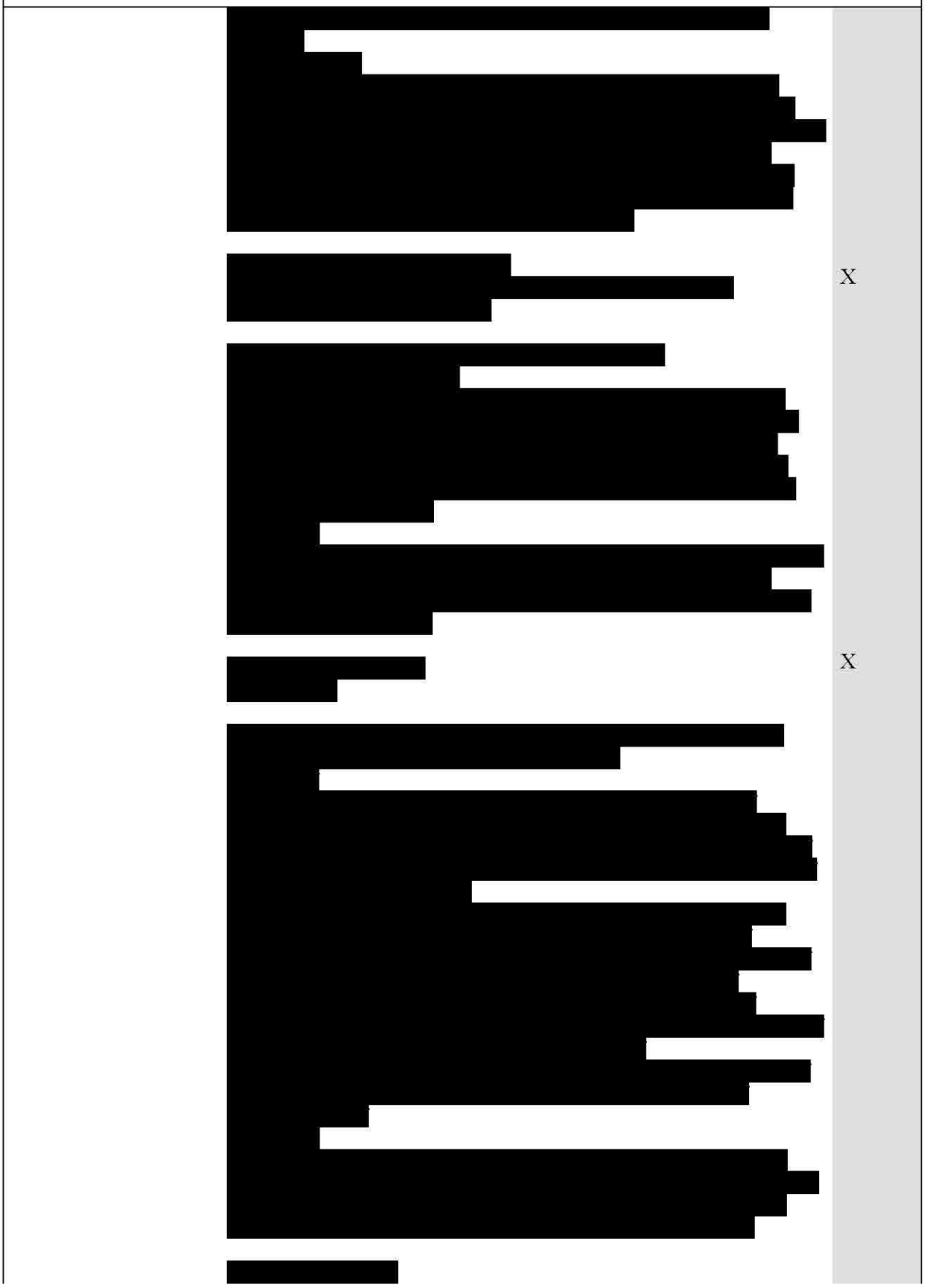
			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2003) 5th Periodic Safety Update Report for: Alcohol solutions for disinfection of intact skin [redacted], 40 pp. [redacted] (2006) Isopropyl alcohol (CAS 67-63-0). Master file for a biocidal substance [redacted], 37 pp. [redacted] (2007) Addendum Report 4 to 5th Periodic Safety Update Report for: Alcohol solutions for disinfection of intact [redacted] 41 pp.	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
Detailed information:		[redacted]	x

Section A6.12/01 **Medical data**

Annex Point IIA 6.12

Section A6.12/01 **Medical data**

Annex Point IIA 6.12



Section A6.12/01 **Annex Point IIA 6.12**	**Medical data**
	[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	2014/02/07
Evaluation of applicant's justification	[redacted]

Section A6.12/01 **Annex Point IIA 6.12**	**Medical data**
	[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 A6.12.2/01 Human Case Report

Annex Point IIA6.12 Studies concerning allergic contact dermatitis after contact with isopropanol containing swabs

Official use only

		1 **REFERENCE**	
1.1	**Reference**	Leow YH & Freeman S (1995) Acute allergic contact dermatitis from Medi-Swabs®, with negative patch tests to the individual ingredients, including isopropyl alcohol. Contact Dermatitis 33, 125 – 126	
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**	Not applicable	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	Not applicable	
		3 **MATERIALS AND METHODS**	
3.1	**Substance**	Medi-Swab® (impregnated with 70 % isopropanol)	
3.2	**Persons exposed**	2	
3.2.1	Sex	1 female	1 male
3.2.2	Age/weight	41 years / no data	43 years / no data
3.2.3	Known Diseases	Morbus Hodgkin	Childhood asthma and hay fever
3.2.4	Number of persons	1	1
3.2.5	Other information	No data	No data
3.3	**Exposure**	Dermal	Dermal
3.3.1	Reason of exposure	in the course of medical treatment	No data
3.3.2	Frequency of exposure	Multiple	No data
3.3.3	Overall time period of exposure	No data	No data
3.3.4	Duration of single exposure	Contact during treatment of recurrent vesicular dermatitis in the cubital fossae	No data
3.3.5	Exposure concentration/dose	No data	No data
3.3.6	Other information	No data	No data
3.4	**Examinations**	Patch testing on the back with Finn chambers on Scanpor tape with complete Medi-Swabs® and single ingredients of Medi-Swabs®: isopropyl alcohol (10 – 95 %) and all other components. Readings after exposure over 2 and 4 days	
3.5	**Treatment**	Avoidance of Medi-Swabs®	
3.6	**Remarks**		

Section A6.12.2/01	Human Case Report
Annex Point IIA6.12	Studies concerning allergic contact dermatitis after contact with isopropanol containing swabs

		4 **RESULTS**
4.1	**Clinical Signs**	No data
4.2	**Results of examinations**	Both subjects showed strongly, vesicular reactions to both types of Medi-Swabs® , while there was no positive reaction to isopropyl alcohol
4.3	**Effectivity of medical treatment**	Not applicable
4.4	**Outcome**	No more symptoms after avoidance of Medi-Swab®
4.5	**Other**	Not applicable
		5 **APPLICANT'S SUMMARY AND CONCLUSION**
5.1	**Materials and methods**	Patch testing on the back with Finn chambers on Scanpor tape
5.2	**Results and discussion**	The dermal reaction is ascribed to a 'compound allergy' without further analysis of compound in question
5.3	**Conclusion**	[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	2008/02/12
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*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*
Remarks	