Table A6_2(4)-3 [^{14}C]-Glutaraldehyde fraction of applied dose in effluents

Table 3

Glutaraldehyde: Species Comparisons of In Vitro Skin Penetration Following a Single Application to the Excised Skin of Humans, [redacted] Rats, [redacted] Mice, [redacted] Guinea Pigs and [redacted] Rabbits

Fraction of Applied Dose Recovered in Effluents After Application of a 0.75% and 7.5% Glutaraldehyde Solution

		0.75% Dose		7.5% Dose	
Species	Sex	% Recovered	Dose Absorbed/Surface Area (mg/cm^2)	% Recovered	Dose Absorbed/Surface Area (mg/cm^2)
Rat	Male	0.06 ± 0.05	0.001 ± 0.001	0.08 ± 0.04	0.01 ± 0.004
	Female	0.05 ± 0.01	0.001 ± 0.0001	0.33 ± 0.10	0.04 ± 0.01
Mouse	Male	1.73 ± 1.65	0.02 ± 0.02	0.39 ± 0.14	0.04 ± 0.01
	Female	0.26 ± 0.04	0.003 ± 0.001	1.43 ± 1.10	0.15 ± 0.12
Guinea Pig	Male	0.53 ± 0.69	0.01 ± 0.01		
	Female	0.17 ± 0.16	0.002 ± 0.002		
Rabbit	Male	0.77 ± 0.20	0.01 ± 0.002	0.85 ± 0.71	0.09 ± 0.08
	Female	0.34 ± 0.11	0.004 ± 0.001	1.55 ± 2.09	0.16 ± 0.22
Human	Female	0.16 ± 0.14	0.002 ± 0.002	0.20 ± 0.08	0.02 ± 0.01

WPC/kam/1916K
03/11/91

Section A6.2(5) Metabolism studies in mammals

Annex Point IIA6.2

IUCLID 7.1.1/05

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2007), Glutaraldehyde: Identification of Metabolites in the [redacted] Rat, [redacted], 29 August 2007 (Also refer to Migneault I, Dartiguenave C, Bertrand M J, Waldron K C (2004) Glutaraldehyde: behaviour in aqueous solution, reaction with proteins and application to enzyme crosslinking, Biotechniques 37:790-802)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Chemical Company[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	USEPA, OPPTS 870.7485 (1998) OECD, Guideline 417 (1984) EEC, Part B.36 (1986)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Radiolabelled and non-radiolabelled glutaraldehyde were used for this study.	
3.1.1	Radiolabelled test material	The following radiolabelled test material was obtained from [redacted] [redacted] Lot no. [redacted] • Chemical name: Glutaraldehyde-[2,4-^{14}C] • Concentration of active ingredient in the solution: 0.878 mg/g • Radiochemical purity: [redacted] • Specific activity 57.5 µCi/mg	X
3.1.2	Non radiolabelled test material	The following non-radiolabelled test material was obtained from [redacted] [redacted] Lot no. [redacted] • Chemical name: Glutaraldehyde • Purity: [redacted]	
3.1.3	Reference Standards	None	
3.2	**Test Animals**	Male and female [redacted] rats [redacted], were obtained from [redacted] All animals were acclimatised to the laboratory environment for at least one week and examined by a laboratory veterinarian prior to entry into the study.	

Section A6.2(5) **Metabolism studies in mammals**

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		At commencement of dosing the animals were approximately 8 weeks old and weighed 200-250g (males) and 160-200g (females).	
3.2.1	Control animals	No	X
3.3	**Administration/ Exposure**	Oral (gavage)	
3.3.1	Dosing regime	Group 1: single oral dose at 5 mg/kg (3M + 3F) Group 2: single oral dose at 75 mg/kg (3M + 3F)	
3.3.2	Dose preparations	Non-radiolabelled test material was added to radiolabelled test material in 0.01N sulphuric acid, this was made up to a final volume with tap water. The formulation was sonicated and stirred to produce a homogeneous preparation. A target dose of 10 mL of dose formualtion/kg bw was used.	
3.3.3	Analysis of dose preparations for radiopurity, homogeneity, and stability	Confirmation of the test material concentration and homogeneity of the dose solutions was conducted according to the standard operating procedures of the Analytical Chemistry Laboratory using liquid scintillation spectroscopy (LSS). Actual values were within 5% of the target values.	
3.4	**Examinations**	*Non entry field*	
3.4.1	Antemortem examinations	Animals were observed once daily for mortality, moribundity, general health and appearance.	
3.4.2	Body weight	Individual body weights were recorded at the time of dosing.	
3.4.3	Sample collection for radioanalysis	<u>Antemortem Sample Collection For Radioanalysis</u> The following samples were collected for radio analysis: **Expired Air (Groups 1 and 2)** Expired air was passed through a solution of monoethanolamine:1-methoxy-2-propanol (3:7,v/v) to trap expired $^{14}CO_2$. One sample was collected at 24 hours. **Urine (Groups 1 and 2)** urine was collected in dry-ice cooled traps at 12, 24 and 48 hours postdose. The weight of each sample was recorded, pooled samples (by timepoint, dose and sex) were prepared and all individual and pooled samples were stored at -80°C. **Faeces (Groups 1 and 2)** faeces were collected in dry-ice cooled containers at 24 and 48 hours postdose. An aqueous homogenate (*ca* 25% w/w) was prepared and weighed aliquots of these homogenates were oxidised and quantified for radioactivity by LSS. Pooled samples were prepared as described above and all samples were stored as described above. <u>Terminal Sacrifice And Collection For Radioanalysis</u> **Terminal Sacrifice and Collection** After the last excreta collection at 48 h post dose, each animal was sacrificed. **Cage Wash (Groups 1 and 2)** Cages were washed. The weight of each sample was recorded and the sample analysed by LSS. <u>Radioanalysis methodology</u> Aliquots of liquid samples (urine, CO_2 trap fluid and cage wash) were mixed with scintillation cocktail and analysed for radioactivity	

without any additional treatment.

Faeces were suspended in distilled water.

Radioactivity was quantified in a liquid scintillation spectrometer equipped with ^{14}C standards to monitor the performance of the LSS.

3.4.4 Sample preparation for metabolite identification

Urine

Pooled urine samples were acidified with glacial acetic acid to 1% (v/v); vortex mixed, centrifuged and analysed by HPLC.

Pooled urine samples from female rats (Group 1) dosed *via* ^{14}C-glutaraldehyde-fortified drinking water at 50 and 1000 ppm were also analysed ([redacted], 2007, TNG Summary 6.2(3)) as detailed above.

Faeces

Pooled faecal homogenates (weighed aliquot of *ca* 0.5g) were extracted with 2 x 5 mL of milli Q water and 1 x 5 mL of acetonitrile:water (1:1, v/v). The extracts were combined and concentrated under a stream of nitrogen prior to analysis by HPLC.

Pooled faecal samples from female rats (Group 1) dosed *via* ^{14}C-glutaraldehyde-fortified drinking water at 50 and 1000 ppm were also analysed ([redacted], 2007, TNG Summary 6.2(3)). The homogenates were centrifuged and the supernatant was taken for analysis.

3.4.5 Analytical methods

Mass spectroscopy

Urine and faecal extracts from the oral gavage study (Zhang *et al*, 2007) were analysed using liquid chromatography/positive electrospray ionisation/mass spectroscopy (LC/PESI-MS) full scan mode and LC/PESI-MS/MS neutral loss scan modes. Glutaric acid was analysed as a metabolite in the LC/PESI-MS/MS multiple reaction monitoring (MRM) scan mode. Urine and faecal homogenates from the drinking water study were not analysed *via* MS.

Sodium dodecyl sulphate polyacrylaminde gel electrophoresis (SDS-PAGE)

Glutaraldehyde has been shown to crosslink with proteins (Migneault *et al* (2004), Ranly and Lazzari (1983), Ranly and Horn (1990)). To explore the possibility that glutaraldehyde or glutaraldehyde derived metabolites may react with proteins *in vivo*, Sodium dodecyl sulphate polyacrylaminde gel electrophoresis (SDS-PAGE) analysis was used to determine the molecular weight range of those various metabolite-HPLC fractions collected from faecal and urine samples from oral gavage-dosed rats. In order to increase the detection of the metabolites/bands of interest (potential metabolites and radioactive bands greater than 5% of the administered dose), 2 female rats were orally dosed with a high radioactivity (*ca* 470 µCi/kg) dose of 50 mg 14C-glutaraldehyde/kg bw and excreta collected for 24 hours. Urines were prepared and the faeces extracted as previously described above. Multiple HPLC injections were made for each matrix and respective fractions of interest were combined and concentrated. The samples were analysed by SDS-PAGE followed by PVDF (polyvinylidene difluoride) membrane transfer, and radioactivity bands were analysed by Kodak Autoradiography BioMax MS film with BioMax TranScreen LE intensifying screen system with up to 18 days

exposure.

3.4.6 Analytical conditions

Representative analytical instrument and conditions used in metabolite profiling and identification from excreta samples from animals treated by oral gavage.

LC-ARC[1]-Stop Flow and Fraction Collection Conditions:
LC System: Agilent 1100 Series (pump, autosampler, UV detector)
LC-ARC Stop Flow System: AIM Research Company, Hockessin, Delaware (S/N: A11748)
[1] = accurate radioisotope counting

LC Conditions

Guard column:	YMC ODS-AQ S-5, 4 x 20 mm; 120A
Column:	YMC ODS-AQ 4.6 x 250 mm; 5µm
Flow Rate:	1.0 mL/min
Mobile phase:	A = 0.05% trifluoroacetic acid (aq) B = 0.05% trifluoroacetic acid in acetonitrile Gradient

Time	Conditions
0 min	100/0 A/B
0-15 min	Ramp to 98/2 A/B
15-40 min	Ramp to 85/15 A/B
40-50 min	Ramp to 60/40 A/B
50-55 min	Ramp to 5/95 A/B
55-60 min	Hold at 5/95 A/B
60-60.5 min	Ramp to 100/0 A/B
60.5-65 min	Hold at 100/0 A/B

UV detection wavelength:	Not reported
Fraction collector:	Foxy 200 (ISCO, Lincoln, NE, S/N 204J20013); fraction size 30 sec

ARC Conditions

Stop-Flow Mode:	Fraction: (level 4, fraction size: 12 sec)
Counting mode:	By time (40 sec)
Background threshold (cpm):	25, background counting time (40 sec)
Count Zone:	0-65 min
Scintillant addition mode:	By Count Zone (0-65 min)

Scintillant/Eluate ratio:	1.5
Waste threshold (cpm):	56.19
Delay volume:	0.00
Radioactive detector:	IN/US (S/N: 1004203)

Representative analytical instrument and conditions used for glutaric acid metabolite identification.

LC/ESI/MS-MS Conditions:
LC System: Agilent 1100 Series (pump and autosampler) and Sciex API 3000 MS/MS System

LC Conditions

Guard column:	YMC ODS-AQ S-5, 4 x 20 mm; 120A
Column:	YMC ODS-AQ 4.6 x 250 mm; 5µm
Flow Rate:	1.0 mL/min (20% into the mass spectrometer)
Mobile phase:	A = 0.05% trifluoroacetic acid (aq) B = 0.05% trifluoroacetic acid in acetonitrile Gradient

Time	Conditions
0 min	100/0 A/B
0-15 min	Ramp to 98/2 A/B
15-40 min	Ramp to 85/15 A/B
40-50 min	Ramp to 60/40 A/B
50-55 min	Ramp to 5/95 A/B
55-60 min	Hold at 5/95 A/B
60-60.5 min	Ramp to 100/0 A/B
60.5-65 min	Hold at 100/0 A/B

UV detection wavelength:	Not reported

MS Conditions

Source temp:	450°C
Curtain gas (N_2):	12 (arbitrary units)
GAS 1 (N_2):	27 (arbitrary units)

GAS 2 (N_2):	31 (arbitrary units)
Ion spray voltage:	-750 V
DP:	-41 V
EP:	-10 V
CE:	-16 V
CXP:	-11 V
Mode:	Multiple Reaction Monitoring (MRM) (positive ions) Glutaric acid: 131 (precursor ion); 87.0 (product ion); dwell time 200 msec

Analysis using SDS-PAGE

Refer to **Table 6.2(5)-4.**

4 RESULTS AND DISCUSSION

4.1 Dose administered

The concentration of GDA and radioactivity in each of the oral dose solutions is shown in Table 6.2(5)-1. The target concentrations of GDA in distilled water dose solutions were 0.5 and 7.5 mg/g; however, the actual concentrations of the dose solutions were 0.5 and 7.27 mg/g. Due to non-linearity of the low standards, the concentration of the low dose solution (5 mg/kg bw) was not determined analytically and was reported at its nominal concentration. Since the purpose of this study was to generate excreta samples for determination and identification of metabolites, using the nominal concentration for the low dose did not impact the results of the study.

The amounts of GDA administered to individual animals in each dose group are presented in Table 6.2(5)-2. The males averaged 5.34 ± 0.06 and 78.6 ± 0.35 mg/kg for the low and high dose groups, respectively. The females averaged 5.33 ± 0.04 and 78.9 ± 0.70 mg/kg for the low and high dose, respectively.

In the drinking water study (Phase I), females (N=4) averaged 8.93 ± 0.60 and 102 ± 40.5 mg/kg for the 50 and 1000 ppm fortified drinking water groups, respectively ([redacted], 2007).

4.2 Excretion pattern

The percentage of radioactivity recovered in different matrices is summarized in Table 6.2(5)-3. During the 2-day collection period for this non-balance study, the average recovery of the administered dose in the combined excreta was between 34 and 58% with 7-11% excreted in urine and 24-47% eliminated in faeces. The low and high doses presented similar excretion patterns in urine with most of the radioactivity being excreted in the first 12 hour urine sample collected post dosing. These total radioactivity recoveries in excreta are fairly comparable with data from a biokinetics studies for ^{14}C-GDA ([redacted], 2004 TNG Summary 6.2(1)), in which 9-14 and 43-59 % of the administered dose were recovered in the urine and faeces of male and female [redacted] rats administered 5 or 75 mg/kg ^{14}C-GDA/kg bw.

$^{14}CO_2$ recoveries of 2-3% of the administered dose were lower than the 19-29% recovered in the [redacted] study ([redacted]

2004), metabolism cage design may have contributed to the low recoveries and were discussed in [redacted] (2007). However, as the objective of this study was to explore the metabolite profile and identification from excreta samples, lower CO_2 recoveries did not affect the results of this study.

Single Low Male Dose (Nominal Dose 5 mg/kg)

For the single low male dose group, recoveries were of 27.28%, 7.18% and 0.08% of the administered dose in faeces, urine, and cage wash, respectively (Table 6.2(5)-3a). Radioactivity in tissues was not determined. In the urine, most of the radioactivity was excreted from rats during the first 12 hours.

Single Low Female Dose (Nominal Dose 5 mg/kg)

For the single female low dose group, recoveries were 23.61%, 7.52%, and 0.15% of the administered dose in faeces, urine, and cage wash, respectively (Table 6.2(5)-3b). In the urine, the majority of the radioactivity was excreted from rats during the first 12 hours.

Single High Male Dose (Nominal Dose 75 mg/kg)

For the single male high dose group, recoveries were 43.75%, 9.09%, and 0.32% of the administered dose in faeces, urine, and cage wash, respectively (Table 6.2(5)-3a). In the urine, most of the radioactivity was excreted from rats during the first 12 hours. Elimination via urine and faeces (as percent of administered dose) was higher than that from low single dose rats. Radioactivity in the tissues was not determined.

Single High Female Dose (Nominal Dose 75 mg/kg)

For the single female high dose group, recoveries were 47.10%, 10.50%, and 0.37% of the administered dose in faeces, urine, and cage wash, respectively (Table 6.2(5)-3b). These data are similar to the single dose male group. In the urine, most of the radioactivity was excreted from rats during the first 12 hours. The majority of the radioactivity in the feces of the low dose rats was excreted during the first 24 hours post-dosing, while the radioactivity measured in the high dose feces was essentially split equally between the 0-24 and 24-48 hours. Radioactivity in tissues was not determined.

4.3 Metabolite identification

Metabolic profiles were determined for excreta samples from male and female rats orally dosed with either 5 or 75 mg ^{14}C-glutaraldehyde/kg bw. In addition, metabolic profiles were also determined for excreta from female rats dose orally *via* drinking water at 50 or 1000 ppm ([redacted] 2007, TNG Summary 6.2(3)).

Radiolabeled components in the pooled urine samples were separated using HPLC and detected with an ARC stop-flow radiochemical detection system in the 12-second fraction mode. Detection of radiolabeled peaks in extracts of pooled fecal samples was performed using LSS analysis of 30-second eluent fractions. The DPM values produced from the stop-flow analyses or LSS analyses were converted into chromatographic data files (reconstructed) compatible with the laboratory data system (Agilent Chem Station). Metabolic profiles were then produced by manual integration of those LSS-derived reconstructed chromatograms. The resulting percent of injected radioactivity for each peak were multiplied by the respective percent of administered dose to

X

determine the respective percent of administered dose for each peak. For the time intervals not analysed, the percent of injected activity from the previous time period for the respective metabolites were multiplied by the respective percent of administered dose in the non-analysed time period. The limit of detection was set at 0.5% of the administered dose.

Urine: Oral Gavage Study (Table 6.2(5)-5 and Figure 6.2(5)-1)

Six-radioactive peaks (A, B, C, D, E and F) were detected in urine at greater than *ca* 0.5% of administrated in the oral gavage study. No time point, dose level, route of administration, or sex contained all six peaks. Only Peak A was detected in all the samples and ranged from 5 to 6% of the administered dose in urine.

Urine: Drinking Water Study (Table 6.2(5)-5 and Figure 6.2(5)-1)

Peak A and Peak B were the only peaks detected at greater than 0.5% of the administered dose and ranged from 6-8 and 4-5%, respectively, for the 50 and 1000 ppm glutaraldehyde fortified drinking water.

Faeces: Oral Gavage Study (Table 6.2(5)-6 and Figure 6.2(5)-2)

A total of four radioactive peaks (A, B, D and E) were detected in faeces at greater than 0.5% of administered dose. No time point, dose level or sex contained all four peaks. Only Peak A was detected in all the samples and ranged from 1 to 2% of the administered dose in the faeces.

In addition to these faecal metabolism peaks, there were also 2 bands of radioactivity in the faecal samples for both sexes and dose levels. The first band extends from 20 to *ca* 30 minutes and the second is from *ca* 30 to 50 min. These were designated Band 1 and Band 2.

Faeces: Drinking Water Study (Table 6.2(5)-6 and Figure 6.2(5)-2)

Only Peak A, Peak D and Peak E were detected in the female faeces from both drinking water dose groups (50 and 1000 ppm) and ranged from 1-3 % of the administered dose. No other peaks were detected at greater than 0.5% of the administered dose. The 24-48 hour faeces (both dose groups) were not analysed. The percent of administered dose in this time period was determined by multiplying the percent of injected activity for the peaks in the 0-24 hour faeces by the percent of administered dose in the 2-48 hour faeces.

In addition to these faecal metabolism peaks, there were also 2 bands of radioactivity in the faecal samples for both sexes and dose levels. The first band extends from *ca* 20 to 39 minutes and the second is from *ca* 39 to 55 minutes. These are designated as Band 1 and Band 2. Band 1 and Band 2 accounted for 8 and 16, and 8 and 27% of the administered dose for the 50 and 1000 mg/kg bw dose, respectively. The broad-radioactive bands (Band 1 and Band 2) likely consist of many unresolved peaks containing glutaraldehyde or glutaraldehyde derived metabolites forming complexes with extraneous and/or endogenous proteins, peptides and amino acids present in the faeces. These data are consistent with Mingeault *et al.* (2004) that determined glutaraldehyde as one of the most effective protein crosslinking reagents. This crosslinking functionality was further demonstrated when control rat faecal homogenate was incubated with ^{14}C- glutaraldehyde and the resulting HPLC profile

showed similar bands of radioactivity in the retention time region of Bands 1 and 2 (Figure 6.2(5)-2).

Metabolite Identification and Molecular Weight Determination

Peak A was detected at greater than 5% of the administered dose in the urine from the orally gavage dosed and fortified drinking water administered rats. Band 1 and Band 2 were detected at greater than 5% of the administered dose in the faeces via both administered routes (Table 6.2(5)-5).

Peak A eluted in the solvent front of the reversed-phase HPLC system, indicating this metabolite is a very polar compound. Various ESI-LC/MS (liquid chromatography electrospray mass spectrometry) or LC/MS-MS (liquid chromatography-electrospray tandem mass spectrometry) analyses were utilized to attempt to obtain a mass spectrum of this metabolite, but no meaningful data were obtained.

Peak E ranged from 1-3% of the administered dose in both the low and high dose urine of the orally gavaged rats. It was also determined to range from 3-6 and 1-3% of the administered dose in the urine and faeces, respectively, in the 50 and 1000 ppm fortified drinking water study. Peak B was found to have the same retention time as an authentic standard of glutaric acid. Multiple-reaction monitoring (MRM) LC/MS/MS analysis of Peak B (female urine, 75 mg/kg bw) showed an MRM response at the same retention time as a standard of glutaric acid (Figure 6.2(5)-4). These data support the identification of Peak B as glutaric acid, Peak E from oral male high dose urine was also analysed *via* by this technique but the presence of glutaric acid was not confirmed. This may be due to the glutaric acid levels being below the limit of detection in the male urine or the peak may also consist of other metabolites. HPLC-MRM analysis also confirmed the formation of glutaric acid after control urine was spiked with ^{14}C-glutaraldehyde (Figure 6.2(5)-4). No glutaric acid was observed in control urine.

As discussed above, the broad HPLC radioactive Bands 1 and 2 were not fully resolvable under the HPLC conditions. In consideration of the high reactivity of GDA with amino functionalities on proteins (Migneault *et al.* 2004), it was postulated that these radioactive-metabolite areas, and perhaps metabolite Peak A, resulted from reaction of GDA with endogenous proteins, peptides or amino acids in the rat, either systemically or present in the excreta. To investigate these possibilities, aliquots of control urine or control faecal homogenates (see Figure 6.2(5)-1 and 6.2(5)-2) were fortified with ^{14}C-GDA and analysed by HPLC with radiochemical detection. These fortified samples were metabolically profiled for comparative purposes only; no mass spectroscopy or SDS-PAGE analyses were conducted.

The results of the urine-fortification experiment showed that the glutaraldehyde can form several adducts with endogenous components (possible proteins, peptides, or amino acids) in control urine, and that the early eluting peak in this fortified sample has a retention time consistent with that of metabolite Peak A (Figure 6.2(5)-1). In a similar fashion, control faecal homogenate-fortification experiments with ^{14}C-GDA also contains a radiolabeled component that co-elutes with Peak A (Figure 6.2(5)-2). These results indicate that peak A may be a very polar adduct of the test

material and one or more extraneous or endogenous proteins, peptides, or amino acids.

The reconstructed profile of the control faeces spiked with ^{14}C-glutaraldehyde also contained a broad band of radioactivity that extending from 20 to 45 minutes (Figure 6.2(5)-2). This band of activity is similar in retention time to the radioactive areas (Bands 1 and 2) seen in the faeces of the oral and drinking water animals. These data would be consistent with adducts of the test material and one or more endogenous proteins, peptide, or amino acids.

To better characterize the molecular weight range of urinary/faecal metabolites Peak A, Peak B, Band 1, and Band 2, these HPLC fractions were isolated from urine and faecal samples from two additional female rats, orally administered ^{14}C-GDA (50 mg/kg) containing a higher level of radiotracer (*ca* 454 µCi/kg bw). These fractions were concentrated then analysed using SDS-PAGE with auto radiographic detection (see Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). As shown in Figure 6.2(5)-3, faecal Peak A (Lane 3) and urinary Peak A (Lane 10) have apparent molecular weights below 2.5 kDa. Urine Peak B and the faecal retention-time range of 10-20 minutes area were included in the SDS-PAGE analysis even though both contained less than 5% of the administered dose (Lane 11 and Lane 4, respectively, Figure 6.2(5)-3) and were determined to have an apparent molecular weight less than 2.5 kDa. These data are consistent with previous urine dialysis results in which Ranly and Horn (1990) demonstrated that 97% of the glutaraldehyde metabolites were less than 1 kDa in size. These authors postulated that this test material is highly conjugated with amino acids or small peptides.

Bands 1 and 2 were also found to be of a low molecular weight range of < 2.5 kDa using SDS-PAGE separation of concentrated HPLC collected fractions (Figure 6.2(5)-3). Although no further characterization data were obtained for these band areas, the wide HPLC retention time of Bands 1 and 2 are consistent with metabolites as a mixture of various adducts of GDA with endogenous proteins, peptides, or amino acids.

In this study, glutaraldehyde has been shown to form very complex radioactive metabolite profiles in both urine and faecal samples in [redacted] rats. These metabolites can be formed by either direct metabolism of glutaraldehyde or indirect metabolism of protein, peptide, or amino acid related adducts of glutaraldehyde. This unusual metabolism can be attributed to the complex reactivity of glutaraldehyde described by Mingeault, *et al.* 2004.

Urine and faecal samples from female [redacted] rats presented with 50 and 1000 ppm of fortified glutaraldehyde drinking water (Hansen, *et al*, 2007) were not analysed using MS or SDS-PAGE.

The proposed metabolic pathway is presented in Figure 6.2(5)-5.

4.4 Statistics

Descriptive statistics were used (i.e. mean ± standard deviation). All calculations in the database were conducted using Microsoft Excel spreadsheets (V.11, Redmond, WA, USA) and databases in full precision mode (15 digits of accuracy).

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.2(5) Metabolism studies in mammals

Annex Point IIA6.2

IUCLID 7.1.1/05

5.1	**Materials and methods**	Three male and three female [redacted] rats were orally dosed with a single administration of either 5 or 75 mg ^{14}C-glutaraldehyde/kg bw and excreta were collected for HPLC profiling and possible metabolite identification. Excreta samples (female rats dosed at 50 or 1000 ppm *via* drinking water) from another study ([redacted] 2007, TNG Summary 6.2(3)) were also profiled using HPLC for comparison.
5.2	**Results and discussion**	There were a total of six and two peaks in the urine of the oral gavage and fortified drinking water rats, respectively. There were a total of four and three peaks in the faeces of the oral gavage and fortified drinking water rats, respectively, in addition to 2 broad bands of radioactivity from each route of administration. Peak A eluted at the solvent front of the HPLC and was the only peak detected in all urine/faecal samples in both sexes, dose levels and route of administration and ranged from 5-8% and 1-3% of the administered dose in the urine and faeces, respectively. Attempts to idetnify Peak A by LC/MS were unsuccessful. Two broad bands of radioactivity (Bands 1 and 2) were detected in the faeces for all doses, sexes and routes of administration and accounted for 8-16% and 8-30% of the administerd dose, respectively. Peak E was identified as glutaric acid by HPLC retention-time match and MS comparison with an authentic standard in the female high dose urine. Peak E ranged from 1-3% and 3% of the administered dose in the oral gavage urines from both dose levels and sexes and in the urine of the 1000 ppm fortified drinking water study. Peak E was only seen in the faeces of the 5 mg/kg oral gavage dosed female rats and the 50 and 1000 ppm fortified drinking water study (1-3% of the administered dose. Control samples: control urine was fortified with ^{14}C-glutaraldehyde and HPLC analysis yielded a peak with a retention time comparable to Peak A along with many other peaks. Control faecal homogenate was fortified with ^{14}C-glutaraldehyde and incubated for 5 h at 37°C, this yielded a HPLC profile similar to the oral gavage and drinking water samples. The HPLC profiles are consistent with the complex reactivity of glutaraldehyde by either direct metabolism of glutataldehyde or indirect metabolism of protein, peptide or amino acid related adducts of glutaraldehyde as described by Mingeault *et al*, 2004. Molecular weight determination by SDS-PAGE indicated that the molecular weight of Peak A was <2.5kDa which is consistent with previous urine dialysis results which demonstrated that 97% of the glutaraldehyde metabolites, even though they were excreted as native molecules, as conjugated forms, or linked to amino acids or small peptides were less than 1 kDa in size. Bands 1 and 2 were shown to be <2.5 kDa in size using SDS-PAGE and are most likely made up of many peaks that are glutaraldehyde or glutaraldehyde derived metabolites cross-linked with extraneous or endogenous proteins, peptides or amino acids and none greater than 5% of the administered dose. The proposec metabolic pathway is presented in Figure 6.2(5)-5.
5.3	**Conclusion**	Glutaraldehyde is one of the most effective cross-linking reagents available due to its high reactivity towards proteins, hence, the difficulty in identifying metabolites.

Section A6.2(5) Metabolism studies in mammals

Annex Point IIA6.2

IUCLID 7.1.1/05

5.3.1	Reliability	1
5.3.2	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 29th, 2010
Materials and Methods	2.3 Deviations. The following exceptions to GLP were reported, which do not affect the quality of the results: 1. The specific activity of the ^{14}C-glutaraldehyde test material was calculated and was not determined via GLP analysis; 2. Characterization of glutaraldehyde was performed concurrently with the study; 3. Radiochemical purity of the ^{14}C-glutaraldehyde and characterization were performed concurrently with the study. 3.1.1 Radiolabelled test material. Specific activity was 50.49 µCi/g (calculated). 3.2.1 Control animals. A single male served as the control animal.
Results and discussion	4.3 Metabolite identification. Correction in the paragraph starting "Peak E ranged from 1-3%...": this whole paragraph concerns Peak E, and all references to Peak B actually refer to Peak E. It is noted that the proposed metabolic pathway presented in Figure 6.2(5)-5 lacks details and cannot be considered a relevant proposal.
Conclusion	Attempts to identify metabolites have been unsuccessful, with the exception of identifying Peak E as glutaric acid.
Reliability	1
Acceptability	Acceptable
Remarks	Please note that the analytical conditions and tabulated numerical results have not been checked in detail by the RMS.
	COMMENTS FROM
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

RMS note 23 December 2010: The tables and figures below have been deleted to reduce the size of the file. These will be present in the final study summary in the form presented by the applicant.

Section A6.3.2 Annex Point IIA, VI.6.3 IUCLID 5.4/09	Repeated dose toxicity Repeated dose toxicity, dermal (28 days)	
	1 REFERENCE (A6.3.2/01)	Official use only
1.1 Reference	[redacted] (1994) Glutaraldehyde: Twenty-Eight Day Repeated Cutaneous Dose Toxicity Study in [redacted] Rats, [redacted], Unpublished, 26 May 1994	
1.2 Data protection	Yes	
1.2.1 Data owner	The Dow Chemical Company	
1.2.2 Companies with letter of access	[redacted]	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes US EPA FIFRA 82-2	X
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	Glutaraldehyde, 50% [redacted]	
3.1.1 Lot/Batch number	[redacted]	
3.1.2 Specification	Not reported	
3.1.2.1 Description	Clear, colorless, low viscosity liquid	
3.1.2.2 Purity	[redacted]	
3.1.2.3 Stability	Assumed to be stable under typical storage conditions.	
3.2 Test Animals		
3.2.1 Species	Rat	
3.2.2 Strain	[redacted]	
3.2.3 Source	[redacted]	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	Approximately 6 weeks of age Weight range (males) 117.8-146.4 grams Weight range (females) 95.8-121.6 grams	
3.2.6 Number of animals per group	15/sex/dose for control and high dose groups 10/sex/dose for low and mid dose groups	
3.2.7 Control animals	Yes, concurrent vehicle	

Section A6.3.2 Annex Point IIA, VI.6.3 IUCLID 5.4/09		Repeated dose toxicity Repeated dose toxicity, dermal (28 days)	
3.3	**Administration/ Exposure**		
3.3.1	Duration of treatment	28 days	
3.3.2	Frequency of exposure	20 applications in 28 days 6 hours each application	
3.3.3	Postexposure period	4 weeks	X
3.3.4	**Dermal**		
3.3.4.1	Type, Test Site Preparation	Hair was clipped from the rats 7 days prior to test. Rats were acclimated to the body wrap for 3 days prior to dosing. Fur was clipped again just prior to dosing. Dosing solution was added directly to the skin, and the entire test site was covered with gauze and bandaging tape. The dosing site was occluded for 6 hours per day, and the site was wiped with a dampened, then a dry cloth.	
3.3.4.2	Concentration	100% water was used as the vehicle.	
3.3.4.3	Vehicle	Water	
3.3.4.4	Concentration in vehicle	2.5, 5, 7.5%	
3.3.4.5	Total volume applied	0, 50, 100, 150 mg/kg/day	X
3.3.4.6	Controls	Yes, concurrent vehicle	
3.4	**Examinations**		
3.4.1	Observations	General observations were made twice daily.	
3.4.1.1	Clinical signs	Detailed clinical observations were made daily during treatment.	
3.4.1.2	Mortality	Observations were made twice daily	
3.4.2	Body weight	Body weights were taken before the first dose, and at test days 4, 8, 15, 22 and 28 prior to sacrifice. Animals were weighed weekly during the recovery phase.	
3.4.3	Food consumption	Food consumption was measured weekly.	
3.4.4	Water consumption	Water consumption was measured twice weekly.	
3.4.5	Ophthalmoscopic examination	Not applicable.	
3.4.6	Haematology	Hematology was conducted at sacrifice, and included: hematocrit, haemoglobin, erythrocyte count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total leukocyte count, differential leukocyte count, platelet count, and reticulocyte count.	
3.4.7	Clinical Chemistry	Clinical chemistry was conducted at sacrifice, and included fasting glucose, urea nitrogen, creatinine, total protein, total bilirubin, direct bilirubin, indirect bilirubin, calcium, phosphorous, sodium, potassium, chloride, aspartate aminotransferase, alanine aminotransferase, creatine kinase, lactate dehydrogenase, gamma-glutamyl transferase, sorbitol dehydrogenase, alkaline phosphatase, and protein electrophoresis.	

Section A6.3.2 **Annex Point IIA, VI.6.3** **IUCLID 5.4/09**		**Repeated dose toxicity** **Repeated dose toxicity, dermal (28 days)**	
3.4.8	Urinalysis	Urinalysis and urine chemistry were evaluated on days 26 and 58. It included osmolality, pH, protein, glucose, ketones, bilirubin, blood, urobilinogen, total volume, color and appearance, microscopic elements, n-acetyl-B-D-glucosaminidase (NAG), alpha-2u-globulin, total protein, creatinine, and calculated creatinine clearance.	X
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Liver, kidneys, brain, heart, adrenals, spleen, ovaries, and testes were weighed at sacrifice for all animals.	
3.5.2	Gross and histopathology	Standard tissues were collected at sacrifice and included the following: gross lesions, lungs with mainstem bronchi, brain, pituitary, thyroid and parathyroid, thymic region, trachea, heart, bone (sternum) with marrow, salivary gland, liver, spleen, kidneys, adrenal gland, pancreas, testes, epididymis, prostate, ovaries, vagina, uterus, aorta, skin (treated and naïve), esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, lymph nodes (mesenteric and nonmesenteric), mammary gland, skeletal muscle, sciatic nerve, eyes, and spinal cord. Histopathology was evaluated for animals of the control and high dose groups. Lungs, liver, kidneys, and treated and naive skin of the low and mid dose groups were also examined.	X
3.5.3	Other examinations	No	
3.5.4	Statistics	The data for quantitative continuous variables were intercompared for the three treatment groups and the control group by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. The t-tests were used when the F value from the ANOVA was significant. When Levene's test indicated similar variances, and the ANOVA was significant, a pooled t-test was used for pairwise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variance, followed, when necessary, by a separate variance t-test for pairwise comparisons. Nonparametric data were statistically evaluated using the Kruskal-Wallis test followed by the Mann-Whitney U-test. Incidence data were compared using Fisher's Exact test. For all statistical tests, the probability value of <0.05 (two-tailed) was used as the critical level of significance.	
3.6	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**	Mild erythema, desquamation, and exfoliation at the test sites was noted in all treatment groups, masked in many cases by a yellow staining from the test material. In some cases, scoring was achieved by evaluating erythema on the peripheri of the test site where staining was less. Skin necrosis was noted in most high dose rats, and scabbing in a few animals.	X
4.1.1	Clinical signs	There were no signs of systemic toxicity in any dose group. Swollen periocular tissue, periocular and perinasal soiling were observed. Excoriation was also noted, but the findings are believed to be from scratching due to the wrapping procedures.	

Section A6.3.2 Annex Point IIA, VI.6.3 IUCLID 5.4/09		Repeated dose toxicity Repeated dose toxicity, dermal (28 days)	
4.1.2	Mortality	There was no treatment-related mortality in any dose group.	
4.2	**Body weight gain**	Male body weights were depressed 5.2% from the controls, but trended towards recovery during the additional 4 week observation phase. Body weight changes in females were seen, but not considered biologically significant due to the small magnitude of the changes. **Table A6.3.2/01-1**	X
4.3	**Food consumption and water consumption**	Food consumption in the high dose group animals was also decreased relative to controls. There were no clear trends in water consumption for males, but were decreased in females at the high and mid dose levels (normal by the end of recovery phase). The authors dismiss the finding as caused by equipment variations in the water valving and air flow in the room. **Table A6.3.2/01-1**	
4.4	**Ophthalmoscopic examination**	Not applicable	
4.5	**Blood analysis**		
4.5.1	Haematology	There were no findings for males. Female platelet counts were increased for all exposure groups (nearly resolved by the end of recovery phase). Reticulocyte counts were elevated in a dose-related manner (resolved by end of recovery phase). **Table A6.3.2/01-1**	X
4.5.2	Clinical chemistry	Urea nitrogen was elevated in mid and high dose males, and was considered associated with skin irritation (resolved at the end of recovery phase). **Table A6.3.2/01-1**	X
4.5.3	Urinalysis	There were no treatment-related findings in urinalysis in male or female rats at any dose level.	X
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	A slight increase in absolute and relative adrenal weights was noted in treated males and females, but is attributed to stress and is frequently seen in studies where an irritant is repeatedly applied to the skin. All other findings were considered spurious and not attributed to direct treatment.	
4.6.2	Gross and histopathology	Significant increases in acanthosis, dermatitis, hyperkeratosis / parakeratosis, epidermitis, and dermal fibrosis were seen in treated animals. Epidermitis (superficial pustular foci) was present in a few animals of each dose group. All skin lesions were graded as minimal to moderate, and were confined to superficial layers of the skin. The severity of the skin lesions generally increased with increasing concentration of the dosing solution. Following the recovery phase, there was minimal to mild dermal fibrosis found in a few mid and high dose animals. There were also notations of increased density of the dermal collagen directly beneath the epidermis, and was intermixed with normal dermal collagen.	X
4.7	**Other**	Analytical confirmation showed that the mean measured concentrations of the 2.5 and 7.5% solutions ranged from 101.1 -104.3% of the nominal concentrations at 7 and 14 days of storage.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	

Section A6.3.2 Annex Point IIA, VI.6.3 IUCLID 5.4/09	Repeated dose toxicity Repeated dose toxicity, dermal (28 days)	
5.1 Materials and methods	Male and female [redacted] rats were obtained from a commercial supplier, and acclimated for 14 days to conditions in the testing facility. All animals were subject to a health evaluation by the testing laboratory veterinary staff. Animals were identified individually and randomized to dose groups. They were housed 2 per cage in steel caging, and provided water and food *ad libitum* in rooms designed to maintain adequate conditions for the species. Groups of 15 male and 15 female rats were assigned to each of 4 dose groups (0, 50, 100, 150 mg/kg/day). Five of the animals for each dose group/sex were maintained an additional 4 weeks post-dosing as a recovery group. Dosing solution was added directly to the skin, and the entire test site was covered with gauze and bandaging tape. The dosing site was occluded for 6 hours per day. Mortality observations were made twice daily. Detailed clinical observations were made daily during treatment. Body weights were taken before the first dose, and at test days 4, 8, 15, 22 and 28 prior to sacrifice; they were recorded weekly during the recovery period. Food consumption was measured weekly, and water consumption was measured twice weekly. Standard batteries of haematology, clinical chemistry, urinalysis, and urine chemistry parameters were evaluated. A complete necropsy was performed on all animals. Standard tissues (full) were collected including treated and naive skin. Histopathology was evaluated for animals of the control and high dose groups. Lungs, liver, kidneys, and treated and naive skin of the low and mid dose groups were also examined.	X
5.2 Results and discussion	**Table A6.3.2/01-1** There were no signs of systemic toxicity or treatment-related mortality in any dose group. Local effects were noted at the dermal test site. Male body weights were depressed 5.6% from the controls, but trended towards recovery during the additional 4 week observation phase. Body weight changes in females were seen, but not considered biologically significant due to the small magnitude of the changes. Food consumption in the high dose group animals was also decreased relative to controls. There were no clinical chemistry or hematology findings for males. Female platelet counts were increased for all exposure groups (nearly resolved by the end of recovery phase). Reticulocyte counts were elevated in a dose-related manner (resolved by end of recovery phase). Urea nitrogen was elevated in mid and high dose males, and was considered associated with skin irritation (resolved at the end of recovery phase). All organ weight findings were considered spurious and not attributed to direct treatment. Significant histopathological findings were noted at the dermal test site. All gross skin lesions were graded as minimal to moderate, and were confined to superficial layers of the skin. The severity of the skin	

Section A6.3.2 **Annex Point IIA, VI.6.3** **IUCLID 5.4/09**	**Repeated dose toxicity** **Repeated dose toxicity, dermal (28 days)**	
	lesions generally increased with increasing concentration of the dosing solution.	
5.3 Conclusion	Glutaraldehyde did not elicit any significant systemic toxicity in rats when dosed via the dermal route for 28 days. Findings were limited to gross and histopathological findings at the dermal test site, and effects secondary to repeated irritation.	X
5.3.1 LOEL	50 mg/kg/day based on dermal effects at the test site.	
5.3.2 NO(A)EL	Not established.	
5.3.3 Other	None	
5.3.4 Reliability	1	
5.3.5 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 23rd, 2010	
Materials and Methods	3.3.3 Postexposure period. This is valid for 5 animals/sex of the high dose group and the control group, while the other animals were sacrificed 3 days after the last treatment. 3.3.4.5 Total volume applied. The volume was adjusted on the base of the animal weight, 2 ml/kg. 3.4.8 Urinalysis. This was performed on day 26 for the animals that were sacrificed 3 days after the end of treatments, and on day 58 for the animals in the 4-week recovery group. 3.5.2 Gross and histopathology. The list includes all tissues collected, even if they were not analysed. The analysed tissues were the following: gross lesions, lungs with mainstem bronchi, brain, pituitary, thyroid, thymic region, heart, bone sternum including marrow, liver, spleen, kidneys, adrenal gland, testes, epididymis, ovaries, uterus, skin (treated and untreated), lymph nodes. All these were analysed for the control and high dose groups, while only the underlined ones were analysed for the low and medium dose groups. 5.1 Materials and methods. Information given on groups is incorrect – please see 3.2.6 for correct information.	

Section A6.3.2 Annex Point IIA, VI.6.3 IUCLID 5.4/09	**Repeated dose toxicity** **Repeated dose toxicity, dermal (28 days)**	
Results and discussion	4.1 Observations. Skin necrosis was seen in all dose groups (3/10, 8/10, 13/15). 4.2 Body weight gain. The 5.2 % difference was observed for the high dose group males, while for all other groups the differences were minimal. 4.5.1 Haematology. Reticulocyte counts were elevated, but not in a dose-related manner. The largest elevation was in the low dose group, and the smallest elevation in the high dose group. 4.5.2 Clinical chemistry. There is no evidence that elevated urea nitrogen was associated with skin irritation. 4.5.3 Urinalysis. It should be mentioned that urine creatinine was elevated in all dose groups for both males and females, reaching statistical significance in all but the male medium dose group. Dose response may exist but is weak. 4.6.2 Gross and histopathology. Following the recovery phase, dermal fibrosis was found in 3/5 males and 5/5 females. There were no medium dose group animals in the recovery phase. 5.2 Results and discussion. The points mentioned above (4.2, 4.5.1, 4.5.3, 4.6.2) should be noted in this box as well.	
Conclusion	5.3 Conclusion. There were two findings that indicate a possibility of kidney damage: a dose-dependent elevation of urea nitrogen in males only, and a non-dose-dependent elevation of urine creatinine in all dose groups in both males and females. There were however no necropsy findings in the kidneys and no statistically significant differences in kidney weights, or kidney weights compared to body weight or to brain weight. It is concluded that there is insufficient evidence to assume kidney damage. There was skin irritation at all dose levels. LO(A)EL (test substance): 50 mg/kg bw/day (lowest dose group) based on dermal effects at the test site. NO(A)EL (test substance): Not established, but NOAEL for systemic effects is 150 mg/kg bw/day (high dose group). LOAEL (GA): 0.51 x 50 = 26 mg/kg bw/day NOAEL (GA): Not established, but NOAEL for systemic effects is 0.51 x 150 = 77 mg/kg bw/day (high dose group).	
Reliability	1	
Acceptability	Acceptable	
Remarks	2.1 Guideline study. This broadly satisfies also the requirements in OECD 410, with some deviations in e.g. haematology examinations. All concentrations in the study are given of the test substance which contains 51 % GA. Therefore, the NOAEL and LOAEL values have been corrected using the factor 0.51.	
	COMMENTS FROM ... (specify)	
Date		
Materials and Methods		
Results and discussion		
Conclusion		

Section A6.3.2 **Annex Point IIA, VI.6.3** **IUCLID 5.4/09**	**Repeated dose toxicity** **Repeated dose toxicity, dermal (28 days)**	
Reliability		
Acceptability		
Remarks		

Table A6.3.2/01-1 Summary of Treatment-Related Findings (28-day Rat)

Parameter	0 mg/kg/day		50 mg/kg/day		100 mg/kg/day		150 mg/kg/day	
	m	f	m	f	m	f	m	f
DOSING PHASE								
Number of animals examined	15	15	10	10	10	10	15	15
In-Life Observations								
Final body weight (grams)	173.6	135.4	169.4	136.1	172.2	135.1	164.5	131.4
Final food consumption (grams)	14.3	12.5	14.7	12.7	14.9	12.8	14.1	12.4
Hemoglobin								
Platelets (10^3/uL)	567	590	571	*646*	589	**653**	608	**658**
Reticulocyte count (% of RBC's)	2.8	2.9	2.8	**3.6**	2.8	*3.4*	3.1	3.3
Clinical Chemistry								
Urea Nitrogen (mg/L)	165	195	180	195	*182*	197	**189**	201
Organ Weights								
Adrenal Weight (grams)	0.037	0.038	0.037	0.040	0.040	0.042	0.038	0.044
Relative Adrenal Weight	0.024	0.032	0.024	0.033	0.026	0.035	0.026	**0.038**
RECOVERY PHASE								
Number of animals examined	5	5	0	0	0	0	5	5
In-Life Observations								
Final body weight (grams)	234.1	162.6	-	-	-	-	221.0	156.6
Final food consumption (grams)	16.5	12.4	-	-	-	-	16.2	13.4
Hemoglobin								
Platelets	588	603	-	-	-	-	613	625
Reticulocyte count	3.2	2.8	-	-	-	-	3.2	2.8
Clinical Chemistry								
Urea Nitrogen (mg/dL)	141	156	-	-	-	-	145	165
Organ Weights								
Adrenal Weight (grams)	0.043	0.040	-	-	-	-	0.38	0.044
Relative Adrenal Weight	0.020	0.027	-	-	-	-	0.019	0.031

m=male
f=female
bold indicates statistically different from control group, $p<0.01$
italics indicates statistically different from control group, $p<0.05$

Section A6.4.1 (1) Annex Point IIA, VI.6.4 IUCLID 5.4/01	Repeated dose toxicity Ninety-day inclusion in drinking water of rats	
	1 REFERENCE (A6.4.1/01)	Official use only
1.1 Reference	[redacted] (1985a) Glutaraldehyde: Ninety-day inclusion in drinking water of rats, [redacted] Unpublished, 13 December 1985	
1.2 Data protection	Yes	
1.2.1 Data owner	The Dow Chemical Company (Dow) [redacted]	
1.2.2 Companies with letter of access	[redacted] Dow	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	No	
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	Glutaraldehyde, 50%	
3.1.1 Lot/Batch number	[redacted]	
3.1.2 Specification	Not reported	
3.1.2.1 Description	Clear liquid	
3.1.2.2 Purity	[redacted]	X
3.1.2.3 Stability	Assumed to be stable under typical storage conditions. The material was stored refrigerated during this study.	
3.2 Test Animals		
3.2.1 Species	Rat	
3.2.2 Strain	[redacted]	
3.2.3 Source	[redacted]	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	Approximately 6 weeks of age Approximately 85-110 grams	
3.2.6 Number of animals per group	0 ppm 30 males; 30 females 50 ppm 30 males; 30 females 250 ppm 30 males; 30 females 1000 ppm 30 males; 30 females	X
3.2.7 Control animals	Yes, concurrent vehicle	

Section A6.4.1 (1) Annex Point IIA, VI.6.4 IUCLID 5.4/01		Repeated dose toxicity Ninety-day inclusion in drinking water of rats	
3.3	**Administration/ Exposure**		
3.3.1	Duration of treatment	90 days	
3.3.2	Frequency of exposure	Continuous in the drinking water	
3.3.3	Postexposure period	Ten animals per sex were added to the 0 and 1000 ppm dose groups for a four week recovery phase. During the recovery phase, animals were placed on municipal tap water.	
3.3.4	**Oral**		
3.3.4.1	Type	Drinking water	
3.3.4.2	Vehicle	Water	
3.3.4.3	Concentration in vehicle	50, 250 or 1000 ppm	
3.3.4.4	Total volume applied	Approximately 0, 5, 25, 100 mg/kg/day for males Approximately 0, 7, 35, 120 mg/kg/day for females	
3.3.4.5	Controls	Yes, concurrent vehicle	
3.4	**Examinations**		
3.4.1	Observations	Daily	
3.4.1.1	Clinical signs	Weekly	
3.4.1.2	Mortality	Daily	
3.4.2	Body weight	Weekly	
3.4.3	Food consumption	Weekly	
3.4.4	Water consumption	Weekly	
3.4.5	Ophthalmoscopic examination	Ophthalmic examinations were performed using an indirect ophthalmoscope prior to dosing and at termination (animals with pre-existing deficiencies were eliminated prior to study start).	
3.4.6	Haematology	Haematology was evaluated on 10 animals per group at 6 weeks and at termination, and included: leukocyte count, erythrocyte count, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corbuscular haemoglobin concentration, platelet count, differential leukocyte count, reticulocyte count).	
3.4.7	Clinical Chemistry	Clinical chemistry parameters that were evaluated included: glucose, urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, total protein, albumin, globulin, total bilirubin, direct bilirubin, gamma-glutamyl transferase, sorbitol dehydrogenase, calcium, phosphorous, sodium, potassium, chloride, and indirect bilirubin.	
3.4.8	Urinalysis	Urine was collected from 10 animals per sex per dose at 6 and 12 weeks over a 24-hour period, and analyzed for volume, colour, turbidity, specific gravity, pH, protein, glucose, ketone, bilirubin, blood, urobilinogen, and microscopic findings. Urinalysis measurements were performed in the 17th week of the study for all control and recovery groups due to chances noted in the 12-week evaluations.	

Section A6.4.1 (1) Annex Point IIA, VI.6.4 IUCLID 5.4/01		Repeated dose toxicity Ninety-day inclusion in drinking water of rats	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Liver, kidney, adrenals, brain, spleen, and gonads	
3.5.2	Gross and histopathology	Gross pathology was completed for the following organs: brain, eyes, Harderian glands, pituitary, salivary glands, heart, aorta, thymic region, thyroid, lungs, trachea, oesophagus, stomach, duodenum, ileum, jejunum, cecum, colon, rectum, adrenals, spinal cord, pancreas, liver, kidneys, urinary bladder, testes, epididymis, prostate, ovaries, uterus, spleen, mesenteric and nonmesenteric lymph nodes, skeletal muscle, mammary gland, skin, bone including marrow, tongue, and any gross lesions. Full histopathology was performed on all control and 1000ppm animals, and the heart, esophagus, stomach, tongue, liver, and kidneys were examined from the 50ppm and 250ppm groups.	
3.5.3	Other examinations	None	
3.5.4	Statistics	Water consumption, food consumption, body weight, and organ weight data were intercompared for the dosage groups and control group by use of Levene's test for homogeneity of variances, by ANOVA, and by individual t-tests. The t-tests were used if F for ANOVA was significant, to delineate which groups differed from the controls. If Levene's test indicated heterogeneous variances, the groups were compared by an ANOVA for unequal variances. This was followed, if necessary, by individual t-tests.	
3.6	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	There were no clinical signs of toxicity noted in any animal.	
4.1.2	Mortality	There was no treatment-related mortality in any dose group.	
4.2	Body weight gain	Body weights decreased for males in the high dose from week 3 forward, and in females at the same dose level from week 11 forward. The body weight decrease was considered a secondary effect of food and water consumption decreases, and were transient. Body weights rapidly returned to normal during the recovery phase.	
4.3	Water consumption	A dose-related reduction in water consumption was observed for male and female rats in the 250 and 1000 ppm groups, but returned to normal in those animals in the recovery groups	
4.4	Food consumption	Statistically-significant decreases were noted in males and females at the 250 and 1000ppm dose levels. Effects on food consumption at the 250 ppm dose level were inconsistent and not considered biologically relevant. Consumption returned to normal in recovery group animals. Effects on food consumption are considered likely due to the decrease in water consumption, and were therefore not assumed to be a direct physiological response to glutaraldehyde toxicity.	
4.5	Ophthalmoscopic examination	A high incidence of superficial corneal dystrophy was observed particularly in females, although seen in males as well but to a lesser	

Section A6.4.1 (1) Annex Point IIA, VI.6.4 IUCLID 5.4/01		Repeated dose toxicity Ninety-day inclusion in drinking water of rats	
		extent. Since the incidences were dispersed throughout the dose levels and control groups, and did not occur in a dose-related manner, they weren't considered directly related to test material toxicity.	
4.6	**Blood analysis**		
4.6.1	Haematology	There were no treatment-related effects on haematology parameters.	
4.6.2	Clinical chemistry	Effects on clinical chemistry were limited to increases in urea nitrogen in females of the mid and high dose groups. The change was considered not indicative of renal damage, as the magnitude of the increase was minimal, and there was no increase in serum creatinine. The findings were transient; levels were similar to the controls after the recovery period.	X
4.6.3	Urinalysis	Changes in urinalysis parameters were seen in treated groups in a dose-related manner, but were considered to be secondary effects of decreased water consumption and not related directly to test material toxicity. Urinalysis findings were reversed during the recovery period.	
4.7	**Sacrifice and pathology**		
4.7.1	Organ weights	***Males*** No differences in absolute organ weights or organ weights relative to brain weight were observed at the 13-week sacrifice of male rats. A dose-related increase in kidney weight relative to final body weight was observed in the 250 and 1000ppm groups. No statistically-significant differences in final body weight or absolute organ weights were observed in the recovery group, although final body weights remained slightly reduced in the recovery groups. This decreased body weight resulted in statistically increased relative organ weights at the 17-week sacrifice. Other statistically significant organ weight changes at the 17 week sacrifice were not considered related to glutaraldehyde exposure. ***Females*** No statistically significant reduction in final body weight of females was observed although a slight reduction in mean weight was seen in the high dose females. Absolute and relative kidney weights and brain weight were increased in a dose-related manner for animals given 250 and 1000ppm, but the changes were not noted in the recovery groups.	
4.7.2	Gross and histopathology	There were no observations associated with glutaraldehyde exposure noted in any animal.	
4.8	**Other**	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Male and female [redacted] rats received nominal concentrations of glutaraldehyde at 0, 50, 250 or 1000 ppm in drinking water for 13 weeks. An additional 10 animals per sex were added to the 0 and 1000 ppm dose groups for a four week recovery phase. During the recovery phase, animals were placed on municipal tap water. Animals were inspected daily for mortality and availability of feed and water, with detailed examination weekly. Food consumption, water	

Section A6.4.1 (1) Annex Point IIA, VI.6.4 IUCLID 5.4/01	Repeated dose toxicity Ninety-day inclusion in drinking water of rats	
	consumption and body weight were assessed weekly. Ophthalmic examinations were performed using an indirect ophthalmoscope prior to dosing and at termination. Urine was collected from 10 animals per sex per dose at 6 and 12 weeks over a 24-hour period, and analyzed for volume, colour, turbidity, specific gravity, pH, protein, glucose, ketone, bilirubin, blood, urobilinogen, and microscopic findings. Urinalysis measurements were performed in the 17th week of the study for all control and recovery groups due to chances noted in the 12-week evaluations. Haematology and clinical chemistry were evaluated on 10 animals per group at 6 weeks and at termination. Organ weights (liver, kidney, adrenals, brain, spleen, and gonads) were taken at necropsy. Gross pathology was completed on a full set of tissues and organs. Full histopathology was performed on all control and 1000ppm animals, and the heart, oesophagus, stomach, tongue, liver, and kidneys were examined from the 50ppm and 250ppm groups.	
5.2 Results and discussion	There were no clinical observations noted at any dose level over the course of the study, nor any mortality. A dose-related reduction in water consumption was observed for male and female rats in the 250 and 1000 ppm groups, but returned to normal in those animals in the recovery groups. Based on previous studies, the authors suggest that the decreases in consumption are likely due to an aversion to the test solutions resulting from odor and/or taste of the test material, and not due to a toxicological effect. Statistically-significant decreases were noted in males and females at the 250 and 1000ppm dose levels. Effects on food consumption at the 250 ppm dose level were inconsistent and not considered biologically relevant. Consumption returned to normal in recovery group animals. Effects on food consumption are considered likely due to the decrease in water consumption, and were therefore not assumed to be a direct physiological response to glutaraldehyde toxicity. Body weights decreased for males in the high dose from week 3 forward, and in females at the same dose level from week 11 forward. The body weight decrease was considered secondary effect of food and water consumption decreases, and was transient. Body weights rapidly returned to normal during the recovery phase. There were no hematological effects at any dose level. Effects on clinical chemistry were limited to increases in urea nitrogen in females of the mid and high dose groups. The change was considered not indicative of renal damage, as the magnitude of the increase was minimal, and there was no increase in serum creatinine. The findings were transient; levels were similar to the controls after the recovery period. Changes in urinalysis parameters were seen in treated groups in a dose-related manner, but were considered to be secondary effects of decreased water consumption and not related directly to test material toxicity. No differences in absolute organ weights or organ weights relative to brain weight were observed at the 13-week sacrifice of male rats. A dose-related increase in kidney weight relative to final body weight was	

Section A6.4.1 (1) **Annex Point IIA, VI.6.4** **IUCLID 5.4/01**	**Repeated dose toxicity** **Ninety-day inclusion in drinking water of rats**	
	observed in males in the 250 and 1000ppm groups. Decreased body weight resulted in statistically increased relative organ weights at the 17-week sacrifice. Absolute and relative kidney weights and brain weight were increased in a dose-related manner for females given 250 and 1000ppm, but the changes were not noted in the recovery groups. There were no gross or histopathological observations associated with glutaraldehyde exposure noted in any animal.	
5.3 Conclusion	Glutaraldehyde when administered to [redacted] rats for 90 days in the drinking water did not elicit any significant signs of systemic toxicity. Body weights were decreased, however it was attributed to decreased food and water consumption decreases (due to unpalatability of the test material). Haematology endpoints were unaffected, and clinical chemistry changes were considered secondary effects of decreased water consumption. Gross and histopathology examinations were unremarkable, and relative organ weight increases were attributed to decreased body weight. Thus, 1000 ppm was considered a minimally toxic dose of glutaraldehyde over 90 days, and 250 ppm was a dose which may have produced a marginal physiological response.	X
5.3.1 LOEL	250 ppm	
5.3.2 NOEL	50 ppm	
5.3.3 Other	None	
5.3.4 Reliability	1	
5.3.5 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 27th, 2010	
Materials and Methods	3.1.2.2 Purity. This is the analytical concentration determined. The solution contained also water (50.8 % ± 1.0 %), methanol (0.1 % ± 0.02 %) and NaH_2PO_4 (0.11 % ± 0.01 %). 3.2.6 Number of animals per group. There were only 20 males and 20 females in the 50 ppm group (not 30 + 30).	
Results and discussion	4.6.2 Clinical chemistry. There was a slight but dose-related increase in urea nitrogen in the females of the mid and high dose groups at 6 and 13 weeks, reported as statistically significant only at 6 weeks (6 weeks: 159/175/185/199 mg/L; 13 weeks: 188/192/203/209 mg/L) (Data from study report Appendix 1 Table 7). For males, such an effect was absent or minimal.	

Section A6.4.1 (1) Annex Point IIA, VI.6.4 IUCLID 5.4/01	**Repeated dose toxicity** **Ninety-day inclusion in drinking water of rats**	
Conclusion	LOEL: approximately 25 and 35 mg/kg bw/day for males and females, respectively (250 ppm – based on increase in kidney weight in males and females, coupled with a slight increase in urea nitrogen) NOAEL: 50 ppm; approximately 5 and 7 mg/kg bw/day for males and females, respectively 5.3 Conclusion. There were two effects suggesting a possibility of kidney damage: 1) there was a slight but dose-related increase in urea nitrogen in the females of the mid and high dose groups, and 2) kidney weight relative to body weight was increased in the mid and high dose groups of both males and females, and absolute kidney weights were increased in females in the mid and high dose groups.	
Reliability	1	
Acceptability	Acceptable	
Remarks		
	COMMENTS FROM ... (specify)	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A6.4.1/01-1 Summary of Treatment-Related Effects

Parameter	0 ppm		50 ppm		250 ppm		1000 ppm	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
DOSING PHASE								
Number of animals examined	30	20	20	20	20	20	30	30
Final body weight (g)	312.4	180.4	312.9	183.1	308.4	177.5	**298.5**	**175.4**
Body weight gain (g)	202.1	90.3	204.0	92.1	198.5	88.0	**189.0**	*86.0*
Food consumption (final) (g)	17.6	12.6	18.0	*13.1*	17.4	12.2	**16.8**	**11.8**
Water consumption (final) (g)	28.4	24.3	28.4	25.1	**24.5**	**21.6**	**22.4**	**18.6**
Urine volume	10.2	10.4	11.1	9.0	*8.0*	*6.9*	*8.0*	**5.4**
Kidney weight (g)	2.201	1.328	2.214	1.359	2.215	*1.379*	2.234	**1.457**
Relative kidney weight (%)	0.696	0.716	0.701	0.723	*0.717*	**0.756**	**0.737**	**0.806**
RECOVERY PHASE								
Number of animals examined	10	10	0	0	0	0	10	10
Final body weight (g)	337.7	192.5	-	-	-	-	331.4	194.5
Body weight gain (g)	225.7	102.4	-	-	-	-	220.4	106.9
Food consumption (final) (g)	18.1	12.8	-	-	-	-	18.5	13.1
Water consumption (final) (g)	26.6	24.5	-	-	-	-	28.1	25.3
Kidney weight (g)	2.305	1.436	-	-	-	-	2.338	1.440
Relative kidney weight (%)	0.683	0.765	-	-	-	-	*0.705*	0.757

- = not evaluated

bold Statistically different from control, alpha < 0.01
* Statistically different from control, alpha < 0.05
m=male
f=female

Section A6.4.1 (2) Annex Point IIA, VI.6.4 IUCLID 5.4/02		Repeated dose toxicity 13-week toxicity study in dogs with administration via the drinking water	
		1 REFERENCE (A6.4.1/02)	Official use only
1.1	Reference	[redacted] (1990a) Glutaraldehyde: 13-week toxicity study in dogs with administration via the drinking water, [redacted] Unpublished, 29 January 1990	
1.2	Data protection	Yes	
1.2.1	Data owner	The Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	Yes OECD 409	X
2.2	GLP	Yes	
2.3	Deviations	No	
		3 MATERIALS AND METHODS	
3.1	Test material	Glutaraldehyde, 50%	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Not reported	
3.1.2.1	Description	Aqueous solution	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Assumed to be stable under typical storage conditions.	
3.2	Test Animals		
3.2.1	Species	Dog	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Approximately 4-5 months of age 6-8 kg	X
3.2.6	Number of animals per group	4	X
3.2.7	Control animals	Yes, concurrent vehicle	
3.3	Administration/ Exposure		
3.3.1	Duration of treatment	13 weeks	

Section A6.4.1 (2) Annex Point IIA, VI.6.4 IUCLID 5.4/02	Repeated dose toxicity 13-week toxicity study in dogs with administration via the drinking water	
3.3.2 Frequency of exposure	Continuous in drinking water	
3.3.3 Postexposure period	No	
3.3.4 Oral		
3.3.4.1 Type	Drinking water	
3.3.4.2 Vehicle	Water	
3.3.4.3 Concentration in vehicle	50, 150 or 250 ppm	
3.3.4.4 Total volume applied	0, 3.3, 9.6, and 14.1 mg/kg/day for males 0, 3.2, 9.9, and 15.1 mg/kg/day for females	
3.3.4.5 Analysis	Homogeneity and stability of dose solutions were established prior to study start using GC. Dose confirmations were accomplished by GC for all dose preparations.	
3.3.4.6 Controls	Yes, concurrent vehicle	
3.4 Examinations		
3.4.1 Observations	Twice daily	
3.4.1.1 Clinical signs	Daily	
3.4.1.2 Mortality	Twice daily	
3.4.2 Body weight	Weekly	
3.4.3 Food consumption	Weekly	
3.4.4 Water consumption	Daily	
3.4.5 Ophthalmoscopic examination	Ophthalmic examinations were performed prior to dosing and at termination (animals with pre-existing deficiencies were eliminated prior to study start).	
3.4.6 Haematology	Haematology was evaluated and included (initially, week 4, and terminal): prothrombin time, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet count, differential leukocyte count, white blood cell count.	
3.4.7 Clinical Chemistry	Clinical chemistry parameters that were evaluated included (initially, week 4, and terminal): glucose, urea nitrogen, creatinine, creatine kinase, aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase, total protein, albumin, globulin, total bilirubin, direct bilirubin, gamma-glutamyl transferase, sorbitol dehydrogenase, cholesterol, calcium, inorganic phosphorous, sodium, potassium, chloride, and indirect bilirubin.	
3.4.8 Urinalysis	Urine was collected and analyzed for volume, physical appearance, specific gravity, pH, protein, glucose, ketone, bilirubin, blood, urobilinogen, and microscopic elements.	
3.5 Sacrifice and pathology		
3.5.1 Organ Weights	Organ weights (liver, kidney, adrenals, brain, spleen, and ovaries, testes, and thyroid) were taken at necropsy.	X
3.5.2 Gross and	Gross pathology was completed for the following organs: brain, eyes,	X

Section A6.4.1 (2) Annex Point IIA, VI.6.4 IUCLID 5.4/02		Repeated dose toxicity 13-week toxicity study in dogs with administration via the drinking water	
	histopathology	Harderian glands, pituitary, salivary glands, heart, aorta, thymus, thyroid with parathyroid, lungs, trachea, oesophagus, stomach, duodenum, ileum, jejunum, cecum, colon, rectum, adrenals, spinal cord, pancreas, liver, kidneys, urinary bladder, testes, epididymis, prostate, ovaries, uterus, spleen, mesenteric lymph nodes, skeletal muscle, mammary gland, skin, sciatic nerve, femur, gall bladder, bone marrow and smear from sternum, tongue, and any gross lesions. Full histopathology was performed on all control and 1000ppm animals, and the heart, oesophagus, stomach, tongue, liver, and kidneys were examined from the 50ppm and 250ppm groups.	
3.5.3	Other examinations	None	
3.5.4	Statistics	Levene's test was done to test for variance homogeneity. In the case of heterogeneity of variance at $p<0.05$, the following transformations were used to stabilize the variance: Log X (analysis following log10 transformation), X2 (analysis following square transformation), x1/2 (analysis following square root transformation), 1/X (analysis following reciprocal transformation), Arcsine X1/2 (analysis following angular transformation), or Rank X (analysis following rank transformation). ANOVA was done on the homogeneous or ranked data. If the ANOVA was significant, Dunnett's t-test, Student's t-test, or the Games and Howell modified Tukey-Kramer test was used for pairwise comparisons between groups. When no transformation established homogeneity of variance at $p<0.001$, the data were examined by nonparametric techniques. These statistics included the Kruskal=Wallis test for multiple comparisons or the Wilcoxon-Mann-Whitney two-sample rank test. Standard one-way ANOVA was used to analyze initial body weights, cumulative body weight changes, food consumptions, water consumptions, clinical chemistry, and haematology values (except red blood cell morphology), urine volume, organ weights, organ-to-body weight percentages, and organ-to-brain weight ratios. Standard one-way analysis of covariance (ANCOVA) was used to analyze body weight, with initial body weight as the covariate. Although Levene's test for variance homogeneity was done, no transformations were used because covariance adjustment removed extraneous heterogeneity. If the ANCOVA was significant, Dunnett's t-test was used for pairwise comparisons between groups. Group comparisons were evaluated at the 5.0% two-tailed probability level.	
3.6	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	An increased incidence of vomiting (fluid and food-like) was observed for both sexes at 150 and 250 ppm. No other treatment-related signs were recorded during the study from either sex in any dose group.	
4.1.2	Mortality	There was no treatment-related mortality in any dose group.	
4.2	Body weights	There were no effects apparent in male body weights in any dose group. Transient differences in body weights were noted throughout the study for females, however they did not appear in a dose- or time-related manner, and were therefore not considered truly related to treatment.	X

Section A6.4.1 (2) Annex Point IIA, VI.6.4 IUCLID 5.4/02		Repeated dose toxicity 13-week toxicity study in dogs with administration via the drinking water	
4.3	Food consumption	There were no statistically-significant decreases for male or female dogs at any dose level tested.	
4.4	Water consumption	A dose-related reduction in water consumption was observed for males (mid and high dose) and females (high dose group) primarily in the first 6 weeks of study. The difference was only statistically significant in week 2 males.	
4.5	Ophthalmoscopic examination	There were no treatment-related eye lesions observed in the study animals.	
4.6	**Blood analysis**		
4.6.1	Haematology	There were no treatment-related effects in any sex or dose group on any parameter evaluated.	
4.6.2	Clinical chemistry	There were no treatment-related effects in any sex or dose group on any parameter evaluated.	X
4.6.3	Urinalysis	There were no treatment-related effects in any sex or dose group on any parameter evaluated.	
4.7	**Sacrifice and pathology**		
4.7.1	Organ weights	No treatment-related findings were observed in any absolute or relative organ weight in males. Increased ovarian weights were noted in females at the high dose, however it was assumed by the authors to be related to estrus cycle and wasn't considered related to treatment.	X
4.7.2	Gross and histopathology	There were no observations associated with glutaraldehyde exposure noted in any animal.	
4.8	**Other**	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Male and female [redacted] dogs received nominal concentrations of glutaraldehyde at 0, 50, 150 or 250 ppm in drinking water for 13 weeks. Animals were inspected twice daily for mortality and availability of feed and water, with detailed examination daily. Food consumption and body weight were assessed weekly. Water consumption was recorded daily. Ophthalmic examinations were performed prior to dosing and at termination (animals with pre-existing deficiencies were eliminated prior to study start). Urine, haematology, and clinical chemistry were evaluated. Organ weights (liver, kidney, adrenals, brain, spleen, and ovaries, testes, and thyroid) were taken at necropsy. Gross pathology was evaluated for all animals, and full histopathology was performed.	X
5.2	**Results and discussion**	Results from the stability study indicated glutaraldehyde remained stable in tap water for at least 14 days. Distribution of glutaraldehyde in dosing solutions were uniform and ranged 91.8-104.5% of the nominal. An increased incidence of vomiting (fluid and food-like) was observed for both sexes at 150 and 250 ppm. No mortality and no other treatment-related signs were recorded during the study from either sex in any dose group. A dose-related reduction in water consumption was observed for males (mid and high dose) and females (high dose group)	

Section A6.4.1 (2) **Annex Point IIA, VI.6.4** **IUCLID 5.4/02**	**Repeated dose toxicity** **13-week toxicity study in dogs with administration via the drinking water**	
	primarily in the first 6 weeks of study. The difference was only statistically significant in week 2 males. There were no statistically-significant decreases for male or female dogs at any dose level tested. Transient differences in body weights were noted throughout the study for females, however they did not appear in a dose- or time-related manner, and were therefore not considered truly related to treatment. There were no statistically-significant decreases for male or female dogs at any dose level tested. Ophthalmology was unremarkable. There were no treatment-related effects on haematology, clinical chemistry, or urinalysis in any sex or dose group on any parameter evaluated. No treatment-related findings were observed in any absolute or relative organ weight in males or females. There were no observations associated with glutaraldehyde exposure noted in any animal.	
5.3 Conclusion	Glutaraldehyde when administered to [redacted] dogs for 90 days in the drinking water did not elicit any significant signs of systemic toxicity other than vomiting. Water consumption was decreased in treated animals, although not statistically significant. Haematology, clinical chemistry, and urinalysis endpoints were unaffected. Gross and histopathology examinations were unremarkable, as were organ weights.	
5.3.1 LOEL	150 ppm	
5.3.2 NOEL	50 ppm	
5.3.3 Other	None	
5.3.4 Reliability	1	
5.3.5 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 29th, 2010	
Materials and Methods	2.1 Guideline study. The OECD 409 guideline is apparently not mentioned, but the study was performed essentially according to this guideline. 3.2.6 Number of animals per group. There were 4 animals/sex/group. 3.2.5 Age/weight at study initiation. The animals were 4.5 months old and the average weight was 8 kg. 3.5.1 Organ Weights. Spleen was not weighed. 3.5.2 Gross and histopathology. Harderian glands are not mentioned in the original report. The dose groups are given erroneously (there is no 1000 ppm group), and all tissues were examined in all dose groups. 5.1 Materials and methods. Same comment as for 3.5.1 above.	

Section A6.4.1 (2) Annex Point IIA, VI.6.4 IUCLID 5.4/02	**Repeated dose toxicity** **13-week toxicity study in dogs with administration via the drinking water**	
Results and discussion	4.2 Body weights. High dose group males had lower mean body weight at week 2 weighing and high- and medium dose group females had lower mean body weighs at several time points. This was probably due to lower palatability of the drinking water. 4.6.2 Clinical chemistry. Many variables included statistically significant changes, but taking into account dose levels, only female calcium levels seem to have been affected by the treatment (all dose levels at week 4; two highest dose levels at week 13). 4.7.1 Organ weights. The kidney weight relative to bw was significantly increased in females of the high dose group. 5.2 Results and discussion. Same comments as 4.2, 4.6.2 and 4.7.1 above.	
Conclusion	LO(A)EL: 150 ppm (9.6 and 9.9 mg/kg bw/day for males and females, respectively) NO(A)EL: 50 ppm (3.3 and 3.2 mg/kg bw/day for males and females, respectively) Other conclusions: The dose levels were chosen in a way that did not cause much toxicity, and few conclusions can be made. High dose females had significantly increased kidney weights, which might be a sign of renal toxicity, but no conclusions can be made in this respect.	
Reliability	1	
Acceptability	Acceptable	
Remarks		
	COMMENTS FROM ... *(specify)*	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A6_3-2. Results of repeated dose toxicity study – Oral Dogs

Parameter	Control		low dose		medium dose		high dose		dose-response +/-	
	m[a]	f[a]	m[a] 3.3 mg/kg bw	f[a] 3.2 mg/kg bw	m[a] 9.6 mg/kg bw	f[a] 9.9 mg/kg bw	m[a] 14.1 mg/kg bw	f[a] 15.1 mg/kg bw	m	f
number of animals examined	NA	NA	4	4	4	4	4	4	-	-
Mortality	NA	NA	0	0	0	0	0	0	-	-
clinical signs*	NA	NA	-	-	-	-	-	-	-	-
body weight	NA	NA	-	↓	-	↓	-	↓	-	↓
food consumption	NA	NA	-	↓	-	↓	-	↓	-	↓
clinical chemistry*	NA	NA	-	-	-	-	-	-	-	-
haematology*	NA	NA	-	-	-	-	-	-	-	-
urinalysis*	NA	NA	-	-	-	-	-	-	-	-
Organ x organ weight*	NA NA	NA NA	-	-	-	-	-	-	-	-
gross pathology*	NA	NA	-	-	-	-	-	-	-	-
microscopic pathology*	NA	NA	-	-	-	-	-	-	-	-
Organ y	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

** specify effects; for different organs give special findings in the order organ weight, gross pathology and microscopic pathology if there are effects*

[a] *give number of animals affected/total number of animals, percentage, or just ↑ or ↓ for increased or decreased*

- = No change

NA = Not applicable

Section A6.4.1(3) Annex Point IIA, VI.6.4 IUCLID 5.4/03		Repeated dose toxicity 90 day drinking water toxicity study in mice	
		1 REFERENCE (A6.4.1/03)	Official use only
1.1	Reference	[redacted] (1989) Glutaraldehyde: Ninety-day drinking water toxicity study in mice, [redacted], Unpublished, 20 March 1989	
1.2	Data protection	Yes	
1.2.1	Data owner	The Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	Yes US EPA FIFRA 82-1 OECD 408	
2.2	GLP	Yes	
2.3	Deviations	No	
		3 MATERIALS AND METHODS	
3.1	Test material	Glutaraldehyde, 25%	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Not reported	
3.1.2.1	Description	Clear, colourless, non-viscous liquid	
3.1.2.2	Purity	[redacted]	X
3.1.2.3	Stability	Assumed to be stable under typical storage conditions.	
3.2	Test Animals		
3.2.1	Species	Mouse	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	49 days of age Approximately 19-41 grams	X
3.2.6	Number of animals per group	30, 20, 20, and 30 animals per sex for controls, 100, 250, and 1000 ppm dose groups, respectively.	
3.2.7	Control animals	Yes, concurrent vehicle	
3.3	Administration/ Exposure		
3.3.1	Duration of treatment	13 weeks	

Section A6.4.1(3) Annex Point IIA, VI.6.4 IUCLID 5.4/03	Repeated dose toxicity 90 day drinking water toxicity study in mice	
3.3.2 Frequency of exposure	Continuous in the drinking water	
3.3.3 Postexposure period	An additional 10 animals per sex were added to the 0 and 1000 ppm dose groups for a six week recovery phase. During the recovery phase, animals were placed on municipal tap water.	
3.3.4 Oral		
3.3.4.1 Type	Drinking water	
3.3.4.2 Vehicle	Water	
3.3.4.3 Concentration in vehicle	100, 250 and 1000 ppm	
3.3.4.4 Total volume applied	Drinking water was offered ad libitum. Males 25, 61, 200 mg/kg/day Females 31, 74, 238 mg/kg/day	
3.3.4.5 Analysis	Dose concentrations were confirmed prior to administration of the dose solutions for the first 4 weeks of the study. Thereafter, one sample from each preparation was analyzed on weeks 8 and 13.	
3.3.4.6 Controls	Yes, concurrent vehicle	
3.4 Examinations		
3.4.1 Observations	Daily	
3.4.1.1 Clinical signs	Weekly	
3.4.1.2 Mortality	Daily	
3.4.2 Body weight	Weekly	
3.4.3 Food consumption	Weekly	
3.4.4 Water consumption	Weekly	
3.4.5 Ophthalmoscopic examination	Ophthalmic examinations were performed using an indirect ophthalmoscope prior to dosing and at termination (animals with pre-existing deficiencies were eliminated prior to study start).	
3.4.6 Haematology	Haematology was evaluated on 10 animals per group at termination, and included: erythrocyte count, haemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corbuscular haemoglobin concentration, platelet count, total and differential leukocyte count, reticulocyte count).	X
3.4.7 Clinical Chemistry	Clinical chemistry parameters that were evaluated (20/sex/dose) included: glucose, urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, albumin, globulin, total bilirubin, direct bilirubin, gamma-glutamyl transferase, sorbitol dehydrogenase, calcium, phosphorous, sodium, potassium, chloride, and indirect bilirubin.	X
3.4.8 Urinalysis	Urine was collected from 10 animals per sex per dose at 12 weeks, and analyzed for volume, specific gravity, pH, protein, glucose, ketone, bilirubin, blood, urobilinogen, and microscopic findings.	
3.5 Sacrifice and pathology		
3.5.1 Organ Weights	Organ weights (liver, kidney, lungs, heart, brain, spleen, and testes/ovaries) were taken at necropsy.	

Section A6.4.1(3) **Annex Point IIA, VI.6.4** **IUCLID 5.4/03**		**Repeated dose toxicity** **90 day drinking water toxicity study in mice**	
3.5.2	Gross and histopathology	Gross pathology was completed for the following organs: brain, eyes, Harderian glands, pituitary, salivary glands, heart, aorta, thymic region, thyroid, lungs with mainstem bronchi, trachea, esophagus, stomach, duodenum, ileum, jejunum, cecum, colon, rectum, adrenals, spinal cord, pancreas, liver, kidneys, urinary bladder, testes, epididymis, prostate, seminal vesicles, ovaries, vagina, uterus, spleen, mesenteric and nonmesenteric lymph nodes, skeletal muscle, mammary gland, skin, bone including marrow, tongue, peripheral nerve, Zymbal gland, exorbital lachrymal glands, nasopharyngeal tissues, sternum with marrow, gall bladder, femur, and any gross lesions. Full histopathology was performed on all control and 1000ppm animals, and the lungs, liver, and kidneys, and gross lesions were examined from the 50ppm and 250ppm groups.	X
3.5.3	Other examinations	None	
3.5.4	Statistics	Water consumption, food consumption, body weight, and organ weight data were intercompared for the dosage groups and control group by use of Levene's test for homogeneity of variances, by ANOVA, and by pooled variance. The t-tests were used if F for ANOVA was significant, to delineate which groups differed from the controls. If Levene's test indicated heterogeneous variances, the groups were compared by an ANOVA for unequal variances. This was followed, if necessary, by individual t-tests. Non-parametric data were analyzed by the Kruskal-Wallis test and, if frequency data, were compared using a Fisher's exact test where appropriate. All statistical tests, except the frequency comparisons, were performed using statistical software. The fiducial limit of 0.05 was used as the critical level of significance for all tests.	
3.6	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	There were no clinical observations noted at any dose level over the course of the study.	
4.1.2	Mortality	There was no treatment-related mortality observed.	X
4.2	Body weights	Transient differences in body weights were noted throughout the study, however they did not appear in a dose- or time-related manner, and were therefore not considered truly related to treatment.	
4.3	Food consumption	There were no statistically-significant decreases for male or female mice at any dose level tested.	
4.4	Water consumption	A dose-related reduction in water consumption was observed for male and female mice in the 1000 ppm group, but returned to normal in those animals in the recovery groups.	
4.5	Analysis	Stability was confirmed at 100 and 1000 ppm for at least 14 days. Homogeneity was also confirmed at all dose levels. Concentration verification of dosing solutions ranged from 91.6-109.0% of the nominal. All solutions were acceptable for use.	
4.6	Ophthalmoscopic examination	There were no treatment-related eye lesions observed in the study animals.	

Section A6.4.1(3) Annex Point IIA, VI.6.4 IUCLID 5.4/03		Repeated dose toxicity 90 day drinking water toxicity study in mice	
4.7	**Blood analysis**		
4.7.1	Haematology	There were no hematological effects at any dose level observed at the end of the dosing period. Due to a slight decrease in monocytes for males at the mid and high dose groups, however, the haematology measurements were made again at the end of the recovery phase. The findings were resolved, and the decreased monocyte finding is not considered biologically significant.	
4.7.2	Clinical chemistry	There were no clinical chemistry effects at any dose level observed at the end of the dosing period.	
4.7.3	Urinalysis	Decreased urine volume and increased osmolality were also noted in the 250 ppm and 1000 ppm dose groups, but the findings were resolved at the end of the 6 week recovery phase.	
4.8	**Sacrifice and pathology**		
4.8.1	Organ weights	No treatment-related findings were observed in any absolute or relative organ weight in males. Relative kidney weights were increased for females (7%) in the high dose group. No other treatment-related findings were noted for females.	X
4.8.2	Gross and histopathology	There were no observations associated with glutaraldehyde exposure noted in any animal.	
4.9	**Other**	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Male and female [redacted] mice received nominal concentrations of glutaraldehyde at 0, 100, 250 or 1000 ppm in drinking water for 13 weeks (groups of 30, 20, 20, and 30 animals per sex, respectively). An additional 10 animals per sex were added to the 0 and 1000 ppm dose groups for a six week recovery phase. During the recovery phase, animals were placed on municipal tap water. Animals were inspected daily for mortality and availability of feed and water, with detailed examination weekly. Food consumption, water consumption and body weight were assessed weekly. Ophthalmic examinations were performed using an indirect ophthalmoscope prior to dosing and at termination. Urine was collected from 10 animals per sex per dose at 12 weeks, and analysed. Haematology was evaluated on 10 animals per group at termination; clinical chemistry parameters were evaluated at the same time. Organ weights (liver, kidney, adrenals, brain, spleen, and gonads) were taken at necropsy (20/sex/dose). Gross pathology was completed for all animals. Full histopathology was performed on all control and 1000ppm animals, and the lungs, liver, and kidneys, and gross lesions were examined from the 50ppm and 250ppm groups.	
5.2	**Results and discussion**	**Table A6.4.1/03-1** There were no clinical observations noted at any dose level over the course of the study, nor any mortality related to glutaraldehyde consumption. A dose-related reduction in water consumption was observed for male and female mice in the 1000 ppm group, but returned	

Section A6.4.1(3) Annex Point IIA, VI.6.4 IUCLID 5.4/03	Repeated dose toxicity 90 day drinking water toxicity study in mice	
	to normal in those animals in the recovery groups. There were no treatment-related differences in body weights for male or female mice at any dose level tested. There were no treatment-related eye lesions observed in the study animals. Transient differences in body weights were noted throughout the study, however they did not appear in a dose- or time-related manner, and were therefore not considered truly related to treatment. There were no hematological effects at any dose level observed at the end of the dosing period. Due to a slight decrease in monocytes for males at the mid and high dose groups, however, the hematology measurements were made again at the end of the recovery phase. The findings were resolved, and the decreased monocyte finding is not considered biologically significant. Decreased urine volume and increased osmolality were also noted in the males at 250 ppm and females in the 1000 ppm dose group, but the findings were resolved at the end of the 6 week recovery phase. No treatment-related findings were observed in any absolute or relative organ weight in males. Relative kidney weights were increased for females (7%) in the high dose group. No other treatment-related findings were noted for females. There were no observations associated with glutaraldehyde exposure noted in any animal during the gross or histopathological examinations.	
5.3 Conclusion	Glutaraldehyde when administered to [redacted] mice for 90 days in the drinking water did not elicit any significant signs of systemic toxicity. Water consumption was decreased in treated animals, but did not significantly alter body weights. Haematology, clinical chemistry, and urinalysis endpoints were mainly unaffected, and changes noted were not attributed to treatment. Gross and histopathology examinations were unremarkable, however an increased relative kidney weight finding was reported for females of the high dose group.	
5.3.1 LOAEL	250 ppm	
5.3.2 NOAEL	100 ppm	
5.3.3 Other	None	
5.3.4 Reliability	1	
5.3.5 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 30th, 2010	
Materials and Methods	3.1.2.2 Purity. The purity of the test material is given as > 99 %. 3.2.5 Age/weight at study initiation. The report gives weights of 19.2 to 35.0 g. 3.4.6 Haematology. Reticulocyte count was not performed. 3.4.7 Clinical Chemistry. Clinical chemistry was performed to 10 animals/sex/group. 3.5.2 Gross and histopathology. Tongue is not mentioned in the original report.	

Section A6.4.1(3) Annex Point IIA, VI.6.4 IUCLID 5.4/03	Repeated dose toxicity 90 day drinking water toxicity study in mice	
Results and discussion	4.1.2 Mortality. One female of the high dose group died on day 8, with no definable cause. The death is not considered treatment related. 4.8.1 Organ weights. The kidney weight of the high dose group females was significantly increased relative to bw and to brain weight, while there was no difference in the absolute weight. The kidney weight (absolute, relative to bw, relative to brain weight) of males in all dose groups was higher than in the control group, but no statistical significance was established. 5.2 Results and discussion. See comment 4.8.1 above.	
Conclusion	LO(A)EL: 1000 ppm, corresponding to 200 mg/kg bw/day for males and 238 mg/kg bw/day for females (based on increased absolute and relative kidney weights) NO(A)EL: 250 ppm, corresponding to 61 mg/kg bw/day for males and 74 mg/kg bw/day for females	
Reliability	1	
Acceptability	Acceptable	
Remarks		
	COMMENTS FROM ... (specify)	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A6.4.1/03-1 Summary of Treatment-Related Effects

Parameter	0 mg/kg/day		24.7 mg/kg/day (100 ppm)		60.8 mg/kg/day (250 ppm)		199.8 mg/kg/day (1000 ppm)	
	m	f	m	f	m	f	m	f
DOSING PHASE								
Number of animals examined	20	20	20	20	20	20	20	20
Final body weight (g)	36.3	31.6	36.5	30.0	37.5	30.5	36.1	30.0
Body weight gain (g)	7.1	7.7	6.6	6.3*	7.7	6.7	6.9	**6.1**
Water Consumption (final) (g)	9.0	9.7	8.5	9.3	8.8	**8.8**	**6.8**	**7.3**
Monocytes (cells / uL)	251	133	300	167	115*	101	190	152
Urine volume	2.4	1.9	2.2	1.3	1.0*	1.6	1.1*	**0.7**
Urine osmolality	2099	2989	1411	3125	2871	2763	3138*	**4132**
Relative kidney weight	1.911	1.619	2.028	1.604	1.942	1.588	2.060	1.729*
RECOVERY PHASE								
Number of animals examined	10	10					10	10
Final body weight	36.2	32.2					37.2	30.5
Body weight gain	7.8	7.9					7.7	7.5
Water Consumption	9.7	10.5					10.1	10.0
Monocytes (cells / uL)	169	282					190	216
Urine volume	1.8	2.5					1.9	1.7
Urine osmolality	2520	2335					2380	2920
Relative kidney weight	2.061	1.693					2.074	1.665

- = below lowest level quantified.

bold Statistically different from control, alpha < 0.01
* Statistically different from control, alpha < 0.05
m=male
f=female

Section A6.4.2(1) Annex Point IIA, VI.6.4 IUCLID 5.4/07	Repeated dose toxicity Dermal: Subchronic Test of Aqueous Glutaraldehyde [redacted] in [redacted] Mice and [redacted] Rats	
	1 REFERENCE (A6.4.2/01)	Official use only
1.1 Reference	[redacted] (1980) Subchronic Test of Aqueous Glutaraldehyde (C55425) in [redacted] Mice and [redacted] Rats, [redacted] Unpublished, 20 June 1980	
1.2 Data protection	Yes	
1.2.1 Data owner	The Dow Chemical Company	
1.2.2 Companies with letter of access	[redacted]	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	No	
2.2 GLP	No	
2.3 Deviations	Not applicable	
	3 MATERIALS AND METHODS	
3.1 Test material	Glutaraldehyde, 2%	
3.1.1 Lot/Batch number	[redacted]	
3.1.2 Specification	Not reported	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	[redacted]	
3.1.2.3 Stability	Assumed to be stable under typical storage conditions. Dose solutions were mixed weekly.	
3.2 Test Animals		
3.2.1 Species	Rat	
3.2.2 Strain	[redacted]	
3.2.3 Source	[redacted]	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	Age not specified Weight range (males) = 120-165 grams Weight range (females) = 110-148 grams	
3.2.6 Number of animals per group	10	X
3.2.7 Control animals	10 concurrent, vehicle control	
3.3 Administration/ Exposure		

Section A6.4.2(1) Annex Point IIA, VI.6.4 IUCLID 5.4/07		Repeated dose toxicity Dermal: Subchronic Test of Aqueous Glutaraldehyde [redacted] in [redacted] Mice and [redacted] Rats	
3.3.1	Duration of treatment	91 days	
3.3.2	Frequency of exposure	5 times per week	
3.3.3	Postexposure period	None	
3.3.4	**Dermal**		
3.3.4.1	Type / Preparation of Test Site	Unoccluded exposures. The dermal test site was a 1 in^2 site on the dorsal region treated with 0.6 mL of a test material solution five times per week for 13 weeks. There is no indication that the material was wiped from the skin or that the animals were restrained to avoid oral ingestion.	
3.3.4.2	Concentration	100% water was used as the vehicle.	
3.3.4.3	Vehicle	Water	
3.3.4.4	Concentration in vehicle	0, 0.125, 0.25, 0.5, 1, and 2% analyzed on all test levels once during the study.	
3.3.4.5	Total volume applied	0.6 mL	
3.3.4.6	Controls	Yes, concurrent vehicle	
3.4	**Examinations**		
3.4.1	Observations	Twice daily	
3.4.1.1	Clinical signs	Twice daily	
3.4.1.2	Mortality	Twice daily	
3.4.2	Body weight	Prior to first dosing, and weekly thereafter	
3.4.3	Food consumption	Not applicable	
3.4.4	Ophthalmoscopic examination	Not applicable	
3.4.5	Haematology	Not applicable	
3.4.6	Clinical Chemistry	Not applicable	
3.4.7	Urinalysis	Not applicable	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Not applicable	
3.5.2	Gross and histopathology	Histopathology was performed on all control and high dose animals. The stomach was examined in doses 0.25% and higher. Bladder, dermal test site, kidney, testes, stomach, thyroid, prostate, gall baldder, liver, adrenal, salivary gland, lung, uterus, and spleen were evaluated.	
3.5.3	Other examinations	None	
3.5.4	Statistics	Statistical methods, if they were used, were not specified in the report.	
3.6	**Further remarks**	None	

Section A6.4.2(1) Annex Point IIA, VI.6.4 IUCLID 5.4/07		Repeated dose toxicity Dermal: Subchronic Test of Aqueous Glutaraldehyde [redacted] in [redacted] Mice and [redacted] Rats	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Fur was stained yellow from the test material, but there were no other clinical observations noted.	
4.1.2	Mortality	There was no treatment-related mortality in any dose group.	
4.2	Body weight gain	**Table A6.4.2/01-1**	
4.3	Food consumption	Not applicable	
4.4	Ophthalmoscopic examination	Not applicable	
4.5	**Blood analysis**		
4.5.1	Haematology	Not applicable	
4.5.2	Clinical chemistry	Not applicable	
4.5.3	Urinalysis	Not applicable	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Not applicable	
4.6.2	Gross and histopathology	Gross and microscopic diagnosis revealed the presence of stomach ulcers in the 1% and 2% groups. Additionally, histological abnormalities in the kidney, adrenal, colon, heart, and stomach were observed. Findings in the kidney were seen in females only, and consisted of crystals in the lumen of a few renal tubules, mainly in collecting ones, and were found in the high dose and control dose groups at approximately the same frequency. Findings at the dermal test site included dermal ulceration with subjacent inflammation and squamous epithelial hyperplasia, both of which were attributed to fighting and not chemical treatment. Glutaraldehyde was not implicated in any significant pathological changes in mice following subchronic dermal exposure.	
4.7	**Other**	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Groups of 10 animals/sex/dose were given dermal doses of 0.6 mL of the test material to a one square inch area of the dorsal region five times weekly for 13 weeks. Vehicle control animals received doses of distilled water. Animals were weighed prior to the first dosing and weekly thereafter. Animals were observed twice daily for pharmacotoxic effects and mortality. They were sacrificed on test day 91. Histopathology was performed on all control and high dose animals.	
5.2	**Results and discussion**	There was no treatment-related mortality in any dose group. Treated animals weighed slightly more than control animals in all dose groups and for both sexes, but were considered within normal variation (Table A6.4.2/01-1). Fur was stained yellow from the test material, but there were no other clinical observations noted.	

Section A6.4.2(1) **Annex Point IIA, VI.6.4** **IUCLID 5.4/07**	**Repeated dose toxicity** **Dermal: Subchronic Test of Aqueous Glutaraldehyde [redacted] in [redacted] Mice and [redacted] Rats**	
	Gross and microscopic diagnosis revealed the presence of stomach ulcers in the 1% and 2% groups. Additionally, histological abnormalities in the kidney, adrenal, colon, heart, and stomach were observed. Findings in the kidney were seen in females only, and consisted of crystals in the lumen of a few renal tubules, mainly in collecting ones, and were found in the high dose and control dose groups at approximately the same frequency. Findings at the dermal test site included dermal ulceration with subjacent inflammation and squamous epithelial hyperplasia, both of which were attributed to fighting and not chemical treatment.	
5.3 Conclusion	Glutaraldehyde, when given dermally to rats for 13 weeks, did not elicit any effects indicative of systemic toxicity, and there were no findings at sacrifice that would indicate target organ toxicity. Signs of ulceration, gross and microscopic, were seen in the gastrointestinal tract. Glutaraldehyde was not implicated in any significant pathological changes in mice following subchronic dermal exposure.	
5.3.1 LOEL	1% in water based on stomach ulceration	
5.3.2 NOEL	0.5% in water	
5.3.3 Other	None	
5.3.4 Reliability	2	
5.3.5 Deficiencies	No clinical chemistry, haematology, urinalysis, organ weights, food consumption, or ophthalmology was conducted. A complete list of tissues and organs on which gross and histopathology was performed was not provided. The animals were group housed and the test sites were not occluded which may have resulted in oral exposure thus resulting in stomach ulceration.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 5th, 2010	
Materials and Methods	3.2.6 Number of animals per group. There were 10 animals/sex/group.	
Results and discussion	See conclusion below.	
Conclusion	No conclusions can be made because 1) occlusion is not mentioned, and 2) the animals were in group cages. It is thus impossible to estimate how much dermal exposure actually occurred, because licking has most probably occurred. This assumption is supported by the findings of stomach ulcers at the two highest dose groups.	
Reliability	3	
Acceptability	Not acceptable	
Remarks	The study summary is generally a valid description of the study, but detailed critical evaluation has not been performed by the RMS because of the conclusion above.	

Section A6.4.2(1) Annex Point IIA, VI.6.4 IUCLID 5.4/07	Repeated dose toxicity Dermal: Subchronic Test of Aqueous Glutaraldehyde [redacted] in [redacted] Mice and [redacted] Rats	
	COMMENTS FROM ... (specify)	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A6.4.2/01-1 Body Weight Summary for Rats

Week	Control		0.125%		0.25%		0.5%		1.0%		2.0%	
	M	F	M	F	M	F	M	F	M	F	M	F
0	144.4	121.4	147.3	121.8	144.7	125.4	140.2	123.6	142.2	120.8	145.4	119.6
1	180.3	124.9	186.9	135.4	185.9	137.4	178.0	145.9	179.4	137.5	162.5	135.8
2	198.6	149.3	213.0	153.4	215.6	141.5	206.5	158.9	197.8	146.2	210.6	148.0
3	245.1	158.4	237.6	158.4	241.5	166.3	234.3	173.9	231.8	160.2	232.3	159.7
4	245.8	163.4	257.5	162.7	247.4	178.1	236.4	163.0	242.2	163.7	247.4	163.1
5	260.7	171.1	268.1	171.2	265.2	176.5	256.8	189.9	255.6	171.8	262.1	170.0
6	265.8	171.1	261.0	169.9	255.1	169.6	249.7	165.7	258.1	165.6	271.5	180.7
7	273.5	179.5	294.2	181.7	286.7	186.2	271.2	190.0	269.9	179.9	284.4	178.2
8	282.7	181.5	293.7	186.0	303.3	187.7	310.8	177.3	282.8	182.7	268.6	180.7
9	298.9	186.0	304.6	188.2	310.3	191.5	293.9	206.0	297.1	185.2	256.0	180.1
10	298.2	189.1	305.7	190.9	315.6	192.0	303.6	208.6	301.4	187.0	292.8	183.0
11	310.5	187.6	327.0	195.8	328.2	196.0	320.6	216.9	324.1	200.4	319.1	192.8
12	315.4	190.4	335.2	197.7	330.7	198.3	325.9	217.6	324.4	201.6	318.8	193.6

M males
F females

Section A6.4.2(2) Annex Point IIA, VI.6.4 IUCLID 5.4/07		A6.4.2 Repeated dose toxicity- dermal Dermal: Subchronic Test of Aqueous Glutaraldehyde (C55425) in B6C3F1 Mice and Fischer 344 Rats	
		1 REFERENCE (A6.4.2/02)	Official use only
1.1	**Reference**	[redacted] 1980) Subchronic Test of Aqueous Glutaraldehyde (C55425) in [redacted] Mice and [redacted] Rats, [redacted] Not GLP, Unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde, 2%	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Not reported	
3.1.2.1	Description	Not reported	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Assumed to be stable under typical storage conditions. Dose solutions were mixed weekly.	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age not specified Weight range (males) = 20-38 grams Weight range (females) = 11-20 grams	
3.2.6	Number of animals per group	10	
3.2.7	Control animals	Yes, concurrent vehicle	
3.3	**Administration/ Exposure**		
3.3.1	Duration of treatment	91 days	

Section A6.4.2(2) Annex Point IIA, VI.6.4 IUCLID 5.4/07		A6.4.2 Repeated dose toxicity- dermal Dermal: Subchronic Test of Aqueous Glutaraldehyde (C55425) in B6C3F1 Mice and Fischer 344 Rats	
3.3.2	Frequency of exposure	Daily	
3.3.3	Postexposure period	None	
3.3.4	**Dermal**		
3.3.4.1	Type / Preparation of Test Site	Unoccluded exposures. The dermal test site was a 1 in^2 site on the dorsal region treated with 0.2mL of the test solutions five times per week for 13 weeks. There is no indication that the material was wiped from the skin or that the animals were restrained to avoid oral ingestion	
3.3.4.2	Concentration	100% water was used as the vehicle.	
3.3.4.3	Vehicle	Water	
3.3.4.4	Concentration in vehicle	0, 0.125, 0.25, 0.5, 1, 2% analyzed on all test solutions once during the study.	
3.3.4.5	Total volume applied	0.2 mL	
3.3.4.6	Controls	Yes, concurrent vehicle	
3.4	**Examinations**		
3.4.1	Observations	Twice daily	
3.4.1.1	Clinical signs	Twice daily	
3.4.1.2	Mortality	Twice daily	
3.4.2	Body weight	Prior to first dosing, and weekly thereafter	
3.4.3	Food consumption	Not applicable	
3.4.4	Ophthalmoscopic examination	Not applicable	
3.4.5	Haematology	Not applicable	
3.4.6	Clinical Chemistry	Not applicable	
3.4.7	Urinalysis	Not applicable	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Not applicable	
3.5.2	Gross and histopathology	Mice were sacrificed on test day 91. Histopathology was performed on all control and high dose animals, and any unscheduled deaths. The stomach was examined in doses 0.25% and higher. Bladder, dermal test site, kidney, testes, stomach, thyroid, prostate, gall bladder, liver, adrenal, salivary gland, lung, uterus, and spleen were evaluated.	
3.5.3	Other examinations	None	
3.5.4	Statistics	Statistical methods, if they were used, were not specified in the report.	
3.6	**Further remarks**	None	

Section A6.4.2(2) Annex Point IIA, VI.6.4 IUCLID 5.4/07		A6.4.2 Repeated dose toxicity- dermal Dermal: Subchronic Test of Aqueous Glutaraldehyde (C55425) in B6C3F1 Mice and Fischer 344 Rats	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	It was reported that sores on the treatment areas of many males were noted, but were considered the result of fighting. There were no other clinical observations noted.	
4.1.2	Mortality	There was one death at the 1% dose level, and two deaths in the 0.5% group.	
4.2	Body weight gain	Treated animals weighed slightly less than control animals across all dose groups and for both sexes, but are considered only possibly related to treatment based on lower starting weights than the control groups and wide variation in the data.	
4.3	Food consumption	Not applicable	
4.4	Ophthalmoscopic examination	Not applicable	
4.5	**Blood analysis**		
4.5.1	Haematology	Not applicable	
4.5.2	Clinical chemistry	Not applicable	
4.5.3	Urinalysis	Not applicable	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Not applicable	
4.6.2	Gross and histopathology	Gross and microscopic diagnosis revealed no findings in mice.	
4.7	**Other**	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Groups of 10 animals/sex/dose were given dermal doses of 0.2 mL of the test material to a one square inch area of the dorsal region five times weekly for 13 weeks. Vehicle control animals received doses of distilled water. Animals were weighed prior to the first dosing, and weekly thereafter. Animals were observed twice daily for pharmacotoxic effects and mortality. They were sacrificed on test day 91. Histopathology was performed on all control and high dose animals and for any unscheduled death.	
5.2	**Results and discussion**	There was one death at the 1% dose level, and two deaths in the 0.5% group, these were not considered to be treatment related. Treated animals weighed slightly less than control animals across all dose groups and for both sexes, but are considered only possibly related to treatment based on lower starting weights than the control groups and wide variation in the data (**Table A6.4.2/02-1**). It was reported that sores on the treatment areas of many males were noted, but were considered the result of fighting. There were no other clinical observations noted.	

Section A6.4.2(2) Annex Point IIA, VI.6.4 IUCLID 5.4/07	A6.4.2 Repeated dose toxicity- dermal **Dermal: Subchronic Test of Aqueous Glutaraldehyde (C55425) in B6C3F1 Mice and Fischer 344 Rats**	
	Gross and microscopic diagnosis revealed no findings.	
5.3 Conclusion	Glutaraldehyde, when given dermally to mice for 13 weeks, did not elicit any effects indicative of systemic toxicity, and there were no findings at sacrifice that would indicate target organ toxicity.	
5.3.1 LOEL	>2% in water	
5.3.2 NOEL	2% in water	
5.3.3 Other	None	
5.3.4 Reliability	2	
5.3.5 Deficiencies	No clinical chemistry, haematology, urinalysis, organ weights, food consumption, or ophthalmology was conducted. A complete list of tissues and organs on which gross and histopathology was performed was not provided.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 5th, 2010	
Materials and Methods	3.2.6 Number of animals per group. There were 10 animals/sex/group.	
Results and discussion	See conclusion below.	
Conclusion	No conclusions can be made because 1) occlusion is not mentioned, and 2) the animals were in group cages. It is thus impossible to estimate how much dermal exposure actually occurred, because licking has most probably occurred.	
Reliability	3	
Acceptability	Not acceptable	
Remarks	The study summary is generally a valid description of the study, but detailed critical evaluation has not been performed by the RMS because of the conclusion above.	
	COMMENTS FROM ... *(specify)*	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Table A6.4.2/02-1 Body Weight Summary for Mice

Week	Control		0.125%		0.25%		0.5%		1.0%		2.0%	
	M	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*
0	26.3	19.3	26.3	19.6	28.9	19.9	25.4	19.8	29.5	16.8	29.2	20.0
1	28.1	20.9	27.6	19.5	27.8	20.1	28.4	20.7	29.2	21.0	28.9	21.0
2	30.1	22.1	29.7	22.8	30.5	21.3	27.8	21.9	29.3	20.7	30.5	21.3
3	30.9	23.7	29.9	24.2	30.3	21.9	31.7	22.8	30.8	22.3	31.7	21.9
4	29.8	22.2	30.6	21.0	32.1	20.8	30.3	22.5	30.8	20.8	30.6	23.2
5	32.2	24.2	32.1	23.1	34.0	23.6	32.7	23.5	32.1	22.8	33.0	23.3
6	31.5	23.4	33.2	24.2	34.9	22.0	29.0	22.1	32.9	21.6	29.6	24.7
7	30.8	24.9	33.1	24.7	34.2	24.1	34.4	25.1	33.5	25.0	34.3	26.4
8	33.6	24.9	33.9	24.9	35.5	23.0	34.0	23.3	33.3	22.6	33.4	22.5
9	34.0	25.3	34.3	24.3	36.0	24.3	35.3	24.8	31.8	24.2	33.4	25.4
10	30.9	23.0	34.5	24.2	34.6	24.1	34.8	24.6	34.7	23.6	30.4	25.2
11	34.9	26.2	32.2	25.0	36.0	25.5	35.7	26.9	34.7	25.5	34.7	27.3
12	35.4	27.1	35.9	25.4	36.0	23.5	33.3	22.5	33.3	25.5	33.5	26.6

M males
F females

Section A6.5(1), 6.7(1) **Annex Point IIA, VI.6.5, 6.7** **IUCLID 5.7/01**		**Chronic Toxicity** **Combined chronic toxicity/oncogenicity study in the drinking water of rats**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] (1994) Glutaraldehyde: Combined chronic toxicity/oncogenicity study in the drinking water of rats, [redacted], GLP, Unpublished, 18 March 1994	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EPA OPP 83-5	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes Verified copies of data collected on heat sensitive paper and some data on calculator tapes were discarded.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde	
3.1.1	Lot/Batch number	Lot not specified	
3.1.2	Specification	Not reported	
3.1.2.1	Description	Clear, colourless, non-viscous liquid	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Assumed to be stable under typical storage conditions.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Approximately 7 weeks of age Weight (males) = approximately 155 grams Weight (females) = approximately 110 grams	
3.2.6	Number of animals per group	100	
3.2.7	Control animals	Yes, concurrent vehicle	
3.2.8	Assignment to dose groups	Rats were assigned to dose groups based on body weight using a non-stratified randomization procedure.	

Section A6.5(1), 6.7(1) Annex Point IIA, VI.6.5, 6.7 IUCLID 5.7/01	Chronic Toxicity Combined chronic toxicity/oncogenicity study in the drinking water of rats	
3.3 Administration/ Exposure		
3.3.1 Duration of treatment	104 weeks	
3.3.2 Frequency of exposure	Continuous in the drinking water	
3.3.3 Postexposure period	None	
3.3.4 Oral		
3.3.4.1 Type	Oral, drinking water	
3.3.4.2 Concentration	100%	
3.3.4.3 Vehicle	Water	
3.3.4.4 Concentration in vehicle	0, 50, 250 or 1000 ppm	
3.3.4.5 Total volume applied	3.6, 17.1, 63.9 mg/kg/day for males 5.5, 250, 85.9 mg/kg/day for females	X
3.3.4.6 Dose Confirmation	Dose solutions were analyzed by gas chromatography. Homogeneity and stability in the drinking water were also verified. Solution concentrations were verified for all dose levels for the first 4 weeks of study prior to administration to the animals. Thereafter, at least one sample from each preparation was analyzed every 4 weeks along with a control sample.	
3.3.4.7 Controls	Yes, concurrent vehicle	
3.4 Examinations		
3.4.1 Observations	Observations for mortality were made twice daily.	
3.4.1.1 Clinical signs	Detailed clinical observations were performed once weekly, with palpitations for masses beginning on the 27th week. Observations for overt clinical signs were made on all other days.	
3.4.1.2 Mortality	Observations for mortality were made twice daily.	
3.4.2 Body weight	Body weights were collected prior to study start, and weekly thereafter until termination.	
3.4.3 Food consumption	Food consumption were measured weekly for the first 13 weeks, and every other week thereafter.	
3.4.4 Water consumption	Water consumption were measured weekly for the first 13 weeks, and every other week thereafter.	
3.4.5 Ophthalmoscopic examination	Eyes were examined by indirect ophthalmoscopy prior to study start, at weeks 52, 78, and 104, and prior to termination for all animals except those chosen for clinical pathology evaluations.	
3.4.6 Haematology	At weeks 13, 26, 52, 78, and 104, animals (10/sex/dose) were fasted and bled for haematology measurements. Reticulocyte smears were made for all animals not evaluated. The following parameters were measured or calculated: hematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin	X

Section A6.5(1), 6.7(1) Annex Point IIA, VI.6.5, 6.7 IUCLID 5.7/01	Chronic Toxicity Combined chronic toxicity/oncogenicity study in the drinking water of rats	
	concentration, erythrocyte count, leukocyte count, differential leukocyte count, platelet count,	
3.4.7 Clinical Chemistry	At weeks 12, 25, 51, 77, and 103, animals (10/sex/dose) were fasted and bled for haematology and clinical chemistry measurements. The following parameters were measured: glucose (fasting), urea nitrogen, creatinine, total protein, albumin, globulin, total bilirubin, direct bilirubin, indirect bilirubin, calcium, phosphorous, sodium, potassium, chloride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase, lactate dehydrogenase, gamma-glutamyl-transferase, sorbitol dehydrogenase, alkaline phosphatase, glutamate dehydrogenase.	X
3.4.8 Urinalysis	On weeks 10, 25, 51, and 103. Ten rats/sex/dose were placed in metabolism cages for urine collection. Urine was evaluated for osmolality, pH, protein, glucose, ketones, bilirubin, blood, urobilinogen, total volume, colour and turbidity, and microscopic constituents.	X
3.5 Sacrifice and pathology	Rats (10/sex/dose) were sacrificed at week 52 and 78, and all remaining animals at 104 weeks. A complete necropsy was performed on all animals.	
3.5.1 Organ Weights	The liver, kidneys, brain, heart, adrenals, and testes were weighed.	
3.5.2 Gross and histopathology	The following tissues were collected and examined grossly and histopathologically: gross lesions, lungs with mainstem bronchi, nasopharyngeal tissue, brain, pituitary, thyroid, thymic region, trachea, heart, sternum, salivary gland, liver, spleen, kidneys, adrenals, pancreas, testes, epididymis, prostate, seminal vesicles, ovaries, vagina, uterus, aorta, skin, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, representative lymph nodes, mammary gland, peripheral nerve, eyes, femur, spinal cord, Zymbal's gland, and exorbital lacrimal gland.	
3.5.3 Other examinations	None	
3.5.4 Statistics	The data for quantitative continuous variables were intercompared for the three treatment groups and the control group by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. The t-tests were used when the f value from the ANOVA was significant. When Levene's test indicated similar variances, and the ANOVA was significant, a pooled t-test was used for pairwise comparisons. When Levene's test indicated a heterogeneous variance, all groups were compared by an ANOVA for unequal variances, followed by a separate variance t-test for pairwise comparisons if needed. Non-parametric data was statistically evaluated using the Kruskal-Wallis test followed by the Mann-Whitney U-test when appropriate. Incidence data were compared using the Fisher's exact test. Additional analyses included a dose-response trend for the incidence of LGL luekemia to determine if increased severity of the leukemia was related to higher doses. All tumour incidence data was used to conduct a single overall statistical test for the presence of any carcinogenic effect. For all statistical tests, the probability value of < 0.05 (two-tailed) was used as the critical level of significance.	
3.6 Further remarks	None	

Section A6.5(1), 6.7(1) Annex Point IIA, VI.6.5, 6.7 IUCLID 5.7/01		Chronic Toxicity Combined chronic toxicity/oncogenicity study in the drinking water of rats	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Sporadic findings of urine soiling, emaciation, laboured respiration, pallor, and yellow cutis was observed, but the relevance of the observations was unclear due to the lack of a dose-response relationship.	
4.1.2	Mortality	There was no treatment-related effect on mortality at any dose level.	
4.2	Body weight gain	Body weight and weight gains were generally decreased throughout the study for 250 and 1000 ppm animals, compared to control values	X
4.3	Food consumption	Food consumption was generally decreased throughout the study for 250 and 1000 ppm animals, compared to control values.	X
4.4	Water consumption	Water consumption was generally decreased throughout the study for 50, 250, and 1000 ppm animals, compared to control values.	X
4.5	**Ophthalmoscopic examination**	No significant, treatment-related effects in any treated group.	
4.6	**Blood analysis**		
4.6.1	Haematology	No significant, treatment-related effects in any treated group.	X
4.6.2	Clinical chemistry	No significant, treatment-related effects in any treated group.	X
4.6.3	Urinalysis	Decreased urine volume and increased urine osmolality were noted in males and females at 250 and 1000 ppm, and are considered to be a compensatory effect associated with decreased water consumption. There were sporadic changes in urine pH. Urinary protein and bilirubin concentrations were increased in high-dose males and females at weeks 12 and 25, respectively. These changes are also considered secondary to decreased water consumption.	
4.7	**Sacrifice and pathology**		
4.7.1	Organ weights	At necropsy (at 52, 78 and 104 weeks), the only statistically significant changes in organ weights were for the kidney. Changes seen in urinary parameters and kidney weights were likely related the decreased water consumption rather than to a direct toxic action of glutaraldehyde.	X
4.7.2	Gross and histopathology	Gross evidence of gastric irritation was present in many 250 and 1000 ppm animals and included thickening of the stomach wall and ulceration of the mucosa, with mucosal hyperplasia in males and females at 104 weeks at 1000 ppm. The main finding of the study was a statistically significant increase in the number of large granular lymphocytis leukemia (LGLL) observed in the liver and spleen of females only. The main cause of death during the study was LGLL. No other significant oncogenic effects were observed.	X
4.8	**Other**	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	

Section A6.5(1), 6.7(1) Annex Point IIA, VI.6.5, 6.7 IUCLID 5.7/01	Chronic Toxicity **Combined chronic toxicity/oncogenicity study in the drinking water of rats**	
5.1 Materials and methods	[redacted] rats (100/sex/group) were dosed Glutaraldehyde at 0, 50, 250 or 1000 ppm in drinking water for 7 days/week for 104 weeks. Observations for mortality were made twice daily. Detailed clinical observations were performed once weekly, with palpitations for masses beginning on the 27th week. Observations for overt clinical signs were made on all other days. Body weights were collected prior to study start, and weekly thereafter until termination. Food and water consumption were measured weekly for the first 13 weeks, and every other week thereafter. Eyes were examined by indirect ophthalmoscopy prior to study start, at weeks 52, 78, and 104, and prior to termination for all animals except those chosen for clinical pathology evaluations. Clinical pathology evaluations were conducted on weeks 12, 25, 51, 77, and 103. Animals were fasted and bled for haematology and clinical chemistry measurements. Urine was evaluated for osmolality, pH, protein, glucose, ketones, bilirubin, blood, urobilinogen, total volume, color and turbidity, and microscopic constituents at weeks 10, 25, 51, and 103. Rats (10/sex/dose) were sacrificed at week 52 and 78, and all remaining animals at 104 weeks. A complete necropsy was performed on all animals.	
5.2 Results and discussion	**Table A6.5,7/01-1** Body weight, weight gains and food consumption were generally decreased throughout the study for 250 and 1000 ppm animals, compared to control values, while for water consumption the dose-related effect was also apparent at 50 ppm. At necropsy (at 52, 78 and 104 weeks), the only statistically significant changes in organ weights were for the kidney. Changes seen in urinary parameters and kidney weights were likely related the decreased water consumption rather than to a direct toxic action of glutaraldehyde. There were no significant, treatment related effects on haematology or clinical chemistry. Gross evidence of gastric irritation was present in many 250 and 1000 ppm animals and included thickening of the stomach wall and ulceration of the mucosa, with mucosal hyperplasia in males and females at 104 weeks at 1000 ppm. The main finding of the study was a statistically significant increase in the number of large granular lymphocyte leukemia (LGLL) observed in the liver and spleen of females only. The main cause of death during the study was LGLL. No other significant oncogenic effects were observed. Although the increase in incidence in female in treatment groups was statistically significant when compared the control value, the toxicological significance of these effect is uncertain. LGLL is a commonly occurring spontaneous neoplasm in [redacted] rats, with an incidence in control female rats varied 6-52%; in this study it was 24 % (low control value). Finally, decreased water consumption throughout the study can have some effect on this condition. A pathology peer review and pathology working group was formed to confirm the incidence and stage involvement of LGL leukemia found in Report 91U0012 (**Ref A6.5/01, Ref A6.7/01**) to render an opinion on	X

Section A6.5(1), 6.7(1) **Annex Point IIA, VI.6.5, 6.7** **IUCLID 5.7/01**	**Chronic Toxicity** **Combined chronic toxicity/oncogenicity study in the drinking water of rats**	
	the biological significance of the findings (**Ref A6.5/02, A6.7/02).** All slides containing sections of spleen, liver, and lung were examined by the reviewing pathologist, and the quality of the slides was considered to be of good quality and prepared according to the study protocol. The working group confirmed an increased incidence of LGL leukemia in all groups compared to controls, but the responses were not proportional to the dose despite the 20-fold increase in doses between low and high dose levels. Furthermore, the percentage of female rats with Stage 3 leukemia was greatest in the control group, while stage 1 and 2 leukemia were most frequent in the high dose group. When a weight-of-evidence approach was applied, the working group concluded that the observed increase in LGL leukemia in female F344 rats had an uncertain relationship to ingestion of glutaraldehyde in drinking water. There was no known mechanism for the increased incidence in female rats, and there was no comparable finding in male rats. There was no evidence of systemic, acute, subchronic, or chronic toxicity involving the hematopoetic system. *In vivo* bone marrow cytogenicity studies were reported to be negative in both sexes, nor did the NTP report similar findings following a 2-year inhalation study. A chronic drinking water study conducted by BASF at the same dose levels in the Wistar rat (a strain with a low spontaneous frequency of background LGL leukemia) also could not repeat the findings. The Working group therefore concluded that the finding of increased LGL leukemia in one sex, one species, and one strain is not toxicologically relevant to human risk assessment, even when the increase was noted as statistically significant.	
5.3 Conclusion	No clear NOEL was established for this study due the decreased water consumption in all dose groups of male and female rats and increased incidence of LGLL in all dose groups of female rats.	
5.3.1 LOEL	No clear LOEL established.	
5.3.2 NO(A)EL	No clear NOEL established.	
5.3.3 Other	None	
5.3.4 Reliability	1	
5.3.5 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 31st, 2010	

Section A6.5(1), 6.7(1) Annex Point IIA, VI.6.5, 6.7 IUCLID 5.7/01	**Chronic Toxicity** **Combined chronic toxicity/oncogenicity study in the drinking water of rats**	
Materials and Methods	3.1.1 Lot/Batch number. The containers used were numbered 51-284A, B and C; 51-528A and B; 52-416A and B; 52-700A and B. 3.2.6 Number of animals per group. There were 100 animals/sex/group. 3.3.4.5 Total volume applied. The mean intake for females in the intermediate dose group is given incorrectly. The correct value is 25.1 mg/kg bw/day. In addition, the RMS concludes that the intake was given to the 50 % test substance and not glutaraldehyde (100 %). This is based on the following arguments: • The test substance was 50 % glutaraldehyde, which in the study is referred to as "glutaraldehyde". • No correction for concentration is mentioned in the study report. Another drinking water study on rats [redacted] yielded very similar intakes at 100 ppm (3.2 mg GA/kg bw/day) as in the current study at 50 ppm (reportedly 3.6 mg GA/kg bw/day).Based on the above, the RMS concludes that the glutaraldehyde (pure) intakes were as follows: • 1.8, 8.6 and 32 mg GA/kg bw/day for males • 2.8, 12.6 and 43 mg GA/kg bw/day for females 3.4.6 Haematology. There were 20 animals/sex/dose (not 10). Reticulocyte smears were reportedly made for all animals but they were not evaluated. 3.4.7 Clinical Chemistry. There were 20 animals/sex/dose (not 10), and the weeks of clinical chemistry are given incorrectly. The correct weeks are the same as for haematology in point 3.4.6. 3.4.8 Urinalysis. The weeks of urinalysis are given incorrectly. The correct weeks were 12, 25, 51, 77 and 103.	
Results and discussion	4.2 Body weight gain. Decreases in bw were seen in males at all dose levels and females of the 1000 ppm group. The difference in the 50 ppm male group was present between weeks 97 and 103 (last weighing), and reached statistical significance only at week 103. 4.3 Food consumption. Food consumption was decreased in the 1000 ppm animals. There were only slight and transient decreases observed in the 250 ppm animals. 4.4 Water consumption. Water consumption was reduced throughout the study in the 1000 ppm animals (9 to 37 %) and 250 ppm animals (6 to 22 %), while for the 50 ppm animals the difference was not significant. 4.6.1 Haematology. Total leukocyte count was increased at week 104 due to increased large granular lymphocytes (large monocytes) in both males and females of the 250 and 1000 ppm groups. This was explained by the increased incidence of LGL leukaemia. Males at the 250 and 1000 ppm groups had an increase in nucleated red cells at week 104, which might be explained similarly. 4.6.2 Clinical chemistry. There were inconsistent changes in serum enzyme activities (ASAT, ALAT, glutamate dehydrogenase and alkaline phosphatase), occurring without clear patterns in different dose groups, males and/or females, and in different time points (13/26/52/78/104 weeks). These were attributed to decreased bw gain and/or decreased food consumption in these groups. 4.7.1 Organ weights. • For conclusions on kidney weights, please see comments on 4.7.2 below. • There were many more changes that were statistically significant, but these were inconsistent and are considered to be unrelated to the treatment.	

Section A6.5(1), 6.7(1) Annex Point IIA, VI.6.5, 6.7 IUCLID 5.7/01	Chronic Toxicity Combined chronic toxicity/oncogenicity study in the drinking water of rats	

4.7.2 Gross and histopathology.

- See Table RMS added below, summarising the stomach and kidney findings.
- Stomach lesions included focal/multifocal colour change (males and females starting at 52 weeks and 250 ppm) and ulceration of the gastric mucosa (males and females at 104 weeks and 1000 ppm). Microscopic findings included keratinized cysts (males 104 weeks, high dose) and hyperplasia of the squamous epithelium (males and females at 104 weeks, high dose). All findings are concluded to be due to a local rather than a systemic effect.
- Kidney findings included an increased incidence of tubular pigmentation in females of the two highest dose groups and males of the high dose group at week 104, and tubular basophilia in females of the two highest dose groups at week 104. These findings may indicate kidney damage, although other findings in kidneys are scarce and nephritis is only slightly more frequent in females (not males) of the high dose group at week 104. As suggested by the applicant, the changes in kidney weight may be a consequence of reduced water consumption and urine volume and not indicate kidney damage.

5.2 Results and discussion.

- Same comments as to points 4.2, 4.3, 4.4, 4.6.1, 4.6.2, 4.7.1 and 4.7.2.
- The discussion provided by the applicant includes details from the following confirmatory study that was done to verify the results and discuss the relevance of the increased LGLL incidence: [redacted] (2003), *Pathology peer review and pathology working group (PWG) review of large granular lymphocyte leukaemia (LGL) in a combined chronic toxicity/oncogenicity study in the drinking water with glutaraldehyde in female* [redacted] *rats*, [redacted] The references given (Ref A6.5/02, A6.7/02) refer to an inhalation study and are not relevant here.
- Male body weights were reduced also at 50 ppm during approximately weeks 97-104 (until termination of study).
- There were effects observed in the stomach and in the kidneys that were not properly discussed in the original study. The RMS agrees with the conclusions however. All the effects seen in the stomach and in the kidneys are put together in Table RMS below.
- It is correct that a higher percentage of the leukaemia cases observed in the control group females were of Stage 3, but there were still fewer stage 3 leukaemia cases in the control group than in any of the dose groups.
- Please see Figure 1 added by the RMS which shows the survival curves for males and females. The decreased survival rate and time of the low dose group females was statistically significant and is probably related with the increased LGLL incidence. Nevertheless, at the two higher doses the survival times were closer to the control group and without significant differences, regardless of the similar (mid-dose) or higher (high dose) LGLL incidences. For both males and females, the difference was small in survival between the high dose group and the control group.

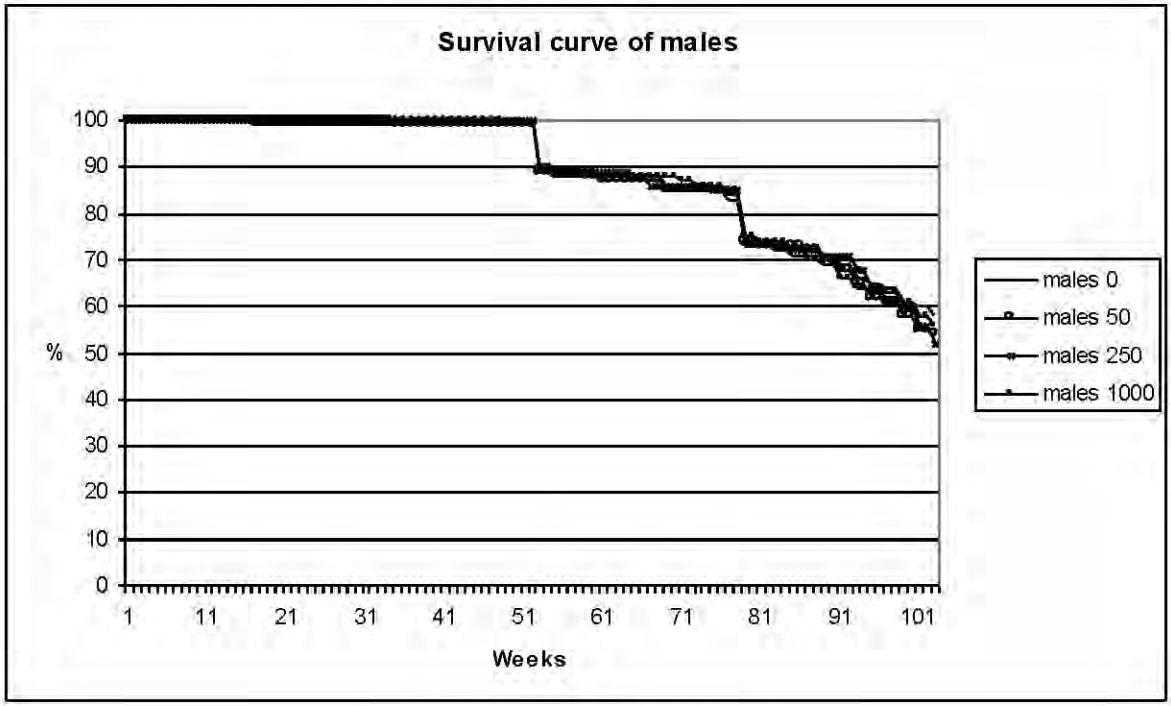
Section A6.5(1), 6.7(1) Annex Point IIA, VI.6.5, 6.7 IUCLID 5.7/01	**Chronic Toxicity** **Combined chronic toxicity/oncogenicity study in the drinking water of rats**
Conclusion	No clear NOAEL or LOAEL can be established based on this study. Apart from the increased incidence of LGL leukaemia in females, the main findings were in the stomach and kidney. The increased LGL leukaemia did not have any major impact on the survival rates of the animals. • The stomach findings are considered as secondary to local irritant effects. • For the kidney findings, a direct effect cannot be excluded but RMS agrees with the applicant that the effects may also be due to a lifetime of reduced water consumption. Findings at the lowest dose level were minimal or non-existent except for the LGL leukemia, and this could be considered an indicative/putative NOAEL (50 ppm, corresponding to 1.8 and 2.8 mg/kg bw/day for males and females, respectively). LO(A)EL: not established NO(A)EL: not established
Reliability	1
Acceptability	Acceptable
Remarks	The data given in Table A6.5,7/01-1 has not been checked in detail by the RMS.
	COMMENTS FROM ... (specify)
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.5,7/01-1 Summary of Treatment-Related Findings

	Control		50 ppm		250 ppm		1000 ppm	
	M	*F*	*M*	*F*	*M*	*F*	*M*	*F*
Number of animals examined	58	65	54	48	52	56	56	58
In-Life Observations- 104-week Sacrifice								
Final Body Weight (grams)	387.0	265.3	*372.7*	264.4	**366.1**	261.7	**351.3**	**244.8**
Final Water Consumption (grams)	28.3	24.4	27.3	24.2	**23.9**	**21.8**	**21.9**	**19.0**
Final Food Consumption (grams)	17.1	13.8	16.6	14.8	16.4	13.5	16.7	13.3
Organ Weights- 104-week Sacrifice								
Brain Weight (grams)	1.968	1.804	1.966	*1.781*	1.983	1.801	1.976	**1.773**
Relative Brain Weight	0.558	0.740	0.570	0.730	*0.588*	0.737	**0.612**	**0.780**
Kidney Weight (grams)	2.994	1.890	*2.791*	1.895	*2.789*	**1.969**	**2.770**	**2.026**
Relative Kidney Weight	0.851	0.774	0.809	0.778	0.825	0.804	0.860	**0.890**
Adrenal Weight (grams)	0.077	0.059	0.066	0.057	0.063	0.080	0.063	0.059
Relative Adrenal Weight	0.022	0.024	0.019	0.023	0.019	0.034	0.020	*0.026*
Urinalysis- 103 weeks								
Total Volume (mL)	16.8	11.2	16.6	14.6	12.1	13.4	*10.1*	9.4
Osmolality (mOSMO/kg)	1190	1434	1257	1345	*1529*	1499	*1611*	*1795*
pH	6.7	6.4	6.7	6.4	6.6	6.3	6.3	6.4
Large Granular Lymphocytis Leukemia								
Incidence in Spleen (# present / # counted)	43/100	**24/100***	51/100	**41/100***	40/100	**41/100***	46/100	**53/100***

bold indicates statistically significant at p<0.01
italics indicates statistically significant at p<0.05
* indicates significant time-to-tumor analyses and significant tumor responses as well as incident significance.

Figure 1. Survival curves of males and females (added by RMS). This data is based on the number of animals weighed at each time point. Note the scheduled sacrifice of 10 animals of each dose group at weeks 52 and 78.



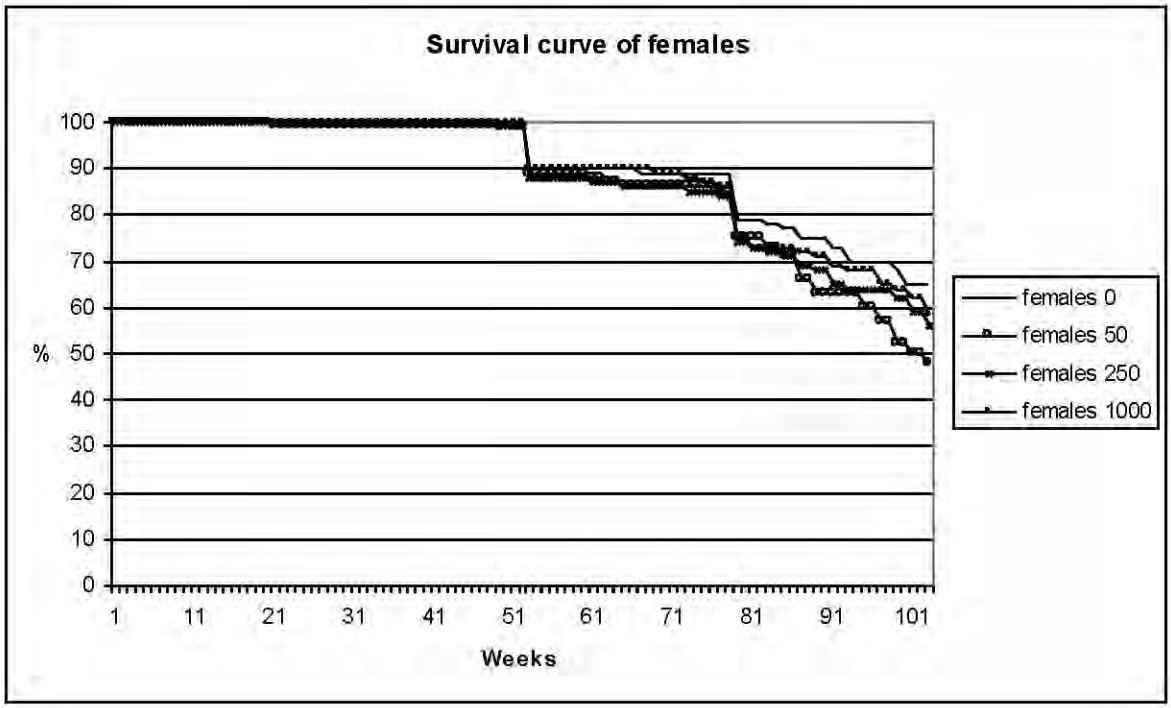


Table RMS. Effects observed in the stomach and in the kidneys.

Effects in the stomach

			Incidence				n				%			
			Control	50 ppm	250 ppm	1000 ppm	Control	50 ppm	250 ppm	1000 ppm	Control	50 ppm	250 ppm	1000 ppm
Colour change, focal/multifocal (gross necropsy)	male	52 weeks	0	0	3	3	10	10	10	10	0 %	0 %	30 %	30 %
		78 weeks	0	0	0	0	9	9	10	9	0 %	0 %	0 %	0 %
		104 weeks	1	3	4	3	56	52	51	51	2 %	6 %	8 %	6 %
		Found dead or moribund	7	6	7	5	25	29	29	30	28 %	21 %	24 %	17 %
	female	52 weeks	0	0	4	0	10	10	10	10	0 %	0 %	40 %	0 %
		78 weeks	0	0	0	2	9	8	9	10	0 %	0 %	0 %	20 %
		104 weeks	2	0	5	5	62	47	52	56	3 %	0 %	10 %	9 %
		Found dead or moribund	2	8	10	10	19	35	29	24	11 %	23 %	34 %	42 %
Ulcerated (gross necropsy)	male	52 weeks	0	0	0	0	10	10	10	10	0 %	0 %	0 %	0 %
		78 weeks	0	0	0	0	9	9	10	9	0 %	0 %	0 %	0 %
		104 weeks	1	3	0	6	56	52	51	51	2 %	6 %	0 %	12 %
		Found dead or moribund	2	0	1	0	25	29	29	30	8 %	0 %	3 %	0 %
	female	52 weeks	0	0	0	0	10	10	10	10	0 %	0 %	0 %	0 %
		78 weeks	0	0	0	0	9	8	9	10	0 %	0 %	0 %	0 %
		104 weeks	1	1	0	5	62	47	52	56	2 %	2 %	0 %	9 %
		Found dead or moribund	0	3	0	8	19	35	29	24	0 %	9 %	0 %	33 %
Mucosal hyperplasia (microscopic diagnoses)	male	52 weeks	0	0	2	0	10	10	10	10	0 %	0 %	20 %	0 %
		78 weeks	0	0	0	0	9	9	10	9	0 %	0 %	0 %	0 %
		104 weeks	1	1	1	7	56	52	51	51	2 %	2 %	2 %	14 %
		Found dead or moribund	1	3	1	3	25	29	29	30	4 %	10 %	3 %	10 %
	female	52 weeks	0	0	0	0	10	10	10	10	0 %	0 %	0 %	0 %
		78 weeks	0	0	0	0	9	8	9	10	0 %	0 %	0 %	0 %
		104 weeks	1	0	1	7	62	47	52	56	2 %	0 %	2 %	13 %
		Found dead or moribund	0	2	0	7	19	35	29	24	0 %	6 %	0 %	29 %
Keratin cyst (microscopic diagnoses)	male	52 weeks	0	0	0	0	10	10	10	10	0 %	0 %	0 %	0 %
		78 weeks	0	1	0	0	9	9	10	9	0 %	11 %	0 %	0 %
		104 weeks	0	0	0	4	56	52	51	51	0 %	0 %	0 %	8 %
		Found dead or moribund	0	0	0	0	25	29	29	30	0 %	0 %	0 %	0 %
	female	52 weeks	0	0	0	0	10	10	10	10	0 %	0 %	0 %	0 %
		78 weeks	0	0	1	0	9	8	9	10	0 %	0 %	11 %	0 %
		104 weeks	0	0	0	0	62	47	52	56	0 %	0 %	0 %	0 %
		Found dead or moribund	0	0	0	0	19	35	29	24	0 %	0 %	0 %	0 %
Lymphoid infiltrates (microscopic diagnoses)	male	52 weeks	0	0	0	0	10	10	10	10	0 %	0 %	0 %	0 %
		78 weeks	0	0	0	0	9	9	10	9	0 %	0 %	0 %	0 %
		104 weeks	1	0	1	6	56	52	51	51	2 %	0 %	2 %	12 %
		Found dead or moribund	1	2	0	0	25	29	29	30	4 %	7 %	0 %	0 %
	female	52 weeks	0	0	0	0	10	10	10	10	0 %	0 %	0 %	0 %
		78 weeks	0	0	0	0	9	8	9	10	0 %	0 %	0 %	0 %
		104 weeks	1	0	0	2	62	47	52	56	2 %	0 %	0 %	4 %
		Found dead or moribund	0	0	0	0	19	35	29	24	0 %	0 %	0 %	0 %

Effects in the kidneys

			Incidence				n				%			
			Control	50 ppm	250 ppm	1000 ppm	Control	50 ppm	250 ppm	1000 ppm	Control	50 ppm	250 ppm	1000 ppm
Colour change, diffuse (gross necropsy)	male	52 weeks	0	0	0	0	10	10	10	10	0 %	0 %	0 %	0 %
		78 weeks	0	0	0	0	9	9	10	9	0 %	0 %	0 %	0 %
		104 weeks	6	7	5	11	56	52	51	51	11 %	13 %	10 %	22 %
		Found dead or moribund	5	2	6	6	25	29	29	30	20 %	7 %	21 %	20 %
	female	52 weeks	0	0	0	0	10	10	10	10	0 %	0 %	0 %	0 %
		78 weeks	0	0	0	0	9	8	9	10	0 %	0 %	0 %	0 %
		104 weeks	5	7	9	7	62	47	52	56	8 %	15 %	17 %	13 %
		Found dead or moribund	3	5	4	8	19	35	29	24	16 %	14 %	14 %	33 %
Tubular basophilia (microscopic diagnoses)	male	52 weeks	6	2	3	7	10	10	10	10	60 %	20 %	30 %	70 %
		78 weeks	8	9	9	9	9	9	10	9	89 %	100 %	90 %	100 %
		104 weeks	53	42	43	45	56	52	51	51	95 %	81 %	84 %	88 %
		Found dead or moribund	12	4	8	9	25	29	29	30	48 %	14 %	28 %	30 %
	female	52 weeks	0	3	3	2	10	10	10	10	0 %	30 %	30 %	20 %
		78 weeks	5	1	6	6	9	8	9	10	56 %	13 %	67 %	60 %
		104 weeks	17	19	26	38	62	47	52	56	27 %	40 %	54 %	68 %
		Found dead or moribund	2	6	3	5	19	35	29	24	11 %	17 %	10 %	21 %
Tubular pigmentation (microscopic diagnoses)	male	52 weeks	0	0	0	0	10	10	10	10	0 %	0 %	0 %	0 %
		78 weeks	0	0	0	0	9	9	10	9	0 %	0 %	0 %	0 %
		104 weeks	4	6	4	15	56	52	51	51	7 %	12 %	8 %	29 %
		Found dead or moribund	2	4	2	10	25	29	29	30	8 %	14 %	7 %	33 %
	female	52 weeks	0	0	0	0	10	10	10	10	0 %	0 %	0 %	0 %
		78 weeks	1	0	0	0	9	8	9	10	11 %	0 %	0 %	0 %
		104 weeks	12	11	29	41	62	47	52	56	19 %	23 %	56 %	73 %
		Found dead or moribund	6	11	11	17	19	35	29	24	32 %	31 %	38 %	71 %

Section A6.6.1(1) Annex Point IIA, VI.6.1 IUCLID 5.5/01		Genotoxicity *in vitro* **Bacterial assay for gene mutation**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] (1993a) [redacted] (Glutaraldehyde, 50% aqueous solution): Mutagenic Potential in the Salmonella/microsome (Ames) assay, [redacted], Unpublished, 15 September 1993	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes US EPA OPP 84-2a	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes The vehicle and positive control substances were not analyzed for chemical purity, stability or uniformity. Analyses for stability and homogeneity of the test and control substances in the dosing solutions were not conducted.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] (Glutaraldehyde 50% aqueous solution)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Not reported	
3.1.2.1	Description	Transparent, colorless, non-viscous liquid	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Assumed to be stable under normal storage conditions	
3.2	**Study Type**	Bacterial mutagenicity assay	
3.2.1	Organism/cell type	***S. typhimurium*** **strains** TA 1535, TA 1537, TA 98, TA 100 and TA 1538	
3.2.2	Deficiencies / Proficiencies	See Section 2.3	
3.2.3	Metabolic activation system	Metabolic activation was accomplished using rat liver S9 homogenate, prepared from Aroclor 1254-induced rats purchased from a commercial supplier. Fresh activation was prepared each day of testing, and stored at 0-4C.	
3.2.4	Positive control	**Strain** **Without S9** **With S9** TA100 NaN_3 2-AA	

Section A6.6.1(1) **Annex Point IIA, VI.6.1** **IUCLID 5.5/01**		**Genotoxicity *in vitro*** **Bacterial assay for gene mutation**	
		TA1535 NaN_3 2-AA TA98 4-NPD 2-AA TA1537 9-AA 2-AA TA1538 4-NPD 2-AA **where :** **4-NPD** = 4-nitro-o-phenylenediamine **NaN_3** = sodium azide **9-AA** = 9-aminoacridine **2-AA** = 2-aminoanthracene	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	**Concentrations of the 50% Glutaraldehyde test material** **Test 1** Without S9 activation 0.003, 0.01, 0.03, 0.10, 0.20 and 0.30 With S9 activation 0.003, 0.01, 0.03, 0.10, 0.20 and 0.30 **Test 2** Without S9 activation 0.003, 0.01, 0.03, 0.05 and 0.10 With S9 activation 0.01, 0.03, 0.10, 0.15 and 0.20	
3.3.2	Way of application	The tested strains were treated in triplicate with the vehicle control, the positive control, and 5-6 dose levels in a preliminary assay and the definitive test. The tests were completed in the presence and absence of S9 activation using the plate incorporation method. Treated cultures were incubated at 37°C for 48-72 hours.	
3.3.3	Pre-incubation time	No pre-incubation, plate incorporation method utilized	
3.3.4	Other modifications	None	
3.4	**Examinations**	Revertant colonies were counted either manually or on a colony counter. The number of colonies per plate was counted and recorded. An examination of background lawn was observed for each plate. If no lawn was present, it was noted, and there was no plate count recorded. If a lawn was sparse, the colonies were counted and the colony count was used to calculate a mean and standard deviation. A reduction in the number of spontaneous revertant colonies is also an indication of toxicity. A dose level was labeled "toxic" when the mean number of colonies / plate was less than one half the mean for the vehicle control.	
3.4.1	Number of cells	Not applicable	

Section A6.6.1(1) Annex Point IIA, VI.6.1 IUCLID 5.5/01		Genotoxicity *in vitro* Bacterial assay for gene mutation	
	evaluated		
		RESULTS AND DISCUSSION	
3.5	**Genotoxicity**	(**Table A6.6.1/01-1 & Table A6.6.1/01-2**)	
3.5.1	without metabolic activation	Negative in all strains (**Table A6.6.1/01-1 & Table A6.6.1/01-2**)	
3.5.2	with metabolic activation	Negative in TA98, TA1535, TA1537 and TA1538 and weakly mutagenic in TA100 (**Table A6.6.1/01-1 & Table A6.6.1/01-2**)	
3.6	**Cytotoxicity**	[redacted] was non-toxic at doses of 0.03mg/plate or less in the absence of S9 to strains TA98 and TA100 (only two strains tested). In the presence of S9 [redacted] was non-toxic at 0.1mg/plate or less.	
		4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	The vehicle control was sterile, distilled, deionized water. Metabolic activation was accomplished using rat liver S9 homogenate, prepared from Aroclor 1254-induced rats purchased from a commercial supplier. Fresh activation was prepared each day of testing, and stored at 0-4 °C. Using the plate incorporation method, Salmonella strains TA98, TA100, TA1537, and TA1538 were treated in triplicate with the vehicle control, the positive control, and 5-6 dose levels (ranging from 0.001-10 mg/plate for the preliminary assay, and 0.003-0.1 mg/plate non-activated or 0.01-0.2 mg/plate activated in the definitive test) of [redacted] in the presence and absence of S9 activation. Concentrations of (50, 75, 100 uL/plate) were tested in the cytotoxicity preliminary test and 50 uL/plate concentration of S9 was utilized for the mutagenicity tests. Treated cultures were incubated at 37°C for 48-72 hours. Two independent repetitions of the complete assay were performed. Growth was recorded as either confluent, sparse, or absent. Confluence indicated nontoxicity, sparse growth indicated moderate toxicity, and no growth indicated extreme toxicity in the preliminary cytotoxicity assay. Revertant colonies were counted either manually or on a colony counter in the mutagenicity assay. The number of colonies per plate was counted and recorded. An examination of background lawn was observed for each plate. If no lawn was present, it was noted, and there was no plate count recorded. If a lawn was sparse, the colonies were counted and the colony count was used to calculate a mean and standard deviation. A reduction in the number of spontaneous revertant colonies is also an indication of toxicity. A dose level was labeled "toxic" when the mean number of colonies / plate was less than one half the mean for the vehicle control.	
4.2	**Results and discussion**	**Table A6.6.1/01-1, 2** [redacted] (Glutaraldehyde 50% Aqueous solution) did not produce consistent, dose related mutagenic effects in Salmonella strains TA98, TA1535, TA1537 or TA1538 either in the presence or absence of S9 metabolic activation. No mutagenic activity was observed in strain TA100 in the absence of S9 but in the presence of S9 [redacted] was weakly mutagenic increasing the frequencies of revertant colonies approximately two fold. These results were obtained in each of the independent tests.	

Section A6.6.1(1) **Annex Point IIA, VI.6.1** **IUCLID 5.5/01**	**Genotoxicity *in vitro*** **Bacterial assay for gene mutation**	
	A full compendium of genotoxicity studies can be found in the following article, provided with the dossier: Zeiger, E., Gollapudi, B., and Spencer, P. (2005) Genetic toxicity and carcinogenicity studies of glutaraldehyde- a review, Mutation Research, 589, 136-151, Published.	
4.3 Conclusion	The test material is considered negative in the bacterial reverse mutation assay for strains TA98, TA1535, TA1537 and TA1538 with and without S9 activation and TA100 without S9 activation. U[redacted] is weakly mutagenic in strain TA100 in the presence of S9 metabolic activation.	
4.3.1 Reliability	1	
4.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 26th, 2010	
Materials and Methods	3.1.2.2 Purity: 50.9 % glutaraldehyde, 0.3 % methanol, probably an adduct of water and formyldihydropyran 0.2 %, no other organic impurity at a concentration greater than 0.1 %. 3.1.2.3 Test substance stability was not confirmed by the test laboratory. 3.2 The study was done in compliance with the OECD 471 Guideline. 3.3.1 Concentrations of the test substance (50 % glutaraldehyde) are given as mg/plate. 3.6 The cytotoxicity results mentioned in this section were determined in a cytotoxicity preliminary test.	
Results and discussion	Applicant's version accepted.	
Conclusion	A 2.3-fold increase in the number of revertant colonies was observed in strain TA100 treated with 0.10 mg/plate [redacted] in the presence of S9 activation in Test 1. In an independent repetition of the first test, there was a 1.9-fold increase in the same strain at the same concentration in the presence of S9. There was an increase in the frequency of revertants with increasing dose of [redacted] over the range of 0.01 to 0.10 mg/plate in both tests. Therefore, [redacted] was weakly mutagenic in strain TA100 in the presence of S9 metabolic activation. It is known that S. typhimurium strains TA 1535, TA1537, TA98, TA100 and TA1538 may not detect cross-linking agents. Hence, in order to detect cross-linking mutagens it would be preferable to include S. typhimurium TA102 or E. coli WP2 or E. coli WP2 (pKM101). As a consequence of the strains tested, the result of this study may be an underestimation of the mutagenic potential of glutaraldehyde.	
Reliability	2	
Acceptability	Acceptable	
Remarks	The study is acceptable but it can not be considered a complete bacterial reverse mutation test because the strains used in the study can not properly detect cross-linking properties of a substance.	
	COMMENTS FROM DOW BENELUX B.V.	

Section A6.6.1(1) **Annex Point IIA, VI.6.1** **IUCLID 5.5/01**	**Genotoxicity *in vitro*** **Bacterial assay for gene mutation**	
Date	3 June 2009; 27 January 2011	
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability	We agree that the study did not use strains that are sensitive to cross-linking agents, thus the inclusion of Wantanabe et al., (1998) [A6.6.1(2)] and Muller et al., (1993) [A6.6.1(3)] that show Glutaraldehyde to be positive when using S. typhimurium strains TA102 and TA2638 and E. coli strains WP2/pKM101 and WP2 uvrA/pKM101. Furthermore, in Zeiger et al., (2005), 14 studies from the literature are reviewed in which the majority reported positive results in S. typhimurium strains TA100, TA102, and TA104 with and/or without liver S9.	
Remarks		

Table A6.6.1/01-1 Ames Bacterial Assay #1 for Gene Mutations in the Absence & Presence of S-9

	TA98		TA100		TA1535		TA1537		TA1538	
mg/Plate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
With S-9										
0.0	28	2.1	113	13.9	14	2.0	10	2.9	23	8.0
PC[b]	1019	95.3	1190	224.9	87	6.4	111	17.5	1072	113.4
0.003	32	8.4	113	16.5	15	3.2	8	0.6	19	5.2
0.01	27	1.7	137	10.6	11	3.2	10	4.9	19	3.8
0.03	33	2.5	143	5.3	15	3.6	8	2.3	24	1.5
0.10	37	5.6	262	55.1	14	0.6	11	4.6	19	3.2
0.20	13(T)	4.2	10(T)	10.6	4(T)	3.8	2(T)	2.5	2(T)	1.7
0.30	T	---	T	---	T	---	T	---	T	---
Without S-9										
0.0	33	4.7	125	7.8	13	5.3	10	4.4	10	1.2
PC[a]	578	12.5	1181	60.3	1164	108.4	392	76.0	855	89.6
0.003	24	6.7	150	12.1	20	2.1	9	4.0	14	4.9
0.01	22	1.2	162	20.0	22	4.0	12	1.5	15	6.6
0.03	44	6.4	229	55.1	17	2.6	9	2.5	11	2.5
0.10	T	---	178	82.1	12	4.9	11	1.2	T	---
0.20	T	---	T	---	T	---	T	---	T	---
0.30	T	---	T	---	T	---	T	---	T	---

T Toxic; absence of background lawn, or mean number of colonies <1/2 solvent control value.
--- No average available

a-Positive Control Compounds Without S-9	
TA98:	4-nitro-o-phenylenediamine (0.01 mg/plate)
TA100:	sodium azide, 0.01 mg/plate
TA1535:	sodium azide, 0.01 mg/plate
TA1537:	9-aminoacridine, 0.06 mg/plate
TA1538:	4-nitro-o-phenylenediamine (0.01 mg/plate)

b-Positive Control Compounds With S-9	
TA98:	2-aminoanthracene, 2.5 ug
TA100:	2-aminoanthracene, 2.5 ug
TA1535:	2-aminoanthracene, 2.5 ug
TA1537:	2-aminoanthracene, 2.5 ug
TA1538:	2-aminoanthracene, 2.5 ug

Table A6.6.1/01-2 Ames Bacterial Assay #2 for Gene Mutations in the Absence & Presence of S-9

	TA98		TA100		TA1535		TA1537		TA1538	
mg/Plate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
With S-9										
0.0	34	4.9	146	12.4	16	4.0	10	1.5	23	2.1
PC[b]	1098	30.1	905	77.1	97	5.7	75	5.1	1013	61.7
0.003	31	4.5	151	9.2	15	5.1	9	0.0	20	0.6
0.01	39	10.7	221	30.8	15	4.6	9	0.0	16	4.7
0.03	52	11.5	275	43.8	16	3.8	11	4.6	20	2.0
0.05	36	9.2	46(T)	16.4	7(T)	1.5	4(T)	3.5	9(T)	3.2
0.10	T	---	(T)	---	T	---	11(T)	0.0	T	---
Without S-9										
0.0	26	10.4	96	8.1	17	7.4	5	2.5	9	4.6
PC[b]	488	28.0	1239	59.5	1155	61.3	86	31.2	728	40.3
0.003	26	4.0	102	11.1	13	4.2	8	1.5	13	6.0
0.01	23	4.2	131	25.0	17	4.2	8	2.6	11	1.5
0.03	33	9.0	133	19.8	18	1.5	7	2.1	15	3.2
0.05	15	3.0	132	8.5	13	3.6	7	3.5	11	0.0
0.10	T	---	46(T)	14.2	10	1.0	4(T)	1.7	T	---

T Toxic; absence of background lawn, or mean number of colonies <1/2 solvent control value.
---No average available

a-Positive Control Compounds Without S-9	
TA98:	4-nitro-o-phenylenediamine (0.01 mg/plate)
TA100:	sodium azide, 0.01 mg/plate
TA1535:	sodium azide, 0.01 mg/plate
TA1537:	9-aminoacridine, 0.06 mg/plate
TA1538:	4-nitro-o-phenylenediamine (0.01 mg/plate)

b-Positive Control Compounds With S-9	
TA98:	2-aminoanthracene, 2.5 ug
TA100:	2-aminoanthracene, 2.5 ug
TA1535:	2-aminoanthracene, 2.5 ug
TA1537:	2-aminoanthracene, 2.5 ug
TA1538:	2-aminoanthracene, 2.5 ug

Section A6.6.2(1) Annex Point IIA, VI.6.2 IUCLID 5.5/07	*In-vitro* Cytogenicity study in mammalian cells Sister chromatid exchange assay in cultured CHO cells	
	1 REFERENCE	Official use only
1.1 Reference	[redacted] (1994a) [redacted] (glutaraldehyde, 50% aqueous solution): Sister chromatid exchange assay in cultured CHO cells, [redacted], Unpublished, 7 April 1994	
1.2 Data protection	Yes	
1.2.1 Data owner	The Dow Chemical Company	
1.2.2 Companies with letter of access	[redacted]	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes US EPA OPP 84-2b	
2.2 GLP	Yes	
2.3 Deviations	Yes The vehicle and positive control substances were not analyzed for chemical purity, stability or uniformity. Analyses for stability and homogeneity of the test and control substances in the dosing solutions were not conducted.	
	3 MATERIALS AND METHODS	
3.1 Test material	[redacted] (Glutaraldehyde 50% aqueous solution)	
3.1.1 Lot/Batch number	[redacted]	
3.1.2 Specification	Not reported	
3.1.2.1 Description	Transparent, colourless, non-viscous liquid	X
3.1.2.2 Purity	[redacted]	
3.1.2.3 Stability	Assumed to be stable under normal storage conditions	X
3.2 Study Type	Sister chromatid exchanges	
3.2.1 Organism/cell type	Cultured Chinese Hamster Ovary Cells	
3.2.2 Deficiencies / Proficiencies	See Section 2.3	
3.2.3 Metabolic activation system	Metabolic activation was accomplished using rat liver S9 homogenate, prepared from Aroclor 1254-induced rats purchased from a commercial supplier.	
3.2.4 Positive control	**In the absence of metabolic activation (S9)** Ethylmethane sulfonate (EMS), Lot-88F-0531 **In the presence of metabolic activation (S9)** Dimethylnitrosamine (DMN), Lot- 38F0882	
3.3 Administration /		

Section A6.6.2(1) **Annex Point IIA, VI.6.2** **IUCLID 5.5/07**		***In-vitro* Cytogenicity study in mammalian cells** **Sister chromatid exchange assay in cultured CHO cells**	
	Exposure; Application of test substance		
3.3.1	Concentrations	**Cytotoxicity study** **In the absence & presence of metabolic activation (S9)** 0, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.10, 0.30, 1.0, 3.0 and 10.0 mg/ml equivalent to 0.0002, 0.0005, 0.002, 0.005, 0.02, 0.05, 0.15, 0.51, 1.5 and 5.1 mg/ml glutaraldehyde, corrected for percent active ingredient. **Main Assay** **In the absence of metabolic activation (S9)** 0.00003, 0.0001, 0.0003, 0.001 and 0.003 mg/ml equivalent to 0.00002, 0.00005, 0.0002, 0.0005 and 0.002 mg/ml glutaraldehyde, corrected for percent active ingredient. **In the presence of metabolic activation (S9)** 0.00001, 0.00003, 0.0001, 0.0003, 0.001 and 0.003 mg/ml equivalent to 0.000005, 0.00002, 0.00005, 0.0002, 0.0005 and 0.002 mg/ml glutaraldehyde, corrected for percent active ingredient.	
3.3.2	Way of application	CHO cells were plated approximately 40 to 48 hours prior to treatment. Duplicate cultures were treated with the test solutions of 5 concentration ranging from 0.00003-0.003 mg/mL in the absence of S9, and 6 concentrations ranging from 0.00001-0.003 mg/mL in the presence of S9. Treatment time was 4 hours for both systems. After treatment, the medium and test substance were removed and fresh F12 medium, dialyzed bovine serum and BrdU was added, and cultures were incubated for an additional 24 to 28 hours. Dosing solutions were prepared daily.	
3.3.3	Pre-incubation time	Not Applicable	
3.3.4	Other modifications	Efforts to dose the test substance at concentrations ranging from 0.001 - 0.01mg/ml in the absence of S9 or at 0.001 - 0.03 mg/ml in the presence of S9 were unsuccessful since they did not yield adequate numbers of metaphase cells containing differentially stained chromosomes.	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	Minimum of 25 cells/duplicate culture	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.1.1	without metabolic activation	Negative (**Table A6.6.2/01-1**)	
4.1.2	with metabolic activation	Negative (**Table A6.6.2/01-1**)	

Section A6.6.2(1) **Annex Point IIA, VI.6.2** **IUCLID 5.5/07**	***In-vitro* Cytogenicity study in mammalian cells** **Sister chromatid exchange assay in cultured CHO cells**	
4.2 Cytotoxicity	[redacted] produced excessive cytotoxicity at concentrations of 0.1mg/ml or greater in the absence of S9 and at concentrations of 0.3mg/ml or greater in the presence of S9. No reduction in relative survival was observed at concentrations less than or equal to 0.001mg/ml in the absence of S9 or at concentrations less than or equal to 0.003 mg/ml in the presence of S9.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Dimethylnitrosamine (DMN) was chosen as the positive control compound for cells treated in the presence of S9. Ethylmethanesulfonate (EMS) was chosen as the positive control compound for cells treated in the absence of S9. S9 activation was prepared from Aroclor 1254-induced rats, and purchased from a commercial supplier. CHO-K1-BH4 cells were obtained from Oak Ridge National Laboratory and treated with the positive control substance, the vehicle control, and the test material in the absence and presence of S9 activation. Cultures were grown from frozen stock, and maintained at the testing facility. Duplicate cultures were used for the test and vehicle control groups, but single cultures were used for the positive control substance. Each dosing solution of the test material was prepared by diluting the appropriate amount of U250 with F12 medium without serum. Concentrations were not adjusted for active ingredient. Dosing solutions were prepared daily. A preliminary cytotoxicity assay was performed with concentrations of U250 from 0.0003-10 mg/mL on CHO cells in the presence and absence of S9 applied to seeded cells in a tissue culture flask. After treatment, the cytotoxicity of the test concentrations was determined by counting the number of cells in each of the cultures. The SCE test was performed by plating CHO cells approximately 40 to 48 hours prior to treatment. Duplicate cultures were treated with the test solutions of 5 concentrations ranging from 0.00003-0.003 mg/mL in the absence of S9, and 6 concentrations ranging from 0.00001-0.003 mg/mL in the presence of S9. Treatment time was 4 hours for both systems. After treatment, the medium and test substance were removed and fresh F12 medium, dialyzed bovine serum and BrdU was added, and cultures were incubated for an additional 24 to 48 hours. Colchicine was added to the flasks prior to harvest. Cells were removed, pelleted by centrifugation, suspended in 0.075 M KCL and incubated for 20-30 mins. The cells were then pelleted again, supernatant decanted and fixed with fixative solution. Slides were made, stained, coded, and read. The number of SCE's and the number of chromosomes in a minimum of 25 cells/duplicate culture were scored for each concentration level. The mean numbers of SCE's/cell and SCE's/chromosome were calculated. Data from the SCE test do not follow a normal distribution. Therefore, the data were analyzed after transformation of the SCE / chromosome values, according to the conversion method of Box and Cox (1964). Data for positive control agents were not compared statistically	X

Section A6.6.2(1) **Annex Point IIA, VI.6.2** **IUCLID 5.5/07**	***In-vitro* Cytogenicity study in mammalian cells** **Sister chromatid exchange assay in cultured CHO cells**	
	whenever they were at least 5 times the concurrent negative control value.	
5.2 Results and discussion	*Cytotoxicity* [redacted] produced excessive cytotoxicity at concentrations of 0.1mg/ml or greater in the absence of S9 and at concentrations of 0.3mg/ml or greater in the presence of S9. No reduction in relative survival was observed at concentrations less than or equal to 0.001mg/ml in the absence of S9 or at concentrations less than or equal to 0.003 mg/ml in the presence of S9. ***Sister Chromatid Exchange (SCE)*** Efforts to dose the test substance at concentrations ranging from 0.001 - 0.01mg/ml in the absence of S9 or at 0.001 - 0.03 mg/ml in the presence of S9 were unsuccessful since they did not yield adequate numbers of metaphase cells containing differentially stained chromosomes. Statistically significant increases ($p<0.05$) in the number of Sister Chromatid Exchanges (SCE's) per chromosome were observed in the absence of S9 at 0.00003 mg/ml and at 0.0001 mg/ml ($p<0.01$). However the mean number of SCE's/Chromosome at the highest concentration tested (0.0003 mg/ml) did not differ significantly from the control. Therefore due to the small magnitude of the increases at the low and mid concentrations as well as the lack of any dose response, the increases in mean SCE's/chromosome were not considered to be treatment related in the absence of S9. Statistically significant increases ($p<0.05$) in the number of SCE's/chromosome were observed in the presence of S9 at 0.0001 mg/ml and at 0.001 mg/ml ($p<0.01$). However the mean number of SCE's/Chromosome at the mid concentration tested (0.0003 mg/ml) did not differ significantly from the control. Therefore due to the small magnitude of the increases at the low and high concentrations as well as the lack of any dose response, the increases in mean SCE's/chromosome were not considered to be treatment related in the presence of S9.	
5.3 Conclusion	[redacted] (glutaraldehyde 50% Aqueous solution) produced statistically significant increases in SCE's both in the absence and presence of S9. However,these increases were not concentration related and were of such small magnitude that they were not considered biologically significant. Therefore [redacted] (glutaraldehyde 50%Aqueous solution) was not considered to be an inducer of reciprocal chromatid interchanges under the conditions of this test.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 26th, 2010	
Materials and Methods	3.1.2.1 It was mentioned in the study report that the test substance is a transparent, colourless liquid. 3.1.2.3 Stability of the test substance under storage conditions or in the dosing solutions was not tested.	

Section A6.6.2(1) Annex Point IIA, VI.6.2 IUCLID 5.5/07	*In-vitro* Cytogenicity study in mammalian cells Sister chromatid exchange assay in cultured CHO cells	
	5.1 Typing error: After treatment, the incubation time was 24 to 28 hours. According to the study report, the number of chromosomes and the number of SCEs in a minimum of 25 cells/duplicate culture were scored for each test substance concentration level when possible. However, the exact numbers were not mentioned in the study report.	
Results and discussion	RMS does not agree with the interpretation of results made by the Applicant and the validity of the study. The highest concentration should cause significant toxicity effects. According to the preliminary cytotoxicity test, dose levels up to 0.001 mg/ml showed no reduction in relative survival in the absence of S9 or at dose levels up to 0.003 mg/ml in the presence of S9, and therefore higher concentrations should have been used. Instead, only concentrations even lower than this were evaluated for the SCEs. • The highest concentration evaluated in the absence of S9 was 0.0003 mg/ml, while even 0.001 mg/ml caused no reduction in relative survival. • The highest concentration evaluated in the presence of S9 was 0.001 mg/ml, while even 0.003 mg/ml caused no reduction in relative survival. **Therefore, the concentrations used were at least 10-fold lower than required.** According to the study report, the percentage of second division cells was at least 95 % at all test substance dose levels tested, both in the absence and presence of metabolic activation. These facts together confirm that there was no difference in the viability of the evaluated and control cultures. Hence, the evaluated test concentrations were not high enough and the study is considered inadequate. The result of this inadequately performed study is equivocal. Statistically significant increases in the number of SCEs/chromosome were observed at two of the three evaluated dose levels, both in the absence and presence of metabolic activation. However, there was no dose response. According to the OECD guideline 479, the result of the test should always be confirmed in an independent experiment. A confirmatory study was not performed.	
Conclusion	The result of this inadequately performed study can be considered equivocally positive.	
Reliability	3	
Acceptability	The study is acceptable as such, but inconclusive.	
Remarks	The comments by the applicant (shown in blue below) have been taken into account in this assessment. 2.1 The study was not performed in compliance with the OECD Guideline 479.	

Section A6.6.2(1) **Annex Point IIA, VI.6.2** **IUCLID 5.5/07**	***In-vitro* Cytogenicity study in mammalian cells** **Sister chromatid exchange assay in cultured CHO cells**	
	COMMENTS FROM DOW BENELUX B.V.	
Date	3 June 2009; 27 January 2011	
Materials and Methods		
Results and discussion	We do not agree that the study used inappropriate doses. 1. Preliminary cytotoxicity is used to establish a top concentration. The second step is determining the concentration at which the compound is not significantly altering cell cycle progression (cell cycle delay). OECD Guideline 479 states, "The highest concentration should give rise to a significant toxic effect but must still allow adequate cell replication to occur." The cytotoxic concentrations for Glutaraldehyde in this test system were 0.1 and 0.3 mg/ml in the absence or presence of metabolic activation (Report Table 1). However, the initial attempt to conduct the assay with concentrations above 0.001 mg/ml was unsuccessful due to inadequate numbers of metaphase cells containing differentially stained chromosomes. In the subsequent assay using lower concentrations, it was determined that concentrations above 0.0003 and 0.001 mg/ml in the absence or presence of metabolic activation (Report Tables 2 and 3) also resulted in insufficient differential staining. These facts resulted in the selection of the final test concentrations of 0.00003, 0.0001, and 0.0003 mg/ml in the absence and 0.0001, 0.0003, and 0.001 mg/ml in the presence of metabolic activation. Thus, we believe the dose selection in this study is logical, rational, and justified. RMS comment: We understand the logic in selecting the doses. However, the resulting concentrations were insufficient and thereby hamper the reliability of the results (please see RMS comments on *Results and discussion*). 2. From the report, the following *a priori* conditions were outlined in evaluating the assay: "*The following responses will be considered positive: Doubling in the SCE frequency at one or more concentrations in duplicate cultures; Statistically significant responses (p < 0.01) at two or more consecutive concentration levels; Induction of a statistically significant (p < 0.05)r concentration-related increase in the number of SCEs. Random statistical indications of increases which do not meet the criteria for a positive test result will be considered negative.*" These criteria were based on the published work of Latt et al. (1981). Within the framework outlined from that publication, one can evaluate the response of the present study. In examining the SCEs in the **absence** of metabolic activation, the mean result/chromosome is: (modified from Table 2)	

mg/ml	rep A	rep B	SCEs/chromosome
0.00003	0.38	0.55	0.465*
0.0001	0.46	0.57	0.515**
0.0003	0.36	0.53	0.445
Control	0.38	0.40	0.390
+ Control	1.15	na	1.15

*Significantly different from control ($p < 0.05$) ** ($p < 0.01$)

Although the two lower concentrations were statistically different from the control, they did not meet the criteria of a doubling in SCE (which would be 0.78). The principal driver for the statistical differences observed in this dataset is

Section A6.6.2(1) Annex Point IIA, VI.6.2 IUCLID 5.5/07	*In-vitro* Cytogenicity study in mammalian cells Sister chromatid exchange assay in cultured CHO cells

the low variability observed between replicates in the control (0.38 and 0.40). Due to the small magnitude of increase (all values were less than 0.78) at the low and mid concentrations, as well as the lack of a dose response, we do not consider this a positive test result as outlined in the *a priori* conditions above.

In examining the SCEs in the **presence** of metabolic activation, the mean result/chromosome is: (modified from Table 3

mg/ml	rep A	rep B	SCEs/chromosome
0.0001	0.47	0.52	0.495*
0.0003	0.44	0.50	0.470
0.001	0.57	0.49	0.530*
Control	0.45	0.40	0.425
+ Control	1.85	na	1.85

*Significantly different from control ($p < 0.05$)

Although the low and high concentrations were statistically different from the control, they did not meet the criteria of a doubling in SCE (which would be 0.85). As stated above, the principal driver for the statistical differences is the low variability observed between replicates in the control (0.45 and 0.40). Due to the small magnitude of increase (all values were less than 0.85) at the low and high concentrations, as well as the lack of a dose response, we do not consider this a positive test result as outlined in the *a priori* conditions above.

Referring to the work of Latt et al. (1981), the current study does not satisfy the criteria for a "clear negative" due to the statistically significant findings both in the absence and presence of metabolic activation, nor does it satisfy the criteria of a "clear positive". The final category to evaluate is the possibility of a "weakly positive" response, which requires a 3-point incremental dose response curve (in the absence of SCE doubling). This was not seen in the study under question.

RMS comment:

Our interpretation is as well that the differences seen do not constitute a clear indication of a positive test result. Nevertheless, evident increase is seen in SCEs and higher concentrations might have yielded positive results. Since we consider the concentrations as insufficient, the RMS is of the opinion that a negative result cannot be concluded.

Conclusion	Although the report is poorly written and confusing at times, it is an adequately performed study in which the results are decidedly negative. Furthermore, this study aids in our understanding of the clastogenic potential of Glutaraldehyde.
Reliability	
Acceptability	
Remarks	

Reference:

Latt SA, Allen J, Bloom SE, Carrano A, Falke E, Kram D, Schneider E, Schreck R, Tice R, Whitfield B, Wolff S. (1981). Sister-chromatid exchanges: a report of the GENE-TOX program. Mutat. Res. 87(1):17-62.

Table A6.6.2/01-1 Sister Chromatid Exchange Data- Treatment with Glutaraldehyde

Concentration (mg/ml)	Total No. of Chromosomes	Total No. of SCEs	SCEs per Cell[a]	Mean SCEs per Chromosome[b]
With S9				
0.00001A	Not Evaluated			
0.00001B	Not Evaluated			
0.00003A	Not Evaluated			
0.00003B	Not Evaluated			
0.0001A	490	230	9.2	0.47 (0.18)*
0.0001B	496	256	10.2	0.52 (0.17)
0.0003A	491	214	8.6	0.44 (0.15)
0.0003B	482	241	9.6	0.50 (0.17)
0.001A	501	284	11.4	0.57 (0.16)**
0.001B	494	243	9.7	0.49 (0.17)
0.003A	Not Evaluated[c]			
0.003B	Not Evaluated[c]			
Controls:				
Vehicle:				
Culture Medium A	485	217	8.7	0.45 (0.18)
Culture Medium B	491	195	7.8	0.40 (0.16)
Positive:				
DMN (300 ug/mL)	493	904	36.2	1.85 (1.14)**
Without S9				
0.00003A	505	189	7.6	0.38 (0.19)*
0.00003B	490	271	10.8	0.55 (0.18)
0.0001A	487	226	9.0	0.46 (0.16)**
0.0001B	489	276	11.0	0.57 (0.27)
0.0003A	493	177	7.1	0.36 (0.14)
0.0003B	491	261	10.4	0.53 (0.16)
0.001A	Not Evaluated[c]			
0.001B	Not Evaluated[c]			
0.003A	Not Evaluated[d]			
0.003B	Not Evaluated[d]			
Controls:				
Vehicle:				
Culture Medium A	487	184	7.4	0.38 (0.14)
Culture Medium B	500	202	8.1	0.40 (0.20)
Positive:				
EMS (300 ug/mL)	495	568	22.7	1.15 (0.23)**

[a] Twenty-five cells examine/treated culture.

[b] Mean SCE/chromosome determined from the values of the individual cells examined. significances are for combined concentration compared to combined vehicle control.

[c] Insufficient differential staining

[d] No mitotic cells

* Significantly different from control group ($p < 0.05$)

** Significantly different from control group ($p < 0.01$)

Section A6.6.2(2) Annex Point IIA, VI.6.2 IUCLID 5.5/08	*In-vitro* Cytogenicity study in mammalian cells *In Vitro* Chromosomal Aberration Assay in Chinese Hamster Ovary Cells	
	1 REFERENCE (A6.6.2/02)	Official use only
1.1 Reference	[redacted] (1991) [redacted] (Glutaraldehyde, 50% Aqueous Solution): *In Vitro* Chromosomal Aberration Assay in Chinese Hamster Ovary Cells, [redacted]. Unpublished, 12 September 1991	
1.2 Data protection	Yes	
1.2.1 Data owner	The Dow Chemical Company	
1.2.2 Companies with letter of access	[redacted]	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes US EPA OPP 84-2	
2.2 GLP	Yes	
2.3 Deviations	Yes The Study Director had no knowledge of the procedures used for physical and chemical characterization of the control substances. Analyses for stability, and homogeneity of the test and control substances in the dosing solutions were not conducted.	
	3 MATERIALS AND METHODS	
3.1 Test material	[redacted] (Glutaraldehyde 50% aqueous solution)	
3.1.1 Lot/Batch number	[redacted]	
3.1.2 Specification	Not reported	
3.1.2.1 Description	Clear liquid, sharp odour.	
3.1.2.2 Purity	[redacted]	
3.1.2.3 Stability	Stable under normal storage conditions	
3.2 Study Type	In Vitro Chromosomal Aberration Assay	
3.2.1 Organism/cell type	Chinese Hamster Ovary Cells	
3.2.2 Deficiencies / Proficiencies	See Section 2.3	
3.2.3 Metabolic activation system	Metabolic activation was accomplished using commercially purchased rat liver S9 homogenate, prepared from Aroclor 1254-induced Sprague Dawley male rats.	
3.2.4 Positive control	**<u>In the absence of metabolic activation (S9)</u>** Triethylenemelamine (TEM) **<u>In the presence of metabolic activation (S9)</u>**	

Section A6.6.2(2) **Annex Point IIA, VI.6.2** **IUCLID 5.5/08**		***In-vitro* Cytogenicity study in mammalian cells** ***In Vitro* Chromosomal Aberration Assay in Chinese Hamster Ovary Cells**	
		Cyclophosphamide (CP)	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	0.3, 0.10, 0.3, 1.0 and 3.0 ug/ml	X
3.3.2	Way of application	**Cytotoxicity assay** A preliminary cytotoxicity assay was performed with concentrations of [redacted] from 0.003-10,000 ug/mL on CHO cells in the presence and absence of S9 applied to cultured cells in a tissue culture flask. Colchicine was added to the cultures during the last 2 hours of culture. Cells were trypsinized and the cytotoxicity of the test concentrations was determined by counting the number of cells in each of the cultures. **Cell Cycle Kinetics** The cell kinetics of treated cultures were assessed by growing those cultures in BrdU. Five dose levels and a solvent control were tested in the presence and absence of S9. Cultures were treated for 4 hours in serum-free medium. The cells were rinsed, BrdU was added, and the chromosomes were harvested. Cells were resuspended, centrifuged, and fixed. Slides were read microscopically for the presence of chromosomes which had undergone only one round of DNA synthesis **Chromosomal Aberration Assay** Each dosing solution of the test material was prepared by diluting the appropriate amount of [redacted] with F12 medium without serum. Concentrations were not adjusted for active ingredient. Dosing solutions were prepared daily. Cultures were established 20 to 24 hours prior to treatment with the test material. Prior to treatment, medium was removed and replaced with serum-free medium. For cells being treated in the presence of S9, the S9 was added to each culture immediately prior to treatment. Duplicate cultures of each dose level was treated with the test material or control substances. All cultures were incubated for approximately 4 hours at 37 ºC.	
3.3.3	Pre-incubation time	Not Applicable	
3.3.4	Other modifications	None	
3.4	**Examinations**	**Table A6.6.2/02-1**	
3.4.1	Number of cells evaluated	The highest three doses that yielded a sufficient number of mitotic cells were evaluated microscopically. Slides were coded prior to evaluation. Fifty metaphase cells were scored for each culture except the CP positive control (only 44 metaphases were scored). Chromosome counts were performed, and only metaphase cells with 18-22 chromosomes were counted.	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**	Negative (**Table A6.6.2/02-1**)	
4.1.1	without metabolic	Negative (**Table A6.6.2/02-1**)	

Section A6.6.2(2) Annex Point IIA, VI.6.2 IUCLID 5.5/08		*In-vitro* Cytogenicity study in mammalian cells *In Vitro* Chromosomal Aberration Assay in Chinese Hamster Ovary Cells	
	activation		
4.1.2	with metabolic activation	Negative (**Table A6.6.2/02-1**)	
4.2	**Cytotoxicity**	**Absence of S9** Relative survivals of 25, 41, and 80% were observed at doses of 10, 3.0, and 1.0 ug/mL, respectively. Dose levels of 30 and 100 ug/mL were not evaluated due to inability to detach cells from the culture flasks. Relative mitotic indices ranged from 0% at 10 ug/mL to 100% at 0.03 ug/mL. **Presence of S9** Relative survivals of 47, 41, and 83% were observed at doses of 100, 30, and 10 ug/mL, respectively. Relative mitotic indices ranged from 89% to 126% at doses 10 to 0.30 ug/mL.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In the absence of S9 Triethylenemelamine (TEM) was chosen as the positive control compound. In the presence of S9 Cyclophosphamide (CP) was chosen as the positive control compound. S9 activation was prepared from Aroclor 1254-induced rats, and purchased from a commercial supplier. CHO-K1-BH4 cells were obtained from Oak Ridge National Laboratory and treated with the positive control substance, the vehicle control, and the test material in the absence and presence of S9 activation. Cultures were grown from frozen stock, and maintained at the testing facility. Duplicate cultures were used for the test and vehicle control groups, but single cultures were used for the positive control substance. Each dosing solution of the test material was prepared by diluting the appropriate amount of [redacted] with F12 medium without serum. Concentrations were not adjusted for active ingredient. Dosing solutions were prepared daily. **Cytotoxicity assay** A preliminary cytotoxicity assay was performed with concentrations of [redacted] from 0.0003-10000 ug/mL on CHO cells in the presence and absence of S9 applied to cultured cells in a tissue culture flask. Colchicine was added to the cultures during the last 2 hours of culture. Cells were trypsinized and the cytotoxicity of the test concentrations was determined by counting the number of cells in each of the cultures. **Cell Cycle Kinetics** The cell kinetics of treated cultures were assessed by growing those cultures in BrdU. Five dose levels and a solvent control were tested in the presence and absence of S9. Cultures were treated for 4 hours in serum-free medium. The cells were rinsed, BrdU was added, and the chromosomes were harvested. Cells were resuspended, centrifuged, and fixed. Slides were read microscopically for the presence of chromosomes which had undergone only one round of DNA synthesis.	

Section A6.6.2(2) **Annex Point IIA, VI.6.2** **IUCLID 5.5/08**	***In-vitro* Cytogenicity study in mammalian cells** ***In Vitro* Chromosomal Aberration Assay in Chinese Hamster Ovary Cells**	
	Chromosomal Aberration Assay Cells were treated with test material, and were harvested 10 hours later to ensure that they were in the first cell cycle after treatment. Cells were removed, centrifuged, resuspended in hypotonic solution, and centrifuged again. Cells were placed on slides, stained, and rinsed. The highest three doses that yielded a sufficient number of mitotic cells were evaluated microscopically. Slides were coded prior to evaluation. Fifty metaphase cells were scored for each culture except the positive control (44). Chromosome counts were performed, and only metaphase cells with 18-22 chromosomes were counted. They were evaluated for chromosome or chromatid-type aberrations including breaks, fragments, rings, minutes, quadriradials, triradials, translocations, and dicentrics. Gaps were noted but not tabulated. Severely damaged cells were scored as 10 breaks/cell, and no attempt was made to classify the type of damage. Breaks, fragments, and minutes were tabulated as breaks. Exchange figures and rings were tabulated as rearrangements.	
	Statistical analyses of the data were performed using Fisher's Exact test. A difference between treated and control cells was considered significant when $p \leq 0.05$. A positive response that appeared to be dose-related was tested for significance using regression analysis.	
5.2 Results and discussion	Treatment of cultured CHO cells with [redacted] did not result in statistically significant or dose-related increases in the frequencies of chromosomal aberrations in the presence or absence of S9 metabolic activation system. A full compendium of genotoxicity studies can be found in the following article, provided with the dossier: Zeiger, E., Gollapudi, B., and Spencer, P. (2005) Genetic toxicity and carcinogenicity studies of glutaraldehyde- a review, Mutation Research, 589, 136-151, Published.	
5.3 Conclusion	[redacted] is not considered to be clastogenic under conditions of the test.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 26th, 2010	
Materials and Methods	3.3.1 Concentrations. The highest dose levels included in the chromosome aberration analysis were 1.0 µg/ml in the absence of S9 and 10 µg/ml in the presence of S9.	
Results and discussion	Dose levels for the chromosome aberration analysis were chosen according to the results of the preliminary cytotoxicity assay: relative survival indices in the absence of S9 were 111, 104, 104, 100, 95, 80, 41 and 25 % at doses of 0.003, 0.01, 0.03, 0.10, 0.30, 1.0, 3.0 and 10 µg/ml, respectively. In the presence of S9, relative survival indices were 107, 106, 99, 106, 97, 100, 101, 83, 41 and 47 % at doses of 0.003, 0.01, 0.03, 0.10, 0.30, 1.0, 3.0, 10, 30 and 100 µg/ml, respectively. Relative mitotic indices in the absence of S9 were 100, 77, 53, 73 and 60 % at	

Section A6.6.2(2) Annex Point IIA, VI.6.2 IUCLID 5.5/08	*In-vitro* Cytogenicity study in mammalian cells *In Vitro* Chromosomal Aberration Assay in Chinese Hamster Ovary Cells	
	doses of 0.03, 0.10, 0.30, 1.0 and 3.0 µg/ml, respectively. In the presence of S9, relative mitotic indices were 126, 105, 121, 89 and 0 % at doses of 0.30, 1.0, 3.0, 10 and 30 µg/ml, respectively. Based on the results of the preliminary toxicity test, the highest concentration chosen for the chromosome aberration test in the absence of S9 should have been at least 3.0 µg/ml. However, this concentration was not included in the results of analysis and there was no explanation for the exclusion. According to the study report, the highest concentration chosen for the chromosome aberration test in the presence of S9 was 30 µg/ml. This concentration was not included in the results of analysis and there was no explanation for the exclusion. According to the OECD Test Guideline No. 473, the highest concentration should show a significant reduction in degree of confluency, cell count or mitotic index, (all greater than 50 %). The highest dose levels chosen for the chromosome aberration analysis were 1.0 µg/ml in the absence of S9 and 10 µg/ml in the presence of S9. According to the study criteria the highest dose level should have been at least 3.0 µg/ml in the absence of S9 and higher than 10 µg/ml in the presence of S9. If this protocol gives negative results both with and without activation, an additional experiment without activation should be done with continuous treatment until sampling at a time equivalent to about 1.5 normal cell cycle lengths. Negative results with metabolic activation need to be confirmed. If confirmation of negative results is not considered necessary, justification should be provided. There are some typing errors concerning test substance concentrations without S9 in the Table A6.6.2/02-1.	
Conclusion	The study does not meet the requirements for a valid chromosome aberration study according to the OECD Guideline 473. Too low test substance concentrations were chosen for the chromosome aberration analysis at least in the absence of metabolic activation and possibly also in the presence of metabolic activation. There are deviations from the Guideline in the study design concerning test substance treatment times and culturing times.	
Reliability	3	
Acceptability	The study is acceptable as such, but inconclusive.	
Remarks		
	COMMENTS FROM DOW BENELUX B.V.	
Date	3 June 2009; 27 January 2011	
Materials and Methods		
Results and discussion	In response to not using 3.0 µg/ml as the top concentration, on page 13 of the current study, it states, "*At 3.0 µg/ml UCARCIDE Antimicrobial 250 in the absence of metabolic activation, there were no mitotic cells.*" Although this dose showed 41% survival in the preliminary toxicity experiment, evidently it produced a level of toxicity in the definitive study as to render this concentration unreadable. In examining the raw study file, on the slide scoring page, the comment was made for the concentration of 3.0 µg/ml "too few mitotics to score". This shows that in the definitive study, 3.0 µg/ml was excessively toxic and thus the use of 1.0 µg/ml as the top concentration. Also, in evaluating the dose selection in the definitive assay with S9, 10 µg/ml was selected as the top concentration due to 30 µg/ml resulting in 100% cell death.	

Section A6.6.2(2) **Annex Point IIA, VI.6.2** **IUCLID 5.5/08**	***In-vitro* Cytogenicity study in mammalian cells** ***In Vitro* Chromosomal Aberration Assay in Chinese Hamster Ovary Cells**	
	RMS comment: In Table 1 of the report, the values are given as referred to in the first paragraph of RMS evaluation, section *Results and discussion* above. The citation given by the applicant in the first paragraph of Dow comments is correct, but this is in contradiction to the data in the table. The mitotic index was 60 % and the relative survival 41 % at 3.0 µg/ml, and therefore it cannot be said that there were no mitotic cells. In the study protocol under, the *a priori* conditions determining sampling time state: *"If there is no cell cycle delay, cells are harvested 10 hr after the onset of treatment to ensure that they are in the first cell cycle after treatment. If a cell cycle delay has been detected in the preliminary test, cells are harvested at the sampling time determined during the preliminary test."* The experimental design of the above study incorporated an extensive and complete assessment of the effect of the test chemical treatment on cell cycle delay to help establish the optimal harvest time for the cultured cells. The demonstration of a lack of effect on cell cycle delay coupled with the fact that the positive control successfully induced a large amount of chromosome aberrations validates that the assay was capable of detecting chemically-induced aberrations after only 10 hr post-treatment. RMS comment: Agree that the harvest time was acceptable, based on the assessment performed (study report, p. 13, 2nd paragraph). The guidance of US EPA is irrelevant in this context. Negative results need to be confirmed as described in RMS comments above.	
Conclusion	**Due to the slight deviation from the current guideline, the present study should be considered reliable with restrictions and used as supplemental information to aid in the evaluation of potential clastogenicity.** RMS conclusion is not changed.	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		