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

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

4.2 Body weight gain, 2-week experiment

RATS				
Test concentration	Mean body weight (g)			Final body weight relative to controls (%)
	Initial	Final	Change[a]	
Males				
0 ppm	107	190	82	
0.16 ppm	108	193	85	102
0.50 ppm	108	183	75	97
1.60 ppm	107	109	2	**58**
5.00 ppm	106	-	-	-
16.00 ppm	109	-	-	-
Females				
0 ppm	101	141	40	
0.16 ppm	100	144	45	102
0.50 ppm	101	138	38	98
1.60 ppm	98	83	-15	**59**
5.00 ppm	101	-	-	-
16.00 ppm	100	-	-	-

[a] refers to surviving animals

MICE				
Test concentration	Mean body weight (g)			Final body weight relative to controls (%)
	Initial	Final	Change	
Males				
0 ppm	24.8	26.2	1.3	
0.16 ppm	24.3	26.6	2.3	102
0.50 ppm	24.2	25.7	1.5	98
1.60 ppm	24.2	-	-	-
5.00 ppm	24.0	-	-	-
16.00 ppm	24.0	-	-	-
Females				
0 ppm	19.3	23.2	4.0	
0.16 ppm	19.6	22.5	2.9	97
0.50 ppm	20.0	22.1	2.1	95
1.60 ppm	19.4	-	-	-
5.00 ppm	19.0	-	-	-
16.00 ppm	19.3	-	-	-

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

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

4.3 Body weight gain, 13-week experiment

RATS				
Test concentration	Mean body weight (g)			Final body weight relative to controls (%)
	Initial	Final	Change[a]	
Males				
0 ppb	102	327	225	
62.5 ppb	102	329	227	101
125 ppb	102	328	226	100
250 ppb	101	323	222	99
500 ppb	101	312	211	95
1000 ppb	103	295	192	**90***
Females				
0 ppb	92	192	100	
62.5 ppb	92	191	99	100
125 ppb	94	195	101	102
250 ppb	91	185	93	97
500 ppb	95	184	89	96
1000 ppb	91	179	88	**93***

[a] refers to surviving animals; *, statistically significant

X

MICE				
Test concentration	Mean body weight (g)			Final body weight relative to controls (%)
	Initial	Final	Change	
Males				
0 ppb	22.5	34.2	11.8	
62.5 ppb	22.8	33.0	10.2	96
125 ppb	22.7	32.8	10.1	96
250 ppb	22.7	31.7	9.0	92
500 ppb	22.2	30.3	8.0	88
1000 ppb[a]	22.8	27.7	4.4	81
Females				
0 ppb	18.9	29.5	10.7	
62.5 ppb	19.4	29.0	9.6	98
125 ppb	19.0	29.4	10.4	100
250 ppb	19.1	27.4	8.4	93
500 ppb	19.4	26.4	7.0	89
1000 ppb	19.4	-	-	-

a, The final body weights were recorded on day 84; at this time one survivor remained in the 1000 ppb group, which died before the end of the experiment.

4.4 Food consumption and compound intake Not considered

4.5 Ophtalmoscopic examination Not performed

4.6 Blood analysis

4.6.1 Haematology, 13-week experiment, rats

The haematological parameters were examined after 4 and 24 days and at the end of the experimental period, in rats only (i.e. day 94).

The main statistically significant changes in haematological parameters can be summarized as follows:

Test conc.		0 (ppb)	62.5 (ppb)	125 (ppb)	250 (ppb)	500 (ppb)	1000 (ppb)
Parameter	D	**Males**					
Hemoglobin (g/dl)	94	14.9+/- 0.1	14.8 +/- 0.1	**15.3** +/- **0.1****	15.0 +/- 0.2	**15.2** +/- **0.1***	15.2 +/- 0.2
Erythrocytes (10^6/µl)	24	8.09 +/-0.08	8.02 +/- 0.1	7.88 +/- 0.11	7.99 +/- 0.10	7.91 +/- 0.09	**8.73** +/- **0.11***

	94	8.83 +/-0.06	8.85 +/- 0.06	**9.17 +/- 0.08****	8.85 +/- 0.07	8.90 +/- 0.03	8.97 +/- 0.08
Mean cell vol. .(fL)	24	58.2 +/- 0.4	57.8 +/- 0.3	58.0 +/- 0.3	58.5 +/- 0.3	57.9 +/- 0.4	**57.0 +/- 0.3***
	94	52.9 +/- 0.3	52.3 +/- 0.2	52.6 +/- 0.2	52.3 +/- 0.3	**52.1 +/- 0.2***	52.3 +/- 0.2
Mean cell haemoglobin conc. (g/dl)	4	31.6 +/- 0.01	32.1 +/- 0.1	**32.2 +/- 0.1****	31.8 +/- 0.1	31.9 +/- 0.1	31.8 +/- 0.1
Leukocytes (10^3/µl)	4	7.68 +/- 0.28	7.29 +/- 0.39	7.86 +/- 0.36	8.36 +/- 0.29	**8.94 +/- 0.41***	8.18 +/- 0.26
	94	7.36 +/- 0.59	7.68 +/- 0.21[a]	8.75 +/- 0.41	7.51 +/- 0.48	**6.31 +/- 0.13****	**6.77 +/- 0.16***
Segmented neutrophils (10^3/µl)	24	0.72 +/- 0.05	0.90 +/- 0.12	**1.19 +/- 0.06****	**0.94 +/- 0.11***	0.90 +/- 0.10	**2.48 +/- 0.36****
Lymphocytes (10^3/µl)	4	6.61 +/- 0.22	6.45 +/- 0.37	6.80 +/- 0.39	7.34 +/- 0.30	**7.63 +/- 0.35***	6.95 +/- 0.17
	94	6.35 +/- 0.24[a]	6.00 +/- 0.40	7.45 +/- 0.36	6.33 +/- 0.38	**5.28 +/- 0.17***	**5.35 +/- 0.21***
Eosinophils (10^3/µl)	24	0.06 +/- 0.01	**0.03 +/- 0.01***	0.04 +/- 0.02	0.02 +/- 0.01	**0.02 +/- 0.02***	**0.03 +/- 0.01***

*, p<=0.05; **, p<=0.01; a, n = 9; D = day

Test conc.		0 (ppb)	62.5 (ppb)	125 (ppb)	250 (ppb)	500 (ppb)	1000 (ppb)
Parameter	D	**Females**					
Vol of packed red cells (ml/dl)	4	44.0 +/- 0.5	43.4 +/- 0.5	44.8 +/- 0.5	44.7 +/- 0.5	**45.6 +/- 0.6***	**45.5 +/- 0.5[a] ***
Erythrocytes (10^6/µl)	4	7.36 +/- 0.12	7.45 +/- 0.06[a]	7.58 +/- 0.11	7.53 +/- 0.12	**7.68 +/- 0.12***	7.64 +/- 0.13[a]
Mean cell vol. .(fL)	4	59.7 +/- 0.4	58.3 +/- 0.2[a] **	59.2 +/- 0.3	59.5 +/- 0.2	59.2 +/- 0.3	59.7 +/- 0.4[a]
	24	59.1 +/- 0.4	58.4 +/- 0.3	58.5 +/- 0.5	58.7 +/- 0.2	58.4 +/- 0.3	**57.3 +/- 0.5****
	94	56.9 +/- 0.1	56.7 +/- 0.2	56.6 +/- 0.2	56.9 +/- 0.2[a]	**56.4 +/- 0.2***	56.5 +/- 0.2
Mean cell haemoglobin conc. (g/dl)	24	31.8 +/- 0.1	31.9 +/- 0.1	32.0 +/- 0.1	32.1 +/- 0.1	31.7 +/- 0.1	**32.3 +/- 0.1***

Platelets (10^3/µl)	24	712 +/- 19.5	705.5 +/- 26.7	679.8 +/- 19.5	666.9 +/- 17.8	**644.9 +/- 17.4***	**571.3 +/- 48.7***
Leukocytes (10^3/µl)	24	8.25 +/- 0.42	7.69 +/- 0.27	8.03 +/- 0.33	8.07 +/- 0.37	7.69 +/- 0.30	**12.01 +/- 0.53****
Segmented neutrophils (10^3/µl)	24	0.75 +/- 0.06	0.80 +/- 0.07	0.77 +/- 0.06	**1.06 +/- 0.12***	0.77 +/- 0.10	**3.11 +/- 0.31****

*, p<=0.05; **, p<=0.01; a, n = 9; D = day

At time point 4 day, no biologically relevant changes in haematological parameters were seen. At day 24, the data in the tables show that for male rats, significant increases in total counts of segmented neutrophils occurred at 125, 250 and 1000 ppb; the same effect was reported for the females of the 250 and 1000 ppb groups. Furthermore, in females, an increase in total leukocyte count was reported for the 1000 ppb group. At the end of the 13-week experimental period (i.e. day 94), no biologically relevant changes in haematology were seen; in fact, effects were sporadic and not treatment-related.

4.6.2 Clinical chemistry, 13-week experiment, rats

The haematological parameters were examined after 4 and 24 days and at the end of the experimental period (i.e. day 94).

The main statistically significant changes in haematological parameters can be summarized as follows:

Test conc.		0 (ppb)	62.5 (ppb)	125 (ppb)	250 (ppb)	500 (ppb)	1000 (ppb)
Parameter	D	**Males**					
Alanine Transferase (IU/l)	24	37 +/-0	38 +/- 1	38 +/- 1	**41 +/- 1***	**45 +/- 4***	**51 +/- 3****
Creatinine (mg/dl)	4	0.63 +/- 0.02	0.59 +/- 0.01	0.63 +/- 0.02	0.62 +/- 0.01	**0.56 +/- 0.02****	**0.57 +/- 0.03***
	24	0.66 +/- 0.03	0.68 +/- 0.02	0.63 +/- 0.02	0.59 +/- 0.02	**0.58 +/- 0.01***	0.60 +/- 0.03
Globulin (g/dl)	24	2.6 +/- 0.1	2.5 +/- 0.1	2.5 +/- 0.1	2.6 +/- 0.1	2.4 +/- 0.1	**2.4 +/- 0.1***
Sorbitol deshydrogenase (IU/l)	94	20 +/- 1	26 +/- 3	23 +/- 2	**16 +/- 0[a]****	19 +/- 1	**14 +/- 0****
Total protein (g/dl)	4	5.6 +/- 0.1	5.6 +/- 0.0	5.5 +/- 0.1	5.7 +/- 0.1	5.6 +/- 0.0	**5.9 +/- 0.1****
	24	6.6 +/- 0.1	6.4 +/- 0.1	**6.3 +/- 0.1****	**6.4 +/- 0.1***	**6.3 +/- 0.1***	**6.3 +/- 0.1***
Urea nitrogen (mg/dl)	4	20.2 +/- 1.0	21.8 +/- 0.9	21.3 +/- 0.5	**23.4 +/- 0.8***	22.0 +/- 0.7	22.4 +/- 0.8

*, p<=0.05; **, p<=0.01; a, n = 9; D = day

X

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

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

Test conc.		0 (ppb)	62.5 (ppb)	125 (ppb)	250 (ppb)	500 (ppb)	1000 (ppb)
Parameter	D	**Females**					
Alanine Transferase (IU/l)	24	31 +/- 1	34 +/- 2	34 +/- 2	**34** +/- **1***	**35** +/- **2***	**52** +/- **5****
Albumin (g/dl)	24	4.0 +/- 0.00	**3.9** +/- **0.1***	**3.8** +/- **0.1***	**3.8** +/- **0.0***	4.0 +/- 0.0	**3.7** +/- **0.1****
Alkaline phosphatase (IU/l)	4	685 +/- 17	662 +/- 9	708 +/- 64	**622** +/- **15***	636 +/- 17	650 +/- 36
	24	416 +/- 11	446 +/- 8	475 +/- 46	454 +/- 15	**488** +/- **13****	**490** +/- **29****
Creatine kinase (IU/l)	4	314 +/- 35	274 +/- 33[a]	244 +/- 33	**184** +/- **10***	307 +/- 49	420 +/- 47
	24	203 +/- 36	198 +/- 37	**123** +/- **14***	**99** +/- **6[a]****	**139** +/- **19***	**134** +/- **19[a]***
Creatinine (mg/dl)	4	0.65 +/- 0.02	0.62 +/- 0.02	**0.59** +/- **0.02***	0.60 +/- 0.02	0.60 +/- 0.02	**0.55** +/- **0.03****
Globulin (g/dl)	24	2.4 +/- 0.1	2.4 +/- 0.1	2.5 +/- 0.1	2.4 +/- 0.1	2.3 +/- 0.0	**2.1** +/- **0.1***
Sorbitol deshydrogenase (IU/l)	4	16 +/- 1	16 +/- 0	16 +/- 1	15 +/- 1	14 +/- 1	**12** +/- **0****
	24	22 +/- 1	23 +/- 1	20 +/- 1	20 +/- 1	20 +/- 1	**17** +/- **1****
Total protein (g/dl)	4	5.7 +/- 0.1	5.9 +/- 0.1	5.6 +/- 0.1	**5.4** +/- **0.1***	5.6 +/- 0.1	5.8 +/- 0.1
	24	6.4 +/- 0.1	6.2 +/- 0.1	6.3 +/- 0.1	6.3 +/- 0.1	6.3 +/- 0.1	**5.8** +/- **0.1****
Urea nitrogen (mg/dl)	4	17.3 +/- 1.1	18.7 +/- 0.9	19.2 +/- 0.5	**20.8** +/- **0.8***	**20.4** +/- **0.6***	**20.7** +/- **0.9***
	24	20.7 +/- 0.6	**23.7** +/- **0.6***	**24.0** +/- **0.7***	22.7 +/- 1.0[a]	22.9 +/- 1.1	22.5 +/- 1.3

*, p<=0.05; **, p<=0.01; a, n = 9; D = day

At time point 4 day, no biologically relevant changes in clinical-chemical parameters were seen. At day 24, minimal to mild statistically significant increased activity of alanine aminotransferase was reported for the male and female rats of the 250, 500 and 1000 ppb groups. The activity of the alkaline phosphatase also was increased in the females of the 500 and 1000 ppb groups. Furthermore, mild decrease in total protein was reported for the males of the 125 and 1000 ppb groups, and for the females of the 1000 ppb group. Albumin also was decreased in the females of the 500 ppb group whereas an increase in urea nitrogen was reported for the females of the 250 and 1000 ppb groups. At the end of the 13-week experimental period (i.e. day 94), no biologically relevant changes in clinical-chemical parameters were seen; in fact, effects were sporadic and not treatment-related.

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

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

4.6.3 Urinalysis — Not performed

4.7 Sacrifice and pathology

4.7.1 Organ weights, 2-week experiment, rats — Decreases in absolute organ weights were observed at 1.6 ppm; however, they were related to the decrease in final body weight.

4.7.2 Organ weights, 2-week experiment, mice — The female mice of the 0.5 ppm group showed significantly lower mean absolute and relative heart weights than controls. The males of the same group showed significantly increased relative liver weight when compared to controls.

4.7.3 Organ weights, 13-week experiment, rats

Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
n	10	10	10	10	10	10
Necropsy body wt	329 ± 8	337 ± 8	334 ± 6	332 ± 6	321 ± 10	309 ± 7*
Heart						
Absolute	0.990 ± 0.028	1.001 ± 0.027	0.984 ± 0.025	0.958 ± 0.014	0.984 ± 0.025	0.958 ± 0.017
Relative	3.01 ± 0.03	2.97 ± 0.05	2.96 ± 0.04	2.92 ± 0.04	3.07 ± 0.05	3.17 ± 0.05**
Right kidney						
Absolute	1.090 ± 0.029	1.169 ± 0.033	1.098 ± 0.034	1.137 ± 0.028	1.082 ± 0.040	1.072 ± 0.031
Relative	3.31 ± 0.03	3.44 ± 0.06*	3.28 ± 0.05	3.43 ± 0.06	3.37 ± 0.04	3.54 ± 0.05**
Liver						
Absolute	11.282 ± 0.414	11.680 ± 0.319	11.484 ± 0.262	11.569 ± 0.296	11.484 ± 0.483	10.685 ± 0.333
Relative	34.22 ± 0.62	34.67 ± 0.45	34.41 ± 0.48	34.83 ± 0.46	35.71 ± 0.59	35.27 ± 0.66
Lungs						
Absolute	1.633 ± 0.072	1.685 ± 0.059	1.681 ± 0.049	1.670 ± 0.058	1.865 ± 0.073	1.688 ± 0.073
Relative	4.95 ± 0.16	5.00 ± 0.14	5.04 ± 0.15	5.04 ± 0.17	5.18 ± 0.18	5.58 ± 0.22*
Right testis						
Absolute	1.390 ± 0.028	1.405 ± 0.025	1.394 ± 0.028	1.348 ± 0.030	1.365 ± 0.026	1.360 ± 0.022
Relative	4.23 ± 0.06	4.18 ± 0.08	4.17 ± 0.04	4.07 ± 0.11	4.27 ± 0.08	4.50 ± 0.08*
Thymus						
Absolute	0.310 ± 0.008	0.325 ± 0.016	0.305 ± 0.009	0.334 ± 0.010	0.346 ± 0.016	0.278 ± 0.012
Relative	0.94 ± 0.02	0.96 ± 0.04	0.91 ± 0.03	1.01 ± 0.03	1.08 ± 0.05*	0.92 ± 0.04
Female						
n	10	10	10	9	10	10
Necropsy body wt	198 ± 3	195 ± 4	198 ± 4	190 ± 5	187 ± 4	175 ± 5**
Heart						
Absolute	0.673 ± 0.015	0.682 ± 0.016	0.683 ± 0.018	0.683 ± 0.017	0.678 ± 0.013	0.667 ± 0.020
Relative	3.44 ± 0.06	3.51 ± 0.06	3.45 ± 0.07	3.60 ± 0.09	3.60 ± 0.06	3.82 ± 0.06**
Right kidney						
Absolute	0.671 ± 0.013	0.687 ± 0.024	0.685 ± 0.016	0.707 ± 0.013	0.678 ± 0.015	0.662 ± 0.027
Relative	3.43 ± 0.04	3.53 ± 0.08	3.45 ± 0.05	3.72 ± 0.05*	3.63 ± 0.08*	3.72 ± 0.08**
Liver						
Absolute	6.484 ± 0.152	6.584 ± 0.309	6.459 ± 0.171	6.418 ± 0.276	6.146 ± 0.151	5.888 ± 0.242
Relative	33.17 ± 0.62	33.74 ± 1.19	32.57 ± 0.62	33.68 ± 1.09	32.80 ± 0.36	33.54 ± 0.65
Lungs						
Absolute	1.192 ± 0.029	1.265 ± 0.055	1.287 ± 0.051	1.290 ± 0.062	1.182 ± 0.037	1.174 ± 0.055
Relative	6.11 ± 0.18	6.48 ± 0.20	6.54 ± 0.21	6.75 ± 0.32	6.31 ± 0.15	6.72 ± 0.25
Thymus						
Absolute	0.278 ± 0.008	0.271 ± 0.007	0.263 ± 0.012	0.257 ± 0.010	0.259 ± 0.005	0.227 ± 0.007[2]**
Relative	1.41 ± 0.03	1.40 ± 0.06	1.32 ± 0.05	1.35 ± 0.05	1.38 ± 0.02	1.27 ± 0.04[2]

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).
[2] n = 9.
* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test.
** Significantly different (P≤0.01) from the control group by Williams' test.

X

4.7.4 Organ weights, 13-week experiment, mice

Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Glutaraldehyde[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
n	10	10	10	10	10	0
Necropsy body wt	36.1 ± 0.4	34.2 ± 0.6*	33.9 ± 0.8*	33.0 ± 0.5**	31.9 ± 0.8**	
Heart						
Absolute	0.161 ± 0.004	0.157 ± 0.003	0.162 ± 0.004	0.159 ± 0.005	0.150 ± 0.002	
Relative	4.46 ± 0.10	4.60 ± 0.08	4.79 ± 0.07**	4.82 ± 0.14**	4.72 ± 0.09**	
Right kidney						
Absolute	0.340 ± 0.007	0.327 ± 0.009	0.316 ± 0.008	0.318 ± 0.008	0.309 ± 0.007	
Relative	9.59 ± 0.16	9.56 ± 0.19**	9.34 ± 0.14**	9.65 ± 0.23**	9.72 ± 0.18**	
Liver						
Absolute	1.690 ± 0.037	1.616 ± 0.043	1.626 ± 0.039	1.570 ± 0.038*	1.569 ± 0.042*	
Relative	46.82 ± 0.94	47.26 ± 0.90	48.06 ± 0.56*	47.62 ± 0.91	49.29 ± 0.99	
Lungs						
Absolute	0.217 ± 0.005	0.224 ± 0.007	0.223 ± 0.006	0.226 ± 0.006	0.218 ± 0.005	
Relative	6.02 ± 0.17	6.56 ± 0.20	6.60 ± 0.16*	6.87 ± 0.22**	6.87 ± 0.22**	
Right testis						
Absolute	0.120 ± 0.001	0.124 ± 0.002	0.125 ± 0.003	0.122 ± 0.002	0.121 ± 0.002	
Relative	3.32 ± 0.06	3.62 ± 0.09**	3.69 ± 0.06**	3.71 ± 0.09**	3.80 ± 0.08**	
Thymus						
Absolute	0.048 ± 0.002	0.049 ± 0.002	0.047 ± 0.002	0.044 ± 0.003	0.040 ± 0.003*	
Relative	1.32 ± 0.06	1.42 ± 0.04	1.38 ± 0.04	1.35 ± 0.09	1.24 ± 0.10	
Female						
n	10	10	10	10	8	0
Necropsy body wt	30.9 ± 0.3	31.1 ± 0.8	30.3 ± 0.8	28.7 ± 0.5**	27.3 ± 0.6**	
Heart						
Absolute	0.143 ± 0.002	0.143 ± 0.002	0.144 ± 0.002	0.137 ± 0.002	0.140 ± 0.004	
Relative	4.63 ± 0.06	4.62 ± 0.12	4.77 ± 0.09	4.78 ± 0.09	5.14 ± 0.12**	
Right kidney						
Absolute	0.203 ± 0.003	0.229 ± 0.006**	0.224 ± 0.004**	0.218 ± 0.003	0.218 ± 0.005	
Relative	6.59 ± 0.16	7.39 ± 0.20**	7.41 ± 0.12**	7.61 ± 0.16**	7.98 ± 0.13**	
Liver						
Absolute	1.538 ± 0.033	1.640 ± 0.044	1.627 ± 0.035	1.524 ± 0.033	1.444 ± 0.060	
Relative	49.76 ± 0.75	52.73 ± 0.54*	53.82 ± 0.88*	53.06 ± 0.71*	52.98 ± 1.77*	
Lungs						
Absolute	0.216 ± 0.003	0.236 ± 0.004**	0.229 ± 0.005	0.224 ± 0.005	0.215 ± 0.004	
Relative	7.00 ± 0.14	7.61 ± 0.14*	7.58 ± 0.18*	7.82 ± 0.20**	7.89 ± 0.10**	
Thymus						
Absolute	0.060 ± 0.003	0.059 ± 0.002	0.058 ± 0.003	0.055 ± 0.001	0.052 ± 0.003*	
Relative	1.94 ± 0.08	1.90 ± 0.04	1.91 ± 0.10	1.91 ± 0.05	1.92 ± 0.12	

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error). Data are not available for the highest dose group (1000 ppb) since there were no survivors in this group at 13 weeks.
* Significantly different (P≤0.05) from the control group by Williams' test
** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test.

4.7.5 Gross pathology, 2-week experiment, rats

Gross pathological effects were seen in rats that died or were sacrificed in extremis before the end of the experimental period. These lesions mainly concerned the respiratory tract and the oral cavity and included crusted exudates at the anterior tip of the nares, a gray and thickened appearance of the laryngeal mucosa and exudates or crusts on the surface of the tongue. In some rats the stomach and the intestine were dilated with air; this was considered to be related to the mouth breathing and the subsequent swallowing of air.

4.7.6 Gross pathology, 2-week experiment, mice

Gross pathological effects were seen in mice treated with 16 ppm test substance and mainly concerned the external nares and the larynx of the animals. Red crusts at the anterior tip of the nares and a gray and thickened appearance of the laryngeal mucosa were reported. Dilation of stomach and intestine was seen in mice that died or were sacrificed in extremis prior the ending of the experiment.

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

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

4.7.7 Histopathology, 2-week experiment, rats

Selected Histopathologic Lesions for F344/N Rats in the 2-Week Inhalation Study of Glutaraldehyde[1]

	Concentration (ppm)					
	0	0.16	0.5	1.6	5.0	16.0
Nasal passages/turbinates						
Hyperplasia	–[2]	–	3/0	5/4	5/5	4/5
Squamous metaplasia	–	–	2/1	5/5	5/5	5/5
Necrosis/inflammation	–	–	–	5/5	5/5	5/5
Larynx						
Necrosis/inflammation	–	–	0/1	2/4	4/3	5/5
Squamous metaplasia	–	–	–	2/5	5/5	5/5
Trachea						
Necrosis/inflammation	–	–	–	–	3/1	5/5
Squamous metaplasia	–	–	–	–	–	5/4
Lung and bronchi						
Inflammation	–	–	–	–	–	5/5
Squamous metaplasia	–	–	–	–	–	5/5
Tongue						
Inflammation/erosion	–	–	–	–	–	4/1

[1] Data are given as male incidence/female incidence for five animals per group.
[2] (–) lesion not present

4.7.8 Histopathology, 2-week experiment, mice

Selected Histopathologic Lesions for B6C3F, Mice in the 2-Week Inhalation Study of Glutaraldehyde[1]

	Concentration (ppm)					
	0	0.16	0.5	1.6	5.0	16.0
Nasal passages/turbinates						
Squamous metaplasia	–[2]	–	–	1/2	2/1	1/3
Necrosis/inflammation	–	–	–	4/4	4/2	5/5
Larynx						
Necrosis/inflammation	–	–	–	0/1	1/2	5/5
Squamous metaplasia	–	–	1/0	5/4	5/4	5/4
Trachea						
Necrosis/inflammation	–	–	–	–	–	3/2

[1] Data are given as male incidence/female incidence for five animals per group.
[2] (–) lesion not present

4.7.9 Gross pathology, 13-week experiment, rats

No gross pathological changes were reported.

4.7.10 Gross pathology, 13-week experiment, mice

Gross pathological changes were seen in mice that died or were sacrificed in extremis before the end of the experiment. These changes included dilation of the stomach and intestines (probably related to the dyspnea and the subsequent swallowing of air) and paler and smaller appearance of the spleen (probably resulting from lymphoid depletion).

Section A6.4.3 _ 01 Repeated dose toxicity

Annex Point IIA6.3 / 6.4 / 6.5

Sub-chronic inhalation toxicity in rats and mice

4.7.11 Histopathology, 13-week experiment, rats

Selected Histopathologic Lesions of the Nasal Passages/Turbinates for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde[1]

	Concentration (ppb)					
	0	62.5	125	250	500	1000
Male						
Respiratory epithelium						
Nasoturbinates/septum						
Hyperplasia	0	0	0	0	0	7 (1.7)
Hyperplasia, goblet cell	0	0	0	1 (1.0)	3 (1.0)	9 (1.4)
Squamous metaplasia	0	0	0	0	0	5 (2.0)
Inflammation	0	0	0	0	0	7 (1.0)
Lateral wall						
Hyperplasia	0	0	1 (1.0)	0	4 (1.0)	7 (1.7)
Squamous metaplasia	0	0	0	0	1 (1.0)	7 (2.5)
Olfactory epithelium						
Degeneration	0	0	0	0	0	1 (2.0)
Nasal vestibule/anterior nares						
Squamous exfoliation	0	0	0	1 (1.0)	4 (1.0)	9 (1.1)
Inflammation	0	1 (1.0)	0	0	0	3 (1.0)
Female						
Respiratory epithelium						
Nasoturbinates/septum						
Hyperplasia	0	0	0	0	0	4 (1.7)
Hyperplasia, goblet cell	0	0	0	0	0	8 (1.2)
Squamous metaplasia	0	0	0	0	0	5 (1.4)
Inflammation	0	0	0	1 (1.0)	0	5 (1.2)
Lateral wall						
Hyperplasia	0	0	0	1 (2.0)	2 (1.0)	8 (1.6)
Squamous metaplasia	0	0	0	1 (3.0)	0	8 (2.0)
Olfactory epithelium						
Degeneration	0	0	0	0	0	2 (1.5)
Nasal vestibule/anterior nares						
Squamous exfoliation	0	0	0	3 (1.3)	7 (1.1)	9 (1.7)
Inflammation	1 (1.0)	0	0	0	0	0
Erosion	0	0	0	0	1 (1.0)	2 (2.0)

[1] The incidence is the number of core-study animals with lesions for groups of 10 animals. Average severity (in parentheses) is based on the number of animals with lesions; 1=minimal, 2=mild, 3=moderate, 4=marked.

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

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

4.7.12 Histopathology, 13-week experiment, mice

Selected Histopathologic Lesions for B6C3F$_1$ Mice In the 13-Week Inhalation Study of Glutaraldehyde[1]

	Concentration (ppb)					
	0	62.5	125	250	500	1000
Male						
Nasal passages/turbinates						
Respiratory epithelium						
Inflammation	0	0	0	0	0	4 (1.0)
Squamous metaplasia	0	0	0	0	0	1 (2.0)
Nasal vestibule/anterior nares						
Squamous exfoliation	0	0	0	1 (1.0)	2 (1.0)	9 (2.8)
Inflammation	0	0	0	0	7 (1.1)	0[2]
Erosion	0	0	0	1 (1.0)	1 (1.0)	0
Larynx						
Squamous metaplasia	0	0	0	0	0	7 (1.6)
Necrosis	0	0	0	0	0	2 (1.0)
Female						
Nasal passages/turbinates						
Respiratory epithelium						
Inflammation	0	0	0	1 (1.0)	1 (1.0)	7 (1.4)
Squamous metaplasia	0	0	0	0	0	3 (1.0)
Nasal vestibule/anterior nares						
Squamous exfoliation	0	0	0	1 (1.0)	2 (2.5)	10 (2.8)
Inflammation	0	5 (1.0)	8 (2.0)	8 (1.6)	8 (2.5)	0[2]
Erosion	0	0	1 (1.0)	0	0	0
Larynx						
Squamous metaplasia	0	0	0	0	0	10 (1.6)
Necrosis	0	0	0	0	0	2 (1.0)

[1] The incidence is the number of core study animals with lesions from groups of 10. Average severity (in parentheses) is based on the number of animals with lesions; 1=minimal, 2=mild, 3=moderate, 4=marked.
[2] Inflammation was a component of "squamous exfoliation" and not diagnosed separately when the latter was present.

4.8 Sperm morphology and vaginal cytology:

No significant differences were seen between treated and control rats.

In mice, the evaluation of the reproductive system revealed significant differences for the females of the 250 and 500 ppb groups, concerning he amount of time spent in estrous stages. In fact, in the females of the 500 ppb group, estrus and diestrus phases were longer than metestrus and proestrus when compared to controls. Reproductive parameters referring to the male mice were inconspicuous.

Summary of estrous cycle characterization in female mice, 13-week experiment:

Estrous phases as % of cycle	0 ppb	62.5 ppb	250 ppb	500 ppb
Diestrus	23.3	25.8	26.7	**31.3**
Proestrus	20.0	21.7	20.0	9.4
Estrus	31.7	31.7	34.2	**37.5**
Metestrus	25.0	20.8	19.2	21.9

4.9 Histoautoradiographic evaluation of respiratory tract

See table E1 to E6

Minimal to mildly severe lesions in the mucosa of the nasal passages were reported for both rats and mice treated with the test substance. These lesions were in accordance with the (histo)pathological findings of the 13-week experiment. The onset and evolution of the lesions were

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summarized in the tables E1 and E2 (see end of the document); the severity of the nasal lesions was summarized in table E3 and E4.

Tables E5 and E6 show the mean cell replication for treated rats and treated mice. The squamous epithelium of the nasal vestibule and to a lesser extent the respiratory epithelium of the atrioturbinate of the dorsal meatus of the treated animals showed treatment-related increase in cell replication rates. In mice and particularly in females (13-week treatment), the increased rate of cell replication in the squamous epithelium of the nasal vestibule was coupled with neutrophilic infiltration of the mucosa. However, the subjective score for severity of neutrophilic infiltrate did not correlate with the degree of S-phase response.

In treated rats, the treatment-related increase in cell replication was almost more important than in mice. Furthermore, the increase in cell replication in the nasal vestibule of rats occurred early and remained elevated or decreased slightly through the study course when compared to mice. In addition to the increased cell replication in the squamous epithelium of the nasal vestibule, the treated rats also displayed increase in cell replication within the respiratory epithelium of the dorsal atrioturbinate; no correspondency with signs of inflammation was evident.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate to toxic potential of glutaraldehyde when administered via inhalation over a period of two and 13 weeks to rats and mice.

Test substance: Glutaraldehyde 50% aqueous solution, [redacted] Clear colourless liquid, purity 50.0% (minor contamination from the polymeric forms of glutaraldehyde and other volatile impurities), stability verified for 2 weeks when stored in the dark at temperatures up to 25 °C

No guideline was mentioned. However the study was guideline-like (OECD 413) and was conducted in accordance with GLP.

The study consisted of a 2-week (range-finding) and a 13-week inhalation experiment and was performed with [redacted] rats and [redacted] mice of both sexes. At test initiation the animals were about 6 to 7 weeks old. The test groups of the 2-week test consisted of 10 animals (5/sex) each, whereas those of the 13-week test consisted of 20 animals (10/sex). The test concentrations used for the 2-week test were: 0, 0.16, 0.50, 1.60, 5.00 and 16.00 ppm for both species. For the 13-week test, the concentrations were: 0, 62.5 ppb, 125 ppb, 250 ppb, 500 ppb and 1000 ppb. The test substance was offered to the test animals as vapour. The animals were whole-body exposed for 6 hours a day, 5 days a week; they were examined for mortality, clinical signs of toxicity and body weight. A series of haematological and clinical-chemical parameters were evaluated after 4 and 24 days and at the end of the experimental period, in rats. At the end of each experiment, the surviving animals were sacrificed for the purpose of necropsy. Animals that died during the experiment or were killed in extremis also were subjected to necropsy. The absolute and relative organ weights were

determined, and the animals were subjected to gross and histopathology.

Following additional evaluations were also performed:

(1) Sperm morphology and vaginal cytology studies were conducted at the end of the 13-week experiment. Male rats of all test groups (0 to 1000 ppb, excepted 125 ppb) and male mice of the 0, 62.5, 250 and 500 ppb groups were evaluated for final body weight and reproductive tissue weights, as well as for spermatozoal data. Female rats of all test groups (0 to 1000 ppb excepted 125 ppb) and female mice of the 0, 62.5, 250 and 500 ppb groups were evaluated for final body weight, estrous cycle length and the percent of cycle corresponding to each of the diestrus, proestrous, estrus and metestrus stage.

(2) Histoautoradiographic evaluation of respiratory tract was performed on rats and mice exposed to all test concentrations from 0 to 1000 ppb for 1 or 4 days, or 6 or 13 weeks. The incidence and severity of nasal lesions were assessed.

5.2 Results and discussion

2-week experiment: 100% mortality was observed for the rats at 5 and 16 ppm; for the mice 100% mortality already was reached at 1.6 ppm. Death was attributed to severe respiratory distress. In rats, clinical signs of toxicity were seen from 1.6 ppm upwards and mainly included labored breathing, ocular and/or nasal discharge and mouth breathing. For mice, such signs also occurred from 1.6 ppm upwards and included respiratory difficulties, ocular and/or nasal discharge and mouth breathing. Body weight (BW) gain of the male rats of the 1.6 ppm group was about 2 g versus 82 g for control animals; for the females, a loss in BW of 15 g was reported, whereas control animals gained about 40 g. BW changes in mice were inconspicuous.

For rats, decrease in absolute organ weights was seen at 1.6 ppm and probably was related to the decrease in final BW. The female mice of the 0.5 ppm group showed significantly lower mean absolute and relative heart weights than controls. The males of the same group showed significantly increased relative liver weight when compared to controls. Gross pathological effects were seen in rats that died or were sacrificed in extremis before the end of the experimental period. These lesions mainly concerned the respiratory tract and the oral cavity (e.g. crusted exudates at the anterior tip of the nares, exudates or crusts on the tongue). In some rats the stomach and the intestine were dilated with air (probably due to mouth breathing and subsequent air swallowing). Gross pathological effects were seen in 16 ppm mice and mainly concerned the external nares and the larynx. Dilation of stomach and intestine also was seen in those mice that died or were sacrificed in extremis. From 0.5 ppm, histopathology of rats mainly revealed lesions in the nasal passages and turbinates, consisting of necrosis, inflammation and squamous metaplasia. Similar lesions were seen in mice (1.6 ppm).

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13-week experiment: All rats survived treatment with up to 1000 ppb glutaraldehyde; mortality was observed in mice from 500 ppb upwards (20% in 500 ppb-females, 100% at 1000 ppb). In rats clinical signs of toxicity were seen during the first 5 weeks of treatment at 1000 ppb, including dyspnea, ruffled fur and emaciation.

In mice, clinical signs of toxicity were seen at 500 ppb (dyspnea during the first weeks of treatment) and 1000 ppb, mainly including dyspnea, emaciation and abnormal posture. Body weight gain for male rats was about 192 g at 1000 ppb versus 225 g in control (final body weight of the 1000 ppb male rats, ca. 90% of control). For the females, body weight gain was about 89 g at 500 ppb and about 88 g at 1000 ppb, versus 100 g in control (final body weight of the 500 and 1000 ppb female rats, ca. 93-96% of control). For male mice of the 125, 250 and 500 ppb groups, a concentration-related reduction in body weight gain was reported (9, 8 and 4.4 g versus ca. 12 g in control); this was also true for the females of the 250 and 500 ppb groups (8.4 and 7 g, versus ca.11 g in control).

Haematological/clinical-chemical parameters were inconspicuous at day 4. At day 24, significant increases in total counts of segmented neutrophils occurred at 125, 250 and 1000 ppb in male rats, and at 250 and 1000 ppb in females. In females, increase in total leukocyte count was reported at 1000 ppb. A statistically significant increased alanine aminotransferase activity was reported for male and female rats of the 250, 500 and 1000 ppb groups; the alkaline phosphatase activity was increased in 500 ppb and 1000 ppb females. Mild decrease in total protein was seen in males of the 125 and 1000 ppb groups, and in 1000 ppb females. Albumin was decreased in 500 ppb females whereas urea nitrogen was increased in 250 and 1000 ppb females. At the end of the 13-week experiment, no biologically relevant changes in haematology and clinical chemistry were seen; effects were sporadic and not treatment-related.

Necropsy revealed some statistically significant increases in relative weights of some organs (heart, kidney, lungs and testis, in rats), which however were small and without any toxicological significance. For the mice, increases in relative weights of some organs also were reported, which also were of no toxicological significance. Gross pathological changes were reported for mice that died during the experiment or were sacrificed in extremis. The more common findings included dilation of the stomach and intestine (probably related to dyspnea and subsequent air swallowing), and paler and smaller spleen (probably due to lymphoid depletion).

Histopathological lesions mainly affected the nasal passages and turbinates of rats and mice. In rats, the lesions mainly concerned the respiratory mucosa (nasal cavity and tips of the turbinates) and the olfactory epithelium (dorsal meatus); hyperplasia, squamous metaplasia, olfactory degeneration, squamous exfoliation (accumulation of keratin, cell debris and bacteria in the lumen of the nasal vestibule) and focal erosions were reported. Neutrophilic inflammation was seen in most 1000 ppb rats. In mice, the spectrum of lesions was similar as above and neutrophilic inflammation was seen from 62.5 ppb upwards. In addition squamous metaplasia and necrosis were seen in the larynx (1000 ppb).

		Considering sperm morphology and vaginal cytology, the treated rats of both sexes and male mice were inconspicuous. The female mice of the 250 and 500 ppb groups displayed longer estrus and diestrus phases and shorter metestrus and proestrus phases when compared to control. Histoautoradiographic evaluation of respiratory tract revealed lesions in the mucosa of the nasal passages of treated rats and mice; the lesions were in accordance with the findings of the 13-week experiment. The squamous epithelium of the nasal vestibule and to a lesser extent the respiratory epithelium of the atrioturbinate of the dorsal meatus of the treated animals showed treatment-related increase in cell replication.
5.3	**Conclusion**	The upper respiratory tract was the major target for toxicity in rats and mice subjected to repeated treatment with glutaraldehyde via vapour inhalation.
5.3.1	NO(A)EL, rats	125 ppb
5.3.2	LO(A)EL, rats	250 ppb
5.3.3	NO(A)EL, mice	< 62.5 ppb
5.3.4	LO(A)EL, mice	62.5 ppb
5.3.5	Other	None
5.3.6	Reliability	**1**
5.3.7	Deficiencies	No guideline was mentioned, however, the study was well-detailed and well-documented, and almost fulfilled the requirements of the OECD guideline 413. Moreover the study followed GLP.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 13th, 2010
Materials and Methods	3.1.2. This refers to Doc IIIA Section A2. 3.3.4.1 Vapor generation. There were 15 fresh air changes per hour (as is appropriate), not just one. 3.4 Further remarks. • Remark 3: the correct reference for the Ames test is [redacted], 1992. • Remark 4: RMS cannot confirm the statement that Reference 6.4.3_01_b ([redacted] 1994) is based on the same study as Reference 6.4.3_01_a ([redacted], 1993), although this seems to be the case.

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

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

Results and discussion

4.3 Body weight gain, 13-week experiment. For mice, the decreased bw gain was statistically significant at all dose levels for males, and at 250 and 500 ppm for females.

4.6.1 Haematology, 13-week experiment, rats. The table appears to be correct (see the last note below in Remarks), but the paragraph summarising the effects is incomplete. Effects at 24 days: Increased segmented neutrophils were seen at all dose levels for males (statistical significance at 125, 250 and 1000 ppb) and at 250 and 1000 ppb (statistically significant) for females. Increased leukocyte counts were seen at 24 days at 1000 ppb and increased counts of segmented neutrophils in males at 125, 250 and 1000 ppb and in females at 250 and 1000 ppb; decreased platelet counts in males at 1000 ppb (not statistically significant) and in females at 125 ppb and above (statistical significance at 500 and 1000 ppb). Effects at 94 days: decreased leukocyte counts in males at 500 and 1000 ppb; decreased lymphocytes in males at 500 and 1000 ppb (statistically significant) and in females at all dose levels (without statistical significance).

4.6.2 Clinical chemistry, 13-week experiment, rats. The table appears to be correct (see the last note below in Remarks), but the paragraph summarising the effects is incomplete. Effects at 24 days: increase in alanine aminotransferase in males at 250, 500 and 1000 ppb (statistically significant) and in females at all dose levels (statistical significance at 250, 500 and 1000 ppb); decreased total protein in males at 125, 250, 500 and 1000 ppb and in females at 1000 ppb; increased alkaline phosphatase in females at all dose levels (statistical significance at 500 and 1000 ppb); decreased creatine kinase in females at 125, 250, 500 and 1000 ppb (statistically significant); increased urea nitrogen in females at all dose levels (statistical significance at 250, 500 and 1000 ppb). Effects at 94 days: decreased sorbitol dehydrogenase in males at 250 and 1000 ppm; increased urea nitrogen in females at all dose levels (statistical significance at 62.5 and 125 ppb).

4.7.3 Organ weights, 13-week experiment, rats. A summary of the results is missing. RMS considers that the differences observed are not toxicologically relevant.

4.7.4 Organ weights, 13-week experiment, mice. A summary of the results is missing. There was a small increase in absolute and relative kidney weight in both males and females, and statistical significance was achieved at all dose levels in one or both of the values (absolute and/or relative weight). The differences in absolute weights were however small and the effect may be largely due to reduced bw of the treated groups. RMS considers that other differences observed are not toxicologically relevant.

4.9 Histoautoradiographic evaluation of respiratory tract.

- To be understandable, the first sentence of the last paragraph should read e.g.: "In treated rats, the treatment-related increase in cell replication was ~~almost more important~~ generally greater than in mice."
- The last sentence of the same paragraph, concerning the respiratory epithelium of the dorsal atrioturbinate, gives a slightly different message than the report which states that "... the increased replication corresponded more with the presence of squamous metaplasia of the respiratory epithelium than with inflammation".

5.2 Results and discussion.

- Fourth paragraph: The body weights of male mice are given incorrectly. See comment 4.3 above.
- Fifth paragraph: See comments 4.6.1 and 4.6.2 above.
- Sixth paragraph: See comments 4.7.3 and 4.7.4 above.

Section A6.4.3 _ 01 Repeated dose toxicity

Annex Point IIA6.3 / 6.4 / 6.5

Sub-chronic inhalation toxicity in rats and mice

Conclusion	Agree with the NOAELs set by the applicant: **Rat** LOAEL: 250 ppb (1.0 µg /l using the conversion factor 0.0041 in ppm→mg/l) NOAEL: 125 ppb (0.51 µg/l using the conversion factor 0.0041 in ppm→mg/l) Other conclusions: Determining the systemic NOAEL for an irritant is questionable, because it is difficult to make the distinction between an adverse effect and a non-adverse effect. There were some effects at all dose levels including the lowest level of 62.5 ppb (increased segmented neutrophils, increased urea nitrogen), but these changes were concluded minimal and not biologically relevant except for possibly demonstrating the exposure. The LOAEL is set based on the histopathologic lesions in the nasal passages and turbinates seen at 250 ppb. **Mouse** LOAEL: 62.5 ppb, lowest dose level (0.26 µg/l using the conversion factor 0.0041 in ppm→mg/l) NOAEL: < 62.5 ppb based on nasal lesions and increased cell replication rates in the nasal vestibule
Reliability	2
Acceptability	Acceptable
Remarks	1.1 Reference. Three publications are cited in this study summary. • Reference 6.4.3_01_a (Kari, 1993) is an NTP (National Toxicology Program, USA) report which should be considered scientifically valid although it is a non-guideline study and the results are not reported to a sufficient detail. This is considered a key study. • Reference 6.4.3._01_b (Gross 1994) is a scientific publication which is reported by the applicant to be based on the same study as Reference 6.4.3_01_a (Kari, 1993). RMS cannot confirm this. The study is scientifically valid although it needs to be considered non-GLP if GLP is not demonstrated. Reporting is insufficient for stand-alone data, but this can be considered supportive data for Reference 6.4.3_01_a (Kari, 1993). • Reference 6.4.3._01_c (Greenspan 1991) is a congress abstract with no detailed information. This reference is unacceptable and no conclusions can be made from it. RMS has considered only Reference 6.4.3_01_a (Kari, 1993) in the evaluation of this study summary. The applicant has submitted a table comparing the NTP test and the OECD 413 guideline (Table F1 added by RMS). Absence of individual animal data reduces the reliability of the study. Note that the numerical values tabulated in this study summary have not been checked in detail by the RMS.
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*

Section A6.4.3 _ 01 Repeated dose toxicity

Annex Point IIA6.3 / 6.4 / 6.5

Sub-chronic inhalation toxicity in rats and mice

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	

Tables E1 – E4: Histoautoradiography, incidence and severity of nasal lesions in treated rats and mice

TABLE E1 Incidence of Nasal Lesions in the Histoautoradiographic Study of F344/N Rats Exposed to Glutaraldehyde by Inhalation[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Nonsuppurative rhinitis						
Day 1	5	5	4[2]	5	5	5
Day 4	5	5	5	5	5	5
Week 6	5	5	5	5	5	3[3]
Week 13	0	1	1	2	3	4
Squamous exfoliation						
Day 1	0	1	0[2]	1	3	5
Day 4	0	0	0	0	3	5
Week 6	0	0	0	0	3	3[3]
Week 13	0	0	0	2	2	2
Intraepithelial neutrophils						
Day 1	0	0	0[2]	1	2	5
Day 4	0	0	0	0	5	5
Week 6	1	0	1	2	4	3[3]
Week 13	5	3	5	5	4	5
Subepithelial neutrophils						
Day 1	0	0	0[2]	3	5	5
Day 4	1	0	2	1	5	5
Week 6	2	3	2	4	5	3[3]
Week 13	5	4	5	5	5	5
Epithelial erosions						
Day 1	0	0	0[2]	1	5	5
Day 4	0	0	0	1	2	5
Week 6	0	0	0	0	4	3[3]
Week 13	1	1	0	1	1	1
Squamous metaplasia						
Day 1	0	0	0[2]	3	1	1
Day 4	0	0	0	0	5	5
Week 6	0	0	0	0	4	3[3]
Week 13	1	0	0	0	5	5
Eosinophilic droplets						
Day 1	0	0	0[2]	0	0	0
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	0	0[3]
Week 13	0	0	0	0	0	0
Olfactory degeneration						
Day 1	0	0	0[2]	0	0	1
Day 4	0	0	0	0	0	4
Week 6	0	0	0	0	0	3[3]
Week 13	0	0	0	0	0	4

TABLE E1 Incidence of Nasal Lesions in the Histoautoradiographic Study of F344/N Rats Exposed to Glutaraldehyde by Inhalation (continued)

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Female						
Nonsuppurative rhinitis						
Day 1	5	5	5	5	5	5
Day 4	5	4	5	5	5	5
Week 6	3	4	4	2	5	2
Week 13	2	3	4	5	5	5
Squamous exfoliation						
Day 1	0	0	0	2	3	4
Day 4	0	0	0	3	5	5
Week 6	0	0	0	3	2	2[4]
Week 13	0	0	0[2]	0	2	4
Intraepithelial neutrophils						
Day 1	0	0	0	0	2	4
Day 4	1	0	0	2	5	5
Week 6	0	1	0	0	2	2[4]
Week 13	1	0	1	3	2	5
Subepithelial neutrophils						
Day 1	0	0	1	1	5	5
Day 4	2	0	0	4	5	5
Week 6	1	2	1	1	5	2[4]
Week 13	2	0	1	3	4	4
Epithelial erosions						
Day 1	0	0	1	0	4	5
Day 4	0	0	0	2	3	5
Week 6	0	0	0	0	4	1[4]
Week 13	0	0	0	0	0	1
Squamous metaplasia						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	1	5	5
Week 6	0	0	0	0	3	2[4]
Week 13	0	0	0	0	3	5
Eosinophilic droplets						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	0	0[4]
Week 13	0	0	0	0	0	0
Olfactory degeneration						
Day 1	0	0	0	0	0	1
Day 4	0	0	0	0	0	1
Week 6	0	1	1	1	1	2[4]
Week 13	0	0	1	0	0	1

[1] n=5 unless otherwise noted.
[2] n=4.
[3] n=3.
[4] n=2.

TABLE E2 Incidence of Nasal Lesions in the Histoautoradiographic Study of B6C3F$_1$ Mice Exposed to Glutaraldehyde by Inhalation[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Nonsuppurative rhinitis						
Day 1	3	0	0	0	0	2
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	0	–[2]
Week 13	0	0	1	0	1	–
Squamous exfoliation						
Day 1	0	0	0	0	4	5
Day 4	0	0	0	4	2	5
Week 6	0	0	2	0	0	–
Week 13	0	0	0	3	1	–
Intraepithelial neutrophils						
Day 1	1	0	1	0	1	5
Day 4	0	0	0	1	4	5
Week 6	0	0	0	1	1	–
Week 13	0	0	1	4	5	–
Subepithelial neutrophils						
Day 1	1	0	1	1	2	5
Day 4	0	0	0	2	4	5
Week 6	0	0	0	1	4	–
Week 13	0	1	2	5	5	–
Epithelial erosions						
Day 1	0	0	0	0	1	2
Day 4	0	0	0	0	1	2
Week 6	0	0	0	0	0	–
Week 13	0	0	0	1	3	–
Squamous metaplasia						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	1	4
Week 6	0	0	0	0	2	–
Week 13	0	0	0	0	1	–
Eosinophilic droplets						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	1	0
Week 6	0	0	0	0	0	–
Week 13	0	0	0	2	5	–
Olfactory degeneration						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	1	–
Week 13	0	0	0	0	1	–

TABLE E2 Incidence of Nasal Lesions in the Histoautoradiographic Study of B6C3F$_1$ Mice Exposed to Glutaraldehyde by Inhalation (continued)

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Female						
Nonsuppurative rhinitis						
Day 1	1	0	0	1	0	0
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	0	–
Week 13	1	0	0	1	0[3]	–
Squamous exfoliation						
Day 1	0	0	0	5	5	4
Day 4	0	0	2	5	5	5
Week 6	0	0	0	2	2	–
Week 13	0	0	0	1[3]	1[3]	–
Intraepithelial neutrophils						
Day 1	0	0	0	0	0	1
Day 4	0	1	0	1	5	4
Week 6	0	1	4	5	5	–
Week 13	0	4	5	4[3]	4[3]	–
Subepithelial neutrophils						
Day 1	0	0	1	0	2	3
Day 4	0	0	0	1	5	5
Week 6	1	1	4	5	5	–
Week 13	2	5	5	5	4[3]	–
Epithelial erosions						
Day 1	0	0	0	0	0	1
Day 4	0	0	0	0	0	2
Week 6	0	0	0	0	0	–
Week 13	0	0	0	0	0[3]	–
Squamous metaplasia						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	3	–
Week 13	0	0	0	0	1[3]	–
Eosinophilic droplets						
Day 1	0	0	0	0	0	0
Day 4	0	1	0	1	0	0
Week 6	1	0	0	1	1	–
Week 13	0	4	2	5	2[3]	–
Olfactory degeneration						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	1	0	0
Week 6	0	0	0	0	0	–
Week 13	0	0	0	0	0	–

[1] n=5 unless otherwise noted.
[2] All animals died before scheduled kill.
[3] n=4.

TABLE E3 Severity of Selected Nasal Lesions in the Histoautoradiographic Study of F344/N Rats Exposed to Glutaraldehyde by Inhalation[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Intraepithelial neutrophils						
Day 1	0	0	0	0.4	0.4	1.2
Day 4	0	0	0	0	1.4	2.6
Week 6	0.2	0	0.2	0.4	0.6	3.0
Week 13	1.2	0.8	1.0	1.2	1.2	1.6
Subepithelial neutrophils						
Day 1	0	0	0	0.8	1.8	2.6
Day 4	0.2	0	0.4	0.2	1.6	3.4
Week 6	0.4	0.6	0.6	0.8	2.0	3.7
Week 13	1.0	1.0	1.2	1.6	1.4	2.0
Squamous metaplasia						
Day 1	0	0	0	0.6	0.2	0.2
Day 4	0	0	0	0	1.2	2.2
Week 6	0	0	0	0	1.6	3.3
Week 13	0.2	0	0	0	2.0	3.0
Female						
Intraepithelial neutrophils						
Day 1	0	0	0	0	0.6	1.0
Day 4	0.2	0	0	0.4	2.2	3.4
Week 6	0	0.2	0	0	0.6	3.5
Week 13	0.2	0	0.4	1.0	0.8	1.4
Subepithelial neutrophils						
Day 1	0	0	0.4	0.2	2.4	2.8
Day 4	0.4	0	0	1.4	2.8	3.0
Week 6	0.6	0.4	0.4	0.4	2.2	4.5
Week 13	0.4	0	0.8	1.0	1.8	2.0
Squamous metaplasia						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0.2	2.0	3.0
Week 6	0	0	0	0	0.6	3.5
Week 13	0	0	0	0	1.2	2.0

[1] Mean for all animals; nonlesion included as 0 in calculation of mean value. Severity scale range: 0 to 5.

TABLE E4 Severity of Selected Nasal Lesions in the Histoautoradiographic Study of B6C3F$_1$ Mice Exposed to Glutaraldehyde by Inhalation[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Intraepithelial neutrophils						
Day 1	0.2	0	0.2	0	0.2	1.0
Day 4	0	0	0	0.2	1.8	2.8
Week 6	0	0	0	0.2	0.8	–[2]
Week 13	0	0	0.2	1.6	2.6	3.0
Subepithelial neutrophils						
Day 1	0.2	0	0.2	0.2	0.4	1.6
Day 4	0	0	0	0.4	1.8	3.2
Week 6	0	0	0	0.4	1.8	–
Week 13	0	0.2	0.8	2.2	2.8	3.0
Squamous metaplasia						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	0.2	0.8
Week 6	0	0	0	0	0.4	0
Week 13	0	0	0	0	0.2	2.0
Female						
Intraepithelial neutrophils						
Day 1	0	0	0	0	0	0.4
Day 4	0	0.2	0	0.4	1.0	0.8
Week 6	0	0.4	1.6	1.8	2.2	–
Week 13	0	2.0	2.4	3.2	3.8	–
Subepithelial neutrophils						
Day 1	0	0	0.2	0	0.4	1.2
Day 4	0	0	0	0.4	1.6	2.0
Week 6	0.2	0.4	2.0	2.4	2.6	–
Week 13	0.4	2.0	2.8	3.2	2.8	–
Squamous metaplasia						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	0.8	–
Week 13	0	0	0	0	0.5	–

[1] Mean for all animals; nonlesion included as 0 in calculation of mean value. Severity scale range: 0 to 5.
[2] All animals died before scheduled kill.

Tables E5, E6: Histoautoradiography, mean cell replication in treated rats and mice

TABLE E5 Mean Cell Replication in F344/N Rats Exposed to Glutaraldehyde by Inhalation in the Histoautoradiographic Study[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Nasal vestibule[2]						
Day 1	9.36	10.91	10.44	15.73	13.44	20.85
Day 4	7.34	9.98	10.59	14.67	21.92	28.04
Week 6	6.52	8.82	6.61	12.37	15.00	15.53
Week 13	14.74	11.74	12.77	10.82	15.18	15.85
Dorsal atrioturbinate[3]						
Day 1	4.53	3.02	4.10	5.38	6.53	14.27
Day 4	4.11	3.66	3.70	5.12	4.51	21.98
Week 6	1.63	2.25	3.40	3.88	15.69	45.00
Week 13	3.30	3.65	6.71	4.37	9.71	36.29
Female						
Nasal vestibule						
Day 1	7.74[4]	8.94	11.56	11.97	14.73[4]	21.78
Day 4	12.09	7.83	10.23	14.52	23.92	37.84
Week 6	8.52	8.99	9.12	11.99	15.02	74.67
Week 13	9.08	12.04	14.04	16.29	19.78	15.85
Dorsal atrioturbinate						
Day 1	3.93[4]	1.93[5]	2.56[6]	3.05[6]	5.16[4]	20.66
Day 4	2.32	1.01	2.07	3.70	2.48	26.33
Week 6	3.14	3.07	3.46	3.83	13.63	89.18
Week 13	2.37	3.48	6.00	9.23	18.86	29.39

[1] Values are expressed as mean for five animals unless otherwise indicated; values are number of labeled cells/mm basement membrane.
[2] Lined by squamous epithelium.
[3] Normally lined by respiratory epithelium. Site of squamous metaplasia (see Table E3).
[4] n=4.
[5] n=2.
[6] n=3.

TABLE E6 Mean Cell Replication in B6C3F$_1$ Mice Exposed to Glutaraldehyde by Inhalation in the Histoautoradiographic Study[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Nasal vestibule[2]						
Day 1	4.52	4.38	3.32	4.65	7.04	13.01
Day 4	7.54	6.54	7.45	11.68	12.27	18.52
Week 6	6.38	5.87	7.03	8.35	15.35	–[3]
Week 13	5.40	5.61	7.71	11.12	17.76	–
Dorsal atrioturbinate[4]						
Day 1	1.54	2.03	1.57	0.95	2.99	2.22
Day 4	1.46	1.98	3.48	1.45	5.37	6.09
Week 6	0.31	0.93	1.13	0.85	2.68	–
Week 13	0.45	0.50	0.35	0.39	0.93	–
Female						
Nasal vestibule						
Day 1	5.82	4.58	3.92	2.52	2.89	5.56
Day 4	8.15	6.11	6.36	8.00	10.05	14.14
Week 6	6.40	8.06	11.56	14.59	21.00	–
Week 13	6.27	16.87	15.33	21.53	23.68	–
Dorsal atrioturbinate						
Day 1	0.82	0.65	0.43	1.31	1.03	1.20
Day 4	1.30	0.73	1.33	2.24	6.60	10.15
Week 6	0.80	0.59	4.03	2.66	4.41	–
Week 13	1.61	2.35	1.65	1.76	0.94	–

[1] Values are expressed as mean for five animals unless otherwise indicated; values are number of labeled cells/mm basement membrane.
[2] Lined by squamous epithelium.
[3] All animals died before scheduled kill.
[4] Normally lined by respiratory epithelium. Site of squamous metaplasia (see Table E4).

Table F1 (Comparison of the NTP test conduct with OECD TG413)

NTP Study: Kari FW (1993) NTP technical report on toxicity studies of glutaraldehyde administered by inhalation to F344/N rats and B6C3F$_1$ mice. US Department of Health and Human Services, Public Health Service, National Institutes of Health NIH, Toxicity Report Series No: 25, NIH Publication No: 93-3348 (Published), BPD ID A6.4.3_01_a				
Parameters	OECD TG 413 Requirements	Fullfilled/Not fulfilled	Deviation	RMS comment (where no comment is given, RMS agrees with the applicant)
Test animals	Rodent (preferred rat)	Yes, rat & mouse	-	-
	Strains must be of common use in laboratory	Yes ([redacted])	-	-
	Healthy animals	Yes (blood analysis for virus and Mycoplasma)	-	Results have not been presented.
	Acclimatisation at least 5 days	Yes (6-7 days)	-	-
	Young adult	Yes (6-7 weeks at test initiation)	-	-
	Initial BW variation ≤± 20%	Yes	-	-
	10/sex/group	Yes	-	-
	If necessary, high dose satellite group for reversibility testing	No, however not compulsory	-	-
Housing and environment	In groups by sex or individually	Yes (indiv. housing)	-	-
	Temperature 22±2°C	Yes (22-25.5 °C)	-	-
	Rel. Hum. 30-70%	Yes (55±15%)	-	-
	Conventional Lab.Feed.	Yes (NIH-07 Open Formula diet in pellet)	-	-
	Drinking water ad libitum	Yes	-	-
	Dynamic air changes in exposure room of 12-15/hr	Yes (15±3)	-	-
	12 hrs light/12 hrs night	Yes	-	-
Test conditions	At least 3 conc. &control & vehicle control if necessary	Yes (5 conc. tested, shame control, no vehicle control as no vehicle used)	-	-
	Highest concentration level selected as to produce toxic effect but no mortality	Rat: yes (based on 2 week-range finding)	Mice: The highest tested level was fatal to mice (100% mortality at 1000 ppb, both sexes)	-

Table F1 (Comparison of the NTP test conduct with OECD TG413) continues

Parameters	OECD TG 413 Requirements	Fullfilled/Not fulfilled	Deviation	RMS comment
Test conditions	Lowest concentration level selected as to produce no effects	Rat and mouse: yes (based on 2 week-range-finding)	-	-
	Concentration levels spaced adequately to demonstrate dose-dependency	Yes	-	-
	Low incidence of mortality in low and intermediate levels to allow meaningful evaluation of results	Yes	-	-
	Whole body exposure accepted	Yes	-	-
	Exposure period 6 hrs	Yes	-	-
	Taking into consideration time for concentration equilibration in chamber	Yes (30 minutes added to the 6 hrs exposure duration)	-	-
	Treatment during 7 days/week preferred but 5 days also accepted	Yes (5 day/week)	-	-
	Fasting during exposure (feed & water)	Yes	-	-
Test system	A dynamic inhalation system with suitable analytical concentration check system	Yes	-	-
	Monitoring of test concentration	Yes	-	-
	Concentrations maintained stable during exposure	Yes (by adjustment of driving pressure)	Remark: variation in overall uniformity of concentration was reported, with relative standard deviations ranging from 4 to 25%	The variation in concentration is great, although RMS recognises the reactive nature of GA, the effects to the study results must be considered.
	Particle seize distribution analysis	No (not relevant as vapour tested)	-	-
Examination	Clinical examination, body weight sacrifice in extremis of moribund animals, necropsy of animals sacrificed prior test ending	Yes (time points as recommended by TG)		-

Table F1 (Comparison of the NTP test conduct with OECD TG413) continues

Parameters	OECD TG 413 Requirements	Fullfilled/Not fulfilled	Deviation	
Examination	Clinical parameters (skin & fur, eyes and mucous membrane, respiratory, autonomic and CNS, somatomotor activity, behaviour)	Yes, however the kind of parameters considered was not specified in details	-	Requirement not fullfilled. The findings should have been reported by sex and concentration.
	Food consumption (weekly measurement)	Not done	-	Requirement not fullfilled.
	Ophthalmological examination	Not done		Requirement not fullfilled.
	Suggested haematological parameters to be examined: Haematocrit, haemoglobin concentration, erythrocyte count, total/differential leukocyte count, clotting potential measurement (clotting time, prothrombin time, thromboplastin time), platelet count	Yes (main parameters were considered, except for clotting)	-	-
	<u>Suggested clinical-chemical parameters to be examined</u>: calcium, phosphorus, chloride, sodium, potassium, fasting glucose, Alanine aminotransferase, aspartate aminotransferase, ornithine decarboxylase, GGT, urea nitrogen, albumen, creatinine, totoal bilirubin, total protein,	Yes (not all as suggested, but selected to cover carbohydrate metabolism, liver function and kidney function)	-	Agree with applicant. Folowing parameters were measured: sorbitol dehydrogenase, alanine aminotransferase, creatine kinase, alkaline phosphatase, concentration of total protein, albumin, globulin, urea nitrogen, creatine and total bile acids.
	Urinanalysis to be done only if indicated	Not done (no indication therefore)	-	-

Table F1 (Comparison of the NTP test conduct with OECD TG413) continues

Parameters	OECD TG 413 Requirements	Fullfilled/Not fulfilled	Deviation	
Pathology	Full gross necropsy required	Yes	-	-
	Weighing of BW & organs (especially liver, kidney, adrenal and gonads)	Yes	-	-
	Organ/Tissue preservation in adequate medium for histopathology	Yes (as recommended by TG)		-
	Full histopathology on respiratory tract & further organs in high dose and control, for gross lesions, respiratory tract and target examination of remaining dose groups required.	Yes In rat: upper respiratory tract (nasal cavity) and gross lesions were also examined in low dose groups; In mouse: all obvious targets found in the high dose group were further examined in the lower dosed groups (i.e. upper resp. tract, thymus, spleen, lymph nodes, bone marrow and epididymides)	Additional examinations done, that were not required by TG: Reproductive tissues and oestrous cycle) (including sperm morphology, vaginal cytology, oestrous cycle) Histoautoradiography of respiratory tract (nasal lesions & cell replication)	-
Data evaluation	Data summaries in tabular form	Yes (Appendix A)	-	-
	Data statistical evaluation	Yes	-	-
	Data must allow a satisfactory estimation of no-effect level	Yes	-	-
	Test report should include description of test conditions (e.g. exposure apparatus), exposure data (e.g., air flow rate, temperature, humidity, nominal and actual test concentrations), Animal data.	Yes	-	Agree with applicant.in reporting the description of test conditions. As individual animal data is not reported the requirements of test reporting are only partly fulfilled.
Conclusion: The test conduct with few exceptions that did not affect the quality and validity of the results was in accordance with almost all requirements of the OECD TG 413 adopted 1981. The study was well-documented and the findings well-described, thus we consider the present study as fully acceptable for use as key study in the BPD dossier.				See Remarks in evaluation box.

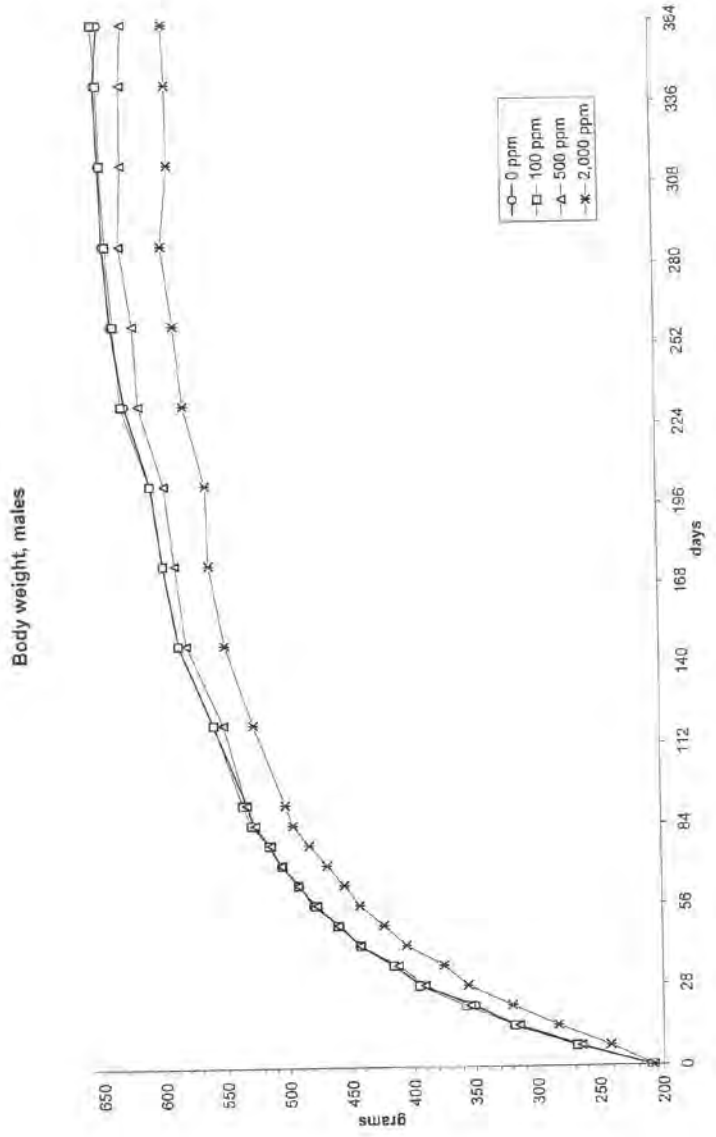
Section A6.5 _ 01 Annex Point IIA6.3 / 6.4 / 6.5		Repeated dose toxicity Chronic oral toxicity in rat	Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2002) [redacted]% Glutaraldehyde) - Chronic toxicity study in [redacted] rats - Administration in the drinking water for 12 months. [redacted] (Unpublished), BPD ID A6.05_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 452 (1981)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]% Glutaraldehyde)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Colorless-clear liquid	
3.1.2.2	Purity	[redacted]% (analysis performed by [redacted])	
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 ([redacted])	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	X
3.2.3	Source	[redacted]	
3.2.4	Sex	Male/Female	
3.2.5	Age/weight at study initiation	At test initiation, the males and females respectively were about 42 and 43 days old. The body weights of the males at test initiation ranged between 191.5 and 231.6 g (group mean: 208.3 g); for the females, the body weights	

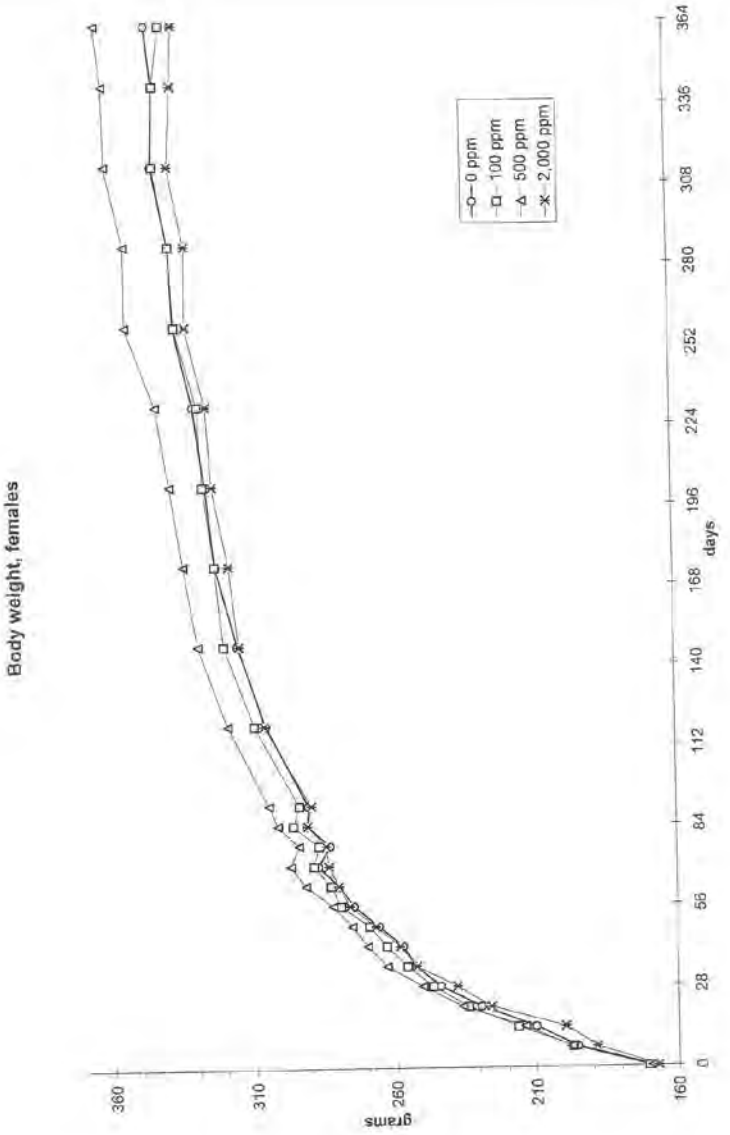
		ranged between 151.2 and 185.3 g (group mean: 169.1 g)
3.2.6	Number of animals per group	20 animals per sex and test group.
3.2.7	Control animals	Yes, untreated animals
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	12 months
3.3.2	Frequency of exposure	Daily (in drinking water)
3.3.3	Post exposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	Drinking water
3.3.4.2	Concentration	0, 100, 500 and 2000 ppm
3.3.4.3	Vehicle	Aqueous solution
3.3.4.4	Preparation of the test concentrations	An amount of drinking water was weighed and the appropriate amount of test substance needed to get the wanted test concentration was added and mixed with a stirrer. The drinking test solutions were prepared twice a week.
3.3.4.5	Controls	Control animals received drinking water without test substance
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	The rats were examined for clinical symptoms of toxicity twice a day on working days and once a day on weekends or public holidays.
3.4.1.2	Mortality	The rats were checked for mortality (dead or moribund animals) twice a day on working days and once a day on weekends or public holidays.
3.4.1.3	Open field observations	Detailled open field observations were conducted on all animals prior test starting and weekly thereafter. For this purpose the animals were transferred to a standard arena (50 x 37.5 cm, 25 cm high) and following parameters were considered: behaviour during handling, fur, skin, posture, salivation, respiration, activity/arousal level, tremors, convulsions, abnormal movements, impaired gait, lacrimation, palpebral closure, exophthalmus, feces, urine and pupillary size. When possible, the findings were ranked according to severity.
3.4.2	Body weight	The rats were weighed prior test initiation, on day 0 (test start), once a week during the first 13 weeks of treatment and thereafter at 4 week-intervals until the end of the study. The last body weight assessment was done prior necropsy. The body weight change was determined for each animal using following formula: $BW_{day\ x} - BW_{day\ 0}$ With $BW_{day\ x}$ = body weight on study day x (in g)
3.4.3	Food consumption	Food consumption was determined once a week over a period of 7 days during the first 13 weeks of treatment and thereafter at 4-week intervals until the end of the study. Food consumption was expressed as grams

		per animal and day.	
3.4.4	Food efficiency	The food efficiency (as group means) was calculated on the basis of the body weight and the food consumption using following formula: $\frac{BW_{day\ x} - BW_{day\ y}}{FC_{y-x}} \times 100$ = Food efficiency on day x BW day x = body weight on test day x; in g BW day y = body weight on test day y (last weighing date prior day x; in g) FC_{y-x} = mean food consumption (g) from day y to day x, calculated as mean daily food consumption on day x, multiplied with the number of days from day y to day x; in g.	
3.4.5	Water consumption	Water consumption was determined once a week over a period of 3 days for males and 4 days for females during the first 13 weeks of treatment and thereafter at 4-week intervals until the end of the study. Water consumption was expressed as grams per animal and day.	
3.4.6	Intake of test substance	The mean daily intake of test substance as group means was calculated according to following formula: $\frac{WTR_x \times C}{BW\ day\ x}$ = Substance intake for day x BW day x = body weight on test day x; in g WTR_x = mean daily water consumption on day x; in g C = concentration of test substance in drinking water on day x; in ppm	
3.4.7	Ophthalmoscopic examination	Respectively 3 days and 2 days prior starting the experiment, the eyes of the male and of the female rats were examined using an ophthalmoscope. On day 359 of treatment, the eyes of the males of the control group and of the 2000 ppm group were again examined for changes in the eyes; the females of these groups also were subjected to ophthalmologic examination, but on day 362 of treatment.	
3.4.8	Haematology	Blood samples were collected from all animals of each test group; they were taken from the retro-orbital venous plexus on following days of treatment: Males: day 93, 177 and 352 Females: day 89, 179 and 354 Following haematological parameters were considered: Haematocrit (HCT), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), erythrocyte count (RBC), leukocyte count (WBC), platelet count (PLT), differential blood count. Clotting analysis was performed and the prothrombin time was determined (Hepato Quick's Test).	X
3.4.9	Clinical Chemisty	Following blood chemistry parameters were considered: alanine aminotransferase, aspartate aminotransferase,alkaline phosphatase, serum gamma glutamyltransferase, sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, and magnesium.	X

3.4.10	Urinalysis	Urine examination was performed for all treated animals of each group at following time points: Males: day 99, 183 and 358 Females: day 102, 186 and 361 Urinalysis consisted of following parameters: volume, color, turbidity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity and sediment.	X
3.5	**Sacrifice and pathology**	The animals were sacrificed for the purpose of necropsy within day 365 to 367 for the males and 367 to 369 for the females	
3.5.1	Organ Weights	Following organs were subjected to weighting: whole body of the anesthetized animals, liver, kidneys, adrenals, testes, epididymides, uterus, ovaries, spleen, brain, and heart.	
3.5.2	Gross and histopathology	Organs and tissues fixed in 4% formaldehyde for purpose of gross/histopathology: Salivary glands, oesophagus, stomach, small and large intestines, liver, pancreas, brain, pituitary, sciatic nerve, spinal cord, eyes, adrenals, thyroid, parathyroid, trachea, lungs, pharynx, larynx, nose and nasal cavity, heart, aorta, lymph nodes, spleen, thymus, femoral bone marrow, lymph nodes, spleen, thymus, kidney, urinary bladder, testes, ovaries, oviduct, uterus, vagina, epididymides, prostate, seminal vesicles, female mammary gland, skin, skeletal muscle, sternum with bone marrow, femur with knee joint, extraorbital lacrimal gland. All gross lesions also were fixed. Organs subjected to light microscopical examination: For purpose of light microscopical examination the tissue section on slides were stained with hematoxylin-eosin. The organs and tissue samples listed above were examined in all animals of both the control and the 2000 ppm groups. The animals of the 100 and the 500 ppm groups were examined for following organs: stomach, small intestines, liver, lungs and kidney. All gross lesions were subjected to examination.	X
3.5.3	Other examinations	None	
3.5.4	Statistics	The statistical assessment of clinical data (food and water consumption, food efficiency, body weight, body weight change) was based on Dunnett's test (two-sided; Dunnett CW, JASA, Vol. 50: 1096-1121, 1955 and Dunnett CW, Biometrics, Vol. 20: 482-491, 1964). The statistical evaluation of the haematological and clinical-chemical parameters was mainly based on the non-parametric one-way analysis using the Kruskal-Wallis test (Siegel S, Non-parametric statistics for the behavioural sciences, McGraw-Hill New York, 1956); urinalysis parameters were assessed by pairwise comparison of each test dose group with the control group using Fisher's exact test for the hypothesis of equal proportions (Siegel S, Non-parametric statistics for the behavioural sciences, McGraw-Hill New York, 1956). Weight parameters were assessed according to the Kruskal-Wallis test, and depending on the p-value, a pairwise comparison of each dose group with the control group was performed using the Wilcoxon test for the hypothesis of equal medians (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 EPA guideline and the German Drinking Water Regulation.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Respiratory sounds were reported for 6 males and 6 females of the 2000 ppm group and were observed from day 14 to day 70 of treatment; this effect was considered to be treatment-related.
4.1.2	Mortality	No treatment-related mortalities were observed. In fact, one male of the control group and one female of the 100 ppm group died respectively on day 235 and day 273.
4.2	**Body weight gain**	Treatment-related effects on body weight and body weight changes were reported for both, males and females of the 2000 ppm group. For the males, a statistically significant decrease in body weight was observed from day 7 until the end of the experimental period; a maximum decrease of – 11.2% was reached at day 14. The body weight change for these males also was statistically significantly decreased over the whole experimental period (-43.1% on day 7, - 11.7% on day 364) For the females, body weight change was statistically significantly decreased only on day 7 and 14 (-21.6%).


Body weight, males
grams
days
0 ppm
100 ppm
500 ppm
2,000 ppm
650
600
550
500
450
400
350
300
250
200
0
28
56
84
112
140
168
196
224
252
280
308
336
364


Body weight, females
grams
360
310
260
210
160
days
0
28
56
84
112
140
168
196
224
252
280
308
336
364
0 ppm
100 ppm
500 ppm
2,000 ppm

4.3 Food consumption

Treatment-related effects on food consumption were seen at the highest tested concentration of 2000 ppm in both males and females. In fact, in males, a statistical significant decrease in food consumption was observed from the very beginning of the treatment until day 35 (up to –18.8%), as well as on days 259 and 287. For the females, a statistically relevant reduction in food consumption was evident during the 2 first weeks of treatment (-9.6%), as well as on days 147, 175, 343 and 364 of treatment. Occasional increases in food consumption were seen in the 500 ppm group, but were incidental and not treatment-related.

The main statistically relevant results for food consumption for males and females respectively are summarized below:

	Mean food consumption (g/animal/day)			
Time point (day)	Males		Females	
	Control	2000 ppm	Control	2000 ppm
7	27.8 +/- 1.8	**22.6** +/- 2.1**	21.5 +/- 1.8	**19.5** +/- 1.4**
14	31.3 +/- 2.1	**26.0** +/- 2.9**	21.5 +/- 1.9	**19.6* +/- 2.0**
21	30.6 +/- 5.3	28.6 +/- 2.6	22.6 +/- 1.7	22.3 +/- 1.7
28	32.6 +/- 2.1	**29.6** +/- 3.0**	23.1 +/- 1.8	22.8 +/- 1.8
35	32.4 +/- 2.3	**29.9** +/- 2.4**	21.8 +/- 1.7	22.2 +/- 1.7
42	31.7 +/- 2.4	30.4 +/-2.0	22.6 +/- 1.6	22.7 +/- 1.6
49	31.7 +/- 2.4	30.3 +/- 2.4	22.4 +/- 1.8	22.1 +/- 1.3
56	30.9 +/-2.1	29.4 +/- 2.1	21.9 +/- 1.6	21.8 +/- 1.4
63	31.2 +/- 2.1	30.0 +/- 2.2	21.8 +/- 1.8	21.3 +/- 1.3
70	30.6 +/- 2.3	29.4 +/- 2.1	20.9 +/- 2.1	21.5 +/- 1.3
77	30.5 +/- 2.2	29.8 +/- 2.4	21.3 +/- 1.9	20.1 +/- 1.3
84	30.1 +/- 2.0	29.3 +/- 2.2	21.2 +/- 1.2	20.7 +/- 1.3
91	29.2 +/- 2.4	28.8 +/- 2.1	19.9 +/- 1.7	19.3 +/- 2.3
119	29.2 +/- 1.8	28.4 +/- 2.3	21.3 +/- 1.5	20.8 +/- 1.3
147	28.2 +/- 2.5	27.4 +/- 2.5	21.1 +/- 1.5	**19.9* +/- 1.4**
175	28.8 +/- 2.0	27.9 +/- 2.4	21.7 +/- 1.5	**20.4** +/- 1.4**
203	28.7 +/- 2.1	27.3 +/- 2.4	21.5 +/- 1.3	20.9 +/- 1.5
231	28.6 +/- 2.6	27.2 +/- 2.1	21.5 +/- 1.6	21.1 +/- 1.3
259	29.4 +/- 2.4	**27.5* +/- 2.1**	22.0 +/- 2.1	20.9 +/- 1.7
287	29.0 +/- 2.7	**26.4** +/- 2.1**	21.5 +/- 1.7	20.5 +/- 1.6
315	28.4 +/- 2.7	26.7 +/- 2.1	22.1 +/- 2.1	20.8 +/- 2.1
343	28.6 +/- 2.2	26.4 +/- 1.9	21.6 +/- 1.8	**20.0* +/- 1.4**
364	26.3 +/- 2.4	24.9 +/- 2.4	22.5 +/- 3.2	**20.5* +/- 1.9**

*, p = 0.05; **, p = 0.01

4.4 Food efficiency

No consistent trend or dose-response relationship was recognizable;

single deviations were incidental.

4.5 Water consumption

Treatment-related effects on water consumption were evident from 500 ppm upwards. In fact, in the 2000 ppm group and for both sexes, water consumption was statistically significantly reduced over the main part of the treatment period (up to 29.5% in males on day 7; up to –34.5% in females on day 343). At 500 ppm, a statistically significant reduction in water consumption was reported for the males only over few days (up to –12.8% on day 56). The reduction in water consumption was related to the bad taste of the test substance and was therefore considered to be of no toxicological relevance.

The main statistically relevant results for water consumption for males and females respectively are summarized below:

	Mean water consumption (g/animal/day)		
Time point (day)	Males		
	Control	500 ppm	2000 ppm
7	36.7 +/- 3.3	**33.9* +/- 3.6**	**25.9** +/- 3.1**
14	39.4 +/- 4.7	**35.3*** +/- 6.4	**28.8** +/- 5.0**
21	36.8 +/- 8.6	35.6 +/- 4.5	**28.8** +/- 5.5**
28	39.1 +/- 4.5	**34.7** +/- 3.0**	**30.3** +/- 5.6**
35	39.6 +/- 3.9	36.9 +/- 4.8	**31.8** +/- 4.4**
42	37.4 +/- 5.8	35.7 +/- 3.8	**31.2** +/- 4.6**
49	37.6 +/- 4.4	34.8 +/- 4.1	**31.0** +/- 3.7**
56	37.5 +/- 5.9	**32.7* +/- 6.6**	**30.8** +/- 4.6**
63	35.7 +/- 5.0	33.8 +/- 4.0	**31.1** +/- 3.8**
70	35.1 +/- 4.9	32.9 +/- 4.7	**29.5** +/- 4.0**
77	34.8 +/- 4.9	32.3 +/- 4.5	**30.8** +/- 4.6**
84	34.0 +/- 4.1	31.1 +/- 4.0	**29.4** +/- 3.8**
91	34.0 +/- 5.0	33.4 +/- 4.4	**29.8* +/- 4.1**
119	35.0 +/- 4.5	32.3 +/- 6.0	**29.6** +/- 4.2**
147	33.5 +/- 5.2	31.6 +/- 4.2	30.0 +/- 4.5
175	32.8 +/- 4.3	30.1 +/- 4.5	**28.7** +/- 3.8**
203	30.9 +/- 5.3	30.0 +/- 4.1	**26.6** +/- 4.0**
231	30.7 +/- 4.5	30.2 +/- 4.5	28.2 +/- 3.8
259	32.0 +/- 4.2	28.0* +/- 3.4	**27.0** +/- 3.3**
287	30.7 +/- 4.1	29.3 +/- 2.7	**27.4* +/- 2.8**
315	30.7 +/- 4.4	29.1 +/- 3.2	**26.1** +/- 3.6**
343	30.0 +/- 4.9	28.0 +/- 6.3	26.6 +/- 3.6
364	31.0 +/- 5.4	28.9 +/- 4.1	**26.6* +/- 4.6**

Time	Mean water consumption (g/animal/day)

point (day)	Females	
	Control	2000 ppm
7	27.8 +/- 3.4	**23.0** +/- 2.7**
14	29.0 +/- 4.4	**20.1** +/- 3.6**
21	28.3 +/- 3.2	**23.0** +/- 2.3**
28	29.3 +/- 4.0	**23.8** +/- 2.8**
35	27.5 +/- 4.2	**22.5** +/- 2.0**
42	28.6 +/- 5.1	**22.7** +/- 2.2**
49	27.4 +/- 3.9	**22.0** +/- 2.4**
56	28.0 +/- 3.8	25.1 +/- 4.0
63	27.1 +/- 4.2	**21.8** +/- 2.8**
70	25.0 +/- 3.2	**20.5** +/- 2.6**
77	26.8 +/- 4.6	**21.6** +/- 2.3**
84	27.4 +/- 3.8	**22.6** +/- 2.6**
91	26.4 +/- 3.6	**20.9** +/- 3.2**
119	26.8 +/- 5.9	**21.7** +/- 2.7**
147	27.8 +/- 4.6	**20.5** +/- 3.3**
175	29.2 +/- 5.6	**22.9** +/- 3.6**
203	31.5 +/- 4.9	**23.1** +/- 4.5**
231	30.2 +/- 7.0	**22.3** +/- 3.7**
259	31.5 +/- 5.9	**23.4** +/- 4.6**
287	32.8 +/- 8.2	**24.1** +/- 4.9**
315	34.2 +/- 8.2	**24.0** +/- 4.5**
343	36.1 +/- 7.5	**23.6** +/- 4.6**
364	37.8 +/- 9.1	**24.9** +/- 4.3**

*, p = 0.05; **, p = 0.01

4.6 Compound intake

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

Test concentration in drinking water (ppm)	Mean daily intake of test substance (mg/kg bw/day)	
	Males	Females
100	6.4	9.6
500	30.5	46.0
2000	116.6	153.2

4.7 Ophtalmoscopic examination

No treatment-related effects were seen.

4.8 Blood analysis

4.8.1 Haematology

In males, a statistically significant decrease in red blood cell count (RBC) was observed at 500 and at 2000 ppm on day 93 of treatment; following values were reported:

Day 93 of treatment	
Test Dose	RBC (Tera/l)
0 ppm	8.66 +/- 0.36
100 ppm	8.54 +/- 0.34
500 ppm	**8.26* +/- 0.49**
2000 ppm	**8.22** +/- 0.33**

*, p<= 0.05; **, p<=0.02; ***, p<=0.002

All other haematological parameters remained inconspicuous.

In females, a statistically significant decrease in red blood cell count, hemoglobin concentration (HGB) and hematocrit value (HCT) was observed at 2000 ppm on day 89 of treatment, with following values: X

Day 89 of treatment			
Test Dose	RBC (Tera/l)	HGB (mmol/l)	HCT (l/l)
0 ppm	8.20 +/- 0.37	9.3 +/- 0.4	0.439 +/- 0.026
100 ppm	8.23 +/- 0.32	9.1 +/- 0.2	0.429 +/- 0.014
500 ppm	8.04 +/- 0.29	**8.8* +/- 0.3**	0.419 +/- 0.016
2000 ppm	**7.76** +/- 0.37**	**8.6** +/- 0.5**	**0.408** +/- 0.017**

*, p<= 0.05; **, p<=0.02; ***, p<=0.002

As shown in table above, haemoglobin concentration also was statistically significantly decreased at 500 ppm. All other haematological parameters remained inconspicuous.

4.8.2 Clinical chemistry

In males, a statistically significant decrease in total protein (TPROT) and in albumin level (ALB) was observed on day 93 of treatment at 2000 ppm. A statistically significant decrease in total bilirubin (TBIL) was reported for the males of the 2000 ppm group on day 93 and 352.

Day 93 of treatment			
Test Dose	TBIL (mymol/l)	TPROT (g/l)	ALB (g/l)
0 ppm	2.51 +/- 0.33	69.14 +/- 3.05	31.98 +/- 1.29
100 ppm	2.38 +/- 0.35	70.44 +/- 4.85	32.22 +/- 1.31
500 ppm	2.51 +/- 0.47	68.99 +/- 2.46	32.13 +/- 1.05
2000 ppm	**2.07 +/- 0.49**	**65.67** +/- 2.42**	**30.65* +/- 1.16**

X

*, p<= 0.05; **, p<=0.02; ***, p<=0.002

Day 352 of treatment	
Test Dose	TBIL (mymol/l)
0 ppm	3.49 +/- 0.61

100 ppm	3.43 +/- 1.39
500 ppm	3.25 +/- 0.50
2000 ppm	**2.72*** +/- 0.40**

*, p<= 0.05; **, p<=0.02; ***, p<=0.002

For sodium and urea values were measured, which were statistically significantly different from control. In fact, for sodium 147.3 +/- 1.7 mmol/l was reported for the females of the 100 ppm group on day 179, versus 145.2 +/- 1.7 for control and 145.7 +/- 2.8 for the 500 ppm group. Urea in the females of the 2000 ppm group on day 354 had a value of 6.82 +/- 0.64 mmol/l, versus 5.91 +/- 0.60 mmol/l in control. In fact, these differences were incidental and/or without any dose response relationship. Therefore the findings referring to sodium and urea measurements were considered to be of no toxicological relevance.

4.8.3	Urinalysis	Urinalysis revealed increased erythrocytes in the urine sediments of the 2000 ppm males on day 99 of treatment. In 2000 ppm females, increased amounts of blood were found in the urine samples on day 102. The remaining urinary parameters were inconspicuous for both, males and females.	X
4.9	**Sacrifice and pathology**		
4.9.1	Organ weights	The mean final body weight of the males of the 2000 ppm group was found to be statistically significantly decreased compared to control (558.27 +/- 43.735 g versus 608.947 +/- 61.55 g for control). The mean absolute weights of the considered organs in the treated rats were not significantly different from control value. A significant increase in relative brain weight was reported for the males of the 2000 ppm group (0.415 +/- 0.024 % versus 0.383 +/- 0.037% for control); this however was related to the decreased final body weight reported for this group.	
4.9.2	Gross and histopathology	<u>Following relevant gross lesions were reported</u>: Thickening of the duodenal wall was reported for 4 of 20 males of the 2000 ppm group; no such thickening was seen in the females. Erosion and ulcer of the glandular stomach were seen in all groups but were clearly more frequent at the highest tested does of 2000 ppm. In fact, erosion/ ulcer of the glandular stomach was seen in one male and one female of the control group, one male and one female of the 100 ppm group, and in one male and one female of the 500 ppm group. In contrast at 2000 ppm, 6 males and 5 females displayed this lesion. An enlargement of the pituitary was reported for the females only, at following frequencies: one case in the control group, one case in the 100 ppm group, 3 cases in the 500 ppm group and 4 cases in the 2000 ppm group. This gross lesion could not be complemented with histopathological findings. Cysts were seen in the spleen of 10 male and 9 female control animals, in 5 males and 9 females of the 100 ppm group, in 6 males and 8 females of the 500 ppm group, and in 3 males and 7 females of the 2000 ppm group. <u>Main histopathological findings</u>: The light microscopical investigations revealed small focal or multifocal erosions in the gastric mucosa, which corresponded to the gross lesions found in the glandular stomach and defined as erosion/ulcer. The incidence of such findings was clearly increased in both males and females of the 2000 ppm (respectively 5and 4 cases). Thickening of the	X

duodenal mucosa also was confirmed histologically; in fact a correlation with the corresponding gross lesion only was verifiable in two cases. This lesion was therefore considered to be incidental. The light microscopical examination of the liver revealed a slight increase in clear cell foci in males only (7 cases in the 2000 ppm group versus 7 cases in each of control, the 100 ppm and the 500 ppm group). Due to this increase, a slight increase in total cellular alterations could be reported for the males (13 cases in the 2000 ppm group versus 8 cases in the control group, 7 cases in the 100 ppm group and 8 cases in the 500 ppm group). A relationship between the increase in clear cell foci and the treatment could not be completely ruled out. The histopathological examination of the larynx of one male of the 2000 ppm group revealed a slight diffuse squamous metaplasia of the epithelium. Because of the reporting of a similar finding within a carcinogenicity study conducted by [redacted]) with the same test substance, this effect was considered to be treatment-related.

4.10 Other

Few neoplasms were detected which were either single occurrences or were similarly distributed between treated and control groups. No treatment-relationship was recognizable. No biologically significant differences in number of tumor bearing animals, and in number of tumors were evident between control and treated groups.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to look for the toxicity potential of glutaraldehyde when administered orally via drinking water to rats over a period of 12 months.

Test substance: [redacted]% Glutaraldehyde), batch No: [redacted]% (analysis performed by [redacted]), 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.

The test substance was administered over a period of 12 months to groups of 40 rats (20/sex) via drinking water at following concentrations: 0, 100, 500 and 2000 ppm. The animals were checked for mortality, clinical symptoms, body weight, food consumption and efficiency and water consumption. Ophthalmological examinations were conducted. Blood samples were collected after 3, 6 and 12 months for the evaluation of a series of haematological and clinical-chemical parameters; urinalysis also was performed. Following necropsy, the absolute and relative weights of a series of organs were assessed, and gross pathological s well as histopathological examinations were done. The statistical assessment of the findings mainly was based on Dunnett's test, the Kruskal-Wallis test and Fisher's exact test.

5.2 Results and discussion

The mean daily intake of test substance via the drinking water was as follows:

Test concentration	100 ppm	500 ppm	2000 ppm
Males	6.4 mg/kg bw/day	30.5 mg/kg bw/day	116.6 mg/kg bw/day
Females	9.6 mg/kg bw/day	46.0 mg/kg bw/day	153.2 mg/kg bw/day

No treatment-related mortalities were observed. Following effects were reported at a test concentration of 2000 ppm, which were considered to be treatment-related: respiratory sounds (both sexes), decrease in body weight (males, up to –11.2%), decrease in body weight change (both sexes, respectively up to -43.1% for males and up to -21.6% for females), decrease in food consumption (both sexes, up to –18.8% for males and up to -9.6% for females), reduced water consumption (both sexes, up to 29.5% for males and up to –34.5% for females), lesions within the glandular stomach (both sexes showed erosion/ulceration of the glandular stomach), increased incidence of clear cell foci in the liver (males), single case of slight diffuse squamous metaplasia in the epithelium of the larynx (male).

The reduction in water consumption also was observed in males of the 500 ppm group; this effect was considered to be due to the bad taste of the test substance and was therefore considered to be of no toxicological relevance. No treatment-related effects were seen at 100 ppm.

Some changes were reported for the haematological and clinical-chemical parameters as well as for the urinalysis. These changes were marginal in nature and transient and were therefore not considered as treatment-related.

The single case of squamous metaplasia reported above was seen as a relevant effect because of the reporting of a similar finding within a carcinogenicity study conducted by [redacted]) with the same test substance (2000 ppm, 12 months).

5.3 Conclusion

5.3.1 LO(A)EL — 2000 ppm (corresponding to respectively 116.6 mg/kg bw/day for male and 153.2 mg/kg bw/day for female rats).

5.3.2 NO(A)EL — 500 ppm (corresponding to respectively 30.5 mg/kg bw/day for male and 46 mg/kg bw/day for female rats).

5.3.3 Other — None

5.3.4 Reliability — **1**

5.3.5 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	September 6th, 2010

Section A6.5 _ 01 **Annex Point IIA6.3 / 6.4 / 6.5**	**Repeated dose toxicity** **Chronic oral toxicity in rat**

Materials and Methods	3.1.2 This refers to Doc IIIA Section A2. 3.2.2 Strain. The strain used is referred to as [redacted] 3.4.8 Haematology. Haematology was not performed to all animals but to 10 animals/sex/dose, which is according to the guideline. 3.4.9 Clinical chemistry. Clinical chemistry was not performed to all animals but to 10 animals/sex/dose, which is according to the guideline. 3.4.10 Urinalysis. Urinalysis was not performed to all animals but to 10 animals/sex/dose, which is according to the guideline. 3.5.2 Gross and histopathology. The extraorbital lacrimal gland was not examined.
Results and discussion	4.8.2 Clinical chemistry. • Total bilirubin was reduced in the high dose group males at all time points, although on day 177 the change was not statistically significant. The effect was not seen in other dose groups. • The total protein and albumin levels were decreased in the high dose group males at all time points, although statistical significance was achieved on day 93 only. • The urea levels were increased on days 179 and 354 in females of the two highest dose groups, with apparent dose dependency. The values on day 179 were 6.03, 6.12, 6.43 and 6.75 and on day 354 they were 5.91, 5.89, 6.35 and 6.82 (for 0, 100, 500 and 2000 ppm, respectively). It is possible that the increased blood urea results from reabsorbed nitrogen compounds from blood as a consequence of bleeding of the ulcers in the glandular stomach. 4.8.3 Urinalysis. It should be mentioned that there were 5 cases of red colour in the urine in the high dose group males (1 case in the control group and low dose group, 0 in mid-dose group), with mean onset on day 75. 4.9.2 Gross and histopathology. • Macroscopic findings: additionally, cystic degeneration in the testes was reported with incidences of 2, 0, 5 and 5 for 0, 100, 500 and 2000 ppm, respectively. This finding is considered treatment related. • The incidence of clear cell foci in the liver is reported incorrectly. The correct numbers are: "~~7~~ **12** cases in the 2000 ppm group versus 7 cases in each of control, the 100 ppm and the 500 ppm group".
Conclusion	LO(A)EL: • 500 ppm for males (corresponding to 15.3 mg GA/kg bw/day), based on diffuse degeneration in the testes. • 2000 ppm for females (corresponding to 76.6 mg GA/kg bw/day), based on focal or multifocal erosion and ulcers in the glandular stomach, respiratory sounds, and changes in haematological and clinical chemistry parameters. NO(A)EL: • 100 ppm for males (corresponding to 3.2 mg GA/kg bw/day) • 500 ppm for females (corresponding to 23.0 mg GA/kg bw/day). The slight changes in haematology and clinical chemistry are not deemed to constitute or demonstrate an adverse effect.
Reliability	1
Acceptability	Acceptable

Section A6.5 _ 01 **Annex Point** **IIA6.3 / 6.4 / 6.5**	**Repeated dose toxicity** **Chronic oral toxicity in rat**

Remarks	Please note that the tabulated numerical data given in this study summary has not been checked in detail by the RMS.
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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 50.0 - 51.3% (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	Male/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.
3.2.7	Control animals	Yes, untreated animals
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	104 weeks
3.3.2	Frequency of exposure	Daily (in drinking water)
3.3.3	Post exposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	Drinking water
3.3.4.2	Concentration	0, 50, 250 and 1000 ppm
3.3.4.3	Vehicle	Aqueous solution
3.3.4.4	Preparation of the test concentrations	A premix of 10000 ppm GA in water was prepared and served for the preparation of the dosing solutions by dilution; the dosing solutions were prepared weekly. The concentrations were verified for all solutions for the first 4 weeks prior test starting; thereafter, at least one sample from each test solution was verified for test substance concentration every 4 weeks. The homogeneity of the solutions was tested and confirmed by removing 3 samples from the top, the middle and the bottom of each test solution. GA in aqueous solutions was analysed by means of gas chromatography (GC, equipped with a flame ionization detector).
3.3.4.5	Controls	Control animals received drinking water without test substance
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	The rats were examined for clinical symptoms of toxicity at least twice a day; additional detailed clinical examinations were conducted once a week. Palpation for masses was started from week 27 of treatment.
3.4.1.2	Mortality	The rats were checked for mortality at least twice a day.
3.4.2	Body weight	The rats were weighed prior test initiation, weekly during the test period, and immediately prior sacrifice.
3.4.3	Food consumption	Food consumption was determined weekly until week 13 of treatment; thereafter food consumption was determined on alternate weeks.
3.4.4	Water consumption	Water consumption was determined weekly until week 13 of treatment; thereafter water consumption was determined on alternate weeks.

3.4.5	Intake of test substance	The mean daily intake of test substance as group means was determined on the basis of water consumption.
3.4.6	Ophthalmoscopic examination	The eyes of the animals were examined prior test starting and thereafter, after week 52, 78 and 104 of treatment.
3.4.7	Haematology	For haematology and clinical chemistry , blood samples were collected from 20 fasted animals per sex and test group; they were taken from the retro-orbital venous plexus on following weeks of treatment: 12, 26, 52, 78 and 104. Following haematological parameters were considered: 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.
3.4.8	Clinical Chemistry	Following blood chemistry parameters were considered: 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.
3.4.9	Urinalysis	For urine sampling, ten animals per sex and group were placed in metabolism cages and urine was collected over 24 hours. Urine sampling was conducted on week 12, 25, 51, 77 and 103 of treatment. Urinalysis consisted of following parameters: volume, color, microscopic elements, osmolality, pH, protein, glucose, ketones, urobilinogen, bilirubin and blood.
3.5	**Sacrifice and 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.
3.5.1	Organ Weights	Liver, kidneys, brain, heart, adrenals and testes were removed for weighing.
3.5.2	Gross and histopathology	In addition to gross pathology, a complete set of standard tissues was collected and fixed in 10% buffered formalin for further histopathological examination.
3.5.3	Other examinations	Particular attention was given to the occurrence and incidence of tumors (see 3.6).
3.5.4	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.
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 oncogenic aspect of the study were reported in a separate robust summary.

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs

An increased incidence of urine stains was reported for the males and females of the low (50 ppm) and high (1000 ppm) groups. The treated females of all groups showed a tendency to increased emaciation, labored breathing, body pallor and yellow cutis; however, these increases were low compared to controls, and no clear dose-response relationship was evident.

4.1.2 Mortality

No significant differences in mortality and survival were seen between control and treated animals of both sexes.

	Drinking water concentrations of GA (ppm)			
	0	50	250	1000
Males				
Total number of rats	100	100	100	100
Number sacrificed	55	71	81	70
Number found dead	9	13	13	12
Number sacrificed moribund	16	16	15	18
Mortality incidence (%)[a]	19	29	29	30
Number of procedural deaths	0	0	0	0
Mean survival time (days)	699	693	697	689
Females				
Total number of rats	100	100	100	100
Number sacrificed	81	68	71	76
Number found dead	7	15	8	[illegible]
Number sacrificed moribund	[illegible]	21	10	[illegible]
Mortality incidence (%)[a]	19	31	18	[illegible]
Number of procedural deaths	0	1	1	1
Mean survival time (days)	720	684	701	711

[a] Estimated (found dead).

4.2 Body weight gain

Treatment-related effects on 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. The findings can be summarized as follows:

<u>Males of the 50 ppm group</u>: no treatment-related effects on body weight and body weight gain.

<u>Males of the 250 ppm group</u>: 2 to 5% decrease in mean absolute body weight, from week 6 of treatment to test ending; 3 to 8% decrease in mean body weight gain throughout the test period.

<u>Males of the 1000 ppm group</u>: 3 to 10% decrease in mean absolute body weight throughout the test period, increasing with time; 8 to 14% decrease in mean body weight gain throughout the test period

<u>Females of the 50 ppm and the 250 ppm groups</u>: no treatment-related effects on body weight and body weight gain.

<u>Females of the 1000 ppm group</u>: 2 to 9% decrease in mean absolute body weight from week 2 of treatment to test ending; 3 to 13% decrease in mean body weight gain throughout the test period.

4.3 Food consumption

Treatment-related effects on food consumption were seen at the highest tested concentration of 1000 ppm for both males and females; in fact, a statistically significant reduction in food consumption reaching 12% throughout the test period was reported. Slight or occasional reductions in food consumption seen in the 250 and the 50 ppm groups only sporadically were of statistical significance.

4.4 Water consumption

Treatment-related effects on water consumption were evident in the 1000 ppm group for both, males and females. In fact, a decrease in water consumption of 9 to 25% and 21 to 37% throughout the test period was reported for the males and females respectively. For the 250 ppm group, a decrease in water consumption up to 16 and to 22% was reported for males and females respectively. In the 50 ppm group, water consumption of the males and females also was slightly below control values, but showed no statistical significance.

4.5 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)

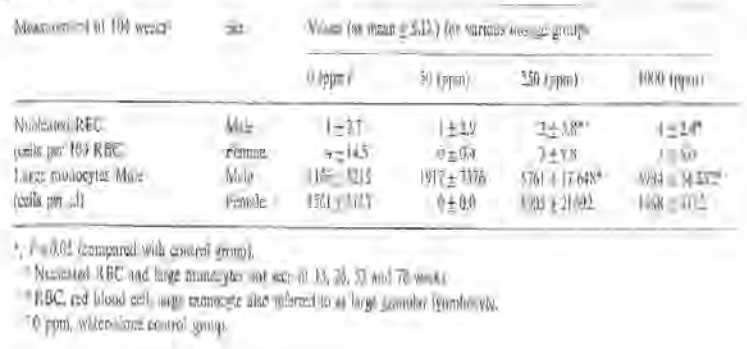
4.6 Ophtalmoscopic examination

No treatment-related effects were reported.

4.7 Blood analysis

4.7.1 Haematology

The main haematological finding seen at the end of the test period (104 weeks) was the appearance of nucleated erythrocytes and of large monocytes (large granular lymphocytes) in all treated groups. The increased incidence of nucleated erythrocytes and of large monocytes was statistically significant for the males of the 250 and the 1000 ppm groups.



4.7.2 Clinical chemistry

Decreases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamate deshydrogenase (GD) was observed from week 13; For ALT and AST, the decreases further appeared dose-

related in the 250 and the 1000 ppm. Statistically significant decreases were observed in the 1000 ppm group at weeks 13 and 52 of treatment. Furthermore, ALT and GD in females were decreased in all treated groups at week 13.

Measurement[a]	Sample time (weeks)	Sex	Values (as mean ± S.D.) for various dosage groups			
			0 ppm[b]	50 ppm	250 ppm	1000ppm
AST(IU/l)	13	Male	81 ±37.9	69 ± 15.7	69 ± 15.6	73 ± 16.4
		Female	69 ± 15.3	56 ± 6.6*	61 ± 12.9	54 ± 8.6*
	26	Male	106 ± 27.6	96 ± 17.5	102 ± 20.1	98 ± 24.3
		Female	68 ± 13.4	70 ± 14.4	66 ± 15.0	60 ± 7.4
	52	Male	109 ± 24.0	111 ± 24.0	104 ± 15.8	86 ± 11.9*
		Female	81 ± 14.8	73 ± 12.3	71 ± 11.4	62 ± 12.1**
	78	Male	58 ± 10.3	57 ± 9.8	54 ± 10.0	49 ± 7.5
		Female	63 ± 13.1	61 ± 12.1	69 ± 31.8	56 ± 14.2
	104	Male	144 ± 251.8	70 ± 40.6	97 ± 85.9	141 ± 217.1
		Female	162 ± 231.9	74 ± 17.0	270 ± 457.2	91 ± 40.0
ALT(IU/l)	13	Male	31 ± 13.3	30 ± 8.1	30 ± 8.1	29 ± 6.7
		Female	29 ± 7.5	22 ± 5.7*	23 ± 5.5*	20 ± 3.6*
	26	Male	46 ± 11.8	41 ± 5.9	44 ± 7.1	37 ± 7.3
		Female	29 ± 6.0	23 ± 11.0	28 ± 9.5	26 ± 6.8
	52	Male	50 ± 8.9	53 ± 8.5	46 ± 6.1	37 ± 4.9**
		Female	41 ± 8.4	35 ± 9.2	34 ± 7.9	29 ± 7.9**
	78	Male	29 ± 4.8	30 ± 4.3	28 ± 5.2	24 ± 3.9
		Female	30 ± 8.6	30 ± 4.4	27 ± 8.1	24 ± 6.4
	104	Male	61 ± 71.4	35 ± 9.9	46 ± 21.0	43 ± 30.6
		Female	69 ± 72.7	47 ± 14.4	86 ± 122.7	47 ± 21.3
GD(IU/l)	13	Male	22 ± 14.4	19 ± 8.5	18 ± 6.6	23 ± 7.9
		Female	33 ± 14.2	24 ± 15.2*	20 ± 8.8*	14 ± 5.0*
	26	Male	25 ± 9.9	22 ± 5.3	23 ± 6.8	20 ± 6.3
		Female	20 ± 9.8	30 ± 18.7	24 ± 17.7	16 ± 5.6
	52	Male	41 ± 16.4	42 ± 15.1	34 ± 6.4	28 ± 5.7
		Female	41 ± 15.9	27 ± 14.3	30 ± 10.2	19 ± 5.7**
	78	Male	22 ± 8.1	23 ± 5.7	18 ± 15.3	18 ± 4.8
		Female	33 ± 14.0	30 ± 7.8	38 ± 14.7	25 ± 12.5
	104	Male	24 ± 31.9	12 ± 5.6	17 ± 6.6	42 ± 96.0
		Female	48 ± 40.7	24 ± 7.3	68 ± 101.9	23 ± 11.1

*, $P<0.05$ (compared with controls), **, $P<0.01$ (compared with controls).
[a] AST, aspartate transaminase; ALT, alanine amino transaminase; GD, glutamate dehydrogenase.
[b] 0 ppm is water-alone control group.

4.7.3 Urinalysis

Urinalysis revealed a dose-related decrease in urine volume throughout the test period for the 250 and the 1000 ppm groups; the decreases were of statistical significance for all except the last week of treatment in the 1000 ppm group whereas in the 250 ppm group, statistically significant decreases in volume were reported for week 12, 25 and 51 of treatment. 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 and was statistically significant thoughout the test period for the 1000 ppm group, and on weeks 12, 25 and 51 for the 250 ppm group. A slight decrease in pH was reported for the urine of the 1000 ppm animals.

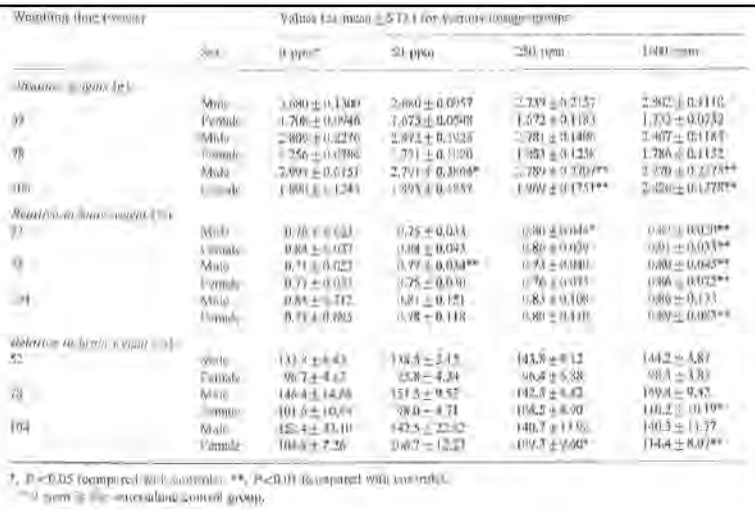
Measurement	Sample time (week)	Sex	Values (as mean ± S.D.) for various dosage groups			
			0 ppm*	50 ppm	250 ppm	1000 ppm
Urine volume (ml)	12	Male	8.8 ± 1.40	8.9 ± 0.66	6.8 ± 0.63**	5.9 ± 0.86**
		Female	9.9 ± 2.21	8.9 ± 1.86	7.2 ± 1.32**	5.8 ± 1.11**
	52	Male	8.4 ± 0.98	9.8 ± 1.26*	7.1 ± 0.69**	6.4 ± 1.05**
		Female	9.5 ± 1.97	8.8 ± 2.70	8.0 ± 1.56*	5.3 ± 0.95**
	78	Male	11.1 ± 1.76	10.8 ± 1.81	8.4 ± 1.10**	8.1 ± 0.72**
		Female	11.3 ± 3.07	11.6 ± 1.59	9.1 ± 1.52	7.3 ± 1.16**
	91	Male	13.3 ± 3.27	15.0 ± 4.53	10.8 ± 2.75	7.9 ± 1.22**
		Female	12.6 ± 3.70	13.0 ± 4.12	11.1 ± 2.30	8.3 ± 2.02**
	102	Male	16.8 ± 7.01	16.6 ± 6.52	12.1 ± 1.66	10.1 ± 5.81
		Female	11.2 ± 4.79	14.6 ± 7.17	13.4 ± 5.63	9.8 ± 1.55
Osmolality (mOsm/kg)	12	Male	1967 ± 293	1872 ± 294	2355 ± 318**	2669 ± 350**
		Female	1685 ± 340	1748 ± 307*	1985 ± 244**	2598 ± 295**
	52	Male	2474 ± 208	2309 ± 219	2708 ± 168**	2827 ± 172**
		Female	1842 ± 258	2017 ± 356	2236 ± 208**	2764 ± 234**
	78	Male	1949 ± 264	1977 ± 233	2290 ± 160**	2473 ± 139**
		Female	1690 ± 273	1712 ± 188	1984 ± 235**	2438 ± 238**
	91	Male	1752 ± 376	1501 ± 378	1965 ± 346	2416 ± 332**
		Female	1580 ± 296	1570 ± 293	1892 ± 318	2226 ± 330**
	103	Male	1490 ± 403	1257 ± 301	1529 ± 185	1811 ± 463**
		Female	1434 ± 319	1345 ± 453	1409 ± 361	1795 ± 287
pH	12	Male	7.2 ± 0.42	7.5 ± 0.53	7.1 ± 0.33	7.5 ± 0.57
		Female	7.3 ± 0.48	7.1 ± 0.32	7.2 ± 0.42	7.1 ± 0.57
	52	Male	6.8 ± 0.42	6.7 ± 0.48	6.8 ± 0.42	6.8 ± 0.42
		Female	6.6 ± 0.52	6.6 ± 0.52	6.7 ± 0.48	6.2 ± 0.42
	78	Male	7.0 ± 0.00	7.0 ± 0.00	7.0 ± 0.00	6.9 ± 0.32
		Female	6.7 ± 0.26	6.9 ± 0.32	6.8 ± 0.26	6.7 ± 0.26
	92	Male	6.9 ± 0.12	6.9 ± 0.16	6.7 ± 0.26*	6.7 ± 0.26*
		Female	6.8 ± 0.26	6.8 ± 0.26	6.7 ± 0.34	6.6 ± 0.28
	103	Male	6.7 ± 0.34	6.7 ± 0.42	6.6 ± 0.16	6.3 ± 0.34
		Female	6.4 ± 0.39	6.40 ± 21	6.3 ± 0.41	6.4 ± 0.28

*, $P<0.05$ (compared with controls), **, $P<0.01$ (compared with controls)
*0 ppm is water-alone control group.

4.8 Sacrifice and pathology

4.8.1 Organ weights

Treatment-related changes in absolute and relative weight were reported for the kidney. In fact, absolute kidney weights were increased, especially in the animals of the 1000 ppm group which were sacrificed after 52 and after 78 weeks of treatment. At sacrifice time point 104 weeks, the absolute kidney weights of the treated males of all groups were decreased compared to control; in contrast, for the treated females, an significant increase in absolute kidney weights was reported for the 250 and the 1000 ppm groups; in the 50 ppm group, the absolute kidney weight of the females only was slightly above control. The relative kidney weight of treated males was significantly increased in the 1000 ppm group except for the last treatment week (i.e.week 104); in the 250 ppm group, the relative kidney weight of the males was significantly increased at sacrifice time point 52 weeks whereas in the 50 ppm group, a significant increase in relative kidney weight was seen at sacrifice time point 78 weeks. For the treated females, relative kidney weight was significantly increased in the 1000 ppm group at all sacrifice time point; in the remaining test groups (50 and 250 ppm), relative kidney weight was within control range.



Weighting (test system)		Values (i.e. mean ± S.D.) for various dosage groups			
	Sex	0 ppm*	50 ppm	250 ppm	1000 ppm
Absolute organ (g)					
52	Male	3.080 ± 0.1300	2.980 ± 0.0957	2.739 ± 0.2157	2.862 ± 0.4110
	Female	1.706 ± 0.0946	1.675 ± 0.0598	1.572 ± 0.1183	1.770 ± 0.0732
78	Male	2.909 ± 0.2276	2.893 ± 0.1928	2.781 ± 0.1486	2.407 ± 0.1187
	Female	1.756 ± 0.0786	1.791 ± 0.1020	1.683 ± 0.1238	1.788 ± 0.1152
104	Male	2.999 ± 0.0151	2.791 ± 0.3068*	2.789 ± 0.2207**	2.370 ± 0.2278**
	Female	1.891 ± 0.1243	1.893 ± 0.0859	1.969 ± 0.1751**	2.026 ± 0.1728**
Relative to body weight (%)					
52	Male	0.70 ± 0.033	0.75 ± 0.033	0.80 ± 0.0484*	0.87 ± 0.0830**
	Female	0.84 ± 0.037	1.00 ± 0.043	0.80 ± 0.0429	0.91 ± 0.0353**
78	Male	0.71 ± 0.025	0.77 ± 0.038**	0.83 ± 0.0401	0.80 ± 0.0455**
	Female	0.77 ± 0.033	0.95 ± 0.070	0.76 ± 0.0455	0.86 ± 0.0525**
104	Male	0.84 ± 0.012	0.81 ± 0.151	0.83 ± 0.108	0.86 ± 0.133
	Female	0.73 ± 0.085	0.78 ± 0.118	0.80 ± 0.110	0.89 ± 0.085**
Relative to brain weight (x)					
52	Male	131.9 ± 6.43	130.5 ± 3.15	143.9 ± 9.12	144.2 ± 5.87
	Female	90.7 ± 4.67	85.8 ± 4.24	96.5 ± 5.38	98.5 ± 3.83
78	Male	146.4 ± 14.86	151.5 ± 9.52	142.3 ± 6.42	139.4 ± 9.42
	Female	101.6 ± 10.64	98.0 ± 4.71	106.2 ± 8.00	101.2 ± 10.19*
104	Male	152.4 ± 13.10	142.5 ± 22.62	140.7 ± 11.96	140.3 ± 11.77
	Female	108.6 ± 7.26	106.7 ± 12.23	109.3 ± 9.60*	114.4 ± 8.07**

*, P<0.05 (compared with controls); **, P<0.01 (compared with controls).
*) term in the recruitment control group.

4.8.2 Gross and histopathology

Gastric irritation was reported as main gross pathological 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 primarly 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.

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.

Dose group (ppm):	0 (Control)		50		250		1000	
Sex:	M	F	M	F	M	F	M	F
Incidence:								
Organ/Finding								
Bone marrow:								
Hyperplasia	1/58	2/62		2/63*		1/62*	6/61*	9/60
Kidney:								
Tubular pigmentation	4/58	2/62				8/62*	13/61*	41/60*

[illegible]
* $P<0.05$
* $P<0.01$

4.9 Other

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, mainly occurred in animals that died and in animals sacrificed at test ending (i.e. after 104 weeks). The finding was discussed in section A 6.7 (see robust summary A6.7.02).

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] ats over a period of 104 weeks; data referring to the oncogenic aspect of the study were reported in a separate robust summary (see A6.7.02).

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 s well as histopathological examinations were done. 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 using Fisher´s Exact test.

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

5.2 Results and discussion

Neither treatment-related mortatilies nor treatment-related clinical symptoms of toxicity were reported. Main effects were observed in the 250 and the 1000 ppm groups and included:

1. Reduction in body weight and body weight gain: males of the 250 ppm group, males and females of the 1000 ppm group.
2. Reduction in food consumption: males and females of the 1000 ppm group.
3. Reduction in water consumption: males and females of the 250 and the 1000 ppm group
4. Increased statistically significant incidence of nucleated erythrocytes and of large monocytes: males of the 250 and the 1000 ppm group.
5. Decreases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamate deshydrogenase (GD) from week 13: For ALT and AST, the decreases further appeared dose-related in the 250 and the 1000 ppm. Statistically significant decreases were observed in the 1000 ppm group at weeks 13 and 52 of treatment. Furthermore, ALT and GD in females were decreased in all treated groups at week 13.
6. Dose-related decrease in urine volume accompanied by a dose-related increase in osmolality: males and females of the 250 and the 1000 ppm group; additionally, slight decrease in pH for the urine of the 1000 ppm animals.
7. Changes in absolute and relative kidney weight: males and females of the 250 and the 1000 ppm group.
8. Gastric irritation: males and females of the 250 and 1000 ppm groups.
9. Increase in bone marrow hyperplasia: 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.
10. Increased incidence of renal tubular pigmentation: females of the 250 ppm group and in males and females of the 1000 ppm group.

The changes in body weight and body weight gain, in food consumption, in water consumption and the gastric lesions can be seen as treatment-related effects.

With regard to the reduced water consumption, similar as in other studies, glutaraldehyde offered to the animals via the drinking water resulted in a decreased water consumption, which probably was related to the bad taste, smell and/or irritancy of the test substance. Therefore the reduction in water consumption was considered to be of no toxicological relevance. As consequence of the reduced water intake, a decrease in urine production coupled with increased osmolality and decreased pH was reported. These effects were indicative of renal physiological adaptative changes resulting from the decreased water intake, and were in accordance with the findings referring to the absolute and relative changes in kidney weight.

Similar as in other studies, the oral treatment with glutaraldehyde resulted in gastric irritation, which the lesions being particularly pronounced at the highest tested concentration of 1000 ppm.

Effects affecting the haematological and clinical-chemical parameters were marginal and of no toxicological relevance. 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.

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.

Test substance intake:

Test concentration in drinking water	Mean daily intake of test substance	
	Males	Females
50 ppm	4 mg/kg bw/day	6 mg/kg bw/day
250 ppm	17 mg/kg bw/day	25 mg/kg bw/day
1000 ppm	64 mg/kg bw/day	86 mg/kg bw/day

5.3 Conclusion

5.3.1	LO(A)EL	250 ppm (corresponding to respectively 17 mg/kg bw/day for male and 25 mg/kg bw/day for female rats)
5.3.2	NO(A)EL	50 ppm (corresponding to respectively 4 mg/kg bw/day for male and 6 mg/kg bw/day for female rats).
5.3.3	Other	An increased incidence of large granular lymphocytic leukaemia (LGLL) was reported as only statistically/biologically relevant neoplastic finding and was discussed in section A 6.7 (see robust summary A6.7.02).
5.3.4	Reliability	**2**
5.3.5	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 were scientifically acceptable.

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.

Section A6.5 _ 02 **Annex Point IIA6.3 / 6.4 / 6.5**	**Repeated dose toxicity** **Chronic oral toxicity and oncogenicity in rat**

Acceptability	See Remarks below.
Remarks	Judging by the details of the study [redacted] (A6.5.1, A6.7.1). Therefore, a detailed assessment of this study summary has not been performed by the RMS. The report as provided here is not applicable for establishing a NOAEL due to insufficient reporting. The report indicates e.g. that there was a tendency for increase of emaciation, labored breathing, body pallor and yellow cutis in all female dose groups. Based on the report, RMS cannot verify whether these and other effects are relevant or not. 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 ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.5 _ 03		**Repeated dose toxicity**	
Annex Point IIA6.3 / 6.4 / 6.5		**Chronic oral toxicity in Beagle dog**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] (2001) ([redacted]% Glutaraldehyde) - Chronic oral toxicity study in Beagle dogs - Administration in drinking water for 12 months. [redacted] (Unpublished), BPD ID A6.05_03	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new active substance (a.s.) for first entry to Annex I authorisation.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 452 (1981)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] ([redacted]% Glutaraldehyde)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Colorless-clear liquid	
3.1.2.2	Purity	[redacted]% (analysis performed by [redacted])	
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 ([redacted])	
3.2	**Test Animals**		
3.2.1	Species	Dog	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male/Female	
3.2.5	Age/weight at study initiation	At test initiation, the males and females respectively were about 5 to 9 months old. The body weights of the males at test initiation ranged between 10.2 and	X

		15.5 kg (group mean: 12.7 kg); for the females, the body weights ranged between 8 and 14.1 kg (group mean: 10.7 kg)
3.2.6	Number of animals per group	10 animals per test group (5 males and 5 females).
3.2.7	Control animals	Yes, untreated animals
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	12 months
3.3.2	Frequency of exposure	Daily (in drinking water)
3.3.3	Post exposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	Drinking water
3.3.4.2	Concentration	0, 20, 100 and 500 ppm test substance, which corresponded to respectively 0, 10, 50 and 250 ppm glutaraldehyde.
3.3.4.3	Vehicle	Aqueous solution
3.3.4.4	Preparation of the test concentrations	An amount of drinking water was weighed and the appropriate amount of test substance needed to get the wanted test concentration was added and mixed with a stirrer. The drinking test solutions were prepared once a week and were stored at room temperature.
3.3.4.5	Controls	Control animals received drinking water without test substance
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	The dogs were examined for clinical symptoms of toxicity at least once on each working day, and if necessary several times a day.
3.4.1.2	Mortality	The dogs were checked for mortality (dead or moribund animals) twice a day on working days and once a day on weekends or public holidays.
3.4.1.3	Open field observations	As the animals were housed under appropriate conditions allowing free moving, abnormal motor activity and alteration in behaviour could easily be assessed.
3.4.2	Body weight	The dogs were weighed at the beginning of the adaptation period (i.e. ca. 1week prior test initiation), on day 0 (test start) and once a week thereafter until the end of the experimental period. The body weight change was determined for each animal as the difference between the body weight on day x and the body weight on day 0 (test start).
3.4.3	Food consumption	Diet was offered to the dogs each day for maximal two hours. Thereafter the feed bowl was removed and the remaining feed in the feed bowl was weighed and subtracted from the initial amount given. Food consumption was determined daily and was expressed as mean food consumption in grams per animal and day. In case of death within at test group, the mean food consumption calculation was based on the surviving animals.
3.4.4	Food efficiency	The food efficiency for each animal was calculated at weekly intervals

		on the basis of the body weight changes and the food consumption using following formula: $\frac{BW_{day\ x} - BW_{day\ x-7}}{FC} \times 100 = \text{Food efficiency on day x}$ BW day x = body weight on test day x; in g BW day x-7 = body weight on test day x-7; in g FC = total daily food consumption (g) over a period of 7 days
3.4.5	Water consumption	Water consumption was determined once a week during the adaptation period prior the test starting. During the experimental period, the water consumption was determined at weekly intervals, over periods of 4 days each, and was expressed in grams as mean water consumption per animals and day.
3.4.6	Intake of test substance	The mean daily intake of test substance per kg bw and per day was calculated according to following formula: $\frac{WC\ (day\ x\text{-}7\ to\ x\text{-}3) \times C}{BW\ day\ x} = \text{Substance intake for day x}$ BW day x = body weight on test day x; in g WC = water consumption (g/day) over 4 days C = concentration of test substance in drinking water; in ppm
3.4.7	Ophthalmoscopic examination	Prior starting the experiment and at the end of the experimental period, the eyes of the male and female dogs were examined for changes (KOWA-RC 2 fundus camera).
3.4.8	Haematology	Blood samples were collected from all animals of each test group. The blood samples were taken from the vena cephalica antebrachii. Blood sampling was conducted on following days: Males: day 11 prior test start, day 94 of treatment, day 185 of treatment and day 360 of treatment. Females: day 10 prior test start, day 95 of treatment, day 186 of treatment and day 361 of treatment. The samples served for the examination of a series of haematological and clinical chemical parameters. Following haematological parameters were considered: haematocrit, haemoglobin, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, erythrocyte count, leukocyte count, platelet count and differential blood count. Clotting analysis was performed and the activated partial thromboplastin time as well as the prothrombin time (Hepato Quick´s Test) was performed.
3.4.9	Clinical Chemistry	Following clinical chemical parameters were considered: alanine aminotransferase, aspartate aminotransferase,alkaline phosphatase, serum gamma glutamyltransferase, sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, and magnesium.
3.4.10	Urinalysis	Urine samples were collected from all animals of each test group. Each animal was kept in a metabolism cage without feed and was given about 500 ml drinking water or drinking test solution; urine was collected overnight. The sampling time points were as follows:

		Males: day 13 prior test start, day 92 of treatment, day 183 of treatment and day 358 of treatment. Females: day 12 prior test start, day 93 of treatment, day 184 of treatment and day 359 of treatment. Following parameters were considered: volume, color, turbidity, pH, protein, glucose, ketones, bilirubin, blood, urobilinogen, specific gravity and sediment.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	The animals were sacrificed by exsanguination from cervical and brachial vessels under anaesthesia, and subjected to necropsy. Following organs were weighed: brain, liver, kidneys, adrenals, testes, ovaries, epididymides, thyroid, parathyroid, heart, spleen and uterus.
3.5.2	Gross and histopathology	Following organs/tissues were fixed (4% formaldehyde) for further examinations: all gross lesions, pituitary, thymus, heart, aorta, liver, kidneys, pancreas, esophagus, larynx, pharynx, nasal cavity (level III), tongue, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, lymph nodes, sciatic nerve, sternum with marrow, femur bone marrow, spinal cord (cervical, thoracic and lumbar parts), femur with knee joint and marrow, brain, thyroids, parathyroids, lungs, trachea, salivary glands, spleen, adrenals, testes, epididymides, ovaries, oviducts, uterus, vagina, prostate, mammary glands, gallbladder, urinary bladder, skeletal muscles, eyes with optic nerve and skin. All gross lesions and tissue/organ samples were processed and stained (hematoxylin-eosin) for light microscopical examination.
3.5.3	Other examinations	None
3.5.4	Statistics	For the statistical assessment of clinical data (food and water consumption, food efficiency, body weight, body weight change), means and standard deviations were calculated. Furthermore the statistical assessment was on Dunnett CW (Dunnett CW, JASA, Vol. 50: 1096-1121, 1955 and Dunnett CW, Biometrics, Vol. 20: 482-491, 1964) and on Winer BJ (Statistical principles in experimental design, MacGraw-Hill New York, 2nd edition, 1971). For blood and plasma data, means and standard deviations were calculated and the analysis of the significance was based on the Kruskal - Wallis test and the U test of Mann-Whitney (Siegel S, Non-parametric statistics for the behavioural sciences, McGraw-Hill New York, 1956). Fischer´s exact test for or the hypothesis of equal proportions (Siegel S, Non-parametric statistics for the behavioural sciences, McGraw-Hill New York, 1956) was used for the statistical assessment of the urinalysis data. For the final body weight and the absolute and relative organ weights, means and standard deviations were calculated; further statistical assessements were based on the Kruskal -Wallis test and the Wilcoxon test.
3.6	**Further remarks**	Tests substance preparations were analysed [redacted] The stability of the test substance in the vehicle (i.e. drinking water) was checked prior test start (1) over a period of 14 days at room temperature in a closed vessel, and (2) over a period of 24 hours at room temperature in an open vessel. The correctness of the test

concentrations also was checked by concentration control analysis at following time points: day 4 of treatment, day 95 of treatment. Day 186 of treatment, day 193 of treatment, day 277 of treatment, day 340 of treatment. At each control time point, all test doses were checked.

Food and drinking water also were subjected to analysis, respectively according to EPA guideline (Fed. Reg. Vol. 44, No. 91, 1979) and to the German Drinking Water Regulation (Trinkwasserverordnung, Bundesgesetztblatt, 1990).

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs

A slight erythema affecting the inguinal region was seen in two cases (one male, one female) at 500 ppm, from day 189 until the end of the experimental period. A similar erythema also was seen in one case (female) at 100 ppm, from day 308 until the end of the experimental period. This effect was attributed to the test substance and probably resulted from incidental skin contact with the test substance preparation by licking. No further treatment-related effects were reported.

4.1.2 Mortality

All animals survived.

4.2 Body weight gain

No treatment-related effects on body weight were observed. In fact, on study day 280, a statistically significant reduction in body weight was reported for the 100 ppm group, which was mainly due to one male dog. Because of the absence of any dose-response relationship, this effect was considered not related to the treatment. No statistically significant deviations of body weigh change could be reported; body weight increase at the end of the experimental period was as follows:

Test group	Mean increase in body weight (kg)
Males	
0 ppm	+ 1.0 kg
20 ppm	+ 1.0 kg
100 ppm	+ 0.8 kg
500 ppm	+ 1.7 kg

Test group	Mean increase in body weight (kg)
Females	
0 ppm	+ 2.9 kg
20 ppm	+ 2.8 kg
100 ppm	+ 3.7 kg
500 ppm	+ 2.9 kg

4.3 Food consumption

No treatment-related effects on food consumption were observed.

4.4 Food efficiency

No treatment-related effects on food efficiency were observed.

4.5 Water

Water consumption of the treated animals during the experimental period was compared to the water consumption observed during the

consumption

adaptation period preceding the test (pretest values) and to the water consumption of the control animals. Following values were reported:

Test group	Mean water consumption (%)	
	Compared to pretest values (100%)	Compared to control group values (100%)
Males		
0 ppm	91%	100%
20 ppm	86%	95%
100 ppm	91%	100%
500 ppm	**70%**	**77%**
Females		
0 ppm	95%	100%
20 ppm	93%	98%
100 ppm	95%	100%
500 ppm	**69%**	**73%**

The table above shows that water consumption for the 0, 20 and the 100 ppm groups was inconspicuous. In contrast within the 500 ppm group, water consumption was clearly reduced during the treatment period and compared to control, for both the males and the females. This effect was related to the bad taste of the drinking solution and/or to an irritant effect of the test substance in the drinking water.

4.6 Compound intake

The mean daily intake of test substance as such (i.e. [redacted]% glutaraldehyde) over the whole experimental period was as follows:

Test concentration in drinking water	Mean daily intake of test substance (mg/kg bw/day)	
	Males	Females
20 ppm	1.5	1.6
100 ppm	7.8	8.1
500 ppm	29.1	33.2

The mean daily intake of the active ingredient glutaraldehyde over the whole experimental period was as follows:

Test concentration in drinking water	Mean daily intake of test substance (mg/kg bw/day)	
	Males	Females
20 ppm	0.8	0.8
100 ppm	3.9	4.1
500 ppm	14.6	16.6

4.7 Ophtalmoscopic examination

The ophthalmoscopic examination of the eyes by means of a KOWA-RC 2 fundus camera revealed no abnormalities.

4.8 Blood analysis

4.8.1 Haematology

No treatment-related changes in haematological parameters were measured.

4.8.2 Clinical chemistry

No treatment-related changes in clinical-chemical parameters were measured.

4.8.3 Urinalysis

The males of the 500 ppm group produced decreased amounts of urine with increased specific gravity over the whole experimental period. The remaining parameters were inconspicuous.

Mean urine volume (ml), male dogs (N = 5)				
Time point	0 ppm	20 ppm	100 ppm	500 ppm
Day 13 prior test start	348 +/- 246	524 +/- 186	416 +/- 169	512 +/- 239
Day 92 of treatment	316 +/- 70	321 +/- 163	274 +/- 92	**147 +/- 212**
Day 183 of treatment	509 +/- 216	418 +/- 226	309 +/- 111	**170 +/- 252**
Day 358 of treatment	440 +/- 138	336 +/- 242	346 +/- 55	**156 +/- 166**

X

Mean specific gravity (g/l), male dogs (N = 5)				
Time point	0 ppm	20 ppm	100 ppm	500 ppm
Day 13 prior test start	1018 +/- 7	1013 +/- 4	1016 +/- 5	1014 +/- 5
Day 92 of treatment	1021 +/- 6	1027 +/- 12	1026 +/- 10	**1039 +/- 21**
Day 183 of treatment	1017 +/- 3	1021 +/- 7	1023 +/- 9	**1036 +/- 12**
Day 358 of treatment	1018 +/- 5	1018 +/- 8	1024 +/- 7	**1034 +/- 10**

4.9 Sacrifice and pathology

4.9.1 Organ weights

In the 20 ppm and the 500 ppm groups, the males displayed statistically significant increases in mean spleen weight. In fact, mean absolute weights of 34.522 +/- 4.495 g and 33.86 +/- 1.91 g respectively were reported for the spleen of the 20 ppm and the 500 ppm treated dogs, versus 25.428 +/- 3.694 g for control dogs. The relative spleen weights of male dogs were statistically significantly increased for all test doses (20, 100, 500 ppm). Following values were reported:

X

0.245 +/- 0.03 % for the 20 ppm male dogs

0.221 +/- 0.019 % for the 100 ppm male dogs

0.235 +/- 0.012 % for the 500 ppm male dogs

versus 0.184 +/- 0.028 % for control male dogs.

4.9.2 Gross and histopathology

Gross pathology revealed following gross lesions:

Test group	Animals affected	Sex	Localisation	Description of the lesions
0 ppm	1	F	Oviduct	Cyst
0 ppm	1	F	Right thyroid	Cyst
20 ppm	2	F + M	Pituitary	Cyst
20 ppm	1	F	Adrenal cortex	Organ-like colored focus (4 mm), slightly thickened
100 ppm	1	F	Skin	Erythema of the inguinal region
500 ppm	2	F + M		
500 ppm	1	M	Pericard	Red-colored focus (10 mm), slightly thickened
500 ppm	1	F	Adrenal cortex	Cyst

Histopathology:

The light microscopic examination of the tissue and organ samples revealed incidental or spontaneous occurring alterations, which were not treatment-related.

4.10 Other

The stability of the test substance in the vehicle (i.e. drinking water) could be verified analytically in both cases: (1) over a period of 14 days at room temperature in a closed vessel, and (2) over a period of 24 hours at room temperature in an open vessel.

The concentration control analysis revealed values, which were in the expected range of the nominal concentrations; recovery was about 94.4 to 109.6%.

The food analysis revealed that food was suitable, with a number of microorganisms that did not exceed 10^5/g food. Water was also found to be suitable according to the considered guideline.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to look for the toxicity potential of glutaraldehyde when administered orally via drinking water to Beagle dogs over a period of 12 months.

Test substance: [redacted]% glutaraldehyde), [redacted] % (analysis performed by [redacted]), 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.

The test substance was administered over a period of 12 months to groups of 10 dogs (5 males and 5 females) via drinking water at following concentrations: 0, 20, 100 and 500 ppm, referring to the test

substance as such. Considering the active ingredient glutaraldehyde, the tested concentrations were 0, 10, 50 and 250 ppm. The animals were checked for mortality, clinical symptoms, body weight, food consumption and efficiency and water consumption. Ophthalmologic examinations were conducted. Blood samples were collected for the evaluation of a series of haematological and clinical-chemical parameters; urinalysis also was performed. Following necropsy, the absolute and relative weights of a series of organs were assessed, and gross pathological s well as histopathological examinations were done

The statistical assessment of the findings mainly was based on Dunnett's test, the Kruskal-Wallis test, Fisher's exact test and the Wilcoxon test.

5.2 Results and discussion

The stability of the test substance in water up to 14 days at room temperature was proven. Since the test substance preparations were stored less than 7 days, and no longer than 24 hrs in a drinking water bowl, stability was assured. The concentration control analyses confirmed the nominal concentrations (recovery: 94.4-107%).

The mean daily intake of the test substance as such via the drinking water was as follows:

Test concentration	20 ppm	100 ppm	500 ppm
Males	1.5 mg/kg bw/day (0.8 mg/kg bw/day GA)*	7.8 mg/kg bw/day (3.9 mg/kg bw/day GA)	29.1 mg/kg bw/day (14.6 mg/kg bw/day GA)
Females	1.6 mg/kg bw/day (0.8 mg/kg bw/day GA)	8.1 mg/kg bw/day (4.1 mg/kg bw/day GA)	33.2 mg/kg bw/day (16.6 mg/kg bw/day GA)

*, Mean daily intake referring to glutaraldehyde

No mortality was observed. A slight erythema affecting the inguinal region was reported for one male and one female of the 500 ppm group, and for one female of the 100 ppm group; this effect was attributed to the test substance and probably resulted from incidental skin contact with the test substance preparation by licking. No treatment-related effects on body weight were observed. No treatment-related effects on food consumption and food efficiency were reported. Within the 500 ppm group, water consumption was clearly reduced during the treatment period for both the males and the females (respectively 23% and 27% below control values); this effect was related to the bad taste of the drinking solution and/or to the irritant effect of the test substance. Water consumption for the 0, 20 and the 100 ppm groups was inconspicuous.

The ophthalmoscopic examination of the eyes revealed no abnormalities.

Neither the haematological nor the clinical chemical parameters were affected by the treatment. Urinalysis revealed a decreased urine production for the males of the 500 ppm group, which was accompanied by an increase in specific gravity. These effects were considered to be treatment-related, resulting from the reduced water consumption mentioned above. The remaining parameters considered within urinalysis were inconspicuous.

In the 20 ppm and the 500 ppm groups, the males displayed statistically significant increases in mean absolute spleen weight (34.522 +/- 4.495 g

and 33.86 +/- 1.91 g respectively for the 20 ppm and the 500 ppm treated dogs, versus 25.428 +/- 3.694 g for control dogs). The relative spleen weights of male dogs were statistically significantly increased for all test doses (0.245 +/- 0.03 % at 20 ppm, 0.221 +/- 0.019 % at 100 ppm and 0.235 +/- 0.012 % at 500 ppm, versus 0.184 +/- 0.028 % for control). The changes in weight affecting the spleen could not be correlated with morphological alterations; therefore these changes were not considered to be of toxicological or biological relevance. A series of incidental gross lesions were reported for all groups, which were not treatment-related.

Summarizing it can be retained that treatment-related effects in dogs treated with [redacted] over a period of 12 months were seen at 500 ppm (corresponding to 250 ppm glutaraldehyde). These effects consisted of a reduction in water consumption seen in both males and females, which again resulted in a decreased urine excretion and increased specific gravity for males only. These effects however rather were due to a palatability problem (bad taste of the drinking solution) than to a toxic effect of the tested substance.

5.3 Conclusion

5.3.1	LO(A)EL	> 500 ppm
5.3.2	NO(A)EL	500 ppm (corresponding to 29.1 mg/kg bw/day for males and to 33.2 mg/kg bw/day for females)
5.3.3	Reliability	**1**
5.3.4	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	September 9th, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2. 3.2.5 Age/weight at study initiation. The age of the animals was 5 to 9 months. No information is given on the relation of age and sex of the animals.
Results and discussion	4.8.3 Urinalysis. The effects described for males (decreased urine volume, increased specific gravity) were also seen in females, although less pronounced. 4.9.1 Organ weights. Both absolute and relative spleen weights were increased in all dose groups in both males and females. Statistical significance was reached in males for absolute weight (high and low dose groups) and relative weight (all dose groups).
Conclusion	LO(A)EL: > 500 ppm NO(A)EL: 500 ppm, corresponding to 14.6 and 16.6 mg GA/kg bw/day for males and females, respectively (29.1 and 33.2 mg test substance/kg bw/day for males and females, respectively) No adverse effects were seen in the study. The effects seen were directly linked to reduced water consumption, resulting from a palatability problem.
Reliability	1

Section A6.5 _ 03	**Repeated dose toxicity**
Annex Point IIA6.3 / 6.4 / 6.5	**Chronic oral toxicity in Beagle dog**

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

Section A6.5 _ 04 — **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 — **Chronic inhalation carcinogenicity study in rats and mice: toxicity data**

			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_a van Birgelen APJM, Chou BJ, Renne RA, Grumbein SL, Roycroft JH, Hailey JR, Bucher JR (2000) Effects of glutaraldehyde in a 2-year inhalation study in rats and mice. Toxicol. Sci. 55(1): 195-205 (Published), BPD ID A6.5_04_b	
1.2	**Data protection**	No	
1.2.1	Data owner	None (published data)	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline mentioned, but the study was well documented and almost in accordance with OECD TG 451 (see chapter 6.7)	
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 A6.5 _ 04	Repeated dose toxicity
Annex Point IIA6.3 / 6.4 / 6.5	Chronic inhalation carcinogenicity study in rats and mice: toxicity data

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]
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 A6.5 _ 04	**Repeated dose toxicity**
Annex Point IIA6.3 / 6.4 / 6.5	**Chronic inhalation carcinogenicity study in rats and mice: toxicity data**

3.3.3	Post exposure period	None
3.3.4	**Inhalation**	

Section A6.5 _ 04 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Chronic inhalation carcinogenicity study in rats and mice: toxicity data**

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.

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

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

<u>Conversion of the ppb values in µg/l</u>:

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

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 theoritical 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	Not considered
3.4.4	Water consumption	Not considered
3.4.5	Ophthalmoscopic examination	Not considered
3.4.6	Haematology	Not considered
3.4.7	Clinical Chemistry	Not considered
3.4.8	Urinalysis	Not considered
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Not considered
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

formalin and were processed and HE-stained for light microscopic examination. These tissues included: brain, pituitary, thyroid, parathyroid, thymus, oesophagus, gall bladder (mice), salivary glands, stomach, small and large intestines, liver, pancreas, pancreatic islets, kidneys, adrenals, spleen, heart, trachea, lungs, larynx, gonads, uterus, mammary gland, clitoral gland, prostate, preputial gland, urinary bladder, lymph nodes, bone marrow, skin and nose.

All tumors as well as all potential target organs (larynx, lung, and nose) of rats and mice were evaluated by a quality assessment pathologist. For the nose, 4 sections of the nasal passage were considered for examination. Tooth degeneration also was evaluated in rats. Brain of rats was examined in case of hydrocephalus or hemorrhage. The kidneys of male mice were examined by the quality assessment pathologist for infarct or nephropathy. The livers of female mice were examined for eosinophilic foci. The thyroid glands of mice of both sexes were re-evaluated from hyperplasia.

The (histo)pathological findings were submitted to the NTP Pathology Working Group chairperson for review.

3.5.3 Other examinations: None

3.5.4 Statistics: The statistical assessment of the findings of the present studies can be summarized as follows:

Endpoint	Statistical Methods
Survival data	Product-limit procedure according to Kaplan and Meier (J. Am. Stat. Assoc. 53: 457-481, 1958), Cox's method (J.R. Stat. Soc. B34: 187-220, 1972) and Tarone's life table test (Biometrika 62: 679-682,1975)
Incidence of neoplasms and non-neoplastic lesions	Poly-k test according to Bailer and Portier (Biometrics 44: 417-431, 1988), Portier and Bailer (Fund. Appl. Toxicol. 12: 731-737, 1989), Piegorsch and Bailer (Statistics for Environmental Biology and Toxicology, Section 6.3.2, Chapman and Hall, London, 1997) and Bieler and Williams (Biometrics 49: 793-801, 1993)
Body weight data	Parametric multiple comparison procedures according to Dunnett (J. Am. Stat. Assoc. 50: 1096-1121, 1955) and Williams (Biometrics 27: 103-117, 1971; Biometrics 28: 519-531, 1972); Mann-Whitney U test according to Hollander and Wolfe (Nonparametric Statistical Methods: 120-123, John Wiley and Sons, NY, 1973)

3.6 Further remarks: None

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs: Some female rats of the 750 ppb group were thin to emaciate and were sacrificed in extremis.

X

No treatment-related clinical symptoms were seen in mice.

4.1.2 Mortality

The main survival data can be summarized as follows:

Test animals	Test group	Survival at study ending (2 years)	
		Males	Females
Rats ($N_{initial}$ = 50/sex)	0 ppb	12/50 (24%; p = 0.032)	26/50 (52%; p < 0.001)
	250 ppb	14/50 (28%; p = 0.395)	31/50 (62%; p = 0.454)
	500 ppb	9/50 (18%; p = 0.788)	**15/50 (30%; p = 0.023)**
	750 ppb*	6/50 (12%; p = 0.094)	**14/50 (28%; p = 0.008)**
Mice ($N_{initial}$ = 50/sex)	0 ppb	31/50 (62%; p = 0.036)	34/50 (68%; p = 0.573)
	62.5 ppb	27/50 (54%; p = 0.464)	37/50 (74%, p = 0.611)
	125 ppb	40/50 (80%; p = 0.091)	35/50 (70%; p = 0.711)
	250 ppb	38/50 (76%; p = 0. 192)	32/50 (64%; p = 0.811)

*, 8 male and 5 female rats of the 750 ppb group were removed from the study between week 13 and 21 because of breathing problems likely related to nasal lesions.

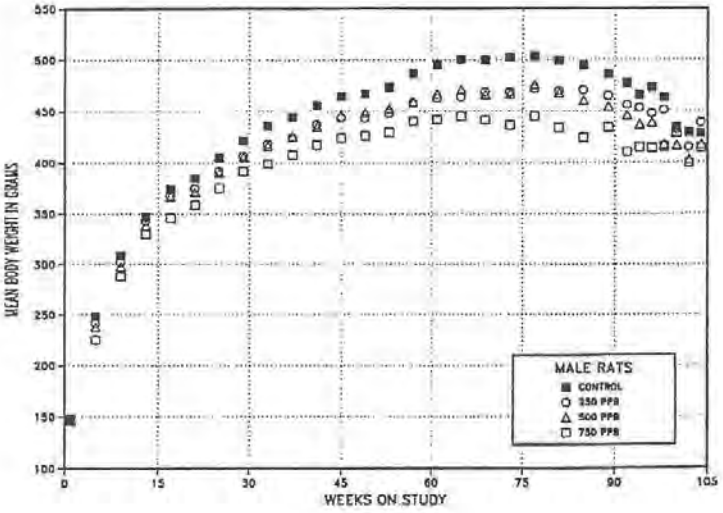
The table above shows that survival of female rats treated with 500 and 7500 ppb test substance was decreased compared to controls; survival of the treated males was similar to control. For the mice, survival in all test groups and for both sexes was similar to controls.

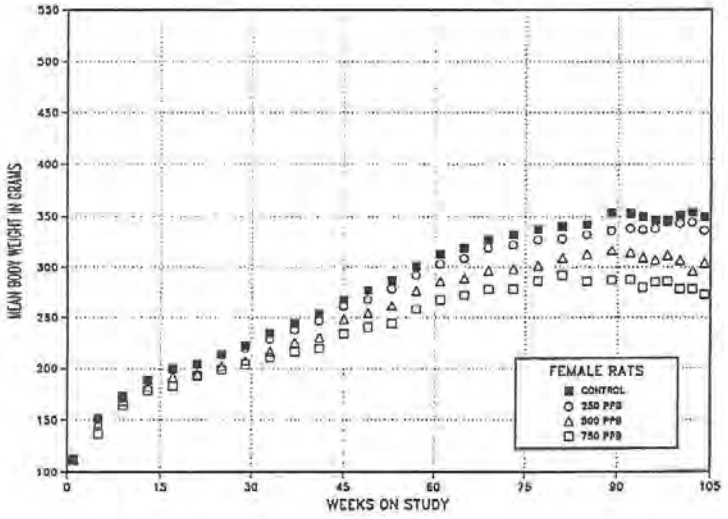
4.2 Body weight gain, Growth curve for rats exposed to glutaraldehyde for 2 years:

Section A6.5 _ 04 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Chronic inhalation carcinogenicity study in rats and mice: toxicity data**

rats





Summary of mean body weights (MBW) data over consecutive intervals of time:

Section A6.5 _ 04 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Chronic inhalation carcinogenicity study in rats and mice: toxicity data**

Male rats							
Weeks	0 ppb	250 ppb		500 ppb		750 ppb	
	MBW (g)	MBW (g)	MBW as % of control	MBW (g)	MBW as % of control	MBW (g)	MBW as % of control
1 - 13	263	258	98%	255	97%	248	94%
14 - 52	428	412	96%	412	96%	394	92%
53 - 104	478	456	95%	449	94%	428	90%

Female rats							
Weeks	0 ppb	250 ppb		500 ppb		750 ppb	
	MBW (g)	MBW (g)	MBW as % of control	MBW (g)	MBW as % of control	MBW (g)	MBW as % of control
1 - 13	156	156	100%	152	97%	148	95%
14 - 52	236	231	98%	219	93%	211	89%
53 - 104	335	325	97%	300	90%	278	83%

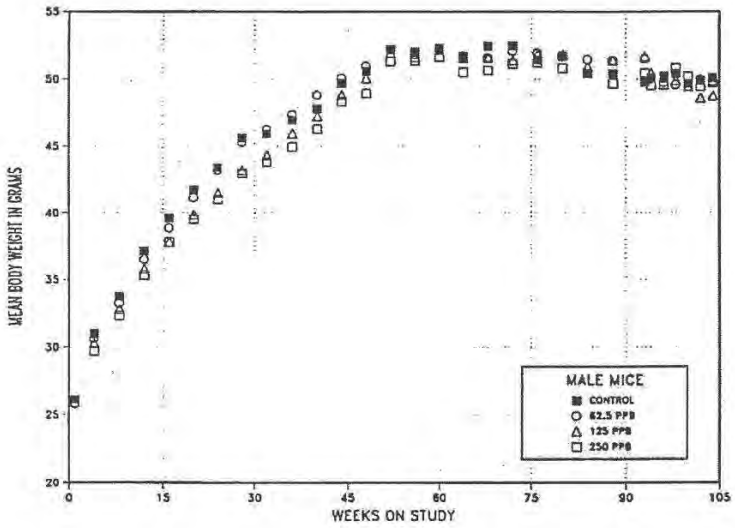
The mean body weights of all treated males and of the females of the 500 and 750 ppb groups were below control values; this effect was considered to be test substance-related.

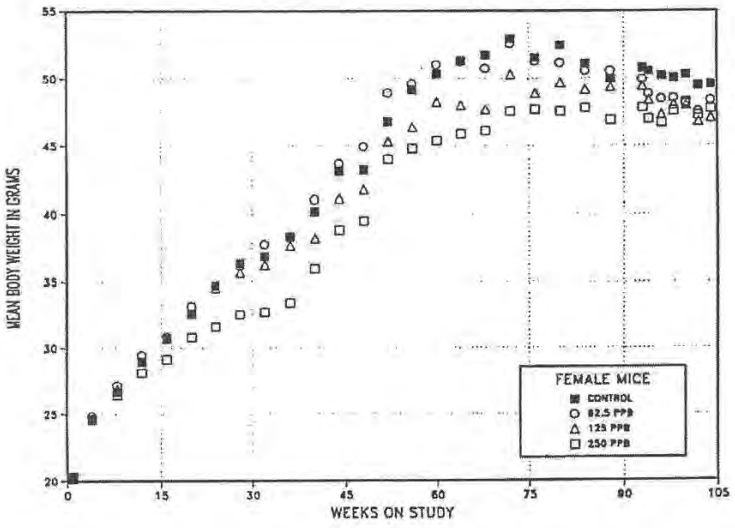
Section A6.5 _ 04 **Repeated dose toxicity**

Annex Point IIA6.3 / 6.4 / 6.5 **Chronic inhalation carcinogenicity study in rats and mice: toxicity data**

4.3 Body weight gain, mice

Growth curve for mice exposed to glutaraldehyde for 2 years:





Summary of mean body weights (MBW) data over consecutive intervals of time:

Male mice							
Weeks	0 ppb	62.5 ppb		125 ppb		250 ppb	
	MBW (g)	MBW (g)	MBW as % of control	MBW (g)	MBW as % of control	MBW (g)	MBW as % of control
1 - 13	32	31.5	98%	31.2	98%	30.8	96%
14 - 52	46.4	46.4	100%	45.0	97%	44.5	96%
53 - 104	50.9	51.0	100%	50.8	100%	50.4	99%

Female mice							
Weeks	0 ppb	62.5 ppb		125 ppb		250 ppb	
	MBW (g)	MBW (g)	MBW as % of control	MBW (g)	MBW as % of control	MBW (g)	MBW as % of control
1 - 13	25.2	25.5	101%	25.2	100%	24.9	99%
14 - 52	38.3	39.0	102%	37.4	98%	34.9	91%
53 - 104	50.7	49.9	98%	48.3	95%	47.0	93%

The male mice showed no treatment-related effects on body weight; in contrast, the mean body weights of the female mice of the 250 ppb group were decreased compared to control.

4.4 Sacrifice and pathology

4.4.1 Rats

Statistically significant and/or biological relevant changes were mainly seen in the nose of the glutaraldehyde-treated rats. No treatment-related neoplastic lesions were observed in the rats, neither in the males, nor in the females. The main non-neoplastic lesions in the nose of the glutaraldehyde-treated rats can be summarized as follows.

Main lesions in the nose of glutaraldehyde-treated rats:

Male rats					
Lesions in the nose		0 ppb	250 ppb	500 ppb	750 ppb
Squamous epithelium	Hyperplasia	3[a] (2.0)[b]	11* (1.6)	39** (2.2)	48** (2.9)
	Inflammation	6 (2.0)	17* (1.5)	41** (2.7)	49** (3.6)
Respiratory epithelium	Hyperplasia	6 (2.0)	5 (2.0)	17** (1.9)	35** (1.9)
	Inflammation	17 (2.1)	10* (1.5)	25 (2.4)	43** (3.2)
	Squamous metaplasia	1 (2.0)	2 (1.5)	11** (2.0)	24** (2.2)
	Goblet cell hyperplasia	1 (1.0)	0	6 (1.8)	6* 81.2)
Olfactory epithelium	Hyaline degeneration	4 (1.0)	8 (1.3)	9 (1.1)	14** (1.1)
Female rats					
Squamous epithelium	Hyperplasia	3 (1.3)	15** (1.7)	29** (2.0)	45** (2.7)
	Inflammation	6 (2.5)	26** (1.5)	42** (2.1)	48** (3.2)
Respiratory epithelium	Hyperplasia	1 (3.0)	6 (1.7)	15** (1.9)	29** (1.9)
	Inflammation	5 (2.2)	9 (1.7)	26** (2.1)	42** (2.5)
	Squamous metaplasia	1 (2.0)	1 (3.0)	11** (1.6)	16** (2.3)
	Goblet cell hyperplasia	1 (2.0)	3 (1.3)	5 (1.4)	8** (1.6)
Olfactory epithelium	Hyaline degeneration	4 (1.0)	5 (1.0)	12* (1.1)	15** (1.1)

*, $p < 0.005$; **, $p < 0.001$; a, Number of animals with lesions (Number of animals examined = 50); b, Average severity grade of lesions (1 = minimal, 2 = mild, 3 = moderate, 4 = marked)

Non-neoplastic lesions were limited primarily to the most anterior region of the nasal cavity. Hyperplasia and inflammation of the squamous epithelium; hyperplasia, goblet cell hyperplasia, inflammation, and squamous metaplasia of the respiratory epithelium; and hyaline degeneration of the olfactory epithelium were observed.

Further effects were seen, which can be summarized as follows.

Main lesions in the lung of glutaraldehyde-treated rats:

One male of the 250 ppb group, one male of the 500 ppb group, two males of the 750 ppb group and one female of the 500 ppb group showed alveolar/bronchiolar adenomas; one 750 ppb male further displayed a carcinoma. These effects however were within the historical control range for inhalation studies and were not related to the treatment with glutaraldehyde. In females, an increased incidence of histiocyte infiltration at 750 ppb and of interstitial fibrosis at 500 and 750 ppb was reported; in males, the incidence of histiocyte infiltration was decreased at 500 ppb and the incidence of fibrosis was increased at 500 ppb. These effects however, were not considered directly related to the treatment and were of little biological relevance.

Main lesions in the thyroid of glutaraldehyde-treated rats:

In two females of the 750 ppb group, the occurrence of thyroid gland follicular cell adenoma was over the historical control range for inhalation studies. As neither hyperplasia nor other treatment-related effects were seen, the two cases of follicular cell adenomas were not related to the treatment with glutaraldehyde.

Main lesions in the mammary gland of glutaraldehyde-treated rats:

Single and multiple fibroadenomas occurred in all groups with a decreasing incidence observed from the 0 ppb (24 cases) to the 750 ppb group (10 cases). The incidence of fibroadenoma or carcinoma (combined) in the females of the 750 ppb group also was significantly decreased compared to the control group (11 cases versus 26 cases). Moreover, the incidences of fibroadenomas or fibroadenoma /carcinoma were below the historical control range for inhalation studies. The decrease in fibroadenomas or fibroadenoma /carcinoma was seen as a consequence of the decrease in body weight and was therefore not seen as a direct effect due to glutaraldehyde.

Main lesions in the pituitary gland of glutaraldehyde-treated rats:

Adenoma occurred in all groups with a decreasing incidence from 0 ppb to 750 ppb; this was associated to the decrease in body weight of the treated animals.

Main lesions in the kidney of glutaraldehyde-treated rats:

Nephropathy is a common spontaneous change seen in almost all male rats surviving to 2 years. In the present case, a decrease in the severity of the nephropathy was observed with increasing test concentration. This effect also was seen as a consequence of the decrease in body weight observed for the treated rats (i.e secondary effect).

4.4.2 Mice

Statistically significant and/or biological relevant changes were mainly seen in the nose of the glutaraldehyde-treated mice. No exposure-related neoplastic lesions were observed in the mice, neither in the males nor in the females. The main non-neoplastic lesions in the nose can be summarized as follows.

X

Main lesions in the nose of glutaraldehyde-treated mice:

Male mice					
Lesions in the nose		0 ppb	62.5 ppb	125 ppb	250 ppb
Respiratory epithelium	Squamous metaplasia	2[a] (1.0)[b]	5 (1.0)	6 (1.2)	9* (1.1)
Turbinate	Necrosis	0	0	2 (2.0)	0
Female mice					
Squamous epithelium	Inflammation	6 (1.2)	7 (1.3)	13 (1.4)	14* (1.4)
Respiratory epithelium	Squamous metaplasia	7 (1.1)	11 (1.0)	16* (1.3)	21** (1.5)
	Hyaline degeneration	16 (1.4)	35** (1.4)	32** (1.3)	30* (1.1)
Turbinate	Necrosis	0	3 (2.0)	1 (1.0)	4 (1.5)

*, $p < 0.005$; **, $p < 0.001$; a, Number of animals with lesions (Number of animals examined = 50, excepted for 0 ppb males: 48 and 62.5 ppb females: 49); b, Average severity grade of lesions (1 = minimal, 2 = mild, 3 = moderate, 4 = marked)

Non-neoplastic lesions were limited primarily to the most anterior region of the nasal cavity. The nasal lesions in the mice were qualitatively similar to those seen in rats. Squamous metaplasia of the respiratory epithelium was observed in both sexes of mice while female mice also had inflammation and hyaline degeneration of the respiratory epithelium.

Further effects were seen, which can be summarized as follows.

Main lesions in the thyroid gland of glutaraldehyde-treated mice:

An increased incidence in hyperplasia of the thyroid gland follicular cells, which was classified as minimal to mild, was reported for the females of the 250 ppb group (37 cases versus 26 in control). This effect however is a common spontaneous effect seen in aged mice. Furthermore, neither increased incidence in adenoma of the thyroid gland follicular cells in males and females, nor treatment-related effects in the thyroid gland of the males were seen. Therefore, the increased incidence in hyperplasia of the thyroid gland follicular cells seen in the 250 ppb females was considered as an incidental, not treatment-related finding.

Main lesions in the pituitary gland of glutaraldehyde-treated mice:

An increased incidence in hyperplasia of the pituitary gland (pars distalis), which was classified as minimal to mild, was reported for the females of the 250 ppb group (28 cases versus 19 in control). The females showed no increased incidence in adenoma of the pituitary gland, and the males were free of treatment-related effects affecting the pituitary gland. Therefore, the increased incidence in hyperplasia of the pituitary gland seen in the 250 ppb females was considered as an

incidental, not treatment-related finding.

Main lesions in the liver of glutaraldehyde-treated mice:

The males of the 62.5 and the 250 ppb groups as well as the females of the 250 ppb group showed decreased incidences in hepatocellular adenoma when compared to control (males: 11 cases seen at 250 ppb versus 19 cases in control; females: 3 cases at 250 ppb versus 11 cases in control). In females, this effect was seen as secondary effect resulting from the decrease in body weight. In males, no such decrease in body weight was seen; therefore the decreased incidence in hepatocellular adenoma seen in males was not considered to be treatment-related.

4.5 Other

None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the carcinogenic potential of glutaraldehyde when administered via inhalation over a period of two years to rats and mice (see A6.7); chronic toxicity also was considered up to a certain point.

Test substance: Glutaraldehyde ca. 25% aqueous solution, [redacted]. A purity of [redacted] relative to the reference standard was reported for the test substance from batch [redacted]. A purity of [redacted] % relative to the reference standard was reported for the test substance of [redacted]). 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.

No guideline was mentioned. However the study was well documented and the conduct was almost similar to OECD 451; the study followed GLP.

Groups of 50 male and female F344/N rats and B6C3F1 mice were whole-body exposed to glutaraldehyde vapour (rats: 0, 250, 500, or 750 ppb; mice: 0, 62.5, 125, or 250 ppb) 6 h/day, 5 days/week, for 104 weeks. The animals were examined for mortality, clinical signs of toxicity and body weight. At study ending the surviving animals were sacrificed for the purpose of necropsy; animals that died during the experiment or were killed in extremis also were subjected to necropsy. All organs and tissues were examined for gross pathology. All major tissues were fixed in 10% neutral buffered formalin and were processed light microscopic examination. These tissues included: brain, pituitary, thyroid, parathyroid, thymus, oesophagus, gall bladder (mice), salivary glands, stomach, small and large intestines, liver, pancreas, pancreatic islets, kidneys, adrenals, spleen, heart, trachea, lungs, larynx, gonads, uterus, mammary gland, clitoral gland, prostate, preputial gland, urinary bladder, lymph nodes, bone marrow, skin and nose. All tumors as well as all potential target organs (larynx, lung, nose) were evaluated by a quality assessment pathologist. For the nose, 4 sections of the nasal passage were considered for examination. Tooth degeneration also was

		evaluated in rats. Brain of rats was examined in case of hydrocephalus or hemorrhage. The kidneys of male mice were examined for infarct or nephropathy by the quality assessment pathologist. The livers of female mice were examined for eosinophilic foci. The thyroid glands of mice of both sexes were re-evaluated from hyperplasia. The (histo)pathological findings were submitted to the NTP Pathology Working Group chairperson for review.
5.2	**Results and discussion**	Survival of 500- and 750-ppb female rats was less than that of controls. Mean body weights of all exposed groups of male rats, 500- and 750-ppb female rats, and 250-ppb female mice were generally less than those of controls. The mean body weights of all treated males and of the females of the 500 and 750 ppb groups were below control values; this effect was considered to be test substance-related. The male mice showed no treatment-related effects on body weight; in contrast, the mean body weights of the female mice of the 250 ppb group were decreased compared to control. No exposure-related neoplastic lesions were observed in either rats or mice. Non-neoplastic lesions were limited primarily to the most anterior region of the nasal cavity. In rats, hyperplasia and inflammation of the squamous epithelium; hyperplasia, goblet cell hyperplasia, inflammation, and squamous metaplasia of the respiratory epithelium; and hyaline degeneration of the olfactory epithelium were observed. In mice, the nasal lesions were qualitatively similar to those in rats. Squamous metaplasia of the respiratory epithelium was observed in both sexes of mice while female mice also had inflammation and hyaline degeneration of the respiratory epithelium.
5.3	**Conclusion**	Chronic exposure to glutaraldehyde by inhalation resulted in considerable non-neoplastic lesions in the noses of rats and mice. No neoplastic lesions were observed.
5.3.1	NO(A)EL, rat	< 250 ppb
5.3.2	LO(A)EL, rat	250 ppb (corresponding to 1.02 µg/l); value based on hyperplasia and inflammation of the squamous epithelium of the nose, seen in both, male and female rats at 250 ppb.
5.3.3	NO(A)EL, mouse	< 62.5 ppb
5.3.4	LO(A)EL, mouse	62.5 ppb (corresponding to 0.255 µg/l); value based on hyaline degeneration of the respiratory epithelium seen in female mice at 62.5 ppb.
5.3.5	Remark	The authors reported following values for male rats, considering **hyperplasia and squamous metaplasia** of the respiratory epithelium: NOAEL: 250 ppb LOAEL : 500 ppb
5.3.6	Reliability	**2**
5.3.7	Deficiencies	The study conduct almost fulfilled the requirements of the OECD TG 451 (Carcinogenicity) and followed GLP. Chronic toxicity was considered up to a certain point (i.e., no data on food /water consumption, haematological, clinical-chemical and urinary parameters,

organs weights), however, toxicity data and non-neoplastic findings were well documented /described and support the findings of the subchronic study. Thus, the chronic toxicity data reported in present study 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	September 23rd, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	4.1.1 Clinical signs. Additionally, 8 male and 5 female rats in the high dose group had breathing difficulties and were euthanized on weeks 13-21. 4.4.2 Mice. In the table, inflammation is marked as occurring in the squamous epithelium of the females. The inflammation was actually within the epithelium and lamina propria, not just on the squamous epithelium.
Conclusion	The study is not suitable for determining a true NOAEL for chronic toxicity because of the lack of some critical data like haematology and clinical chemistry. The effects described below may be secondary to local irritant effects. Rats: LO(A)EL: 250 ppb, based on significantly increased incidences of hyperplasia (♂, ♀) and inflammation (♂, ♀) of the squamous epithelium. Furthermore, trends of increasing incidence without statistical significance at this dose level were seen for hyperplasia (♀) and inflammation (♀) of the respiratory epithelium, and for hyaline degeneration of the olfactory epithelium (♂). NO(A)EL: < 250 ppb Mice: LO(A)EL: 62.5 ppb, based on significantly increased incidence of hyaline degeneration of the respiratory epithelium (♀), squamous metaplasia which was statistically significant only above 125 ppb (♂, ♀) and few cases of turbinate necrosis (♀) which is not a common spontaneous lesion. The latter was seen at all dose levels in females (0, 3, 1, 4) and at 250 ppb in males (0, 0, 0, 2). NO(A)EL: < 62.5 ppb
Reliability	2
Acceptability	Acceptable as supplementary data only.
Remarks	The study summary is presented for both chronic toxicity and for oncogenicity, but the study concerns only carcinogenicity. 2.2 GLP. There is no GLP certificate, but the study is reported to be in compliance with GLP. Please note that the tabulated numerical results copied to the study summary have not been checked in detail by the RMS.

Section A6.5 _ 04	**Repeated dose toxicity**
Annex Point IIA6.3 / 6.4 / 6.5	**Chronic inhalation carcinogenicity study in rats and mice: toxicity data**

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

Section A6.6.1 _ 01 Genotoxicity in vitro

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3 **Ames test with Salmonella typhimurium**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994) Ames/Salmonella plate incorporation assay on [redacted] (Unpublished), ([redacted]), BPD ID A6.06.1_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, 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.	
3.2	**Study Type**	Bacterial reverse mutation test (Ames test)	
3.2.1	Organism/cell type	S. typhimurium: TA 1535, TA 1537, TA 1538, TA 98, TA 100, TA 102 and TA 104	

Section A6.6.1 _ 01 **Genotoxicity in vitro**

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3 **Ames test with Salmonella typhimurium**

3.2.2 Deficiencies / Proficiencies

All strains had an rfa mutation (affecting membrane permeability)

All strains excepted TA 102 had a uvrB deletion mutation (affecting DNA excision repair)

TA 98, TA 100, TA 102 and TA 104 had a plasmid pKM101 (enhancing the error-prone DNA repair system)

TA 1535 and TA 100 detect base pair substitution mutations affecting the hisG46 allele

TA 1538 and TA 98 detect frameshift mutations affecting the hisD3052 allele

TA 1537 detects frameshift mutations affecting the hisC3076 allele

TA 102 and TA 104 detect a wide variety of genetic damage affecting the hisG428 allele.

3.2.3 Metabolic activation system

The S9 mix was prepared according to Maron DM and Ames BN (Mut. Res. 113(3-4): 173-215, 1983) and was obtained by mixing a series of cofactors ($MgCl_2$, KCl, NADP, glucose-6-phosphate, Na_2HPO_4) with the S9 liver fraction of male Sprague-Dawley rats, which had been treated with Aroclor 1254.

3.2.4 Positive control

In the absence of S9 mix:

Tester Strains	Positive control
TA 1535, TA 100	Sodium azide (10 µg/plate)
TA 1537	9-Aminoacridine (150 µg/plate)
TA1538, TA 98	2-Nitrofluorene (5 µg/plate); mitomycin C (2.5 µg/plate)
TA 104	Methylglyoxal (250 µg/plate)

In the presence of S9 mix:

Tester strains	Positive control
TA 1535, TA 1537, TA1538, TA 98, TA 100	2-Anthramine (2.50 µg/plate)
TA 102, TA 104	2-Acetylaminofluorene (20 µg/plate)

3.3 Administration / Exposure; Application of test substance

3.3.1 Concentrations

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

50, 167, 500, 1670 and 5000 µg/plate

Ames test (with and without S9 mix):

0.5, 1.67, 5.0, 16.7, 50 and 100 µg/plate

Section A6.6.1 _ 01 **Genotoxicity in vitro**

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3 **Ames test with Salmonella typhimurium**

3.3.2	Way of application	0.1 ml of the tester strain was added to 0.1 ml of test solution in 2 ml molten agar (supplemented with 0.5 mM histidine and 0.5 mM biotin) in test tubes. In case of metabolic activation, 0.5 ml of the S9 mix was added. The tubes were vortexed and the contents were poured onto minimal glucose plates and allowed to solidify. Within one hour the plates were inverted and incubated in the dark at 37°C over 48 hours.
3.3.3	Pre-incubation time	None (no preincubation)
3.3.4	Other modifications	None
3.4	**Examinations**	Toxicity pre-screen test:
		Following incubation, the background lawn and spontaneous revertants were scored for normal, inhibited (i.e. no confluent bacterial lawn and/or presence of pindot colonies) and no growth.
		Ames test:
		The revertant colonies were assessed by means of an Artek electronic colony counter interfaced with an IBM PC/AT computer.
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	

Section A6.6.1 _ 01 Genotoxicity in vitro

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3 **Ames test with Salmonella typhimurium**

4.1.1 Original Assay (with/without S9 mix)

TABLE 2. Summary Data - Original Assay

CONTROLS		S9	AVERAGE REVERTANTS/PLATE TA1535	TA1537	TA1538	TA98	TA100	TA102	TA104
DI-H2O (100 UL)		(-)	8 (5)	9 (3)	8 (3)	28 (3)	117 (10)	275 (1)	431 (48)
DI-H2O (100 UL)		(+)	8 (5)	8 (4)	14 (7)	36 (8)	139 (23)	336 (12)	560 (62)
SODIUM AZIDE	10.0	(-)	1136*(48)	--- (---)	--- (---)	--- (---)	1303*(72)	--- (---)	--- (---)
9-AMINOACRIDINE	150	(-)	--- (---)	1559*(234)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)
2-NITROFLUORENE	5.00	(-)	--- (---)	--- (---)	763*(39)	626*(36)	--- (---)	--- (---)	--- (---)
MITOMYCIN C	2.50	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	1383*(118)	--- (---)
METHYLGLYOXAL	500	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	1309*(36)
2-ANTHRAMINE	2.50	(+)	125*(42)	453*(176)	1574*(41)	2363*(213)	2389*(191)	--- (---)	--- (---)
2-AMINOFLUORENE	20.0	(+)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	887*(64)	1761*(170)
Sepacid GA 50 (UG/PL)									
	0.500	(-)	8 (0)	10 (2)	6 (3)	25 (2)	131 (5)	307 (37)	484 (14)
	1.67	(-)	7 (4)	10 (5)	4 (1)	28 (2)	120 (16)	301 (37)	479 (20)
	5.00	(-)	8 (5)	12 (3)	7 (2)	31 (6)	125 (16)	330 (21)	586 (28)
	16.7	(-)	11 (2)a	12 (4)	10 (2)	31 (4)	137 (4)	358 (20)	712 (19)
	50.0	(-)	10 (2)a/b	12 (2)a	11 (2)a/b	39 (4)a	183 (25)a	623*(53)	1105*(17)a
	100	(-)	5 (1)c	15 (6)a/b	12 (5)b/c	52 (1)a/b	27 (9)c	856*(113)a/b	642 (420)b/c
	0.500	(+)	6 (2)	13 (5)	13 (4)	45 (6)	128 (18)	360 (37)	571 (50)
	1.67	(+)	6 (1)	13 (5)	10 (3)	44 (6)	129 (21)	330 (8)	606 (37)
	5.00	(+)	9 (4)	12 (2)	11 (6)	47 (13)	122 (11)	408 (36)	678 (52)
	16.7	(+)	6 (1)	16*(3)	10 (4)	45 (16)	149 (20)	500 (30)	895 (48)
	50.0	(+)	12 (1)	10 (9)	15 (2)a/b	59 (3)	172 (6)	772*(71)	1203*(138)a
	100	(+)	14 (3)a/b	17*(5)a/b	23 (6)b	71 (4)a/b	162 (12)a/b	1104*(69)a	1272*(122)a/b

*Positive Response: ±2X Solvent (TA1535, TA1537, TA1538, TA98, TA100, TA102, TA104).
Data Reported as: Mean (Standard Deviation).
a/b/c = slight/moderate/severe toxicity.
No precipitate.

Section A6.6.1 _ 01 **Genotoxicity in vitro**

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3 **Ames test with Salmonella typhimurium**

4.1.2 Retest (with/without S9 mix)

TABLE 3. Summary Data - Retest

CONTROLS		S9	AVERAGE REVERTANTS/PLATE TA1535	TA1537	TA1538	TA98	TA100[d]	TA102	TA104
DI-H2O (100 UL)		(-)	8 (5)	13 (1)	7 (4)	29 (3)	113 (13)	262 (24)	421 (43)
DI-H2O (100 UL)		(+)	12 (3)	11 (5)	12 (4)	34 (7)	107 (7)	291 (19)	519 (50)
SODIUM AZIDE	10.0	(-)	962*(109)	--- (---)	--- (---)	--- (---)	1199*(103)	--- (---)	--- (---)
9-AMINOACRIDINE	150	(-)	--- (---)	2147*(212)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)
2-NITROFLUORENE	5.00	(-)	--- (---)	--- (---)	972*(165)	606*(18)	--- (---)	--- (---)	--- (---)
MITOMYCIN C	2.50	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	1571*(44)	--- (---)
METHYLGLYOXAL	500	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	1700*(34)
2-ANTHRAMINE	2.50	(+)	179*(44)	708*(264)	1939*(70)	1989*(93)	1847*(198)	--- (---)	--- (---)
2-AMINOFLUORENE	20.0	(+)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	831*(24)	1116*(89)
Sepacid GA 50 (UG/PL)									
0.500		(-)	11 (5)	9 (2)	6 (1)	28 (3)	102 (2)	233 (18)	453 (47)
1.67		(-)	5 (2)	7 (3)	8 (4)	31 (5)	95 (9)	229 (31)	436 (32)
5.00		(-)	8 (1)	16 (2)	7 (3)	34 (3)	98 (7)	264 (37)	572 (23)
16.7		(-)	10 (5)	7 (2)	5 (1)	40 (2)	103 (23)a	307 (33)	716 (191)
50.0		(-)	6 (2)a/b	12 (5)a/b	6 (4)b	41 (12)a/b	124 (9)a/b	580*(25)	1002*(73)a/b
100		(-)	0 (0)c	4 (3)b/c	0 (1)c	9 (9)c	14 (3)b/c	612*(137)a/b	119 (148)c
0.500		(+)	8 (3)	8 (0)	13 (4)	34 (15)	115 (20)	274 (28)	534 (67)
1.67		(+)	10 (2)	8 (2)	11 (3)	31 (10)	121 (5)	268 (19)	524 (21)
5.00		(+)	13 (4)	9 (4)	13 (1)	38 (12)	136 (36)	279 (25)	676 (60)
16.7		(+)	9 (4)	9 (1)	13 (3)	42 (4)	145 (10)	303 (54)	775 (54)
50.0		(+)	14 (5)a	11 (3)	11 (3)	63 (6)	183 (24)a/b	496 (69)	1367*(52)
100		(+)	3 (2)b/c	14 (2)a/b	9 (4)a/b	66 (29)a/b	86 (27)b	802*(114)a/b	1150*(359)a/b

*Positive Response: ≥2X Solvent (TA1535, TA1537, TA1538, TA98, TA100, TA102, TA104).
Data Reported as: Mean (Standard Deviation).
a/b/c = slight/moderate/severe toxicity.
d = Strain TA100 was evaluated separately from the other strains due to a technical error during the second treatment.
No precipitate.

Section A6.6.1 _ 01 Genotoxicity in vitro

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3

Ames test with Salmonella typhimurium

4.2 Cytotoxicity

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

Dose (µg/plate)	Background growth (no S9 mix)		
	TA 1538	TA 100	TA 102
0.00*	Normal	Normal	Normal
50	Inhibited, slight to moderate toxicity	Inhibited, slight to moderate toxicity	Inhibited, slight to moderate toxicity
167	Inhibited to no growth, severe toxicity	No growth	Inhibited, severe toxicity
500	No growth	No growth	Inhibited, severe toxicity
1670	No growth	No growth	No growth
5000	No growth	No growth	No growth

*: Solvent control (100 µl/plate di-H_2O)

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Section A6.6.1 _ 01 **Genotoxicity in vitro**

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3 **Ames test with Salmonella typhimurium**

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 against bacteria in the in vitro Ames test.

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

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

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 [redacted] rats). Following Salmonella typhimurium tester strains were used in this assay: TA 1535, TA 1537, TA 1538, TA 98, TA 100, TA 102 and TA 104.

First, a toxicity pre-screen test was conducted, without S9 mix, using TA 1538, TA 100 and TA 102 as tester strains. Following concentrations of the test substance were used: 50, 167, 500, 1670 and 5000 µg/plate. On the basis of the results of the pre-screen test, the test concentrations for the Ames test (original assay + retest; with and without S9 mix) were selected as follows: 0.5, 1.67, 5.0, 16.7, 50 and 100 µg/plate. The main tests were conducted in triplicate cultures with all tester strains. The test series were accompanied by a solvent control (diH_2O) and by the following positive controls:

In the absence of S9 mix:

Tester Strains	Positive control
TA 1535, TA 100	Sodium azide (10 µg/plate)
TA 1537	9-Aminoacridine (150 µg/plate)
TA1538, TA 98	2-Nitrofluorene (5 µg/plate); mitomycin C (2.5 µg/plate)
TA 104	Methylglyoxal (250 µg/plate)

In the presence of S9 mix:

Tester strains	Positive control
TA 1535, TA 1537, TA1538, TA 98, TA 100	2-Anthramine (2.50 µg/plate)
TA 102, TA 104	2-Acetylaminofluorene (20 µg/plate)

The revertant colonies were assessed by means of an Artek electronic colony counter interfaced with an IBM PC/AT computer.

Section A6.6.1 _ 01	Genotoxicity in vitro Ames test with Salmonella typhimurium	
Annex Point IIA6.6.1 / 6.6.2 / 6.6.3		
5.2 Results and discussion	Toxicity pre-screen test: The results showed that the test substance affected the bacterial growth of all tester strains at all tested concentrations, resulting in inhibition to complete absence of growth. Main assay: An inhibited growth was observed in all tester strains at 16.7, 50 and/or 100 µg/plate, with and without S9 mix. A statistically significant and dose-dependent increase in the frequency of revertants (ca. 1.2 to 3,3 fold control values) was observed for the tester strains TA 1535, TA 98, TA 100, TA 102 and TA 104 in presence of S9 mix, and for TA 98, TA 100, TA 102 and TA 104 in absence of S9 mix. Such an increase (ca. 2.3 fold control values) also was reported for TA 1537 in the presence of S9 mix, but it was neither statistically significant nor dose-dependent. Retesting: The retesting resulted in quite similar findings. In fact, an inhibited growth was observed in all tester strains at 50 and/or 100 µg/plate, with and without S9 mix. A statistically significant and dose-dependent increase in the frequency of revertants (ca. 1.7 to 2.8 fold control values) was observed for the tester strains TA 98, TA 100, TA 102 and TA 104 in presence of S9 mix, and for TA 100, TA 102 and TA 104 in absence of S9 mix. All positive and negative controls were within acceptable limits.	X
5.3 Conclusion	The results of the Ames test were indicative of the mutagenicity of [redacted] in the bacterial reverse mutation assay with S. typhimurium under the experimental conditions chosen	
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	**3.1.2** This refers to Doc IIIA Section A2.
Results and discussion	5.2 Results and discussion. A statistical analysis was not performed.
Conclusion	Genotoxicity was tested in an Ames test with seven *S.typhimurium* strains with and without metabolic activation. A dose-dependent increase in the frequency of revertants (ca. 1.2- to 3.3-fold control values) was observed as follows: • In the presence of S9 mix: TA 98, TA 100, TA 102, TA 104, TA 1535 • In the absence of S9 mix: TA 98, TA 100, TA 102, TA 104 More than 2-fold increases were seen for strains TA102 and TA104 both in the absence and presence of S9 mix. Glutaraldehyde was mutagenic in the Ames test.
Reliability	1
Acceptability	Acceptable

Section A6.6.1 _ 01 Genotoxicity in vitro

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3

Ames test with Salmonella typhimurium

Remarks	2.3 Deviations. Analyses of dosing solutions were not performed to verify the homogeneity or stability of the dosing solutions.
	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 A6.6.1 _ 02 **Genotoxicity in vitro**

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3 **Salmonella typhimurium reverse mutation assay**

			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	[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 Haworth S et al., Environ. Mutagen. 5(1): 3-142, 1983 and to Zeiger E et al., Environ. Mol. Mutagen. 19(21): 2-41, 1982)	X
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 ([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**	Bacterial reverse mutation test	
3.2.1	Organism/cell type	S. typhimurium: TA 98, TA 100, TA 1535, TA 1537, TA 102 and TA 104	

3.2.2	Deficiencies / Proficiencies	TA 98, TA 100, TA 1535, TA 1537, TA 102, TA 104 All strains had an rfa mutation (affecting membrane permeability) All strains excepted TA 102 had a uvrB deletion mutation (affecting DNA excision repair) TA 98, TA 100, TA 102 and TA 104 had a plasmid pKM101 (enhancing the error-prone DNA repair system) TA 1535 and TA 100 detect base pair substitution mutations affecting the hisG46 allele TA 98 detect frameshift mutations affecting the hisD3052 allele TA 1537 detects frameshift mutations affecting the hisC3076 allele TA 102 and TA 104 detect a wide variety of genetic damage affecting the hisG428 allele.
3.2.3	Metabolic activation system	The S9 mix was obtained by mixing a series of appropriate cofactors with the S9 liver fraction of male Sprague-Dawley rats or Syrian hamsters, which had been treated with Aroclor 1254.
3.2.4	Positive control	In the absence of S9 mix:

Tester strains	Positive control
TA 1535, TA 100	Sodium azide
TA 1537	9-Aminoacridine
TA 98	4-Nitro-o-phenylenediamine
TA 102	Mitomycin C
TA 104	Methyl methanesulfonate

In the presence of S9 mix:

Tester strains	Positive control
All strains	2-Aminoanthracene
TA 102	2-Aminoanthracene/Sterigmatocystin

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	The test substance was sent coded to 3 different laboratories (EG&G Mason Research Institute, Case Western Reserve University, Inveresk Research International) for testing of mutagenicity. Concentrations tested at the EG&G Mason Research Institute: 0 (negative control), 3.3, 10, 20, 33, 50, 75, 100, 150, 200, 333 ug/plate Concentrations tested at the Case Western Reserve University: 0, 10, 33, 100, 333, 1000, 3333 ug/plate Concentrations tested at the Inveresk Research International: 0, 25, 50, 100, 200, 300 ug/plate
3.3.2	Way of application	The test substance was incubated with each tester strain in presence or absence of S 9 mix for 20 minutes at 37 °C. Top agar supplemented with l-histidine and d-biotin was added to each test tube and the content was mixed prior to being poured onto minimal glucose agar plates. Each trial consisted of triplicate plates.

3.3.3	Pre-incubation time	None (no preincubation)
3.3.4	Other modifications	None reported
3.4	**Examinations**	The number of revertant colonies was counted after 2 days of incubation at 37 °C. The assessment of the test results, i.e. of the number of revertants was based on following definitions: A positive response was defined as a reproducible, dose-related increase in revertant colonies in any strain, in presence or absence of S9 mix. An equivocal response was defined as an increase in revertants that was not dose-related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A negative response was defined as the absence of revertant colonies.

4 RESULTS AND DISCUSSION

4.1 Genotoxicity

4.1.1 Results obtained from EG&G Mason Research Institute

TABLE D1 Mutagenicity of Glutaraldehyde in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at EG&G Mason Research Institute							
TA100	0	120 ± 6.9	116 ± 8.6	124 ± 10.4	76 ± 0.9	148 ± 4.4	122 ± 1.2
	3.3	133 ± 1.7	134 ± 3.8	126 ± 10.1		134 ± 2.4	
	10	140 ± 9.8	130 ± 10.1	132 ± 4.4	91 ± 4.7	135 ± 8.7	135 ± 2.9
	20		159 ± 7.2				
	33	192 ± 11.7	229 ± 8.4	124 ± 7.8	88 ± 8.5	178 ± 8.2	178 ± 13.3
	50		227 ± 23.6[3]				218 ± 13.6
	75						219 ± 1.8
	100	70 ± 8.6[3]		179 ± 5.5	146 ± 9.8	182 ± 12.6[3]	147 ± 11.3[3]
	150				163 ± 4.9[3]		
	200				75 ± 2.9[3]		
	333	Toxic		Toxic		75 ± 7.5[3]	
Trial summary		Equivocal	Positive	Equivocal	Positive	Equivocal	Positive
Positive control[4]		1498 ± 14.6	1949 ± 20.1	1326 ± 58.7	1337 ± 47.2	972 ± 24.8	1262 ± 69.9
TA1535	0	19 ± 2.5	19 ± 4.6	12 ± 1.5	10 ± 0.3	11 ± 3.9	11 ± 2.2
	3.3	29 ± 1.9	19 ± 1.5	10 ± 2.4	10 ± 0.7		
	10	27 ± 2.3	17 ± 0.6	10 ± 1.5	10 ± 0.9	9 ± 1.2	12 ± 1.3
	20		23 ± 1.5				
	33	22 ± 2.3	19 ± 1.3	9 ± 2.0	13 ± 2.0	9 ± 1.5	11 ± 1.8
	50		19 ± 3.0[3]				11 ± 1.5
	75						13 ± 1.3
	100	Toxic		14 ± 1.9	11 ± 0.9	9 ± 0.7[3]	13 ± 1.3
	150				10 ± 2.1		
	200				9 ± 1.7[3]		
	333	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1521 ± 10.7	1467 ± 30.2	170 ± 40.8	123 ± 17.7	38 ± 6.1	71 ± 6.4
TA1537	0	9 ± 0.9	9 ± 2.0	8 ± 1.2	11 ± 0.3	7 ± 1.0	10 ± 1.9
	3.3	8 ± 1.2	6 ± 0.0	8 ± 1.9		7 ± 0.3	
	10	11 ± 2.5	7 ± 1.2	9 ± 2.3	10 ± 1.2	8 ± 1.3	8 ± 2.3
	20		11 ± 0.7				
	33	10 ± 1.2	11 ± 0.9	8 ± 1.5	9 ± 1.7	11 ± 1.9	11 ± 0.9
	50		9 ± 2.0				9 ± 1.5
	75						8 ± 1.8
	100	Toxic		9 ± 1.0	11 ± 1.7	8 ± 1.2	15 ± 3.2
	150				15 ± 3.3		
	200				9 ± 0.9[3]		
	333	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		509 ± 84.3	447 ± 21.4	71 ± 6.1	129 ± 1.5	34 ± 8.1	125 ± 9.8

TABLE D1 Mutagenicity of Glutaraldehyde in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA98	0	25 ± 5.7	27 ± 5.6	28 ± 2.5	32 ± 2.8	27 ± 1.9	32 ± 3.2
	3.3	22 ± 0.7	28 ± 2.7	19 ± 2.1		23 ± 1.5	
	10	25 ± 1.5	32 ± 2.1	23 ± 4.3	30 ± 4.6	24 ± 1.2	28 ± 2.5
	20		30 ± 3.6				
	33	32 ± 3.3[3]	37 ± 7.4	26 ± 2.8	28 ± 3.2	34 ± 5.2	38 ± 2.7
	50		38 ± 4.1				42 ± 4.0
	75						54 ± 4.6
	100	Toxic		27 ± 2.5	28 ± 3.7	35 ± 0.9[3]	36 ± 3.8[3]
	150				44 ± 3.0		
	200				32 ± 1.5[3]		
	333	Toxic		16 ± 2.1[3]		Toxic	
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Equivocal
Positive control		2245 ± 98.6	1434 ± 19.3	1121 ± 62.3	1093 ± 20.9	469 ± 32.3	1007 ± 55.1

4.1.2 Results obtained from Case Western Reserve University

TABLE D1 Mutagenicity of Glutaraldehyde in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at Case Western Reserve University							
TA100	0	92 ± 2.8	98 ± 2.6	117 ± 20.8	113 ± 6.4	85 ± 2.7	111 ± 9.1
	10		91 ± 3.6		116 ± 0.3		175 ± 10.0
	33	99 ± 2.7	93 ± 0.6	101 ± 12.0	114 ± 6.9	95 ± 2.6	137 ± 12.5
	100	96 ± 8.5	93 ± 6.2	98 ± 2.8	160 ± 17.0	114 ± 7.1	163 ± 6.6
	333	99 ± 6.4	87 ± 5.0	130 ± 12.0	Toxic	133 ± 8.7	Toxic
	1000	Toxic	95 ± 3.5	Toxic	4 ± 4.0	65 ± 9.5	3 ± 3.3
	3333	0 ± 0.0		0 ± 0.0		0 ± 0.0	
Trial summary		Negative	Negative	Negative	Negative	Equivocal	Equivocal
Positive control		307 ± 18.1	394 ± 78.3	2397 ± 104.0	2104 ± 81.4	2363 ± 61.5	1230 ± 27.7
TA1535	0	10 ± 2.0	5 ± 1.2	9 ± 2.0	11 ± 0.6	10 ± 1.0	3 ± 0.3
	10		7 ± 0.6		9 ± 1.5		3 ± 1.2
	33	8 ± 2.7	8 ± 0.6	7 ± 0.3	9 ± 0.3	10 ± 1.3	8 ± 0.7
	100	9 ± 1.9	2 ± 0.3	7 ± 1.0	6 ± 0.9	10 ± 1.8	3 ± 0.6
	333	8 ± 1.7	2 ± 0.3	9 ± 2.5	5 ± 1.2	7 ± 0.3	3 ± 0.6
	1000	5 ± 1.5	0 ± 0.3	4 ± 1.2	2 ± 1.7	4 ± 0.3	3 ± 1.5
	3333	0 ± 0.0		0 ± 0.0		0 ± 0.0	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		97 ± 46.8	310 ± 33.8	39 ± 8.1	41 ± 3.8	37 ± 8.4	42 ± 4.3
TA1537	0	4 ± 1.2	3 ± 1.2	6 ± 2.6	8 ± 1.5	8 ± 1.9	8 ± 1.8
	10		4 ± 0.9		7 ± 2.0		5 ± 0.9
	33	2 ± 1.2	2 ± 0.3	7 ± 0.3	6 ± 0.3	7 ± 1.2	8 ± 1.2
	100	4 ± 0.9	2 ± 0.3	7 ± 2.4	3 ± 1.2	12 ± 2.3	5 ± 0.7
	333	4 ± 1.2	1 ± 0.3	6 ± 2.1	2 ± 0.9	10 ± 1.2	1 ± 0.6
	1000	1 ± 0.7	0 ± 0.3	5 ± 0.9	1 ± 0.6	8 ± 0.9	0 ± 0.3
	3333	0 ± 0.0		0 ± 0.0		0 ± 0.0	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		148 ± 19.5	72 ± 38.0	141 ± 4.4	163 ± 27.8	210 ± 58.3	72 ± 8.7
TA98	0	12 ± 1.2	11 ± 0.9	24 ± 1.5	21 ± 3.0	26 ± 1.9	17 ± 1.9
	10		13 ± 1.9		25 ± 1.8		17 ± 4.1
	33	14 ± 1.5	10 ± 0.3	23 ± 3.2	22 ± 1.8	27 ± 2.7	26 ± 2.7
	100	14 ± 1.5	7 ± 3.8	31 ± 5.5	22 ± 5.0	37 ± 11.3	13 ± 1.2
	333	15 ± 1.7	4 ± 1.2	33 ± 2.9	20 ± 2.1	43 ± 9.0	18 ± 1.5
	1000	8 ± 1.2	5 ± 1.5	19 ± 7.5	17 ± 2.7	Toxic	18 ± 0.6
	3333	0 ± 0.0		0 ± 0.0		0 ± 0.0	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		118 ± 11.8	150 ± 24.2	1775 ± 121.2	1590 ± 52.8	2141 ± 79.2	561 ± 12.0

4.1.3 Results obtained from Inveresk Research International

TABLE D1 Mutagenicity of Glutaraldehyde in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at Inveresk Research International									
TA102	0	257 ± 9.2	136 ± 10.4	—[5]	305 ± 21.0	327 ± 4.5	226 ± 27.0	279 ± 19.4	274 ± 9.0
	25	259 ± 5.0	187 ± 6.5	—	353 ± 24.8	322 ± 15.9	257 ± 21.6	346 ± 10.3	309 ± 53.3
	50	297 ± 25.9	214 ± 4.7	—	333 ± 6.5	364 ± 13.3	365 ± 5.9	394 ± 41.4	389 ± 34.3
	100	275 ± 7.3	278 ± 21.3	—	417 ± 5.0	441 ± 38.4	473 ± 27.0	485 ± 34.7	535 ± 15.2
	200	232 ± 11.3	192 ± 13.8	—	570 ± 19.7	741 ± 35.8	504 ± 58.2	379 ± 57.0	481 ± 39.8
	300	46 ± 29.7[3]	27 ± 1.8[3]	—	352 ± 17.3[3]	743 ± 23.2[3]	250 ± 24.5[3]	608 ± 8.7[3]	268 ± 68.4[3]
Trial summary		Negative	Positive	—	Positive	Positive	Positive	Positive	Positive
Positive control		634 ± 95.3	896 ± 38.0	—	443 ± 14.0	562 ± 25.8	478 ± 28.4	454 ± 3.0	497 ± 15.3
TA104	0	453 ± 13.3	338 ± 12.5	338 ± 8.7	482 ± 21.2	406 ± 7.5	—	417 ± 21.7	495 ± 15.7
	25	632 ± 10.8	321 ± 3.8	464 ± 36.1	726 ± 40.3	605 ± 36.6	—	506 ± 15.4	689 ± 50.0
	50	732 ± 13.5	452 ± 17.0	602 ± 6.6	893 ± 25.5	771 ± 19.9	—	543 ± 14.6	1003 ± 40.8
	100	1018 ± 21.1	600 ± 16.2	715 ± 22.2	1074 ± 56.5	1020 ± 21.0	—	1185 ± 121.4	1174 ± 31.8
	200	807 ± 44.3	815 ± 33.5	783 ± 28.9	754 ± 20.2	854 ± 123.9	—	667 ± 15.7	861 ± 51.0
	300	296 ± 68.7[3]	861 ± 14.4[3]	522 ± 110.3[3]	477 ± 55.0[3]	620 ± 65.0[3]	—	173 ± 23.2[3]	541 ± 107.8[3]
Trial summary		Positive	Positive	Positive	Positive	Positive	—	Positive	Positive
Positive control		232 ± 4.7[3]	653 ± 43.5	818 ± 50.4	1371 ± 21.4	1052 ± 14.9	—	1133 ± 60.4	976 ± 42.8
TA100	0	65 ± 5.5	82 ± 9.0	91 ± 3.6	84 ± 2.0	112 ± 3.6	90 ± 2.7	83 ± 1.7	94 ± 5.7
	25	106 ± 6.5	116 ± 4.9	108 ± 6.4	116 ± 6.2	122 ± 2.9	114 ± 4.3	119 ± 4.9	121 ± 4.2
	50	84 ± 7.4	135 ± 2.2	159 ± 5.4	139 ± 9.7	151 ± 1.7	146 ± 14.0	163 ± 3.5	180 ± 3.1
	100	131 ± 1.2	197 ± 27.4	330 ± 14.7	146 ± 19.5	224 ± 18.2	261 ± 8.2	255 ± 3.8	259 ± 16.7
	200	149 ± 13.0	356 ± 18.1	355 ± 35.7	151 ± 11.0	256 ± 19.6	296 ± 6.5	96 ± 9.0	177 ± 11.1
	300	89 ± 3.8[3]	152 ± 4.4[3]	117 ± 9.1[3]	90 ± 3.5[3]	158 ± 13.2[3]	86 ± 6.8[3]	85 ± 4.3[3]	133 ± 10.7[3]
Trial summary		Positive	Positive	Positive	Weakly positive	Positive	Positive	Positive	Positive
Positive control		182 ± 5.3	338 ± 12.5	456 ± 4.4	512 ± 15.5	1308 ± 105.9	1253 ± 78.9	408 ± 17.6	829 ± 38.0

[1] The detailed protocol and the data from the first two studies are presented in Haworth *et al.* (1983). The protocol for the third study (Inveresk Research International) is presented in Zeiger *et al.* (1992). 0 µg/plate is the solvent control.
[2] Revertants are presented as mean ± standard error from three plates.
[3] Slight toxicity.
[4] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), 4-nitro-o-phenylenediamine (TA98), mitomycin C (TA102), and methyl methanesulfonate (TA104). The positive control for metabolic activation with all strains was 2-aminoanthracene, and 2-aminoanthracene/sterigmatocystin was used for TA102.
[5] Trial was not performed for this strain.

4.1.4 Summary of the main results obtained from all test laboratories

Laboratory	Strain	Without S9		S9 (hamster)		S9 (rat)	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
EG&G Mason Research Institute	TA 100	+/-	+	+/-	+	+/-	+
	TA 1535	-	-	-	-	-	-
	TA 1537	-	-	-	-	-	-
	TA 98	-	-	-	+/-	-	+/-
Positive results were obtained with the tester strain TA 100, with and without S 9 mix, independently of the source of the S9 fraction (Aroclor 1254-induced hamster or rat). For TA 1535 and TA 1537, the results were unambiguously negative. For TA 98, the results almost were negative.							

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

Laboratorie	Strain	Without S9		S9 (hamster)		S9 (rat)	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Case Western Reserve University	TA 100	-	-	-	-	+/-	+/-
	TA 1535	-	-	-	-	-	-
	TA 1537	-	-	-	-	-	-
	TA 98	-	-	-	-	-	-
A small increase in revertant colonies was obtained for the tester strain TA 100 in presence of S9 mix gained from Aroclor 1254-treated rats; the results however were considered equivocal. For TA 1535, TA 1537 and TA 98, the results were unambiguously negative.							

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

Laboratorie	Strain	Without S9			S9 (hamster)			S9 (rat)	
		Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2
Inveresk Research International	TA 102	-	+	NP	+	+	+	+	+
	TA 104	+	+	+	+	+	NP	+	+
	TA 100	+	+	+	+ (weak)	+	+	+	+
The results were clearly positive for all tester strain, with and without S 9 mix, independently of the source of the S9 fraction (Aroclor 1254-induced hamster or rat).									

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

Section A6.6.1 _ 02		**Genotoxicity in vitro**
Annex Point IIA6.6.1 / 6.6.2 / 6.6.3		**Salmonella typhimurium reverse mutation assay**
4.2	**Cytotoxicity**	EG&G Mason Research Institute: Glutaraldehyde 50% was tested at concentrations ranging from 0 to 333 µg/plate; cytotoxicity was reported from 100 µg/plate upwards. Case Western Reserve University: Glutaraldehyde 50% was tested at concentrations ranging from 0 to 3333 µg/plate; Cytotoxicity was reported from 333 µg/plate upwards. Inveresk Research International: Glutaraldehyde 50% was tested at concentrations ranging from 0 to 300 µg/plate; no cytotoxicity was observed.

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 bacterial reverse mutation test using Salmonella typhimurium as test organism.

Test substance: Glutaraldehyde 50% aqueous solution, [redacted]% glutaraldehyde (minor contamination from the polymeric forms of glutaraldehyde and other volatile impurities)

The test was conducted according to Haworth S et al., Environ. Mutagen. 5(1): 3-142, 1983 and to Zeiger E et al., Environ. Mol. Mutagen. 19(21): 2-41, 1982); it was not recognizable whether the tests were conducted in accordance with GLP or not.

The test substance was sent coded to 3 different laboratories (EG&G Mason Research Institute, Case Western Reserve University, Inveresk Research International) for testing of mutagenicity. 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 Institute, the test concentrations were as follows: 0, 3.3, 10, 20, 33, 50, 75, 100, 150, 200, 333 ug/plate; at the Case Western Reserve University, the test concentrations were: 0, 10, 33, 100, 333, 1000, 3333 ug/plate, and at the Inveresk Research International the test concentrations were 0, 25, 50, 100, 200, 300 ug/plate.

The assessment of the test results, i.e. of the number of revertants was based on following definitions: (1) a positive response was defined as a reproducible, dose-related increase in revertant colonies in any strain, in presence or absence of S9 mix; (2) an equivocal response was defined as an increase in revertants that was not dose-related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity; (3) a negative response was defined as the absence of revertant colonies. The test series were accompanied by following positive controls:

In the absence of S9 mix: Sodium azide (TA 1535, TA 100), 9-Aminoacridine (TA 1537), 4-Nitro-o-phenylenediamine (TA 98), Mitomycin C (TA 102), Methyl methanesulfonate (TA 104).

In the presence of S9 mix: 2-Aminoanthracene (all strains), 2-Aminoanthracene/Sterigmatocystin (TA 102).

5.2	**Results and discussion**	Summary of the results obtained at the EG&G Mason Research Institute: Positive results were obtained with the tester strain TA 100, with and without S 9 mix, independently of the source of the S9 fraction (Aroclor 1254-induced hamster or rat). For TA 1535 and TA 1537, the results were unambiguously negative. For TA 98, the results almost were negative. Cytotoxicity was reported from 100 µg/plate upwards. Summary of the results obtained at the Case Western Reserve University: A small increase in revertant colonies was obtained for the tester strain TA 100 in presence of S9 mix gained from Aroclor 1254-treated rats; the results however were considered equivocal. For TA 1535, TA 1537 and TA 98, the results were unambiguously negative. Cytotoxicity was reported from 333 µg/plate upwards. Summary of the results obtained at Inveresk Research International: The results were clearly positive for all tester strain, with and without S 9 mix, independently of the source of the S9 fraction (Aroclor 1254-induced hamster or rat). No cytotoxicity was reported.
5.3	**Conclusion**	The results were indicative of a genotoxic potential for glutaraldehyde 50% in the bacterial reverse mutation assay with S. typhimurium under the experimental conditions chosen.
5.3.1	Reliability	**2**
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	**3.1.2** This refers to Doc IIIA Section A2.
Results and discussion	The combined overall results of the tests in different laboratories are presented in Table 6.6.1/02-01, added by RMS.
Conclusion	Glutaraldehyde tested clearly positive in TA102, TA104 and TA 100, and negative in TA 98, TA 1535 and TA 1537. All results were independent of metabolic activation.
Reliability	3
Acceptability	Acceptable
Remarks	Detailed protocols and data were not provided for assessment.

Section A6.6.1 _ 02 **Genotoxicity in vitro**

Annex Point IIA6.6.1 / 6.6.2 / 6.6.3 **Salmonella typhimurium reverse mutation assay**

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

Table 6.6.1/02-1 Mutagenicity of glutaraldehyde in Salmonella typhimurium (added by RMS)

Strain	Dose (ug/plate)	Laboratorie	Results	
			with S9-mix	without S9-mix
TA 100	0 - 333	EG&G Mason Research Institute	positive	positive
TA 1535	0 - 333	EG&G Mason Research Institute	negative	negative
TA 1537	0 - 333	EG&G Mason Research Institute	negative	negative
TA 98	0 - 333	EG&G Mason Research Institute	negative	negative
TA 100	0 - 3333	Case Western Reserve University	equivocal	negative
TA 1535	0 - 3333	Case Western Reserve University	negative	negative
TA 1537	0 - 3333	Case Western Reserve University	negative	negative
TA 98	0 - 3333	Case Western Reserve University	negative	negative
TA 102	0 - 300	Inveresk Research International	positive	positive
TA 104	0 - 300	Inveresk Research International	positive	positive
TA 100	0 - 300	Inveresk Research International	positive	positive