Section A6.6.2 _ 01	**Genotoxicity in vitro**
Annex Point IIA6.6.1 / 6.6.2 / 6.6.3	**In-vitro chromosomal aberration test with Chinese hamster V79 cells**

			Official use only
		1 REFERENCE	
1.1	**Reference**	(2002) In vitro chromosome aberration assay with % Glutaraldehyde). (Unpublished), BPD ID A6.06.2_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access		
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 guideline 473 (1997)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	% glutaraldehyde in water)	
3.1.1	Lot/Batch number		
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Colorless liquid.	
3.1.2.2	Purity	% a.i. in water	
3.1.2.3	Stability	The stability of the test substance at room temperature in water over a period of 14 days had been verified analytically ( )	
3.2	**Study Type**	Cytogenetic assay measuring chromosomal aberrations	
3.2.1	Organism/cell type	<u>Mammalian cell lines:</u> Chinese Hamster V 79 cells	
3.2.2	Deficiencies / Proficiencies	Thymidine kinase (TK) deficiency	
3.2.3	Metabolic activation system	The S-9 mix was prepared according to Ames et al. (Mut. Res. 31: 347-364, 1975) from the S-9 fraction, which was collected from the liver of Aroclor 1254-treated male Sprague-Dawley rats. The S9 mix was prepared freshly prior each experiment by mixing a defined amount of the S9 fraction with S9-supplement (cofactors) in a 1:9 ratio.	

3.2.4	Negative control	Untreated control: The untreated negative controls were performed with and without S9 mix and contained cells and culture medium* only, i.e. no test substance was added. Vehicle control: As culture medium was used as vehicle, the vehicle control was identical to the untreated control. *, culture medium: MEM medium with Earle's salt
3.2.5	Positive control	Without metabolic activation: Ethyl methanesulfate (EMS; SIGMA, M-0880), 350 µg/ml culture medium added in a volume of 1 ml. With metabolic activation: Cyclophosphamide (CPP; Endoxan, ASTA MEDICA, Reg. Nr. E432-1), 0.5 µg/ml culture medium added to a volume of 1 ml.
3.3	**Pre-test**	A range-finding cytotoxicity test was performed prior to the main test.
3.4	**Administration / Exposure; Application of test substance**	
3.4.1	Concentrations	The test concentrations were chosen on the basis of the results of the pre-test, and were as follows: Without S9 mix: 0, 0.25, 0.5 and 1 µg/ml With S9 mix: 0, 2.5, 5 and 10 µg/ml The test concentrations refer to the test substance as such and not to the active ingredient.
3.4.2	Way of application	The test substance was dissolved in 1ml serum-free culture medium and was then added to the culture medium with or without 1 ml S9 mix.

3.4.3 Test design

The test design can be summarized as follows:

Without S9 mix			
Exposure	Harvest*	Test group	Metaphases analysed
4 h	18 h	Negative control	200
4 h	18 h	0.25 µg/ml TS**	200
4 h	18 h	0.50 µg/ml TS	200
4 h	18 h	1.00 µg/ml TS	200
4 h	18 h	Positive control (EMS, 350 µg/ml)	100
With S9 mix			
4 h	18 h	Negative control	200
4 h	18 h	2.50 µg/ml TS	200
4 h	18 h	5.00 µg/ml TS	200
4 h	18 h	10.00 µg/ml TS	200
4 h	18 h	Positive control (CPP, 0.5 µg/ml)	100

*, The first sampling time of 18 h was within the 1 to 1.5 x the normal cycle time as recommended by the guideline

**, TS = [redacted]% glutaraldehyde in water)

3.4.4 Other modifications None

3.5 Examinations

Chromosomal aberrations were analyzed in the first metaphase to avoid loss during mitoses or conversion of the initial aberrations into more complex derivatives during subsequent cell cycles.

Following exposure of the cells to the test substance (4 hours), the culture medium was replaced by fresh medium supplemented with 10% fetal calf serum and the cells were incubated again for further 14 hours until harvesting. Approximatively 2 to 3 hours prior harvesting, 0.2 µg/ml Colcemid in culture medium was added to the cells in order to stop mitosis in the metaphase. For harvesting, the culture medium was completely removed and the cells were subjected to an hypotonic treatment (0.4% KCl, 37 °C, 20 minutes); thereafter they were fixed (methanol: glacial acetic acid, 3:1), dried and stained on the slides with a solution of Giemsa and Titrisol. The cell preparations were mounted using Corbit-Balsam.

200 (100 for positive controls) well-spread metaphases of each culture were counted. Cells displaying 20 to 22 chromosomes were examined for structural chromosome aberrations, such as gaps, breaks, fragments, segment loss, multiple aberrations, pulverization (break down of chromosomes into irregular particles) and/or exchanges. Numerical aberrations such as e.g. hyperploidy (metaphase with additional chromosomes) also were considered. Furthermore, the mitotic index was determined and the cells were counted for determination of cytotoxicity using additional cultures treated as those of the main test. The definitions used within the chromosomal analysis were based on following references:

(1) Evans HJ, O'Riordan ML (1975) Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. Mutat Res. 31(3): 135-48 (published)

(2) Savage JRK (1975) Classification and relationships of induced chromosomal structural changes. J. Med. Genet. 12: 103-122 (published)

(3) Standard-Protokoll zur cytogenetischen Auswertung von Mitose- und Meiose-chromosomen bei der Routineuntersuchung; ausgearbeitet von der Arbeitsgruppe der Industrie, Cytogenetik“, 1987

The statistical evaluation of the findings was carried out using the MUCHAN program system ([redacted]). Fisher's exact test for the hypothesis of equal proportions was used for the comparison of each test dose with the control. The test was Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one-sided.

4 RESULTS AND DISCUSSION

4.1 Genotoxicity

4.1.1 Without metabolic activation

Yes, the test substance caused a statistically significant and dose-dependent increase in the number of structurally aberrant metaphases (with and without gaps).

Dose (µg/ml)	Metaphases with aberrations[a]							
	Incl. Gaps (%)	Excl. Gaps (%)	EX (%)	MA (%)	CD (%)	AP (%)	PP (%)	EP (%)
Neg. Cont.	3.0	2.0	1.0	0.0	0.0	0.0	0.5	0.0
0.25	4.0	4.0	2.5	0.0	0.0	0.0	0.0	0.0
0.50	5.0	4.0	3.0	0.0	0.0	0.0	0.5	0.0
1.00	12**	11**	11**	0.0	0.0	0.0	0.0	0.0
Pos. Cont. (EMS)	19**	19**	12**	0.0	0.0	0.0	0.0	0.0

[a], Total number of metaphases: 200 (except for positive control, 100)

EX, exchanges

MA, multiple aberrations

CD, chromosomal disintegration

AP, aneuploidy

PP, polyploidy

EP, endoploidy

*: p<=0.05; **: p<=0.01

4.1.2 With metabolic activation

Yes, the test substance caused a statistically significant and dose-dependent increase in the number of structurally aberrant metaphases (with and without gaps).

Dose (µg/ml)	Metaphases with aberrations[a]							
	Incl. Gaps (%)	Excl. Gaps (%)	EX (%)	MA (%)	CD (%)	AP (%)	PP (%)	EP (%)
Negative Control	3.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0
2.50	3.5	2.5	2.5*	0.0	0.0	0.0	0.0	1.0
5.00	6.5	6.5*	5.0**	0.0	0.0	0.0	0.5	0.5
10.00	8.0	7.5**	6.5**	0.0	0.0	0.0	0.0	0.0
Positive Control (CPP)	19**	17**	12**	0.0	0.0	0.0	0.0	0.0

[a], Total number of metaphases: 200 (except for positive control, 100)

EX, exchanges

MA, multiple aberrations

CD, chromosomal disintegration

AP, aneuploidy

PP, polyploidy

EP, endoploidy

*: p<=0.05; **: p<=0.01

4.1.3 Mitotic index

Mitotic index (based on 1000 cells/culture):

A weak suppression of the mitotic activity was observed under all experimental conditions, as shown in following table.

Without S9 mix	
Test groups	Relative percentage of mitotic cells (i.e. versus 100% for negative control)
Negative control	100%
0.25 µg/ml	69.2%
0.50 µg/ml	77.1%
1.00 µg/ml	71.8%
Positive control (EMS)	74.0%
With S9 mix	
Negative control	100%
2.50 µg/ml	65.9%
5.00 µg/ml	75%
10.0 µg/ml	54.1%
Positive control (CPP)	56.3%

4.1.4	Cell attachment to the slides / Quality of the metaphases	The treatment of the cells with the different doses of test substance did not disturb cell attachment on the slides. Within the dose-range that was tested, the quality of the metaphases was sufficiently good to allow chromosomal evaluation.

4.2 Cytotoxicity

Cell count:

A slight growth inhibition of the cells was observed under all experimental conditions, as shown in following table.

Without S9 mix	
Test groups	Cell count as percentage of negative control
Negative control	100%
0.25 µg/ml	106.6%
0.50 µg/ml	107.6%
1.00 µg/ml	64.0%
2.00 µg/ml	73.1%
4.00 µg/ml	57.9%
With S9 mix	
Negative control	100%
1.25 µg/ml	101.5%
2.50 µg/ml	91.3%
5.00 µg/ml	98.0%
10.0 µg/ml	82.1%
20.0 µg/ml	55.9%

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to assess the clastogenic and aneugenic potential of glutaraldehyde in Chinese Hamster V79 cells in vitro, with and without metabolic activation.

Test substance: [redacted]% glutaraldehyde in water), [redacted] [redacted], colorless liquid.

The test was conducted according to OECD 473 (1997), with GLP.

The S-9 mix was prepared according to Ames et al. (Mut. Res. 31: 347-364, 1975) from the S-9 fraction, which was collected from the liver of Aroclor 1254-treated male Sprague-Dawley rats.

A range-finding cytotoxicity test was performed prior to the main test. On the basis of the results of the pre-test, the test concentrations for the main test were chosen as follows: (1) 0, 0.25, 0.5 and 1 µg/ml without S9 mix, and (2) 0, 2.5, 5 and 10 µg/ml with S9 mix. The test concentrations were prepared by diluting the test substance in culture medium. The negative controls (with and without S9 mix) contained cells and culture medium only, and served also for vehicle control. Ethyl methanesulfate (EMS; 350 µg/ml) was used as positive control in absence of S9 mix whereas cyclophosphamide (CPP; 0.5 µg/ml) was used as positive control in the presence of S9 mix. The cells were incubated in the test solutions over a period of 4 hours; thereafter the test solutions were replaced by medium and the cells were allowed to incubate for a further 14 hours in culture medium. About 2 to 3 hours prior harvesting, 0.2µ g/ml colcemid was added in the culture medium to stop mitosis in the metaphase; the cells were harvested after a total period of 18 hours, corresponding to 1 - 1.5 x the normal cycle time of the cells. Following fixation, drying and staining with Giemsa and Titrisol, 200 (100 for positive controls) well-spread metaphases of each culture were counted and cells displaying 20 to 22 chromosomes were examined for structural and numerical chromosome aberrations. The mitotic index was determined and the cells were counted for determination of cytotoxicity using additional cultures treated as those of the main test. The statistical evaluation of the findings was based on the Bonferroni-Holm corrected Fisher's exact test for the hypothesis of equal proportions.

Section A6.6.2 _ 01	Genotoxicity in vitro
Annex Point IIA6.6.1 / 6.6.2 / 6.6.3	In-vitro chromosomal aberration test with Chinese hamster V79 cells

5.2	**Results and discussion**	In both case, with and without S9 mix, [redacted] caused a statistically significant and dose-dependent increase in the number of structurally aberrant metaphases (with and without gaps). Without S9 mix, 12% structurally aberrant metaphases including gaps (11% excluding gaps) were seen at 1 µg/ml test substance; 11% of these aberrations were identified as chromosomal exchanges. With S9 mix, 8% structurally aberrant metaphases including gaps (7.5% excluding gaps) were seen at 10 µg/ml test substance; 6.5% of these aberrations were chromosomal exchanges. In fact, with S9 mix, chromosomal exchanges, which were statistically significant, already occurred at the lowest tested concentration of 2.5 µg/ml test substance (2.5%). The determination of the mitotic index revealed a weak suppression of the mitotic activity at all tested concentrations, with and without S9 mix. Growth inhibition of the cells indicative of cytotoxicity was observed from 1 µg/ml upward in the absence of S9 mix, and at the highest tested concentration of 20 µg/ml in the presence of S9 mix. No aneugenic activity of the test substance on V 79 cells could be shown.
5.3	**Conclusion**	[redacted]% glutaraldehyde in water) was found to be clastogenic against V 79 cells under in vitro test conditions.
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	October 28th, 2010
Materials and Methods	Agree with applicant's version.
Results and discussion	**3.1.2** This refers to Doc IIIA Section A2. 5.2 Results and discussion. Two clarifications in paragraphs 2 and 3: • Without S9 mix, 12% structurally aberrant metaphases including gaps (11% excluding gaps) were seen at 1 µg/ml test substance; 11% of these aberrations were identified as chromosomal exchanges. • With S9 mix, 8% structurally aberrant metaphases including gaps (7.5% excluding gaps) were seen at 10 µg/ml test substance; 6.5% of these aberrations were chromosomal exchanges.
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	Acceptable

Section A6.6.2 _ 01 Annex Point IIA6.6.1 / 6.6.2 / 6.6.3	**Genotoxicity in vitro** **In-vitro chromosomal aberration test with Chinese hamster V79 cells**
Remarks	This is a key study for Doc IIIA section 6.6.2 (*In vitro* cytogenicity in mammalian cells).

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

Section A6.6.2 _ 02 Annex Point IIA6.6.1 / 6.6.2 / 6.6.3		**Genotoxicity in vitro** **In-vitro cytogenicity study in Chinese hamster ovary cells (CHO) measuring sister chromatid exchange and chromosome aberrations**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Kari FW (1993) NTP technical report on toxicity studies of glutaraldehyde administered by inhalation to F344/N rats and B6C3F1 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 A6.04.3_01	
1.2	**Data protection**	No	
1.2.1	Data owner	Not relevant (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, no guideline was mentioned but the test was conducted according to Galloway SM et al. (Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: comparison of results for 22 compounds in two laboratories. Environ Mutagen. 7(1)1-51 (Published), Environ. Mutagen. 7: 1-51, 1985)	
2.2	**GLP**	Not specified	
2.3	**Deviations**	Not relevant	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde 50% aqueous solution, [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X
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	**Study Type**	In Vitro mammalian sister chromatid exchange and chromosome aberration test	
3.2.1	Organism/cell type	Mammalian cell lines: Chinese hamster Ovary (CHO)	

3.2.2 Deficiencies / Proficiencies

Thymidine kinase (TK) deficiency, BrdU sensitivity

3.2.3 Metabolic activation system

The S9 mix was prepared by mixing the S-9 fraction of the liver of Aroclor 1254-treated male Sprague-Dawley rats with appropriate cofactors.

3.2.4 Positive control

Triethylenemelamine (without S-9 mix) and cyclophosphamide (with S-9 mix). For further details, see 3.3.1 and 3.3.2

3.3 Administration / Exposure; Application of test substance

3.3.1 Sister chromatid exchange, test concentrations

The test substance was sent as coded aliquots to Litton Bionetics, Inc. and to the Columbia University for testing.

Laboratory	S-9 mix	Trial No.	Glutaraldehyde test concentrations
Litton Bionetics, Inc.	-	1	0.36, 1.08, 3.6 and 10.8 µg/ml
	+	1	1, 3.6 and 10.8 µg/ml
	+	2	10, 12.5 and 15 µg/ml
Columbia University	-	1	0.5, 1.6, 5 and 16 µg/ml
	+	1	1.6, 5 and 16 µg/ml

Laboratory	S-9 mix	Trial No.	Negative control	
			Substance name	Test concentration
Litton Bionetics, Inc.	-	1	Distilled water	-
	+	1	Distilled water	-
	+	2	Distilled water	-
Columbia University	-	1	Dimethylsulfoxide (DMSO)	-
	+	1	Dimethylsulfoxide (DMSO)	-

Laboratory	S-9 mix	Trial No.	Postive control	
			Substance name	Test concentration
Litton Bionetics, Inc.	-	1	Triethylenemelamine	15 µg/ml
	+	1	Cyclophosphamide	1.5 µg/ml
	+	2	Cyclophosphamide	1.5 µg/ml
Columbia University	-	1	Triethylenemelamine	0.015 µg/ml
	+	1	Cyclophosphamide	1 µg/ml

3.3.2 Chromosomal aberrations, test concentrations

The test substance was sent as coded aliquots to Litton Bionetics, Inc. and to the Columbia University for testing.

Laboratory	S-9 mix	Trial No.	Glutaraldehyde test concentrations
Litton Bionetics, Inc.	-	1	0.3, 1. 3, 10 µg/ml
	+	1	1, 3, 10, 15 and 30 µg/ml
Columbia University	-	1	1.6, 5 and 16 µg/ml
	+	1	1.6, 5 and 16 µg/ml

Laboratory	S-9 mix	Trial No.	Negative control	
			Substance name	Test concentration
Litton Bionetics, Inc.	-	1	Distilled water	-
	+	1	Distilled water	-
Columbia University	-	1	Dimethylsulfoxide (DMSO)	-
	+	1	Dimethylsulfoxide (DMSO)	-

Laboratory	S-9 mix	Trial No.	Positive control	
			Substance name	Test concentration
Litton Bionetics, Inc.	-	1	Triethylenemelamine	50 µg/ml
	+	1	Cyclophosphamide	50 µg/ml
Columbia University	-	1	Triethylenemelamine	0.15 µg/ml
	-	1	Cyclophosphamide	15 µg/ml

3.3.3 Way of application

Sister chromatid exchange test in absence of S-9 mix:

In absence of S-9 mix, the CHO cells were incubated for 26 hours in the appropriate medium supplemented with fetal bovine serum, l-glutamine and antibiotics, and containing the test substance. BrdU was added 2 hours after test initiation. At the end of the incubation period, the medium was removed and replaced by fresh one, containing BrdU and colcemid; the cultures were subjected to a further incubation, this time for 2 hours. Thereafter the cells were harvested, fixed and stained (Hoechst 33258 and Giemsa) on slides.

Sister chromatid exchange test in presence of S-9 mix:

In presence of S-9 mix, the CHO cells were incubated in serum free medium containing the test substance and S-9 mix for 2 hours. The medium was then removed and replaced by fresh one, containing serum and BrdU; the cells were incubated for further 26 hours. Two hours prior ending the incubation time, colcemid was added. Thereafter the cells were harvested, fixed and stained (Hoechst 33258 and Giemsa) on slides.

Chromosomal aberration test in absence of S-9 mix:

In absence of S-9 mix, the CHO cells were incubated for 8.5 to 12 hours in the appropriate medium containing the test substance. Colcemid was added and the cells were incubated for further two hours. Thereafter the cells were harvested, fixed and stained (Hoechst 33258 and Giemsa) on slides. Cells were selected for scoring on the basis of good morphology and complete karyotype (i.e., 21 +/- 2 chromosomes). 100 first division metaphase cells were scored per dose level. Following classes of aberrations were considered:

Aberration classes	
Simple aberrations	Breaks, terminal deletions
Complex aberrations	Rearrangements, trasnlocations
Other aberrations	Pulverized cells, despiralized chromosomes, cells with 10 or more aberrations

Chromosomal aberration test in presence of S-9 mix:

In presence of S-9 mix, the CHO cells first were incubated in medium containing the test substance and S-9 mix for 2 hours. The medium was then removed and replaced by fresh one, and the cells were incubated again for 8.5 to 12 hours. Two hours prior ending the incubation time, colcemid was added. Thereafter the cells were harvested, fixed and stained (Hoechst 33258 and Giemsa) on slides. Scoring was conducted as described for the chromosomal aberration test in absence of S-9 mix.

3.3.4 Pre-incubation time None

3.3.5 Other modifications The cell cultures were handled under gold light to prevent photolysis of BrdU-substituted DNA

3.4 Examinations

3.4.1	Number of cells evaluated	Sister chromatid exchange test: Statistical assessment of the results was based on the slopes of the dose-response curve, according to Galloway SM et al. (Environ. Mutagen. 7: 1-51, 1985). Chromosomal aberration test: Statistical assessment of the results was based on the dose-response curve and individual dose points, according to Galloway SM et al. (Environ. Mutagen. 7: 1-51, 1985).
3.5	**Remark**	For evaluation of the results, following two evaluation systems were considered: (1) Galloway SM et al. (1985) Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: comparison of results for 22 compounds in two laboratories. Environ Mutagen. 7(1)1-51 (Published) (2) Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S,Rimpo J, Margolin BH, Resnick MA, Anderson B, Zeiger E (1987) Chromosome aberrations and sister chromatid exchanges in chinese hamster ovary cells: evaluations of 108 chemicals. Environ. Mol. Mutagen. 10(suppl. 10): 1-175 (Published)
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	

Section A6.6.2 _ 02 — Genotoxicity in vitro

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3 — In-vitro cytogenicity study in Chinese hamster ovary cells (CHO) measuring sister chromatid exchange and chromosome aberrations

4.1.1 Sister chromatid exchange - results obtained from Litton Bionetics, Inc. and from the Columbia University

TABLE D3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Glutaraldehyde[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)[2]
Study performed at Litton Bionetics, Inc.								
-S9								
Trial 1 Summary: Positive								
Distilled water		50	1026	390	0.38	7.8	25.5	
Triethylenemelamine	15	50	1010	2079	2.05	41.6	25.5	441.53
Glutaraldehyde	0.36	50	1031	475	0.46	9.5	25.5	21.20*
	1.08	50	1034	400	0.38	8.0	25.5	1.77
	3.6	50	1028	539	0.52	10.8	25.5	37.94*
	10.8	0					25.5	
								P<0.001[3]
+S9								
Trial 1 Summary: Weak positive								
Distilled water		50	1035	477	0.46	9.5	25.5	
Cyclophosphamide	1.5	50	1023	1348	1.31	27.0	25.5	185.92
Glutaraldehyde	1	50	1046	501	0.47	10.0	25.5	3.93
	3.6	50	1045	535	0.51	10.7	25.6	11.09
	10.8	50	1035	713	0.68	14.3	25.5	49.48*
								P<0.001
Trial 2 Summary: Positive								
Distilled water		50	1026	394	0.38	7.9	26.0	
Cyclophosphamide	1.5	50	1052	1691	1.60	33.8	26.0	318.59
Glutaraldehyde	10	50	1028	451	0.43	9.0	26.0	14.24
	12.5	50	1019	560	0.54	11.2	26.0	43.11*
	15	50	1025	652	0.63	13.0	26.0	65.64*
								P<0.001

TABLE D3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Glutaraldehyde (continued)

Compound	Dose (µg/mL)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)
Study performed at Columbia University								
-S9								
Trial 1 Summary: Negative								
Dimethylsulfoxide		50	1050	524	0.49	10.5	26.0	
Triethylenemelamine	0.015	50	1050	1437	1.36	28.7	26.0	174.24
Glutaraldehyde	0.5	50	1050	545	0.51	10.9	26.0	4.01
	1.6	50	1049	483	0.46	9.7	26.0	-7.74
	5	50	1048	531	0.50	10.6	26.0	1.53
	16	25	524	321	0.61	12.8	26.0	22.75*
								P=0.035
+S9								
Trial 1 Summary: Weak positive								
Dimethylsulfoxide		100	2097	915	0.43	9.2	26.0	
Cyclophosphamide	1	100	2095	2593	1.23	25.9	26.0	183.66
Glutaraldehyde	1.6	50	1048	484	0.46	9.7	26.0	5.84
	5	50	1047	484	0.46	9.7	26.0	5.95
	16	100	2092	1167	0.55	11.7	26.0	27.85*
								P<0.001

[1] SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. The protocol and these data are published in Galloway *et al.* (1985).
[2] Percentage increase in SCEs/chromosome of culture exposed to glutaraldehyde relative to those of culture exposed to solvent.
[3] Significance was tested by the linear regression trend test vs. log of the dose.
* Positive (>20% increase over solvent control).

Section A6.6.2 _ 02 — Genotoxicity in vitro

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3 — **In-vitro cytogenicity study in Chinese hamster ovary cells (CHO) measuring sister chromatid exchange and chromosome aberrations**

4.1.2 Chromosomal aberrations - results obtained from Litton Bionetics, Inc. and from the Columbia University

TABLE D4 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Glutaraldehyde[1]

Study performed at Litton Bionetics, Inc.

-S9 — Trial 1 – Harvest time: 10.5 hours; Summary: Negative

Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Distilled water				
	100	3	0.03	3.0
Triethylenemelamine				
50	100	19	0.19	18.0
Glutaraldehyde				
0.3	100	0	0.00	0.0
1	100	1	0.01	1.0
3	100	1	0.01	1.0
10	0			
				P=0.843[2]

+S9 — Trial 1 – Harvest time: 10.5 hours; Summary: Negative

Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Distilled water				
	100	8	0.08	6.0
Cyclophosphamide				
50	100	43	0.43	23.0
Glutaraldehyde				
1	100	2	0.02	2.0
3	100	2	0.02	2.0
10	100	5	0.05	5.0
15	0			
30	0			
				P=0.631

Study performed at Columbia University

-S9 — Trial 1 – Harvest time: 14.0 hours; Summary: Weak positive

Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Dimethylsulfoxide				
	100	1	0.01	1.0
Triethylenemelamine				
0.15	100	23	0.23	20.0
Glutaraldehyde				
1.6	100	4	0.04	4.0
5	100	6	0.06	5.0
16	100	12	0.12	11.0*
				P=0.001

+S9 — Trial 1 – Harvest time: 14.0 hours; Summary: Negative

Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Dimethylsulfoxide				
	100	1	0.01	1.0
Cyclophosphamide				
15	100	23	0.23	19.0
Glutaraldehyde				
1.6	100	1	0.01	1.0
5	100	4	0.04	3.0
16	100	7	0.07	7.0*
				P=0.004

[1] Abs=aberrations. The protocol and these data are presented by Galloway *et al.* (1985).
[2] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose.
* Positive (P≤0.05).

4.1.3 Summary of the main results obtained from all test laboratories

Sister chromatid exchange (SCE):

Laboratory	Without S9	S9 (rat)	
	Trial 1	Trial 1	Trial 2
Litton Bionetics, Inc.	+	+ (weak)	+
Columbia University	-	+ (weak)	NP

-, negative; +, positive; +/- equivocal; NP, not performed;

The results of the first laboratory showed that glutaraldehyde clearly induced SCE in the cells, with and without S-9 mix. The increase in SCE was statistically significant at 0.36 µg/ml (21.20 % over neg. control) and 3.6 µg/ml (37.94%) in the absence of S-9 mix, and at 10.8 µg/ml (49.48%), 12.5 µg/ml (43.11%) and 15 µg/ml (65.64%) in the presence of S-9 mix. The 2nd laboratory reported a positive response with S-9 mix only (27.85% at 16 µg/ml). In absence of S-9 mix, an increase in SCE frequency was seen at 16 µg/ml (22.75%) but the trend test resulted in $p = 0.035$ (i.e. $p > 0.025$); according to the evaluation system of Galloway et al. (Environ. Mutagen. 7: 1-51, 1985) the trial was evaluated as negative. According to the newer evaluation system of this author (Galloway SM et al., Environ. Mol. Mutagen. 10(10): 1-175, 1987), the results of this trial would be “equivocal”.

Chromosomal aberrations (CA):

Laboratory	Without S9	S9 (rat)
	Trial 1	Trial 1
Litton Bionetics, Inc.	-	-
Columbia University	+ (weak)	-

-, negative; +, positive; +/- equivocal

The results of the first laboratory were negative, with and without S-9 mix. In absence of S-9 mix, the % of CA in treated cultures ranged from 0 (0.3 µg/ml) to 1% (3 and 10 µg/ml) and was therefore < the negative control value (3%). In presence of S-9 mix, the % of CA ranged from 2 to 5 and was therefore within negative control range (6%). According to the results of the 2nd laboratory, a statistically significant increased % of CA was seen at 16 µg/ml without S9 mix (11% versus 1% in the neg. control). With S-9 mix and at 16 µg/ml a value of 7% was given (neg. control: 1%); this value was not of sufficient magnitude for positive evaluation when considering the evaluation system of Galloway et al. (1985). According to the more recent evaluation system of Galloway et al. (1987) the result given above would be interpreted as “weakly positive”.

4.2 Cytotoxicity

The highest test concentration chosen was limited by toxicity. Therefore a cytotoxic effect was expected at concentrations > 16 µg/ml test substance.

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.6.2 _ 02	Genotoxicity in vitro
Annex Point IIA6.6.1 / 6.6.2 / 6.6.3	In-vitro cytogenicity study in Chinese hamster ovary cells (CHO) measuring sister chromatid exchange and chromosome aberrations

5.1 Materials and methods

The aim of the present study was to look for the genotoxic potential of glutaraldehyde in an in vitro cytogenicity study with CHO cells measuring sister chromatid exchange and chromosome aberrations.

Test substance: Glutaraldehyde 50% aqueous solution, from Union Carbide (South Charleston, WV) batch No: 95296, purity 50.0%

The test was conducted according to Galloway SM et al. (Environ. Mutagen. 7: 1-51, 1985); it was not recognizable whether the tests were conducted in accordance with GLP or not. For testing, GA aliquots were sent to Litton Bionetics, Inc. and to the Columbia University.

Sister chromatid exchange test: Without S-9 mix, the CHO cells were incubated for 26 h in medium with GA. BrdU was added 2 hours after test start. The medium was replaced by fresh medium with BrdU and colcemid; the cells were incubated for 2 h. With S-9 mix, the cells were incubated in medium with GA and S-9 mix (2 h). The medium was replaced by fresh medium with BrdU; the cells were incubated for 26 h. 2 h prior the end of incubation colcemid was added.

Laboratory	S-9 mix	Trial No.	Glutaraldehyde test concentrations
Litton Bionetics, Inc.	-	1	0.36, 1.08, 3.6 and 10.8 µg/ml
	+	1	1, 3.6 and 10.8 µg/ml
	+	2	10, 12.5 and 15 µg/ml
Columbia University	-	1	0.5, 1.6, 5 and 16 µg/ml
	+	1	1.6, 5 and 16 µg/ml

Chromosomal aberration test: Without S-9 mix, the cells were incubated for 8.5 - 12 h in medium with GA. Colcemid was added and the cells were incubated for 2 h. With S-9 mix, the cells were incubated in medium with GA and S-9 mix (2 h). Fresh one replaced the medium; the cells were incubated for 8.5 to 12 h. Colcemid was added 2 h prior the end of incubation. Cells were selected for scoring on the basis of good morphology and complete karyotype. 100 first division metaphases were scored/dose. Following classes of aberrations were considered: Simple aberrations (e.g. breaks), complex aberrations (e.g. rearrangements), and further aberrations (e.g. pulverized cells).

Laboratory	S-9 mix	Trial No.	Glutaraldehyde test concentrations
Litton Bionetics, Inc.	-	1	0.3, 1. 3, 10 µg/ml
	+	1	1, 3, 10, 15 and 30 µg/ml
Columbia University	-	1	1.6, 5 and 16 µg/ml
	+	1	1.6, 5 and 16 µg/ml

The results were assessed according to the evaluation system of Galloway SM et al. (Environ. Mutagen. 7: 1-51, 1985). Some results also were re-assessed with the newer evaluation system of Galloway SM et al. (Environ. Mol. Mutagen. 10(10): 1-175, 1987).

Positive control substances: Triethylenemelamine (without S-9 mix) and cyclophosphamide (with S-9 mix).

The test substance was tested for mutagenicity in the reverse mutation assay on bacteria with and without metabolic activation (S9-mix prepared from the liver S9 fraction of Aroclor 1254-treated male Sprague-Dawley rats or Syrian hamsters). Following Salmonella typhimurium tester strains were used in this assay: TA 98, TA 100, TA 1535, TA 1537, TA 102 and TA 104. At the EG&G Mason Research

5.2 Results and discussion

Sister chromatid exchange (SCE):

Laboratory	Without S9	S9 (rat)	
	Trial 1	Trial 1	Trial 2
Litton Bionetics, Inc.	+	+ (weak)	+
Columbia University	-	+ (weak)	NP

-, negative; +, positive; +/- equivocal; NP, not performed;

The results of the first laboratory showed that glutaraldehyde (GA) clearly induced SCE in the cells, with and without S-9 mix. The increase in SCE was statistically significant at 0.36 µg/ml (21.20 % over neg. control) and 3.6 µg/ml (37.94%) in the absence of S-9 mix, and at 10.8 µg/ml (49.48%), 12.5 µg/ml (43.11%) and 15 µg/ml (65.64%) in the presence of S-9 mix. The 2nd laboratory reported a positive response with S-9 mix only (27.85% at 16 µg/ml). In absence of S-9 mix, an increase in SCE frequency was seen at 16 µg/ml (22.75%) but according to the evaluation system of Galloway et al. (1985) the trial was evaluated as negative. According to the newer evaluation system of Galloway et al. (1987), the results of this trial would be "equivocal".

Chromosomal aberrations (CAb):

Laboratory	Without S9	S9 (rat)
	Trial 1	Trial 1
Litton Bionetics, Inc.	-	-
Columbia University	+ (weak)	-

-, negative; +, positive; +/- equivocal

The results of the first laboratory were negative, with and without S-9 mix. In absence of S-9 mix, the % of CAb in treated cultures ranged from 0 (0.3 µg/ml) to 1% (3 and 10 µg/ml) and was therefore < the negative control value (3%). In presence of S-9 mix, the % of CAb ranged from 2 to 5 and was therefore within negative control range (6%). According to the results of the 2nd laboratory, a statistically significant increased % of CAb was seen at 16 µg/ml without S9 mix (11% versus 1% in the neg. control). With S-9 mix and at 16 µg/ml a value of 7% was given (neg. control: 1%); this value was not of sufficient magnitude for positive evaluation when considering the evaluation system of Galloway et al. (1985). According to the more recent evaluation system of Galloway et al. (1987) the result given above were interpreted as "weakly positive" in the absence of S9 mix.

X

5.3 Conclusion

Glutaraldehyde produced sister chromatid exchanges as well as chromosomal aberrations in mammalian cells.

5.3.1 Reliability

2

5.3.2 Deficiencies

For the chromosomal aberrations test, percentages of chromosomal aberrations were reported (quantitative data), but no data on the type and distribution of the aberrations (qualitative data) were provided.

Section A6.6.2 _ 02	**Genotoxicity in vitro**
Annex Point IIA6.6.1 / 6.6.2 / 6.6.3	**In-vitro cytogenicity study in Chinese hamster ovary cells (CHO) measuring sister chromatid exchange and chromosome aberrations**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 28th, 2010
Materials and Methods	**3.1.2** This refers to Doc IIIA Section A2.
Results and discussion	5.2 Results and discussion. The results are given mistakenly for the Lab 2 results of chromosomal aberrations. The correct result is as follows: Without S9: positive With S9: weakly positive
Conclusion	Agree with applicant's version.
Reliability	3
Acceptability	Acceptable as supplementary data
Remarks	The test conditions were different in the two laboratories, e.g. test concentrations were different, and the vehicle control was either distilled water (lab 1) or dimethyl suofoxide (lab 2). The study is not described in sufficient detail to properly evaluate its reliability.
	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	

			Official use only
		1 REFERENCE	
1.1	**Reference**	(1994) AS52/XPRT Mammalian cell forward gene mutation assay on . (Unpublished), ( ), BPD ID A6.06.3_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access		
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, EPA OPP 84-2	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	(a.i. glutaraldehyde)	
3.1.1	Lot/Batch number		
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Clear colorless liquid	
3.1.2.2	Purity	About % glutaraldehyde	
3.1.2.3	Stability	The test substance was obtained from the sponsor and was stored over the study period at room temperature as received (an amber glass bottle). No physical changes indicative of instability were observed.	
3.2	**Study Type**	In vitro mammalian cell gene mutation test	
3.2.1	Organism/cell type	AS52 Chinese hamster ovary cells, clone –1.3, designated AS52	
3.2.2	Deficiencies / Proficiencies	Xanthine-guanine phosphoribosyl transferase locus (XPRT). The wild-type cells can metabolize 6-thioguanine (TG) to its toxic derivative as they possess XPRT activity. In case of XPRTmutation, the mutants are identifiable by giving them TG; in fact, presumptive mutants, due to the loss of XPRT activity are unable to convert the purine-analogue TG to its toxic monophosphate metabolite and therefore they are resistant to the lethal effect of this metabolite.	
3.2.3	Metabolic activation system	S9 mix containing S9 liver fraction obtained from Aroclor 1254-treated male Sprague-Dawley rats, and cofactors ($MgCl_2$, KCl, NADP, glucose-6-phosphate, Na_2HPO_4).	

3.2.4 Positive control

In the absence of S9 mix:

Ethyl methanesulfonate (EMS, 200 µg/ml)

In the presence of S9 mix:

Dimethylnitrosamine (DMN, 100 µg/ml)

3.3 Administration / Exposure; Application of test substance

3.3.1 Concentrations

Preliminary toxicity pre-screen test (with and without S9 mix):

0.167, 0.5, 1.67, 5, 16.7, 50, 167, 500, 1670 and 5000 µg/ml

Main mutation assay:

Without S9-mix:

5, 16.7, 50, 100, 167, 250, 333, 500, 667, 833 and 1000 ng/ml

With S9-mix:

167, 500, 1670, 2500, 3330, 5000, 6670, 8330, 10,000, 13,300 and 16,700 ng/ml

Confirmatory test:

Without S9-mix:

50, 167, 333, 500, 1000, 1670,2500, 3330, 5000, 6670, 8330, 10,000, 13,300 and 16,700 ng/ml

With S9-mix:

167, 500, 1670, 2500, 3330, 5000, 6670, 8330, 10,000, 13,300 and 16,700 ng/ml

All test series were accompanied by untreated and solvent controls (diH_2O, 20 µl/ml).

3.3.2 Way of application

The way of application was similar for both, the toxicity pre-screen test and the main mutation assay.

The tester cells were plated in the appropriate medium at a density of about 8 x 10^5 cells/flask in 25 cm^2 tissue culture flasks. They were incubated at 37 °C for 16 to 24 hours prior treatment. At the end of the incubation period and following a series of steps including medium exchanges and washing of the cultures, the cells, which were in exponential growth state (1 to 1.2 x 10^6 cells/flask), were treated with the test substance by addition of 50 to 100 µl of test or control solution (solvent: di-H_2O) to each flask. The contents of the flasks were thoroughly mixed and incubated over 5 hours under standard conditions. At the end of the incubation period, the test or control solutions were replaced by medium via a series of washing and medium exchange steps. The cultures were subjected to a further incubation over 19 hours. In case of metabolic activation, 1 ml of S9 mix was added to the appropriate flasks. At the end of the 19-hour incubation period, the medium was aspired and the cultures were washed twice prior to being harvested by trypsinisation. 0.2 ml diluted cell aliquots were prepared and added to each of three 60-mm plates containing 5 ml of medium, resulting in a density of 200 cells/plate. The plates were incubated for 7 days and the cell colonies were fixed (ethyl alcohol), stained (crystal violet) and counted.

For the main assay, duplicate cultures were used for each test concentration as well as each positive of negative control.

3.3.3	Pre-incubation time	None
3.3.4	Other modifications	None
3.3.5	Statistical assessment	The statistical assessment of the findings was based on Snee RD and Irr JD (Mut. Res. 85: 77-93, 1981).
3.4	**Examinations**	Toxicity pre-screen test: The cells were examined for their ability to form colonies. Therefore, the reduction in colony forming ability of the cells was used as indicator for the cytotoxic potential of [redacted] at defined test concentrations. Main mutation assay: Following treatment, the relative survival was determined for each culture. After a growth period of 8 days to allow expression of the mutant phenotype, 10^6 from each culture were plated in medium containing 6-thioguanine to select mutant cells. The mutant frequency was expressed as TG^r mutants/10^6 clonable cells and was obtained by dividing the total number of mutant clones by the number of cells plated, corrected for the cloning efficiency (average of 3 plates) of the cells at the time of mutant selection.

4 RESULTS AND DISCUSSION

4.1 Genotoxicity

4.1.1 Main test without metabolic activation

Control cultures, main results:

Compound	Dose (µg/ml)	Relative survival (%)	Cloning efficacity (%)	Average mutant frequency (mutants/10^6 clonable cells)
Untreated	0	67.90	82.50	40.60
	0	93.14	76.17	
Di-H_2O (µl/ml)	20	125.39	79.50	37.01
	20	89.09	75.17	
EMS	200	57.47	59.17	213.54**
	200	75.82	54.67	

EMS, ethyl methanesulfonate, positive control; *, $p<0.05$; **, $p<0.01$

Treated cultures, main results:

[redacted] Dose (ng/ml)	Relative survival (%)	Cloning efficacity (%)	Average mutant frequency (mutants/10^6 clonable cells)
5	100.81	71.00	91.71**
5	101.30	64.00	
16.7	80.96	67.67	77.10*
16.7	68.92	67.17	
50	104.43	64.83	71.13*
50	66.41	67.17	
100	65.11	69.17	75.85*
100	110.68	71.83	
167	102.41	72.83	87.64**
167	51.48	69.00	
250	92.27	68.67	49.85
250	120.47	69.83	
333	74.25	70.17	68.74
333	99.94	64.00	
500	102.63	73.00	108.55**
500	79.99	65.67	
667	89.97	66.00	102.12**
667	87.63	71.67	
833	76.07	93.17	70.98*
833	76.01	92.83	
1000	76.50	88.33	68.71
1000	77.00	85.00	

4.1.2 Main test with metabolic activation

Control cultures, main results:

Compound	Dose (µg/ml)	Relative survival (%)	Cloning efficacity (%)	Average mutant frequency (mutants/10^6 clonable cells)
Untreated	0	116.51	67.83	53.40
	0	103.84	59.50	
Di-H_2O (µl/ml)	20	92.28	69.17	59.05
	20	111.85	85.00	
DMN	100	35.04	51.83	304.47**
	100	40.20	55.17	

DMN, Dimethylnitrosamine, positive control; *, $p<0.05$; **, $p<0.01$

Treated cultures, main results:

[redacted] Dose (ng/ml)	Relative survival (%)	Cloning efficacity (%)	Average mutant frequency (mutants/10^6 clonable cells)
167	99.72	66.00	97.52**
167	111.05	52.00	
500	111.14	62.83	72.93
500	135.59	63.33	
1670	85.14	50.17	79.37*
1670	110.53	66.17	
2500	95.30	68.00	81.13*
2500	111.98	72.00	
3330	111.37	56.00	116.80**
3330	93.65	44.00	
5000	92.39	57.17	134.21**
5000	91.59	53.83	
6670	43.78	63.00	81.61*
6670	49.55	54.00	
8330	65.16	66.33	155.26**
8330	52.68	55.50	
10,000	51.24	70.83	133.29**
10,000	18.38	58.83	
13,300	21.58	56.50	120.47**
13,300	15.66	58.83	
16,700	8.33	56.67	96.80 (excluded from mutation evaluation because of high tox.)
16,700	3.33	57.17	

4.1.3 Confirmatory test without metabolic activation

Control cultures, main results:

Compound	Dose (µg/ml)	Relative survival (%)	Cloning efficacity (%)	Average mutant frequency (mutants/10^6 clonable cells)
Untreated	0	88.91	61.67	32.67
	0	86.74	63.83	
Di-H_2O (µl/ml)	20	90.73	72.67	32.70
	20	87.23	66.67	
EMS	200	59.60	56.33	199.38**
	200	66.10	47.67	

EMS, ethyl methanesulfonate, positive control; *, p<0.05; **, p<0.01

Treated cultures, main results:

[redacted] Dose (ng/ml)	Relative survival (%)	Cloning efficacity (%)	Average mutant frequency (mutants/10^6 clonable cells)
50	73.87	58.00	22.46
50	60.37	78.67	
167	66.88	73.83	46.10*
167	79.56	56.33	
333	54.70	71.50	30.38
333	67.59	59.50	
500	81.15	66.17	50.38*
500	55.90	64.67	
1000	62.11	64.50	37.99
1000	64.62	81.83	
1670	18.01	78.67	48.68*
1670	17.78	84.00	
2500	2.75	66.83	88.83 (excluded from mutation evaluation because of high tox.)
2500	1.47	65.83	
3330	0.26	68.67	213.86 (excluded from mutation evaluation because of high tox.)
3330	0.15	67.17	

Remark: due to the extreme immediate cytotoxicity, the test concentrations > 3330 ng/ml were not taken into consideration.

4.1.4 Confirmatory test with metabolic activation

Control cultures, main results:

Compound	Dose (µg/ml)	Relative survival (%)	Cloning efficacity (%)	Average mutant frequency (mutants/10^6 clonable cells)
Untreated	0	108.18	91.50	20.86
	0	119.36	75.00	
Di-H_2O (µl/ml)	20	108.15	75.50	21.82
	20	110.69	75.67	
DMN	100	36.13	52.50	312.26**
	100	37.10	39.33	

DMN, Dimethylnitrosamine, positive control; *, $p<0.05$; **, $p<0.01$

Treated cultures, main results:

[redacted] Dose (ng/ml)	Relative survival (%)	Cloning efficacity (%)	Average mutant frequency (mutants/10^6 clonable cells)
167	91.13	61.33	31.29
167	74.82	56.17	
500	101.75	54.67	29.15
500	83.01	67.00	
1670	72.23	71.00	25.94
1670	66.58	68.00	
2500	84.13	66.83	37.06
2500	85.82	68.33	
3330	81.40	69.83	23.75
3330	88.56	69.17	
5000	77.34	56.33	40.94
5000	75.21	63.33	
6670	53.95	69.67	56.22**
6670	42.24	55.33	
8330	43.13	53.33	68.85**
8330	44.93	60.00	
10,000	19.03	65.67	38.87
10,000	19.71	57.67	
13,300	10.80	44.17	63.18**
13,300	10.13	44.50	
16,700	2.72	50.33	75.48 (excluded from mutation evaluation because of high tox.)
16,700	0.47	61.00	

Section A6.6.3 _ 01 — Genotoxicity in vitro

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3 — **Mammalian cell forward gene mutation assay**

4.2 Cytotoxicity

The results of the toxicity pre-screen test were as follows:

Cytotoxicity Prescreen

Compound	µg/mL	S9 (±)	Day 1 Cell Count	Absolute Cloning Efficiency(%)	Viable Cells per Culture[a]	Relative Survival(%)[b]
Untreated	0.00	-	99	86.83	1.72	74.45
Untreated	0.00	+	169	79.17	2.68	115.89
di-H_2O[c]	20.0	-	140	77.50	2.17	93.98
di-H_2O	20.0	+	155	86.17	2.67	115.68
	0.167	-	160	78.17	2.50	108.33
	0.500	-	10	23.33	0.05	2.02
	1.67	-	17	24.83	0.08	3.66
	5.00	-	12	0.67	0.00	0.07
	16.7	-	10	0.00	0.00	0.00
	50.0	-	7	-[d]	-	-
	167	-	3	-	-	-
	500	-	2	-	-	-
	1670	-	3	-	-	-
	5000	-	2	-	-	-
	0.167	+	175	81.00	2.84	122.77
	0.500	+	162	79.33	2.57	111.31
	1.67	+	188	65.67	2.47	106.93
	5.00	+	107	29.67	0.63	27.50
	16.7	+	93	8.83	0.16	7.11
	50.0	+	12	0.33	0.00	0.03
	167	+	14	-[c]	-	-
	500	+	3	-	-	-
	1670	+	6	-	-	-
	5000	+	3	-	-	-

[a]Viable Cells/Culture = (Day 1 count)(Absolute cloning efficiency)(2)(10^4); pooled negative controls = 3.05×10^6.

[b]Relative Survival = Relative viable cells/culture (i.e., viable cells/culture as compared to the pooled negative controls).

[c]Not determined; discarded on Day 1 due to extreme immediate cytotoxicity.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to test glutaraldehyde for mutagenicity using the Mammalian cell forward gene mutation assay.

Test substance: [redacted] (a.i. glutaraldehyde), [redacted]

The test was conducted according to EPA 84-2, with GLP.

The test substance was tested on AS52 Chinese hamster ovary cells. The wild-type cells can metabolize 6-thioguanine (TG) to its toxic monophosphate derivative as they possess xanthine-guanine phosphoribosyl transferase-activity (XPRT). In case of XPRTmutation, the cells lose their XPRT activity and become unable to convert TG to its toxic metabolite; therefore they are resistant to the lethal effect of this metabolite. The testing was conducted both with and without metabolic activation (S9-mix prepared from the liver S9 fraction of Aroclor 1254-treated male Sprague-Dawley rats); each experiment was performed in duplicate.

First, a toxicity pre-screen test was conducted; in fact, the cells were examined for the reduction in colony forming ability, as parameter indicative of the cytotoxic potential of Sepacid GA 50 at defined test concentrations. Following concentrations were tested, with and without S9-mix: 0.167, 0.5, 1.67, 5, 16.7, 50, 167, 500, 1670 and 5000 µg/ml.

On the basis of the results of the pre-screen test, the test concentrations for the main mutation test were selected as follows:

Without S9-mix: 5, 16.7, 50, 100, 167, 250, 333, 500, 667, 833 and 1000 ng/ml

With S9-mix: 167, 500, 1670, 2500, 3330, 5000, 6670, 8330, 10,000, 13,300 and 16,700 ng/ml

For the confirmatory retest, following concentrations were tested:

Without S9-mix: 50, 167, 333, 500, 1000, 1670,2500, 3330, 5000, 6670, 8330, 10,000, 13,300 and 16,700 ng/ml

With S9-mix: 167, 500, 1670, 2500, 3330, 5000, 6670, 8330, 10,000, 13,300 and 16,700 ng/ml

Within the main mutation test and the confirmatory test, the following parameters were considered: the relative survival, the number of mutants, the cloning efficacity and the mutant frequency. The statistical assessment of the findings was based on Snee RD and Irr JD (Mut. Res. 85: 77-93, 1981).

All test series were accompanied by untreated and solvent controls (diH_2O, 20 µl/ml) as well as by following positive controls: ethyl methanesulfonate (EMS, 200 µg/ml; without S9 mix) and dimethylnitrosamine (DMN, 100 µg/ml; with S9 mix).

5.2	**Results and discussion**	Controls: All negative and positive controls were within acceptable ranges. Toxicity pre-screen test: Within the pre-screen test, [redacted] was found to be cytotoxic to AS52 Chinese hamster ovary cells in the absence and presence of S9 mix. In fact, without S9 mix, no more viable cells were present in the test cultures from 16.7 µg/ml test substance upwards (relative survival, 0%); in the presence of S9 mix, the same result was reported for test concentrations >=50 µg/ml (relative survival, 0.03%). For the negative untreated and solvent controls, a relative survival of 74 – 116% and 94 – 116% respectively was reported. Main mutation assay: The average mutant frequencies of the [redacted] treated cultures (excepted for 16,700 ng/ml with S9 mix, which was excluded from the mutation evaluation because of the high immediate cytotoxicity that was observed) ranged from 49.85 to 155.26 TG^r mutants/10^6 clonable cells, versus 37 to 59 for the negative control cultures. The increase in mutant frequencies related to the treatment with [redacted] was statistically significant and dose-dependent in both cases, with and without S9 mix. The treatment-related increase was >= 2 fold control values, and represented net increases in average mutant frequencies of >= 30 TG^r mutants/10^6 clonable cells. The re-evaluation of the test substance in a confirmatory test resulted in findings, which were in accordance with those reported above.
5.3	**Conclusion**	The test substance [redacted] (a.i. ca. [redacted]% glutaraldehyde) was found to be positive in the AS52/XPRT Mammalian cell forward gene mutation assay.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	The study report shows unit-errors in some tables. In fact, in these tables (e.g. page 23 of 166), µg/ml was inscribed by inadvertency instead of the correct ng/ml-unit. However, these errors did not affect the validity of the study.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 28th, 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. The test substance was mutagenic to mammalian cells *in vitro*.
Reliability	1
Acceptability	Acceptable

Section A6.6.3 _ 01 **Annex Point IIA6.6.1 / 6.6.2 / 6.6.3**	**Genotoxicity in vitro** **Mammalian cell forward gene mutation assay**
Remarks	This is a key study for Doc IIIA section 6.6.3 (*In vitro* gene mutation assay in mammalian cells).
	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	

			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 B6C3F1 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 A6.04.3_01	
1.2	**Data protection**	No	
1.2.1	Data owner	Not relevant (published data)	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, no guideline was mentioned but the test was conducted according to the protocol of McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Caspary WJ (1988) Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay. II: 18 coded chemicals. Environ Mol Mutagen. 11(1): 91-118 (Published)	
2.2	**GLP**	Not specified	
2.3	**Deviations**	Not relevant	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde 50% aqueous solution,	
3.1.1	Lot/Batch number		
3.1.2	Specification	As given in section 2	X
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	
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	
3.2	**Study Type**	Mouse lymphoma assay	
3.2.1	Organism/cell type	Mammalian cell lines: Mouse lymphoma L5178Y cells	

3.2.2	Deficiencies / Proficiencies	Thymidine kinase (TK) deficiency, BrdU sensitivity
3.2.3	Metabolic activation system	Test performed without S9 mix.
3.2.4	Positive control	Ethyl methanesulfonate was used as positive control substance and was tested at a concentration of 250 µg/ml.
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	The test substance was sent coded to Inveresk Research International for testing of mutagenicity; the test concentrations were as follows: 0 (negative control), 0.5, 1, 2, 4, 8 and 16 µg/ml.
3.3.2	Way of application	The tester cells were maintained in suspension in Fischer's medium supplemented with l-glutamine, sodium pyruvate, pluronic F68, antibiotics and heat-inactivated horse serum. In order to reduce the number of spontaneously occurring trifluorothymidine-resistant cells, subcultures were incubated once with medium supplemented with thymidine, hypoxanthine, methotrexate and glycine (THMG) for 1 day, followed by an incubation in medium containing thymidine, hypoxanthine and glycine (THG) for a further day; this was followed by incubation in normal medium for 3 to 5 days. For cloning, horse serum content was increased and Noble agar was added. All tests were performed in replicate, without S9 mix. For each treatment, 6 x 10^6 cells/10 ml medium were incubated for 4 hours with the test substance. Thereafter, then medium with the test substance was removed and the cells were resuspended into fresh medium and incubated for 2 days to allow expression of the mutant phenotype. The cell density was monitored for maintenance of a log-phase growth. After the two days incubation period, the cells (3 x 10^6) were plated in medium and soft agar supplemented with trifluorothymidine (TFT) for selection of the TFT-resistant cells. 600 cells were plated in non-selective medium and soft agar for determination of cloning efficiency. All the plates were incubated at 37°C for 10 to 12 days. Depending on the results, a further test series with S9 was added or not.
3.3.3	Pre-incubation time	None
3.3.4	Other modifications	None
3.4	**Examinations**	

3.4.1 Number of cells evaluated

Calculation of toxicity was based on the formula given below. For data evaluation the quality control criteria given in table I were considered. For evaluation of the results, the four response categories given in table II and III were considered.

$$RTG = \frac{\text{(total suspension growth} \times \text{cloning efficiency) in dosed culture}}{\text{(total suspension growth} \times \text{cloning efficiency) in control culture}}$$

Mutant fraction (MF) was calculated as follows:

$$MF = 200 \times \frac{\text{mutant clones per plate (usually a mean of 3)}}{\text{total clones per plate (usually a mean of 3)}}$$
$$= \text{mutants}/10^6 \text{ clonable cells.}$$

TABLE I. Technical Quality Control Criteria*

1. Solvent control
   (a) A solvent control count was rejected if the cloning efficiency was <50% or >115%. Between 50% and 60%, judgment was used in accepting the result.
   (b) The average mutant fraction of the solvent controls had to be >15 mutants per 10^6 surviving cells and <110 mutants per 10^6 surviving cells. The range was extended to >10– <150 mutants per 10^6 surviving cells for experiments that were positive.
   (c) Unless at least two solvent control cultures were accepted, the experiment was rejected.
   (d) The experiment was rejected if a chi-square test for consistency of the acceptable mutant fractions showed P < 5%.
2. Positive control
   (a) A positive control culture was rejected if the cloning efficiency was <10% or >115%.
   (b) A positive control culture was rejected if the relative total growth (RTG) was <1%.
3. Doses
   (a) A culture was rejected if the cloning efficiency was <10% or >115%.
   (b) A culture was rejected if the RTG was <1%.
   (c) A culture was rejected if the relative suspension growth for the second day of expression was <40%.
   (d) If the RTG was between 1%, and 5% and/or the cloning efficiency was between 10% and 20%, then the mutant count was examined. If a significant increase in the mutant fraction was not supported by an increase in the mutant count, then the culture was rejected.
   (e) A culture was rejected if the day two count was <3 × 10^5 per ml.
   (f) A dose was rejected if the compound was not soluble at that dose.
   (g) No dose greater than 5 mg/ml was tested.
   (h) A dose set was rejected if the chi-square test for consistency of the acceptable mutant fractions within that dose set showed P < 5%.
   (i) Each dose set had to contain two or more acceptable cultures.
   (j) If fewer than three dose sets were accepted, then the experiment was rejected unless the reason for dose set rejection was precipitation and there was no mutagenic response.

*Quality control criteria are those minimum criteria that had to be met before an experiment was further evaluated. Experiments that met these minimum criteria might have been rejected upon further evaluation.

TABLE II. Response Categories for Experiments

Positive response (+)
The dose-related trend and the response at one of the three highest acceptable doses (see Table I) were statistically significant.

Negative response (−)
Two categories were used. In both there was:
(a) No dose-related trend.
(b) No statistically significant response at any dose.
(c) An acceptable positive control response.

Nontoxic, negative response (=)
There was an RTG among the acceptable doses of >30% (approximately), higher toxicities being unattainable owing to intrinsic properties of either the compound or the system.

Toxic, negative response (−)
There was either an RTG of <30% (approximately) at the maximum acceptable dose, or the lethal concentration was no greater than 1.5 times a lower concentration at which the RTG was >30%.

Inconclusive (i)
There was:
(a) No dose-related trend and a statistically significant dose was any other than one of the highest three doses.
(b) A response which would have been negative, but the lowest RTG at acceptable doses was >35%.
(c) A response which would have been negative, but there were no acceptable positive controls.

Questionable (?)
There was either:
(a) No dose-related trend, but a statistically significant response occurred at one of the highest three doses, or
(b) A statistically significant dose-related trend, but none of the acceptable doses was statistically significant on its own.

TABLE III. Response Categories for Tests

Positive (+)
A test was considered positive when, out of three trials, a positive trial was reproducible.

Negative (−)
A test was considered negative when, out of three trials, a positive response or a positive dose was not reproducible.

Questionable (?)
A test was considered questionable when, out of three trials, neither a positive nor a negative response was reproduced.

4 RESULTS AND DISCUSSION

4.1 Genotoxicity

4.1.1 Without metabolic activation

TABLE D2 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by Glutaraldehyde[1]

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[2]	Average Mutant Fraction
-S9						
Trial 1						
Distilled water						
		68	98	68	33	
		71	89	68	32	
		58	102	55	31	
		77	111	41	18	29
Ethyl methanesulfonate						
		70	91	312	149	
	250	80	84	353	147	148*
Glutaraldehyde						
	0.5	64	106	105	55	
		68	153	48	23	39
	1	62	96	87	47	
		83	154	80	32	40
	2	44	71	120	91	
		80	199	67	28	59*
	4	69	100	98	47	
		75	128	97	43	45
	8	29	26	236	270	
		67	22	285	142	206*
	16	Lethal				
		Lethal				

TABLE D2 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by Glutaraldehyde (continued)

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 2						
Distilled water						
		74	102	79	36	
		59	110	66	38	
		70	88	95	45	39
Ethyl methanesulfonate						
		75	55	725	324	
	250	63	62	615	325	324*
Glutaraldehyde						
	0.5	92	88	160	58	
		80	93	88	37	47
	1	86	98	92	36	
		66	90	57	29	32
	2	64	90	76	40	
		79	87	107	45	43
	4	89	64	187	70	
		72	69	89	41	56
	8	21	2	385	611	
		27	5	283	352	481*
	16	Lethal				
		Lethal				

[1] Study performed at Inveresk Research International. The experimental protocol and these data are presented in McGregor *et al.* (1988).
[2] Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF/1 × 10^6 cells treated); MF=mutant fraction.
* Significant positive response (P≤0.05).

4.1.2 With metabolic activation — Because of the clear positive results obtained in absence of S9 mix, no further assay in presence of S9 mix was conducted.

4.2 Cytotoxicity — Cytotoxicity was observed at 16 µg/ml.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The aim of the present study was to investigate the genotoxic potential of glutaraldehyde in the in vitro mouse lymphoma assay using mouse lymphoma L5178Y cells as test model. Test substance: Glutaraldehyde 50% aqueous solution, [redacted] [redacted], purity 50.0% glutaraldehyde (minor contamination from the polymeric forms of glutaraldehyde and other volatile impurities) The test was conducted according to McGregor DB et al. (Environ. Mol. Mutagen. 11: 91-118, 1988); it was not recognizable whether the tests were conducted in accordance with GLP or not. The test substance was sent coded to Inveresk Research International for testing of mutagenicity. The test substance first was tested without metabolic activation, in two trials. The test concentrations were as follows: 0 (negative control), 0.5, 1, 2, 4, 8 and 16 µg/ml. The assessment of the test results was based on Caspary WJ et al. (Environ. Mol. Mutagen. 12 (suppl. 13): 19-36, 1988). A positive control was conducted with ethyl methanesulfonate (250 µg/ml).
5.2	**Results and discussion**	Negative control (distilled water), without S9 mix: average mutant fractions of 29 and 39 were reported for the respective trials. Positive control, without S9 mix: as expected, average mutant fractions of 148 and 324 for the respective trials were reported; both values were statistically significant (p<= 0.05). Glutaraldehyde treated cells, without S9 mix: within the first trial, average mutant fractions were > than negative control, with statistically significant values observed at 2 µg/ml (59) and 8 µg/ml (206). Similar results were obtained from the second trial; the average mutant fraction at 8 µg/ml was statistically significantly different from control (481 versus 39). Cytotoxicity: for both trials, 16 µg/ml was reported as lethal concentration. Assay in presence of S9 mix: as the results obtained in the absence of S9 mix clearly were positive, there was no need to conduct a further assay with S9 mix.
5.3	**Conclusion**	Glutaraldehyde was found to induce mutations at the thymidine kinase locus of the mouse lymphoma L5178Y cells and therefore was genotoxic under the experimental conditions chosen.
5.3.1	Reliability	**2**
5.3.2	Deficiencies	No guideline was mentioned but the test was conducted according to the protocol of McGregor DB et al. (Environ. Mol. Mutagen. 11: 91-118, 1988); it was not specified whether the study followed GLP or not.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE

Section A6.6.3 _ 02 | **Genotoxicity in vitro**

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3 | **Mouse lymphoma assay**

Date	October 28th, 2010
Materials and Methods	See remarks below
Results and discussion	See remarks below
Conclusion	See remarks below
Reliability	See remarks below
Acceptability	See remarks below
Remarks	This study summary has not been assessed in detail by the RMS, because: 1. The results are given only briefly in the provided documentation. 2. The original study is a scientific publication, and this has not been provided. 3. It is a non-guideline, non-GLP study. 4. It is concluded that another forward mutation assay is not required, and therefore a study summary is not necessary.
	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	

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994) In vivo micronucleus test with [redacted] in mouse bone marrow erythropoietic cells. [redacted] (Unpublished), ([redacted]), BPD ID A6.06.4_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, EPA OPP 84-2	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] (a.i. glutaraldehyde)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Clear colorless liquid	
3.1.2.2	Purity	About [redacted]% glutaraldehyde	
3.1.2.3	Stability	The test substance was obtained from the sponsor and was stored over the study period at room temperature as received (an amber glass bottle). No physical changes indicative of instability were observed.	
3.1.2.4	Max. tolerable dose	20 mg/kg bw.	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
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 the mice were ca. 7 weeks old and their weights ranged from 28 to 33 g for males, and 21 to 26 g for females.	
3.2.6	Number of animals per group	Preliminary dose range-finding study (DRF): 2/sex/group, 9 treated groups and one negative control group (deionized water) Micronucleus test (MNT): 5/sex/group, 9 treated groups, 3 negative	

		control groups (deionized water), one positive control group (triethylenemelamine, TEM, 0.5 mg/kg bw)	
3.2.7	Control animals	Yes, see above	
3.3	**Administration/ Exposure**	**Intraperitoneal**	
3.3.1	Nr. of applications	One single application	
3.3.2	Interval between applications	Not relevant	
3.3.3	Post-exposure period	24, 48 and 72 h after treatment	
3.3.4	Vehicle	Deionized water	
3.3.5	Total volume applied	A constant volume of test solution of 10 ml/kg bw was given.	
3.3.6	Dose applied	DRF: 0.5, 5, 25, 50, 100, 250, 500, 1000 and 2500 mg/kg bw MNT: 2, 10 and 20 mg/kg bw	
3.3.7	Substance used as Positive Control	Triethylenemelamine (TEM), dosed 0.5 mg/kg bw	
3.3.8	Controls	Control substances were applied at the same volume as [redacted]	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Tissue	Bone marrow	
		Nr. of animals:	All animals
		Number of cells:	1000 poly-chromatic erythrocytes (PCEs) per mouse, i.e., 10,000 PCEs per test group
		Time points:	24, 48 and 72 h after treatment
		Type of cells	Erythropoietic cells in bone marrow
		Parameters:	Micronucleated polychromatic erythrocytes/ polychromatic erythrocytes (MPCEs/PCEs) Polychromatic erythrocytes/normochromatic erythrocytes ratio (PCEs/NCEs) for 1000 erythrocytes
3.5	**Further remarks**	The whole study consisted of a dose range-finding study (DRF) for the determination of the adequate test doses for the micronucleus test, which was followed by the ultimate MNT. Because of the results of the MNT, an additional confirmatory test using female mice only was conducted.	

4 RESULTS AND DISCUSSION

Section A6.6.4 _ 01	**Genotoxicity in vivo**
Annex Point IIA6.6.4 / 6.6.5 / 6.6.6	**In vivo Micronucleus test using mouse bone marrow erythropoietic cells.**

4.1 Clinical signs

DRF (0.5 to 2500 mg/kg bw): All mice treated with doses >= 100 mg/kg bw died within 24 hours; at 50 mg/kg bw, 1 male and 1 female died after 72 h. No mortality was observed in lower dosed groups. Symptoms of toxicity were observed at 5, 25 and 50 mg/kg bw and mainly included writhing, abnormal gait, distended abdomen and piloerection. At the lowest tested dose of 0.5 mg/kg bw, no such symptoms were seen.

MNT (2, 10 and 20 mg/kg bw): As expected, no mortally was observed at the chosen test doses. In fact, symptoms of toxicity included piloerection (all doses), writhing (all doses) and abnormal gait (at 10 and 20 mg/kg bw). No such symptoms were seen in the controls (both, positive and negative).

4.2 Frequency of MPCEs in mice treated with [redacted] after 24 hours

Mean percentage of micronucleated polychromatic erythrocytes (MPCE) for male (M) and female (F) mice at time point 24 h:

Sex	TS	Dose (mg/kg bw)	Total MPCE/ 5000 PCE[b]	Range	% MPCE
M	DeH_2O	0	1	0-1	0.02+/-0.045
	[redacted]	2	4	0-2	0.08+/-0.084
	[redacted]	10	3	0-2	0.06+/-0.089
	[redacted]	20	5	0-3	0.10+/-0.123
	TEM[a]	0.5	93	8-29	1.86+/-0.783**
F	DeH_2O	0	4	0-2	0.08+/-0.084
	[redacted]	2	1	0-1	0.02+/-0.045
	[redacted]	10	9	1-3	0.18+/-0.084*
	[redacted]	20	1	0-1	0.02+/-0.045
	TEM[a]	0.5	95	17-21	1.90+/-0.187**

*, one-tailed t-tests, p<=0.05; **, p<=0.01;a, positive control triethylenemelamine; b, 1000 PCE per mouse and 5000 per sex and group

4.3 Frequency of MPCEs in mice treated with after 48 hours

Mean percentage of micronucleated polychromatic erythrocytes (MPCE) for male (M) and female (F) mice at time point 48 h:

Sex	TS	Dose (mg/kg bw)	Total MPCE/ 5000 PCE[a]	Range	% MPCE
M	DeH$_2$O	0	4	0-2	0.08+/-0.084
		2	0	0-0	0.00+/-0.000
		10	1	0-1	0.02+/-0.045
		20	4	0-3	0.08+/-0.130
F	DeH$_2$O	0	2	0-1	0.04+/-0.055
		2	1	0-1	0.02+/-0.045
		10	2	0-1	0.04+/-0.055
		20	6	0-2	0.12+/-0.084

*, one-tailed t-tests , p<=0.05; **, p<=0.01; a, 1000 PCE per mouse and 5000 per sex and group

4.4 Frequency of MPCEs in mice treated with after 72 hours

Mean percentage of micronucleated polychromatic erythrocytes (MPCE) for male (M) and female (F) mice at time point 72 h:

Sex	TS	Dose (mg/kg bw)	Total MPCE/ 5000 PCE[a]	Range	% MPCE
M	DeH$_2$O	0	2	0-1	0.04+/-0.055
		2	4	0-1	0.08+/-0.045
		10	2	0-2	0.04+/-0.089
		20	3	0-2	0.06+/-0.089
F	DeH$_2$O	0	4	0-3	0.08+/-0.130
		2	3	0-1	0.06+/-0.055
		10	2	0-2	0.04+/-0.089
		20	2	0-1	0.04+/-0.055

*, one-tailed t-tests , p<=0.05; **, p<=0.01; a, 1000 PCE per mouse and 5000 per sex and group

4.5 Frequency of MPCEs in female mice treated with confirmatory test, 24 h

Mean percentage of micronucleated polychromatic erythrocytes (MPCE) for female mice in the confirmatory test (24 h):

Sex	TS	Dose (mg/kg bw)	Total MPCE/ 5000 PCE[a]	Range	% MPCE
F	DeH$_2$O	0	6	0-2	0.06+/-0.07
		10	9	0-2	0.09+/-0.088

*, one-tailed t-tests , p<=0.05; **, p<=0.01; a, 1000 PCE per mouse and 5000 per sex and group

4.6 PCE/NCE ratios in mice treated with 50 after 24 hours

PCE/NCE ratios for male (M) and female (F) mice at time point 24 h:

Sex	TS	Dose (mg/kg bw)	Range	Mean +/- SD
M	DeH$_2$O	0	1.304-2.040	1.681+/-0.306
		2	0.988-1.833	1.373+/-0.340
		10	1.294-1.597	1.494+/-0.125
		20	0.901-1.994	1.397+/-0.400
	TEM	0.5	0.842-1.907	1.340+/-0.414
F	DeH$_2$O	0	1.008-2.571	1.619+/-0.596
		2	1.899-3.504	2.541+/-0.819
		10	0.748-1.915	1.352+/-0.452
		20	0.667-1.174	0.954+/-0.194*
	TEM	0.5	0.681-2.257	1.407+/-0.637

*, one-tailed t-tests , p<=0.05; **, p<=0.01

4.7 PCE/NCE ratios in mice treated with 50 after 48 hours

PCE/NCE ratios for male (M) and female (F) mice at time point 48 h:

Sex	TS	Dose (mg/kg bw)	Range	Mean +/- SD
M	DeH$_2$O	0	1.336-1.833	1.647+/-0.200
		2	0.848-2.257	1.552+/-0.526
		10	1.336-2.584	1.905+/-0.456
		20	0.250-1.793	1.114+/-0.570
F	DeH$_2$O	0	1.053-1.841	1.551+/-0.327
		2	1.336-1.786	1.548+/-0.194
		10	1.049-2.333	1.651+/-0.486
		20	0.416-2.040	1.138+/-0.643

*, one-tailed t-tests , p<=0.05; **, p<=0.01

4.8 Genotoxicity

As neither a reproducible statistically significant increase nor a clear dose-related increase in the frequency of MPCE were observed, the test substance was considered as non-clastogenic in the in vivo micronucleus test.

4.9 Other

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the in vivo genotoxic potential of glutaraldehyde using the micronucleus test (MNT)

Test substance: [redacted] (a.i. glutaraldehyde), batch No [redacted]

The test was conducted according to EPA 84-2, with GLP.

The whole study consisted of a dose range-finding study (DRF; determination of the adequate test doses for the MNT), which was followed by the MNT, and an additional confirmatory test using female animals only. [redacted] mice of both sex were used; at test initiation they were ca. 7 weeks old and their body weights ranged from 28 to 33 g for the males, and 21 to 26 g for the females. The mice received single application of the test solution via i.p. injection; the application volume was 10 ml/kg bw; the vehicle was deionized water. The DRF was conducted with following concentration: 0.5, 5, 25, 50, 100, 250, 500, 1000 and 2500 mg/kg bw. On the basis of the results of the DRF test, the experimental design of the MNT was chosen as follows:

Group	Dose Level (mg/kg bw)	Number of mice/sacrifice time		
		24 h	48 h	72 h
Deionized water	0	20	10	10
[redacted]	2	10	10	10
[redacted]	10	20	10	10
[redacted]	20	10	10	10
TEM *	0.5	10	0	0

*, Triethylenemelamine, positive control

Preparation of the bone marrow: The preparation of the bone marrow was based on the method of Schmid W (Mut. Res. 19:109-117, 1975) and the main steps of this preparation can be summarized as follows:

- Dissection/removal of the soft tissues from the femora
- Extraction of the bone marrow and sampling in 5 ml round bottom culture tubes containing fetal bovine serum
- Mixing and centrifugation of the bone marrow suspension
- Removal of the supernatant and resuspension of the precipitate
- Application of a drop of the suspension onto slides for microscopy, rapid drying of the slides on a slide warmer (ca. 56°C), short dipping of the slides in absolute methanol and air-drying
- Staining of the preparations on the slides with a modified Wrights Stain Pak (4481; polychrome methylene blue-eosin), air-drying
- Coverslipping of the slided with Permaslip.

Cytogenic analysis: 1000 PCE were scored for MPCE, and the PCE/NCE ratio was determined for 1000 erythrocytes.

Section A6.6.4 _ 01	Genotoxicity in vivo
Annex Point IIA6.6.4 / 6.6.5 / 6.6.6	In vivo Micronucleus test using mouse bone marrow erythropoietic cells.

5.2	**Results and discussion**	The main results of the present study can be summarized as follows: A statistically significant increase in the frequency of MPCE in mice treated with 10 mg/kg bw [redacted] was observed at sacrifice time point 24 h; this was mainly due to the females. A statistically significant decrease of the PCE/NCE ratio was reported for the females at sacrifice time point 24 h; when combined together with the results for the males, a similarly significant decrease was observed at 20 mg/kg bw after 24 and 48 hours. The decrease of the PCE/NCE ratio was indicative of the toxicity of the test substance to bone marrow, even though it did not increase the frequency of MPCE. Within the confirmatory test, the statistically significant findings of the main micronucleus test could neither be reproduced nor confirmed.
5.3	**Conclusion**	[redacted] was not clastogenic in the *in vivo* micronucleus test
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	October 28th, 2010
Materials and Methods	2.3 Deviations. No analysis of the dosing solution was performed. 3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	Agree with applicant's version.
Conclusion	There was an inconsistent pattern of slight increases in micronucleated polychromatic erythrocytes (PCE), which is interpreted to indicate a moderate genotoxic effect. The reduced PCE/NCE ratio at 20 mg/kg bw is interpreted as an indication that the test substance has reached the bone marrow.
Reliability	2
Acceptability	Acceptable
Remarks	There were slight increases in the numbers of micronucleated PCEs, but these were neither reproducible nor dose related. The confirmatory assay was performed without a positive control, in one sex only, and not at the highest concentration. The results of the confirmatory test are therefore considered inconclusive. Please note that the tabulated numerical values in the tables above have not been checked in detail by the RMS. This is a key study for Doc IIIA section 6.6.4 (*In vivo* genotoxicity study).

Section A6.6.4 _ 01	**Genotoxicity in vivo**
Annex Point IIA6.6.4 / 6.6.5 / 6.6.6	**In vivo Micronucleus test using mouse bone marrow erythropoietic cells.**

	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	

			Official use only
		1 REFERENCE	
1.1	**Reference**	████ (2002) In vivo unscheduled DNA synthesis (UDS) assay with ████% Glutaraldehyde) in rat hepatocytes - single oral administration. BASF AG, Department of Product Safety, Ludwigshafen/Rhein, Germany, Report No: 80M0447/974255 (Unpublished), BPD ID A6.06.5_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	████	
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 486 (1997)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	████% Glutaraldehyde)	
3.1.1	Lot/Batch number	████	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Colorless liquid	
3.1.2.2	Purity	About ██% glutaraldeyhde as aqueous solution	
3.1.2.3	Stability	The stability of ████ at room temperature in the vehicle (water) over a period of 14 days was verified analytically (████).	
3.1.2.4	Maximum tolerable dose	400 mg/kg bw. The maximum tolerable dose was selected on the basis of the results of an acute oral toxicity pretest.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	████	
3.2.3	Source	████	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	Mean weight: about 256 g Age: about 10 to 12 weeks	

3.2.6	Number of animals per group	3 males per test group
3.2.7	Control animals	Yes, vehicle control (i.e. distilled water)
3.3	**Administration/ Exposure**	
3.3.1	Number of applications	Single application
3.3.2	Interval between applications	None
3.3.3	Post-exposure period	3 and 14 hours
		Oral
3.3.4	Type	Gavage
3.3.5	Concentration	200 and 400 mg/kg bw (the test concentrations refer the test substance as such and not to the active ingredient)
3.3.6	Vehicle	Purified water
3.3.7	Concentration in vehicle	Respectively 2 and 4 g/100 ml
3.3.8	Total volume applied	10 ml test solution/ kg bw
3.3.9	Controls	Negative control: vehicle control (purified water, 10 ml/kg bw) Positive control: 2-acetylaminofluorene (2-AAF), 50 mg/kg bw suspended in corn oil, 10 ml/kg bw test solution

3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Tissue	Liver	
		Number of animals:	3 animals/test group
		Number of cells:	For microscopical evaluation and quantification of UDS, a total of 100 cells/animal were considered.
		Time points:	3 and 14 hours after treatment
		Type of cells	Isolated hepatocytes (isolation procedure based on the method described by Butterworth BE et al., Mutat. Res. 189(2): 123-133, 1987)
		Parameters:	Net nuclear grain (NNG) count/cell Mean nuclear grain (NG) count Mean cytoplasmic grain count (CG) count Mean net nuclear grain count Percentage of cells in repair (cells with NNG >= 0) Percentage of cells in repair (cells with NNG >= 5)
		Additional parameters	Cytoxicity (i.e. cell viability as measured by the trypan blue exclusion technique) and conspicuous changes in cell morphology.

3.5 Acceptance and Evaluation criteria for the *in vitro* UDS assay

Acceptance criteria:

-Clearly negative results in the untreated and in the vehicle controls in the range of historical control data.

-Clearly positive results in the positive control group (>=30% of cells in repair, as mean of 3 animals) in the range of historical control data.

-Viability (trypan blue staining) of at least 70% in hepatocytes from negative control animals.

Evaluation criteria:

-Positive response: a positive response implicates a dose-related increase in mean number of NNG counts (> 0 at one of the test points) and in percentage of cells in repair (i.e. cells with NNG >= 5), which must be >= 20.

-Marginal response: a response is considered marginal when the dose-related increase in percentage of cells in repair is >= 5 outside the values of both the concurrent negative control and the historical control (>= 5 < 20), and when the dose-related increase in mean number of NNG counts is near to but without exceeding 0. In this case, an additional confirmatory test is needed.

-Negative response: a negative response implicates that both, the NNG counts and the percentage of cells in repair are within the range of negative control.

3.6 Further remarks

Samples of the test solutions were subjected to analytical monitoring for verification of the test concentrations.

4 RESULTS AND DISCUSSION

4.1 Clinical signs

Evident signs of toxicity were seen in animals treated with the test substance at both test concentrations. These signs mainly included piloerection, squatting posture and bloody snout.

Signs of toxicity were neither seen in the negative nor in the positive control animals.

4.2 Body weight data

Mean body weight/test group after 3 hours following treatment:

Doses (ml or mg/kg bw)	Mean body weight/group* (g)
Vehicle control	260.9 +/- 18.3
200	256.7 +/- 9.4
400	257.9 +/- 14.2
Positive control (2-AAF50 mg/kg bw)	266.5 +/- 13.8

*, N = 3

Mean body weight/test group after 14 hours following treatment:

Doses (ml or mg/kg bw)	Mean body weight/group* (g)
Vehicle control	260.7 +/- 18.7
200	261.2 +/- 12.2
400	230.7 +/- 9.1
Positive control (2-AAF50 mg/kg bw)	254.8 +/- 22.6

*, N = 3

4.3 Cytotoxicity data

Viability of the hepatocytes (trypan blue exclusion technique)

After 3 hours following treatment:

Doses (ml or mg/kg bw)	Viability of the cells as mean values *	Viability of the cells as % of control
Vehicle control	82.5 +/- 10.2	100
200	82.0 +/- 5.8	99.4
400	82.5 +/- 8.42	100
Positive control (2-AAF50 mg/kg bw	84.5 +/- 3.5	102.4

*, N = 3

Viability of the hepatocytes (trypan blue exclusion technique)

After 14 hours following treatment:

Doses (ml or mg/kg bw)	Viability of the cells as mean values *	Viability of the cells as % of control
Vehicle control	85.2 +/- 8.3	100
200	85.3 +/- 6.1	100.1
400	79.8 +/- 4.4	93.7
Positive control (2-AAF50 mg/kg bw	84.1 +/- 2.4	98.6

*, N = 3

4.4 Genotoxicity, DNA repair activity, 3 hours after treatment

Test groups	Vehicle control water			200 mg/kg			400 mg/kg			Positive control 50 mg/kg 2-AAF		
No. of cells	100	100	100	100	100	100	100	100	100	100	100	100
NG counts Mean ± SD*	6.06 ±3.67	6.38 ±2.81	7.92 ±4.18	8.45 ±4.18	8.58 ±4.04	6.34 ±3.73	6.23 ±3.05	6.42 ±3.05	6.76 ±3.91	20.13 ±6.46	20.22 ±9.15	23.33 ±10.50
CG counts Mean ± SD*	11.55 ±4.06	10.94 ±3.36	13.72 ±4.41	14.82 ±4.42	14.68 ±5.05	14.32 ±4.35	12.67 ±3.79	12.31 ±4.23	12.37 ±3.93	17.02 ±5.33	14.30 ±4.69	15.07 ±5.34
NNG counts Mean ± SD*	-5.49 ±3.24	-4.56 ±3.22	-5.80 ±3.70	-6.37 ±3.64	-6.10 ±4.92	-7.98 ±3.91	-6.44 ±3.27	-5.89 ±3.62	-5.61 ±2.87	3.12 ±5.52	5.93 ±6.84	8.26 ±8.15
% cells in repair NNG > 0	8	9	8	5	10	3	2	5	3	70	84	87
% cells in repair NNG ≥ 5	0	0	0	0	0	0	0	0	0	34	55	63
NG counts Mean ± SD**	6.79 ± 0.99			7.79 ± 1.26			6.47 ± 0.27			21.23 ± 1.82		
CG counts Mean ± SD**	12.07 ± 1.46			14.61 ± 0.26			12.45 ± 0.19			15.46 ± 1.40		
NNG counts Mean ± SD**	-5.28 ± 0.65			-6.82 ± 1.02			-5.98 ± 0.42			5.77 ± 2.57		
% cells in repair NNG ≥ 0 Mean ± SD**	8.33 ± 0.58			6.00 ± 3.61			3.33 ± 1.53			80.33 ± 9.07		
% cells in repair NNG ≥ 5 Mean ± SD**	0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			50.67 ± 14.98		

NG, nuclear grains; CG, cytoplasmic grains, NNG, net nuclear grains; *, mean per animals (100 cells); **, mean per group (3 animals); SD, standard deviation

Section A6.6.5 _ 01 | **Genotoxicity in vivo**

Annex Point IIA6.6.4 / 6.6.5 / 6.6.6 | **In-vivo rat hepatocyte unscheduled DNA synthesis assay**

4.5 Genotoxicity, DNA repair activity, 14 hours after treatment

Test groups	Vehicle control water			200 mg/kg			400 mg/kg			Positive control 50 mg/kg 2-AAF		
No. of cells	100	100	100	100	100	100	100	100	100	100	100	100
NG counts Mean ± SD*	6.77 ±3.46	10.54 ±4.34	6.34 ±3.27	7.22 ±3.67	7.68 ±3.83	8.07 ±4.30	6.37 ±3.14	7.16 ±3.22	5.90 ±3.04	17.95 ±9.46	19.02 ±8.85	22.50 ±9.44
CG counts Mean ± SD*	11.21 ±3.35	16.12 ±4.04	11.71 ±3.15	12.36 ±3.81	14.03 ±4.20	14.61 ±4.40	11.31 ±3.19	13.04 ±3.65	10.36 ±3.10	12.92 ±5.17	12.49 ±4.13	15.48 ±5.73
NNG counts Mean ± SD*	-4.44 ±3.21	-5.58 ±3.85	-5.37 ±3.12	-5.14 ±3.55	-6.35 ±3.84	-6.54 ±4.57	-4.94 ±3.19	-5.88 ±3.56	-4.46 ±2.80	5.03 ±7.32	6.54 ±8.13	7.03 ±8.07
% cells in repair NNG > 0	9	8	8	11	2	6	8	4	7	76	81	83
% cells in repair NNG ≥ 5	0	0	0	0	0	0	0	0	0	47	54	56
NG counts Mean ± SD**	7.88 ± 2.31			7.66 ± 0.43			6.48 ± 0.64			19.82 ± 2.38		
CG counts Mean ± SD**	13.01 ± 2.70			13.67 ± 1.17			11.57 ± 1.36			13.63 ± 1.62		
NNG counts Mean ± SD**	-5.13 ± 0.61			-6.01 ± 0.76			-5.09 ± 0.72			6.20 ± 1.04		
% cells in repair NNG ≥ 0 Mean ± SD**	8.33 ± 0.58			6.33 ± 4.51			6.33 ± 2.08			80.00 ± 3.61		
% cells in repair NNG ≥ 5 Mean ± SD**	0.00 ± 0.00			0.00 ± 0.00			0.00 ± 0.00			52.33 ± 4.73		

4.6 Other

<u>Analytical monitoring of the test concentration:</u>

Theoritical values	Analytically determined values	
	Individual values	Means
20 mg/ml	15.9 mg/ml *	16.6 mg/ml
	17.4 mg/ml **	
	16.5 mg/ml ***	
40 mg/ml	41.5 mg/ml *	42.3 mg/ml
	42.5 mg/ml **	
	42.8 mg/ml ***	

*, data of sample1; **, data of sample 2, ***, data of sample 3

Analytically recovery was about 79.5 to 107 % of the theoretical values.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The ability of █% glutaraldehyde) to cause DNA damage and repair was examined in the *in vivo* UDS assay using male █ rats.

Test substance: █% Glutaraldehyde), batch No: █, purity ca. █% glutaraldeyhde (aqueous solution), The stability of █ at room temperature in the vehicle (water) over a period of 14 days was verified analytically (█).

The assay was conducted according to OECD 486 (1997), with GLP.

On the basis of the results of a pretest, 400 mg/kg bw was selected as maximal tolerable dose and the assay was performed using following two test concentrations: 200 and 400 mg/kg bw (referring to the test substance as such). 3 animals were used per test group The test doses were prepared using purified water as vehicle; the rats received single oral application of test solution at an application volume of 10 ml/kg bw. Purified water was tested as negative control; 2-acetylaminofluorene (2-AAF; 50 mg/kg bw suspended in corn oil) was tested as positive control. The test animals were examined for clinical signs of toxicity and for body weight. Hepatocytes were prepared 3 and 14 hours after test substance administration and were examined for cell viability as measured by the trypan blue exclusion technique. DNA damage and repair was measured by incorporation of ^{3}H-Thymidine using autoradiography technique. In fact, for microscopical evaluation and quantification of UDS, a total of 100 cells/animal was examined and following parameters were considered:

Net nuclear grain (NNG) count/cell
Mean nuclear grain (NG) count
Mean cytoplasmic grain count (CG) count
Mean net nuclear grain count
% of cells in repair (cells with NNG >= 0; cells with NNG >= 5)

The results were assess on the basis of following evaluation criteria:

Positive response	Dose-related increase in mean number of NNG counts (> 0 at one of the test points) and in percentage of cells in repair (i.e. cells with NNG >= 5), which must be >= 20.
Marginal response	Dose-related increase in percentage of cells in repair >= 5 outside the values of both the concurrent negative control and the historical control (>= 5 < 20), and dose-related increase in mean number of NNG counts near to but without exceeding 0. In this case, an additional confirmatory test is needed.
Negative response	NNG counts and percentage of cells in repair within the range of negative control.

The test concentrations were subjected to an analytical monitoring.

Section A6.6.5 _ 01	Genotoxicity in vivo
Annex Point IIA6.6.4 / 6.6.5 / 6.6.6	In-vivo rat hepatocyte unscheduled DNA synthesis assay

5.2 Results and discussion

In contrast to the negative and positive controls, evident signs of toxicity including piloerection, squatting posture and bloody snout, were reported for the animals treated with [redacted].

For all treated animals including those serving for positive control, the cell viability after 3 and 14 hours was within 94 - 102% of control.

The microscopical evaluation and quantification of UDS showed that for the negative controls, the frequencies of mean nuclear grain counts were within historical control range. For the positive controls, the increase in unscheduled DNA synthesis was as expected. The results obtained for the [redacted]-treated animals were within the range of negative controls, as can be seen from following summary-table:

Test group	Negative Control.	[redacted] 200 mg/kg bw	[redacted] 400 mg/kg bw	Positive control
Cells harvested after 3 hours following treatment				
NG counts*	6.79 +/- 0.99	7.79 +/- 1.26	6.47 +/- 0.27	21.23 +/- 1.82
CG counts*	12.07 +/- 1.46	14.61 +/- 0.26	12.45 +/- 0.19	15.46 +/- 1.40
NNG counts**	- 5.28 +/- 0.65	- 6.82 +/- 1.02	-5.98 +/- 0.42	5.77 +/- 2.57
% cells in repair (NNG >= 0)	8.33 +/- 0.58	6.00 +/- 3.61	3.33 +/- 1.53	80.33 +/- 9.07
% cells in repair (NNG >= 5)	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	50.67 +/- 14.98
Cells harvested after 14 hours following treatment				
NG counts*	7.88 +/- 2.31	7.66 +/- 0.43	6.48 +/- 0.64	19.82 +/- 2.38
CG counts*	13.01 +/- 2.70	13.67 +/- 1.17	11.57 +/- 1.36	13.63 +/- 1.62
NNG counts**	-5.13 +/- 0.61	-6.01 +/- 0.76	-5.09 +/- 0.72	6.20 +/- 1.04
% cells in repair (NNG >= 0)	8.33 +/- 0.58	6.33 +/- 4.51	6.33 +/- 2.08	80.00 +/- 3.61
% cells in repair (NNG >= 5)	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	52.33 +/- 4.73

NG, nuclear grains; CG, cytoplasmic grains, NNG, net nuclear grains; *, mean per group (3 animals) with standard deviation

The analytical monitoring of the test solutions revealed a test substance recovery of about 79.5 to 107 %.

5.3 Conclusion

The test substance [redacted] was negative in the *in vivo* UDS assay with rat hepatocytes.

5.3.1 Reliability — **1**

5.3.2 Deficiencies — No

http://www.ateneum.fi/	**Evaluation by Competent Authorities**
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 29th, 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: GA was negative in the *in vivo* UDS assay. It is however not established that the test substance has reached the target organ, and therefore the result is not conclusive.
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 A6.6.6 _ 01 Annex Point IIA6.6.4 / 6.6.5 / 6.6.6	**Genotoxicity in vivo** **In vivo genotoxicity test for assessment of possible germ cell effects**	
	1 REFERENCE	Official use only
1.1 Reference	Kari FW (1993) NTP technical report on toxicity studies of glutaraldehyde administered by inhalation to F344/N rats and B6C3F1 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	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. In fact, the study followed the method described by Yoon JS et al. (Environ Mutagen. 7(3):349-367, 1985) and by Zimmering S et al. (Environ. Mol. Mutagen. 14(4):245-251, 1989), and was in accordance with the OECD guideline 477.	X
2.2 GLP	Yes	X
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	Glutaraldehyde 50% aq. solution, [redacted]	X
3.1.1 Lot/Batch number	[redacted]	X
3.1.2 Specification	As given in section 2	X
3.1.2.1 Description	Clear colorless liquid	X
3.1.2.2 Purity	50.0% (minor contamination from the polymeric forms of glutaraldehyde and other volatile impurities)	X
3.1.2.3 Stability	No data given	
3.1.2.4 Maximum tolerable dose	Not relevant	
3.2 Test Animals		
3.2.1 Species	Drosophila melanogaster	
3.2.2 Strain	Canton-S wild-type	
3.2.3 Source	No data	
3.2.4 Control	Untreated	
3.2.5 Sex	First approach: Male	

Section A6.6.6 _ 01 Annex Point IIA6.6.4 / 6.6.5 / 6.6.6		Genotoxicity in vivo In vivo genotoxicity test for assessment of possible germ cell effects	
		Second approach: Male/Female Third approach: Male	
3.2.6	Age/weight at study initiation	First approach: Adult male flies (24 to 72 h old) Second approach: Eggs Third approach: Adult male flies (<= 24 h old)	
3.3	**Administration/ Exposure**	First approach: Injection into the thorax under the wing Second approach: Oral feed Third approach: Oral feed	
3.3.1	Number of applications	First approach: Single injection Second approach: Oral feed over the larval development; corn meal food with glutaraldehyde in solvent (5% ethanol). Third approach: Oral feed over 3 days; solution of test substance in 5% sucrose.	X
3.3.2	Principle of the SLRL test	Mutations in the X-chromosome of D. melanogaster are phenotypically expressed in males carrying the mutant gene. When the mutation is lethal in the hemizygous condition, its presence is inferred from the absence of one class of male offspring out of the two that are normally produced by a heterozygous female. The SLRL test takes advantage of these facts by means of specially marked and arranged chromosomes.	

Section A6.6.6 _ 01 | **Genotoxicity in vivo**

Annex Point IIA6.6.4 / 6.6.5 / 6.6.6 | **In vivo genotoxicity test for assessment of possible germ cell effects**

3.3.3 Test procedure	First approach, according to Yoon JS et al. (Environ Mutagen. 7(3):349-367, 1985): Adult male flies (24 to 72 h old) received single injection of the test substance and were allowed to recover for 24 hours. Each of the treated males was then mated with 3 Basc females for 3 days; the fresh females were given for mating at 2-days intervals to produce 3 matings of 3, 2 and 2 days. The offspring of the females were scored for lethal effects corresponding to the effects on mature sperm, mid or late stage spermatids, early spermatids, spermatocytes and spermatogonia at the time of treatment. The heterozygous female offspring were mated individually to their brothers and were then placed in individual vials. The F1 females of the same parental male were kept together to identify clusters. In the next generation the progeny from each separate dose was scored for phenotypically wild-type males. The presence of less than 5% of the expected number of wild-type males per vial after 17 days was indicative of a sex-linked recessive lethal mutation that had occurred in a germ cell of the parental male following treatment with glutaraldehyde. Second approach according to Zimmering S et al. (Environ. Mol. Mutagen. 14(4):245-251, 1989): Adult male and female flies were mated and the eggs were placed into vials containing corn meal food with glutaraldehyde in solvent (5% ethanol). The adult emergent males (ca. 24 h old) were mated with 2 successive harems of 3 to 5 Basc females to produce 2 single-day broods. The offspring of these females were scored for lethal effects corresponding to the effects on mature sperm, mid or late stage spermatids, early spermatids, spermatocytes and spermatogonia at the time of treatment. The heterozygous female offspring were mated individually to their brothers and were then placed in individual vials. The F1 females of the same parental male were kept together to identify clusters. In the next generation the progeny from each separate dose was scored for phenotypically wild-type males. The presence of less than 5% of the expected number of wild-type males per vial after 17 days was indicative of a sex-linked recessive lethal mutation that had occurred in a germ cell of the parental male following treatment with glutaraldehyde. Third approach according to Yoon JS et al. (Environ Mutagen. 7(3):349-367, 1985): Adult male flies (<= 24 h old) were fed a solution of test substance in 5% sucrose for 3 days. A control group consisted of flies fed with 5% sucrose only. Each of the treated males was then mated with 3 Basc females for 3 days; the fresh females were given for mating at 2-days intervals to produce 3 matings of 3, 2 and 2 days. The offspring of these females were scored for lethal effects corresponding to the effects on mature sperm, mid or late stage spermatids, early spermatids, spermatocytes and spermatogonia at the time of treatment. The heterozygous female offspring were mated individually to their brothers and were then placed in individual vials. The F1 females of the same parental male were kept together to identify clusters. In the next generation the progeny from each separate dose was scored for phenotypically wild-type males. The presence of less than 5% of the expected number of wild-type males per vial after 17 days was indicative of a sex-linked recessive lethal mutation that had occurred in a germ cell of the parental male following treatment with glutaraldehyde.	X

Section A6.6.6 _ 01 **Genotoxicity in vivo**

Annex Point IIA6.6.4 / 6.6.5 / 6.6.6 **In vivo genotoxicity test for assessment of possible germ cell effects**

3.3.4 Test concentrations

First approach: 0, 3000 ppm, 4000 ppm

Second approach: 0, 3500 ppm

Third approach: 0, 7500, 10000 ppm

3.4 Statistics

The SLRL data were assessed statistically by simultaneous comparison with the concurrent and historical controls using a normal approximation to the binomial test according to Margolin BH et al (Environ. Mutagen. 5: 705-716, 1983).

4 RESULTS AND DISCUSSION

4.1 First approach

Summary of the main results (for details, see Table 4.1):

Dose	Deaths	Sterility	Sex-linked lethal mutation	Result of the test
0 ppm	-	-	0.06 – 0.24%	Negative
3000 ppm	2%	0%	0.22%	
4000 ppm	22%	54%	0.10%	

4.2 Second approach

Summary of the main results (for details, see Table 4.1):

Dose	Deaths	Sterility	Sex-linked lethal mutation	Result of the test
0 ppm	-	-	0.08%	Negative
3500 ppm	10%	0%	0.11%	

4.3 Third approach

Summary of the main results (for details, see Table 4.1):

Dose	Deaths	Sterility	Sex-linked lethal mutation	Result of the test
0 ppm	-	-	0.07 – 0.21%	Negative
7500 ppm	27%	37%	0.10%	
10000 ppm	68%	2%	0.00%	

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Kari FW (1993) reported the data of three Drosophila SLRL tests conducted with glutaraldehyde 50% for the assessment of possible germ cell effects.

Test substance: Glutaraldehyde [redacted], batch No: [redacted], purity 50.0% (minor contamination from the polymeric forms of glutaraldehyde and other volatile impurities).

The study was conducted according to Yoon JS et al. (Environ Mutagen. 7(3):349-367, 1985) and to Zimmering S et al. (Environ. Mol. Mutagen. 14(4):245-251, 1989); the test conduct was similar to OECD 477. The study followed GLP.

For the first test, adult male flies (Drosophila melanogaster; 24-72 h old) received single injection of the test substance. After 24 h, each treated male was mated with 3 Basc females for 3 days; the fresh females were given for mating at 2-days intervals to produce 3 matings The offspring were scored for lethal effects (effects on mature sperm, mid or late stage spermatids, early spermatids, spermatocytes and spermatogonia). The heterozygous female offspring were mated individually to their brothers and were placed in individual vials; F1 females of the same parental male were kept together to identify clusters. In the next generation the progeny from each separate dose was scored for phenotypically wild-type males.

For the second test, adult male and female flies were mated and the eggs were placed into vials with corn meal food and test substance. The adult emergent males (ca. 24 h old) were mated with 2 successive harems of 3 to 5 Basc females to produce 2 single-day broods. The offsprings were scored for lethal effects (effects on mature sperm, mid or late stage spermatids, early spermatids, spermatocytes and spermatogonia). The heterozygous female offspring were mated individually to their brothers and were then placed in individual vials. The F1 females of the same parental male were kept together to identify clusters. In the next generation the progeny from each separate dose was scored for phenotypically wild-type males

For the third test, Adult male flies (<= 24 h old) were fed a solution of test substance in 5% sucrose for 3 days. Control flies were fed 5% sucrose only. Each of the treated males was then mated with 3 Basc females for 3 days; the fresh females were given for mating at 2-days intervals to produce 3 matings. The offspring of these females were scored for lethal effects (see above) and the heterozygous female offspring were mated individually to their brothers and were then placed in individual vials; the F1 females of the same parental male were kept together to identify clusters. In the next generation the progeny from each separate dose was scored for phenotypically wild-type males.

The SLRL data were assessed statistically by simultaneous comparison with the concurrent and historical controls using a normal approximation to the binomial test according to Margolin BH et al. (Environ. Mutagen. 5: 705-716, 1983).

Section A6.6.6 _ 01 Annex Point IIA6.6.4 / 6.6.5 / 6.6.6	Genotoxicity in vivo In vivo genotoxicity test for assessment of possible germ cell effects
5.2 Results and discussion	For all three tests, the percentage of sex-linked lethal mutations was within control range (0.06 – 0.24%) and the tests were therefore negative.
5.3 Conclusion	Glutaraldehyde has no germ cell mutagenic potential
5.3.1 Reliability	**2**
5.3.2 Deficiencies	According to BPD, an assessment of possible germ cell effects only is required when the results of section A6.6.4 were positive. Glutaraldehyde was found to neither be clastogenic in vivo (negative in the Micronucleus test), nor it resulted in unscheduled DNA synthesis (negative in the UDS test). Nevertheless, the results of the NTP Drosophila SLRL tests reported by Kari FW (1993) were included in the BPD dossier as supplementary information, as the test conduct for each of the three experiments was almost in accordance with the requirements of the OECD TG 477 adopted 1984, and the results were well-documented and reported. Thus, the data are of scientific acceptability.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 29th, 2010
Materials and Methods	See Remarks below. 2.1 Guideline study. The studies are performed similarly to the OECD 477 guideline, but details are not given to verify whether the methodology follows this guideline. 2.2 GLP. There is no indication of GLP in the original reports. 3.1 Test material. The test material was Glutaraldehyde [redacted]. 3.1.1 [redacted] 3.1.2 Specification. See comments to 3.1 and 3.1.2 above. 3.1.2.1 Description. No information is given. 3.1.2.2 Purity. No information is given. 3.3.1: The solvent in the second approach was water, not ethanol. 3.3.3: The solvent in the second approach was water, not ethanol. All details of the procedures could not be confirmed using references indicated in 1.1 and 2.1.
Results and discussion	See Remarks below.

Section A6.6.6 _ 01	**Genotoxicity in vivo**
Annex Point IIA6.6.4 / 6.6.5 / 6.6.6	**In vivo genotoxicity test for assessment of possible germ cell effects**

Conclusion	The results were clearly negative, but the reliability of the study is poor. This is a non-GLP study that has not been reported to a sufficient detail, and the test material has not been properly characterised. See Remarks below.
Reliability	3
Acceptability	Acceptable as supportive information only.
Remarks	1.1 Reference. The reference is given to the NTP study BPD ID A6.4.3_01, where the results of earlier studies are merely cited. The correct references for the studies are the ones cited under point 2.1. The details of the procedures and of the results are poorly documented, and critical evaluation of the study is not possible. The applicant has submitted a comparison of the NTP test and OECD guideline 477 (see Table 2 added by RMS, with RMS comments).
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.6 _ 01 **Genotoxicity in vivo**

Annex Point IIA6.6.4 / 6.6.5 / 6.6.6 **In vivo genotoxicity test for assessment of possible germ cell effects**

Table 4.1. Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* following treatment with Glutaraldehyde

Route of Exposure	Dose (ppm)	Incidence of Deaths (%)	Incidence of Sterility (%)	No. of Lethal/No. of X Chromosomes Tested Mating 1	Mating 2	Mating 3	Total[2]
Injection	3000	2	0	6/1792	1/1146	3/1688	10/4626 (0.22%)
	0			4/1125	3/1146	1/1133	8/3404 (0.24%)
Injection	4000	22	54	0/486	2/855	0/578	2/1919 (0.10%)
	0			1/1009	1/1297	0/865	2/3171 (0.06%)
Feeding	7500	27	37	2/1947	3/2194	1/1828	6/5969 (0.10%)
	0			3/1858	0/1832	1/2090	4/5780 (0.07%)
Feeding	10000	68	2	0/742	0/618	0/698	0/2058 (0.00%)
	0			2/724	1/706	1/443	4/1873 (0.21%)
Larva Feeding	3500	10	0	4/2694	2/2686	0/000	6/5380 (0.11%)
	0			2/2598	2/2630	0/000	4/5228 (0.08%)

[1] Study performed at Brown University. The protocol and the data from the adult feeding and injection studies are presented in Yoon *et al.* (1985). The protocol and the data from the larval feeding study are presented in Zimmering *et al.* (1989). Results were not significant at the 5% level (Margolin *et al.*, 1983).

[2] Total number of lethal mutations/total number of X chromosomes tested for three mating trials.

Table 2. Comparison of NTP and OECD TG 477

NTP Study: Kari FW (1993) NTP technical report on toxicity studies of glutaraldehyde administered by inhalation to F344/N rats and B6C3F1 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

Parameter	OECD TG 477 Requirements	Fulfilled/Not fulfilled	Deviations	RMS comment
Test substance	Must be well characterized (purity & impurities, relevant physical-chemical properties, stability data)	Yes	-	The test substance was % pure GA from . This is all the information available, and therefore the test material has not been properly characterised.
Positive control substances	Ethyl methanesulphonate and N-nitrosodimethylamine are sited by the OECD TG as examples of substances which might be used as a positive control.	No; however not a must	-	Agree with applicant.

Parameter	OECD TG 477 Requirements	Fulfilled/Not fulfilled	Deviations	RMS comment
Test animals	Males *Drosophilia melanogaster* must be of welldefined wild type; Females of the Muller-5 stock may be used. Other appropriate female stocks with multiply inverted X-chromosomes may also be used.	Yes; Male *Drosophilia melanogaster* of accepted Canton-S-wildtype were used. Basc females were used.	-	Agree with applicant.
	Recommended age: 3 to 5 days	Partly	For the first experiment (single injection), the males were ca. 24 to 72 hours old, i.e., 1 to 3 days old. For the second experiment (feeding during development), larvae were used. For the third experiment (feeding of adult), the males were ca. 24 hours old.	Agree with applicant.
Treatment	Administration of the test substance by the oral route, by injection or by exposure to gases or vapour is accepted.	Yes; 3 experiments were conducted, based on single, feeding of larvae, and feeding of adults.	-	Agree with applicant.
	Feeding of the test substance may be done in sugar solution.	Yes (third experiment)	-	Agree with applicant,
	When necessary, substances may be dissolved in a 0.7% NaCl solution and injected into the thorax or abdomen.	It was not specified whether a vehicle was used for injection (first experiment) or not. However it can be assumed that glutaraldehyde 50% aq.sol. was injected as such.	-	Agree with applicant.
Dose level selection	For the initial assessment of mutagenicity, one exposure of the test substance is used, that exposure being the maximum-tolerated concentration or that producting some indication of toxicity, where possible.	Yes	Toxicity tests were performed to set concentrations of glutaraldehyde at a level that would induce 30% mortality after 72 hours of feeding, while keeping induced sterility at an acceptable level.	Agree with applicant.
Negative controls	Negative (vehicle) controls should be included. However, if appropriate laboratory historical control data are available, no concurrent controls are needed.	Yes; in fact, both, concurrent and historical controls, were included and considered.	-	Agree with applicant,

Parameter	OECD TG 477 Requirements	Fulfilled/Not fulfilled	Deviations	RMS comment
Test design	Wild-type males are treated with the test substance and mated individually to an excess of virgin females from appropriate stock (i.e. with multiply inverted X-chromosomes). The females are replaced with fresh virgins every two to three days to cover the entire germ cell cycle. The offspring of these females are scored for lethal effects corresponding to the effects on mature sperm, mid or late-stage spermatids, early spermatids, spermatocytes and spermatogonia at the time of treatment. Heterozygous F1 females from the above crosses are allowed to mate individually with their brothers. In the F2 generation, each culture is scored for the absence of wild-type males.	Yes, (experiment 1 and 3)	An additional experiment was conducted, which in principle was similar to the remaining two, except for the fact that eggs obtained from untreated parents were allowed to develop in presence of corn meal food enriched with test substance, and the adult emergent males (ca. 24 h old) were mated with untreated females.	Agree with applicant.
	If a culture appears to have arisen from an F1 female carrying a lethal in the parental X-chromosome (i.e. no males with the treated chromosome are observed), a daughter of that female with the same genotype should be tested to ascertain whether the lethality is repeated at the next generation.	No, as relevant.	-	Agree with applicant,
Test results	Test results should be confirmed in a separate experiment.	Yes (three experiments were conducted, all resulted in negative finding)	-	Agree with applicant.
Test results	Data should be tabulated to show the number of chromosomes tested, the number of non fertile males and the number of lethal chromosomes at each exposure concentration and for each mating period for each male treated. Numbers of clusters of different sizes per male should be reported.	Yes (original table was subsequently included in the robust study summary A.6.06.6_01)	-	Number of males treated, number of F_2 cultures established, number of F_2 cultures without prgeneny should have been reported.

Parameter	OECD TG 477 Requirements	Fulfilled/Not fulfilled	Deviations	RMS comment
	Several statistical techniques are acceptable for evaluation sex-linked recessive lethal tests. Clustering of recessive lethals originating from one male should be considered and evaluated in an appropriate statistical manner.	Yes; The SLRL data were assessed statistically by simultaneous comparison with the concurrent and historical controls using a normal approximation to the binomial test according to Margolin BH et al. (Environ. Mutagen. 5: 705-716, 1983).	-	Agree with applicant.
Validation criteria	Positive response: 1)- Statistically significant dose-related increase in the frequency of sex-linked recessive lethal mutations; 2)- Reproducibility and statistical significance of the positive response for at least one of the test points	No		Agree with applicant.
	Negative response: 1)- No statistically significant dose-related increase in the frequency of sex-linked recessive lethal mutations; 2)- No statistically significant and reproducible positive response at any one of the test points	Yes		Agree with applicant.
Test reporting	The test report should include informations on the Drosophila stock/strain used, the age of insects, the number of males treated, the number of sterile males, the number of F2 cultures established, the number of F2 cultures without progeny, the number of chromosomes tested, the number of chromosomes carrying a lethal mutation detected at each germ cell stage	Yes	-	Agree with applicant.
	The test report should include a detailed description of treatment and sampling schedule, exposure levels, toxicity data, negative (solvent) and positive controls, if appropriate.	Yes	-	Reporting is not sufficiently detailed for proper evaluation of the original data.

Parameter	OECD TG 477 Requirements	Fulfilled/Not fulfilled	Deviations	RMS comment
	The test report should include the criteria for scoring of lethal mutations, data on exposure/effect relationship where relevant, and data on the statistical evaluation.	Yes	-	Agree with applicant.
Conclusion: According to BPD, an assessment of possible germ cell effects only is required when the results of section A6.6.4 were positive. Glutaraldehyde was found to neither be clastogenic in vivo (negative in the Micronucleus test), nor it resulted in unscheduled DNA synthesis (negative in the UDS test). Nevertheless, the results of the NTP Drosophila SLRL tests reported by Kari FW (1993) were included in the BPD dossier as supplementary information, as the test conduct for each of the three experiments was almost in accordance with the requirements of the OECD TG 477 adopted 1984, and the results were well-documented and reported. Thus, the data are of scientific acceptability.				The studies were performed using [redacted] This is all the information available, and therefore the test material has not been properly characterised. The scientific evidence is taken into account as supportive data, but no conclusive evidence is provided by these experiments.

Section A6.7 _ 01 Annex Point IIA6.7		Carcinogenicity Carcinogenicity in Wistar rats following administration in the drinking water for 24 months	

			Official use only
		1 REFERENCE	
1.1	**Reference**	(2003) % glutaradeldehyde) – Carcinogenicity study in rats - Administration in the drinking water for 24 months. (Unpublished), BPD ID A6.07_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access		
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 Guideline 451 (1981)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	% Glutaraldehyde)	
3.1.1	Lot/Batch number		
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Colorless-clear liquid	
3.1.2.2	Purity	% (analysis performed by)	
3.1.2.3	Stability	The stability of the test substance in drinking water over a period of 14 days at room temperature had been proven prior to starting the experiment. The stability of the test substance was proven by reanalysis ( )	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain		

3.2.3	Source	[redacted]
3.2.4	Sex	Males /Female
3.2.5	Age/weight at study initiation	42 +/- 1 days old at study start Mean body weight for the males: 211.3 g (187.1 – 235.6 g) Mean body weight for the females: 166 g (147.6 – 183.9 g)
3.2.6	Number of animals per group	50 animals per sex and test group were used.
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	24 months
3.3.2	Interim sacrifice(s)	None
3.3.3	Final sacrifice	After 24 months
3.3.4	Frequency of exposure	The animals received the test substance in drinking water continuously throughout the 104 weeks of treatment.
3.3.5	Post-exposure period	None
		Oral
3.3.6	Type	In drinking water
3.3.7	Concentration	

Test group	Test concentration (ppm)
0	0 ppm (control)
1	100 ppm
2	500 ppm
3	2000 ppm

3.3.8	Controls	Controls received drinking water as such.

3.4	**Examinations**	
3.4.1	Body weight	Yes, determined (1) prior starting the treatment, (2) on day 0 (test start), (3) weekly during the first 13 weeks of treatment and (4) at 4 -week intervals until study termination The body weight gain was calculated according to following formula: $BW_{day\ x} - BW_{day\ 0}$ $BW_{day\ x}$ = body weight on study day x (g) $BW_{day\ 0}$ = body weight on day 0 (g)
3.4.2	Food consumption	Yes, determined (1) once a week over a period of 7 days during the first 13 weeks of treatment and (2) at 4 -week intervals until study termination.
3.4.3	Food efficiency	Food efficiency was calculated according to following formula: $\frac{BW_{day\ x} - BW_{day\ y}}{FC_{y\text{-}x}} \times 100$ = Food efficiency on day x BW day x = body weight on day x (following day y; in g) BW day x-y = body weight on day y (in g) $FC_{y\text{-}x}$ = mean food consumption (g) from day y to day x; calculated as mean food consumption on day x, multiplied with number of days between day y and day x.
3.4.4	Water consumption	Yes, determined (1) once a week over a period of 7 days during the first 13 weeks of treatment and (2) at 4 -week intervals until study termination
3.4.5	Test substance intake	The mean daily intake of test substance as group means was calculated according to following formula: $\frac{WC_x \times C}{BW_x}$ = Substance intake for day x BW_x = body weight on day x (in g) WC_x = mean daily water consumption on day x (in g) C = concentration in the drinking water
3.4.6	Clinical signs and mortality	The animals were checked for symptoms of toxicity and mortality twice a day on working days, and once a day on Saturdays, Sundays and public holidays
3.4.7	Macroscopic investigations	A comprehensive clinical examination including palpation was conducted once a week.
3.4.8	Ophthalmoscopic examination	No
3.4.9	Haematology	Yes Blood samples were collected from all animals at necropsy; differential blood

			smears were prepared. The blood smears of the control and the highest test group (2000 ppm) were evaluated. Blood smears of all animals, which were killed in extremis during the administration period, also were evaluated, independently from the test group.	
		Number of animals:	All animals	
		Time points:	End of the study.	
		Parameters:	Leukocyte differential count, white and red cell morphology (light microscopical examination)	
3.4.10	Clinical Chemistry		None	
3.4.11	Urinalysis		None	
3.4.12	Pathology	Yes, the sacrificed animals were assessed for gross pathology, organ weight and histopathology. Animals that died during the administration period were subjected to necropsy as soon as possible following death.		
3.4.12.1	Organ Weights	Yes		
		From:	All animals at terminal sacrifice.	
		Organs:	Liver, kidneys, adrenals, testes, epididymes, uterus, ovaries, spleen, brain, heart	
		Body	The terminal body weight of the anesthetized animals was determined.	
3.4.13	Histopathology	Yes		
		Organs:	The following organs and tissues were fixed in 4% formaldehyde: All gross lesions, the target organs, brain, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, pharynx, larynx, nasal cavity, aorta, gonads, uterus, female mammary gland, prostate, urinary bladder, lymph nodes, bone, bone marrow, femur with knee joint, eyes, epididymes, sciatic nerve, spinal cord, seminal vesicles, skeletal muscle, skin.	X
		Examination	All gross lesions were subjected to light microscopical examination. Following organs and tissues were examined in the control and the high dose (i.e. 2000 ppm) sacrificed animals: Brain, cecum, cervical cord, colon, duodenum, epididymides, esophagus, eyes, mammary glands, heart, ileum, jejunum, mandibular	

gland, mandibular lymphnode, mesenteric lymph nodes, ovaries, oviduct, uterus, vagina, pituitary, thyroid, parathyroid, thymus, pancreas, adrenals, spleen, nasal cavity, aorta, prostate, urinary bladder, bone marrow, femur with knee joint, lumbar cord, rectum, sternum with marrow, sublingual gland, thoracic cord, sciatic nerve, seminal vesicles, skeletal muscle, skin.

Following organs and tissues were examined in the control and all treated groups (100, 500 and 2000 ppm) sacrificed animals: Forestomach, glandular stomach, kidneys, larynx, liver, lungs, pharynx, testes, trachea.

Animals that died intercurrently: These animals were assessed like control animals.

3.5 Statistics

Statistical evaluation of the clinical parameters (food consumption, water consumption, body weight, body weight change, food efficiency):

Calculation of the means and standard deviation.

Assessment of the statistical significance of the differences observed versus control by means of Dunnett's test (Dunnett CW, JASA, Vol. 50: 1096-1121, 1955; Dunnett CW, Biometrics, Vol. 20: 482-491, 1964).

Haematological parameters:

Means and standard deviations were calculated.

Pathological parameters:

For terminal body weight, and the absolute and relative organ weights, means and standard deviation were calculated. Further statistical assessments were based on the Kruskal-Wallis test and if necessary, on the Wilcoxon test (Hettmannsperger TP, Statistical inference based on ranks, John Wiley & Sons, New York, 132-140, 1984; International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1 – nakl-3; Miller RG, Simultaneous statistical inference, Springer-Verlag, New York Inc., 165-167, 1981; Nijenhuis A & Wilf SW, Combinatorial Algorithms, Academic Press, New York, 32-33, 1978)

3.6 Further remarks

Stability, homogeneity and concentration control analysis of the test substance were performed. Food as well as drinking water also was subjected to analyses according to the EPA guideline (Fed. Reg. Vol. 44, No.91, 1979) and to the German Drinking Water Regulation (Trinkwasserverordnung, Bundesgesetzblatt, 1990).

4 RESULTS AND DISCUSSION

4.1 Body weight, males

Summary of the most relevant body weight data in males:

Time point (days)	Mean body weight (g)			
	0 ppm	100 ppm	500 ppm	2000 ppm
7	268.9 +/- 10.2 (N=50)	267.4 +/- 12.2 (N=49)	269.1 +/- 13.8 (N=50)	**244.5**** +/- **14.9** (N=49)
14	321.0 +/- 18.0 (N=50)	316.7 +/- 16.1 (N=50)	317.5 +/- 13.7 (N=50)	**290.4**** +/- **16.6** (N=50)
21	364.5 +/- 17.9 (N=48)	362.5 +/- 18.8 (N=49)	361.9 +/- 17.6 (N=48)	**335.3**** +/- **18.1** (N=50)
28	400.5 +/- 18.4	397.1 +/- 23.8	396.0 +/- 19.9	**369.2**** +/- **22.0**

	(N=50)	(N=50)	(N=50)	(N=50)
56	484.5 +/- 25.8 (N=50)	478.7 +/- 30.7 (N=50)	478.7 +/- 26.0 (N=50)	447.2** +/- 32.0 (N=50)
70	515.1 +/- 30.7 (N=50)	507.7 +/- 35.2 (N=50)	503.2 +/- 27.2 (N=50)	470.1** +/- 35.3 (N=50)
91	546.8 +/- 32.1 (N=49)	543.0 +/- 39.6 (N=49)	533.8 +/- 30.2 (N=50)	506.4** +/- 32.5 (N=50)
147	588.6 +/- 39.1 (N=50)	588.0 +/- 45.5 (N=49)	572.3 +/- 34.4 (N=50)	545.6** +/- 39.8 (N=50)
259	637.7 +/- 47.4 (N=50)	643.9 +/- 56.1 (N=50)	625.1 +/- 42.1 (N=49)	588.9** +/- 46.4 (N=49)
343	652. 4 +/- 52.7 (N=49)	654.6 +/- 59.7 (N=49)	640.0 +/- 47.8 (N=49)	601.4** +/- 48.0 (N=49)
455	648.8 +/- 67.2 (N=44)	658.7 +/- 61.8 (N=48)	644.5 +/- 50.6 (N=46)	604.6** +/- 53.8 (N=48)
511	654.7 +/- 62.3 (N=44)	656.8 +/- 62.8 (N=49)	639.1 +/- 58.0 (N=45)	605.0** +/- 55.4 (N=48)
595	651.6 +/- 68.2 (N=37)	653.7 +/- 62.4 (N=46)	644.3 +/- 63.8 (N=43)	592.5** +/- 67.1 (N=45)
707	593.1 +/- 72.1 (N=29)	599.0 +/- 85.8 (N=33)	597.7 +/- 89.1 (N=33)	567.6 +/- 55.2 (N=36)
728	591.6 +/- 72.5 (N=27)	600.3 +/- 60.1 (N=30)	611.8 +/- 78.4 (N=25)	549.7* +/- 63.8 (N=35)

N, number of animals; * = 0.05; ** = 0.01

Over the whole experimental period, the 2000 ppm male rats showed a statistically significant impairment of body weight when compared to control. In fact, the maximum of reduction (ca. 9.6%) was seen on day 14 of treatment (mean weight of ca. 290 g, versus 321 g in control). Body weight change also was statistically significantly below control value for the 2000 ppm males (body weight gain 39% below control for the period ranging from day 7 to day 595). Body weight of male rats in the remaining test groups was inconspicuous. The effects on body weight were considered to be treatment-related.

4.2 Body weight, females

Summary of the most relevant body weight data in females:

Time point (days)	Mean body weight (g)			
	0 ppm	100 ppm	500 ppm	2000 ppm
7	196.7 +/- 10.0 (N=50)	196.2 +/- 9.3 (N=50)	187.5** +/-9.1 (N=50)	182.7** +/- 9.1 (N=50)
14	241.7 +/- 13.2 (N=50)	214.7 +/- 13.3 (N=50)	207.2* +/- 13.3 (N=50)	198.2** +/- 13.1 (N=50)
21	232.6 +/- 14.5 (N=50)	233.9 +/- 15.8 (N=49)	225.2* +/- 15.5 (N=50)	219.9** +/- 15.1 (N=50)
28	248.2 +/- 14.3 (N=50)	246.7 +/- 16.0 (N=50)	241.6 +/- 16.0 (N=50)	235.7** +/- 16.8 (N=50)
56	280.4 +/- 15.2 (N=50)	277.8 +/- 19.6 (N=50)	277.1 +/- 19.4 (N=49)	268.9** +/- 19.8 (N=50)
70	291.9 +/- 16.9 (N=50)	291.6 +/- 18.9 (N=50)	285.6 +/- 18.4 (N=50)	279.4** +/- 21.9 (N=50)
91	295.5 +/- 17.4 (N=50)	295.3 +/- 19.4 (N=50)	294.7 +/- 19.2 (N=50)	285.8* +/- 22.0 (N=50)

147	313.9 +/- 18.1 (N=49)	317.7 +/- 20.3 (N=49)	321.0 +/- 22.3 (=50)	307.4+/- 23.0 (N=50)
259	335.8 +/- 23.4 (N=50)	336.8 +/- 24.9 (N=50)	340.0 +/- 26.8 (N=50)	325.8 +/- 28.6 (N=50)
343	344.9 +/- 26.8 (N=47)	344.5 +/- 27.4 (N=48)	347.8 +/- 32.2 (N=50)	329.3 +/- 31.0 (N=49)
455	365.9 +/- 33.2 (N=47)	361.2 +/-38.3 (N=48)	363.4 +/- 40.0 (N=49)	343.6* +/- 35.4 (N=48)
511	377.5 +/- 39.3 (N=44)	375.7 +/- 44.4 (N=44)	372.9 +/- 45.0 (N=48)	**346.6**** +/- **37.0** (N=48)
595	390.4 +/- 44.5 (N=42)	384.7 +/- 59.4 (N=38)	380.3 +/- 52.4 (N=46)	**347.1**** +/- **41.9** (N=44)
707	390.7 +/- 45.8 (N=34)	367.6 +/- 65.8 (N=28)	364.3 +/- 63.4 (N=40)	**352.2*** +/- **37.5** (N=29)
728	381.3 +/- 60.4 (N=34)	373.3 +/- 66.4 (N=26)	360.8 +/- 64.3 (N=36)	347.0 +/- 39.6 (N=27)

N, number of animals; * = 0.05; ** = 0.01

Over the whole experimental period, the 2000 ppm female rats showed a statistically significant impairment of body weight when compared to control. In fact, the maximum of reduction (ca. 11.1%) was seen on day 595 of treatment (mean weight of ca. 347 g, versus 390 g in control). In the 500 ppm group, body weight was statistically significantly reduced from day 7 to day 21 only (ca. 5% below control). Body weight change also was statistically significantly below control value for the 2000 ppm females (body weight gain 39.6% below control on day 7). In the 500 ppm group, body weight change was also significantly decreased on days 7, 14, 147 and 175 of treatment (maximum of ca. 22.4% below control). The effects on body weight were considered to be treatment-related.

4.3 Food consumption in males, food efficiency

Summary of the most relevant food consumption data in males:

Time point (days)	Mean food consumption (g/animal/day)			
	0 ppm	**100 ppm**	**500 ppm**	**2000 ppm**
7	29.7 +/- 1.6 (N=50)	29.7 +/- 1.9 (N=49)	**28.5**** +/- **1.9** (N=50)	**24.3**** +/- **1.9** (N=49)
28	34.8 +/- 1.8 (N=50)	34.9 +/- 3.0 (N=50)	**33.5*** +/- **2.0** (N=50)	**32.1**** +/- **2.4** (N=50)
56	33.3 +/- 1.7 (N=50)	33.1 +/- 2.0 (N=50)	**31.7**** +/- **2.1** (N=50)	**30.6**** +/- **2.2** (N=50)
63	34.1 +/- 2.4 (N=49)	33.8 +/- 2.4 (N=48)	**31.6**** +/- **2.0** (N=49)	**31.2**** +/- **2.4** (N=50)
70	33.0 +/- 2.3 (N=50)	32.9 +/- 2.5 (N=50)	**31.3**** +/- **2.1** (N=50)	**30.3**** +/- **2.5** (N=50)
91	32.5 +/- 1.8 (N=49)	32.5 +/- 2.3 (N=49)	**30.5**** +/- **1.9** (N=50)	**30.5**** +/- **1.9** (N=50)
119	32.0 +/- 2.3 (N=50)	32.4 +/- 2.2 (N=50)	**30.4**** +/- **2.0** (N=50)	**30.1**** +/- **1.9** (N=50)
147	31.5 +/- 1.9 (N=50)	31.5 +/- 2.4 (N=49)	**29.9**** +/- **2.1** (N=50)	**28.8**** +/- **2.9** (N=50)
259	30.7 +/- 2.2 (N=50)	30.2 +/- 2.5 (N=50)	29.5 +/- 1.9 (N=49)	**27.7**** +/- **3.1** (N=49)
371	28.9 +/- 2.9 (N=49)	29.2 +/- 3.2 (N=50)	28.4 +/- 2.9 (N=47)	**27.3*** +/- **2.1** (N=47)

455	29.8 +/- 4.1 (N=44)	29.8 +/- 2.3 (N=48)	29.5 +/- 2.2 (N=46)	**27.0*** +/- **2.0** (N=48)
483	29.1 +/- 4.7 (N=46)	29.6 +/- 3.0 (N=49)	29.1 +/- 2.3 (N=47)	**27.3*** +/- **2.2** (N=48)
539	30.1 +/- 3.0 (N=43)	29.7 +/- 2.0 (N=49)	**28.4*** +/- **3.8** (N=45)	**27.4**** +/- **2.0** (N=48)
595	29.5 +/- 2.9 (N=37)	28.8 +/- 4.5 (N=46)	28.4 +/- 3.1 (N=43)	**25.9**** +/- **2.8** (N=45)
651	27.8 +/- 6.3 (N=33)	28.5 +/- 5.7 (N=37)	28.1 +/- 4.2 (N=39)	26.8 +/- 2.3 (N=40)
728	28.2 +/- 4.4 (N=27)	27.7 +/- 4.0 (N=30)	29.1 +/- 4.4 (N=25)	**24.2**** +/- **5.6** (N=35)

N, number of animals; * = 0.05; ** = 0.01

Food consumption was statistically significantly reduced on several days for the 2000 ppm males. A maximum of reduction of 18.1% below control was reported for day 7. In the 500 ppm group, food consumption of male rats was statistically significantly reduced on some days only; the maximum of reduction (7.4% below control) was seen on day 63 of treatment. Food consumption in the 100 ppm group was inconspicuous. The findings reported for the male rats of the 500 and the 2000 ppm groups were considered to be treatment-related. No treatment-related effect on food efficiency could be assessed. In fact, differences between treated and control groups only occurred spontaneously and were within the normal biological range of variation.

4.4 Food consumption in females, food efficiency

Summary of the most relevant food consumption data in females:

Time point (days)	Mean food consumption (g/animal/day)			
	0 ppm	100 ppm	500 ppm	2000 ppm
7	23.2 +/- 1.7 (N=50)	23.3 +/- 1.3 N=50	**22.0****+/- **1.6** (N=50)	**19.7**** +/- **1.6** (N=50)
28	24.1 +/- 1.5 (N=49)	24.4 +/- 1.7 (N=50)	23.6 +/- 1.8 (N=50)	23.5 +/- 1.9 (N=50)
56	23.3 +/- 1.7 (N=50)	23.8 +/- 1.7 (N=50)	22.9 +/- 1.6 (N=49)	23.2 +/- 1.6 (N=50)
70	22.3 +/- 1.5 (N=50)	22.6 +/- 1.2 (N=50)	21.8 +/- 1.6 (N=50)	21.7 +/- 1.7 (N=50)
91	20.9 +/- 1.6 (N=50)	21.3 +/- 1.7 (N=50)	21.5 +/- 1.6 (N=50)	20.7 +/- 1.5 (N=50)
119	22.0 +/- 1.4 (N=50)	21.6 +/- 1.3 (N=50)	21.7 +/- 1.6 (N=50)	21.7 +/- 1.7 (N=50)
147	21.1 +/- 1.8 (N=49)	21.2 +/- 1.8 (N=49)	21.1 +/- 1.8 (N=50)	21.1 +/- 1.4 (N=50)
259	21.4 +/- 3.1 (N=49)	21.2 +/- 1.5 (N=50)	21.0 +/- 1.7 (N=50)	20.8 +/- 1.6 (N=50)
371	22.2 +/- 2.1 (N=47)	21.8 +/- 2.2 (N=49)	21.6 +/- 2.4 (N=50)	**21.0*** +/- **1.8** (N=48)
455	22.8 +/- 2.6 (N=47)	22.9 +/- 2.8 (N=48)	22.4 +/- 2.1 (N=49)	**21.4**** +/- **1.8** (N=48)
483	22.4 +/- 2.8 (N=45)	21.7 +/- 2.6 (N=47)	21.9 +/- 2.3 (N=49)	**21.1*** +/- **2.1** (N=48)
539	22.6 +/- 3.0 (N=44)	22.5 +/- 3.2 (N=44)	21.7 +/- 3.3 (N=47)	**20.6**** +/- **2.3** (N=48)

595	23.7 +/- 3.8 (N=42)	23.7 +/- 3.9 (N=38)	22.9 +/- 2.7 (N=46)	**21.3**** +/- **2.6** (N=44)
651	24.6 +/- 4.1 (N=39)	23.2 +/- 4.6 (N=33)	**22.4*** +/- **3.2** (N=43)	**21.7**** +/- **2.8** (N=40)
728	23.0 +/- 6.9 (N=34)	23.4 +/- 5.1 (N=26)	22.1 +/- 5.8 (N=36)	21.6 +/- 2.7 (N=27)

N, number of animals; * = 0.05; ** = 0.01

Food consumption was statistically significantly reduced on several days for the 2000 ppm females. A maximum of reduction of 15.2% below control was reported for day 7. In the 500 ppm group, food consumption of female rats was statistically significantly reduced on some days only; the maximum of reduction (8.9% below control) was seen on day 651of treatment. Food consumption in the 100 ppm group was found to be statistically significantly reduced for the females on day 287 only (4.9% below control). The findings reported for the 2000 ppm and the 500 ppm females were considered to be treatment-related; the isolated finding at 100 ppm was incidental. No treatment-related effect on food efficiency could be assessed. In fact, differences between treated and control groups only occurred spontaneously and were within the normal biological range of variation.

4.5 Water consumption, males

Summary of the most relevant water consumption data in males:

Time point (days)	Mean water consumption (g/animal/day)			
	0 ppm	100 ppm	500 ppm	2000 ppm
7	38.9 +/- 3.5 (N=50)	37.6 +/- 5.6 (N=50)	**35.9**** +/- **5.0** (N=50)	**28.2**** +/- **5.3** (N=50)
35	42.6 +/- 5.6 (N=50)	40.6 +/- 5.0 (N=49)	**36.7*** +/- **4.1** (N=50)	**33.3**** +/- **5.5** (N=49)
56	39.5 +/- 5.8 (N=50)	37.5 +/- 4.9 (N=50)	**36.3****+/- **5.3** (N=50)	**31.6**** +/- **3.8** (N=49)
63	38.6 +/- 5.5 (N=49)	36.7 +/- 6.0 (N=50)	**33.9****+/- **5.7** (N=49)	**31.2**** +/- **4.2** (N=50)
70	38.6 +/- 5.8 (N=50)	37.4 +/- 4.9 (N=49)	**33.7****+/- **5.4** (N=49)	**30.4**** +/- **4.5** (N=50)
91	36.5 +/- 5.3 (N=50)	35.6 +/- 4.5 (N=49)	**31.9****+/- **5.1** (N=50)	**28.8**** +/- **3.3** (N=50)
119	37.0 +/- 6.0 (N=50)	36.8 +/- 4.6 (N=50)	**32.8****+/- **5.7** (N=50)	**30.3**** +/- **3.9** (N=50)
147	37.1 +/- 6.2 (N=50)	35.6 +/- 5.4 (N=49)	**32.5****+/- **5.3** (N=50)	**29.9**** +/- **3.6** (N=49)
259	35.2 +/- 7.7 (N=50)	32.3 +/- 6.3 (N=50)	**31.2****+/- **6.0** (N=48)	**26.6**** +/- **4.8** (N=49)
371	32.8 +/- 6.6 (N=49)	32.8 +/- 7.6 (N=49)	**30.0****+/- **8.1** (N=49)	**26.4**** +/- **3.9** (N=48)
427	35.0 +/- 6.0 (N=47)	32.8 +/- 6.0 (N=48)	**31.0****+/- **4.0** (N=47)	**29.0**** +/- **4.5** (N=48)
539	36.4 +/- 7.2 (N=43)	35.6 +/- 6.2 (N=49)	**32.3****+/- **6.2** (N=45)	**29.1**** +/- **4.5** (N=48)
595	39.4 +/- 11.2 (N=37)	38.3 +/- 9.3 (N=46)	35.5 +/- 8.1 (N=43)	**30.2**** +/- **6.5** (N=45)
623	36.6 +/- 14.4 (N=35)	38.8 +/- 11.0 (N=43)	34.4 +/- 10.0 (N=42)	**30.0**** +/- **4.6** (N=42)
707	41.5 +/- 11.0	38.9 +/- 11.1	35.8 +/- 13.3	**31.6**** +/- **8.9**

	(N=28)	(N=31)	(N=33)	(N=36)
728	45.2 +/- 13.7 (N=27)	42.3 +/- 14.4 (N=30)	43.6 +/- 9.9 (N=25)	**32.0**** +/- **9.3** (N=35)

N, number of animals; * = 0.05; ** = 0.01

Water consumption was statistically significantly reduced on nearly every day over the whole experimental period for the males of the 2000 ppm group. A maximum of reduction of 29.2% below control was reported for day 728. In the 500 ppm group, water consumption of male rats was statistically significantly reduced on several days over the whole experimental period; the maximum of reduction (14% below control) was seen on day 35 of treatment. Water consumption within the 100 ppm group was inconspicuous. The findings reported for the male rats of the 500 and the 2000 ppm groups were considered to be treatment-related.

4.6 Water consumption, females

Summary of the most relevant water consumption data in females:

Time point (days)	**Mean water consumption (g/animal/day)**			
	0 ppm	**100 ppm**	**500 ppm**	**2000 ppm**
7	30.1 +/- 3.3 (N=50)	30.7 +/- 3.2 (N=50)	**26.4****+/- **3.5** (N=50)	**23.1**** +/- **3.4** (N=49)
35	28.8 +/- 3.9 (N=50)	29.3 +/- 3.6 (N=50)	**25.5*** +/- **3.4** (N=49)	**23.5**** +/- **3.5** (N=50)
56	27.8 +/- 4.3 (N=50)	28.0 +/- 4.1 (N=49)	**25.5***+/- **5.1** (N=50)	**24.0**** +/- **4.7** (N=49)
91	36.6 +/- 4.6 (N=50)	26.3 +/- 4.8 (N=49)	**23.9****+/- **4.1** (N=50)	**21.7**** +/- **3.4** (N=50)
119	27.8 +/- 5.7 (N=50)	27.0 +/- 4.2 (N=50)	**24.7****+/- **4.5** (N=50)	**22.6**** +/- **4.0** (N=50)
147	26.9 +/- 5.8 (N=50)	26.5 +/- 5.0 (N=50)	**23.7****+/-**3.9** (N=50)	**22.2**** +/- **3.6** (N=50)
175	30.1 +/- 5.8 (N=50)	**26.3****+/- **4.5** (N=50)	**25.8****+/-**6.0** (N=50)	**22.7**** +/- **4.2** (N=50)
203	32.4 +/- 6.9 (N=50)	**28.6**** +/- **5.9** (N=50)	**26.9****+/-**7.7** (N=50)	**24.9**** +/- **4.0** (N=50)
231	32.5 +/- 8.2 (N=50)	**28.0**** +/- **5.7** (N=50)	**27.0****+/-**5.0** (N=50)	**24.3**** +/- **4.5** (N=50)
259	32.4 +/- 8.5 (N=50)	30.4 +/- 6.4 (N=50)	**27.4****+/- **6.2** (N=50)	**24.5**** +/- **4.6** (N=50)
343	37.4 +/- 9.9 (N=49)	**33.5*** +/- **7.5** (N=49)	**30.3****+/- **8.4** (N=49)	**27.2**** +/- **4.7** (N=49)
455	42.7 +/- 11.0 (N=47)	38.4 +/- 9.6 (N=48)	**34.7****+/- **9.0** (N=49)	**29.6**** +/- **5.8** (N=48)
595	52.2 +/- 18.8 (N=41)	46.1 +/- 16.4 (N=37)	**39.5****+/- **8.4** (N=46)	**32.1**** +/- **8.0** (N=44)
623	55.4 +/- 17.6 (N=39)	**45.6****+/-**16.2** (N=38)	**42.0****+/- **11.5** (N=45)	**35.6**** +/- **6.9** (N=42)
707	56.9 +/- 15.4 (N=34)	**44.7***+/- **21.8** (N=28)	**46.1***+/- **16.8** (N=40)	**36.3**** +/- **9.3** (N=29)
728	51.4 +/- 22.4 (N=33)	41.7 +/- 17.4 (N=25)	43.8 +/- 16.9 (N=36)	**35.7**** +/- **11.0** (N=26)

N, number of animals; * = 0.05; ** = 0.01

Water consumption was statistically significantly reduced on nearly every day over the whole experimental period for the females of the 2000 ppm group. A maximum of reduction of 38.5% below control was reported for day 595. In the 500 ppm group, water consumption of female rats was statistically significantly reduced on several days over the whole experimental period; the maximum of reduction (24.3% below control) was seen on day 595 and day 623 of treatment. Water consumption of the 100 ppm females was statistically significantly reduced on few days over the experimental period, with a maximum reduction of 21.4% below control observed on day 707 of treatment. Reduction in water consumption was treatment-related but was not seen as an adverse effect; this effect probably was due to the bad taste of the test substance.

4.7 Test substance intake

Mean daily test substance intake (mg/kg bw/day):

Concentration in the drinking water	Mean daily test substance intake (mg/kg bw/day)	
	Males	Females
100 ppm	6.1	10.5
500 ppm	31.9	48.5
2000 ppm	120.7	176.4

X

4.8 Mortality

Mortality rate over the whole experimental period (i.e. 24 months):

Concentration	Mortality (males)		Mortality (females)	
0 ppm	24	48%	17	34%
100 ppm	22	44%	25	50%
500 ppm	25	50%	15	30%
2000 ppm	15	30%	23	46%

The mortality rate was quite similar for all groups, indicating that the treatment as such did not affect survival of the animals of both sexes. However, at the highest tested concentration of 2000 ppm, 2 males and 9 females died from asphyxia, which was considered to be a test substance-related effect.

4.9 Clinical signs

Treatment-related symptoms of toxicity were seen in the 2000 ppm group. In fact, 13 males and 13 females produced respiratory sounds on several days of the study. These sounds were often observed at study beginning and in most cases, an association with metaplasic changes in the larynx and/or the trachea became evident. This finding was considered to be treatment-related. Furthermore, reddish-discolored urine was seen in all groups, in both sexes. The incidence of this finding was particularly high in the 2000 ppm group when compared to control, affecting 13 males and 7 females. The increase incidence of reddish-discolored urine at 2000 ppm was considered to be treatment-related.

4.10 Haematology

Differential blood count:

Type	Males		Females	
	0 ppm (N=25)	2000 ppm (N=35)	0 ppm (N=33)	2000 ppm (N=27)
Eosinophils	1.04 +/- 0.93%	1.29 +/-1.60%	1.39 +/- 2.15%	1.33 +/-1.49%
Basophils	0.16 +/- 0.37%	0.26 +/- 0.44%	0.27 +/- 0.57%	0.41 +/- 0.80%
Neutrophilic bands	0.08 +/- 0.28%	0.66 +/- 3.05%	0.00 +/- 0.00%	0.15 +/- 0.36%
Polymorphonuclear	34.92 +/-	30.60 +/-	31.18 +/-	32.93 +/-

neutrophils	16.06%	13.03%	12.72%	12.91%
Lymphocytes	57.76 +/- 17.35%	61.54 +/- 12.63%	62.18 +/- 12.76%	60.15 +/- 12.94%
Monocytes	6.04 +/- 3.34%	5.66 +/- 2.76%	4.97 +/- 2.30%	5.04 +/- 2.58%

N, number of values

Summary of some morphological variations in white blood cells:

Morphological variations	Assessment limits	Males		Females	
		0 ppm (N=25)	2000 ppm (N=35)	0 ppm (N=33)	2000 ppm (N=27)
Metamyelocytes	>0	0/25	1/35	1/33	0/27
Lymphoblasts	>1	0/25	1/35	1/33	1/27
Juvenile lymphocytes	>1	0/25	1/35	0/33	1/27
Changes in nucleus of lymphocytes	>0	2/25	6/35	0/33	3/27
Changes in plasma of lymphocytes	>3	0/25	1/35	2/33	2/27
Juvenile monocytes	>0	0/25	3/35	0/33	1/27

N, number of values

Summary of some morphological variations in red blood cells:

Morphological variations	Assessment limits	Males		Females	
		0 ppm (N=25)	2000 ppm (N=35)	0 ppm (N=33)	2000 ppm (N=27)
Polychromasia	>1	1/25	1/35	1/33	2/27
Hypochromasia	>0	1/25	2/35	0/33	1/27
Anisocytosis	>0	1/25	4/35	1/33	2/27

No conspicuous haematological findings were seen at 2000 ppm (males and females).

4.11 Absolute organ weights

Treatment-related findings:

Males				
Organ	0 ppm	100 ppm	500 ppm	2000 ppm
Whole body (g)	550.58 +/- 65.9 (N=26)	561.9 +/- 58.2 (N=28)	572.4 +/- 75.7 (N=25)	**516.8*** +/- **61.8** (N=35)
Liver (g)	15.31 +/- 2.2 (N=26)	14.76 +/- 2.2 (N=28)	15.55 +/- 4.02 (N=25)	**13.8*** +/- **2.2** (N=35)
Heart (g)	2.1 +/- 0.3 (N=26)	2.12 +/- 0.35 (N=28)	2.09 +/- 0.26 (N=25)	**1.84**** +/- **0.2** (N=35)
Brain (g)	2.37 +/- 0.08 (N=26)	2.34 +/- 0.08 (N=28)	2.47 +/- 0.08 (N=25)	**2.3**** +/- **0.07** (N=35)
Females				
Whole body (g)	353.55 +/- 54.9 (N=33)	336.8 +/- 56.6 (N=25)	333.59 +/- 56.6 (N=35)	**318.12**** +/- **34.5** (N=27)
Uterus (g)	2.04 +/- 4.00 (N=33)	1.8 +/- 2.18 (N=25)	**2.37*** +/- **2.84** (N=35)	**3.57**** +/- **8.6** (N=27)

Heart (g)	1.54 +/- 0.21 (N=33)	1.48 +/- 0.15 (N=25)	1.47 +/- 0.19 (N=35)	**1.39**** +/- 0.15 (N=27)
Other organs	Inconspicuous in both males and females			

N, number of animals; *, p<=0.05; **, p<=0.01

The data above show that the mean terminal body weight of both the 2000 ppm males and females was statistically significantly decreased compared to control (respectively about 6.1% and 10% below control). In the 2000 ppm males, the absolute weight of liver, heart and brain was statistically significantly decreased compared to control (respectively 9.6%, 12.5% and 2.9% below control). In the 2000 ppm females, the absolute weight of the heart also was statistically significantly below control value (9.5%); in contrast, the absolute weight of the uterus was found to be statistically significantly increased in both, the 2000 ppm and the 500 ppm females (respectively 74.9% and 16.2% above control).

The decrease in body weight was considered to be treatment-related; the different decreases in organ weights reported above (liver, heart, brain) were considered to rather be due to the overall decrease in body weight than to the treatment itself (indirect consequence).

4.12 Relative organ weights

For the 100 ppm males, a slight but statistically relevant decrease in relative testes weight (ca. 0.62 +/- 0.16 g, versus 0.72 +/- 0.11 g for control; ca. 13.8% below control)) was reported, which was not treatment-related. The females of both, the 2000 and the 500 ppm groups showed statistically significantly increased relative uterus weights (respectively ca. 1.08 +/- 2.4 g and 0.75 +/- 1.02 g, versus 0.61 +/- 1.26 g for control; respectively 75.9% and 22.3% above control). A statistically significant increase in relative weight also was reported for the spleen of the 2000 ppm and 500 ppm females (0.29 +/- 0.1 g at 2000 ppm and 0.30 +/- 0.13 g at 500 ppm, versus 0.25 +/- 0.05 g for control; respectively 19% and 23.5% above control). For the spleen, no clear dose-relationship was discernible, indicating that this effect was not treatment-related.

4.13 Gross pathology

Gross lesions were found in almost all investigated organs. Some lesions showed an increased incidence in treated animals compared to controls. These lesions and their incidences are summarized in following table:

Concentration	0 ppm		100 ppm		500 ppm		2000 ppm	
Sex (Number of animals)	M (50)	F (50)	M (50)	F (50)	M (50)	F (50)	M (50)	F (50)
Gross lesions								
Erosion/ulcer in the glandular stomach	12	18	8	18	14	17	**17**	**29**
Retraction in the kidney (retraction)	11	3	11	6	14	4	**26**	**13**
Various foci in the liver	20	32	**28**	31	**24**	34	**27**	30
Cysts in the spleen	9	11	**13**	15	**19**	15	**17**	8
Calcification in the testes	12	-	**14**	-	**14**	-	**18**	-
Cystic degeneration in the testes	5	-	**13**	-	**9**	-	**14**	-

X

Horn dilation in the uterus	-	17	-	16	-	25	-	25

The incidence of erosion/ulcer in the mucosa of the glandular stomach was particularly increased in the 2000 ppm females; in the 2000 ppm males, the increase in incidence was marginal. This finding was considered to be treatment-related. The incidence of retraction in the kidneys was clearly increased in the 2000 ppm females and males when compared to control. Retraction in the kidney is seen as a step in the progression of chronic nephropathy. However, the authors of the study considered this finding not to be a specific treatment-related effect but a fortuitously increased constituent of chronic progressive nephropathy. Foci in the liver occurred more often in treated than in control males. However there was no evidence of a dose-relationship and in treated females, no such increased incidence was seen. The proliferation of cysts (various sizes between 2 and 8 mm) in the spleen is a strain specific finding. In the treated males, the incidence of such cysts was increased but without clear dose-relationship. In contrast, in the treated females, a decrease in incidence was seen at 2000 ppm (8 cases versus 11 cases in control). Therefore a clear treatment-relationship could not be assessed. The effects reported for the testes occurred more often in the treated than in the control rats, but without clear dose-relationship. This was also true of the horn dilation in the uterus reported for the females.

The remaining lesions, which were reported, were incidental and showed no treatment-relationship.

4.14 Comparison of gross lesions in sacrificed rats and rats that died or were killed in extremis

Some lesions were found to only occur in rats that were sacrificed at study terminations whereas other lesions were only seen in rats that died or were killed in extremis during the experimental period.

<u>Following series of lesions only occurred in sacrificed rats</u>: Foci in the adrenal cortex, cysts and retraction in the kidney, foci and cysts in the liver, foci in lung and pituitary, cysts in the ovaries, calcification and cysts in the testes, dilation and wall-thickening in the uterus. These lesions almost were related to the aging and an obvious treatment-related cumulation could be seen in none of the groups.

<u>Following series of lesions only occurred in rats that died or were killed in extremis</u>: Erosion/ulcer of the mucosa in the forestomach, enlarged kidneys, reduced organ sizes of epididymides, seminal vesicle and/or testes, effusion of fluid in the body cavities. These lesions almost were related to the early death of the animals and an obvious treatment-related cumulation could be seen in none of the groups.

4.15 Gross lesions suspected to represent tumors

Gross lesions defined as “mass” were seen in different organs and were listed in table A6_7-1.

In many cases, the incidence of such masses was <= 5% (i.e. less than 2 cases per group, each group consisting of 50 animals) and no conspicuous difference was seen between treated and control groups. Within the organs displaying masses with incidences >= 5%, the testes and the vagina stand out: 3 cases of masses in the testes were reported for the 2000 ppm males versus 0 in control, 3 cases of masses in the vagina versus 0 in control were reported for the females of the same group. The remaining masses with an incidence >=5% were found to be equally distributed over all test groups (treated and control; e.g. liver) and in some cases, the incidence in control even was higher than in the treated groups (e.g. mammary gland).

Moreover, the summarized incidence of all masses per group was quite similar for all test groups (i.e. treated and control), without any conspicuous sex-related difference. The majority of the masses (54%; 59% in females and 40% in males) were found in sacrificed animals, indicating that they almost were related to aging.

4.16 Histopathology

No conspicuous findings were reported.

X

4.17 Neoplastic findings, summary

The tumors with an incidence >5% (i.e. >= 3 cases per group of 50 animals) are listed in table A6_7-2.

The distribution of all recorded tumors was very similar in all groups. No indication for a treatment-related increase in tumor incidence was evident; occasionally seen differences were within the range of biological variability as shown by comparison with historical control data. The comparison between sacrificed rats and rats that died or were killed in extremis revealed that most neoplastic lesions (61%) occurred in rats sacrificed at study termination; this indicates that these lesions almost were age-related. In both cases, sacrificed rats and rats that died or were killed in extremis, there was no indication of a treatment-related cumulated neoplasm incidence. The number of 2000 ppm males displaying neoplasms (39/50) was similar to that of control (39/50); the number of 2000 ppm females with neoplasms was below that of control (44/50 versus 49/50). The number of 2000 ppm males with benign neoplasms was slightly increased compared to control (37/50 verus 31/50); the number of 2000 ppm females with benign neoplasms was quite similar to control (43/50 versus 44/50). The number of 2000 ppm males with malignant neoplasms was below control (9/50 versus 16/50); this also was true for the females (16/50 versus 26/50). The total number of primary neoplasms was similar for the2000 ppm and the control males (73 each). The total number of benign neoplasm was slightly increased in the 2000 ppm males compared to control (62 versus 56), but the number of malignant neoplasm was decreased (11 versus 17). The total number of systemic neoplasms was similar for the 2000 ppm and the control males (2 each), whereas the total number of metastasized neoplasms was lower in the 2000 ppm males than in control (1 versus 3). For the 2000 ppm females, each of the total number of primary neoplasms, of benign neoplasms, of malignant neoplasms, of systemic neoplasms as well as of metastasized neoplasms was below control (respectively 101 versus 103, 76 versus 77, 25 versus 26, 2 versus 4 and 1 versus 6).

Summarizing it can be retained that all neoplasms observed were spontaneous in origin and showed no treatment-relationship.

X

4.18 Non-neoplastic findings, summary

Following non-neoplastic findings were considered to be treatment-related:

(1)-Squamous metaplasia of the epithelium of the larynx, which was diffuse, was seen in 18/50 males and 25/50 females of the 2000 ppm group (control: 0 cases). In 6 2000 ppm females (control: 0 cases) squamous metaplasia was focal. Diffuse squamous metaplasia was accompanied by accumulation of keratin in the laryngeal lumen (2 males and 11 females of the 2000 ppm group; control: 0 cases). One single case of focal nodular hyperplasia within metaplasic tissue was reported (one 2000 ppm female); this finding is considered to be a precursor for neoplastic growth.

(2)-Squamous metaplasia of the epithelium of the trachea was reported for 4/50 males and 12/50 females of the 2000 ppm group (control: 0 cases). In 1male and 6 females, the squamous metaplasia was diffuse and always occurred in association with diffuse squamous metaplasia of the larynx. In 3 males and 6 females, the squamous metaplasia was focal and associated to diffuse squamous metaplasia of the larynx in all males and 5/6 females. Focal squamous metaplasia of the trachea was considered to be treatment-related when its presence was associated to diffuse squamous metaplasia of the larynx. Accumulation of keratin in the tracheal lumen was seen in 3 of the females with diffuse squamous metaplasia in the trachea. Moderate to severe inflammation with/without purulent contents in the tracheal lumen of 2000 ppm females (5/50) with metaplasia in the larynx and/or trachea was considered to be treatment-related.

X

(3)- Squamous metaplasia of the bronchial epithelium of the lung was found in one 2000 ppm male; as this finding was associated with both squamous metaplasia in larynx and in trachea, it was regarded as treatment-related. The lung of 4 males and 6 females of the 2000 ppm group contained foreign body granuloma; only in those animals that also displayed squamous metaplasia in larynx and/or trachea, this finding was considered to be treatment-related (1 male and 5 females). One 2000 ppm female

died prematurely from purulent broncho-pneumonia, which was related to the aspiration of keratinized material and pus from the detritus found in the lumen of the metaplastic larynx and/or trachea. This finding therefore was considered to be treatment-related.

(4)- Purulent inflammation in the nasal cavity was seen in 6 2000 ppm females (control: 1 case); all these females also displayed squamous metaplasia in larynx a/or trachea and all excepted for one had keratin detritus in the lumen of the larynx and/or trachea. The authors suggested that the inflammation may be due to abnormal feed regurgitation into the lower parts of the nasal cavity as a consequence of loss of elasticity of the pharynx wall or coughing, coughing being due to the obstruction of the pharynx and/or trachea by the desquamated detritus. Three of the 2000 ppm males displayed slight to moderate purulent inflammation in the nasal cavity, associated to squamous metaplasia of the larynx and/or the trachea; although no keratin detritus were seen in these animals, these findings were considered to be treatment-related.

(5)- The combined incidence of grossly and/or histopathological confirmed erosion/ulcer of the glandular stomach was increased in the 2000 ppm group (females: 29 cases versus 18 in control; males: 20 cases versus 13 in control).

4.19 Remark

The stability of the test substance in the drinking water over a period up to 2 weeks at room temperature was verified and could be confirmed. The correctness of the test concentrations could be demonstrated; in fact, the analytical concentrations were within 90.5 to 109.3% of the nominal concentrations. Analysis revealed that both, food and water were suitable.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to test glutaraldehyde for its carcinogenic potential in Wistar rats following chronic administration in the drinking water over a period of 24 months.

Test substance: % Glutaraldehyde), % (analysis performed by), stability in drinking water over a period of 14 days at room temperature confirmed.

The study was conducted according to OECD Guideline 452 (1981), with GLP.

Glutaraldehyde was administered to 50 male and 50 female rats per test group via the drinking water; the test concentrations were as follows: 0, 100, 500 and 2000 ppm. Food and water consumption as well as body weight were determined once a week for the first 13 weeks of treatment, and at 4-week intervals thereafter. The state of health of the animals was checked daily (clinical symptoms and mortality). Detailed clinical examinations including palpation were performed once a week. At the end of the administration period, differential blood counts were obtained from all surviving animals; differential blood counts also were conducted on animals that died or were killed in extremis during the experimental period. At study termination all animals were assessed by gross pathology and histopathology and selected organs were weighed; particular attention was given to neoplastic and non-neoplastic findings.

Stability, homogeneity and concentration control analysis of the test substance were performed. Food and drinking water also were tested for suitability according to EPA guideline and to the German Drinking Water Regulation. The stability of the test substance in the drinking water over a period of 2 weeks at room temperature was confirmed. The correctness of the test concentrations was demonstrated (recovery of 90.5 to 109.3% of the nominal concentrations). Both, water and food were found to be suitable.

Taking the water consumption within the different groups into account, the test substance intake was calculated be as follows:

Test concentration	Mean daily test substance intake (mg/kg bw/day)	
	Males	Females
100 ppm	6.9	10.5
500 ppm	31.9	48.5
2000 ppm	120.7	176.4

5.2 Results and discussion

Following findings were considered to be treatment-related:

Body weight: Compared to control the 2000 ppm rats showed statistically significantly decreased body weights (BW) and BW changes (BWC). For the males the maximum BW reduction of 9.6% was seen on day 14, and the BWC reached –39% on day 7; for the females, the max. BW reduction of 11.1% was seen on day 595 and BWC reached -39.6% on day 7. For the 500 ppm females, BW and BWC also were significantly decreased.

Mortality and symptoms of toxicity: At 2000 ppm, 2 males and 9 females died from asphyxia. At 2000 ppm respiratory sounds brought in association with metaplasic changes in larynx and/or trachea as well as increased incidence in reddish-discolored urine were reported.

Food and water consumption: Food consumption was significantly reduced for the 2000 and 500 ppm groups. The maximum reduction was 18.1% and 7.4% below control for the 2000 and the 500 ppm males respectively, and 15.2% and 8.9% below control for the 2000 and the 500 ppm females. Water consumption was significantly

reduced at 2000 ppm (males: max. 29.2% below control on day 728; females: max. 38.5% below control on day 595) and at 500 ppm (males: max. 14% below control on day 35; females: max. 24.3% below control on day 595 and 623). At 100 ppm, water consumption was significantly reduced for females only (max. 21.4% below control on day 707).

Terminal body weight, organ weights and pathology: The mean terminal body weight of the 2000 ppm rats was significantly decreased (6.1 and 10% below control respectively for males and females). Consequently, liver, hear and brain showed decreased organ weights. An increased incidence of erosions/ulcer in the mucosa of the glandular stomach was reported for the females of the 2000 ppm group.

Non-neoplastic findings: Squamous epithelial metaplasia in the larynx was reported for the 2000 ppm group (18/50 males and 30/50 females, versus 0 cases in control). Squamous metaplasia in trachea also was seen at 2000 ppm (4/50 males and 11/50 females, versus 0 cases in control). Metaplasia was nearly always accompanied by accumulation of keratin detritus in the laryngeal and/or tracheal lumen. In five 2000 ppm females with laryngeal/tracheal metaplasia, inflammation in the tracheal lumen with/without purulent contents was seen. One 2000 ppm male with squamous metaplasia in larynx and trachea also showed squamous metaplasia of the bronchial epithelium. Some 2000 ppm animals with squamous laryngeal/tracheal metaplasia also displayed foreign body granuloma in their lungs. Purulent inflammation in the nasal cavity was seen in 6 females and 3 males of the 2000 ppm group. Furthermore, an increased combined incidence of grossly and/or histopathologically confirmed erosions/ulcer of the glandular stomach was reported for the 2000 ppm group (males: 20 cases versus 13 in control; females: 29 cases versus 18 in control).

All remaining parameters and findings were inconspicuous or incidental, and without any relationship to the treatment with glutaraldehyde. Neoplastic findings were spontaneous in origin and showed no treatment-relationship.

5.3 Conclusion

The findings of the present study revealed no carcinogenic potential for glutaraldehyde administered to Wistar rats in the drinking water over a period of 24 months. In fact no treatment-related neoplastic changes were seen, but the non-neoplastic findings clearly identified the larynx, the trachea, the lung and bronchi, the nasal cavity and the glandular stomach as target organs for glutaraldehyde in rat. A NOAEL of 100 ppm could be determined, which corresponded to ca. 7 mg test substance /kg bw/day for males and ca. 10 mg test substance/kg bw/day for females. Referring to the active ingredient glutaraldehyde, the NOAEL was ca. 3.5 mg a.i./kg bw/day and ca. 5 mg/kg bw/day for males and females, respectively.

5.3.1	Reliability	**1**
5.3.2	Deficiencies	No

Section A6.7 _ 01 Annex Point IIA6.7	**Carcinogenicity** **Carcinogenicity in Wistar rats following administration in the drinking water for 24 months**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

October 25th, 2010

Materials and Methods

3.1.2 This refers to Doc IIIA Section A2.

3.4.13 Histopathology. As indicated, the organs were fixed in 4 % formaldehyde. However, fixation of the testes in formaldehyde/formalin causes severe artefacts due to shrinking of the tissues. It is difficult to estimate the degree of vacuolization in such samples, and histopathological examinations remains unreliable. Guidelines for reproductive toxicology specifically warn about this problem and recommend other fixatives, such as Bouin's fluid or Stieve's fixative.

Results and discussion

4.7 Test substance intake. There is an error in the table – the correct figures are given in 5.1 (mean daily test substance intake for the 100 ppm males was 6.9 mg/kg bw/day).

4.13. Gross pathology.

- Retraction in the kidney is considered as a treatment related effect by the RMS.
- Cystic degeneration (5, 13, 9, 14) and calcification (12, 14, 14, 18) show dose related increases in the incidences in the gross pathology of the testes.

4.16 Histopathology. Unlike stated, there were many histopathological findings that are reported under points 4.17 and 4.18.

4.17 Neoplastic findings, summary. The table referred to (A6_7-2) has not been given, but is included in the original report. The effects are reported correctly in the text.

- The incidence of Leydig cell adenomas were as follows: 0, 3, 3, 4. The incidences are comparable to historical controls.

4.18 Non-neoplastic findings, summary.

- Squamous metaplasia of the larynx epithelium: the incidences (of 50 examined in each group) were as follows:
  - Diffuse, males: 0, 0, 1, 18
  - Diffuse, females: 0, 0, 0, 25
  - Focal, females only: 0, 3, 2, 6
- Squamous metaplasia of the trachea. In the study report, this was considered spontaneous and not treatment-related when it was not associated with squamous metaplasia of the larynx. This assumption was made based on the larynx being more sensitive than the trachea. The RMS questions this conclusion, and will consider all cases of squamous metaplasia as treatment-related in the absence of cases in the control group. The incidences of squamous metaplasia of the trachea epithelium (of 50 examined in each group) were as follows:
  - Diffuse, males: 0, 0, 0, 1
  - Diffuse, females: 0, 0, 0, 6
  - Focal, males: 0, 0, 1, 3
  - Focal, females: 0, 0, 1, 6
- Leydig cell hyperplasia: The incidence of focal hyperplasia in the Leydig cells were as follows: 4, 3, 6, 10.
- Microscopic findings in the testes were as follows: focal degeneration (16, 17, 23, 13), diffuse degeneration (15, 17, 14, 22), focal tubular mineralization (15, 16, 18, 22)

Carcinogenicity

Carcinogenicity in Wistar rats following administration in the drinking water for 24 months

	5.2 Results and discussion. The large difference between the uterus weights of the control group and the high dose group (75.9 %) and the mid-dose group (22.3 %) has not been properly explained. A possible partial explanation given in the report is that cystic uterine glands and dilation of the uterine horns were more frequent in the two highest dose groups (18, 14, 27, 26 and 12, 12, 15, 18, respectively). These findings reflect morphologic changes related to the oestrus cycle. Apparently this effect is not connected with any tumours or metaplasia. **Non-neoplastic findings**: Please refer to the numbers given above. The applicant's version contains some errors and numbers that will not be used in the risk assessment.
Conclusion	GA did not have any clear oncogenic potential when administered to Wistar rats in the drinking water for 24 months. Leydig cell adenoma incidence was increased at all dose levels, but within historical controls (4.17 above). There were several other types of dose related effects in the testes: Leydig cell hyperplasia (4.18 above), cystic degeneration and calcification (4.13 above), diffuse degeneration and focal tubular mineralization (4.18 above). All these effects are common findings and the increases in the incidences are not remarkable, but the increased incidence might be suggestive of a common mechanism. The RMS does not consider the findings sufficient for conclusions on oncogenicity, but the effects should be clearly stated in the risk assessment. Based on cystic degeneration in the testes at all dose levels that was coupled with an increased incidence of Leydig cell hyperplasia at the highest dose level, no NOAEL is established. Non-oncogenic effects were located in the larynx, trachea, lungs, bronchi, nasal cavity, testes and the glandular stomach. The LOAEL could be established at 100 ppm, corresponding to 6.9 and 10.5 mg test substance/kg bw/d in males and females, respectively. This corresponds to 3.5 and 5.3 mg GA/kg bw/d.
Reliability	1
Acceptability	Acceptable
Remarks	2.3 Deviations. The applicant has indicated that there are deviations, but this is apparently an error, as none were specified nor could be identified by the RMS. Please note that the tabulated numerical results copied to the study summary have not been checked in detail by the RMS.

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

Table A6_7-1. List of gross lesions defined as "mass" seen in different organs:

Organs with gross lesion "mass"

Organ	Male rats				Female rats			
	Dose groups							
	0	1	2	3	0	1	2	3
abdominal cavity	0	1	1	0	0	0	0	1
adrenal cortex	0	0	0	0	1	0	1	0
adrenal medulla	0	1	1	1	0	0	0	0
bone (all sites)	0	0	1	0	0	0	0	0
brain	0	1	0	0	0	0	0	1
colon	1	0	0	1	0	0	0	0
diaphragm	1	0	0	0	1	0	0	1
duodenum	1	0	0	0	0	0	0	0
esophagus	0	0	1	0	0	0	0	0
forestomach	0	0	0	1	0	0	0	0
glandular stomach	0	1	0	1	0	0	0	0
jejunum	0	0	1	0	0	0	1	1
kidneys	0	0	1	0	0	0	1	1
liver	1	3	1	2	2	1	0	0
lungs	0	2	1	0	2	1	1	0
mammary gland	1	0	1	0	21	19	17	19
mandibular lymph node	0	0	0	0	1	0	0	0
mediastinum	0	0	1	0	0	0	0	0
mesentery	1	0	0	0	1	0	0	1
pancreas	2	0	1	0	4	2	0	2
pituitary gland	10	10	9	14	22	28	27	27
prostate gland	0	0	2	0				
rectum	0	0	0	0	0	0	1	0
seminal vesicles	0	0	1	0				
skeletal muscle	2	1	0	0	0	1	1	0
skin	4	8	6	5	3	3	1	2
spleen	1	2	0	0	1	0	1	0
testes	0	2	1	3				
thymus	0	0	0	1	1	2	1	0
thyroid glands	0	1	0	2	1	0	0	0
uterus					10	9	13	11
vagina					0	1	2	3
vena cava caudalis	0	1	0	0	0	0	0	0
sum of all masses/group	**25**	**34**	**30**	**31**	**71**	**67**	**68**	**70**
Sum of all masses/sex	**120**				**276**			

			Official use only
		1 REFERENCE	
1.1	**Reference**	Van Miller JP, Hermansky SJ, Losco PE, Ballantyne B (2002) Chronic toxicity and oncogenicity study with glutaraldehyde dosed in the drinking water of Fischer 344 rats. Toxicology 175: 177-189 (Published), BPD ID A6.05_02	
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 was specified; however, the study design was well described and similar to guideline.	
2.2	**GLP**	Not specified	
2.3	**Deviations**	No	
		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	Aqueous solution	
3.1.2.2	Purity	purity [redacted]% (w/w)	
3.1.2.3	Stability	The stability of the 50 and 1000 ppm solutions of glutaraldehyde (GA) was confirmed by analysis directly after preparation and after 7, 14 and 21 days of storage at room temperature. GA was found to be stable for at least 21 days. GA in aqueous solutions was analysed by means of gas chromatography (GC, equipped with a flame ionization detector).	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Males /Female	

3.2.5 Age/weight at study initiation

At test initiation, the males and females were about 48 days old (ca. 27 days old at arrival, 3 weeks of acclimatisation prior test starting).

The body weights of the males at test initiation ranged between 131.03 and 185.23 g ; for the females, the body weights ranged between 98.48 and 124.36 g .

3.2.6 Number of animals per group

100 animals per sex and test group were used.

3.2.7 Control animals

Yes

3.3 Administration/ Exposure

3.3.1 Duration of treatment

104 weeks

3.3.2 Interim sacrifice(s)

After 52 and after 78 weeks of treatment

3.3.3 Final sacrifice

After 104 weeks

3.3.4 Frequency of exposure

Daily

3.3.5 Post-exposure period

None

Oral

3.3.6 Type

In drinking water

3.3.7 Concentration

Test group	**Test concentration (ppm)**
0	0 ppm (control)
1	50 ppm
2	250 ppm
3	1000 ppm

3.3.8 Controls

Controls received drinking water as such.

3.4	**Examinations**		
3.4.1	Body weight	The rats were weighed prior test initiation, weekly during the test period, and immediately prior sacrifice.	
3.4.2	Food consumption	Food consumption was determined weekly until week 13 of treatment; thereafter food consumption was determined on alternate weeks.	
3.4.3	Water consumption	Water consumption was determined weekly until week 13 of treatment; thereafter water consumption was determined on alternate weeks.	
3.4.4	Test substance intake	The mean daily intake of test substance as group means was determined on the basis of water consumption.	
3.4.5	Clinical signs and mortality	The rats were checked for mortality at least twice a day. The rats were examined for clinical symptoms of toxicity at least twice a day.	
3.4.6	Macroscopic investigations	Additional detailed clinical examinations were conducted once a week; palpation for masses was started from week 27 of treatment	
3.4.7	Ophthalmoscopic examination	The eyes of the animals were examined prior test starting and thereafter, after week 52, 78 and 104 of treatment.	
3.4.8	Haematology	Yes	For haematology and clinical chemistry , blood samples were collected from the retro-orbital venous plexus of rat's eye.
		Number of animals:	20 fasted animals/sex/group
		Time points:	2, 26, 52, 78 and 104 weeks of treatment.
		Parameters:	Haematocrit (HCT), haemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), erythrocyte count (RBC), leukocyte count (WBC), platelet count (PLT), differential leukocyte count.
3.4.9	Clinical Chemistry	Yes	See 3.4.8
		Number of animals:	See 3.4.8
		Time points:	See 3.4.8
		Parameters:	Alanine aminotransferase, aspartate aminotransferase,alkaline phosphatase, serum gamma glutamyl transferase, creatine kinase, lactate deshydrogenase, sorbitol deshydrogenase, glutatamate deshydrogenase, sodium, potassium, chloride, calcium, urea nitrogen, creatinine, glucose, total bilirubin, total protein, albumin, globulins.
3.4.10	Urinalysis	Yes	For urine sampling, the animals were placed in metabolism cages and urine was collected over 24 hours.
		Number of animals:	Ten animals per sex and group
		Time points:	Urine sampling was conducted on week 12, 25, 51, 77 and 103 of treatment.
		Parameters:	Volume, color, microscopic elements, osmolality, pH, protein, glucose, ketones, urobilinogen, bilirubin and blood.

3.4.11 Pathology	Ten rats per sex and group were sacrificed for the purpose of necropsy after 52 and after 78 weeks of treatment; the remaining animals were sacrificed at test ending, i.e. after 104 weeks of treatment.
3.4.11.1 Organ Weights	Yes
	From: Sacrificed animals .
	Organs: Liver, kidneys, brain, heart, adrenals and testes were removed for weighing.
	Body The terminal body weight was determined (see 3.4.1).
3.4.12 Histopathology	Yes
	Organs: In addition to gross pathology, a complete set of standard tissues was collected and fixed in 10% buffered formalin for further histopathological examination.
	Examination Particular attention was given to the occurrence and incidence of tumors
3.5 Statistics	The statistical assessment of quantitative continuous variables was based on Levene's test for equality of variance, analysis of variance (ANOVA) and t-tests. The statistical evaluation of non-parametric data (e.g. haematological and clinical-chemical parameters) was based on the one-way analysis using the Kruskal-Wallis test followed by the Mann-Whitney U-test. Incidence data were compared using Fisher's Exact test and incidental fatal tumor analysis according to Haseman JK (Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ Health Perspect. 58:385-392, 1984). Time to tumor analyses were based on Mantel-Cox, Tarone-Ware and Breslow trend tests (Dixon WJ, BMDP Statistical Software. University of California Press, Berkeley, California, 1990). Additional statistical assessments considered the dose-response trend for the incidence of large granular lymphocytic leukaemia (LGLL) severity grade in spleen in order to determine if increased severity of leukaemia was associated with higher dosages. The severity of LGLL in spleen was determined by evaluating the degree of alteration of the normal spleen morphology. All tumor incidence data were used for a single overall statistical test for the presence of any carcinogenic effect found in the study.
3.6 Further remarks	Within the present study, the potential of glutaraldehyde to produce chronic toxicity as well as oncogenic effects was investigated. Data referring to the chronic systemic toxicity were reported in a separate robust summary (see A6.5.02).
	4 RESULTS AND DISCUSSION
4.1 Body weight gain	Treatment-related decreases in body weight and body weight gain were reported for the males of the 250 and 1000 ppm groups, and for the females of the 1000 ppm group (for details, see A 6.5.02).
4.2 Food consumption	Treatment-related reduction in food consumption was observed in the 1000 ppm group for both males and females; slight or occasional reductions in food consumption were seen in the 250 and the 50 ppm groups, which only sporadically were of statistical significance (for details, see A 6.5.02).

4.3 Water consumption

Treatment-related reduction in water consumption was seen in the 1000 and the 250 ppm groups, for both, males and females. In the 50 ppm group, water consumption of the males and females also was slightly below control values, but showed no statistical significance (for details, see A 6.5.02).

4.4 Test substance intake

The mean daily intake of test substance over the whole test period was as follows:

Test concentration in drinking water	Mean daily intake of test substance (mg/kg bw/day)	
	Males	Females
50 ppm	4 (range: 2.9 – 6.9)	6 (range: 4.4 – 7.9)
250 ppm	17 (range: 14.5 – 31.8)	25 (range: 20.1 – 35.8)
1000 ppm	64 (range: 54.7 – 104.6)	86 (range: 72.2 – 121.0)

4.5 Mortality

No significant differences in mortality and survival were seen between control and treated animals of both sexes (for details, see A.6.5.02).

4.6 Clinical signs

No treatment-related clinical signs of toxicity were reported (for details, see A.6.5.02).

4.7 Haematology

No relevant, treatment-related effects or changes were reported (for details, see A.6.5.02). In fact, the main haematological finding seen at the end of the test period and which consisted of the appearance of nucleated erythrocytes and large monocytes in all treated groups (statistically significant for the males of the 250 and the 1000 ppm groups) was related to the incidence of large granular lymphocytic leukemia (LGLL) in the spleen.

4.8 Clinical chemistry

No relevant, treatment-related effects or changes were reported (for details, see A.6.5.02).

4.9 Urinalysis

Urinalysis revealed a dose-related decrease in urine volume throughout the test period for the 250 and the 1000 ppm groups. The decrease in urine volume was accompanied by an increase in osmolality, which also was dose-related in the 250 and the 1000 ppm groups. A slight decrease in pH was reported for the urine of the 1000 ppm animals (for details, see A 6.5.02).

4.10 Sacrifice and pathology

4.10.1 Organ weights

Treatment-related changes in absolute and relative weight were reported for the kidney (for details, see A 6.5.02).

Section A6.7 _ 02	**Carcinogenicity**
Annex Point IIA6.7	**Carcinogenicity in Fischer 344 rats following administration in the drinking water for 104 weeks**

4.10.2 Gross and histopathology, non-neoplastic findings

Gastric irritation was reported as main non-neoplastic finding in the males and females of the 250 and 1000 ppm groups, at each of the 3 sacrifice time points (52, 78 and 104 weeks), as well as in animals that died during the experiment. Gastric irritation was indicated by multifocal color changes, mucosal thickening, and by nodules and ulcerations primarily affecting the non-glandular mucosa. Histopathological examination revealed lesions in the stomach which were in accordance with the gastric irritation mentioned above; these lesions included gastritis, edema, squamous hyperplasia and, in 1000 ppm males only, keratinized cysts. Gastric irritation mainly was seen in animals that died during the experiment and in those sacrificed at test ending, i.e. after 104 weeks. No local tumorigenic potential could be evidenced in the stomach.

Histopathological examination also revealed an increased incidence in bone marrow hyperplasia in all groups including control; this effect was seen in animals that died as well as in animals that were sacrificed after 104 weeks. However, bone marrow hyperplasia was significantly increased compared to control for all treated females (i.e.,50, 250 and 1000 ppm) and for the males of the 1000 ppm group, which were sacrificed after 104 weeks. Histopathological examination further revealed renal tubular pigmentation in males and females of all groups that died, as well as in animals sacrificed after 104 weeks. The incidence of renal tubular pigmentation was significantly increased in females of the 250 ppm group and in females and males of the 1000 ppm group which were sacrificed at test ending. Both, bone marrow hyperplasia and renal tubular pigmentation were put into relation with the occurrence/incidence of large granular lymphocytic leukemia (LGLL), and were considered by the authors of the study as being secondary to a low grade hemolytic anemia in animals with LGLL.

Dose group (ppm)	0 (Control)		50		250		1000	
Sex	M	F	M	F	M	F	M	F
Incidence[a] Organ/Finding								
Bone marrow Hyperplasia	[illegible]	[illegible]		[illegible]		[illegible]	[illegible]	[illegible]
Kidney Tubular pigmentation	[illegible]	[illegible]				[illegible]	[illegible]	[illegible]

[a] Incidence; number affected/number examined.
[b] [illegible]
[c] [illegible]

4.10.3 Gross and histopathology, neoplastic findings

An increased incidence of large granular lymphocytic leukaemia (LGLL) was reported as only statistically/biologically relevant neoplastic finding. LGLL was seen in animals of both sexes, in all groups including the control. LGLL which was diagnosed in spleen and liver (see table below), mainly occurred in animals that died and in animals sacrificed at test ending (i.e. after 104 weeks); particular attention was given to the spleen, which is considered to be the most reliable and sensitive indicator for the occurrence of LGLL as LGLL originates from there (Losco PE and Ward JM, The early stage of large granular lymphocyte leukaemia in the F344 rat. Vet. Pathol. 21(3): 286-291, 1984).

[illegible]	Sex	Incidence of LGLL in various groups			
		0 ppm	50 ppm	250 ppm	1000 ppm
[illegible]					
[illegible]	Male	0/10	0/10	0/10	0/10
	Female	0/10	0/10	0/10	0/10
[illegible]	Male	[illegible]	[illegible]	0/10	[illegible]
	Female	0/9	1/8	[illegible]	0/10
[illegible]	Male	[illegible]	[illegible]	[illegible]	[illegible]
	Female	13/62	[illegible]	[illegible]	[illegible]
[illegible]	Male	[illegible]	13/29	17/29	16/30
	Female	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	Male	[illegible]	[illegible]	[illegible]	[illegible]
	Female	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]					
[illegible]	Male	0/10	[illegible]	0/10	0/10
	Female	0/10	[illegible]	0/10	0/10
[illegible]	Male	[illegible]	[illegible]	[illegible]	[illegible]
	Female	0/9	[illegible]	[illegible]	[illegible]
[illegible]	Male	[illegible]	[illegible]	[illegible]	[illegible]
	Female	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	Male	[illegible]	[illegible]	[illegible]	[illegible]
	Female	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	Male	[illegible]	[illegible]	[illegible]	[illegible]
	Female	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

The statistical assessment of the LGLL findings revealed that the incidence of LGLL in treated male rats was not significantly increased. In contrast, the treated females showed a significantly increased incidence of LGLL; this was particularly true for those females that were sacrificed at test ending, i.e. after 104 weeks of treatment. Analysis for dose-response trend for the severity of LLGL in the spleen revealed an increased severity in females at the higher dosages; no such observation could be made for the males. Time to tumor analysis also revealed a significant difference to control for the females only, which mainly was due to the findings reported for the 104 weeks sacrifice time point. The single overall statistical test for the presence of any carcinogenic effect found in the study revealed a statistically significant trend for total tumor incidence in the females, but not in the males; the combination of male and female data resulted in a statistical significance for total tumor response of the entire population.

4.11 Remark

The stability, homogeneity and concentration of glutaraldehyde in the test solutions were verified.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to look for the toxic and the oncogenic potential of glutaraldehyde when administered via drinking water to [redacted] rats over a period of 104 weeks; data referring to the chronic systemic toxicity were reported in a separate robust summary (see A6.5.02).

Test substance: Glutaraldehyde [redacted], purity [redacted]% (w/w); the stability was tested and confirmed for a period of at least 21 days.

No guideline was mentioned; however, the study design was well described and similar to guideline. It was not specified in the publication whether the study followed GLP or not.

The test substance was administered over a period of 104 weeks to groups of 200 [redacted] rats (100/sex) via drinking water at following concentrations: 0, 50, 250 and 1000 ppm. The animals were checked for mortality, clinical symptoms, body weight, food consumption and water consumption. Ophthalmological examinations were conducted. Blood samples were collected for the evaluation of a series of haematological and clinical-chemical parameters; urinalysis also was performed. Ten rats per sex and group were sacrificed for the purpose of necropsy after 52 and after 78 weeks of treatment; the remaining animals were sacrificed at test ending, i.e. after 104 weeks of treatment.Following necropsy, the absolute and relative weights of a series of organs were assessed, and gross pathological as well as histopathological examinations were done. Particular attention was given to neoplastic changes.

The statistical assessment of quantitative continuous variables was based on Levene's test, analysis of variance (ANOVA) and t-tests. The statistical evaluation of non-parametric data was based on the one-way analysis using the Kruskal-Wallis test followed by the Mann-Whitney U-test. Incidence data were compared with Fisher's Exact tes,t and incidental fatal tumor analysis according to Haseman JK (Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ Health Perspect. 58:385-392, 1984). Time to tumor analyses were based on Mantel-Cox, Tarone-Ware and Breslow trend tests (Dixon WJ, BMDP Statistical Software. University of California Press, Berkeley, California, 1990). Additional statistical assessments considered the dose-response trend for the incidence of large granular lymphocytic leukaemia (LGLL) severity grade in spleen, in order to determine if increased leukaemia severity was associated with higher dosages. The severity of LGLL in spleen was determined by evaluating the degree of alteration of the normal spleen. All tumor incidence data were used for a single overall statistical test for the presence of any carcinogenic effect found in the study.

The stability, homogeneity and concentration of glutaraldehyde in the test solutions were verified.

<u>Test substance intake was determined on the basis of water consumption and was as follows</u>: 50 ppm corresponded to 4 mg/kg bw/day in males and 6 mg/kg bw/day in females.250 ppm corresponded to respectively 17 and 25 mg/kg bw/day, and 1000 ppm corresponded to respectively 64 and 86 mg/kg bw/day.

5.2 Results and discussion

Systemic toxicity: Findings referring to the systemic toxicity were presented in section A 6.5, in a further robust summary (see A 6.5.02).

5.2.1	Neoplastic findings	With regard to neoplastic findings, an increased incidence of large granular lymphocytic leukaemia (LGLL) was reported as only statistically/biologically relevant finding. LGLL was seen in males and females, in all groups including the control. LGLL which was diagnosed in spleen and liver mainly occurred in animals that died and in animals sacrificed after 104 weeks. The statistical assessment of the LGLL findings revealed that the incidence of LGLL in treated male rats was not significantly increased. In contrast, the treated females showed a significantly increased incidence of LGLL; this was particularly true for those females that were sacrificed at test ending, i.e. after 104 weeks of treatment. Analysis for dose-response trend for the severity of LLGL in the spleen revealed an increased severity in females at the higher dosages (53% in spleen and 54% in liver versus respectively 20% and 23% in untreated females); no such observation were made for the males. Time to tumor analysis revealed a significant difference to control for the females only, which mainly was due to the findings reported for the 104 weeks sacrifice time point. The single overall statistical test for the presence of any carcinogenic effect found in the study revealed a statistically significant trend for total tumor incidence in the females, but not in the males; the combination of male and female data resulted in a statistical significance for total tumor response of the entire population. Particular attention was given to the spleen, which is considered to be the most reliable and sensitive indicator for the occurrence of LGLL as LGLL originates from there (Losco PE and Ward JM, The early stage of large granular lymphocyte leukaemia in the F344 rat. Vet. Pathol. 21(3): 286-291, 1984); the main haematological finding seen at the end of the test period and which consisted of the appearance of nucleated erythrocytes and large monocytes in all treated groups was related to the incidence of large granular lymphocytic leukemia (LGLL) in the spleen. Furthermore, both, bone marrow hyperplasia and renal tubular pigmentation were put into relation with the occurrence/incidence of large granular lymphocytic leukemia (LGLL), and were considered by the authors of the study as being secondary to a low grade hemolytic anemia in animals with LGLL.

5.2.2	Discussion	LGLL is known to be a tumor with high incidence in untreated [redacted] rats, which further is the most common cause of spontaneous death for this strain . Hermansky et al.(Hermansky SJ, Longhorn KA, Ballantyne B, Large granular lymphocytes leukemia in [redacted]) rats: summary of incidence from several laboratories. J. Am. Coll. Toxicol. 12: 115-116, 1992) reviewed the LGLL incidence [redacted] rats in various laboratories and reported an overall incidence in untreated animals of 29% (34% for males, 24% for females), however with variations between laboratories. Considering the findings of present study, it is rather unclear whether the increased occurrence of LGLL in treated groups and especially in treated females after 104 weeks of treatment was due to glutaraldehyde or was an artifact. Arguments rejecting the relationship between finding and glutaraldehyde treatment are the following: LGLL was seen in all groups including control; the incidence of LGLL in the 1000 ppm group was high compared to control, but no clear dose-response relationship was evident; LGLL mainly affected the treated females whereas the incidence in treated males was within control range. The authors suggested that the statistical significance of the findings in treated animals might have result from the lower incidence seen in control animals, which again might have been related to the known biological variability of LGLL incidence in untreated [redacted] rats. A further possibility considered by the authors was that the increased incidence of LGLL in females following chronic treatment with GA might have result from the influence of the chronic GA supply on factors influencing the expression of LGLL in [redacted] rats.
5.3	**Conclusion**	Following chronic treatment of [redacted] rats with glutaraldehyde via drinking water over a period of 104 weeks, a statistically significant increased incidence of large granular lymphocytic leukaemia (LGLL) in females treated with 1000 ppm glutaraldehyde was reported as only statistically/biologically relevant neoplastic finding. LGLL is a type of neoplasm occurring commonly and spontaneously in [redacted] rats. LGLL was seen in all groups including control. The incidence of LGLL in the 1000 ppm group was high compared to control, but no clear dose-response relationship was evident. The LGLL mainly affected the treated females whereas the incidence in treated males was within control range. These facts taken together lead to the assumption that there was no clear and/or direct relationship between increased incidence of LGLL and glutaraldehyde treatment.
5.3.1	Reliability	**2**
5.3.2	Deficiencies	No guideline was mentioned and it was not specified in the publication whether the study followed GLP or not. However, the study design was well described and similar to guideline, and the results and discussion were scientifically acceptable.

Section A6.7 _ 02	**Carcinogenicity**
Annex Point IIA6.7	**Carcinogenicity in Fischer 344 rats following administration in the drinking water for 104 weeks**
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 8th, 2010
Materials and Methods	See Remarks below.
Results and discussion	See Remarks below.
Conclusion	See Remarks below.
Reliability	See Remarks below.
Acceptability	See Remarks below.
Remarks	Judging by the details of the study, this scientific publication describes [redacted]. Therefore, a detailed assessment of this study summary has not been performed by the RMS. No conclusions can be made on the relevant result, i.e. the increased incidence of LGL leukaemia. Furthermore, the report only indicates the effects considered relevant by the authors. Some detailed information is given on these effects, and any other details are omitted.
	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 A6.7 _ 03	Carcinogenicity
Annex Point IIA6.3 / 6.4 / 6.5	Chronic inhalation in rats and mice

			Official use only
		1 REFERENCE	
1.1	**Reference**	van Birgelen APJM (1999) NTP technical report on the toxicology and carcinogenesis of glutaraldehyde (CAS No. 111-30-8) administered in F344/N rats and B6C3F1 mice (inhalation studies). US Department of Health and Human Services, Public Health Service, National Institutes of Health NIH, NTP TR No: 490, NIH Publication No: 99-3980 (Published), BPD ID A6.5_04	
1.2	**Data protection**	No	
1.2.1	Data owner	Not relevant (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 the study was well documented and almost in accordance with OECD TG 451	
2.2	**GLP**	Not specified	X
2.3	**Deviations**	Not relevant	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde ca. 25% aqueous solution, [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X

Section A6.7 _ 03 **Carcinogenicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Chronic inhalation in rats and mice**

3.1.2.1	Description	Liquid
3.1.2.2	Purity	The purity of the test substance was determined by gas chromatography, functional group titration and by pH determination (at the study laboratory) as well as by Karl Fischer moisture and elemental analyses (Galbraith Laboratories, Knoxville, TN). The main results for each lot were as follows: Lot No: IS-611699: A purity of 91.2 to 92.9 % relative to the reference standard was reported (< 0.6% methanol) Lot No: IS-678984: A purity of 94.6 to 94.8 % relative to the reference standard was reported (< 0.3% methanol)
3.1.2.3	Stability	Stability studies conducted with Glutaraldehyde 50% aqueous solution, from [redacted] revealed that the test substance was stable as a bulk chemical for 2 weeks when stored in the dark at temperature up to 25 °C. To ensure stability, the bulk chemical used in present studies was stored under N_2 headspace at ca. 0°C in 1-gallon amber glass bottles. The stability of the bulk material was monitored during the 2-years study by gas chromatography with flame ionization detection and by ultraviolet/visible spectroscopy. No degradation of the bulk chemical was detected.
3.2	**Test Animals**	
3.2.1	Species	Rat Mouse
3.2.2	Strain	[redacted] [redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	Males/Females
3.2.5	Age/weight at study initiation	The animals were 6 to 7 weeks old at test initiation Mean weight of the male rats at test initiation: about 150 g Mean weight of the female rats at test initiation: about 112 g Mean weight of the male mice at test initiation: about 26 g Mean weight of the female mice at test initiation: about 20 g
3.2.6	Number of animals per group	Each test group comprised 50 animals per sex
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	104 weeks
3.3.2	Frequency of exposure	6 hours + 25 minutes (T_{90}) per day, 5 days a week

Section A6.7 _ 03 **Annex Point IIA6.3 / 6.4 / 6.5**	**Carcinogenicity** **Chronic inhalation in rats and mice**
3.3.3 Post exposure period	None
3.3.4 **Inhalation**	

Section A6.7 _ 03 **Carcinogenicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Chronic inhalation in rats and mice**

3.3.4.1 Vapor generation

Glutaraldehyde (GA) vapour was generated with a rotary evaporation system with a hot-water operated at 44 °C and modified to included a heated stream of N_2 metered into the flask; the GA and water vapors arising from the flask were carried through the generator by the N_2. The temperature of the generator was sufficient to prevent condensation of the vapour during passage through the generator. Because of the evaporation rate of water (> than that of GA), ultrapure water was pumped into the evaporation flask throughout the generation period to maintain a constant volume in the flask. The vapour was conducted through a distribution manifold and was diluted with heated HEPA-and charcoal-filtered air. The flow into each exposure-chamber was controlled by means of vaccum pumps. Vapor flowed through separate metering valves for each chamber and was further diluted with filtered air to get the appropriate concentration. In order to maintain uniform exposure concentrations, chamber air circulation was increased by means of a recirculating system, which was added to each exposure chamber. The total active mixing volume of each chamber was 1.7 m^3. A small particle detector was used to check that glutaraldehyde was present in the exposure chamber as vapour and not as aerosol, both in presence and absence of test animals. No particle counts above the minimum resolvable level of about 200 particles/cm^3 were detected.

3.3.4.2 Monitoring of the test substance-vapour concentration

Monitoring of the GA vapour concentrations in the distribution system was based on gas chromatography and showed that these concentrations were stable. Monitoring of the GA vapour concentrations in the exposure chambers was based on online gas chromatography, with the monitor being coupled to the exposure chamber via a computer-controlled 12-port steam select valve. Each chamber was samples approximately every 45 minutes.

The mean chamber concentrations of glutaraldehyde during the 2-year inhalation studies were as follows:

Target concentration (ppb)		Total Number of Readings	Average concentration (ppb) with standard deviation
Rat chambers	250	3890	253 +/- 25
	500	3788	503 +/- 49
	750	3813	754 +/- 75
Mice chambers	62.5	3937	62.4 +/- 7.4
	125	3826	127 +/- 12
	250	3847	252 +/- 24

Conversion of the ppb values in µg/l:

The ppb values were converted into µg/l, resulting in following values.

Concentration in ppb	62.5	125	250	500	750
Conversion in µg/l	0.255	0.51	1.02	2.04	3.06

Section A6.7 _ 03 Annex Point IIA6.3 / 6.4 / 6.5		Carcinogenicity Chronic inhalation in rats and mice
3.3.4.3	Characterization of the vapour concentration in the exposure chambers	Build up and decay rates for the test concentrations in the chambers were determined in the 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}. The theoretical value of T_{90} and T_{10} under 15-air changes/hour condition is ca. 12.5 minutes. During pre-start testing, T_{90}-values ranged between 25 and 40 minutes in rat chambers whereas the T_{10} values were about 6 to 10 minutes. In mice chambers, the T_{90} values ranged between 18 and 31 minutes whereas the T_{10} values ranged between 9 and 11 minutes. During the studies, T_{90} was found to range between 9 and 24 minutes for rat chambers, and between 7 and 20 minutes for mice chambers; T_{10} was about 7 to 10 minutes for the rats and about 4 to 7 minutes for the mice. On the basis of these values, T_{90} was given a value of 25 minutes.
3.3.4.4	Type of exposure	Whole body exposure
3.3.4.5	Vehicle	The test substance was offered to the animals as vapour.
3.3.4.6	Concentration in vehicle	Rats: 0, 250, 500, 750 ppb Mice: 0, 62.5, 125, 250 ppb
3.3.4.7	Duration of exposure	Each exposure had a duration of 6 hours + 25 minutes (T_{90})
3.3.4.8	Controls	Sham exposed
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs and mortality	All animals were observed twice daily. Clinical observations were recorded at test initiation and thereafter, every 4 weeks from week 5 to week 89, and every 2 weeks from week 92 (rats or 93 (mice) until test ending.
3.4.2	Body weight	Body weights were recorded at test initiation and thereafter, every 4 weeks from week 5 to week 89, and every 2 weeks from week 92 (rats or 93 (mice) until test ending.
3.4.3	Food consumption	_
3.4.4	Water consumption	_
3.4.5	Ophthalmoscopic examination	_
3.4.6	Haematology	_
3.4.7	Clinical Chemistry	_
3.4.8	Urinalysis	_
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	No
3.5.2	Gross and histopathology	Complete necropsy as well as complete histopathology was conducted on all test animals. All organs and tissues were examined for gross pathology. All major tissues were fixed in 10% neutral buffered