Section A6.2 _ 02 _ b Percutaneous absorption (in vivo test)

Annex Point IIA6.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2004) Glutaraldehyde: pharmacokinetics in [redacted] rats following oral gavage or dermal application. [redacted] (unpublished), ([redacted]), BPD ID A6.02_02	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline was mentioned; however, the study was well-conducted and well-documented.	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde, [redacted]% solution in water from [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Colorless liquid	
3.1.2.2	Purity	[redacted]% glutaraldehyde, [redacted]% water, [redacted]% impurities [redacted]	
3.1.2.3	Stability	Not specified	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Females	
3.2.5	Age/weight at study initiation	Age: 11 weeks old	

Section A6.2 _ 02 _ b Percutaneous absorption (in vivo test)

Annex Point IIA6.2

		Weight: 155 – 171 g
3.2.6	Number of animals per group	4 animals/group
3.2.7	Control animals	Yes (not treated with glutaraldehyde)
3.3	**Administration/ Exposure**	Dermal
3.3.1	Preparation of test site	About 16 to 24 hours prior application of the test substance, the rats were anaethetized with isoflurane and the fur was removed from the back by clipping. The application site was protected from access by means of a protective appliance made of ca. 1.5 mm thick Teflon (4 cm x 5 cm) with a cutout opening (3 cm x 4 cm), formed into a saddle shape. The protective was maintained in place by means of a Permabond Industrial Grade 910 adhesive. The application site was semi-occluded by means of a Teflon Spectra/Mesh macroporous filter material, which was attached to the protective appliance using Velcro strips. In addition, the rats were fitted with rodent jackets with dermal inserts.
3.3.2	Concentration of test substance	7.5% and 0.75% solution in 0.9% physiological saline
3.3.3	Volume applied	10 µl/cm^2 (total: 120 µl)
3.3.4	Size of test site	About 12 cm^2
3.3.5	Exposure period	24 hours (sacrifice of the rats after 24 h)
3.3.6	Sampling time	10, 20, 30 and 45 minutes, 1, 2, 4, 6, 8, 12 and 24 hours after initiation of skin contact.
3.3.7	Samples	0.2 ml blood collected at each sampling time point
3.3.8	Samples analysis	The whole blood samples were analyzed for the parent compound glutaraldehyde using a method based on a previous gas chromatographic–mass spectrometric analysis method (GC/MS) developed for malondialdehyde and which used glutaraldehyde as an internal standard (Chiesa LM et al., Archiv für Lebensmittelhygiene, 50:41-43, 1999). The main steps of the method can be summarized as follows: (1)-The samples were acidified by addition of acidified water containing a ^{13}C-glutaraldehyde internal standard, (2)-The vials were capped, chilled and vortexed, (3)-The samples were derivatized with pentafluorobenzyl hydroxylamine, vortexed, extracted with toluene and centrifuged (10 min., 2350 x g), (4)- Toluene was removed, (5)-The samples were subjected to GC/MS.
3.3.9	Statistics and estimation of pharmacokinetic parameters	Statistical assessment was based on the calculation of mean and standard deviation. Estimation of pharmacokinetic parameters (Cmax, AUC, elimination half-lives) was based on the pharmacokinetic computer modelling program PK Solutions (Montrose, Colorado).

Section A6.2 _ 02 _ b Percutaneous absorption (in vivo test)

Annex Point IIA6.2

3.3.10 Remark

The whole study was conducted in two phases, an *in vivo* and an *in vitro* phase. The in vivo phase consisted of two experiments; within one experiment, the test substance was administered to the cannulated rats by oral gavage whereas in the second experiment, the cannulated rats were subjected to dermal application of the test substance. We here only report the data referring to the *in vivo* dermal application experiment.

4 RESULTS AND DISCUSSION

4.1 Actual test concentrations in the dose solutions and amounts of test substance applied

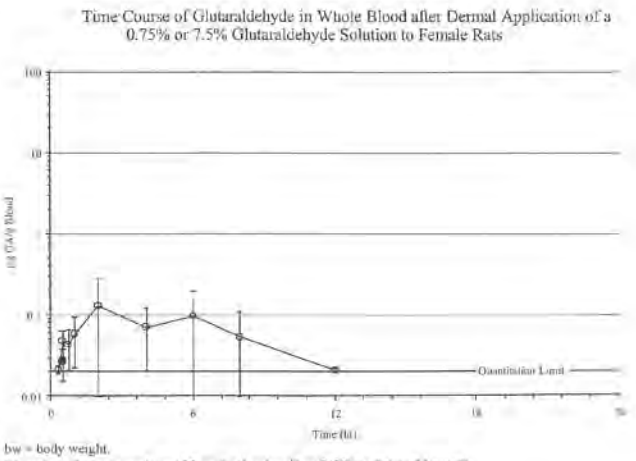
	0.75% Dermal application	7.5% Dermal application
Target concentration of the dose solution	7.5 mg/g	75.00 mg/g
Actual concentration of the dose solution	6.85 mg/g	70.90 mg/g
Body weight (mean +/-SD)	0.162 kg +/- 0.004	0.162 kg +/- 0.004
Dose solution administered (mean +/- SD)	0.127 g +/- 0.005	0.124 g +/- 0.007
Glutaraldehyde administered (mean +/- SD; mg)	0.9 mg +/- 0.0	8.8 mg +/- 0.5
Glutaraldehyde administered (mean +/- SD; mg/kg bw)	5.4 mg/kg bw +/- 0.2	54.4 mg/kg bw +/- 4.3

The actual doses applied to the skin were about 95 to 112% of the target amounts applied.

Section A6.2 _ 02 _ b Percutaneous absorption (in vivo test)

Annex Point IIA6.2

4.2 Glutaraldehyde time course in blood



bw = body weight.
Closed markers: Low dose of 5 mg/kg bw [oral] or 0.75% solution [dermal]
Open markers: High dose of 75 mg/kg bw [oral] or 7.5% solution [dermal].

Time course of Glutaraldehyde in Blood following Dermal Application

µg Glutaraldehyde/g Blood

	0.75% Dermal Application					
Time (hour)	Animal Number					
	03A2433	03A2434	03A2435	03A2436	Mean	SD
0	NQ	NQ	NQ	NQ	NQ	-
0.17	NS	NQ	NQ	NS	NQ	-
0.33	NQ	NQ	NQ	NQ	NQ	-
0.50	NS	NQ	NQ	0.039	NQ (0.026)	0.011
0.75	NS	NQ	NQ	NS	NQ	-
1	NQ	NQ	NQ	NQ	NQ	-
2	NQ	NQ	NQ	NQ	NQ	-
4	NQ	NQ	NQ	NS	NQ	-
6	NQ	NQ	NQ	NS	NQ	-
8	NQ	NQ	NQ	NQ	NQ	-
12	NQ	NQ	NQ	NS	NQ	-
24	NQ	NQ	NQ	NQ	NQ	-

	7.5% Dermal Application					
Time (hour)	Animal Number					
	03A2437	03A2438	03A2439	03A2440	Mean	SD
0	NQ	NQ	NQ	NQ	NQ	-
0.17	NQ	NQ	NQ	NQ	NQ	-
0.33	NQ	NQ	0.024	NQ	NQ (0.021)	0.002
0.50	0.041	0.070	0.044	0.031	0.047	0.017
0.75	0.046	0.026	0.072	0.026	0.043	0.022
1	0.042	0.038	0.111	0.039	0.058	0.036
2	0.350	0.024	0.098	0.039	0.128	0.152
4	0.034	0.115	0.109	NQ	0.070	0.049
6	0.237	0.069	0.059	NQ	0.096	0.096
8	0.135	0.021	0.035	NQ	0.053	0.055
12	NQ	0.020	0.023	NQ	0.021	0.001
24	NQ	NQ	NQ	NQ	NQ	

NS = No sample
NQ = Non-quantifiable

Section A6.2 _ 02 _ b Percutaneous absorption (in vivo test)

Annex Point IIA6.2

4.3 Percutaneous absorption

A percutaneous absorption of glutaraldehyde following dermal application was evident at the test dose of 7.5%. In fact, glutaraldehyde was recovered and quantified in blood in one rat after 20 minutes following application (0.024 µg glutaraldehyde/g blood), and in all rats after 30 minutes (ranging from 0.031 to 0.070µg/g; mean = 0.047 µg/g +/- 0.017). The blood levels of glutaraldehyde remained measurable almost in all animals during a period of 12 hours following application, with peak concentrations seen about 2 hours post-application (mean = 0.128 µg/g). At time point 12 hours, glutaraldehyde was no more quantifiable in 2 of 4 rats; at time point 24 hours, glutaraldehyde was quantifiable in none of the rats. The half-life of glutaraldehyde for dermal absorption was about 1 hour; the terminal half-life of elimination ($t_{1/2\beta}$) was about 4 hours. At the test dose of 0.75%, only one rat showed a quantifiable level of glutaraldehyde in blood (0.039 µg/g).

Following parameters were calculated (PK Solutions computer modelling software):

Dermal application	0.75% (i.e. 5.9 mg/kg bw)	7.5% (i.e. 58 mg/kg bw)
T ½ abs	_	1.17 h
T ½ ß	_	4.09 h
Cmax	_	0.128 µg/g
AUC (0-12h)	_	0.785 µg-h/ml

Dermal absorption estimated from 20 minutes to 1 hour post-treatment.

Elimination half-life estimated from 2 to 12 hours post-treatment.

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.2 _ 02 _ b Percutaneous absorption (in vivo test)

Annex Point IIA6.2

5.1	**Materials and methods**	The aim of the present study was to provide toxicokinetic data on glutaraldehyde in blood. The whole study was conducted in two phases, an *in vivo* and an *in vitro* phase. The *in vivo* phase consisted of two experiments; within one experiment, the test substance was administered to rats by oral gavage whereas in the second experiment, the rats were subjected to dermal application of the test substance. The present summary only refers to the *in vivo* dermal application experiment. Test substance: Glutaraldehyde ([redacted]% aq. sol. from [redacted], purity [redacted]% glutaraldehyde, [redacted]% water, [redacted]% impurities ([redacted]) No guideline was given, however the study was guideline-like and was conducted in accordance with GLP. Jugular vein-cannulated female [redacted] rats were purchased from [redacted]. The test substance was applied under semi-occlusive conditions on a 12 cm^2 clipped area of the back of each animal for 24 hours. Two doses were tested: 0.75 and 7.5% test substance in physiological saline; the application volume was 10 $\mu l/cm^2$. Each test group comprised 4 animals. Blood samples (0.2 ml) were collected over the whole treatment period at following time points: 10, 20, 30 and 45 minutes, 1, 2, 4, 6, 8, 12 and 24 hours after initiation of skin contact. After 24 hours, the rats were sacrificed. Sampling analysis for glutaraldehyde was based on gas chromatography and mass spectrometry.
5.2	**Results and discussion**	The actual doses applied to the skin were about 95 to 112% of the target amounts applied. Percutaneous absorption: A percutaneous absorption of glutaraldehyde following dermal application was evident at the test dose of 7.5%. In fact, glutaraldehyde was recovered and quantified in blood in one rat after 20 minutes following application (0.024 µg glutaraldehyde/g blood), and in all rats after 30 minutes (ranging from 0.031 to 0.070µg/g; mean = 0.047 µg/g +/- 0.017). The blood levels of glutaraldehyde remained measurable almost in all animals during a period of 12 hours following application, with peak concentrations seen about 2 hours post-application (mean = 0.128 µg/g). At time point 12 hours, glutaraldehyde was no more quantifiable in 2 of 4 rats; at time point 24 hours, glutaraldehyde was quantifiable in none of the rats. The half-life of glutaraldehyde for dermal absorption was about 1 hour; the terminal half-life of elimination ($t_{1/2\beta}$) was about 4 hours. At the test dose of 0.75%, only one rat showed a quantifiable level of glutaraldehyde in blood (0.039 µg/g).
5.3	**Conclusion**	In rats, following percutaneous absorption, glutaraldehyde was found to be systemically available in the blood, but also was rapidly removed from there, either by macromolecular binding or by metabolism.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	No guideline was mentioned; however, the study was well conducted and well documented and followed GLP.

Section A6.2 _ 02 _ b Percutaneous absorption (in vivo test)

Annex Point IIA6.2

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 26th, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.2 _ 02 _ c Toxicokinetics in blood, in vitro experiment

Annex Point IIA6.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2004) Glutaraldehyde: pharmacokinetics in [redacted] rats following oral gavage or dermal application. [redacted] (unpublished), (sponsor: [redacted]), BPD ID A6.02_02	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline was mentioned; however, the study was well conducted and well documented.	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde, [redacted]% solution in water from [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Colorless liquid	
3.1.2.2	Purity	[redacted]% glutaraldehyde, [redacted]% water, [redacted]% impurities ([redacted])	
3.1.2.3	Stability	Not specified	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Females	
3.2.5	Age/weight at study initiation	Age: 11 weeks old Weight: 155 – 171 g	
3.2.6	Number of animals	15 animals	

Section A6.2 _ 02 _ c Toxicokinetics in blood, in vitro experiment

Annex Point IIA6.2

	per group	
3.3	**In vitro experiment**	
3.3.1	Preparation of the blood samples	All rats were anasthetized and sacrificed by exsanguinations via cardiac puncture. Blood was collected, separated into 3 equal portions, and kept at 37°C in a water bath. Defined amounts of test substance were added to 20 ml-blood samples and the mixtures were allowed to equilibrate (1-2 minutes at 37 °C under gentle agitation). At defined time points, aliquots of these samples were taken and acidified water containing a ^{13}C-glutaraldehyde internal standard was added to each aliquot. A further series of aliquots was taken and centrifuged 5 minutes to obtain the plasma fraction. An aliquot of plasma was then taken and acidified water containing a ^{13}C-glutaraldehyde internal standard was added. The remaining plasma was centrifuged again, and an aliquot of the protein-free filtrate was removed and acidified water containing a ^{13}C-glutaraldehyde internal standard was added. Thereafter, all the samples were analysed for glutaraldehyde.
3.3.2	Concentration of test substance	250, 2500 and 25000 ng glutaraldehyde/ml blood.
3.3.3	Sampling time points	0, 5, 10, 120 and 480 minutes following inoculation of the blood with the test substance.
3.3.4	Samples analysis	The whole blood samples were analyzed for the parent compound glutaraldehyde using a method based on a previous gas chromatographic–mass spectrometric analysis method (GC/MS) developed for malondialdehyde and which used glutaraldehyde as an internal standard (Chiesa LM et al., Archiv für Lebensmittelhygiene, 50:41-43, 1999). The main steps of the method can be summarized as follows: (1)-The samples were acidified by addition of acidified water containing a ^{13}C-glutaraldehyde internal standard, (2)-The vials were capped, chilled and vortexed, (3)-The samples were derivatized with pentafluorobenzyl hydroxylamine, vortexed, extracted with toluene and centrifuged (10 min., 2350 x g), (4)- Toluene was removed, (5)-The samples were subjected to GC/MS.
3.3.5	Remark	The whole study was conducted in two phases, an *in vivo* and an *in vitro* phase. The in vivo phase consisted of two experiments; within one experiment, the test substance was administered to the cannulated rats by oral gavage whereas in the second experiment, the cannulated rats were subjected to dermal application of the test substance. We here report the data referring to the *in vitro* experiment.

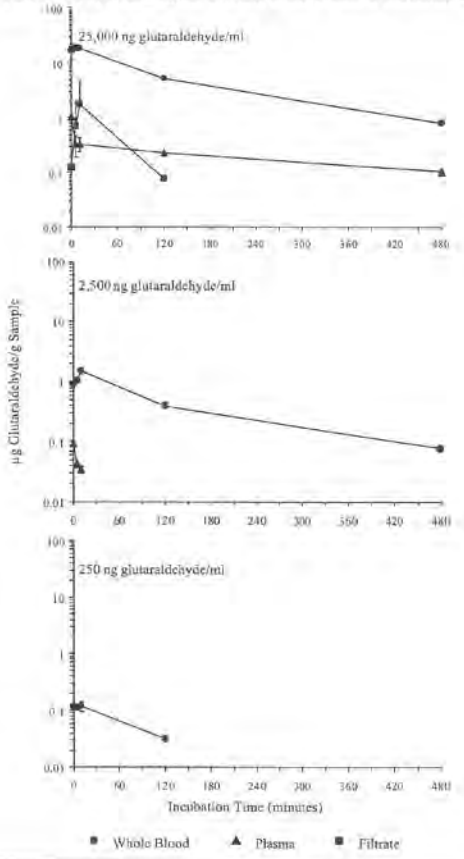
4 RESULTS AND DISCUSSION

Section A6.2 _ 02 _ c Toxicokinetics in blood, in vitro experiment

Annex Point IIA6.2

4.1 Glutaraldehyde time course in blood

Time Course of Glutaraldehyde in Whole Blood, Plasma or Ultracentrifugation Filtrate.



Glutaraldehyde Time Course in Blood, Plasma and Ultracentrifugation Filtrate Following Inoculation of Blood, *in vitro*, with Indicated Amounts of Glutaraldehyde.

250 ng glutaraldehyde/ml

	Blood	Plasma	Filtrate
Time (Min)	Mean SD	Mean SD	Mean SD
0	0.116 ± 0.011	NQ	NQ
5	0.112 ± 0.013	NQ	NQ
10	0.118 ± 0.023	NQ	NQ
120	0.032 ± 0.004	NQ	NQ
480	NQ	NQ	NS

2,500 ng glutaraldehyde/ml

	Blood	Plasma	Filtrate
Time (Min)	Mean SD	Mean SD	Mean SD
0	0.892 ± 0.116	0.095 ± 0.010	NQ
5	1.030 ± 0.036	0.044 ± 0.001	NQ
10	1.525 ± 0.167	0.035 ± 0.005	NQ
120	0.396 ± 0.030	NQ	NQ
480	0.077 ± 0.003	NQ	NS

25,000 ng glutaraldehyde/ml

	Blood	Plasma	Filtrate
Time (Min)	Mean SD	Mean SD	Mean SD
0	16.500 ± 4.969	1.038 ± 0.115	0.122 ± 0.023
5	18.091 ± 1.373	0.334 ± 0.139	0.721 ± 1.130
10	18.397 ± 1.066	0.334 ± 0.091	1.746 ± 2.883
120	5.230 ± 0.244	0.228 ± 0.002	0.079 ± 0.001
480	0.816 ± 0.011	0.104 ± 0.000	NS

NS = No sample
NQ = Non-quantifiable

Section A6.2 _ 02 _ c Toxicokinetics in blood, in vitro experiment

Annex Point IIA6.2

4.2	**Glutaraldehyde quantification in whole blood, in the plasma fraction and in the protein-free filtrate**	At 250 ng/ml: Glutaraldehyde was quantifiable in whole blood during 2 hours following inoculation with the test substance; at time point 2 hours, a mean amount of 0.032 µg glutaraldehyde/g sample was measured. Neither the plasma nor the filtrate showed quantifiable amounts of glutaraldehyde. At 2500 ng/ml: Glutaraldehyde was quantifiable in whole blood during 8 hours following inoculation with the test substance; at time point 8 hours, a mean amount of 0.070 µg glutaraldehyde/g sample was measured. Quantifiable amounts also were found in the plasma fraction 5 and 10 minutes following inoculation (respectively 0.044 and 0.035 µg/g). In the protein-free filtrate no quantifiable amounts of glutaraldehyde could be detected. At 25000 ng/ml: Glutaraldehyde was quantifiable in whole blood at all time points following inoculation with the test substance; the highest amounts of test substance were found at time points 5 and 10 minutes (respectively 18.09 and 18.4 µg/g). Glutaraldehyde also was quantifiable in the plasma fraction, however at lesser extent than in whole blood. The highest amounts of test substance were found immediately after inoculation (time point 0 min, 1.038 µg/g), after which a rapid decline was observed reaching 0.104 µg/ml at time point 8 hours. In the protein-free filtrate, very small amounts of glutaraldehyde could be quantified, with the highest level (1.746 µg/g) being reached at time point 10 minutes following inoculation. **5 APPLICANT'S SUMMARY AND CONCLUSION**	X

Section A6.2 _ 02 _ c Toxicokinetics in blood, in vitro experiment

Annex Point IIA6.2

5.1 Materials and methods

The aim of the present study was to provide toxicokinetic data on glutaraldehyde in blood. The whole study was conducted in two phases, an *in vivo* and an *in vitro* phase. The *in vivo* phase consisted of two experiments; within one experiment, the test substance was administered to rats by oral gavage whereas in the second experiment, the rats were subjected to dermal application of the test substance. The present summary refers to the *in vitro* experiment that was undertaken in order to determine if glutaraldehyde was bound to red blood cells or plasma protein, or if glutaraldehyde was present in blood as free glutaraldehyde.

Test substance: Glutaraldehyde ([redacted]% aq. sol. from [redacted] [redacted]% glutaraldehyde, [redacted]% water, [redacted]% impurities ([redacted])

No guideline was given, however the study was guideline-like and was conducted in accordance with GLP.

Non-cannulated rats were purchased from [redacted]. All rats were anaesthetized and sacrificed; blood was collected, separated into 3 equal portions, and kept at 37°C. Defined amounts of test substance were added to 20 ml-blood samples in order to get following test concentrations: 250, 2500 and 25000 ng glutaraldehyde/ml blood. At defined time points, aliquots of these samples were taken and acidified water containing a ^{13}C-glutaraldehyde internal standard was added to each aliquot. A further series of aliquots was taken and centrifuged 5 minutes to obtain the plasma fraction. An aliquot of plasma was then taken and acidified water containing a ^{13}C-glutaraldehyde internal standard was added. The remaining plasma was centrifuged again, and an aliquot of the protein-free filtrate was removed and acidified water containing a ^{13}C-glutaraldehyde internal standard was added. The sampling time points were 0, 5, 10, 120 and 480 minutes following inoculation of the blood with the test substance. Sampling analysis for glutaraldehyde was based on gas chromatography and mass spectrometry.

Section A6.2 _ 02 _ c Toxicokinetics in blood, in vitro experiment

Annex Point IIA6.2

5.2	**Results and discussion**	At a test concentration of 250 ng/ml, glutaraldehyde was quantifiable in whole blood following inoculation with the test substance until time point 2 hours (mean amount of 0.032 µg glutaraldehyde/g sample). Neither the plasma nor the filtrate showed quantifiable amounts of glutaraldehyde. At 2500 ng/ml, glutaraldehyde was quantifiable in whole blood following inoculation with the test substance until time point 8 hours (mean amount of 0.070 µg glutaraldehyde/g sample). Quantifiable amounts also were found in the plasma fraction 5 and 10 minutes following inoculation (respectively 0.044 and 0.035 µg/g). In the protein-free filtrate no quantifiable amounts of glutaraldehyde could be detected. At 25000 ng/ml, glutaraldehyde was quantifiable in whole blood at all time points following inoculation with the test substance; the highest amounts of test substance were found at time points 5 and 10 minutes (respectively 18.09 and 18.4 µg/g). Glutaraldehyde also was quantifiable in the plasma fraction, however at lesser extent than in whole blood. The highest amounts of test substance were found immediately after inoculation (time point 0 min, 1.038 µg/g), after which a rapid decline was observed reaching 0.104 µg/ml at time point 8 hours. In the protein-free filtrate, very small amounts of glutaraldehyde could be quantified, with the highest level (1.746 µg/g) being reached at time point 10 minutes following inoculation.
5.3	**Conclusion**	The results of the *in vitro* experiment suggest that most of the glutaraldehyde in blood is bound to blood proteins, with only small amounts of free glutaraldehyde remaining in the protein-free fraction.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	No guideline was mentioned; however, the study was well conducted and well documented and followed GLP.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 26th, 2010
Materials and Methods	Agree with applicant's version.
Results and discussion	4.2 Glutaraldehyde quantification in whole blood, in the plasma fraction and in the protein-free filtrate. • Correction: "At 2500 ng/ml: … at time point 8 hours, a mean amount of ~~0.070~~ **0.077** µg glutaraldehyde/g sample was measured." • At 25 000 ng/ml, there was an increasing amount of glutaraldehyde in the protein-free filtrate during the first 10 minutes, having decreased to about 5 % of the maximum by 2 h.
Conclusion	Agree with applicant's version.
Reliability	2
Acceptability	Acceptable

Section A6.2 _ 02 _ c Toxicokinetics in blood, in vitro experiment

Annex Point IIA6.2

Remarks	The value of the study is questionable as it concerns *ex vivo* blood, and the description of the fractioning of blood is insufficient. It is difficult to interpret the relevance of the study, but it is concluded to show that a low amount of free glutaraldehyde is present in the blood.
	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 A6.02_5
Annex Point IIA6.2

Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (2007) Glutaraldehyde: Identification of metabolites in the [redacted] rat. [redacted] (Unpublished), ([redacted])
1.2	**Data protection**	Yes
1.2.1	Data owner	The Dow Chemical Company and BASF SE
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes; the study was conducted according to US EPA OPPTS 870.7485 (1998), OECD Guideline 417 (1984), and EEC, Part B.36 (1986)
2.2	**GLP**	Yes
2.3	**Deviations**	The study conduct followed GLP, however with following exceptions: 1. The specific activity of the ^{14}C-glutaraldehyde test material was calculated and was not determined via GLP analysis; 2. Characterization of glutaraldehyde was performed concurrently with the study; 3. Radiochemical purity of the ^{14}C-glutaraldehyde and characterization were performed concurrently with the study.

3 MATERIALS AND METHODS

3.1	**Test material**	2,4-^{14}C-Glutaraldehyde and Glutaraldehyde
3.1.1	Lot/Batch number	
3.1.1.1	Radiolabelled test material	[redacted]
3.1.1.2	Non-radiolabelled test material	[redacted]
3.1.2	Specification	As given in section 2

X

Section A6.02_5 Annex Point IIA6.2		Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA
3.1.2.1	Description	Clear, colourless liquid
3.1.2.2	Purity	
3.1.2.2.1	Radiolabelled test material	[redacted]% (impurities: [redacted]% identified [redacted])
3.1.2.2.2	Non-radiolabelled test material	[redacted]% aqueous sol.
3.1.2.3	Stability	No data
3.1.2.4	Radiolabelling	Specific activity: 1.87 MBq/g solution; 50.49 µCi/g solution; 0.878 mg ^{14}C-Glutaraldehyde/g solution; 5.76 mCi/mmol
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	[redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	Males and Females
3.2.5	Age/weight at study initiation	At test initiation both sexes were about 8 weeks old The males weighed between 200 and 250 g The females weighed between 160 and 200 g
3.2.6	Number of animals per group	Three animals per sex and group
3.2.7	Control animals	One male rat served control
3.3	**Administration/ Exposure**	**Oral**
3.3.1	Way	Single gavage
3.3.2	Test doses	Low dose:5 mg ^{14}C-glutaraldehyde/kg bw High dose: 75 mg ^{14}C-glutaraldehyde/kg bw

Section A6.02_5 Annex Point IIA6.2		Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA
3.3.3	Preparation of the test dose	The oral doses were prepared according to [redacted] (2004). ^{14}C-Glutaraldehyde solutions were prepared in tap water. In order to achieve the required radiolabelled doses, non-radiolabelled material was added to the stock solutions of radiolabelled material and distilled water was added to the final volumes. The targeted radioactive dose per animal was about 67 µCi/kg bw. Confirmation of the test material concentration in the dose suspensions was conducted in accordance with the standard operating procedures of the Analytical Chemistry Laboratory at TERC. Radioactivity in the dose solutions was quantified by liquid scintillation spectroscopy (LSS) as described below. LSS analysis of aliquots of the ^{14}C-labeled dosing solution taken from various locations in the solution container was used to confirm the concentration of radioactivity and the homogeneity of the ^{14}C-GDA in the dosing solutions.
3.3.4	Specific activity of test substance	The targeted radioactive dose per animal was about 67 µCi/kg bw
3.3.5	Volume applied	Ten ml/kg bw of these preparations were administered to rats via oral gavage.
3.3.6	Excreta examined	Urine, feces, expired CO_2, final cage wash (FCW)
3.3.7	Sampling	

Section A6.02_5 Annex Point IIA6.2	Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA	
3.3.7.1 Urine	All urine voided during the study was collected in dry-ice cooled traps. The urine traps were changed at 12, 24, and 48 hour post-dosing. Each urine specimen and rinse was weighed, and a weighed aliquot of each sample was analyzed for radioactivity by LSS as described below. Equal volume aliquots of urine samples from the 0-12 hour, 12-24 hour, and 24-48 hour collection intervals were pooled, by dose and sex, and all individual and pooled samples stored at -80 °C.	
3.3.7.2 Feces	Feces were collected in dry-ice chilled containers at 0-24 hour and 24-48 hour intervals. An aqueous homogenate (~25% w/w) was prepared and weighed aliquots of these homogenates were oxidized ([redacted]) and quantitated for radioactivity by LSS. In addition, equal volume aliquots of fecal homogenates from each animal were pooled. The individual and pooled samples were stored at -80 °C.	
3.3.7.3 Expired CO_2	After exiting the metabolism Roth cage, the expired air was passed through a solution of monoethanolamine:1-methoxy-2-propanol (3:7 v/v) to trap expired CO_2. The CO_2 trap was changed at 24 hour post-dosing and a weighed aliquot of each sample was analyzed for radioactivity by LSS as described below.	
3.3.7.4 FCW	At 48 hours post-dosing the animals were anaesthetized with CO_2/O_2 and sacrificed by exsanguination. Following the terminal sacrifice, a final cage wash (FCW) was performed. The FCW was collected and weight of the sample was determined. A weighed aliquot of the final cage was analyzed for radioactivity via LSS.	
3.3.7.5 Methods for sample analysis	Liquid scintillation spectrometry (LSS) and High Performance Liquid Chromatography (HPLC)	
3.3.7.6 Samples from control animal	Control urine and feces were collected in dry-ice cooled traps from the male control rat, which had not been administered with ^{14}C-glutaraldehyde. The control animal was sacrificed and blood collected by the same procedure as the dosed animals. 550 µl of control urine collected from male rats was fortified with ca. 2.5 µCi of ^{14}C-glutaraldehyde; an aliquot of this mixture was prepared for and subjected to HPLC. Approximately 1 g of control male fecal homogenate was spike with ca. 2.5 µCi of ^{14}C-glutaraldehyde; an aliquot of a clear supernatant obtained from centrifugation was prepared for and subjected to HPLC.	X
3.3.8 Metabolite identification	Radiolabeled components in the pooled urine samples were separated via HPLC and detected with an ARC stop-flow radiochemical detection system in the 12-second fraction mode. Detection of radiolabelled peaks in extracts of pooled fecal samples was performed via LSS analysis of 30-second eluent fractions Glutaric acid was analyzed as a metabolite in the Liquid Chromatography/Positive Electrospray Ionization/Mass Spectroscopy (LC/PESIMS) scan mode. Urine and fecal homogenates from the drinking water study (see below) were not analyzed via MS.	

Section A6.02_5 Annex Point IIA6.2		Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA
3.3.9	Glutaraldehyde or glutaraldehyde-derived metabolites cross-linking with proteins	To evaluate the possibility that glutaraldehyde or glutaraldehyde-derived metabolites may react with proteins *in vivo*, sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE) analysis was used to determine the molecular weight range of those various metabolite-HPLC fractions collected from fecal and urine samples from the oral gavaged rats In order to increase the detection of the metabolites/bands of interest (i.e., potential metabolites and radioactive bands greater than 5% of the administered dose), two female rats were orally dosed with a high radioactivity (ca. 470µCi/kg) dose of 50 mg ^{14}C-glutaraldehyde/kg bw and excreta were collected for 24 hours. Urines and feces were prepared for and subjected to HPLC. The samples also were analyzed by SDS-PAGE followed by PVDF (polyvinylidene difluoride) membrane transfer. The radioactivity bands were analyzed by Autoradiography.
3.3.10	Additional experiment, drinking water	In a further approach, female [redacted] rats received either 50 or 1000 ppm ^{14}C-glutaraldehyde fortified drinking water. The excreta were also profiled *via* HPLC for comparison to the oral gavage profiles (no more details provided, but it was referred to [redacted] (2007). Glutaraldehyde: Pharmacokinetics of Drinking Water Administered Glutaraldehyde in [redacted] Rats. [redacted] (Unpublished). Pooled urine and pooled fecal homogenates from female rats receiving ^{14}C-glutaraldehyde *via* drinking water study (50 or 1000 ppm) were analyzed as described above. In addition, metabolic profiles were also determined.
3.3.11	Statistics	Descriptive statistics were used (*i.e.*, mean ± standard deviation).
		4 RESULTS AND DISCUSSION
4.1	**Actual ingested amounts of test material**	Gavage experiment: The male rats averaged 5.34 ± 0.06 and 78.6 ± 0.35 mg/kg bw for the low and high dose groups, respectively. The females averaged 5.33 ± 0.04 and 78.9 ± 0.70 mg/kg bw for the low and high dose, respectively. Drinking water experiment: The females (N=4) averaged 9.83 ± 0.60 and 102 ± 40.5 mg/kg bw for the 50 and 1000 ppm fortified drinking water groups, respectively.
4.2	**Recovery of labelled compound following single gavage**	The average recovery of the administered dose in the combined excreta ranged between 34 and 58% with 7-11% excreted in urine and 24-47% eliminated in feces. The excretion patterns in urine was similar for both dose levels, with most radioactivity being excreted in the first 12 hour urine sample collected post-dosing. ^{14}C- recoveries in expired CO_2 was 2-3% of the administered dose

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Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA

4.2.1 In males gavaged with 5 mg/kg bw of ^{14}C-glutaraldehyde

Sum of % Admin			Sex	Animal			
			Male				
Sample Class	Sample	Time (hr)	04A4708	04A4709	04A4710	Mean	SD
CO2	CO2	24	1.34	0.76	2.28	1.46	0.77
		48	0.12	0.09	0.12	0.11	0.02
CO2 Total			1.46	0.85	2.40	1.57	0.78
FCW	FCW		0.05	0.10	0.08	0.08	0.03
Feces	Feces	24	25.43	22.50	23.17	23.70	1.54
		48	2.91	5.94	1.90	3.58	2.10
Feces Total			28.34	28.43	25.07	27.28	1.92
Urine/Rinse	Rinse	12	0.27	0.24	0.34	0.28	0.05
		24	0.06	0.04	0.06	0.05	0.01
		48	0.02	0.03	0.08	0.04	0.03
	Urine	12	6.78	4.90	5.36	5.68	0.98
		24	0.90	0.96	0.52	0.79	0.24
		48	0.37	0.32	0.31	0.34	0.03
Urine/Rinse Total			8.39	6.48	6.67	7.18	1.05

For the single low male dose group, the mean recoveries of the administered dose were:
27.28% in feces,
7.18% in urine and FCW, there from 5.68% in urine produced during the first 12 hours following dosing,
1.57 % in expired CO_2

4.2.2 In males gavaged with 75 mg/kg bw of ^{14}C-glutaraldehyde

Sum of % Admin			Sex	Animal			
			Male				
Sample Class	Sample	Time (hr)	04A4711	04A4712	04A4713	Mean	SD
CO2	CO2	24	5.61	2.18	0.63	2.81	2.55
		48	0.40	0.15	0.16	0.24	0.14
CO2 Total			6.01	2.33	0.79	3.04	2.68
FCW	FCW		0.74	0.17	0.06	0.32	0.36
Feces	Feces	24	15.31	16.51	40.93	24.25	14.46
		48	20.07	29.88	8.57	19.50	10.67
Feces Total			35.38	46.39	49.50	43.75	7.42
Urine/Rinse	Rinse	12	0.19	0.51	0.12	0.27	0.21
		24	0.04	0.10	0.09	0.08	0.03
		48	0.07	0.05	0.07	0.07	0.01
	Urine	12	4.96	5.88	6.83	5.89	0.94
		24	1.62	2.34	1.99	1.98	0.36
		48	0.84	0.97	0.59	0.80	0.19
Urine/Rinse Total			7.71	9.86	9.70	9.09	1.20

For the single high male dose group, the mean recoveries of the administered dose were:
43.75% in feces,
9.09% in urine and FCW, there from 5.89% in urine produced during the first 12 hours following dosing,
3.04 % in expired CO_2

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Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA

4.2.3 In females gavaged with 5 mg/kg bw of ^{14}C-glutaraldehyde

Sum of % Admin			Sex Animal				
			Female				
Sample Class	Sample	Time (hr)	04A4714	04A4715	04A4716	Mean	SD
CO2	CO2	24	1.52	3.94	2.33	2.60	1.23
		48	0.13	0.37	0.21	0.24	0.12
CO2 Total			1.65	4.31	2.54	2.83	1.35
FCW	FCW		0.16	0.12	0.17	0.15	0.03
Feces	Feces	24	22.07	18.25	25.19	21.84	3.48
		48	1.99	2.09	1.23	1.77	0.47
Feces Total			24.06	20.34	26.42	23.61	3.07
Urine/Rinse	Rinse	12	1.02	0.71	0.86	0.86	0.15
		24	0.06	0.03	0.05	0.05	0.01
		48	0.04	0.06	0.06	0.05	0.01
	Urine	12	5.69	5.80	5.19	5.56	0.32
		24	0.73	0.77	0.64	0.71	0.07
		48	0.28	0.29	0.24	0.27	0.03
Urine/Rinse Total			7.82	7.67	7.05	7.52	0.41

For the single low female dose group, the mean recoveries of the administered dose were:
23.61% in feces,
7.52% in urine and FCW, there from 5.56% in urine produced during the first 12 hours following dosing,
2.83 % in expired CO_2

4.2.4 In females gavaged with 75 mg/kg bw of ^{14}C-glutaraldehyde

Sum of % Admin			Sex Animal				
			Female				
Sample Class	Sample	Time (hr)	04A4717	04A4718	04A4719	Mean	SD
CO2	CO2	24	1.18	4.44	3.00	2.88	1.63
		48	0.06	NS*	0.37	0.21	
CO2 Total			1.24	4.44	3.37	3.02	1.63
FCW	FCW		0.27	0.29	0.56	0.37	0.16
Feces	Feces	24	32.68	0.06	38.76	23.83	20.81
		48	20.38	40.46	8.96	23.27	15.95
Feces Total			53.06	40.52	47.72	47.10	6.29
Urine/Rinse	Rinse	12	0.73	0.32	0.56	0.54	0.21
		24	0.25	0.20	0.22	0.23	0.02
		48	0.11	NS*	0.09	0.10	
	Urine	12	7.05	8.65	6.22	7.31	1.23
		24	2.43	2.01	1.52	1.99	0.46
		48	0.69	NS*	0.45	0.57	
Urine/Rinse Total			11.26	11.18	9.07	10.50	1.24

*- NS - sample collected but no sample analyzed for radioactivity

For the single high female dose group, the mean recoveries of the administered dose were:
47.10% in feces,
10.50% in urine and FCW, there from 7.31% in urine produced during the first 12 hours following dosing,
3.02 % in expired CO_2

4.3 Metabolites

Section A6.02_5 Annex Point IIA6.2		Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA
4.3.1	Gavaged rats experiment, urine	For details see table 6_2-1 A total of 6 radioactive peaks (A, B, C, D, E, and F) were detected in urine at greater than 0.5% of the administrated dose in the oral gavage study. From these peaks, only one (peak A) was common to all urine samples and ranged between 5 and 6% of the administered dose.
4.3.2	Gavaged rats experiment, feces	For details see table 6_2-2 A total of four radioactive peaks (A, B, D, and E) were detected in feces at greater than 0.5% of administrated dose. Only one peak (Peak A) was detected in all the samples and ranged from 1 to 2% of the administered dose. In addition to the metabolism peaks reported above, there were also 2 bands of radioactivity in the fecal samples for both sexes and dose levels. The first band extended from 20 to 30 minutes and the second, from 30 to 50 minutes. They were designated as Band 1 and Band 2 and respectively accounted for 8 to 15 and 14 to 31% of the administered dose for the 5 and 75 mg/kg bw dose, respectively, for both sexes (see table 6_2-2).
4.3.3	Drinking water experiment, urine	For details see table 6_2-3 Peak A and Peak E were the only peaks detected at greater than 0.5% of the administered dose and ranged from 6-8 and 4-5%, respectively, for the 50 and 1000 ppm glutaraldehyde fortified drinking water.
4.3.4	Drinking water experiment, feces	For details see table 6_2-4 Only Peak A, Peak D, and Peak E were detected in the female feces from both drinking water dose groups (50 and 1000 ppm) and ranged from 1-3 % of the administered dose. No other peaks were detected at greater than 0.5% of the administered dose. In addition to these fecal metabolism peaks, there were also 2 bands of radioactivity in the fecal samples for both dose levels. The first band extends from 20 to 39 minutes and the second is from 39 to 55 minutes. These are designated as Band 1 and Band 2 and accounted for 8 and 16, and 8 and 27% of the administered dose for the 50 and 1000 ppm dose, respectively. The broad-radioactive bands were found to consist of many unresolved peaks containing glutaraldehyde or glutaraldehyde derived metabolites forming complexes with extraneous and/or endogenous proteins, peptides and amino acids present in the feces. This cross-linking functionality between glutaraldehyde/glutaraldehyde derived metabolites and proteins was further demonstrated when control rat fecal homogenate was incubated with ^{14}Cglutaraldehyde (see 3.3.7.6). In fact, the resulting HPLC profile showed similar bands of radioactivity in the retention time region of Bands 1 and 2.
4.4	**Metabolite Identification**	

Section A6.02_5 Annex Point IIA6.2		Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA
4.4.1	Peaks A and E	Peak A was detected at greater than 5% of the administered dose in the urine from the orally gavaged and fortified drinking water administered rats. HPLC revealed that the Peak A metabolite was a very polar compound. Unfortunately various ESI-LC/MS (liquid chromatographyelectrospray mass spectrometry) or LC/MS-MS (liquid chromatography-electrospray tandem mass spectrometry) analyses were utilized to attempt to obtain a mass spectrum of this metabolite, but no meaningful data were obtained. In consideration of the high reactivity of glutaraldehyde with amino functionalities on proteins it was postulated that metabolite Peak A (see 4.4.1), perhaps resulted from reaction of glutaraldehyde with endogenous proteins, peptides or amino acids in the rat, either systemically or present in the excreta. This possibility was examined (see 3.3.7.6) and it was shown that glutaraldehyde can form several adducts with endogenous components (possible proteins, peptides, or amino acids) in control urine. Furthermore, an early eluting peak was detected in the urine of control animals fortified with ^{14}C-glutaraldehyde which had a retention time consistent with that of metabolite Peak A. In control fecal homogenate a radiolabeled component also was found, which co-eluted with Peak A. Thus the findings indicate that peak A may be a very polar adduct of the test material and one or more extraneous or endogenous proteins, peptides, or amino acids. Peak E ranged from 1-3% of the administered dose in both the low and high dose urine of the orally gavaged rats. It was also found to range from 3-6 and 1-3% of the administered dose in the urine and feces, respectively, in the 50 and 1000 ppm drinking water experiment. Peak E was found to have the same retention time as an authentic standard of glutaric acid and was therefore identified as glutaric acid.
4.4.2	Band 1 and Band 2	Band 1 and Band 2 were detected at greater than 5% of the administered dose in the feces via both administered routes (gavage, drinking water). The broad HPLC radioactive Bands 1 and 2 were not fully resolvable under the HPLC conditions. In consideration of the high reactivity of GDA with amino functionalities on proteins it was postulated that, similarly as for Peak A, these radioactive-metabolite areas resulted from reaction of glutaraldehyde with endogenous proteins, peptides or amino acids in the rat, either systemically or present in the excreta.

Section A6.02_5 Annex Point IIA6.2		Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA	
4.4.3	Molecular weight characterization	For characterization of the molecular weight range of the urinary/fecal metabolites, the HPLC fractions were isolated from urine and fecal samples from the two additional female rats, orally administered 50 mg/kg bw test substance containing a higher level of ^{14}C-glutaraldehyde (see 3.3.9). Referring to Peak A, Band 1 and Band 2, Analysis by SDS-PAGE and autoradiography revealed that each of fecal and urinary Peak A, Band 1 and Band 2 had a molecular weight <2.5 kDa. Peak B (see 4.3.1 and 4.3.2) also was analyzed and also had a molecular weight < 2.5 kDa. These data are in accordance with the results of a previous study conducted by Ranly and Horn (Ranly DM, Horn D (1990) Distribution, metabolism, and excretion of [14C]-glutaraldehyde. J. Endodontics 16(3):135-139 (Published), BPD ID A6.02_04, IUCLID entry) which demonstrated that 97% of the glutaraldehyde metabolites were less than 1 kDa in size. These authors postulated that this test material is highly conjugated with amino acids or small peptides. **5 APPLICANT'S SUMMARY AND CONCLUSION**	X

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Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA

5.1 Materials and methods

The aim of present study was to investigate the metabolites resulting from glutaraldehyde following oral administration of ^{14}C-labelled test substance to [redacted] rats.

Test substances:

(1) Glutaraldehyde-[2,4-^{14}C], purity [redacted]% (impurities: [redacted]), specific activity1.87 Mbq/g solution. (2) Unlabelled glutaraldehyde, [redacted]% aqueous sol.

The study was conducted according to OECD 417 (1984) with GLP.

In the main experiment, the radioactive test substance was administered orally to male and female rats (3/sex/group) by single gavage; the tested doses were 75 and 5 mg/kg bw. Urine, feces, expired CO_2, final cage wash (FCW) were sampled. In fact, the urine traps were changed at 12, 24, and 48 hour post-dosing. Each urine specimen and rinse was weighed, and a weighed aliquot of each sample was analyzed for radioactivity by LSS as described below. Equal volume aliquots of urine samples from the 0-12 hour, 12-24 hour, and 24-48 hour collection intervals were pooled, by dose and sex, and all individual. Feces were collected at 0-24 hour and 24-48 hour intervals, and equal volume aliquots of fecal homogenates from each animal were pooled. Expired air was passed through a solution of monoethanolamine:1-methoxy-2-propanol (3:7 v/v) to trap expired CO_2. The CO_2 trap was changed at 24 hour post-dosing. At 48 hours post-dosing and following sacrifice of the animals, a final cage wash (FCW) was performed.

A male control rat was added to the experiment and urine and feces were collected from this animal. A defined amount urine and of feces was fortified with ca. 2.5 µCi of ^{14}C-glutaraldehyde; aliquots were prepared for and subjected to HPLC.

For the purpose of comparison, an additional experiment was conducted with female Wistar rats received either 50 or 1000 ppm ^{14}C-glutaraldehyde fortified drinking water; urine and feces were sampled as described above (no details provided data separately reported in Hansen et al. (2007).

To evaluate the possibility that glutaraldehyde or glutaraldehyde-derived metabolites may react with proteins *in vivo*, sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE) analysis was used to determine the molecular weight range of those various metabolite-HPLC fractions collected from fecal and urine samples from the oral gavaged rats

In order to increase the detection of the metabolites/bands of interest (i.e., potential metabolites and radioactive bands greater than 5% of the administered dose), two female rats were orally dosed with a high radioactivity (ca. 470µCi/kg) dose of 50 mg ^{14}C-glutaraldehyde/kg bw and excreta were collected for 24 hours. Urine and feces sampled.

The analytical methods used analysis of the excreta samples were in principle based on Liquid scintillation spectrometry (LSS) and High Performance Liquid Chromatography (HPLC).

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Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA

5.2 Results and discussion

^{14}C recovery:
Following single gavage, the average recovery of the administered dose in the combined excreta ranged between 34 and 58% with 7-11% excreted in urine and 24-47% eliminated in feces.
The excretion patterns in urine was similar for both dose levels, 5 and 75 mg/ kg bw, with most radioactivity being excreted in the first 12 hour urine sample collected post-dosing. ^{14}C- recoveries in expired CO_2 was 2-3% of the administered dose.
These total radioactivity recoveries in excreta were fairly comparable with data from biokinetics studies with ^{14}C-glutaraldehyde (see A6.02_1; [redacted] 2004), in which 9-14 and 43-59 % of the administered dose were recovered in the urine and feces of male and female [redacted]r rats administered 5 or 75 mg/kg bw ^{14}C-GDA/kg bw. In contract, recoveries of ^{14}C in expired CO_2 were lower than the 19-29% recovered in the BASF study mentioned above.

Metabolite identification and characterisation:
Referring to metabolite indentification, in the single gavage experiment, 6 radioactive peaks (A, B, C, D, E, and F) were detected in urine at > 0.5% of the administrated dose; only peak A was common to all urine samples and ranged between 5 and 6% of the administered dose. In feces, 4 radioactive peaks (A, B, D, and E) were detected at > 0.5% of administrated dose, with only Peak A being present in all samples at levels of 1 to 2% of the administered dose. In feces, 2 additionally bands (Band 1 and Band 2) were found for both sexes and dose levels. They respectively accounted for 8 to 15 and 14 to 31% of the administered dose for the 5 and 75 mg/kg bw dose, respectively, for both sexes. In the comparative drinking water experiment conducted with female rats, Peak A and Peak E were the only peaks detected in urine at > 0.5% of the administered dose and they ranged from 6-8 and 4-5%, respectively, for the 50 and 1000 ppm glutaraldehyde fortified drinking water. In feces, Peak A, Peak D, and Peak E were detected and ranged from 1-3 % of the administered dose. Similar as in the single gavage study, the 2 bands described above also were found in the fecal samples of this experiment for both dose levels; Band 1 and Band 2 and accounted for 8 and 16, and 8 and 27% of the administered dose for the 50 and 1000 ppm dose, respectively.

Peak E was identified as glutaric acid.

Peak A was identified/described as a very polar adduct of the test material and one or more extraneous or endogenous proteins, peptides, or amino acids.

Band 1 and Band 2were two broad-radioactive bands consisting of many unresolved peaks containing glutaraldehyde or glutaraldehyde derived metabolites forming complexes with extraneous and/or endogenous proteins, peptides and amino acids present in the feces. The cross-linking functionality between glutaraldehyde/glutaraldehyde derived metabolites and proteins was further demonstrated when control rat fecal homogenate was incubated with ^{14}C-glutaraldehyde. Fecal and urinary Peak A, Band 1 and Band 2 had a molecular weight <2.5 kDa.

5.3 Conclusion

In addition to glutaric acid, following oral uptake, glutaraldehyde has been shown to form very complex radioactive metabolite profiles in both urine and fecal samples in Wistar rats. These metabolites can be formed by either direct metabolism of glutaraldehyde or indirect

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metabolism of protein, peptide, or amino acid related adducts of glutaraldehyde. This unusual metabolism can be ascribed to the complex reactivity of glutaraldehyde.

5.3.1 Reliability **1**

5.3.2 Deficiencies No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 29th, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2. 3.3.7.6 Samples from control animal. Correction: "~~550~~ **450** µl of control urine collected from male rats was fortified..."
Results and discussion	4.2.1, 4.2.2, 4.2.3, 4.2.4. In each of these points, final cage wash (FCW) is not reported. The value given is the recovery in urine, and the FCW values are as follows: 0.08 %, 0.15 %, 0.32 % and 0.37 %, respectively. 4.4.3 Molecular weight characterization. Note that Peak A, Band 1 and Band 2 were migrating in the buffer front and were quite clearly below the smallest band in the molecular size standard (2.5 kDa). Furthermore, the protein nature of these bands has not been established, and the size estimates would not be valid for other types of molecules.
Conclusion	Attempts to identify metabolites have been unsuccessful, with the exception of identifying Peak E as glutaric acid.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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Annex Point IIA6.2

Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA

Table A6_2-1. Radioactive distribution from HPLC separation of pooled urine from groups of male and female [redacted] rats orally administered ^{14}C-Glutaraldehyde by gavage:

Matrix	Sex	Route	dose	Time (hr)	Urinary Peaks as % of Administered Dose							
					A	B	C	D	E	F	Band 1	Band 2
Urine	Male	Oral	5 mg/kg	0-12	3.29	ND	0.78	ND	1.89	ND	ND	ND
				12-24	0.84	ND	ND	ND	ND	ND	ND	ND
				24-48*	0.38	ND	ND	ND	ND	ND	ND	ND
				Urinary Totals	4.51	ND	0.78	ND	1.89	ND	ND	ND
Urine	Male	Oral	75 mg/kg	0-12	2.83	0.65	0.70	ND	0.96	1.03	ND	ND
				12-24	2.06	ND	ND	ND	ND	ND	ND	ND
				24-48*	0.87	ND	ND	ND	ND	ND	ND	ND
				Urinary Totals	5.75	0.65	0.70	ND	0.96	1.03	ND	ND
Urine	Female	Oral	5 mg/kg	0-12	3.45	ND	1.28	ND	1.69	ND	ND	ND
				12-24	0.76	ND	ND	ND	ND	ND	ND	ND
				24-48*	0.33	ND	ND	ND	ND	ND	ND	ND
				Urinary Totals	4.54	ND	1.28	ND	1.69	ND	ND	ND
Urine	Female	Oral	75 mg/kg	0-12	2.76	ND	0.93	0.74	2.87	0.55	ND	ND
				12-24	2.21	ND	ND	ND	ND	ND	ND	ND
				24-48*	0.45	ND	ND	ND	ND	ND	ND	ND
				Urinary Totals	5.42	ND	0.93	0.74	2.87	0.55	ND	ND

Table A6_2-2. Radioactive distribution from HPLC separation of pooled feces homogenate from groups of male and female [redacted] rats orally administered ^{14}C-Glutaraldehyde by gavage:

Sex	Route	dose	Time (hr)	Fecal Peaks as % of Administered Dose							
				A	B	C	D	E	F	Band 1	Band 2
Male	Oral	5 mg/kg	0-24	1.91	1.96	ND	ND	ND	ND	6.62	12.08
			24-48*	0.29	0.30	ND	ND	ND	ND	1.00	1.82
			Fecal Totals	2.19	2.26	ND	ND	ND	ND	7.62	13.90
			Urine and Feces Totals	6.71	2.26	0.78	ND	1.89	ND	7.62	13.90
Male	Oral	75 mg/kg	0-24	0.70	0.56	ND	0.62	ND	ND	6.70	15.67
			24-48	0.65	ND	ND	ND	ND	ND	6.01	12.85
			Fecal Totals	1.35	0.56	ND	0.62	ND	ND	12.71	28.52
			Urine and Feces Totals	7.11	1.21	0.70	0.62	0.96	1.03	12.71	28.52
Female	Oral	5 mg/kg	0-24	1.96	2.05	ND	ND	0.54	ND	7.48	9.81
			24-48	0.16	0.17	ND	ND	0.04	ND	0.61	0.79
			Fecal Totals	2.12	2.21	ND	ND	0.58	ND	8.09	10.60
			Urine and Feces Totals	6.67	2.21	0.70	0.62	2.27	ND	8.09	10.60
Female	Oral	75 mg/kg	0-24	0.69	ND	ND	ND	ND	ND	7.15	15.99
			24-48	1.09	ND	ND	ND	ND	ND	7.95	14.23
			Fecal Totals	1.78	ND	ND	ND	ND	ND	15.10	30.22
			Urine and Feces Totals	7.20	ND	0.93	0.74	2.87	0.55	15.10	30.22

Section A6.02_5

Annex Point IIA6.2

Identification of glutaraldehyde (GA) metabolites in Wistar rats following oral administration of ^{14}C-labelled GA

Table A6_2-3. Radioactive distribution from HPLC separation of pooled urine from groups of female [redacted] rats administered ^{14}C-Glutaraldehyde *via* the drinking water:

Matrix	Sex	Route	dose	Time (hr)	Urinary Peaks as % of Administered Dose							
					A	B	C	D	E	F	Band 1	Band 2
Urine	Female	Drinking water	50 ppm	0-12	4.68	0.62	ND	ND	4.01	ND	ND	ND
				12-24	2.18	ND	ND	ND	0.63	ND	ND	ND
				24-48*	1.14	ND	ND	ND	0.33	ND	ND	ND
				Urinary Totals	7.99	ND	ND	ND	4.98	ND	ND	ND

Matrix	Sex	Route	dose	Time (hr)	Urinary Peaks as % of Administered Dose							
					A	B	C	D	E	F	Band 1	Band 2
Urine	Female	Drinking water	1000 ppm	0-12	3.03	ND	ND	ND	2.53	ND	ND	ND
				12-24	1.59	ND	ND	ND	0.81	ND	ND	ND
				24-48*	1.02	ND	ND	ND	0.52	ND	ND	ND
				Urinary Totals	5.64	ND	ND	ND	3.86	ND	ND	ND

* - The 24-48 hour samples not analyzed. Percentage of administered dose determined by multipying the per cent of injected activity of the respective peaks in the 12-24 hour time interval by the per cent of administered dose in the 24-48 hour interval.

Table A6_2-4. Radioactive distribution from HPLC separation of pooled feces homogenate from groups of female [redacted] rats administered ^{14}C-Glutaraldehyde *via* the drinking water:

Sex	Route	dose	Time (hr)	Fecal Peaks as % of Administered Dose							
				A	B	C	D	E	F	Band 1	Band 2
Female	Drinking water	50 ppm	0-24	2.44	ND	ND	1.57	0.81	ND	5.36	7.21
			24-48*	0.79	ND	ND	0.51	0.27	ND	1.75	2.35
			Fecal Totals	3.23	ND	ND	2.09	1.08	ND	7.10	9.56
			Urine and Feces Totals	11.23	ND	ND	2.09	6.06	ND	7.10	9.56

Sex	Route	dose	Time (hr)	Fecal Peaks as % of Administered Dose							
				A	B	C	D	E	F	Band 1	Band 2
Female	Drinking water	1000 ppm	0-24	1.32	ND	ND	0.56	1.21	ND	5.31	8.84
			24-48*	1.67	ND	ND	0.70	1.53	ND	6.72	11.18
			Fecal Totals	3.00	ND	ND	1.26	2.74	ND	15.91	27.33
			Urine and Feces Totals	8.64	ND	ND	1.26	6.60	ND	15.91	27.33

* - Sample not analyzed. Per cent of administered dose determined by multipying the per cent of injected activity of the respective peaks/bands in the 0-24 hr time interval by the per cent of administered dose in the 24-48 hr interval.

Section A6.3.1 _ 01 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Oral short-term repeated dose toxicity**

14 day-study with rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1985) Glutaraldehyde: Two-week inclusion in drinking water of rats. [redacted] (Unpublished), [redacted], BPD ID A6.03.1_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline was mentioned, however the study was well documented/described and basic data were given.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Not relevant	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted], from [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Clear liquid	
3.1.2.2	Purity	[redacted] a.i.	
3.1.2.3	Stability	The analyses conducted at 10 and 1000 ppm revealed that glutaraldehyde was stable for 28 days when stored at room temperature in plastic-capped, clear glass bottles.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male/Female	
3.2.5	Age/weight at study initiation	At test initiation the rats were about 6 weeks old. Body weight range at test initiation for the males: 112.9 – 129.0 g Body weight range at test initiation for the females: 91.6 – 103.5 g	
3.2.6	Number of animals	Each test group consisted of 10 male and 10 female rats	

Section A6.3.1 _ 01	Repeated dose toxicity
Annex Point IIA6.3 / 6.4 / 6.5	Oral short-term repeated dose toxicity 14 day-study with rats

	per group	
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	14 days
3.3.2	Frequency of exposure	Daily
3.3.3	Post exposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	The test substance was offered to the animals in the drinking water
3.3.4.2	Concentration	10, 100 and 1000 ppm (w/w) The high dose solution (1000 ppm) was prepared by direct addition of test substance the appropriate amount of water. The lower doses were then prepared by diluting the appropriate amount of the 1000 ppm solution. Test solutions were prepared each week. The applied test-doses were checked for correctness.
3.3.4.3	Vehicle	Tap water (pH adjusted between 6 and 7)
3.3.4.4	Controls	Controls received drinking water without addition of test substance.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Observations for clinical signs of toxicity were performed daily
3.4.1.2	Mortality	Observations for mortality were performed daily
3.4.2	Body weight	Body weights were measured weekly
3.4.3	Food consumption	Food consumption was monitored daily
3.4.4	Water consumption	Water consumption was monitored daily
3.4.5	Ophthalmoscopic examination	Not considered
3.4.6	Haematology	Prior to sacrifice the animals in the 0, 100 and 1000 ppm groups were anesthetized with methoxyflurane and blood for hematology and clinical chemistry measurements was collected by retroorbital bleeding. The 10 ppm animals were not considered. The following heamatological parameters were considered: Total leukocyte count (WBC), erythrocyte count (RBC), hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCII), mean corpuscular hemoglobin concentration (MCHC) and platelets.
3.4.7	Clinical Chemistry	The following clinical chemistry parameters were considered: Creatinine, total bilirubin, direct bilirubin, indirect bilirubin, aspartate aminotransferase, alanine aminotransferase, y-glutamyl transpeptidase, sorbitol dehydrogenase
3.4.8	Urinalysis	Not considered

Section A6.3.1 _ 01		**Repeated dose toxicity**
Annex Point IIA6.3 / 6.4 / 6.5		**Oral short-term repeated dose toxicity** **14 day-study with rats**
3.5	**Sacrifice and pathology**	At the end of the 14 days exposure period, the animals were sacrificed for the purpose of necropsy.
3.5.1	Organ Weights	The heart, lungs, liver, kidneys, spleen, gonads and brain of each animal were weighed. Body weights were recorded prior to sacrifice for expression of relative organ weight.
3.5.2	Gross and histopathology	A complete necropsy was performed for all animals. Histopathological examination of the tongue, stomach and liver was performed for all animals from the 0, 100 and 1000 ppm groups.
3.5.3	Other examinations	None
3.5.4	Statistics	The statistical assessment of the findings was based on following tests: Levene's test for homogeneity of variances, Analysis of variance, Individual t-tests.
3.6	**Further remarks**	All measurements of the test substance and water were made by weight. Calculations were corrected for purity of glutaraldehyde at 51.6% (actual purity 49.8 ± 0.5%). This discrepancy resulted from an incorrect value for purity obtained prior to the study. For convenience, nominal concentrations were considered as 10, 100 and 1000 ppm in this report. Analytical monitoring of the test doses: Homogeneity of the test solutions was determined for all dosage level by measurement of glutaraldehyde concentrations in three samples each from the top, middle and bottom of the reservoir used for preparation. The test mixtures were found to be homogeneous at all tested concentrations. Verification of the test concentrations revealed a recovery of test substance of 97%, 101% and 98% of nominal for the 10, 100 and 1000 ppm concentrations, respectively, for day 1 Recovery was 96%, 97% and 96 % after 8 days. The actual concentrations (corrected to a purity of 49.8% a.i.) were calculated to be 9.4, 98 and 950 ppm for day 1, and 9.3, 94 and 928 ppm for day 8. The analysis of drinking water samples collected from the bottles offered to the animals of the 100 and the 1000 ppm groups revealed that the test solutions were stable. For the 1000 ppm solution, the measured concentrations of test substance at days 7 and 14 were 101.4% and 100.2% of the nominal value, respectively. For the 100 ppm solution, the measured concentrations at days 7 and 14 were 97.1% and 99.5% of the nominal values, respectively. In the 10 ppm solution, recovery of glutaraldehyde at day 7 ranged between 93 and 0%, and at days 14, 18 and 20, the concentrations were below limit detection. Further testing revealed that the loss of test substance in the 10 ppm solution was most likely due to the contact with the neoprene rubber stoppers of the water bottles (unpublished observations).
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No clinical signs of toxicity were observed. A single case of urine staining in a female from the 1000 ppm group was reported.
4.1.2	Mortality	No mortality was observed.

X

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Annex Point IIA6.3 / 6.4 / 6.5

Repeated dose toxicity

Oral short-term repeated dose toxicity

14 day-study with rats

4.2 Body weight gain

Males:

No treatment-related differences in absolute body weight or weight gain were observed for males exposed to glutaraldehyde. In fact, statistically significant increases in weight gain were reported at 10 and 100 ppm, but they were not dose-related and were of sufficiently small magnitude to be considered not biologically significant.

GLUTARALDEHYDE: TWO-WEEK WATER INCLUSION STUDY IN RATS
BODY WEIGHT (G): SUMMARY OF MEANS
MALES

GLUTARALDEHYDE CONCENTRATIONS		0 PPM	10 PPM	100 PPM	1000 PPM
DAY 0					
	MEAN	121.8	121.7	122.3	121.6
	S.D.	3.57	2.45	4.91	3.45
	N	10	10	10	10
7					
	MEAN	131.4	134.7	133.4	149.2
	S.D.	5.84	4.30	7.10	4.59
	N	10	10	10	10
14					
	MEAN	187.6	193.3	184.5	187.1
	S.D.	9.06	6.41	10.47	9.02
	N	10	10	10	10

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP

GLUTARALDEHYDE: TWO-WEEK WATER INCLUSION STUDY IN RATS
BODY WEIGHT GAINS (G): FROM INTERVAL ZERO: SUMMARY OF MEANS
MALES

GLUTARALDEHYDE CONCENTRATIONS		0 PPM	10 PPM	100 PPM	1000 PPM
DAY 0 TO 7					
	MEAN	29.6	33.20	33.18	27.6
	S.D.	3.81	3.50	2.90	3.32
	N	10	10	10	10
0 TO 14					
	MEAN	65.7	71.6	72.18	65.5
	S.D.	7.78	5.58	6.61	5.83
	N	10	10	10	10

* = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT 0.05 LEVEL

Females:

In females, absolute body weight was not affected during the two-week exposure period. However, body weight gain was reduced in females from the high dose group by day 14 of the study.

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14 day-study with rats

GLUTARALDEHYDE, TWO-WEEK WATER INCLUSION STUDY IN RATS
BODY WEIGHT (G), SUMMARY OF MEANS
FEMALES

GLUTARALDEHYDE CONCENTRATION:		0 PPM	10 PPM	100 PPM	1000 PPM
DAY 0					
	MEAN	99.2	99.4	98.6	99.5
	S.D.	2.65	3.44	2.62	3.62
	N	10	10	10	10
7					
	MEAN	113.9	113.2	113.0	112.7
	S.D.	5.11	4.41	3.21	4.63
	N	10	10	10	10
14					
	MEAN	130.3	128.7	129.5	126.4
	S.D.	3.95	3.83	2.29	3.98
	N	10	10	10	10

** SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP

GLUTARALDEHYDE, TWO-WEEK WATER INCLUSION STUDY IN RATS
BODY WEIGHT GAINS (G), FROM INTERVAL ZERO, SUMMARY OF MEANS
FEMALES

GLUTARALDEHYDE CONCENTRATION:		0 PPM	10 PPM	100 PPM	1000 PPM
DAY 0 TO 7					
	MEAN	14.7	13.9	14.5	13.3
	S.E.	1.59	1.55	1.55	1.98
	N	10	10	10	10
0 TO 14					
	MEAN	31.1	29.4	30.6	27.188
	S.E.	3.27	3.43	2.35	2.49
	N	10	10	10	10

** = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT 0.01 LEVEL

4.3 Food consumption Food consumption for males and females (see tables below) was depressed for the 1000 ppm at day 1. Statistically significant reductions were noted through day 3 for females and day 7 for males of this dosage group. Food consumption remained lower for males of the high dose group throughout the study. No other effects an food consumption were noted.

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Annex Point IIA6.3 / 6.4 / 6.5 **Oral short-term repeated dose toxicity**

14 day-study with rats

GLUTARALDEHYDE: TWO-WEEK WATER INCLUSION STUDY IN RATS
FOOD CONSUMPTION (GRAMS/ANIMAL/DAY): SUMMARY OF MEANS
MALES

GLUTARALDEHYDE CONCENTRATION:		0 PPM	10 PPM	100 PPM	1000 PPM
DAY 1	MEAN	14.4	14.4	14.2	12.2**
	S.D.	0.91	0.73	0.78	0.61
	N	10	10	10	10
2	MEAN	13.8	14.1	14.2	12.3**
	S.D.	1.00	0.97	1.40	0.91
	N	10	10	10	10
3	MEAN	14.0	13.4	13.2	11.1**
	S.D.	1.06	0.99	1.15	0.86
	N	10	10	10	10
4	MEAN	13.3	13.2	13.3	11.3**
	S.D.	1.38	0.62	0.98	1.35
	N	10	10	10	10
5	MEAN	14.3	13.9	14.0	14.5**
	S.D.	0.63	0.83	1.04	0.69
	N	10	10	10	10

** = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT 0.01 LEVEL

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GLUTARALDEHYDE: TWO-WEEK WATER INCLUSION STUDY IN RATS
FOOD CONSUMPTION (GRAMS/ANIMAL/DAY): SUMMARY OF MEANS
MALES

GLUTARALDEHYDE CONCENTRATION:		0 PPM	10 PPM	100 PPM	1000 PPM
DAY 6	MEAN	14.5	14.1	14.4	14.9**
	S.D.	1.31	0.98	1.26	1.32
	N	10	10	10	10
7	MEAN	14.5	17.0	16.9	15.2*
	S.D.	1.13	1.40	1.26	1.30
	N	10	10	10	10
8	MEAN	14.9	14.8	14.8	14.0
	S.D.	1.13	1.12	1.34	1.30
	N	10	10	10	10
9	MEAN	16.3	15.9	15.5	14.1**
	S.D.	1.16	0.74	1.43	1.26
	N	10	10	10	10
10	MEAN	16.7	16.9	16.8	15.6
	S.D.	1.43	1.11	1.32	1.43
	N	10	10	10	10

* = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT 0.05 LEVEL
** = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT 0.01 LEVEL

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GLUTARALDEHYDE: TWO-WEEK WATER INCLUSION STUDY IN RATS
FOOD CONSUMPTION (GRAMS/ANIMAL/DAY): SUMMARY OF MEANS
MALES

GLUTARALDEHYDE CONCENTRATION:		0 PPM	10 PPM	100 PPM	1000 PPM
DAY 11	MEAN	17.9	18.4	18.0	16.9
	S.D.	1.06	1.21	0.99	1.47
	N	10	10	10	10
12	MEAN	17.0	17.4	17.5	16.1*
	S.D.	0.87	0.89	1.09	1.05
	N	10	10	10	10
13	MEAN	16.4	17.3	16.6	16.2
	S.D.	1.40	0.71	1.24	1.20
	N	10	10	10	10
14	MEAN	16.6	16.6	17.2	15.9
	S.D.	1.52	1.30	1.38	0.97
	N	10	10	10	10

* = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT 0.05 LEVEL

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Oral short-term repeated dose toxicity

14 day-study with rats

GLUTARALDEHYDE: TWO-WEEK WATER INCLUSION STUDY IN RATS
FOOD CONSUMPTION (GRAMS/ANIMAL/DAY): SUMMARY OF MEANS
FEMALES

GLUTARALDEHYDE CONCENTRATION:		0 PPM	10 PPM	100 PPM	1000 PPM
DAY 1	MEAN	10.1	11.088	10.3	9.588
	S.D.	0.58	0.50	0.35	0.28
	N	10	10	10	10
2	MEAN	11.2	11.4	11.1	10.088
	S.D.	0.95	0.32	0.39	0.84
	N	10	10	10	10
3	MEAN	11.2	11.2	11.3	10.288
	S.D.	0.53	0.70	0.46	0.98
	N	10	10	10	10
4	MEAN	11.6	12.0	12.4	11.0
	S.D.	0.83	0.74	0.79	0.62
	N	10	10	10	10
5	MEAN	12.0	11.8	11.8	12.0
	S.D.	0.91	0.85	0.96	0.52
	N	10	10	10	10

88 = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT 0.01 LEVEL

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GLUTARALDEHYDE: TWO-WEEK WATER INCLUSION STUDY IN RATS
FOOD CONSUMPTION (GRAMS/ANIMAL/DAY): SUMMARY OF MEANS
FEMALES

GLUTARALDEHYDE CONCENTRATION:		0 PPM	10 PPM	100 PPM	1000 PPM
DAY 11	MEAN	12.0	12.1	12.0	12.1
	S.D.	0.73	0.61	0.55	0.48
	N	10	10	10	10
12	MEAN	11.7	12.0	12.1	12.0
	S.D.	1.27	0.74	0.66	0.68
	N	10	10	10	10
13	MEAN	11.7	11.9	11.7	10.9
	S.D.	1.02	0.67	0.71	0.79
	N	10	10	10	10
14	MEAN	10.9	11.7	10.8	10.1
	S.D.	0.88	1.14	1.07	0.94
	N	10	10	10	10

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP

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Annex Point IIA6.3 / 6.4 / 6.5

Repeated dose toxicity

Oral short-term repeated dose toxicity

14 day-study with rats

4.4 Water consumption

Water consumption was reduced each day of the study for males and females from the high dose group (1000 ppm; see tables 1 & 2). Females from the 100 ppm group had a slightly depressed water consumption which was significantly different from controls only at days 2, 3, 5, 6, 7 and 12.

Water consumption (g/animal/day) of males					
Treatment period (2 weeks)					
Week		0 ppm	10 ppm	100 ppm	1000 ppm
1	Mean (N)/SD	19.4 (10)/ 1.15	19.2 (9)/ 1.46	18.6 (10)/ 1.13	**10.7** (9)/ 0.92**
2	Mean (N)/SD	17.4 (10)/ 1.22	18.1 (10)/ 1.76	18.1 (10)/ 1.12	**13.1** (10)/1.11**
3	Mean (N)/SD	20.2 (10)/ 1.45	19.6 (10)/ 2.11	19.4 (10)/ 1.00	**14.1** (10)/ 1.59**
4	Mean (N)/SD	19.8 (10)/ 1.47	19.5 (10)/ 2.24	20.1 (10)/ 1.20	**14.1** (10)/ 0.94**
5	Mean (N)/SD	20.4 (10)/ 1.61	19.6 (10)/ 2.34	20.1 (10)/ 1.68	**15.2** (10)/ 1.20**
6	Mean (N)/SD	20.7 (10)/ 1.93	19.4 (10)/ 2.53	20.2 (10)/ 1.24	**15.4** (10)/ 1.58**
7	Mean (N)/SD	21.3 (9)/ 2.15	20.8 (10)/ 2.01	211 (10)/ 1.52	**15.9** (10)/ 1.60**
8	Mean (N)/SD	19.3 (9)/ 1.69	18.9 (10)/ 2.57	18.6 (10)/ 1.61	**14.9** (10)/ 1.66**
9	Mean (N)/SD	18.2 (10)/ 1.80	18.4 (10)/ 1.89	19.1 (10)/ 1.78	**14.5** (10)/ 1.49**
10	Mean (N)/SD	22.6 (10)/ 2.40	20.9 (10)/ 2.57	22.6 (10)/ 2.31	**17.4** (10)/ 2.38**
11	Mean (N)/SD	23.1 (10)/ 2.33	23.0 (10)/ 2.07	23.0 (10)/ 2.13	**19.0** (10)/ 4.22**
12	Mean (N)/SD	21.4 (10)/ 1.95	21.5 (10)/ 1.93	22.3 (10)/ 1.65	18.9 (10)/ 4.88
13	Mean (N)/SD	22.1 (10)/ 1.94	22.0 (10)/ 2.42	22.5 (10)/ 2.33	**18.5** (10)/ 2.22**
14	Mean (N)/SD	23.4 (10)/ 2.60	22.7 (10)/ 2.28	22.6 (10)/ 2.42	**18.5**(10)/ 2.05**

**, significantly different from control at 0.01 level

Water consumption (g/animal/day) of females					
Treatment period (2 weeks)					
Week		0 ppm	10 ppm	100 ppm	1000 ppm
1	Mean (N)/SD	16.2 (10)/ 1.55	16.9 (10)/ 1.61	15.3 (10)/ 0.57	**10.0** (10)/ 1.11**
2	Mean (N)/SD	17.3 (10)/ 1.73	16.4 (9)/ 1.64	**15.1** (10)/ 1.04**	**11.5** (10)/1.07**
3	Mean (N)/SD	16.8 (10)/ 2.43	15.4 (9)/ 1.67	**15.1* (10)/ 1.06**	**10.8** (10)/ 1.39**

4	Mean (N)/SD	16.9 (10)/ 2.00	16.9 (10)/ 2.06	16.4 (10)/ 1.69	**12.9** (10)/ 1.21**
5	Mean (N)/SD	18.0 (10)/ 1.96	**15.7** (10)/ 1.44**	**15.6**(10)/ 1.02**	**12.8** (10)/ 1.94**
6	Mean (N)/SD	17.8 (10)/ 1.80	16.8 (10)/ 1.24	**16.3* (10)/ 1.24**	**12.8** (10)/ 1.40**
7	Mean (N)/SD	17.7 (10)/ 1.55	17.1 (10)/ 1.67	**15.6**(10)/ 1.20**	**12.6** (10)/ 1.36**
8	Mean (N)/SD	15.9 (10)/ 1.77	14.9 (10)/ 1.97	14.5 (10)/ 1.56	**12.1** (10)/ 1.57**
9	Mean (N)/SD	16.8 (10)/ 1.20	17.0 (10)/ 2.69	16.3 (10)/ 2.09	**12.4** (10)/ 1.52**
10	Mean (N)/SD	17.9 (10)/ 2.06	16.8 (10)/ 1.61	16.5 (10)/ 2.12	**12.3** (10)/ 1.38**
11	Mean (N)/SD	17.4 (10)/ 1.26	16.8 (10)/ 1.46	16.1 (10)/ 1.62	**12.9** (10)/ 1.73**
12	Mean (N)/SD	17.6 (10)/ 1.88	16.6 (10)/ 1.69	**15.6* (10)/ 1.86**	**13.1** (10)/ 1.32**
13	Mean (N)/SD	17.1 (10)/ 1.93	16.3 (10)/ 1.54	15.7 (10)/ 1.32	**12.3** (10)/ 1.73**
14	Mean (N)/SD	17.9 (10)/ 1.64	17.1 (10)/ 2.82	16.9 (10)/ 1.32	**12.8**(10)/ 1.12**

*, significantly different from control at 0.05 level

**, significantly different from control at 0.01 level

4.5 Blood analysis

4.5.1 Haematology — No biologically significant changes were observed in the 100 or 1000 ppm groups. Due to the uncertainty of dosage, clinical pathology measurements were not performed for the 10 ppm group.

4.5.2 Clinical chemistry — No biologically significant changes were observed in the 100 or 1000 ppm groups. Due to the uncertainty of dosage, clinical pathology measurements were not performed for the 10 ppm group.

4.5.3 Urinalysis — Not performed

4.6 Sacrifice and pathology

4.6.1 Organ weights — The final body weights, absolute organ weights and relative organ weights showed no biologically significant effects an any measurements for either sex. In fact, an increase in relative kidney weight was reported for the females of the 1000 ppm group, which was of sufficiently small magnitude to be considered not biologically significant. Other statistically significant findings were considered not biologically significant.

4.6.2 Gross and histopathology — Necropsy revealed no treatment-related gross lesions in the 100 and the 1000 ppm groups; animals of the 10 ppm group were not necropsied. No treatment-related findings were obvious from histopathological examination of the tongue, stomach and liver of the treated animals.

Section A6.3.1 _ 01 **Repeated dose toxicity**

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14 day-study with rats

Mild mucus gland hyperplasia was seen in the stomachs of two males of the 1000 ppm group, which indicated early lesions resulting from glutaraldehyde treatment.

4.7 Other

Based on water consumption, the mean calculated dose of glutaraldehyde (based an 49.8% purity) for the males of the 1000 ppm and 100 ppm groups was calculated to be 100.7 and 12.8 mg/kg body weight/day, respectively. The corresponding values for females were 105.5 and 13.6 mg/kg body weight/day.

Due to the loss of glutaraldehyde, dose was not determined for the 10 ppm group (see 3.6)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The authors investigated the effects of 50 % glutaraldehyde on rats following continuous treatment over 14 consecutive days; the test substance was offered to the animals in the drinking water.

Test substance: [redacted]
Identification No: [redacted] % (w/w; [redacted]

No guideline was mentioned, however the study was well documented/described and basic data were given. The study followed GLP.

[redacted] rats were purchased from [redacted]; at test starting they were about 6 weeks old and the body weight ranged from 112.9 to 129 g for the males and from 91.6 to 103.5 g for the females. The animals received [redacted] in the drinking water for a period a 2 weeks at following test concentrations:0, 10, 100 and 1000 ppm (w/w). The animals were observed for mortality, clinical symptoms of toxicity, body weight and food and water consumption. Haematological and clinical-chemical parameters were assessed. At necropsy, the animals were examined (histo)pathologically and organ weights were assessed.

The test substance/test solutions were analyzed for homogeneity and stability and the correctness of the test concentrations was verified.

5.2 Results and discussion

The main findings can be summarized as follows:

Actual dosages: Based on water consumption, the mean calculated dose of glutaraldehyde (based an [redacted]% purity) for the males of the 1000 ppm and 100 ppm groups was calculated to be 100.7 and 12.8 mg a.i./kg body weight/day, respectively. The corresponding values for females were 105.5 and 13.6 mg/kg body weight/day.

Mortality and clinical signs of toxicity: Neither mortality nor clinical signs of toxicity related to the treatment were observed. A single case of urine staining in a female from the 1000 ppm group was reported.

Body weight changes: Body weight and body weight gain were inconspicuous in males. In females, absolute body weight was not affected by the treatment; however body weight gain was reduced in females of the 1000 ppm group by day 14 of the study.

Food consumption: Food consumption for males and females was

Section A6.3.1 _ 01 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Oral short-term repeated dose toxicity**

14 day-study with rats

depressed for the 1000 ppm at day 1. Statistically significant reductions were noted through day 3 for females and day 7 for males of this dosage group. Food consumption remained lower for males of the high dose group throughout the study. No other effects an food consumption were noted.

Water consumption: Water consumption was reduced each day of the study for males and females from the high dose group (1000 ppm).

Haematology and clinical chemistry: All considered parameters were inconspicuous.

Necropsy: Organ and body weight (absolute and relative) at necropsy were inconspicuous, and no gross abnormalities were seen. Histopathology revealed mild mucus gland hyperplasia in the stomachs of two males of the 1000 ppm group, which indicated early lesions resulting from glutaraldehyde treatment.

5.3 Conclusion

5.3.1	LO(A)EL	1000 ppm, corresponding to 100.7 mg a.i./kg bw/day for males and 105.5 mg a.i./kg bw/day for females.
5.3.2	NO(A)EL	100 ppm, corresponding to 12.8 mg a.i./kg bw/day for the males and 13.6 mg a.i./kg bw/day for the females.
5.3.3	Reliability	**2**
5.3.4	Deficiencies	In the 10 ppm solution, recovery of glutaraldehyde at day 7 ranged between 93 and 0%, and at days 14, 18 and 20, the concentrations were below limit detection. Further testing revealed that the loss of test substance in the 10 ppm solution was most likely due to the contact with the neoprene rubber stoppers of the water bottles (unpublished observations). Due to the uncertainty of dosage, clinical pathology measurements were not performed for the 10 ppm group and animals of the 10 ppm group were not necropsied.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 28th, 2010
Materials and Methods	Agree with applicant's version. **3.1.2** This refers to Doc IIIA Section A2.
Results and discussion	Agree with applicant's version.
Conclusion	LO(A)EL: 1000 ppm, corresponding to 100.7 mg a.i./kg bw/day for males and 105.5 mg a.i./kg bw/day for females (based on mild mucus gland hyperplasia in the stomach of two males, on reduced body weight gain for females and on slightly increased kidney weight in females) NO(A)EL: 100 ppm, corresponding to 12.8 mg a.i./kg bw/day for the males and 13.6 mg a.i./kg bw/day for the females
Reliability	2

Section A6.3.1 _ 01	**Repeated dose toxicity**
Annex Point IIA6.3 / 6.4 / 6.5	**Oral short-term repeated dose toxicity** **14 day-study with rats**

Acceptability	Acceptable
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 A6.3.2 _ 01 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Dermal short-term repeated dose toxicity**

10 applications on the dorsal skin of mice

			Official use only
		1 REFERENCE	
1.1	**Reference**	Ballantyne B (1986) Glutaraldehyde review of toxicological studies and human health effects. Union Carbide Corporation, Specialty Chemicals Division, Danbury (Published), BPD ID A6.03.2_01	
1.2	**Data protection**	No	
1.2.1	Data owner	None (published data)	
1.2.2	Companies with letter of access	[redacted]	X
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline was mentioned, however the study was well documented/described and basic data were given.	
2.2	**GLP**	GLP was not compulsory at the time the study was conducted.	
2.3	**Deviations**	Not relevant	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde [redacted]	
3.1.1	Lot/Batch number	No data	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	The test material was an aqueous solution	
3.1.2.2	Purity	50% a.i.	
3.1.2.3	Stability	No data on stability provided	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	No data	
3.2.6	Number of animals per group	Ten mice/group	
3.2.7	Control animals	Yes	

Section A6.3.2 _ 01	**Repeated dose toxicity**
Annex Point IIA6.3 / 6.4 / 6.5	**Dermal short-term repeated dose toxicity** **10 applications on the dorsal skin of mice**

3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	14 days
3.3.2	Frequency of exposure	Each animal received 10 applications
3.3.3	Post exposure period	None
3.3.4	**Dermal**	
3.3.4.1	Area covered	Each application consisted of 50 µl of test solution, which were applied to the clipped dorsal skin of each mouse
3.3.4.2	Occlusion	None
3.3.4.3	Vehicle	Deionized water was used for dilution.
3.3.4.4	Concentration in vehicle	0.05, 0.25, 0.5, 2.5, 5, 25 and 50% glutaraldehyde (w/w)
3.3.4.5	Total volume applied	50 µl /application
3.3.4.6	Duration of exposure	Continuous
3.3.4.7	Removal of test substance	None
3.3.4.8	Controls	Deionized water
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	The mice were observed for clinical signs indicative of toxicity as well as for skin changes.
3.4.1.2	Mortality	The mice were observed for mortality.
3.4.2	Body weight	Body weights were recorded.
3.4.3	Food consumption	Not considered
3.4.4	Water consumption	Not considered
3.4.5	Ophthalmoscopic examination	Not considered
3.4.6	Haematology	Not considered
3.4.7	Clinical Chemistry	Not considered
3.4.8	Urinalysis	Not considered
3.5	**Sacrifice and pathology**	Animals that died during the test period were subjected to necropsy as soon as possible; at test ending, the surviving animals were sacrificed for the purpose of necropsy.
3.5.1	Organ Weights	Not considered
3.5.2	Gross and	The animals were necropsied for the purpose of gross pathology and

	histopathology	histopatholgy
3.5.3	Other examinations	None
3.5.4	Statistics	Not specified
3.6	**Further remarks**	Original reference: [redacted] (1981) Evaluation of the subacute dermal toxicity of glutaraldehyde in mice. [redacted] *cited in* [redacted] (1986) Glutaraldehyde review of toxicological studies and human health effects. [redacted]
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	At applied concentrations of less than 5%, there were no signs of toxicity.
4.1.2	Mortality	All animals receiving 25 or 50% glutaraldehyde died after 4 to 9 applications.
4.2	**Body weight gain**	All animals receiving 25 or 50% glutaraldehyde lost weight. Mice receiving 5% glutaraldehyde had decreased body weights after 4 and 6 doses, but not thereafter. At applied concentrations of less than 5%, there were no effects on body weight
4.3	**Food consumption and compound intake**	Not performed
4.4	**Ophtalmoscopic examination**	Not performed
4.5	**Blood analysis**	
4.5.1	Haematology	Not performed
4.5.2	Clinical chemistry	Not performed
4.5.3	Urinalysis	Not performed
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Not performed
4.6.2	Gross and histopathology	Necropsy of animals that died and of those that were sacrificed revealed no abnormalities
4.7	**Other**	No data
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The short-term repeated percutaneous toxicity ofglutaraldehyde was investigated in mice by applying 50 µl of various concentrations of glutaraldehyde in water to the clipped dorsal skin. Test substance: Glutaraldehyde 50% No guideline was mentioned and it was not specified whether the study followed GLP.

Section A6.3.2 _ 01	**Repeated dose toxicity**
Annex Point IIA6.3 / 6.4 / 6.5	**Dermal short-term repeated dose toxicity** **10 applications on the dorsal skin of mice**

		Each test group consisted of 10 male mice. Following concentrations were tested: 0.05, 0.25, 0.5, 2.5, 5, 25 and 50% (w/w). These concentrations corresponded to 1.4, 7.0. 14.0, 70.0, 140, 700 and 1400 mg/kg bw/day.
5.2	**Results and discussion**	All animals receiving 25 or 50% glutaraldehyde lost weight and died after 4 to 9 applications, indicating that cumulative toxicity is possible by repeated dermal contact with 25 or 50% solutions of glutaraldehyde. No consistent features were seen an necropsy of these animals. Mice receiving 5% glutaraldehyde had decreased body weights after 4 and 6 doses, but not thereafter. At applied concentrations of less than 5%, there was no evidence of short-term cumulative toxicity.
5.3	**Conclusion**	
5.3.1	LO(A)EL	25% glutaraldehyde, i.e. about 700 mg a.i./kg bw/day
5.3.2	NO(A)EL	5% glutaraldehyde, i.e. about 140 mg/kg bw/day
5.3.3	NOEL	2.5 % glutaraldehyde, i.e. about 70 mg/kg bw/day
5.3.4	Reliability	**2**
5.3.5	Deficiencies	The data were reported in an acknowledged toxicological review on glutaraldehyde, published 1996 by Ballantyne B.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 28th, 2010
Reference	1.2.2 Apparently this is a mistake, as it is also reported that this is published data with no data owner.
Materials and Methods	There is very little information on the test substance, animals and methodology. The information given in the study summary is misleading or is taken from other sources. The full information on materials and methods, as given in the original report, is as follows: "*The short-term repeated percutaneous toxicity of glutaraldehyde was investigated by applying 50 ul of various concentrations of glutaraldehyde in water to the clipped dorsal skin of male C3H/HeJ mice for a total of 10 applications. There were 10 mice for each concentration tested: 0.05, 0.25, 0.5, 2.5, 5, 25 and 50 % (w/w).*"
Results and discussion	There is very little information on the results. The full information on results, as given in the original report, is as follows: "*All animals receiving 25 or 50 % glutaraldehyde lost weight and died after 4 to 9 applications. No consistent features were seen on necropsy of these animals. Mice receiving 5 % glutaraldehyde had decreased body weights after 4 and 6 doses, but not thereafter. At applied concentrations of less than 5 %, there were no signs of toxicity and no effects on body weight.*"
Conclusion	No conclusions can be made because the original data is not available and reporting is poor.

Section A6.3.2 _ 01	**Repeated dose toxicity**
Annex Point IIA6.3 / 6.4 / 6.5	**Dermal short-term repeated dose toxicity** **10 applications on the dorsal skin of mice**

Reliability	4
Acceptability	Not acceptable
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 A6.3.3 _ 01 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Short-term repeated dose toxicity, Inhalation**

9 day-study with rats

Official use only

		1 REFERENCE
1.1	**Reference**	Ballantyne B, Greenspan BJ, Fowler EH, Snellings WM (1985) Subchronic inhalation toxicity of glutaraldehyde. The Toxicologist 5: 29, Abstract 115 (Published), BPD ID A6.03.3_01 Ballantyne B (1986) Glutaraldehyde review of toxicological studies and human health effects. Union Carbide Corporation, Specialty Chemicals Division, Danbury (Published), BPD ID A6.03.1_01
1.2	**Data protection**	No
1.2.1	Data owner	None (published data)
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No guideline was mentioned, however the study was well documented/described and basic data were given.
2.2	**GLP**	GLP was not compulsory at the time the study was conducted.
2.3	**Deviations**	Not relevant
		3 MATERIALS AND METHODS
3.1	**Test material**	Glutaraldehyde [redacted]
3.1.1	Lot/Batch number	No data
3.1.2	Specification	As given in section 2
3.1.2.1	Description	The test material was an aqueous solution
3.1.2.2	Purity	About [redacted]% water)
3.1.2.3	Stability	The test substance was subjected to a compositional analysis at the end of the study, which confirmed the stability of glutaraldehyde throughout the experimental period.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	[redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	Male/Female
3.2.5	Age/weight at study initiation	At test starting the animals were 55 days old.

Section A6.3.3 _ 01	Repeated dose toxicity
Annex Point IIA6.3 / 6.4 / 6.5	Short-term repeated dose toxicity, Inhalation 9 day-study with rats

3.2.6	Number of animals per group	Ten animals per sex were assigned to each of the three test groups and the control group.
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	The rats were exposed to glutaraldehyde vapor during 9 days over a period of 11 days according to following regimen: - 6 hours of exposure per day for 5 consecutive days, - Resting period of 2 days, - 6 hours of exposure per day for 4 consecutive days.
3.3.2	Frequency of exposure	See above
3.3.3	Post exposure period	None
3.3.4	**Inhalation**	
3.3.4.1	Vapor generation	The glutaraldehyde vapor was generated by means of vapor generators developed by the Special Instrumentation Division of Union Carbide Corporation. Air was drawn through a dehumidifier and was then heated and conducted into a rotating evaporator tube into which the liquid solution to be vaporized was metered. The hot air evaporated the sample and the vapor further was diluted at the chamber air intake.
3.3.4.2	Monitoring of the test substance-vapour concentration	Approximatively once an hour, air samples from the inhalation chamber were taken and were subjected to concentration analysis by means of a Perkin Elmer 3920B dual column gas chromatograph. The temperature and humidity within the inhalation chamber were monitored at least 4 times during each 6-hour exposure period.
3.3.4.3	Test concentrations	The test concentrations were: 0.2, 0.63 and 2.1 ppm (corresponding to 0.00083, 0.0026 and 0.0087 mg/l)
3.3.4.4	Controls	Control was treated similarly as the test groups but without test substance.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	The animals were observed for clinical symptoms of toxicity.
3.4.1.2	Mortality	The animals were observed for mortality.
3.4.2	Body weight	The animals were observed for changes in body weight.
3.4.3	Food consumption	The animals were observed for food consumption.
3.4.4	Water consumption	Not considered.
3.4.5	Ophthalmoscopic examination	The animals were subjected to ophthalmologic examinations.
3.4.6	Haematology	Not considered.
3.4.7	Clinical Chemistry	Not considered.

Section A6.3.3 _ 01	Repeated dose toxicity
Annex Point IIA6.3 / 6.4 / 6.5	Short-term repeated dose toxicity, Inhalation 9 day-study with rats

3.4.8	Urinalysis	Not considered.
3.4.9	Further examination	The animals were subjected to a functional observational battery (modified Irwin Screen Test). The modified Irwin Screen Test was conducted on 5 rats/sex of the 2.09 ppm group (tested prior and after the first, second, fifth and sixth exposure, and prior sacrifice) and on 5 rats/sex of the 0.63 ppm group (tested prior sacrifice). The animals of the 0.20 ppm and the control groups were not screened. Following parameters were considered: corneal response, pupil response, tail pinch, toe pinch, righting reflex, locomotor activity, impaired gait, respiration, tremors, convulsions, salivation, piloerection, diarrhea, tail elevation, lacrimation, stereotypy.
3.5	**Sacrifice and pathology**	Animals that died or had to be sacrificed in extremis because of bad state of health during the experiment were subjected to necropsy; survivors were sacrificed at the end of the experiment and were also subjected to necropsy.
3.5.1	Organ Weights	Necropsy included organ weighing (absolute and relative) of liver, lungs, kidneys and testes (males).
3.5.2	Gross and histopathology	Necropsy included gross pathological and histopathological examinations of organs and tissues.
3.5.3	Other examinations	None.
3.5.4	Statistics	The statistical assessment of the findings was based on Bartlett´s test for homogeneity of variance, on the analysis of variance and on Duncan´s multiple range test. Depending on the results of these tests, Student´s test, the Cochran t-test and Fisher´s exact test were considered
3.6	**Further remarks**	Original reference: Greenspan BJ (1983) Glutaraldehyde nine-day inhalation study on rats. Bushy Run Research Center, Pennsylvania, Report No: 46-95 (sponsor: Union Carbide Corporation), ***cited in*** Ballantyne B (1986) Glutaraldehyde review of toxicological studies and human health effects. Union Carbide Corporation, Specialty Chemicals Division, Danbury (Published)
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	The rats of both sexes treated with 2.1 and 0.63 ppm displayed a series of symptoms including lacrimation, nasal discharge, salivation, mouth breathing and labored respiration. These symptoms were more prominant in the 2.1 ppm group and became more severe towards the end of the experimental period. Intermittent and slight nasal discharge, lacrimation and salivation also were noticed in the 0.20 ppm group.
4.1.2	Mortality	9/10 males and 7/10 females of the 2.1 ppm group and 1/10 males of the 0.63 ppm group died, predominantly during the second half of the experimental period.
4.2	**Body weight gain**	Both males and females of the 2.1 ppm group showed statistically significant reductions in body weight change when compared to controls (7 to 8% body weight loss after the first exposure; 38% loss for the only surviving male and 34% loss for the surviving females at the end of the experiment). A statistically significant reduction in body weight gain

		also was reported for the 0.63 and 0.20 ppm males and for the 0.63 ppm females.
4.3	**Food consumption**	Food consumption for males and females of the 2.1 and the 0.63 ppm groups was significantly reduced compared to control (respectively 5% of control values after 4 exposures for the 2.09 ppm group, and 26% of control values after 9 exposures for the 0.63 ppm group). In the 0.20 ppm group, reduction in food consumption only was reported for the males.
4.4	**Ophtalmoscopic examination**	Dullness of the cornea was seen in 5/9 males and 2/7 females of the 2.1 ppm group that died during the experimental period. The only male survivor of the 2.1 ppm group exhibited keratitis.
4.5	**Blood analysis**	
4.5.1	Haematology	Not performed
4.5.2	Clinical chemistry	Not performed
4.5.3	Urinalysis	Not performed
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Because of the reduced number of male survivors in the 2.1 ppm group, organ weight was not considered. For the males of the 0.63 ppm group, following findings were reported: Significant decrease in absolute liver weight (49%), absolute lung weight (15%), absolute kidney weight (22%) and absolute testes weight (17%); significant decrease in relative liver weight (20%) and significant increase in relative lung weight (37%), relative kidney weight (25%) and relative testes weight (30%). For the 0.63 ppm females, a significant decrease in relative liver weight (16%) and increase in relative lung weight (27%) were reported.
4.6.2	Gross and histopathology	The main pathological findings reported for the animals of the 2.1 ppm group were findings that are commonly seen in case of autolysis (9/10 males and 7/10 females died during the experiment). Females of the 2.1 and the 0.63 ppm groups displayed gas in their stomach and intestines; this finding was seen as a consequence of mouth breathing and gasping. In males of the 2.1 ppm group, necropsy revealed a significantly increased incidence of corneal dullness, nasal discharge and congestion of the nasal cavity. Because of autolysis or congestion, no histopathological assessment of the findings in the nasal cavity was possible.
4.7	**Other**	Modified Irwin Screen Test: The males and females of the 2.09 ppm group showed hypoactivity, abnormal righting reflex, impaired gait, slow corneal response, small pupils and slow locomotor activity. In the 0.63 ppm group, two males and three females suffered from abnormal breathing (abdominal and mouth); one female further showed lacrimation, small pupils, showed corneal response and decreased locomotor activity.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and**	The author investigated the effects of glutaraldehyde 50% in male and

Section A6.3.3 _ 01	**Repeated dose toxicity**
Annex Point IIA6.3 / 6.4 / 6.5	**Short-term repeated dose toxicity, Inhalation** **9 day-study with rats**
methods	female [redacted] rats, which were exposed to vapor of the test substance 6 hours /day, nine days, over a period of 11 days. The study also was a range-finding study for the determination of the adequate exposure concentration to be used for a 90-day inhalation study. Test substance: Glutaraldehyde [redacted] No guideline was mentioned, however the study was well documented/described and basic data were given. GLP was not compulsory at the time the study was conducted. The rats were obtained from Microbiological Associates (Walkersville, MD). Ten animals per sex were assigned to each of the three test groups and the control group. At test starting the animals were 55 days old. The glutaraldehyde vapor was generated by means of vapor generators developed by the Special Instrumentation Division of Union Carbide Corporation. Air was drawn through a dehumidifier and was then heated and conducted into a rotating evaporator tube into which the liquid solution to be vaporized was metered. The hot air evaporated the sample and the vapor further was diluted at the chamber air intake. Approximatively once an hour, air samples from the inhalation chamber were taken and were subjected to concentration analysis by means of a Perkin Elmer 3920B dual column gas chromatograph. The rats were exposed to the glutaraldehyde vapor according to following regimen: - 6 hours of exposure per day for 5 consecutive days, - Resting period of 2 days, - 6 hours of exposure per day for 4 consecutive days. The test concentrations were: 0.2, 0.63 and 2.1 ppm (corresponding to 0.00083, 0.0026 and 0.0087 mg/l) The animals were observed for mortality and clinical symptoms of toxicity and were subjected to a functional observational battery (modified Irwin Screen Test). The modified Irwin Screen Test was conducted on 5 rats/sex of the 2.09 ppm group (tested prior and after the first, second, fifth and sixth exposure, and prior sacrifice) and on 5 rats/sex of the 0.63 ppm group (tested prior sacrifice). Furthermore, ophthalmologic examinations, body weight measurements and evaluation of food consumption were performed. Animals that died or had to be sacrificed in extremis because of bad state of health during the experiment were subjected to necropsy; survivors were sacrificed at the end of the experiment and were also subjected to necropsy. Necropsy included gross pathological and histopathological examinations of organs and tissues, as well as organ weighing. The statistical assessment of the findings was based on Bartlett's test for homogeneity of variance, on the analysis of variance and on Duncan's multiple range test. Depending on the results of these tests, Student's test, the Cochran t-test and Fisher's exact test were considered. The test substance was checked for purity and stability. The temperature and humidity within the inhalation chamber were monitored at least 4 times during each 6-hour exposure period.

Section A6.3.3 _ 01 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Short-term repeated dose toxicity, Inhalation**

9 day-study with rats

5.2 Results and discussion

The main findings can be summarized as follows:

Mortality: 9/10 males and 7/10 females of the 2.1 ppm group and 1/10 males of the 0.63 ppm group died, predominantly during the second half of the experimental period.

Clinical symptoms of toxicity: The rats of both sexes treated with 2.1 and 0.63 ppm displayed a series of symptoms including lacrimation, nasal discharge, salivation, mouth breathing and labored respiration. These symptoms were more prominant in the 2.1 ppm group and became more severe towards the end of the experimental period. Intermittent and slight nasal discharge, lacrimation and salivation also were noticed in the 0.20 ppm group.

Modified Irwin Screen Test: The males and females of the 2.09 ppm group showed hypoactivity, abnormal righting reflex, impaired gait, slow corneal response, small pupils and slow locomotor activity. In the 0.63 ppm group, two males and three females suffered from abnormal breathing (abdominal and mouth); one female further showed lacrimation, small pupils, showed corneal response and decreased locomotor activity.

Ophthalmology: Dullness of the cornea was seen in 5/9 males and 2/7 females of the 2.1 ppm group that died during the experimental period. The only male survivor of the 2.1 ppm group exhibited keratitis.

Body weight: Both males and females of the 2.1 ppm group showed statistically significant reductions in body weight change when compared to controls (7 to 8% body weight loss after the first exposure; 38% loss for the only surviving male and 34% loss for the surviving females at the end of the experiment). A statistically significant reduction in body weight gain also was reported for the 0.63 and 0.20 ppm males and for the 0.63 ppm females.

Food consumption: Food consumption for males and females of the 2.1 and the 0.63 ppm groups was significantly reduced compared to control (respectively 5% of control values after 4 exposures for the 2.09 ppm group, and 26% of control values after 9 exposures for the 0.63 ppm group). In the 0.20 ppm group, reduction in food consumption only was reported for the males.

Necropsy: The main pathological findings reported for the animals of the 2.1 ppm group were findings that are commonly seen in case of autolysis (9/10 males and 7/10 females died during the experiment). Females of the 2.1 and the 0.63 ppm groups displayed gas in their stomach and intestines; this finding was seen as a consequence of mouth breathing and gasping. In males of the 2.1 ppm group, necropsy revealed a significantly increased incidence of corneal dullness, nasal discharge and congestion of the nasal cavity. Because of autolysis or congestion, no histopathological assessment of the findings in the nasal cavity was possible. Because of the reduced number of male survivors in the 2.1 ppm group, organ weight was not considered. For the males of the 0.63 ppm group, following findings were reported:

Significant decrease in absolute liver weight (49%), absolute lung weight (15%), absolute kidney weight (22%) and absolute testes weight (17%); significant decrease in relative liver weight (20%) and significant increase in relative lung weight (37%), relative kidney

Section A6.3.3 _ 01	Repeated dose toxicity
Annex Point IIA6.3 / 6.4 / 6.5	Short-term repeated dose toxicity, Inhalation 9 day-study with rats

		weight (25%) and relative testes weight (30%). For the 0.63 ppm females, a significant decrease in relative liver weight (16%) and increase in relative lung weight (27%) were reported.
5.3	**Conclusion**	
5.3.1	LO(A)EL	0.2 ppm
5.3.2	NO(A)EL	< 0.2 ppm
5.3.3	Reliability	**2**
5.3.4	Deficiencies	The present study was conducted in 1979, i.e. prior to the implementation of guidelines; furthermore, at that time GLP was not compulsory. However, the method and results were well described and well documented, and are scientifically acceptable quality. The data also were reported within the acknowledged toxicological review on glutaraldehyde, published 1996 by Ballantyne B.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 28th, 2010
Materials and Methods	
Results and discussion	
Conclusion	No conclusions can be made.
Reliability	4
Acceptability	Not acceptable
Remarks	The study summary has not been evaluated because no original data has been provided.
	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 A6.4.1 _ 01 Repeated dose toxicity

Annex Point IIA6.3 / 6.4 / 6.5

Sub-chronic oral toxicity in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2001) [redacted] % Glutaraldehyde) - Subchronic oral toxicity and neurotoxicity study in [redacted] rats - Administration in drinking water for 3 months. [redacted] (Unpublished) & Amendment, BPD ID A6.04.1_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 408 (1981); EPA OPPTS 870.6200	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, due to an error in the protocol, no ophtalmological examinations were conducted prior starting the treatment; in fact, ophtalmological examinations only were performed at the end of the treatment period.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] % Glutaraldehyde)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Homogeneous liquid, colorless-clear	
3.1.2.2	Purity	[redacted] % glutaraldehyde (w/w)	
3.1.2.3	Stability	The stability of the test substance was proven by reanalysis at the end of the experimental period; the reanalysis revealed [redacted] % a.i. (w/w).	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male / Female	
3.2.5	Age/weight at study initiation	All animals were 49 days old at test initiation. Body weight for the males, group mean: 251.6 g (228.9 - 272) g Body weight for the females, group mean: 169.4 g (152.8 – 187.5) g	
3.2.6	Number of	Each dose group comprised 15 animals per sex and was subdivided into 3	

	animals per group	sections as follows: Section A comprised the first 5 animals of each sex and group. Section B comprised the further 5 animals of each sex and group. Section C comprised the remaining 5 animals of each sex and group. The animals of section A and B were subjected to the functional observational batteries (FOB) and the motor activity assessment (MA); The animals of section A were sacrificed at the end of the experimental period and were then perfusion-fixed for neuropathological examination. The animals of section B and C were subjected to urinalysis and ophthalmological examination, and after sacrifice, they were subjected to necropsy and blood examination (see study design, 3.2.8).
3.2.7	Control animals	Yes
3.2.8	Study design	

Section A*, males	Section B**, males	Section C***, males	Section A*, females	Section B**, females	Section C***, females	Phase of study/ examination	Study day
						Arrival of the animals and start of acclimatization period	
March 2, 1999			March 9, 1999				-13
	March 2, 1999	March 2, 1999		March 9, 1999	March 9, 1999		-14
March 4, 1999			March 11, 1999			Randomization	-11
	March 4, 1999	March 4, 1999		March 11, 1999	March 11, 1999		-12
March 8, 1999	March 9, 1999		March 15, 1999	March 16, 1999		FOB, MA	-7
March 15, 1999	March 16, 1999	March 16, 1999	March 22, 1999	March 23, 1999	March 23, 1999	Day of test substance administration	0
April 6, 1999	April 7, 1999		April 13, 1999	April 14, 1999		FOB, MA	22
May 4, 1999	May 5, 1999		May 11, 1999	May 12, 1999		FOB, MA	50
June 8, 1999	June 9, 1999		June 15, 1999	June 16, 1999		FOB, MA	85
	June 11, 1999	June 11, 1999		June 18, 1999	June 18, 1999	Urinalysis	87
	June 15, 1999	June 15, 1999		June 22, 1999	June 22, 1999	Ophthalmological examination	91
June 14, 1999	June 15, 1999	June 15, 1999	June 21, 1999	June 22, 1999	June 22, 1999	Last determination of body weight	91
June 15, 1999			June 22, 1999			Perfusion fixation	92
	June 16, 1999	June 16, 1999		June 23, 1999	June 23, 1999	Blood examination and Necropsy	92

* = first 5 animals of each dose group
** = second 5 animals of each dose group
*** = third 5 animals of each dose group
FOB = functional observational battery
MA = motor activity measurement

3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	90 days
3.3.2	Frequency of exposure	Daily, 7 days per week
3.3.3	Post-exposure period	None

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

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**

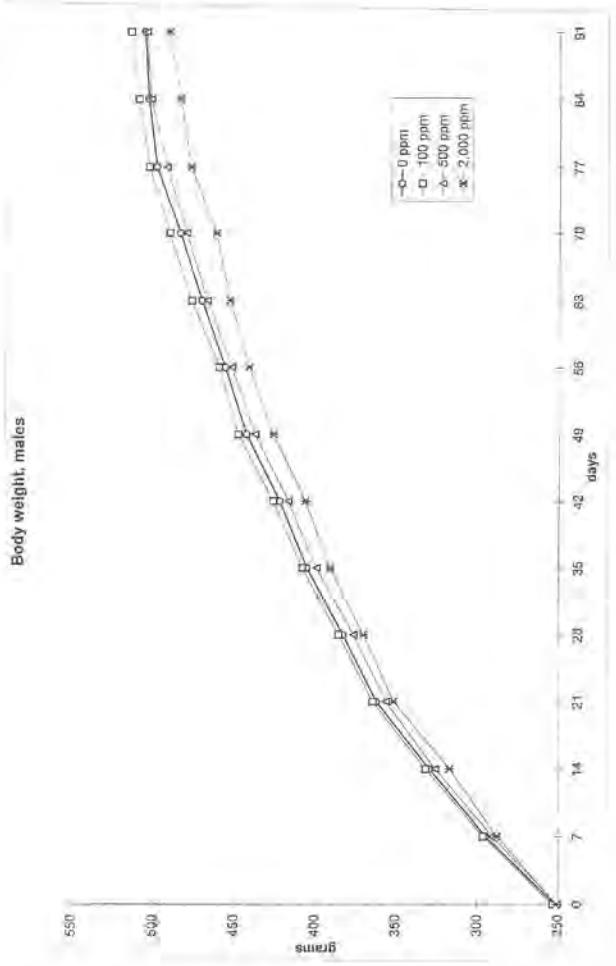
3.3.4	**Oral**	
3.3.4.1	Type	In drinking water
3.3.4.2	Concentration	0, 100, 500 and 2000 ppm
3.3.4.3	Vehicle	The test substance was administered as aqueous solution in the drinking water.
3.3.4.4	Controls	Drinking water without test substance
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	The rats were checked twice daily for clinical signs of toxicity from Mondays to Fridays, and once daily on Saturdays, Sundays and public holidays; general clinical examinations were furthermore carried out daily.
3.4.1.2	Mortality	The rats were checked twice daily for mortality from Mondays to Fridays, and once daily on Saturdays, Sundays and public holidays.
3.4.2	Body weight	The body weight of the rats was determined prior to the first neurofunctional test for randomized distribution of the animals in the test groups. This parameter was again determined at test initiation (day 0) and thereafter once a week. A further body weight measurement was done on the days when functional observational batteries (FOB) were conducted. The body weight parameter was expressed as body weight change (i.e. difference between BW on day X and BW on day 0).
3.4.3	Food consumption	The food consumption (as grams/animals/day) of the rats was determined once a week during the experiment.
3.4.4	Food efficiency	The food efficiency was calculated on the basis of the body weight and the food consumption on days where both parameters were determined simultaneously, using following formula: $\frac{\text{BW day x} - \text{BW day y}}{\text{FC}} \times 100$ BW day x = body weight on test day x (in g) BW day y = body weight on test day y (last weighing before x; in g) FC = mean food consumption between day y and day x
3.4.5	Water consumption	Water consumption of the rats was determined once a week over a period of 4 days, and was calculated as mean water consumption in grams per animal per day.
3.4.6	Intake of test substance	The mean daily intake of test substance (group mean) was calculated on the basis of following formula: $\frac{WTR_x \times C}{BW_x}$ WTR_x = mean daily water consumption on day x (in g) C = concentration of TS in drinking water on day x (in mg/kg) BW_x = Body weight on day x (in g)
3.4.7	Ophthalmoscopic	The eyes of the control and of the high-dose animals of section B and C

	examination	were examined at the end of the treatment period.	
3.4.8	Hematology	See study design Parameters: Leukocyte count (WBC; peroxidase method) Erythrocyte count (RBC; flow cytometric laserlight scattering) Hemoglobin (HGB; cyanmethehoglobin method) Hematocrit (HCT; calculated on the basis of MCV and RBC) Mean corpuscular volume (MCV; RBC/PLT method) Mean corpuscular hemoglobin (MCH; calculated on the basis of hemoglobin/ erythrocytes) Mean corpuscular hemoglobin concentration (MCHC; calculated on the basis of hemoglobin/hematocrit) Platelet count (PLT; flow cytometric laserlight scattering) Differential blood count (cytochemistry coupled with flow cytometry). Clotting analysis was performed and the prothrombin time (Hepato Quick's Test; HQT) was determined (citrated blood with calcium thromboplastin method).	
3.4.9	Clinical Chemistry	See study design Parameters: Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum-gamma-glutamyl transferase, sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulina, triglycerides, cholesterol and magnesium.	
3.4.10	Urinalysis	See study design Parameters: Volume, colour, turbidity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity and sediment.	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	The weight of following organs was determined: Anesthetized animal, liver, kidneys, adrenals, testes/ovaries, epididymides, uterus, thymus, spleen, brain and heart.	
3.5.2	Gross and histopathology	Gross lesions: All gross lesions were examined in all affected non-perfused animals per sex and group, for each test group (0, 100, 500 and 2000 ppm). Following organs and tissues were fixed with neutral buffered 4% formaldehyde for the purpose of (histo)pathological examination: All gross lesions, salivary glands, oesophagus, stomach, duodenum/jejunum/ileum, caecum/colon/rectum, liver, pancreas, brain, pituitary, sciatic nerve, spinal cord, eyes with optic nerve, adrenals, thyroid, parathyroid, trachea, lungs, pharynx, larynx, nose/nasal cavity, aorta, heart, bone marrow, lymph nodes, spleen, thymus, kidneys, urinary	X

		bladder, ureter, testes/ovaries, oviducts/uterus/vagina, epididymides/prostate/seminal vesicle, female mammary gland, skin, skeletal muscle, sternum with marrow, femur with knee joint and marrow, extraorbital lacrimal glands. The following organ samples of all non-perfused animals per sex and group of the control-and the 2000 ppm group were processed for histological assessment (i.e. paraffin embedding, sectioning and staining): Brain, pituitary, thyroid, parathyroid, thymus, trachea, lungs, pharynx, larynx, nasal cavity, aorta, heart, salivary glands, liver, spleen, kidneys, adrenals, pancreas, testes/ovaries, oviducts/uterus/vagina, epididymides/prostate/seminal vesicle, skin, oesophagus, stomach, duodenum/jejunum/ileum, caecum/colon/rectum, urinary bladder, ureter, mesenteric and mandibular lymphnodes, female mammary gland, skeletal muscle, sciatic nerve, sternum with marrow, femur bone marrow, eyes with optic nerve, femur with knee joint all spinal cord.
3.5.3	Other examinations	The animals were subjected to functional observational batteries (FOB) as well as to motor activity measurements. Five animals per sex and group were examined for neuropathology. The data referring to the neurotoxicity part of the study are presented in a separated chapter (see section 6.9).
3.5.4	Statistics	Statistical assessment of clinical data: Calculation of means and standard deviations. Statistical assessment of food/water consumption, body weight, body weight change, food efficiency: Analysis of variance (ANOVA) followed by Dunnett's test Statistical assessment of feces, rearing, grip strength (forelimbs, hindlimbs), landing foodsplay test, motor activity, hematological and clinical-chemical data (excepted differential blood count): Non-parametric one-way analysis using the Kruskal-Wallis test (two-sided) followed when necessary by the Mann-Whitney U-test (two-sided). Statistical assessment of urinalysis data (excepted volume, color, turbidity and specific gravity: Fisher's exact test. Statistical assessment of organ weight data: Non-parametric one-way analysis using the Kruskal-Wallis test (two-sided), followed when necessary by the Wilcoxon test.
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Excepted for one male control animal that showed a skin lesion on the left hindlimb with swelling of the wound region, no clinical signs of toxicity were seen.
4.1.2.	Mortality	No cases of mortality were reported.
4.2	**Body weight gain**	

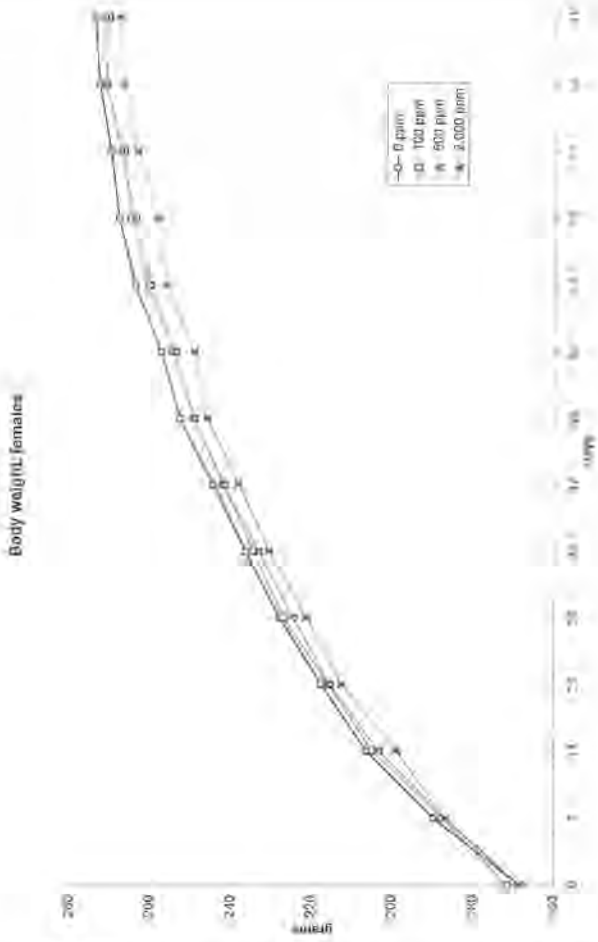
Section A6.4.1 _ 01 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**



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

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**



The figures clearly show a test substance-related effect on body weight. At 2000 ppm and at the end of the testing period (day 91), the body weight values were 3 and 2.3% below controls for males and females respectively; the corresponding body weight change values were respectively 4.5 and 8.8% below control.

4.3 Food consumption & efficiency

Food consumption:

Compared to control, a slight decrease in food consumption was observed over the whole experimental period, for both males and females treated with 2000 ppm. The decrease was statistically significant on day 70 for the males (-7%) and on day 14 for the females (-8.1%).

Food efficiency:

Food efficiency was not affected by the treatment. In fact, a statistically significant decrease in food efficiency only was seen in the 500 ppm males on day 84, and was therefore considered to be accidental (no dose-relationship).

4.4 Water consumption and compound intake

Water consumption:

A statistically significant decrease in water consumption was observed for the 2000 ppm treated males over the whole experimental period (excepted X

for day 56), with values that were up to 22.4% below control.

A statistically significant decrease in water consumption over the whole experimental period was observed for the 500 ppm treated females (excepted for day 63, 77 and 91) and the 2000 ppm treated females. In fact, for the 500 ppm females, the values were up to 19.5% below control whereas .for the 2000 ppm females, the values were up to 27.2% below control. At the lowest tested dose of 100 ppm, the water consumption of the females also was decreased compared to control (up to 16.4%), but a statistical significance only could be observed on day 14 and 49.

Mean daily TS intake over the whole experimental period:

Test group	Test concentration in water	Intake of test substance (50% glutaraldehyde solution) (mg/kg bw/day)		Intake of active ingredient (glutaraldehyde) (mg a.i./kg bw/day)	
		Males	Females	Males	Females
1	100 ppm	6.1	8.2	3.05	4.2
2	500 ppm	29.9	38.5	14.95	19.25
3	2000 ppm	106.9	144.4	53.5	72.2

4.5 Ophtalmoscopic examination

No substance-related effects were seen.

4.6 Blood analysis

4.6.1 Haematology

All parameters were inconspicuous; there were no treatment-related changes.

4.6.2 Clinical chemistry

All parameters were inconspicuous; there were no treatment-related changes. In fact, only one parameter, the triglycerides, was statistically significantly different from control in the subgroup Section B of the 500 ppm group. This finding was incidental, without any dose-response relationship, and was considered to be of no toxicological relevance.

4.6.3 Urinalysis

All parameters were inconspicuous; there were no treatment-related changes.

4.7 Sacrifice and pathology

4.7.1 Absolute organ weights

The statistically significant findings for the absolute organ weights of males and females can be summarized as follows:

Sex		Males			
Dose group (ppm)		0	100	500	2000
Terminal body weight (g)	Mean	475.11	479.31	480.03	451.32
	SD	23.01	41.71	35.07	23.26
Liver (g)	Mean	16.41	15.16	**18.2***	15.85
	SD	1.73	2.02	1.75	2.43
Brain (g)	Mean	2.10	2.08	2.03	**2.01****
	SD	0.06	0.11	0.08	0.08

Other organs	Inconspicuous

N = 10; *, p<0.05; **, p<0.01

Sex		Females			
Dose group (ppm)		0	100	500	2000
Terminal body weight (g)	Mean	260.92	257.61	254.66	253.34
	SD	20.25	16.78	23.17	11.36
All organs		Inconspicuous			

N = 10; *, p<0.05; **, p<0.01

A statistically significant increase in absolute mean liver weight was seen for the males of the 500 ppm group (ca. 10%). A statistically significant decrease in the absolute weight of the brain was reported for the males of the 2000 ppm group (ca. 4%).

4.7.2 Relative organ weights

The statistically significant findings for the relative organ weights of males and females can be summarized as follows:

Sex		Males			
Dose group (ppm)		0	100	500	2000
Terminal body weight (%)	Mean	100	100	100	100
Liver (%)	Mean	3.46	3.16	**3.79***	3.51
	SD	0.37	0.30	0.27	0.50
Other organs		Inconspicuous			

N = 10; *, p<0.05; **, p<0.01

Sex		Females			
Dose group (ppm)		0	100	500	2000
Terminal body weight (%)	Mean	100	100	100	100
All organs		Inconspicuous			

N = 10; *, p<0.05; **, p<0.01

A statistically significant increase in relative mean liver weight was seen for the males of the 500 ppm group (ca. 11%).

4.7.3 Gross and histopathology

Gross lesions:

One male of the control group showed a lesion of the skin. One male of the 100 ppm group showed a lesion within the forestomach which was defined as "Margo plicatus thickened". One male of the 100 ppm group showed a prominent acinar pattern in the liver. One male of the 500 ppm group showed deposition in the spleen. One male and two females of the 2000 ppm group showed erosion and ulceration of the glandular stomach.

Histopathological findings:

The acinar pattern seen in the liver of one male of the 100 ppm group correlated histopathologically with a moderate portal fatty change in the liver.

The skin lesion of the male control animal correlated with a benign hair follicle tumor.

4.8 Other

All findings related to the neurotoxicological assessment of the test substance are reported in section 6.9. Summarized, it can be retained that no effects indicative of a neurotoxic potential of [redacted]% glutaraldehyde) in the rat were found.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to look for the systemic toxicity and the neurotoxic potential of [redacted]% Glutaraldehyde) in rats following repeated administration over a period of 3 months. x

Test substance: [redacted]% Glutaraldehyde), [redacted]%, homogeneous, colorless-clear liquid, stability confirmed

The test was conducted according to OECD 408 (1981) with GLP.

[redacted] rats were used for the experiment and were 49 days old at test initiation; the mean group body weight was 251.6 g and 169.4 g respectively for the males and females. Each test group comprised 15 animals per sex and was further subdivided into 3 sections of 5 animals/sex each (section A, B and C). The animals of section A and B were subjected to the functional observational batteries (FOB) and the motor activity assessment (MA); The animals of section A were sacrificed at the end of the experimental period and were then perfusion-fixed for neuropathological examination. The animals of section B and C were subjected to urinalysis and ophthalmological examination, and after sacrifice, they were subjected to necropsy and blood examination.

The test substance was administered as aqueous solution in the drinking water. 0, 100, 500 and 2000 ppm; the animals were treated daily, 7 days per week, over a period of 90 days.

The rats were checked for clinical signs of toxicity, mortality, body weight, food consumption and water consumption. The food efficiency and the mean daily intake of test substance were calculated. The eyes of the control and of the high-dose animals were examined at the end of the treatment period. Hematological, clinical-chemical and urinary parameters were examined. Following sacrifice and necropsy, the absolute and relative weights of a series of organs (liver, brain, kidneys and so on) were determined and the animals were examined for gross lesions and histopathological changes.

The statistical assessment of the findings was based on a series of tests including Analysis of variance (ANOVA) followed by Dunnett's test, the Kruskal-Wallis test and Fisher's exact test.

Neurotoxicological investigation: The animals also were subjected to functional observational batteries (FOB) as well as to motor activity measurements. Five animals per sex and group (section A) were examined for neuropathology. The data referring to the neurotoxicity part of the study are presented in a separated chapter (see section 6.9).

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

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**

5.2 Results and discussion

The mean daily TS intake over the whole experimental period was calculated to be as follows:

Test group	Test concentration in water	Intake of test substance (50% glutaraldehyde solution) (mg/kg bw/day)		Intake of active ingredient (glutaraldehyde) (mg a.i./kg bw/day)	
		Males	Females	Males	Females
1	100 ppm	6.1	8.2	3.05	4.2
2	500 ppm	29.9	38.5	14.95	19.25
3	2000 ppm	106.9	144.4	53.5	72.2

Excepted for one control animal (male) showing a skin lesion, no clinical signs of toxicity were seen, and all animals survived the experiment. A test substance-related effect on body weight was recognizable; at 2000 ppm (day 91), the body weight values were 3 and 2.3% below controls for males and females respectively, and the corresponding body weight change values were respectively 4.5 and 8.8% below control. Compared to control, a slight decrease in food consumption was observed over the whole experimental period, for both males and females treated with 2000 ppm. The decrease was statistically significant on day 70 for the males (-7%) and on day 14 for the females (-8.1%). Food efficiency was not affected by the treatment. A statistically significant decrease in water consumption was observed for the 2000 ppm treated males over the whole experimental period (excepted for day 56), with values up to 22.4% below control. A statistically significant decrease in water consumption over the whole experimental period was observed for the 500 ppm treated females (up to 19.5% below control; excepted for day 63, 77 and 91) and the 2000 ppm treated females (up to 27.2% below control). At 100 ppm, the water consumption of the females also was decreased compared to control (up to 16.4%), but a statistical significance only could be observed on day 14 and 49. The examination of the eyes revealed no substance-related effects. The hematological and clinical-chemical parameters were inconspicuous.

A statistically significant increase in absolute and relative mean liver weight was seen for the males of the 500 ppm group (ca. 10-11%). A statistically significant decrease in the absolute weight of the brain was reported for the males of the 2000 ppm group (ca. 4%). Necropsy furthermore revealed following gross lesions: One case of skin lesion (male, control group), one case of "Margo plicatus thickened" in the forestomach (male, 100 ppm), one case of prominent acinar pattern in the liver (male, 100 ppm), one case of deposition in the spleen (male, 500 ppm), and 3 cases of erosion and ulceration of the glandular stomach (1male, 2 females, 2000 ppm). The acinar pattern seen in the liver of one male of the 100 ppm group correlated histopathologically with a moderate portal fatty change in the liver. The skin lesion of the male control animal correlated with a benign hair follicle tumor.

All findings related to the neurotoxicological assessment of the test substance are reported in section 6.9. Summarized, it can be retained that no effects indicative of a neurotoxic potential of [redacted]% glutaraldehyde) in the rat were found.

Section A6.4.1 _ 01	Repeated dose toxicity
Annex Point IIA6.3 / 6.4 / 6.5	Sub-chronic oral toxicity in rats

5.3	Conclusion	
5.3.1	LO(A)EL	2000 ppm for both males and females, corresponding to 106.9 mg test substance /kg bw/day for males and 144.4 mg test substance /kg bw/day for females.
5.3.2	NO(A)EL	500 ppm for both, males and females, corresponding to 29.9 mg test substance /kg bw/day for males and to 38.5 mg test substance /kg bw/day for females.
5.3.3	Other	Impairment of water consumption was assessed to be due to a palatability problem rather than treatment-related.
5.3.4	Reliability	**1**
5.3.5	Deficiencies	The study showed following deviation from the guideline 408 (10981): due to an error in the protocol, no ophtalmological examinations were conducted prior starting the treatment; in fact, ophtalmological examinations only were performed at the end of the treatment period. However, as no treatment-related effects were seen, the validity of the study was not affected by this deviation.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2nd, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2. 3.5.2 Gross and histopathology. The last words should read "femur with knee joint, spinal cord and all gross lesions" (not "femur with knee joint all spinal cord"). Otherwise agree with applicant's version.
Results and discussion	4.4 Water consumption and compound intake. The a.i. intake for 100 ppm females was 4.1 (not 4.2) mg/kg bw/day. Otherwise agree with applicant's version.
Conclusion	Agree with applicant's version. NOAEL and LOAEL are based on the effects seen in the 2000 ppm group: reduced body weight gain, erosion/ulcer in the glandular stomach of 1 male and 2 females and mean brain weight reduction in males (the last finding being possibly incidental). LOAEL (test substance): 2000 ppm (106.9 mg/kg bw/day for males and 144.4 mg/kg bw/day for females) LOAEL (Glutaraldehyde): 1010 ppm (53.5 mg/kg bw/day for males and 72.2 mg/kg bw/day for females) NOAEL (test substance): 500 ppm (29.9 mg/kg bw/day for males and 38.5 mg/kg bw/day for females) NOAEL (Glutaraldehyde): 252.5 ppm (15.0 mg/kg bw/day for males and 19.3 mg/kg bw/day for females)

Section A6.4.1 _ 01 Repeated dose toxicity

Annex Point IIA6.3 / 6.4 / 6.5

Sub-chronic oral toxicity in rats

Reliability	1
Acceptability	Acceptable
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 A6.4.1 _ 02 Repeated dose toxicity

Annex Point IIA6.3 / 6.4 / 6.5

Sub-chronic oral toxicity in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1985) Glutaraldehyde: ninety-day inclusion in drinking water of rats. [redacted] (Unpublished), ([redacted]), & Addendum, BPD ID A6.04.1_02	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, however the study was guideline-like	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	x
3.1.2.1	Description	Clear liquid	
3.1.2.2	Purity	[redacted]% (w/w) glutaraldehyde as a.i.	x
3.1.2.3	Stability	Storage in the refrigerator, test substance determined to be indefinitely stable at normal refrigerator temperature.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male/Female	
3.2.5	Age/weight at study initiation	At test initiation the rats were 6 weeks old. Body weight range at test initiation for the males: 98 – 128.6 g Body weight range at test initiation for the females: 80.1 – 100.1 g	

Section A6.4.1 _ 02 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**

3.2.6 Number of animals per group

Test group	Test Dose	Number of animals		
		Male	Female	Total
1	0 ppm	20 + 10 (for recovery phase)	20 + 10 (for recovery phase)	60
2	50 ppm	20	20	40
3	250 ppm	20 + 10 (alternative for recovery phase*)	20 + 10 (alternative for recovery phase*)	60
4	1000 ppm	20 + 10 (for recovery phase)	20 + 10 (for recovery phase)	60

*, the additional 10 animals/sex added in the 250 ppm group were intended to serve for the recovery study phase in case of excessive mortality at 1000 ppm.

3.2.7 Control animals: Yes

3.3 Administration/ Exposure

3.3.1 Duration of treatment: 90 days

3.3.2 Frequency of exposure: Daily

3.3.3 Post-exposure period: 4 weeks (week 14 to week 17)

3.3.4 Oral

3.3.4.1 Type: In drinking water

3.3.4.2 Concentration: An aqueous stock solution of 2000 ppm glutaraldehyde was prepared using the pH adjusted water. Starting from this stock solution and using the same water for dilution, following test concentrations were prepared:

50, 250 and 1000 ppm.

3.3.4.3 Vehicle: Tap water with a pH adjusted to 6 – 7 with phosphoric acid was used as vehicle

3.3.4.4 Controls: One group of rats received the vehicle without test substance. (0 ppm glutaraldehyde)

3.4 Examinations

3.4.1 Observations

3.4.1.1 Clinical signs including mortality: Detailed clinical observations were performed once/week whereas examinations for overt symptoms of toxicity were conducted on the other days.

3.4.2 Body weight: Body weights were measured once a week over the 13 weeks of treatment and over the post exposure period.

3.4.3 Food consumption: Food consumption was measured once a week over the 13 weeks of treatment and over the post exposure period.

3.4.4 Water: Water consumption was measured once a week over the 13 weeks of

	consumption	treatment and over the post exposure period.
3.4.5	Ophthalmoscopic examination	Ophthalmoscopic examinations were performed at test initiation and at test ending (i.e. sacrifice for the rats for necropsy).
3.4.6	Haematology	Blood samples were collected via orbital sinus puncture from 10 animals per sex and group after 6 weeks of treatment and at the end of the experimental period (i.e. after 13 weeks, prior sacrifice). These samples served for the evaluation of the haematological and clinical-chemical parameters. Blood samples further were collected from the animals that were subjected to an additional post-exposure period (4 additional weeks without treatment) for the assessment of recovery; sampling here was done at the end of the recovery period. Following haematological parameters were considered: Leukocyte count, erythrocyte count, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular hemoglobin concentration, platelet count, differential leukocyte count (only control and high dose) and reticulocyte count.
3.4.7	Clinical Chemistry	Following clinical-chemical parameters were considered: Glucose, urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, total protein, albumin, globulin, total and direct bilirubin, serum-gamma-glutamyltransferase, sorbitol deshydrogenase, calcium, phosphorus, sodium, potassium, chloride, and indirect bilirubin.
3.4.8	Urinalysis	At week 6 and 12 of treatment, urine was collected from 10 animals/sex/group over a period of 24 hours. Urine from the animals of the recovery phase also was sampled (17^{th} week). Following urinary parameters were considered: Volume, color, turbidity, specific gravity, pH, protein, glucose, ketones, bilirubin, blood and urobilinogen. The samples collected after 12 weeks were further subjected to microscopical examination.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	The weight of following organs was determined: Anesthetized animal, liver, kidneys, adrenals, brain, spleen and gonads.
3.5.2	Gross and histopathology	Following tissues were saved for possible histological examination: Brain (cerebellar and cerebral cortex, medulla/pons), eyes with Harderian glands, pituitary, salivary glands, heart, aorta, thymic region, thyroid, parathyroid, lungs, trachea, oesophagus, stomach, duodenum/jejunum/ileum, cecum/colon/rectum, adrenals, spinal cord, pancreas, liver, kidneys, urinary bladder, testes, epididymides, prostate, ovaries, uterus, spleen, lymph nodes, skeletal muscle, skin, sciatic nerve, mammary gland (female), bone (with marrow), tongue and all gross lesions. Following tissues from animals of the control and the 1000 ppm groups were examined for histopathology: All sampled tissues. Following tissues from animals of the 50 and the 250 ppm groups were examined histopathology: Heart, esophagus, stomach, tongue, liver and kidneys.

Animals subjected to a recovery phase:

No histopathology was performed.

3.5.3 Other examinations: None

3.5.4 Statistics: The statistical assessment of the findings was based on following tests:

Levene's test for homogeneity of variances,

Analysis of variance,

Individual t-tests.

3.6 Further remarks

Analytical monitoring of the test doses:

Analytical monitoring revealed glutaraldehyde concentrations of 96.5 +/- 2, 98.8 +/- 2.7 and 97 +/- 1.9% of nominal for the 50, 250 and 1000 ppm samples. In the control samples, no peaks at the retention time of glutaraldehyde were seen.

The analysis of samples collected from the drinking bottles offered to the rats of the 50 ppm group showed that glutaraldehyde was stable. In fact, the glutaraldehyde content in 20 randomly selected bottles following one week of exposure (different time points over the test period) ranged from 91.6 +/- 1.79 to 96.7 +/- 2.54% of nominal.

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs: No effects were seen.

4.1.2 Mortality: No mortality was observed.

Section A6.4.1 _ 02 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**

4.2 Body weight gain

Both, the absolute body weight and the body weight gain were reduced in males of the 1000 ppm group over the whole treatment period. The reduction became statistically relevant from week 3 upwards. During the recovery period, an increase in both the absolute body weight and the body weight gain was observed, but the values still remained below control values. For the females of the 1000 ppm group, the absolute body weight and the body weight gain were statistically significantly reduced from week 11 of treatment upwards. During the recovery period an adjustement of the values to controls was observed. Absolute body weight and body weight gain were inconspicuous in the 50 and 250 ppm groups for both males and females.

Summary of the statistically significant changes in absolute body weight for male rats:

Absolute body weight (g) of males during treatment period, starting from week 3 (N = 30)			
Week		0 ppm	1000 ppm
3	Mean (SD)	202.2 (12.52)	**193.2*** (11.15)
4	Mean (SD)	226.1 (13.76)	**215.0**** (11.64)
5	Mean (SD)	241.2 (15.26)	232.7 (11.74)
6	Mean (SD)	252.3 (16.68)	242.9 (12.07)
7	Mean (SD)	261.5 (17.46)	**249.2**** (11.97)
8	Mean (SD)	275.0 (17.67)	**261.7**** (12.12)
9	Mean (SD)	286.7 (19.53)	**273.4**** (13.35)
10	Mean (SD)	293.5 (20.95)	**280.9**** (13.42)
11	Mean (SD)	303.3 (20.65)	**286.8**** (13.17)
12	Mean (SD)	309.0 (21.83)	**292.0**** (14.87)
13	Mean (SD)	312.4 (21.73)	**298.5**** (15.59)
Body weight (g) of males during recovery period (N= 10)			
14	Mean (SD)	315.3 (14.40)	306.6 (9.75)
15	Mean (SD)	320.5 (14.44)	313.1 (10.73)
16	Mean (SD)	326.6 (13.86)	319.5 (10.38)
17	Mean (SD)	337.7 (14.65)	331.4 (11.43)

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

Summary of the statistically significant changes in body weight gain for male rats:

Body weight gain (g) of males over the whole experimental period			
Week		0 ppm	1000 ppm
0 - 13	Mean (SD)	202.1 (19.12)	**189.0**** (14.19)
0 - 17	Mean (SD)	225.7 (13.23)	220.4 (11.39)

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

Section A6.4.1 _ 02 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**

Summary of the statistically significant changes in absolute body weight for female rats:

Absolute body weight (g) of females during treatment period, starting from week 3 (N = 30)			
Week		0 ppm	1000 ppm
11	Mean (SD)	177.5 (8.00)	**171.9*** (7.66)
12	Mean (SD)	180.0 (8.53)	**174.5*** (8.18)
13	Mean (SD)	180.4 (8.47)	**175.4*** (8.20)
Body weight (g) of females during recovery period (N= 10)			
14	Mean (SD)	179.9 (5.72)	179.0 (10.46)
15	Mean (SD)	180.3 (6.67)	182.9 (9.83)
16	Mean (SD)	183.7 (6.73)	186.1 (9.70)
17	Mean (SD)	192.5 (6.53)	194.5 (9.05)

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

Summary of the statistically significant changes in body weight gain for female rats:

Body weight gain (g) of males over the whole experimental period			
Week		0 ppm	1000 ppm
0 - 13	Mean (SD)	90.3 (6.61)	**86.0*** (5.82)
0 - 17	Mean (SD)	102.4 (3.87)	106.9 (5.57)

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

The changes affecting the body weight ran parallel to those reported for the food consumption and were therefore considered to be a direct consequence of the changes seen in food consumption rather than being due to the treatment.

Section A6.4.1 _ 02 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**

4.3 Food consumption

Food consumption was reduced for both males and females of the 1000 ppm group. At 250 ppm, a slight and intermittent reduction in food consumption was reported for the females whereas for the males, statistically significant effects on food consumption at this test dose were inconsistent and without any biological relevance. No effects on food consumption were reported for the males of the 50 ppm group; for the females, statistically significant increases in food consumption were reported as spurious findings and therefore were of no biological relevance.

Food consumption (g/animal/day) of males					
Treatment period (13 weeks)					
Week		0 ppm	50 ppm	250 ppm	1000 ppm
1	Mean (N)/SD	15.1(30)/ 0.86	15.3 (19)/ 0.64	15.2 (30)/ 1.22	**13.8**** (30)/ 0.66
2	Mean (N)/SD	16.0 (30)/ 1.09	16.4 (19)/ 0.70	**16.6*** (30)/ 0.93	16.0 (29)/ 0.86
3	Mean (N)/SD	18.1 (30)/ 1.34	18.3 (19)/ 0.88	**17.4*** (30)/ 1.08	**16.4**** (29)/ 0.95
4	Mean (N)/SD	18.0 (30)/ 1.35	18.4 (19)/ 0.68	**17.3*** (30)/ 1.17	**16.4**** (30)/ 0.90
5	Mean (N)/SD	17.6 (30)/ 1.21	17.7 (19)/ 0.69	17.8 (30)/ 0.88	**16.9**** (30)/ 0.93
6	Mean (N)/SD	16.7 (30)/ 1.25	16.9 (20)/ 0.97	16.5 (30)/ 1.05	**15.7**** (30)/ 0.97
7	Mean (N)/SD	16.7 (30)/ 1.38	16.2 (20)/ 1.06	16.7 (30)/ 1.33	**15.7**** (30)/ 1.17
8	Mean (N)/SD	15.8 (30)/ 1.17	15.6 (20)/ 0.89	15.3 (30)/ 1.12	**14.8**** (30)/ 1.07
9	Mean (N)/SD	16.7 (30)/ 1.35	17.3 (20)/ 1.14	17.2 (30)/ 1.15	16.5 (30)/ 1.07
10	Mean (N)/SD	16.0 (30)/ 1.42	16.5 (20)/ 1.06	16.5 (30)/ 1.37	16.3 (30)/ 1.24
11	Mean (N)/SD	17.3 (30)/ 1.29	17.3 (20)/ 1.01	**16.2**** (30)/ 1.22	**15.5**** (30)/ 1.03
12	Mean (N)/SD	17.9 (30)/ 1.40	18.3 (20)/ 0.91	**16.8**** (30)/ 1.24	**16.0**** (30)/ 1.17
13	Mean (N)/SD	17.6 (30)/ 1.28	18.0 (20)/ 0.97	17.4 (30)/ 1.06	**16.8**** (30)/ 1.21

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

Food consumption (g/animal/day) of females					
Treatment period (13 weeks)					
Week		0 ppm	50 ppm	250 ppm	1000 ppm
1	Mean (N)/SD	12.5 (28)/ 0.58	**13.0*** (19)/ 0.59	12.7 (30)/ 0.79	**11.8**** (30)/ 0.51
2	Mean (N)/SD	12.9 (28)/ 0.61	13.2 (19)/ 0.55	**12.6*** (29)/ 0.61	**12.4**** (30)/ 0.48
3	Mean (N)/SD	13.0 (28)/ 0.58	13.2 (20)/ 0.66	13.1 (28)/ 0.58	12.6 (30)/ 0.58

4	Mean (N)/SD	12.7 (28)/ 0.76	**13.2*** (20)/ 0.90	12.9 (28)/ 0.07	12.6 (30)/ 0.62
5	Mean (N)/SD	13.4 (29)/ 0.90	**14.1**** (20)/ 0.88	**12.9*** (29)/ 0.67	**12.6**** (30)/ 0.72
6	Mean (N)/SD	12.7 (29)/ 0.79	13.0 (20)/ 1.04	**12.1**** (29)/ 0.75	**11.9**** (30)/ 0.66
7	Mean (N)/SD	12.1 (29)/ 1.16	11.9 (20)/ 1.44	11.9 (29)/ 1.63	11.9 (30)/ 0.91
8	Mean (N)/SD	11.6 (29)/ 0.86	11.3 (20)/ 1.13	11.6 (29)/ 0.66	11.4 (30)/ 0.59
9	Mean (N)/SD	12.3 (29)/ 0.73	12.3 (20)/ 0.98	**11.7**** (29)/ 0.93	**11.7**** (30)/ 0.75
10	Mean (N)/SD	11.8 (29)/ 0.67	11.8 (20)/ 0.85	**11.3**** (29)/ 0.82	**11.4*** (30)/ 0.72
11	Mean (N)/SD	12.4 (29)/ 0.76	12.2 (20)/ 1.04	**11.6**** (29)/ 0.73	**11.5**** (30)/ 0.88
12	Mean (N)/SD	12.5 (29)/ 0.76	28.8 (19)/ 0.78	12.3 (29)/ 0.66	**11.8**** (30)/ 0.91
13	Mean (N)/SD	12.6 (29)/ 0.81	**13.1*** (20)/ 0.71	12.2 (29)/ 0.75	**11.8**** (30)/ 0.72

*, p<0.05; **, p<0.01

Food consumption (g/animal/day) of males			
Recovery period (4 weeks)			
Week		0 ppm	1000 ppm
14	Mean (N)/SD	17.3 (9)/ 0.97	17.7 (10)/ 1.49
15	Mean (N)/SD	17.3 (10)/ 0.76	17.8 (10)/ 1.05
16	Mean (N)/SD	18.0 (10)/ 0.81	18.2 (10)/ 1.06
17	Mean (N)/SD	18.1 (10)/ 1.52	18.5 (10)/ 0.84

*, p<0.05; **, p<0.01

Food consumption (g/animal/day) of females			
Recovery period (4 weeks)			
Week		0 ppm	1000 ppm
14	Mean (N)/SD	12.3 (10)/ 0.88	12.4 (10)/ 0.85
15	Mean (N)/SD	12.3 (10)/ 0.84	12.8 (10)/ 0.92
16	Mean (N)/SD	12.8 (10)/ 0.70	12.8 (10)/ 0.78
17	Mean (N)/SD	12.8 (10)/ 0.75	13.1 (10)/ 0.72

*, p<0.05; **, p<0.01

As the changes in food consumption ran in parallel to the changes in water consumption (see 4.4), these changes were not considered to be related to the treatment with the test substance.

4.4 Water consumption

A dose-related reduction in water consumption was reported for the males of the 250 and the 1000 ppm groups over the whole treatment period. A similar reduction in water consumption was reported for the females of the 1000 ppm group over the whole treatment period; for the females of the 250 ppm

group this was also true excepted for week 4 of treatment. Water consumption of the 1000 ppm males and females allowed to recover (week 14 to 17 post exposure) returned to normal.

Water consumption (g/animal/day) of males					
Treatment period (13 weeks)					
Week		0 ppm	50 ppm	250 ppm	1000 ppm
1	Mean (N)/SD	22.9 (20)/ 3.53	21.6 (17)/ 1.70	**20.5**** (25)/ 1.67	**17.4**** (24)/ 1.66
2	Mean (N)/SD	23.2 (25)/ 1.77	23.8 (19)/ 1.98	**21.9*** (25)/ 1.56	**19.3**** (30)/ 1.07
3	Mean (N)/SD	24.9 (29)/ 1.95	25.3 (20)/ 2.44	**22.6**** (28)/ 1.79	**19.9**** (30)/ 1.60
4	Mean (N)/SD	27.0 (27)/ 3.07	27.5 (19)/ 2.43	**24.8**** (26)/ 1.88	**22.0**** (28)/ 1.80
5	Mean (N)/SD	25.0 (26)/ 2.29	25.4 (20)/ 2.38	**23.9*** (28)/ 1.73	**20.8**** (29)/ 1.68
6	Mean (N)/SD	26.3 (26)/ 1.74	25.9 (18)/ 2.61	**24.8**** (27)/ 2.02	**21.2**** (30)/ 1.95
7	Mean (N)/SD	27.0 (27)/ 2.33	**25.5*** (17)/ 2.66	**25.1**** (24)/ 2.09	**21.7**** (26)/ 2.28
8	Mean (N)/SD	25.6 (28)/ 2.05	25.7 (19)/ 2.69	**22.7**** (28)/ 1.80	**20.9**** (28)/ 2.02
9	Mean (N)/SD	26.7 (28)/ 2.12	26.4 (20)/ 2.53	**24.2**** (29)/ 2.04	**21.9**** (29)/ 2.24
10	Mean (N)/SD	26.7 (27)/ 2.46	26.2 (18)/ 2.55	**24.1**** (27)/ 2.31	**21.1**** (30)/ 2.74
11	Mean (N)/SD	29.0 (27)/ 2.12	29.9 (20)/ 2.67	**24.9**** (28)/ 2.40	**23.5**** (30)/ 2.34
12	Mean (N)/SD	28.0 (28)/ 2.35	29.1 (19)/ 2.56	**24.6**** (29)/ 2.69	**22.2**** (30)/ 1.93
13	Mean (N)/SD	28.4 (28)/ 2.01	28.4 (19)/ 3.00	**24.5**** (28)/ 2.15	**22.4**** (30)/ 3.07

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

Water consumption (g/animal/day) of females					
Treatment period (13 weeks)					
Week		0 ppm	50 ppm	250 ppm	1000 ppm
1	Mean (N)/SD	20.9 (19)/ 4.11	19.7 (15)/ 2.41	**18.2**** (25)/ 2.76	**15.1**** (25)/ 0.92
2	Mean (N)/SD	21.2 (24)/ 1.88	21.1 (15)/ 1.88	**18.4**** (24)/ 1.76	**15.8**** (25)/ 0.79
3	Mean (N)/SD	20.3 (21)/ 2.68	19.4 (14)/ 3.18	**18.5*** (26)/ 2.47	**16.5**** (24)/ 1.73
4	Mean (N)/SD	21.6 (21)/ 1.99	20.7 (16)/ 5.15	**21.9**** (23)/ 2.44	**18.2**** (28)/ 1.70
5	Mean (N)/SD	21.9 (23)/ 2.55	21.3 (16)/ 1.95	**19.1**** (24)/ 2.75	**15.6**** (23)/ 3.19
6	Mean (N)/SD	23.6 (24)/ 3.41	21.3* (16)/ 5.32	**20.6**** (24)/ 1.79	**17.3**** (23)/ 1.37

7	Mean (N)/SD	23.0 (18)/ 1.59	**22.8*** (11)/ 1.92	**21.2**** (21)/ 2.78	**18.1**** (23)/ 1.56
8	Mean (N)/SD	23.4 (20)/ 2.76	22.2 (13)/ 2.82	**20.8**** (24)/ 2.45	**18.2**** (24)/ 1.45
9	Mean (N)/SD	23.9 (24)/ 3.32	22.1 (14)/ 2.30	**19.1**** (22)/ 2.14	**17.2**** (23)/ 1.18
10	Mean (N)/SD	22.8 (19)/ 3.18	22.0 (13)/ 2.43	**18.7**** (21)/ 1.88	**16.7**** (23)/ 1.86
11	Mean (N)/SD	24.1 (18)/ 2.69	23.4 (13)/ 2.56	**21.2**** (22)/ 1.92	**19.0**** (25)/ 1.63
12	Mean (N)/SD	23.0 (19)/ 2.13	23.7 (14)/ 2.23	**19.8**** (21)/ 1.56	**17.7**** (22)/ 1.34
13	Mean (N)/SD	24.3 (20)/ 2.55	25.1 (16)/ 3.15	**21.6**** (23)/ 1.76	**18.6**** (24)/ 1.73

*, p<0.05; **, p<0.01

Water consumption (g/animal/day) of males			
Recovery period (4 weeks)			
Week		0 ppm	1000 ppm
14	Mean (N)/SD	27.0 (9)/ 2.54	27.5 (9)/ 2.44
15	Mean (N)/SD	24.4 (9)/ 1.92	**27.0**** (10)/ 1.57
16	Mean (N)/SD	26.1 (10)/ 1.73	27.2 (10)/ 2.46
17	Mean (N)/SD	26.6 (10)/ 2.07	28.1 (10)/ 1.74

*, p<0.05; **, p<0.01

Water consumption (g/animal/day) of females			
Recovery period (4 weeks)			
Week		0 ppm	1000 ppm
14	Mean (N)/SD	21.8 (8)/ 1.61	22.6 (5)/ 2.57
15	Mean (N)/SD	21.5 (8)/ 2.69	23.0 (6)/ 2.24
16	Mean (N)/SD	22.2 (10)/ 3.08	24.2 (10)/ 2.36
17	Mean (N)/SD	24.5 (10)/ 2.67	25.3 (10)/ 2.01

*, p<0.05; **, p<0.01

Despite of the statistically significant differences observed above, the effects on water consumption were considered to by related to an aversion of the rats against the odor or taste of the test solutions rather than being treatment-related.

Section A6.4.1 _ 02 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**

4.5 Compound intake

Mean daily TS intake over the whole experimental period:

Test group	Test concentration in water	TS intake (mg/kg bw/day)	
		Males	Females
1	0 ppm	0	0
2	50 ppm	5	7
3	250 ppm	25	35
4	1000 ppm	100	120

4.6 Ophtalmoscopic examination

An increased incidence of superficial corneal dystrophy was reported for 15, 12, 14 and 12 females of the control, the 50 ppm, the 250 ppm and the 1000 ppm group respectively. Such findings also were found in males of all groups but the incidence in males was lesser than in females. These findings were not considered to be treatment-related.

4.7 Blood analysis

4.7.1 Haematology

The haematological parameters were inconspicuous.

4.7.2 Clinical chemistry

A dose-related increase in urea nitrogen was seen in the females of the 250 and the 1000 ppm groups at the 6-week measurement (respectively 185 +/- 19 mg/l and 199 +/- 27mg/l, versus 159 +/-18 mg/l for control). After 13 weeks and after the 4 weeks of recovery, the values for this parameter turned back to control values. X

The increase in urea nitrogen was considered as minor and was furthermore not accompanied by an increase in serum creatinine; therefore the changes observed were not considered to be indicative of renal damage.

4.7.3 Urinalysis

A series of parameters showed alterations in both sexes and at both measurement time points 6 week and 12 week. These parameters included a dose-related decrease in total volume and an increase in specific gravity seen at 250 and 1000 ppm. These findings can be summarized as follows:

Dose (ppm)	**Measurments at time point 6 week**			
	Males		**Females**	
	Specific gravity (SD)	**Total volume (SD**	**Specific gravity (SD)**	**Total volume (SD)**
0	1.054 (0.007)	10.9 ml (2.0)	1.045 (0.01)	11.0 ml (2.3)
50	1.059 (0.005)	9.9 ml (1.6)	1.044 (0.009)	11.0 ml (1.8)
250	**1.064** (0.01)**	**7.8 ml** (2.5)**	1.054 (0.015)	**8.4 ml** (2.2)**
1000	**1.072***(0.009)**	**7.9 ml** (1.7)**	**1.071*** (0.011)**	**6.0 ml***(1.1)**

*, 0.01>p>0.001; **, p<0.001

Dose	**Measurments at time point 12 week**

Section A6.4.1 _ 02 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**

(ppm)	Males		Females	
	Specific gravity (SD)	Total volume (SD	Specific gravity (SD)	Total volume (SD)
0	1.045 (0.005)	10.2 ml (2.3)	1.037 (0.009)	10.4 ml (4.6)
50	1.049 (0.008)	11.1 ml (1.1)	1.041 (0.008)	9.0 ml (1.1)
250	1.049 (0.009)	**8.0 ml* (1.0)**	**1.056*** (0.007)**	**6.9 ml* (1.2)**
1000	1.049 (0.009)	**8.0 ml* (1.0)**	**1.059*** (0.011)**	**5.4 ml**(0.8)**

*; 0.05>p>0.01; **; 0.01>p>0.001; ***, p<0.001

Furthermore, the protein and ketone concentrations were slightly increased. During the recovery period, the males of the 1000 ppm group showed a slightly decreased specific gravity and an increased volume; all other parameters were control-like.

These effects were related to the decreased water consumption and were therefore not treatment-related.

4.8 Sacrifice and pathology

Section A6.4.1 _ 02 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**

4.8.1 Organ weights, male rats

Organ weights, main findings in male rats:

At the end of the treatment period (13 weeks) the mean final body weight for the 1000 ppm males was slightly but not statistically significantly decreased compared to control.

Dose	0 ppm	50 ppm	250 ppm	1000 ppm
Mean final BW (g) for the males (N=20)	316.7 +- 23.93	316.0 +/- 13.19	309.3 +/- 18.13	303.2 +/- 17.99

A dose-related increase in kidney weight relative to the final body weight was reported at 250 and 1000 ppm (0.717 +/- 0.0335% and 0.737 +/- 0.0273% respectively, versus 0.696 +/-0.0330% for control).

In the male rats sacrificed after 17 weeks (i.e.after recovery), no statistically significant differences in body weight and absolute organ weights were seen in rats sacrificed at the end of the recovery period. In fact, the final body weights of the treated animals were slightly decreased compared to controls, resulting in statistically increased relative organ weights.

Section A6.4.1 _ 02 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**

4.8.2 Organ weights, female rats

Organ weights, main findings in female rats:

At the end of the treatment period (13 weeks) the mean final body weight for the 1000 ppm females was slightly but not statistically significantly decreased compared to control.

Dose	0 ppm	50 ppm	250 ppm	1000 ppm
Mean final BW (g) for the females (N=20)	185.7 +/- 10.23	188.1 +/- 9.44	182.9 +/-10.58	180.9 +/- 8.42

The absolute and relative kidney weights as well as the kidney weights relative to the brain weight were increased in a dose-related manner for the females of the 250 and the 1000 ppm groups. No such changes were seen at the end of the recovery period (17 weeks). In the 50 ppm group, a statistically significant increase in adrenals weight was observed at the end of the treatment period, but was not considered to be of biological relevance.

Absolutr organ weight (g)	Dose	0 ppm	50 ppm	250 ppm	1000 ppm
Brain	Mean (N=20)	1.713 +/- 0.0537	1.697 +/- 0.0465	1.698 +/- 0.0627	1.710 +/- 0.0563
Kidney	Mean (N=20)	1.328 +/- 0.0907	1.359 +/- 0.0903	**1.379*** +/- 0.0683	**1.457**** +/- 0.0723
Adrenals	Mean (N=20)	0.055 +/- 0.0046	**0.059*** +/- 0.0072	0.053 +/- 0.0086	0.055 +/- 0.0078

*, p<0.05; **, p<0.01

Relative organ weight (% of final BW)	Dose	0 ppm	50 ppm	250 ppm	1000 ppm
Brain	Mean (N=20)	0.925 +/- 0.0502	0.904 +/- 0.0427	0.931 +/- 0.0637	0.947 +/- 0.0525
Kidney	Mean (N=20)	0.716 +/- 0.0396	0.723 +/- 0.0336	**0.756**** +/- 0.0567	**0.806**** +/- 0.0378
Adrenals	Mean (N=20)	0.029 +/- 0.0025	0.032 +/- 0.0039	0.029 +/- 0.0046	0.030 +/- 0.0039

*, p<0.05; **, p<0.01

Relative organ weight (% of brain weight)	Dose	0 ppm	50 ppm	250 ppm	1000 ppm
Kidney	Mean (N=20)	77.53 +/- 5.093	80.117 +/- 4.7238	**81.344*** +/- 4.6173	**85.295**** +/- 5.2787
Adrenals	Mean (N=20)	3.19 +/- 0.2423	**3.502**** +/- 0.411	3.122 +/- 0.5347	3.204 +/- 0.4716

*, p<0.05; **, p<0.01

Section A6.4.1 _ 02 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic oral toxicity in rats**

4.8.3	Gross and histopathology	The findings were inconspicuous in both, the animals sacrificed after 13 weeks and those sacrificed after 17 weeks (recovery).
4.9	**Other**	The histopathological examination of three additional tissues in the females (skin, submandibular lymph nodes and Harderian glands) was described in the "Addendum". There were no treatment-related effects. In fact, the only lesion observed was an adenitis of Harderian glands, whose incidence was the same in the control and test substance groups excepted for the 50 ppm group, in which there was no adenitis. A statistically significant increase in lymphatic ectasia of the submandibular lymph nodes was reported; this effect was regarded as biologically not significant.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to look for the systemic toxicity of [redacted]% glutaraldehyde as a.i.) in [redacted] rats following repeated administration over a period of 13 weeks.

Test substance: [redacted], stability at normal refrigerator temperature confirmed.

No guideline was cited but the test was conducted in accordance with GLP.

The [redacted] rats used for the experiment were 6 weeks old at test initiation; their body weights ranged from 98 to 128.6 g for the males and from 80.1 to 100.1 g

The study consisted of 4 test groups corresponding to following test doses: 0, 50, 250 and 1000 ppm test substance in pH adjusted tap water. The control group (0 ppm) and the 1000 ppm group comprised 30 animals per sex each; 10 animals per sex were planned for the recovery period. The 250 ppm group also comprised 30 animals were sex with 10 planned as alternative for the recovery period in case of high mortality in the 1000 ppm group. The 50 ppm group consisted of 20 animals per sex.

The test substance was administered as aqueous solution in the drinking water; the animals were treated daily, over a treatment period of 13 weeks and were sacrificed at the end of this period. Parts of the animals were allowed to survive further 4 weeks (i.e. until week 17) without treatment for the purpose of recovery testing.

The rats were checked for clinical signs of toxicity, mortality, body weight, food consumption and water consumption. The mean daily intake of test substance was determined. Ophthalmoscopic examinations were performed at test initiation and at test ending. Blood samples were collected via orbital sinus puncture from 10 animals per sex and group after 6 weeks of treatment and at the end of the experimental period (i.e. after 13 weeks, prior sacrifice). These samples served for the evaluation of the haematological and clinical-chemical parameters. Blood samples further were collected from the animals that were subjected to an additional post-exposure period (4 additional weeks without treatment) for the assessment of recovery; sampling here was done at the end of the recovery period. At week 6 and 12 of treatment, urine was collected from 10 animals/sex/group over a period of 24 hours. Urine from the animals of the recovery phase also was sampled (17^{th} week) for urinalysis. Following sacrifice and necropsy, the absolute and relative weights of a series of organs (final body weight, brain, liver, kidneys and so on) were determined and the animals were examined for

gross lesions and histopathological changes.

The statistical assessment of the findings was based on Levene´s test for homogeneity of variances, the Analysis of variance, and individual t-tests.

5.2 Results and discussion

Mean daily TS intake over the whole experimental period:

Test group	Test concentration in water	TS intake (mg/kg bw/day)	
		Males	Females
1	0 ppm	0	0
2	50 ppm	5	7
3	250 ppm	25	35
4	1000 ppm	100	120

Neither clinical signs of toxicity nor mortality were observed.

A dose-related reduction in water consumption was reported for the males at 250 and 1000 ppm over the whole treatment period. A similar reduction in water consumption was reported for the females of the 1000 ppm; for the females of the 250 ppm group this was also true excepted for week 4 of treatment. Water consumption of the 1000 ppm males and females allowed to recover (week 14 to 17 post exposure) returned to normal. Despite of the statistically significant differences observed, the effects on water consumption were considered to by related to an aversion of the rats against the odor or taste of the test solutions rather than being treatment-related.

Food consumption was reduced for both males and females at 1000 ppm group. At 250 ppm, a slight and intermittent reduction was reported for the females whereas for the males, statistically significant effects on food consumption at this test dose were inconsistent and without any biological relevance. No effects were reported for the males of the 50 ppm group; for the females, statistically significant increases in food consumption were reported as spurious findings and were biologically not relevant. As the changes in food consumption ran parallel to the changes in water consumption (see 4.4), they were not considered to be related to the treatment with the test substance.

Both, the absolute body weight and the body weight gain were reduced in the 1000 ppm-males over the whole treatment period. The reduction became statistically relevant from week 3 upwards. During recovery, an increase in both the absolute body weight and the body weight gain was observed, but the values still remained below control values. For the females of the 1000 ppm group, the absolute body weight and the body weight gain were statistically significantly reduced from week 11 of treatment upwards. During the recovery period an adjustment of the values to controls was observed. Absolute body weight and body weight gain were inconspicuous in the 50 and 250 ppm groups for both males and females. The changes affecting the body weight ran parallel to those reported for the food consumption and were therefore considered to be a direct consequence of the changes seen in food consumption rather than being due to the treatment. Cases of superficial corneal dystrophy were seen in all groups and were not treatment-related. The hematological parameters were inconspicuous. A dose-related increase in urea nitrogen was seen in the females of the 250 and the 1000 ppm groups at the 6-week; this parameter turned back to control values after 13 weeks and after recovery. As there was no increase in serum

creatinine, this effect was not considered to be indicative of renal damage. Urinalysis particularly revealed a dose-related decrease in total volume and an increase in specific gravity seen at 250 and 1000 ppm for both, males and females. However, these effects were related to the decreased water consumption and were therefore not considered to be treatment-related. Necropsy mainly revealed a dose-related increase in kidney weight relative to the final body weight at 250 and 1000 ppm for the males. In females, the absolute and relative kidney weights as well as the kidney weights relative to the brain were increased in a dose-related manner at 250 and 1000 ppm. No such changeswere seen at the end of the recovery period. The increase in kidney weight was dose-related and occurred in both sexes. It also was seen in the 250 ppm group where neither food consumption nor body weight were significantly affected. The fact that in females, both, the absolute and the relative kidney weights were increased suggests that the effect was not related to body weight alterations. Furthermore, reduction in water consumption, which was the only factor correlating with the effect in the kidney is not expected to induce increase in kidney weight. On the basis of all these aspects, the increase in kidney weight has to be considered as treatment-related.

The findings of gross and histopathology were inconspicuous in both, the animals sacrificed after 13 weeks and those sacrificed after 17 weeks (recovery).

5.3	**Conclusion**	
5.3.1	LO(A)EL	250 ppm, corresponding to 25 mg/kg bw/day for males and 35 mg/kgbw/day for females.
5.3.2	NO(A)EL	50 ppm, corresponding to 5 mg/kg bw/day for males and 7 mg/kg bw/day for females.
5.3.3	Other	None
5.3.4	Reliability	**1**
5.3.5	Deficiencies	N

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 4th, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2. 3.1.2.2 Purity. This is the analytical concentration determined. The solution contained also water (50.8 % ± 1.0 %), methanol (0.1 % ± 0.02 %) and NaH_2PO_4 (0.11 % ± 0.01 %). Otherwise agree with applicant's version.

Section A6.4.1 _ 02 Repeated dose toxicity

Annex Point IIA6.3 / 6.4 / 6.5

Sub-chronic oral toxicity in rats

Results and discussion	4.7.2 Clinical chemistry. There was a slight but dose-related increase in urea nitrogen in the females of the mid and high dose groups at 6 and 13 weeks, reported as statistically significant only at 6 weeks (6 weeks: 159/175/185/199 mg/L; 13 weeks: 188/192/203/209 mg/L) (Data from study report Appendix 1 Table 7). For males, such an effect was absent or minimal. Otherwise agree with applicant's version.
Conclusion	LOAEL: 250 ppm, corresponding to 25 and 35 mg/kg bw/day for males and females, respectively; based on increase in kidney weight in males and females, coupled with a slight increase in urea nitrogen. NOAEL: 50 ppm, corresponding to 5 mg/kg bw/day for males and 7 mg/kg bw/day for females. There were two effects suggesting a possibility of kidney damage: 1) there was a slight but dose-related increase in urea nitrogen in the females of the mid and high dose groups, and 2) kidney weight relative to body weight was increased in the mid and high dose groups of both males and females, and absolute kidney weights were increased in females in the mid and high dose groups.
Reliability	1
Acceptability	Acceptable
Remarks	Key study Note that the numerical values tabulated in this study summary have not been checked in detail by the RMS.

	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 A6.4.2 _ 01

Annex Point IIA6.3 / 6.4 / 6.5

Repeated dose toxicity

Sub-chronic dermal toxicity in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2000) 13-week toxicity study by cutaneous route in rats. [redacted] (Unpublished), ([redacted]), BPD ID A6.04.2_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new active substance (a.s.) for first entry to Annex I authorisation.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 411 (1981)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Colorless clear liquid	
3.1.2.2	Purity	[redacted]% glutaraldehyde in water	
3.1.2.3	Stability	Storage at + 4°C (N_2 - Gas), stability until February 2000 confirmed/guaranteed by the sponsor.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male/Female	
3.2.5	Age/weight at study initiation	About 9 weeks old at test initiation Mean body weight of the males: 340 g (320 g- 362 g) Mean body weight of the females: 221 g (207 g – 243 g)	
3.2.6	Number of animals per group	A total of 90 animals were obtained for the breeder (45 males and 45 females); there from 40 animals per sex were used for the experiment and were distributed in 4 test groups.	

Section A6.4.2 _ 01

Annex Point IIA6.3 / 6.4 / 6.5

Repeated dose toxicity

Sub-chronic dermal toxicity in rats

3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	13 weeks (i.e. 93 days), with a total of 67 days of test substance-administration.
3.3.2	Frequency of exposure	Daily, 5 days per week
3.3.3	Post-exposure period	None
3.3.4	**Dermal**	
3.3.4.1	Area covered	About 10 % of body surface
3.3.4.2	Occlusion	Semi-occlusive
3.3.4.3	Vehicle	Water
3.3.4.4	Concentration in vehicle	Following concentrations were tested:

Test group	Dose-level (mg/kg bw/day	Test concentration referring to the test substance (g/100g)	Test concentration referring to the active ingredient glutaraldehyde (g/100g)
1 (N= 20, 10/sex)	0	0	0
2 (N= 20, 10/sex)	50	5	2.5
3 (N= 20, 10/sex)	100	10	5.0
4 (N= 20, 10/sex)	150	15	7.5

3.3.4.5	Total volume applied	2 ml/kg bw/day
3.3.4.6	Duration of exposure	6 hours
3.3.4.7	Removal of test substance	At the end of the exposure period the residual test substance was removed with a gauze pads moistened with water.
3.3.4.8	Controls	Tested with the vehicle as such, i.e. water.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Starting from the day prior test initiation, the animals were checked at least once daily for clinical signs of toxicity (including mortality) and signs of skin irritation. In addition to these standard examinations, the animals also were subjected once a week to a more detailed examination of following parameters: changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions, autonomic activity, changes in gait and posture, reaction to handling, presence of clonic or tonic movements, and stereotypical or bizarre behaviour.
3.4.1.2	Mortality	See above
3.4.2	Body weight	The body weight of each animal was recorded prior allocation of the

		animals to each test group, on the first day of treatment and thereafter once a week over the whole treatment period.
3.4.3	Food consumption	Food consumption for each animal was recorded once a week over a 7-day period, from test initiation until test ending.
3.4.4	Water consumption	-
3.4.5	Ophthalmoscopic examination	All animals were subjected to ophthalmologic examinations prior test initiation. At test ending, the eyes of the control animals and of the animals of the high-dose group were examined.
3.4.6	Haematology	Blood samples were collected via orbital sinus puncture from all surviving animals at the end of the experimental period (week 13). Prior to blood sampling, the animals were fasted overnight. The blood samples served for the evaluation of the haematological and clinical-chemical parameters. Following haematological parameters were considered: Erythrocytes, hemoglobin, mean cell volume, packed cell volume, mean cell hemoglobin concentration, mean cell hemoglobin, thrombocytes, leucocytes, differential white cell count with cell morphology (neutrophils, eosinophils, basophils, lymphocytes, monocytes), and prothrombin time.
3.4.7	Clinical Chemisty	Following clinical-chemical parameters were considered: Sodium, potassium, chloride, calcium, inorganic phosphorus, glucose, urea, creatinine, total bilirubin, total protein, albumin, albumin/globulin ratio, cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase.
3.4.8	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	The animals were weighed a last time prior sacrifice. After sacrifice, following organs were taken for weighing: Adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus, uterus. In addition to the absolute weights, the weights of these organs relative to the body weight also were determined.
3.5.2	Gross and histopathology	Following sacrifice, all the animals were first subjected to a complete macroscopic examination, including the external surfaces, all orifices, the cranial cavity, the brain surface, the surface of the spinal cord, the thoracic, abdominal and pelvic cavities with their associated organs and tissues and the neck with its associated organs and tissues. Following organs/tissues were preserved for the purpose of histopathology: All gross lesions, adrenals, aorta, brain (cerebellar and cerebral cortex, medulla/pons), caecum, colon, duodenum, epididymes, eyes with Harderian glands, femoral bone with articulation, heart, ileum, jejunum, kidneys, larynx, liver, lung with bronchi, lymph nodes, mammary glands, nose, oesophagus, optic nerve, ovaries, pancreas, pharynx, pituitary gland, prostate, rectum, salivary gland, sciatic nerve, seminal

vesicles, skeletal muscle, skin, spinal cord, spleen, sternum with bone marrow, stomach with forestomach, testes, thymus, thyroids and parathyroids, tongue, trachea, urinary bladder uterus and vagina.

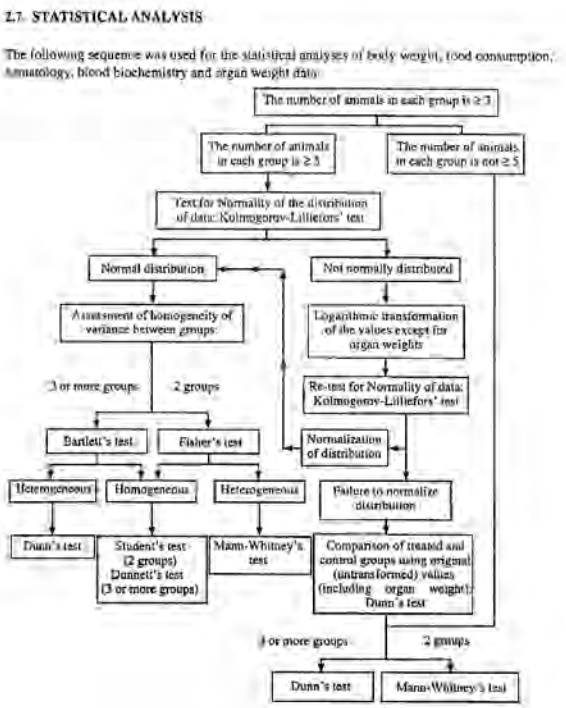
Following organs/tissues were embedded in paraffin wax, sectioned and stained with hematoxylin-eosin for microscopical examination:
All organs/tissues listed above for all sacrificed animals of the control group and of the high-dose group (excepted for the femoral bone, the skeletal muscle, the tongue and the vagina).

All organs/tissues listed above for all animals that died or had to be killed prematurely during the experimental period.

All gross lesions.

3.5.3 Other examinations None

3.5.4 Statistics



3.6 Further remarks Samples taken from each dosage form (including control) prepared for use in weeks 1, 4, 8 and 13 were subjected to analytical monitoring for concentration check.

Section A6.4.2 _ 01

Annex Point IIA6.3 / 6.4 / 6.5

Repeated dose toxicity

Sub-chronic dermal toxicity in rats

4 RESULTS AND DISCUSSIONS

4.1 Observations

4.1.1 Clinical signs

Systemic toxicity:

Poor condition including pallor of eyes and/or extremities, piloerection, round back, tremors, staggering gait, half-closed eyes and/or dyspnea, was seen in week 11 or 13 in 4 animals (1male, 3 females) of the 100 mg/kg bw group. Soft feces were seen in one male of the 50 mg/kg bw group, cutaneous lesions on the abdomen were reported for a further male of this group (from week 12). Exophthalmus, and hair loss on one forelimb were respectively reported for one male (from week 11) and one female (from week 10) of the 150 mg/kg bw group.

Local skin reactions:

A yellowish skin coloration was noted at the site of application in all animals (i.e. including controls) from first days of the treatment period. Modest local irritation was indicated by scabs and very light to well-defined erythema in almost all treated animals. The incidence and severity of the skin irritation increased with increasing dose, indicating that this effect was treatment-related. Desquamation was also seen in all treated animals, but without any dose-dependency.

Signs of skin irritation	Dose level (mg/kg bw/day)			
	0	50	100	150
Males				
Yellowish coloration of skin	9/10[a]	10/10	10/10	10/10
Very slight erythema	-	5/10	5/10	5/10
Well-defined erythema	-	1/10	1/10[b]	2/10[b]
Moderate to severe erythema	-	-	-	-
Scabs	-	8/10	10/10	10/10
Desquamation	-	-	1/10	-
Females				
Yellowish coloration of skin	10/10	10/10	10/10	10/10
Very slight erythema	-	8/10	6/10	7/10[d]
Well-defined erythema	-	2/10[b]	3/10[b]	-
Moderate to severe erythema	-	-	-	1/10[c]
Scabs	-	10/10	10/10	10/10
Desquamation	-	7/10	4/10	6/10

a, One animal died on week 7; b, these animals first displayed very slight erythema, which turned into well-defined; c, in one animal, erythema varied from well-defined to very slight and then to moderate over the treatment period; d, in two case, erythema first was well defined and then turned into very slight.

4.1.2 Mortality

Control group: One male rat died on day 44 from acute rhinitis. A female was killed in extremis on day 46 for ethical reasons.

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Repeated dose toxicity

Sub-chronic dermal toxicity in rats

50 mg/kg bw group: One male was found dead on day 86; no clinical signs had been seen prior to death.

100 mg/kg bw group: One male was found dead on day 80; prior to death the animal showed poor clinical condition.

4.2 Body weight gain

Summary of the mean body weight data:

Body weight	Dose level (mg/kg bw/day)			
	0	50	100	150
Males				
Initial body weight (g/animal)	341	341	339	341
Final body weight (g/animal)	491	500	501	513
Body weight gain (g/animal; week 1 to13)	150	159	162	172
Body weight gain (%)	44	47	48	50
Females				
Initial body weight (g/animal)	224	223	219	217
Final body weight (g/animal)	305	302	**293***	291
Body weight gain (g/animal; week 1 to 13)	81	79	74	74
Body weight gain (%)	36	35	34	34

*, $p<0.05$

For the males, no significant differences between treated and control groups were seen. For the females, the mean final body weight was slightly decreased compared to control in both the 100 and the 150 mg/kg bw/day groups, implicating decreased body weight gain over the whole experimental period.

4.3 Food consumption

Summary of the mean food consumption data:

Food consumption (week 1 to 13)	Dose level (mg/kg bw/day)			
	0	50	100	150
Males				
Mean food consumption (g/animal/day)	31.9	32.2	31.7	32.3
Differences from control (as %)	-	+1	-0.6	+1.3
Females				
Mean food consumption (g/animal/day)	24.7	24.5	24.0	24.1
Differences from control (as %)	-	-0.8	-2.8	-2.4

The mean food consumption (g/animal/day) for both sexes was similar for all groups including control.

4.4 Ophtalmoscopic examination

Ophthalmologic examinations prior test initiation revealed findings such as variation in corneal thickness and vacuolization of the cornea, which mainly were seen in the control group. On week 13, ophthalmological examination revealed hyphemia, exophthalmia and luxation of the lens in one male of the 150 mg/kg bw/day group.

4.5 Blood analysis

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Repeated dose toxicity

Sub-chronic dermal toxicity in rats

4.5.1	Haematology	Following parameters in treated animals were statistically significantly different from control:	
		In males of the 50 mg/kg bw/day group, slightly increased mean cell volume and mean cell haemoglobin as well as slightly decreased erythrocyte count and mean cell haemoglobin concentration were reported.	
		In males of the 100 and 150 mg/kg bw/day groups and in females of the 50 mg/kg bw/day group, increased eosinophil count.	X
		In females of the 150 mg/kg bw/day group, increased thrombocyte count.	
		All these effects however were slight, without any dose-relationship and had values in the range of historical control data. They therefore were considered to be of no toxicological relevance.	
4.5.2	Clinical chemistry	Following differences to control were reported:	
		In males and females of the 100 and 150 mg/kg bw/day groups, lower glucose levels.	
		In females of the 100 and 150 mg/kg bw/day groups, lower calcium levels.	
		These effects were slight, without any dose-relationship and without any pathological significance. They therefore were considered to be of no toxicological relevance.	
4.5.3	Urinalysis	Not performed	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Differences in organ weights were minimal and without any dose-relationship or correspondency with microscopical findings.	
4.6.2	Gross and histopathology	Yellowish coloration of the skin was seen in control and test animals. Due to lack of corresponding microscopic findings this was regarded as being of no toxicological importance. Scabs were seen in all treated animals; they were associated with microscopic findings and were considered to be treatment-related. Changes in liver (yellowish or greyish areas, colored foci, changes in size or consistency) were seen both in control and treated animals (similar incidence and degree of severity). These changes were therefore not considered to related to the treatment.	
		Histopathologically, slight to moderate acanthosis together with the presence of inflammatory cells in the dermis was reported for the skin of treated animals. Furthermore ulceration and scabs also were reported for the treated animals. The incidence and severity of these skin findings did not differ notably between the individual treated groups. These skin findings were considered to be treatment-related.	
		Histopathologically further revealed lesions in the liver including coagulative hepatocellular necrosis, interlobular fibroplasia, tension lipidosis and macrophages with yellow pigment contents. These findings were in accordance with the macroscopical findings in the liver described above, and were found with similar incidence and severity in both, treated and control animals. The effects affecting the liver possibly	

were related to the wearing of bandage and slight compression at the abdomen.

4.7 Other

Analytical monitoring revealed a good agreement between the nominal and measured concentrations of the test substance in all dosage forms. In fact the deviation of the measured from the nominal values did not exceed 6%.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to evaluate the potential toxicity of [redacted] following repeated cutaneous application to rats over a period of 13 weeks.

Test substance: [redacted], colorless clear liquid, purity [redacted]%, homogeneity confirmed by analytical monitoring, storage at + 4°C (N_2 - Gas)

The study was conducted according to OECD 411 (1981) with GLP.

Nine weeks old male and female [redacted] rats were used for the test; each test group consisted of 10 animals per sex. [redacted] was diluted with water to give concentrations of 5, 10 and 15% test concentrations, corresponding to 2.5, 5, and 7.5% active ingredient (glutaraldehyde). The test volume was 2 ml/kg bw and the dose level referring to the test material was as follows: 0, 50, 100 and 150 mg/kg bw/day. The test solution was applied to the clipped dorsal skin of each animal; the site of application was about 10% of the total body surface. The application site was then covered with a gauze held in place with a semi-occlusive dressing for 6 hrs. Following removal of the dressing, residual test substance on the skin was removed using a gauze pad moistened with water. The control animals were treated similarly as above, but with the vehicle only (i.e. water). The animals were treated 5 days a week over a period of 13 weeks, implying 67 days of test substance-administration.

The animals were checked for clinical signs of toxicity, mortality and skin irritation. The body weight of each animal was recorded and the food consumption for each animal was determined. All animals were subjected to ophthalmologic examinations prior test initiation; at test ending, the eyes of the control animals and of the animals of the high-dose group were examined. Blood samples were collected via orbital sinus puncture from all surviving animals at the end of the experimental period (week 13). These samples served for the evaluation of the haematological and clinical-chemical parameters. The animals were weighed a last time prior sacrifice. After sacrifice, a series of organs (e.g., adrenals, brain, liver, kidneys) were taken for weighing (absolute and relative weights). All the animals were first subjected to a complete macroscopic examination. A series of organs/tissues of the control animals and of those of the high-dose group, which had been sacrificed, were examined for histopathology. Histopathological examination also was performed on organs/tissues of animals that died or had been killed prematurely during the experimental period. All gross lesions were examined. A series of tests were used for statistical assessment of the findings, including among other Kolmogorov-Lilliefors´ test, Fisher´s test, Bartlett´s test and Student´s test.

For analytical monitoring of the test concentrations, samples taken from

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Repeated dose toxicity

Sub-chronic dermal toxicity in rats

		each dosage form (including control) prepared for use in weeks 1, 4, 8 and 13 were subjected to analysis.
5.2	**Results and discussion**	Analytical monitoring revealed a good agreement between the nominal and measured concentrations of the test substance in all dosage forms (deviation of the measured from the nominal values <= 6%). Two cases of mortality were seen in the control group. One case of mortality (male) was reported for respectively the 50 mg/kg bw/day and the 100 mg/kg bw/day group. The low incidence of mortality, the absence of a dose-relationship and the occurrence of mortality in the control group indicated that mortality was not treatment-related. The clinical examination revealed poor condition including pallor of eyes and/or extremities, piloerection, round back, tremors, staggering gait, half-closed eyes and/or dyspnea, in 4 animals (1male, 3 females) of the 100 mg/kg bw group. Soft feces were seen in one male of the 50 mg/kg bw group whereas cutaneous lesions on the abdomen were reported for a further male of this group. Exophthalmus, and hair loss on one forelimb were respectively reported for one male and one female of the 150 mg/kg bw group. The low incidence of clinical signs and the absence of a dose-relationship indicated that the clinical effects were not treatment-related. Examination of the skin revealed local irritation as indicated by scabs and erythema in almost all treated animals. The incidence and severity of the skin findings increased with increasing dose, indicating that skin irritation was treatment-related. Slight changes in body weight were observed (females of the 100 and the 150 mg/kg bw/day groups), which were not treatment-related; food consumption was inconspicuous. Some ophthalmologic effects were reported, which mainly affected control animals, indicating that these effects were not treatment-related. Some changes in haematological and clinical-chemical parameters were reported (e.g. an increased eosinophil count or lower glucose levels), which were slight, without any dose-relationship and had values in the range of historical control data. Therefore these changes were of no toxicological relevance. Necropsy confirmed that the skin findings were treatment-related.
5.3	**Conclusion**	The repeated cutaneous application of [redacted] over a period of 13 weeks resulted in no systemic toxicity in rats at the tested doses.
5.3.1	LO(A)EL	-
5.3.2	NO(A)EL	150 mg/kg bw/day for both, males and females
5.3.3	Other	The repeated cutaneous application of [redacted] over a period of 13 weeks resulted in dose-related signs of local skin irritation at all tested concentrations.
5.3.4	Reliability	**1**
5.3.5	Deficiencies	No

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Sub-chronic dermal toxicity in rats

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 6th, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	4.1.1 Clinical signs. There are several errors in the Table. A corrected Table is inserted below. 4.5.1 Haematology. The eosinophil count was increased in all dose groups, which could be an indication of an allergic reaction.
Conclusion	Agree with applicant's version. LOAEL: not established. NOAEL (systemic): 150 mg/kg bw/day. Skin irritation occurred at all dose levels.
Reliability	1
Acceptability	Acceptable
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	

4.1.1 Clinical signs – Table corrected by RMS. Underlining indicates a corrected value.

Signs of skin irritation	Dose level (mg/kg bw/day)			
	0	50	100	150
Males				
Yellowish coloration of skin	9/10	10/10	10/10	10/10
Very slight erythema	-	5/10	6/10	7/10
Well-defined erythema	-	1/10	1/10	2/10
Moderate to severe erythema	-	-	-	-
Scabs	-	8/10	10/10	10/10
Desquamation	-	-	1/10	-
Females				
Yellowish coloration of skin	10/10	10/10	10/10	10/10
Very slight erythema	-	8/10	9/10	9/10
Well-defined erythema	-	2/10	3/10	4/10
Moderate to severe erythema	-	-	-	1/10
Scabs	-	10/10	10/10	10/10
Desquamation	-	7/10	4/10	6/10

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Sub-chronic inhalation toxicity in rats and mice

			Official use only
		1 REFERENCE	
1.1	**Reference**	Kari FW (1993) NTP technical report on toxicity studies of glutaraldehyde administered by inhalation to F344/N rats and B6C3F$_1$ mice. US Department of Health and Human Services, Public Health Service, National Institutes of Health NIH, Toxicity Report Series No: 25, NIH Publication No: 93-3348 (Published), BPD ID A6.4.3_01_a Gross EA, Mellick PW, Kari FW, Miller FJ, Morgan KT (1994) Histopathology and cell replication responses in the respiratory tract of rats and mice exposed by inhalation to glutaraldehyde for up to 13 weeks. Fundam. Appl. Toxicol. 23(3):348-362 (Published), BPD ID A6.4.3_01_b Greenspan BJ, Gieschen AW, Westerberg RB, Goehl TJ, Roycroft JH (1991) Generation, monitoring, and concentration verification of ppb concentrations of glutaraldehyde for inhalation studies. The Toxicologist 11(1): 105, Abstract 334 of the 30th Annual Meeting (Published), BPD ID A6.4.3_01_c	X
1.2	**Data protection**	No	
1.2.1	Data owner	None (published data)	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline mentioned, but well-detailed and well-documented study, almost in accordance with OECD guideline 413.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Not relevant	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X

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

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic inhalation toxicity in rats and mice**

3.1.2.1	Description	Clear colorless liquid
3.1.2.2	Purity	50.0% glutaraldehyde as a.i.; minor contamination from the polymeric forms of glutaraldehyde and other volatile impurities (chemical analyses performed by the [redacted]
3.1.2.3	Stability	Glutaraldehyde 50% aqueous solution was stable for 2 weeks when stored in the dark at temperatures up to 25 °C (stability tested by the [redacted]
3.2	**Test Animals**	
3.2.1	Species	Rat Mouse
3.2.2	Strain	[redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	Male/Female
3.2.5	Age/weight at study initiation	All animals were about 6 to 7 weeks old at test initiation (both studies).

Mean initial body weight (BW) per test group, rats:

Test animals	Mean initial body weight (g)		
	Test group	Males	Females
Rats (N = 5/sex) 2-week experiment	0 ppm	107	101
	0.16 ppm	108	100
	0.50 ppm	108	101
	1.60 ppm	107	98
	5.00 ppm	106	101
	16.00 ppm	109	100
Rats (N = 10/sex) 13-week experiment	0 ppb	102	92
	62.5 ppb	102	92
	125 ppb	102	94
	250 ppb	101	91
	500 ppb	101	95
	1000 ppb	103	91

Mean initial body weight (BW) per test group, mice:

Test animals	Mean initial body weight (g)		
	Test group	Males (g)	Females (g)
Mice (N = 5/sex) 2-week experiment	0 ppm	24.8	19.3
	0.16 ppm	24.3	19.6
	0.50 ppm	24.2	20.0
	1.60 ppm	24.2	19.4
	5.00 ppm	24.0	19.0
	16.00 ppm	24.0	19.3
Mice (N = 10/sex) 13-week experiment	0 ppb	22.5	18.9
	62.5 ppb	22.8	19.4
	125 ppb	22.7	19.0
	250 ppb	22.7	19.1
	500 ppb	22.2	19.4
	1000 ppb	22.8	19.4

3.2.6	Number of animals per group	2-week experiment: 10 animals (5/sex) for each test group. 13-week experiment: 20 animals (10/sex) for each test group.
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	

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

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic inhalation toxicity in rats and mice**

3.3.1	Duration of treatment	The study consisted of following two experiments: Experiment 1: 2 weeks Experiment 2: 13 weeks The test concentrations using within the second experiment were based on the results of the first experiment.
3.3.2	Frequency of exposure	Experiment 1: 6 hours + 20 minutes per day, 5 days a week Experiment 2: 6 hours + 30 minutes per day, 5 days a week
3.3.3	Post-exposure period	None
3.3.4	**Inhalation**	

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

Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic inhalation toxicity in rats and mice**

3.3.4.1	Vapor generation	The test substance first was diluted with deionized water to a 25% w/w aqueous glutaraldehyde solution, which was pumped into a rotating glass evaporation column. HEPA-filtered and compressed air was heated and passed through the column from the base at a flow rate of ca. 3 cubic feet /min. The glass column rotated at ca. 15 revolutions/minute and the test solution was spread over the internal surface of the column by means of a glass rod extending the length of the column. The spreading enhanced the evaporation of the test material and was necessary because of the low vapour pressure of glutaraldehyde. The temperature of the air exiting the evaporator was maintained at ca. 60 °C. The glass column and the associated tubing were insulated to prevent heat loss. Excess, unvaporized material was collected at the base of the column in an Erlenmeyer flask.	
		In the two-week experiment, the vapour-laden air was passed through a water-cooled condensing column (18°C) to bring the temperature closer to room temperature prior to being distributed to the exposure chambers. In the 13-week experiment, the vapour-laden air was conducted to a mixing chamber, where it was diluted with conditioned air prior to being distributed to the exposure chambers. Monitoring was performed using a Miran infrared analyzer, prior distribution to the individual chambers. The delivery of the test vapour into the chambers was ensured by means of a driving vaccum, which was generated by Air-Vac pumps. The exposure concentration in each chamber was adjusted by adjustment of the driving pressure to the appropriate Air-Vac pump. An effective air-flow of ca. 72 cubic feed per minute through the chamber and 1 fresh air changes per hour were ensured.	X

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Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic inhalation toxicity in rats and mice**

3.3.4.2 Monitoring of the test substance-vapour concentration

Monitoring of the test substance-vapour concentration was based on an automated gas sampling gas chromatograph system equipped with a flame ionization detector. Test substance-concentration was measured in each exposure chamber including the chamber with the control animals, and in the exposure room; an on-line standard as well as blank samples (nitrogen blank in the 2-week experiment, carbon-filtered air in the 13-week blank) also were considered, and sampling was done at different positions.

The mean chamber concentrations of glutaraldehyde during both experiments were calculated from daily monitoring data and were as follows:

Mean Chamber Concentrations of Glutaraldehyde in the 2-Week and 13-Week Inhalation Studies in F344/N Rats and B6C3F$_1$ Mice

Target Concentration	Mean ± SD	Target ± RSD[1]	Maximum	Minimum	Samples within Range[2] (%)
F344/N RATS					
2-WEEK STUDY					
0.0 ppm	-0.022 ± 0.017	–	0.00	-0.044	–
0.16 ppm	0.161 ± 0.018	100 ± 11	0.193	0.111	68
0.50 ppm	0.497 ± 0.082	99 ± 17	0.803	0.278	59
1.60 ppm	1.53 ± 0.233	96 ± 15	1.83	0.826	66
5.00 ppm	4.96 ± 0.226	99 ± 5	5.39	4.17	98
16.00 ppm	15.4 ± 0.916	97 ± 6	17.3	13.4	88
13-WEEK STUDY					
0.0 ppb	MDL[3]	–	MQL[4]	0.0	–
62.5 ppb	63.4 ± 10.4	102 ± 16	106	22.2	71
125 ppb	131 ± 25.9	105 ± 20	307	18.1	63
250 ppb	263 ± 43.5	105 ± 16	506	95.4	70
500 ppb	503 ± 73.7	100 ± 15	867	182	78
1000 ppb	990 ± 123	99 ± 12	1460	274	85
B6C3F$_1$ MICE					
2-WEEK STUDY					
0.0 ppm	-0.022 ± 0.017	–	0.0	-0.044	–
0.16 ppm	0.161 ± 0.017	101 ± 11	0.193	0.111	69
0.50 ppm	0.499 ± 0.081	100 ± 16	0.803	0.278	61
1.60 ppm	1.51 ± 0.229	94 ± 15	1.78	0.924	65
5.00 ppm	4.95 ± 0.237	99 ± 5	5.39	4.17	98
16.00 ppm	15.4 ± 0.752	96 ± 5	17.3	14.1	92
13-WEEK STUDY					
0.0 ppb	MDL[3]	–	MQL[4]	MDL[3]	–
62.5 ppb	63.2 ± 10.3	101 ± 16	106	22.2	72
125 ppb	130 ± 25.6	104 ± 20	307	18.1	64
250 ppb	261 ± 41.2	104 ± 16	412	95.4	71
500 ppb	502 ± 73.7	100 ± 15	867	182	78
1000 ppb	988 ± 121	99 ± 12	1430	274	86

[1] Target concentration ± relative standard deviation as a percent of target concentration.
[2] Samples were considered to be in range if they were within 10% of target concentrations during the 2-week studies and if they were within 15% of target concentrations in the 13-week studies.
[3] MDL = minimum detectable limit, 13 ppb.
[4] MQL = minimum quantifiable limit, 43 ppb.

Conversion of the ppm and ppb values in mg/l:

The values in the study are reported as ppm and ppb; for conversion into mg/l, use a conversion factor of 0.0041 (Derelanko MJ, Toxicologist´s pocket handbook, CRC Press, Boca Raton London New York Washington DC, 2000).

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Sub-chronic inhalation toxicity in rats and mice

3.3.4.3 Experimental design, overview

EXPERIMENTAL DESIGN	
Study Laboratory	Battelle Pacific Northwest Laboratories, Richland, WA
Size of Study Groups	2-Week Studies: 5 males and 5 females of each species per dose group 13-Week Studies: Core Studies: 10 males and 10 females of each species per dose group Clinical Pathology Study (rats only): 10 males and 10 females per dose group Histoautoradiographic Studies: 5 males and 5 females of each species per dose group at each of four time points
Exposure Concentrations/ Duration	2-Week Studies: Duration: 6 hours plus 20 minutes per day, 5 days per week for 12 exposure days Exposure concentrations: Rats: 0, 0.16, 0.5, 1.6, 5.0, or 16.0 ppm Mice: 0, 0.16, 0.5, 1.6, 5.0, or 16.0 ppm 13-Week Studies: Duration: 6 hours plus 30 minutes per day, 5 days per week for 13 weeks Exposure concentrations: Rats: 0, 62.5, 125, 250, 500, or 1000 ppb Mice: 0, 62.5, 125, 250, 500, or 1000 ppb
Date of First Exposure	2-Week Studies: Rats: 3 August 1987 Mice: 4 August 1987 13-Week Studies: Rats: males, 18 September 1989; females, 19 September 1989 Mice: 19 September 1989
Date of Last Exposure	2-Week Studies: Rats: 18 August 1987 Mice: 19 August 1987 13-Week Studies: Rats: males, 18 December 1989; females, 19 December 1989 Mice: males, 20 December 1989; females, 21 December 1989
Type and Frequency of Observation	2-Week Studies: Observed two times per day, 7 days per week, for mortality/morbidity and up t three times per day for clinical signs of toxicity on each exposure day. Animal were weighed on Days 1 and 8 and at necropsy. 13-Week Studies: Observed two times per day. Clinical signs of toxicity were recorded weekly. Body weights were recorded at study initiation, weekly thereafter, and at necropsy.

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Annex Point IIA6.3 / 6.4 / 6.5 Sub-chronic inhalation toxicity in rats and mice

3.3.4.4 Necropsy

Necropsy and Histologic Examinations

All animals received a complete necropsy.

2-Week Studies:

All tissues from all animals were saved in 10% neutral buffered formalin. In addition to organs with gross lesions, histopathologic evaluations were conducted on the following tissues from all treated and control animals: larynx (transverse sections), lungs and attached tracheobronchial lymph nodes, nasal cavity (three sections), and trachea (longitudinal and transverse sections).

13-Week Studies:

The protocol required that tissues be examined microscopically in all control animals, all animals in the highest dose group with at least 60% survivors, and all animals in the higher dose groups. In addition to any gross lesions, tissue masses, or suspect tumors, along with regional lymph nodes, tissues to be examined were: adrenal glands, bone (femur, diaphysis with marrow cavity and epiphyseal cartilage plate, articular cartilage and articular surface), brain (3 sections), clitoral glands, esophagus, eyes (if grossly abnormal), gallbladder (mice), heart, intestine (large: cecum, colon, rectum; small: duodenum, jejunum, ileum), kidneys, larynx, liver, lungs and mainstem bronchi, lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary glands (including surface skin), muscle (thigh), nasal cavity and turbinates (3 sections), ovaries, pancreas, parathyroid glands, pituitary gland, preputial glands, prostate gland, salivary glands, seminal vesicles, spinal cord and sciatic nerve (if neurologic signs present), spleen, stomach (forestomach and glandular stomach), testes with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus. In rats at all lower dose levels, the following target organs were examined microscopically: nasal cavity and all gross lesions. In mice, target organs identified during examination of the high-dose group were examined at lower doses and included: larynx, nasal cavity, thymus, spleen, lymph nodes, bone marrow, and epididymides.

Supplemental Evaluations

Clinical Pathology (rats only):

Clinical pathology studies were performed on designated male and female rats at 4 and 24 days and on core-study rats at the end of the study (Day 94). Hematology studies included erythrocyte count and morphologic assessment, hematocrit, hemoglobin concentration, mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), leukocyte count with differential, platelet count and morphologic assessment, reticulocyte count, and volume of packed red blood cells (VPRC). Clinical chemistry studies included alanine aminotransferase (ALT), albumin, albumin/globulin (A/G) ratio, alkaline phosphatase, creatine kinase (CK), creatinine, globulin, sorbitol dehydrogenase (SDH), total bile acids, total protein, and urea nitrogen (UN).

Sperm Morphology/Vaginal Cytology:

Sperm morphology/vaginal cytology studies were performed at the end of the 13-week studies. Male rats and mice exposed to 0, 62.5, 250, 500 (mice), or 1000 ppb (rats) were evaluated for necropsy body and reproductive tissue weights and spermatozoal data. Female rats and mice exposed to 0, 62.5, 250, 500 (mice), or 1000 ppb (rats) were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spend in the various stages.

Histoautoradiographic Evaluation of Respiratory Tract:

Rats and mice exposed to 0, 62.5, 125, 250, 500, or 1000 ppb for 1 or 4 days or 6 or 13 weeks were evaluated for incidence and severity of nasal lesions. Cell replication data for the nasal vestibule and dorsal atrioturbinate were also determined. Complete details are provided in Appendix E. These studies were performed in collaboration with the Chemical Industry Institute of Toxicology.

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Annex Point IIA6.3 / 6.4 / 6.5 **Sub-chronic inhalation toxicity in rats and mice**

3.3.4.5 Histoautoradiographic evaluation of respiratory tract

Histoautoradiographic evaluation of respiratory tract was performed on rats and mice exposed to all test concentrations from 0 to 1000 ppb for 1 or 4 days, or 6 or 13 weeks. Five animals per sex were examined for the incidence and severity of nasal lesions at each of the time points mentioned above. 18 hours after the last treatment with the test substance, each animal was treated with tritiated thymidine, a DNA precursor used to detect cells in S-phase, by means of a single intraperitoneal injection of 2 µCi tritiated thymidine/g bw. After further 2 hours, the animals were sacrificed and all tissues including gross lesions, duodenum, respiratory tract and tail were fixed in 10% buffered formalin. Trimmed tissue samples covering the complete respiratory tract as well as of the duodenum were embedded in paraffin wax, sectioned, and either HE-stained for light microscopical examination or prepared for light microscopic autoradiography. The findings were entered into a computerized database (XIBION PATHTOX); they were given a subjective score for severity based on following definitions:

Score	Definition
1	Minimal, of doubtful biological significance
2	Mild, a clear lesion but very limited extent and/or severity
3	Moderately severe
4	Severe
5	Very severe

Based on the findings of the light microscopical examination, sections were selected for autoradiography and first were deparaffinized. These section were then dipped into Kodak autoradiography emulsion (NTB-2, Dupont) and were exposed for 10 weeks at –20 °C. Thereafter, the slides were developed (Kodak D-19 developer) and HE-stained. Two sites were selected for histoautoradiographic evaluation: 1) the pseudostratified respiratory epithelium of the dorsal atrioturbinate and 2) the squamous epithelial lining of the most anterior section of the nasal vestibule. Cells of these regions with > 10 silver grains over the nucleus were considered as being in the S-phase; the basement membrane lengths for these regions also were measured (Zeisss Videoplan). The results were expressed as the number of labelled cells per mm basement membrane (according to Monticello TM et al., Toxicol. Pathol. 18. 24-31, 1990).

3.3.5 Statistics

Organ and body weight data were statistically assessed by means of the Williams (Williams DA, Biometrics 27:103-117, 1971 and Biometrics 28: 519-531, 1972) or Dunnett's test (Dunnett CW, J. Am. Stat. Assoc. 50: 1096-1121, 1955).

Hematological and clinical-chemical data were assessed by the methods of Shirley (Shirley E, Biometrics 33: 386-389, 1977) or Dunn (Dunn OJ, Technometrics 6: 241-252, 1964).

The assessment of the significance of dose-response trends was based on Jonckheere's test (Jonckheere AR, Biometrika 41: 131-145, 1954),

which also was used to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable to detect departures from monotonic dose response (Dunnett, Dunn). For the detection of extreme values, the outlier test of Dixon and Massey (Dixon WJ, Massey FJ Jr, Introduction to statistical analysis, 1st ed.: 145-147, McGraw-Hill Book Company, NY).

3.4 Further remarks

(1) During each of the two experiments, the uniformity of vapour concentration through each exposure chamber was measured. The chamber uniformity data for the 2-week experiment were within the limits (+/-5 %) established by the NTP for all chambers. During the 13-week experiment, an increased variation in overall uniformity of concentration was obtained, with relative standard deviations ranging from 4 to 25%.

(2) Build up and decay rates for the test concentrations in the chambers were determined in the absence and presence of test animals. The time needed to reach 90% of the final stable concentration in the chamber was defined as T_{90}, whereas the time needed for the exposure to decrease to 10% of the stable concentration was defined as T_{10}. For the two-week experiment, a T_{90} and a T_{10} of 20 minutes respectively were reported. For the 13-week experiment, a T_{90} of 30 minutes respectively was reported. For the two-week experiment, the animals were therefore whole-body exposed to the test substance vapour for 6 hours + 20 minutes (i.e.T_{90}) per day, 5 days a week. For the 13-week experiment, the animals were whole-body exposed to the test substance vapour for 6 hours + 30 minutes per day, 5 days a week (see study design).

(3) The authors also assessed the genotoxicity of the test substance with the present inhalation study, using following assays: Ames test according to Haworth S et al., Environ. Mutagen. 5(1): 3-142, 1983 and to Zeiger E et al., Environ. Mol. Mutagen. 19(21): 2-41, 1982; Mouse lymphoma assay according to McGregor DB et al., Environ. Mol. Mutagen. 11: 91-118, 1988; Chinese hamster ovary cell cytogenetic assay for induction of sister chromatid exchanges and chromosomal aberration, according to Galloway SM et al., Environ. Mol. Mutagen. 10(10): 1-175, 1985. The in vivo genotoxicity of the test substance was assessed by the authors using the Drosophila melanogaster sex-linked recessive lethal test with adult flies as described by Yoon JS et al. (Environ. Mutagen. 7: 349-367, 1985) and with larvae, as described by Zimmering S et al. (Environ. Mol. Mutagen. 14: 245-251, 1989). The results however are presented within the sections referring to genotoxicity. X

(4) Gross EA and coworkers (1994) published parts of the work of Kari FW (1993) and focused on the histopathologic and histoautoradiographic findings in two selected sites of the nasal passage: (1) the squamous epithelium of the nasal vestibule designated as squamous epithelium, and (2) the respiratory epithelium of the dorsal atrioturbinate, designated as respiratory epithelium.

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Mortality, 2-week experiment

Test animals	Survival		
	Test group	Males	Females
Rats (N = 5/sex)	0 ppm	5/5	5/5
	0.16 ppm	5/5	5/5
	0.50 ppm	5/5	5/5
	1.60 ppm	5/5	5/5
	5.00 ppm	**0/5**	**0/5**
	16.00 ppm	**0/5**	**0/5**
Mice (N = 5/sex)	0 ppm	5/5	4/5
	0.16 ppm	5/5	5/5
	0.50 ppm	5/5	5/5
	1.60 ppm	**0/5**	**0/5**
	5.00 ppm	**0/5**	**0/5**
	16.00 ppm	**0/5**	**0/5**

4.1.2 Clinical signs, 2-week experiment

Rats:

Clinical signs of toxicity were seen at 1.6, 5 and 16 ppm and included labored breathing, ocular and/or nasal discharge, mouth breathing and rough haircoat.

Mice:

Clinical signs of toxicity were seen at 1.6, 5 and 16 ppm and included marked respiratory difficulties, ocular and/or nasal discharge and mouth breathing; the animals of these groups did not appear to eat or drink during the experiment.

4.1.3 Mortality, 13-week experiment

Test animals	Survival		
	Test group	Males	Females
Rats (N = 10/sex)	0 ppb	10/10	10/10
	62.5 ppb	10/10	10/10
	125 ppb	10/10	10/10
	250 ppb	10/10	9/10
	500 ppb	10/10	10/10
	1000 ppb	10/10	10/10
Mice	0 ppb	10/10	10/10

(N = 10/sex)	62.5 ppb	10/10	10/10
	125 ppb	10/10	10/10
	250 ppb	10/10	10/10
	500 ppb	10/10	**8/10**
	1000 ppb	**0/10**	**0/10**

4.1.4 Clinical signs, 13-week experiment

Rats:

Clinical signs of toxicity were seen during the first 5 weeks of treatment at 1000 ppb and included dyspnea and ruffled fur in all animals, as well as emaciation in many animals.

Mice:

Clinical signs of toxicity were seen at 1000 ppb and included dyspnea, emaciation, abnormal posture, hypoactivity, ruffled fur, paraphimosis and tachypnea. At 500 ppb, dyspnea was observed during the first weeks of the experiment.