Section A6.1.2 Annex Point IIA, VI.6.1.2 IUCLID 5.1.3/01	Acute Toxicity Percutaneous toxicity	
3.2.9 Occlusion	Yes	
3.2.10 Vehicle	None	
3.2.11 Concentration in vehicle	100% (material was administered as received)	
3.2.12 Total volume applied	Single application. Dosage of 4.0, 2.0, 1.0 and 0.5 ml/kg of the 50% Glutaraldehyde solution	
3.2.13 Duration of exposure	24-hour contact period	
3.2.14 Removal of test substance	Not Reported	
3.2.15 Controls	No	
3.3 Examinations	14 day observation period, specifics not reported.	
3.4 Method of determination of LD_{50}	The LD50's were calculated by the moving average method based on a 14-day observation period.	
3.5 Further remarks	None	
	RESULTS AND DISCUSSION	
3.6 Clinical signs	All animals dosed at 4 mL/kg (2 mL a.i./kg) died on test day 1 with edema and necrosis. One animal dosed at 2 mL/kg (1 mL a.i./kg) died on test day one, and survivors had edema, ecchymosis, necrosis, and scabs at test day 14. Two animals died when dosed with 1 mL/kg (0.5 mL a.i./kg). Survivors at this dose had higher terminal body weights than the higher dose levels, with similar observations at the test sites. There was one death at the low dose, 0.5 mL/kg (0.25 mL a.i./kg), higher overall bodyweights than higher dose levels, and similar test site observations.	X
3.7 Pathology	***Spontaneous deaths*** Lungs red; livers mottled red and light red or tan; spleens dark red; kidneys mottled red and tan; kidney sections red; intestines opaque. ***Survivors*** Nothing remarkable.	X
3.8 Other	None	
3.9 LD_{50}	The LD_{50}'s were calculated based on a formulation density of 1.1 g/mL and 50% active in the formulation is 875 (386-1975) mg/kg	
	4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	Male rabbits (3-5 months old) were immobilized during a 24-hour contact period. The sample was retained in contact with the skin by impervious sheeting on the clipped, intact skin of the trunk. The LD50's were calculated by the moving average method based on a 14-day observation period.	

Section A6.1.2 Annex Point IIA, VI.6.1.2 IUCLID 5.1.3/01	Acute Toxicity Percutaneous toxicity	
4.2 Results and discussion	All animals dosed at 4 mL/kg (2 mL a.i./kg) died on test day 1 with edema and necrosis. One animal dosed at 2 mL/kg (1 mL a.i./kg) died on test day one, and survivors had edema, ecchymosis, necrosis, and scabs at test day 14. Two animals died when dosed with 1 mL/kg (0.5 mL a.i./kg). Survivors at this dose had higher terminal body weights than the higher dose levels, with similar observations at the test sites. There was one death at the low dose, 0.5 mL/kg (0.25 mL a.i./kg), higher overall bodyweights than higher dose levels, and similar test site observations.	
4.3 Conclusion	Moderately toxic. The LD50's calculated based on a formulation density of 1.1 g/mL and 50% active in the formulation is 875 (386-1975) mg/kg.	X
4.3.1 Reliability	2	
4.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	May 10th, 2010	
Materials and Methods	In general, agree with applicant's version although only the highest concentration tested (50 %) is reported here. Experiments were done with 25 % and 5 % glutaraldehyde as well. These results are briefly summarised below.	
Results and discussion	**50 % glutaraldehyde.** **3.6. Clinical signs**. Clarification: All animals in all dose groups had oedema and necrosis at unspecified time points. All animals in all but the highest dose group had ecchymosis as well, again at unspecified time points. Survivors had scabs at 14 days. **3.7. Pathology**. In addition to the findings described, testes were injected. Otherwise agree with applicant's version. **25 % glutaraldehyde.** The results for mortality, clinical signs and pathology were similar to those obtained with 50 % glutaraldehyde. The volumes applied were 4-fold higher at each dose level (2-16 ml/kg) **5 % glutaraldehyde.** The volume applied was 16 ml/kg (only one dose level, 6 animals). There were no mortalities. Clinical signs: one animal had erythema and ecchymosis at 24 h, and one had desquamation and fissuring at 14 days. Nothing remarkable was reported in the pathological examination.	

Section A6.1.2 Annex Point IIA, VI.6.1.2 IUCLID 5.1.3/01	Acute Toxicity Percutaneous toxicity
Conclusion	Other conclusions: Three concentrations of glutaraldehyde were tested. The effects were found to be connected to the concentration applied instead of the dose level, since the effects seen with the 5 % solution were significantly milder than at the lowest dose level of the 50 % solution, although the dose level was lower in the latter one. The conclusion is also supported by the finding that different dose levels of the same dilution result in similar effects, while larger differences are seen between concentrations tested. LD_{50} is highly dependent on the concentration applied.
Reliability	2
Acceptability	Acceptable
Remarks	Key study. The study also included testing of eye irritation, which is not reported in this study summary or elsewhere except in the original study report. This is considered acceptable because the present study was not performed under GLP, and there is another study which was performed under GLP (6.1.4E).
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.2/01-1 Table for Acute Toxicity (dermal)

Dose [ml/kg]	*Number of dead / number of investigated*	*Time of death (Day)*	*Observations*
0.5	1/4	8	Edema, ecchymosis, necrosis; scabs on survivors at 14 days
1.0	2/4	1,2	Edema, necrosis; scabs on survivors at 14 days
2.0	1/4	1	Edema, ecchymosis, necrosis; scabs on survivors at 14 days
4.0	4/4	1,1,1,1	Edema, necrosis.
LD_{50}	*875 (386-1975) mg/kg*		

Section A6.1.3/02 Annex Point IIA, VI.6.1.3 IUCLID 5.1.2/01	Acute Inhalation Toxicity Four-Hour LC_{50} Inhalation Study on Rats	
	1 REFERENCE	Official use only
1.1 Reference	Glutaraldehyde: Acute Vapour Inhalation Toxicity Study in Rats, , GLP, unpublished, 29 June 1995.	
1.2 Data protection	Yes	
1.2.1 Data owner	The Dow Chemical Company	
1.2.2 Companies with letter of access		
1.2.3 Criteria for data protection	Data 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 Pesticide Assessment Guidelines Subdivision F (81-3) and OECD 403	
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	50% w/w Glutaraldehyde in water	
3.1.1 Lot/Batch number		
3.1.2 Specification	As given in Section 2	
3.1.2.1 Description	Transparent colourless liquid	X
3.1.2.2 Purity		
3.1.2.3 Stability	Stability not confirmed as part of this study	
3.2 Test Animals		
3.2.1 Species	Rat	
3.2.2 Strain		
3.2.3 Source		
3.2.4 Sex	Male and Female	
3.2.5 Age/weight at study initiation	Approximately 7 weeks (Males 224-273, females 160-193)	X
3.2.6 Number of animals per group	5/sex/dose	
3.2.7 Control animals	No	

Section A6.1.3/02 Annex Point IIA, VI.6.1.3 IUCLID 5.1.2/01	Acute Inhalation Toxicity Four-Hour LC_{50} Inhalation Study on Rats	
	Inhalation	
3.2.8 Concentrations	27 ppm (non heated); 11, 28, 37, 60 and 94 ppm (heated)	
3.2.9 Particle size	Not reported	
3.2.10 Type or preparation of particles	Room-temperature vapor was generated by passing compressed air through a bubbler into a flask containing steel wool, into a fritted bubbler, through a filter, and into the chamber. The heated vapor was generated by pumping glutaraldehyde into the outlet end of the rotating evaporator tube by a FMI pump. A blower drew ambient air through a dehumidifier and heated the air to 60C. Air was passed into a rotating evaporator tube in which the glutaraldehyde was metered. Hot air with the glutaraldehyde vapor was passed into a bell jar to cool before introduction into the chamber. Chamber concentrations were measured 6-7 times each exposure by sampling the chamber with impingers containing water. Concentration of glutaraldehyde in the impinger solutions was determined using a spectrophotometric analytical method. The means and standard deviations were 27.1± 2.6, 27.4 ± 4.3 (non heated) , 11.0 ± 3.6, 28.0 ± 7.4, 37.0 ± 6.3 ppm, 59.7 ± 9.3 and 94.3 ± 13.5 ppm (heated) for target concentrations of 27 ppm (non heated) and 11, 28, 37, 60 an 94 ppm (heated), respectively. Visible inspection of the atmosphere was performed with a laser for particles. Additional confirmation of absence of particles was done using an aerodynamic particle sizer four times each exposure. Chamber temperature and relative humidity was measured during the exposure.	
3.2.11 Type of exposure	Whole body	
3.2.12 Vehicle	Water was used as a diluent in order to obtain a 10% solution which was then used for vapour generation.	X
3.2.13 Concentration in vehicle	See Section 3.2.12.	
3.2.14 Duration of exposure	4 hours	
3.2.15 Controls	No	
3.3 Examinations	Body weights were measured at day 0, 7, and 14. Complete necropsy was performed on all animals. Gross lesions, lungs with mainstem bronchi, nasal cavity, trachea, and larynx were evaluated for gross and histopathology. No statistical comparisons of body weight or necropsy data were made.	
3.4 Method of determination of LC_{50}	The 4-hour LC50 was calculated by a probit analysis method of Finney for males, females, and both sexes combined using all exposure groups from the heated generation system	X
3.5 Further remarks	None	X
	4 RESULTS AND DISCUSSION	

Section A6.1.3/02 Annex Point IIA, VI.6.1.3 IUCLID 5.1.2/01	Acute Inhalation Toxicity Four-Hour LC_{50} Inhalation Study on Rats	
4.1 Clinical signs	Clinical signs were similar for unheated and heated exposures. Signs included blepharospasm, hypoactivity, perioral wetness, mouth breathing, general facial soiling, decreased respiration rate, and/or abdominal breathing. High dose animals also exhibited urogenital soiling.	X
4.2 Pathology	No exposure-related findings were observed in rats sacrificed on day 14. Of the spontaneous deaths, most showed inflammation and necrosis of the larynx, trachea, and/or lungs. Pulmonary congestion was sporadically noted.	X
4.3 Other	None	
4.4 LC_{50}	**The LC50 for rats exposed to non heated glutaraldehyde was >27 ppm.** The LC50 for rats (combined sexes) exposed to heated glutaraldehyde (60°C) was 44.37 ppm with 95% CI 34.86-56.46 ppm.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The acute inhalation toxicity of glutaraldehyde was investigated using [redacted] rats obtained from a commercial supplier. were examined for health status upon arrival at the testing facility. Animals were individually identified, and randomly assigned to dose groups. Animals were housed in steel cages three per cage in rooms designed to maintain adequate environmental conditions for rats. Water and food was provided *ad libitum* except during inhalation exposure. Animals were observed for 14 days following the exposure. Body weights and clinical signs of toxicity were recorded throughout the period. At necropsy on test day 14, the lungs, larynx, trachea, and nasal turbinates and gross lesions were collected and retained in 10% buffered formalin. Collected tissues from all animals that died and for 2 surviving rats/sex/group were processed histologically and examined microscopically.	X
5.2 Results and discussion	There was no mortality in the 27 ppm non heated group. For the heated exposures, the percentages of lethality for the male rats were 0, 20 (caging accident), 40, 100 and 100 for the 11, 28, 37, 59.7 and 94.3 ppm groups, respectively. The percentages of lethality for the female rats were 0, 20, 0, 80 and 80 for the 11, 28, 37, 59.7 and 94.3 ppm groups, respectively. For the non heated group, clinical signs included blepharospasm, periocular wetness, and mouth breathing. During subsequent postexposure observations, all rats appeared normal, except audible breathing was observed for 2 male rats 1 day post exposure. For all heated exposure groups clinical signs included blepharospsm, hypoactivity, perioral wetness, and/or mouth breathing during the exposure and on the same day following exposure. These clinical signs persisted throughout the 14-day postexposure period in all groups except the 11 ppm group which appeared normal. Urogenital area wetness was also observed in females from the 94.3 ppm group.	X

Section A6.1.3/02 Annex Point IIA, VI.6.1.3 IUCLID 5.1.2/01	**Acute Inhalation Toxicity** **Four-Hour LC_{50} Inhalation Study on Rats**	
	Body weight gains were observed for male and female animals from all exposure groups at both 7 and 14 days following exposure, except males from the 28 ppm group on Day 7, the surviving female from 59.7 ppm group on Day 7 and the surviving female from the 94.3 ppm group on Day 7 and Day 14. No exposure-related findings were observed in rats sacrificed on day 14. Of the spontaneous deaths, most showed inflammation and necrosis of the larynx, trachea, and/or lungs. Pulmonary congestion was sporadically noted.	
5.3 Conclusion	**The LC50 for rats exposed to non heated glutaraldehyde was >27 ppm.** The LC50 for rats (combined sexes) exposed to heated glutaraldehyde (60°C) was 44.37 ppm with 95% CI 34.86-56.46 ppm.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	January 12th, 2011	
Materials and Methods	3.1.2.1 Description. The test substance is described only as a liquid. 3.2.5 Age/weight at study initiation. The numbers in parenthesis refer to weight in grams. 3.2.12 Vehicle. Information on dilution before vapour generation appears not to be given in the original report. 3.4 Method of determination of LC_{50}. Note that LC_{50} was determined for the heated glutaraldehyde exposure only, because there was only one concentration of non-heated glutaraldehyde. 3.5 Further remarks. • Oxygen content in the chamber was not assured • The relative humidity was 76 to 77 %, while it should be 30 to 70 %. 5.1 Materials and methods. • The animals were not examined for health status before the test. • There were 5 animals/cage (not 3).	

Section A6.1.3/02 Annex Point IIA, VI.6.1.3 IUCLID 5.1.2/01	Acute Inhalation Toxicity Four-Hour LC_{50} Inhalation Study on Rats
Results and discussion	4.1 Clinical signs. The clinical signs resulting from non-heated glutaraldehyde exposure were as follows: blepharospasm, periocular wetness and mouth breathing during the exposure, and periocular wetness, unkempt fur, mouth breathing, audible breathing and decreased respiratory rate after exposure on the same day. Audible breathing was observed in two males on day 1 after exposure, and thereafter there were no clinical signs. 4.2 Pathology. • Non-heated exposure: 3 out of 4 investigated males and 2 out of 4 females had goblet cell hyperplasia in the nasal cavity (day 14) • Non-heated exposure: 3 out of 4 investigated males and 2 out of 4 females had rhinitis (day 14) • Heated exposure: Pulmonary congestion was seen in all rats that were found dead, except for one male. 5.2 Results and discussion. Comments to 4.1 and 4.2 also concern this section.
Conclusion	The LC_{50} was not determined for non-heated glutaraldehyde, and is above 27 ppm (0.11 mg/L) for both males and females (using the conversion factor of 4.09 for ppm to mg/m^3).
Reliability	2 Based on the general organisation of the study with a single dose level in the non-heated exposure and no control group, and because the relative humidity was too high, which might have diminished the irritant effects.
Acceptability	Acceptable as supportive information.
Remarks	The data that is usable for the purpose of BPD Annex I inclusion only concerns one dose level and no control group.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.3/02 Annex Point IIA, VI.6.1.3 IUCLID 5.1.2/01	Acute Inhalation Toxicity Four-Hour LC_{50} Inhalation Study on Rats

Table A6.1.3/02-1 Table for Acute Toxicity (inhalation)

Dose [ppm]	Number of dead / number of investigated	Time of death (Day)	Treatment-Related Observations
27.1	Males 0/5 Females 0/5	---	blepharospasm, hypoactivity, perioral wetness, mouth breathing, general facial soiling, decreased respiration rate, and/or abdominal breathing.
27.4	Males 0/5 Females 0/5	---	blepharospasm, hypoactivity, perioral wetness, mouth breathing, general facial soiling, decreased respiration rate, and/or abdominal breathing.
11.0	Males 0/5 Females 0/5	---	blepharospasm, hypoactivity, perioral wetness, mouth breathing, general facial soiling, decreased respiration rate, and/or abdominal breathing.
28.0	Males 1/5 Females 1/5	Males: Day 0[@] Females: 1 on Day 2	blepharospasm, hypoactivity, perioral wetness, mouth breathing, general facial soiling, decreased respiration rate, and/or abdominal breathing.
37.0	Males 20/5 Females 0/5	Males: 2 on Day 2	blepharospasm, hypoactivity, perioral wetness, mouth breathing, general facial soiling, decreased respiration rate, and/or abdominal breathing.
59.7	Males 5/5 Females 4/5	Males: 1 during exposure, 1 on Day 0 and 2 on Day 2 Females: 2 on Day 2, 1 on Day 4 and 1 on Day 5	blepharospasm, hypoactivity, perioral wetness, mouth breathing, general facial soiling, decreased respiration rate, and/or abdominal breathing.
94.3	Males 5/5 Females 4/5	Males: 1 during exposure, 2 on Day 1 and 2 on Day 2 Females: 2 on Day 1, 1 on Day 2 and 1 on Day 4	blepharospasm, hypoactivity, perioral wetness, mouth breathing, general facial soiling, decreased respiration rate, and/or abdominal breathing and urogenital soiling..
LC_{50}	**The LC50 for rats exposed to non heated glutaraldehyde was >27 ppm.** The LC50 for rats (combined sexes) exposed to heated glutaraldehyde (60°C) was 44.37 ppm with 95% CI 34.86-56.46 ppm.		

[@] One male rat died during exposure due to a caging accident.

* Day of Exposure = Study Day 0

Section A6.1.3 Annex Point IIA, VI.6.1.3 IUCLID 5.1.2/01	Acute Inhalation Toxicity Four-Hour LC_{50} Inhalation Study on Rats	
	1 REFERENCE	Official use only
1.1 Reference	[redacted] (1982) Glutaraldehyde Four-Hour LC_{50} Inhalation Study on Rats, [redacted], Not GLP, unpublished, 7 January 1982.	
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, but comparable to OECD Guideline 403 with deviations listed in section 2.3, these were considered to be minor in nature.	
2.2 GLP	No	
2.3 Deviations	• The humidity range was slightly outside of that specified in the guideline (guideline 30-70%; range recorded during the study 20-66%). • The temperature at which the exposure was carried out at was slightly outside of that specified in the guideline (guideline 22±2°C; range recorded during the study 21-26°C). • The equipment used for measuring the temperature and humidity was not described. • Nominal concentrations (total amount of test substance fed into the inhalation equipment divided by the volume of air) were not reported. • A dose-mortality curve was not reported.	
	3 MATERIALS AND METHODS	
3.1 Test material	50% w/w Glutaraldehyde in water	
3.1.1 Lot/Batch number	[redacted]	
3.1.2 Specification	Not given	
3.1.2.1 Description	Clear liquid, sharp odour	
3.1.2.2 Purity	[redacted]	
3.1.2.3 Stability	Not reported	
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	55 days old, Males Mean Bodyweight = 179.45g; Females Mean	

Section A6.1.3 Annex Point IIA, VI.6.1.3 IUCLID 5.1.2/01	Acute Inhalation Toxicity Four-Hour LC_{50} Inhalation Study on Rats	
initiation	Bodyweight = 129.03g	
3.2.6 Number of animals per group	6/sex/dose	
3.2.7 Control animals	Yes	
	Inhalation	
3.2.8 Concentrations	0, 10, 20 and 50 ppm (0, 0.04, 0.08 and 0.2 mg/L)	
3.2.9 Particle size	Not applicable	
3.2.10 Type or preparation of particles	A 10% dilution was made with water prior to vapour generation. Vapours were generated by metering the solution into the outlet of a rotating evaporator. Liquid sample flowed counter-current to the airflow. A baffle was inserted to create turbulence, and a blower forced ambient air through a heater (heated to approx. 65°C) that was exhausted through the rotating evaporator tube and generated the vapour into the chamber. Liquid flow rate and chamber air exhaust rates were adjusted to maintain the target concentrations. Chamber vapour concentrations were measured by GC and a Tenax trapping technique. For the 4-hour inhalation exposure the chambers at different dose levels were analyzed 3 to 4 times after the chamber reached equilibrium concentration. The means and standard deviations were 42.7 ± 0.2, 23.0 ± 0.1, 10.6 ± 0.2 and 0.3 ± 0.0 ppm for target concentrations of 50, 20, 10 and 0 ppm, respectively.	
3.2.11 Type of exposure	Whole body	
3.2.12 Vehicle	Water was used as a diluent in order to obtain a 10% solution which was then used for vapour generation.	
3.2.13 Concentration in vehicle	See Section 3.2.12.	
3.2.14 Duration of exposure	4 hours	
3.2.15 Controls	Yes, a negative control group was run concurrent with the study.	
3.3 Examinations	Animals were observed during exposure, and for 14 days following the exposure. Food consumption, body weights, and clinical signs of toxicity were recorded throughout the period. At necropsy on test day 14, the lungs, larynx, trachea, and nasal turbinates were retained, fixed, and examined from three rats per sex per dose, and all animals dying prior to the scheduled necropsy.	
3.4 Method of determination of LC_{50}	Quantitative continuous variables were compared by ANOVA, Bartlett's homogeneity of variance, and Duncan's multiple range test between dose groups. Depending on the outcome of variance testing, further statistics were used.	
3.5 Further remarks	None	
	4 RESULTS AND DISCUSSION	
4.1 Clinical signs	There was no mortality in the 10 ppm group. Two male and two female rats died during the 14 day post-exposure period following exposure to 20 ppm. All male and three female rats died after exposure to 50 ppm in the following 14 days.	X

Section A6.1.3 Annex Point IIA, VI.6.1.3 IUCLID 5.1.2/01	Acute Inhalation Toxicity Four-Hour LC_{50} Inhalation Study on Rats	
	Periocular soiling, lacrimation, mouth breathing, and labored breathing were seen in all dose groups, most frequently and severely at 50ppm. Following exposure, animals in the 50ppm group exhibited decreased respiration, loss of coordination, ungroomed appearance, perioral soiling, and perinasal wetness, perianal soiling, and decreased motor activity. Animals in the 20ppm group also showed perioral soiling and perinasal wetness, and one female appeared emaciated. Increased respiration was noted in one female at 10ppm. By test day 14, only two females (20ppm) had unkempt fur, and all other observations appeared to be largely resolved. Additionally, decreased motor activity, emaciation, fur discoloration, and perianal soiling were noted in 20 and 50 ppm animals during the 14-day observation period. Statistically significant decriments in body weights were seen in all three test groups at all time points, corresponding to decreased food and water consumption in the same groups.	
4.2 Pathology	Patchy colour change in the lungs was noted in most animals at the high dose, and in three rats at 20ppm. Corneal opacity was noted in one high-dose female. There were no findings in the control or 10ppm groups.	
4.3 Other	None	
4.4 LC_{50}	The LC50 for male rats was 23.5 ppm with 95% CI 16.8-32.8 ppm. For female rats, the LC50 was 40.1ppm with 95% CI 15.2-105.8 ppm.	X
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	A 10% dilution was made with water prior to vapour generation. Vapours were generated by metering the solution into the outlet of a rotating evaporator. Liquid sample flowed counter-current to the airflow. A baffle was inserted to create turbulence, and a blower forced air and vapour into the chamber. Liquid flow rate and chamber air exhaust rates were adjusted to maintain the target concentrations. Chamber vapour concentrations were measured by GC and a Tenax trapping technique. Male and female ███ rats were obtained from a commercial supplier and were examined for health status upon arrival at the testing facility. Animals were individually identified, and randomly assigned to dose groups. Animals were housed in steel cages three per cage in rooms designed to maintain adequate environmental conditions for rats. Water and food was provided *ad libitum* except during inhalation exposure. Animals were observed for 14 days following the exposure. Food consumption, body weights, and clinical signs of toxicity were recorded throughout the period. At necropsy on test day 14, the lungs, larynx, trachea, and nasal turbinates were saved, fixed, and examined from three rats per sex per dose, and all animals dying prior to the scheduled necropsy.	
5.2 Results and discussion	There was no mortality in the 10ppm group. Two male and two female rats died during the 14 day post-exposure period following exposure to 20ppm. All male and three female rats died after exposure to 50ppm in the following 14 days. Periocular soiling, lacrimation, mouth breathing, and labored breathing were seen in all dose groups, most frequently and severely at 50ppm.	

Section A6.1.3 Annex Point IIA, VI.6.1.3 IUCLID 5.1.2/01	**Acute Inhalation Toxicity** **Four-Hour LC_{50} Inhalation Study on Rats**	
	Following exposure, animals in the 50ppm group exhibited decreased respiration, loss of coordination, ungroomed appearance, perioral soiling, and perinasal wetness, perianal soiling, and decreased motor activity. Animals in the 20ppm group also showed perioral soiling and perinasal wetness, and one female appeared emaciated. Increased respiration was noted in one female at 10ppm. By test day 14, only two females (20ppm) had unkempt fur, and all other observations appeared to be largely resolved. Additionally, decreased motor activity, emaciation, fur discoloration, and perianal soiling were noted in 20 and 50 ppm animals during the 14-day observation period. Statistically significant decriments in body weights were seen in all three test groups at all time points, corresponding to decreased food and water consumption in the same groups. Patchy colour change in the lungs was noted in most animals at the high dose, and in three rats at 20ppm. Corneal opacity was noted in one high-dose female. There were no findings in the control or 10ppm groups.	
5.3 Conclusion	The LC_{50} for male rats was 23.5 ppm with 95% CI 16.8-32.8 ppm. For female rats, the LC_{50} was 40.1 ppm with 95% CI 15.2-105.8 ppm. The LC_{50} expressed in mg/L was 0.096 mg/L for males and 0.16 mg/L for females (1ppm = 4.09mg/m^3 based on conversion for standard temperature and pressure).	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	May 11th, 2010	
Materials and Methods	Agree with Applicant's version.	
Results and discussion	**This study is not acceptable because it concerns heated glutaraldehyde and is therefore not usable for risk assessment.** The conclusions on heated glutaraldehyde are as follows: The LC_{50} values can be derived using two different analytical concentrations obtained using two different analytical methods. The concentration was determined using both gas chromatography [redacted] and Tenax trapping technique [redacted] LC_{50} (male) was 0.096 mg/L when using [redacted] analytical data. LC_{50} (male) was 0.090 mg/L when using [redacted] analytical data. LC_{50} (female) was 0.16 mg/L when using either [redacted] or [redacted] analytical data. It is not possible to estimate which analytical data is more reliable, and therefore the [redacted] data is chosen, resulting in lower LC_{50}. Otherwise agree with Applicant's version.	
Conclusion	**The study concerns heated glutaraldehyde and is therefore not usable for risk assessment.**	
Reliability	3	
Acceptability	Not acceptable	

Section A6.1.3 Annex Point IIA, VI.6.1.3 IUCLID 5.1.2/01	Acute Inhalation Toxicity Four-Hour LC_{50} Inhalation Study on Rats
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.3/01-1 Table for Acute Toxicity (inhalation)

Dose [ppm]	Number of dead / number of investigated	Time of death (Day)	Treatment-Related Observations
0	Males 0/6 Females 0/6	---	---
10	Males 0/6 Females 0/6	---	Periocular encrustation, periocular wetness, perioral wetness, salivation , mouth breathing, audible respiration , increased respiration
20	Males 2/6 Females 2/6	Males: 2 on Day 1 Females: 1 on Day 1 and 1 on Day 7	Periocular encrustation, periocular wetness, lacrimation, perinasal encrustation, perinasal wetness, perioral encrustation, perioral wetness, salivation , mouth breathing, audible respiration
50	Males 6/6 Females 3/6	Males: 1 during exposure, 2 on Day 1, 2 on Day 2 and 1 on Day 3 Females: 2 on Day 1 and 1 on Day 3	Periocular encrustation, periocular wetness, lacrimation, perinasal encrustation, perinasal wetness, perioral encrustation, perioral wetness, salivation , mouth breathing, audible respiration , abdominal breathing, decreased respiration , fur unkempt, coordination loss/slow righting reflex
LC_{50}	Male rats- 23.5 ppm (16.8-32.8 ppm) = 0.096 mg/L Female rats- 40.1 ppm (15.2-105.8 ppm) = 0.16 mg/L		

Section A6.1.4(e) Annex Point IIA, VI.6.1.4 IUCLID 5.2.2/01	Acute Eye Irritation - Rabbit	
	1 REFERENCE	Official use only
1.1 Reference	1987a) : Primary eye irritancy in the rabbit, , GLP, Unpublished, 3 September 1987	
1.2 Data protection	Yes	
1.2.1 Data owner	The Dow Chemical Company	
1.2.2 Companies with letter of access		
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 FIFRA 81-4	
2.2 GLP	Yes	
2.3 Deviations	The sponsor provided the purity of the test material and indicated that it would be stable for the duration of the study. No analysis of the stability of the test material was performed by the testing facility.	
	3 MATERIALS AND METHODS	
3.1 Test material	45% Glutaraldehyde	
3.1.1 Lot/Batch number		
3.1.2 Specification	Not reported	
3.1.2.1 Description	Clear low viscosity liquid	
3.1.2.2 Purity	See 2.3	
3.1.2.3 Stability	Assumed to be stable under normal storage conditions See 2.3	
3.2 Test Animals		
3.2.1 Species	Rabbit	
3.2.2 Strain		
3.2.3 Source		
3.2.4 Sex	Male and Female	
3.2.5 Age/weight at study initiation	12 to 18 weeks Weight range 2.0 to 3.0 kg	
3.2.6 Number of animals per group	3/sex	
3.2.7 Control animals	The untreated eye of each rabbit served as the control.	

Section A6.1.4(e) Annex Point IIA, VI.6.1.4 IUCLID 5.2.2/01		Acute Eye Irritation - Rabbit	
3.3	**Administration/ Exposure**	0.1 ml of test material administered as received placed in the conjunctival sac of the eye and lids were held together for one second.	
3.3.1	Preparation of test substance	The material was instilled as received.	
3.3.2	Amount of active substance instilled	0.1 ml	
3.3.3	Exposure period	The material was not rinsed from the eye, and therefore the exposure period was not specified.	
3.3.4	Postexposure period	Observed for 21 days following instillation	
3.4	**Examinations**		
3.4.1	Ophthalmoscopic examination	Ocular effects were recorded after 1 hour and 1, 2, 3, 7, 10, 14, 17 and 21 days post instillation.	
3.4.1.1	Scoring system	Table 6.1.4-1	
3.4.1.2	Examination time points	See Section 3.4.1	
3.4.2	Other investigations	None	
3.5	**Further remarks**	None	
		RESULTS AND DISCUSSION	
3.6	**Clinical signs**	Severe corneal injury, iritis and severe conjunctival irritation in each of 6 rabbits. Ocular effects included a purulent discharge, conjunctival necrosis, adhesion of the nictitating membrane and cornea and pannus. Corneal opacity and conjunctival swelling were so severe that complete scoring of the cornea and iritis was not possible for most of the observation period. Adhesion of the nictitating membrane to the cornea as well as the purulent discharge further interfered with the ocular examinations. One rabbit died 17 days after dosing, but did not appear related to treatment. Eyes were severely affected through day 21.	
3.7	**Average scores**		
3.7.1	Cornea	Table A6.1.4-2	
3.7.2	Iris	Table A6.1.4-2	
3.7.3	Conjunctival Discharge	Table A6.1.4-2	
3.7.3.1	Redness	Table A6.1.4-2	
3.7.3.2	Chemosis	Table A6.1.4-2	
3.8	**Reversibility**	No. In the surviving animals, all eyes were severely affected through 21 days of observation.	
3.9	**Other**	None	

Section A6.1.4(e) Annex Point IIA, VI.6.1.4 IUCLID 5.2.2/01	Acute Eye Irritation - Rabbit	
3.10 Overall result	Severe corneal injury, iritis and severe conjunctival irritation in each of 6 rabbits. In the surviving animals, all eyes were severely affected through 21 days of observation.	
	4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	Male and female [redacted] rabbits (12-18 weeks old) were received from a commercial supplier, and were examined for health status and uniquely identified upon arrival to the testing facility. They were housed singly in cages with wire floors, and offered food and water *ad libitum*. Rooms were designed to maintain adequate environmental conditions for the species. Pre-study staining of the cornea was done 24 hours prior to dosing to exclude any animal with pre-existing conditions. A dose of 0.1ml of [redacted] was instilled into one eye of each of 6 [redacted] rabbits (3 per sex per dose). Ocular effects were recorded after 1 hour and 1, 2, 3, 7, 10, 14, 17 and 21 days post instillation. Fluorescein staining was included at one day and subsequent days. Grading and scoring were performed according to predefined scales.	
4.2 Results and discussion	Severe corneal injury, iritis and severe conjunctival irritation in each of 6 rabbits. Ocular effects included a purulent discharge, conjunctival necrosis, adhesion of the nictitating membrane and cornea and pannus. Corneal opacity and conjunctival swelling were so severe that complete scoring of the cornea and iritis was not possible for most of the observation period. Adhesion of the nictitating membrane to the cornea as well as the purulent discharge further interfered with the ocular examinations. One rabbit died 17 days after dosing, but did not appear related to treatment. In the surviving animals, all eyes were severely affected through 21 days of observation.	
4.3 Conclusion	45% Glutaraldehyde [redacted] is severely irritating to the eyes of rabbits.	
4.3.1 Reliability	1	
4.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 16th, 2010	
Materials and Methods	Agree with applicant's version.	
Results and discussion	Agree with applicant's version.	
Conclusion	Agree with applicant's version. Classification as R41 '*Risk of serious damage to eyes*' is warranted. CLP: Classification in Category 1 for irreversible effects on the eye is warranted.	
Reliability	1	

Section A6.1.4(e) **Annex Point IIA, VI.6.1.4** **IUCLID 5.2.2/01**	**Acute Eye Irritation - Rabbit**	
Acceptability	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table 6.1.4-1 GRADES FOR OCULAR LESIONS

CORNEA	
No ulceration or opacity..	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details or iris clearly visible...	1
Easily discernible translucent areas, details of iris slightly obscured................................	2
Necreous (opalescent) areas, no details or iris visible, size of pupil barely discernible	3
Opaque, iris not discernable through the opacity..	4
IRIS	
Normal	0
Markedly deepened rugae (folds), congestion, swelling, moderate circumcorneal hyperemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)...	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2
CONJUNCTIVAE	
Redness (refers to palpebral and bulbar conjunctivae, cornea and iris)	
Blood vessels normal	0
Some blood vessels definitely hyperemic; injected	1
Diffuse crimson color, individual vessels not easily discernible	2
Diffuse beefy red	3
Chemosis: lids and/or nictitating membranes	
No swelling	0
Any swelling above normal; includes nictitating membranes [slight]	1
Obvious swelling with partial eversion of lids [moderate]	2
Swelling with lids about half closed [moderate]	3
Swelling with lids more than half closed [marked]	4
Discharge	
No discharge	0
Any amount different from normal	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs considerable area around the eye	3

Table 6.1.4-2 Irritation Scoring

	1 hour	1 day	2 days	3 days	7 days	10 days	14 days	17 days	21 days
Cornea:									
Opacity	1.0	4.0	4.0	4.0	4.0	4.0@	4.0@	4.0@	*
Area	4.0	4.0@	*	*	*	*	*	*	*
Iris:									
Inflammation	1.0	*	*	*	*	*	*	*	*
Conjunctiva:									
Redness	1.0	1.0	2.0	2.0	1.8	1.0@	1.0@	1.0@	1.0
Chemosis	1.7	4.0	3.8	4.0	3.7	4.0	4.0	4.0	4.0
Discharge	3.0	2.8	2.8	3.0	3.0	3.0	2.5	2.4	2.0
Fluorescein Exam.	---	*	*	*	*	*	*	*	*

@ Mean value reflects a $N \leq 5$ due to inability to score as defined below.
* Scoring not possible because of severe corneal opacity, swelling, adhesion and/or discharge.
--- Not conducted

Section A6.1.4(s) Annex Point IIA, VI.6.1.4 IUCLID 5.2.1/01	Acute Dermal Irritation – Rabbit 50% aqueous glutaraldehyde samples, Primary dermal irritancy studies in the rabbit	
	1 REFERENCE	Official use only
1.1 Reference	[redacted] (1988) 50% aqueous glutaraldehyde samples, Primary dermal irritancy studies in the rabbit, [redacted], Not GLP, Unpublished, 2 June 1988	
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 404 Also meets US Department of Transportation Corrosion Test Classification.	
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	[redacted] AND [redacted] 50% Glutaraldehyde	
3.1.1 Lot/Batch number	[redacted] [redacted] **50% Glutaraldehyde** [redacted]	
3.1.2 Specification	Not reported	
3.1.2.1 Description	Clear, non-viscous liquids	
3.1.2.2 Purity	Not Reported	
3.1.2.3 Stability	Assumed to be stable under typical storage conditions.	
3.2 Test Animals		
3.2.1 Species	Rabbit	
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 12 to 18 weeks of age Weight Range 2.0 to 3.0 kg	

Section A6.1.4(s) Annex Point IIA, VI.6.1.4 IUCLID 5.2.1/01		Acute Dermal Irritation – Rabbit 50% aqueous glutaraldehyde samples, Primary dermal irritancy studies in the rabbit	
3.2.6	Number of animals per group	6/sex/group	X
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**		
3.3.1	Application	A dose of 0.5 mL was applied to one intact site on each of 6 rabbits. A gauze square was placed over the test site, and secured in place for the duration of the contact period (four hours, one hour and/or 3 mins). Animals were restrained during the contact period as well.	
3.3.1.1	Preparation of test substance	Test materials were administered as received.	
3.3.1.2	Test site and Preparation of Test Site	The dorsal area of the trunk of each rabbit was clipped just prior to dosing. A dose of 0.5 mL was applied to one intact site on each of 6 rabbits. A gauze square was placed over the test site, and secured in place for the duration of the contact period (four hours, one hour and/or 3 mins). Animals were restrained during the contact period as well.	X
3.3.2	Occlusion	Yes	
3.3.3	Vehicle	None	
3.3.4	Concentration in vehicle	Not Applicable	
3.3.5	Total volume applied	0.5 mL of the test materials	
3.3.6	Removal of test substance	At the end of the exposure period, the gauze was removed, and excess test material was removed.	
3.3.7	Duration of exposure	[redacted] - 60 minutes and 4 hours [redacted] **50% Glutaraldehyde** - 3 minutes, 60 minutes and 4 hours	
3.3.8	Postexposure period	**3 and 60 minutes** - 7 day observation period **4 hour** - 10 day observation period	
3.3.9	Controls	No	
3.4	**Examinations**		
3.4.1	Clinical signs	Animal care technicians examined the rabbits at the time of receipt from the supplier. Additional examinations, including weight, were completed by the investigators at least twice prior to dosing. The rabbits were weighed and inspected on the day of the test.	
3.4.2	Dermal examination	The application sites were evaluated for erythema, eschar, edema, desquamation, necrosis, scab formation, fissuring and alopecia.	
3.4.2.1	Scoring system	**Table 6.1.4-1**as indicated in the 1981 OECD Guideline 404	
3.4.2.2	Examination time points	The sites were evaluated at 1 hour, and 1, 2, 3, 7, and 10 days according to a predefined scoring system.	
3.4.2.3	Other examinations	Additional examinations, including weight, were completed by the investigators at least twice prior to dosing. The rabbits were weighed and inspected on the day of the test. Only those exhibiting a healthy state and demonstrating weight gain were used. Qualifications for a healthy animal included: appeared alert, active and well-groomed, with no evidence of discharge, diarrhea, breathing difficulties or locomotor abnormalities. The rabbits were randomly assigned to cages and were	

Section A6.1.4(s) Annex Point IIA, VI.6.1.4 IUCLID 5.2.1/01	**Acute Dermal Irritation – Rabbit** **50% aqueous glutaraldehyde samples, Primary dermal irritancy studies in the rabbit**	
	designated for dosing according to need and availability.	
3.5 Further remarks	None	
	RESULTS AND DISCUSSION	
3.6 Average scores		
3.6.1 Erythema	**Table 6.1.4-2**	
3.6.2 Edema	**Table 6.1.4-2**	
3.7 Reversibility	[redacted] 4 hours Effects of moderate erythema, severe edema (noted by day 1), necrosis (noted in 4 animals by day 2). Necrosis, desquamation, scabs, alopecia present on day 10. 60 minutes Effects of minor to moderate erythema resolved by day 7, necrosis present on day 7 in one animal. [redacted] **50% Glutaraldehyde** 4 hours Effects of erythema, edema, and necrosis by day 7. Scabs and alopecia remaining on day 10. 60 minutes Effects of minor to moderate erythema, edema (in 2 animals) resolved by day 7. Two animals were noted with diffuse necrosis on day 10. 3 minutes Effects of minor erythema in 1/6 rabbits.	X
3.8 Other examinations	None reported	
3.9 Overall result	For a 4 hour contact period both test materials were concluded to be corrosive according to the United States Department of Transportation definition.	
	4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	Additional examinations, including weight, were completed by the investigators at least twice prior to dosing. The rabbits were weighed and inspected on the day of the test. Only those exhibiting a healthy state and demonstrating weight gain were used. Qualifications for a healthy included: appeared alert, active and well-groomed, with no evidence of discharge, diarrhea, breathing difficulties or locomotor abnormalities. The rabbits were randomly assigned to cages and were designated for dosing according to need and availability. The dorsal area of the trunk of each rabbit was clipped just prior to dosing. A dose of 0.5 mL was applied to one intact site on each of 6 rabbits. A gauze square was placed over the test site, and secured in place for the duration of the contact period (four hours, one hour and/or 3 mins.). Animals were restrained during the contact period as well. At	X

Section A6.1.4(s) Annex Point IIA, VI.6.1.4 IUCLID 5.2.1/01	**Acute Dermal Irritation – Rabbit** **50% aqueous glutaraldehyde samples, Primary dermal irritancy studies in the rabbit**	
	the end of the exposure period, the gauze was removed, and excess test material was removed. The sites were evaluated at 1 hour, and 1, 2, 3, 7, and 10 days according to a predefined scoring system.	
4.2 Results and discussion	[redacted] Four hours of contact with 0.5 mL resulted in moderate erythema on all 6 rabbits. One animal developed only minor edema, but the other 5 exhibited moderate to severe edema. Necrosis was present on 4 animals at 24 hours. Two rabbits were dead by test day 9, but there were no indications that death was related directly to treatment. No erythema or edema was noted after 10 days. Scabbing, alopecia, necrosis, and desquamation persisted through test day 10. A volume of 0.5 mL applied to occluded skin for 60 minutes produced minor to moderate erythema on all 6 rabbits tested, and was resolved by test day 7. One rabbit had persistent, mild necrosis continuing through the end of the test. The material was considered corrosive to skin for the four-hour test. **[redacted] 50% Glutaraldehyde** Four hours of contact with 0.5 mL resulted in moderate to severe erythema and edema on all 6 rabbits. Scabbing, alopecia, necrosis, and desquamation persisted through test day 10. No erythema or edema was noted after 10 days. A volume of 0.5 mL applied to occluded skin for 60 minutes produced minor to moderate erythema on all 6 rabbits tested. Minor transient edema was noted on 2 of 6 animals. One animal died on day 5, but there were no indications that death was related directly to treatment. Erythema and edema were resolved by test day 7. Three rabbits had persistent, mild necrosis continuing through the end of the test. Four rabbits also showed signs of desquamation on test day 7. All signs of erythema and edema were resolved after 7 days. A volume of 0.5 mL applied to occluded skin for 3 minutes produced minor erythema on one of six rabbits. No edema was noted. No irritation effects were reported after day one.	
4.3 Conclusion	For a 4 hour contact period both test materials were concluded to be corrosive according to the United States Department of Transportation definition.	
4.3.1 Reliability	1	
4.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 15th, 2010	
Materials and Methods	3.2.6 There were not 6 animals/sex/group, but 6 animals per group, 3 males and 3 females. OECD 404 calls for a total of 3 animals or less. 3.3.1.2 The clipping was not done just prior to dosing, but according to the guideline, i.e. on the previous day. Otherwise agree with applicant's version.	

Section A6.1.4(s) Annex Point IIA, VI.6.1.4 IUCLID 5.2.1/01	**Acute Dermal Irritation – Rabbit** **50% aqueous glutaraldehyde samples, Primary dermal irritancy studies in the rabbit**
Results and discussion	3.7. Reversibility • For [redacted], after 60 min exposure, there was also desquamation in all 6 animals at 7 days. • For [redacted] 50 % Glutaraldehyde, after 4 h exposure, there was necrosis, desquamation, alopecia and scab formation at 10 days. • For [redacted] 50 % Glutaraldehyde, after 60 min exposure, there was also desquamation in 4 of the 5 surviving animals at 7 days. • For [redacted] 50 % Glutaraldehyde, after 3 min exposure, minor effects were noted during the first day only (fully reversible). 4.1. The same comment as for point 3.3.1.2 above. 4.1 For [redacted] 50 % Glutaraldehyde, after 60 min exposure, there was necrosis in 2 (not 3) of the 5 surviving animals at 7 days.
Conclusion	Both test substances are corrosive.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.4-1 Skin Irritation Scoring

	Value
Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm, and extending beyond the area of exposure)	4

Table A6.1.4-2 Skin Irritation Summary

			Average Dermal Scores					
Group	*Material*	*Dermal Endpoint*	*5 hours*	*1 Day*	*2 Days*	*3 Days*	*7 Days*	*10 Days*
4 hr	[redacted]	Erythema & Eschar Formation	1.0	2.0	2.5	2.3	1.6*	0.0*
		Edema Formation	1.5	3.2	1.5	1.2	1.2*	0.0*
		Other Irritation or Effects @	0.0	N	N	N	N,D*	N,D, A,S*
60 min.	[redacted]	Erythema & Eschar Formation	0.0	1.3	1.2	0.8	0.0	---
		Edema Formation	0.0	0.0	0.0	0.0	0.0	---
		Other Irritation or Effects @	NO	NO	NO	NO	N,D	---
4 hr	[redacted] 50% Glutaraldehyde	Erythema & Eschar Formation	1.0	2.8	3.0	2.7	1.7	0.0
		Edema Formation	1.0	3.2	2.7	2.0	1.3	0.0
		Other Irritation or Effects @	NO	N	N	N,F	N,F D,S	N,D,S, AL
60 min.	[redacted] 50% Glutaraldehyde	Erythema & Eschar Formation	0.5	2.0	2.2	1.7	0.0*	---
		Edema Formation	0.0	0.3	0.0	0.0	0.0*	---
		Other Irritation or Effects @	NO	N	N	N	N,D*	---
3 min.	[redacted] 50% Glutaraldehyde	Erythema & Eschar Formatio n	0.2	0.0	0.0	0.0	0.0	---
		Edema Formation	0.0	0.0	0.0	0.0	0.0	---
		Other Irritation or Effects @	NO	NO	NO	NO	NO	---

All animals in all groups of both test materials exhibited a brown stain at the dose site throughout all test days.

* N = 5 due to spontaneous death

@ Other Irritation and Effects does not reflect a mean, but an account of all effects observed.

Specific Effects/Remarks: NO = None, D = desquamation; N = necrosis; S = scab formation; F = fissuring
A = alopecia

--- No examinations conducted.

Section A6.1.5(01) Annex Point IIA, VI.6.1.5 IUCLID 5.3/01	Skin sensitisation Guinea pig maximisation test	
	1 REFERENCE	Official use only
1.1 Reference	(1993) Guinea pig maximization test with glutaraldehyde, , GLP, Unpublished, 24 September 1993	
1.2 Data protection	Yes	
1.2.1 Data owner	The Dow Chemical Company	
1.2.2 Companies with letter of access		
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 Section 798.4100 (1985)	
2.2 GLP	Yes	
2.3 Deviations	Assays to verify concentrations, stability and homogeneity of the aqueous 2% glutaraldehyde dilutions and alkalinized 2% glutaraldehyde and its dilutions were not performed. Archival samples of alkalinized 2% glutaraldehyde and sodium phosphate were not taken.	
	3 MATERIALS AND METHODS	
3.1 Test material	2% aqueous glutaraldehyde and alkalinized 2% glutaraldehyde in propylene glycol.	
3.1.1 Lot/Batch number	2% aqueous glutaraldehyde- Alkalinized 2% glutaraldehyde in propylene glycol-prepared from 2% aqueous glutaraldehyde.	
3.1.2 Specification	Not reported	
3.1.2.1 Description	Clear liquid	
3.1.2.2 Purity		
3.1.2.3 Stability	Assumed to be stable under normal storage conditions, material was used well within the shelf-life specified by the Sponsor.	
3.1.2.4 Preparation of test substance for application	**Induction- Intradermal** Site 1- 0.5 ml/ml mixture of FCA and distilled water. Site 2- Test or control materials were added to propylene glycol to make the following concentrations; DNCB- 0.001 g/ml, Test materials: 2% glutaraldehyde- 0.05 ml/ml and 2% alkalinized glutaraldehyde- 0.05 ml/ml. Site 3 – Test or control materials were mixed as follows in FCA/Water dependent upon the dose group: 0.01 g of DNCB to 5 ml of distilled water and 5 ml of FCA to produce 0.001 g/ml, or 0.5 ml of either test material (2% glutaraldehyde or 2% alkalinized glutaraldehyde) into 4.75 mL distilled water and 4.75 mL FCA to produce 0.05 ml/ml mixture, or 0.5 mL of propylene glycol into 4.75 mL distilled water and 4.75 mL	

Section A6.1.5(01) Annex Point IIA, VI.6.1.5 IUCLID 5.3/01	Skin sensitisation Guinea pig maximisation test	
	FCA to produce 0.5 mL/mL mixture. **Induction- Topical** DCNB added to 70% ethanol to produce 0.001 g/ml mixture. 2% aqueous glutaraldehyde and alkalinized 2% glutaraldehyde -no preparation required. Negative Control- 70% ethanol-no preparation required. **Challenge- Topical** DCNB-added to 70% ethanol to produce 0.001 g/ml mixture. 2% aqueous glutaraldehyde and alkalinized 2% glutaraldehyde-distilled water added to glutaraldehyde solution to create a 0.1 ml/ml mixture. **Rechallenge- Topical** 2% aqueous glutaraldehyde and alkalinized 2% glutaraldehyde-distilled water added to glutaraldehyde solution to create a 0.1 ml/ml mixture.	
3.1.2.5 Pretest performed on irritant effects	**Intradermal Pre-test** 2% Aqueous Glutaraldehyde and 2% Alkalinized Glutaraldehyde were tested (2 injections of each solution) intradermally. Observations were made 24 and 48 hours post-injection to determine the concentrations that did not display extensive necrosis or ulceration or severe systemic toxicity. **Topical Pre-test** A topical irritation test was also conducted to determine the concentration at which each material produces mild irritation (the concentration used for induction) and the highest concentration that caused no irritation (used for the challenges). Exposures were 24 hours, occluded. Observations for erythema, edema, and eschar formation were recorded 24 and 48 hours after removal of the patches according to a standard scoring scale.	
3.2 Test Animals		
3.2.1 Species	Guinea Pigs	
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	***Range-finding*** Intradermal- 3-4 weeks of age. Weights not reported. Topical: 9-10 weeks of age. Weights not reported. ***Sensitization Study*** 5-6 weeks of age. Weight range (males) 366-466 grams Weight range (females) 287-406 grams	
3.2.6 Number of animals per group	***Range-finding*** 14 males ***Sensitization study*** 50 (25 males, 25 females) ***Irritation Control***: Challenge: 10 (5 males, 5 females) Re-challenge: 10 (5 males, 5 females)	
3.2.7 Control animals	Yes	

Section A6.1.5(01) Annex Point IIA, VI.6.1.5 IUCLID 5.3/01	**Skin sensitisation** **Guinea pig maximisation test**	
	FCA Controls (in distilled water) *Irritation Controls* (To differentiate dermal reactions of irritation from those produced by sensitization, subjected to an injection of FCA/water emulsion and vehicle only, and then to the same methods as in the Challenge procedures.) *Positive Control* (induced and challenged with 2,4-dinitrochlorobenzene (DNCB))	
3.3 Administration/ Exposure		
3.3.1 Induction schedule	*Table A6.1.5/01-1*	
3.3.2 Way of Induction	Induction was accomplished by intradermal injection of 5% v/v concentrations of 2% aqueous glutaraldehyde (with and without FCA) and alkalinized 2% glutaraldehyde (with and without FCA) in propylene glycol. Injections of 0.1 mL per site were made in the clipped shoulder area. One row of three injections on each side for a total of six injections. Injections 1 and 2 were given close together and nearest to the head; injection 3 was given most caudally. Injections were made in an 8 cm^2 area covered with a patch for a week. Topical applications of vehicle, test or control material were applied to a 2x4 filter paper to saturation (approximately 0.2 mL). The filter paper was placed on the clipped shoulder region and covered by overlapping impermeable plastic, which was firmly secured by an elastic adhesive bandage. The patches were left in place for 48 hours after which the patches were removed, and the skin was wiped free of any excess material with distilled water and gauze. DCNB and propylene glycol were used as the positive control and negative controls, respectively, and were given to the animals in the same manner concurrently.	
3.3.3 Concentrations used for induction	***Intradermal*** Site 1- 0.1 mL of a 0.5 ml/ml mixture of FCA and distilled water. Site 2- 0.1 mL of either propylene glycol alone, test or control materials in propylene glycol; positive control (DNCB)- 0.001 g/ml Test materials: 0.05 ml/ml of either 2% glutaraldehyde or 2% alkalinized glutaraldehyde. Site 3 – 0.1 mL of either test or control materials mixed in FCA/Water. Positive control (DNCB)- 0.001 g/ml Test materials (either 2% glutaraldehyde or 2% alkalinized glutaraldehyde) 0.05 ml/ml mixture. ***Topical*** DCNB- 0.001 g/ml mixture in 70% ethanol 2% aqueous glutaraldehyde or alkalinized 2% glutaraldehyde each - no preparation required. Negative Control- 70% ethanol-no preparation required.	
3.3.4 Concentration Freunds Complete Adjuvant (FCA)	0.5 ml/ml mixture of FCA and distilled water (emulsion)	

Section A6.1.5(01) Annex Point IIA, VI.6.1.5 IUCLID 5.3/01	Skin sensitisation Guinea pig maximisation test	
3.3.5 Challenge schedule	**Table A6.1.5/01-1**	
3.3.6 Concentrations used for challenge	DCNB- 0.001 g/ml mixture in 70% ethanol. 2% aqueous glutaraldehyde or alkalinized 2% glutaraldehyde-0.1 ml/ml mixture in distilled water.	
3.3.7 Rechallenge	2% aqueous glutaraldehyde or alkalinized 2% glutaraldehyde-0.1 ml/ml mixture in distilled water.	
3.3.8 Scoring schedule	24 and 48 hours following each induction, challenge and/or rechallenge.	
3.3.9 Removal of the test substance	Removed after a 48-hour contact period	
3.3.10 Positive control substance	2,4-dinitrochlorobenzene (DNCB)	
3.4 Examinations	Mortality checks were performed twice daily. Bodyweights were taken prior to the first induction and at termination (three days after Challenge). Animals were observed prior to treatment and weekly during the study for general health and any unusual observations were recorded. There were no post-mortem examinations performed on surviving animals. The severity index was determined for the response readings by dividing the sum total of grades in a given group by the number of animals exposed.	
3.4.1 Pilot study	Not applicable	
3.5 Further remarks	None	
	RESULTS AND DISCUSSION	
3.6 Results of pilot studies	5% v/v of 2% Aqueous Glutaraldehyde in propylene glycol displayed local necrosis and moderate to very slight erythema. 5% v/v of 2% Alkalinized Glutaraldehyde in propylene glycol displayed local necrosis and slight to very slight erythema.	X
3.7 Results of test	Positive	
3.7.1 24h after challenge	***Tables A6.1.5/01-2***	
3.7.2 48h after challenge	***Tables A6.1.5/01-2***	
3.7.3 Other findings	None	
3.8 Overall result	Test materials were sensitizers in guinea pigs.	
	4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	Male and Female [redacted] guinea pigs were obtained from a commercial supplier. Male bodyweights ranged from 366 to 466 grams and females ranged from 287 to 406 grams. All animals were subjected to a health examination prior to treatment. They were housed individually, and given food and water *ad libitum* in rooms designed to maintain adequate conditions for the species. Animals were arbitrarily assigned to groups based on cage position, and any animals considered unsuitable (based on health status or body weight or unacceptable skin conditions) were excluded. Animals were identified with an ear tag. A range-finding test was conducted via intradermal application to	

Section A6.1.5(01) Annex Point IIA, VI.6.1.5 IUCLID 5.3/01	Skin sensitisation Guinea pig maximisation test	
	determine the concentrations that did not display extensive necrosis or ulceration or severe systemic toxicity, and topically to determine the concentration of each material which produced mild irritation (used for induction at the highest concentration which did not produce irritation for challenge). As a result the test materials were administered as follows in the main assay: intradermal induction-5%, topical induction-100%, Challenge/Rechallenge-10%. On test day 0, animals were induced by intradermal injection, and on test day 7 by topical administration. Animals were challenged on test day 21 by topical administration of the test material. On test day 28, animals were re-challenged by topical administration ***Induction*** Induction was accomplished by intradermal injection of 5% v/v concentrations of 2% aqueous glutaraldehyde with and without FCA or alkalinized 2% glutaraldehyde in propylene glycol with and without FCA. Injections of 0.1 mL per site were made in the clipped shoulder area. One row of three injections on each side for a total of six injections. Injections 1 and 2 were given close together and nearest to the head; injection 3 was given most caudally. Topical applications of vehicle, test or control material were applied to a 2x4 filter paper to saturation (approximately 0.2 mL). The filter paper was placed on the clipped shoulder region and covered by overlapping impermeable plastic, which was firmly secured by an elastic adhesive bandage. The patches were left in place for 48 hours after which the skin was wiped free of any excess material with distilled water and gauze. DCNB and propylene glycol were used as the positive control and negative controls, respectively, and were given to the animals in the same manner concurrently. Irritation controls received the FCA/water emulsion or vehicle only. ***Challenge*** Challenges and Rechallenges were by topical application of a 10% dilution of the 2% aqueous solution on the clipped skin of the flanks. The test material was applied in the same manner as the topical applications during induction. The test sites were evaluated at 24 and 48 hours post administration according to a predefined scoring system (***Table A6.1.5/01-3).*** The severity index was determined for the response readings by dividing the sum total of grades in a given group by the number of animals exposed.	

Section A6.1.5(01) **Annex Point IIA, VI.6.1.5** **IUCLID 5.3/01**	**Skin sensitisation** **Guinea pig maximisation test**	
4.2 Results and discussion	Animals were exposed to 2% glutaraldehyde or alkalinized 2% glutaraldehyde. Intradermal induction was to 5% solution (of the 2% solution). Topical induction was 100% of the 2% solution. Challenge was a 10% solution (of the 2% solution). The Incidence Index of Sensitization to Aqueous 2 % glutaraldehyde at Challenge was 68 % and at Re-challenge was 32 %. The Challenge Severity Indices (maximum attainable Index is 3.0) for Aqueous 2 % glutaraldehyde at 24 and 48 hours are 0.8 and 0.4, and Re-Challenge Severity Indices are 0.5 and 0.0, respectively. The Incidence Index of Sensitization to Alkalinized 2 % glutaraldehyde at Challenge was 30 % and at Re-challenge was 5 %. The Challenge Severity Indices (maximum attainable Index is 3.0) for Alkalinized 2 % glutaraldehyde at 24 and 48 hours are 0.4 and 0.2, and Re-Challenge Severity Indices are 0.2 and 0.0, respectively. All ten animals treated with the positive control 2,4-dinitrochlorobenzine DNCB exhibited dermal responses at challenge to a non-irritating concentrations, as confirmed by a lack of response by irritation control animals to the same concentration. The Incidence Index of Sensitization to DNCB was 100 %	X
4.3 Conclusion	Aqueous 2 % glutaraldehyde exhibited a moderate to strong potential to produce dermal sensitization in Guinea Pigs. Alkalinized 2 % glutaraldehyde exhibited a weak to moderate potential to produce dermal sensitization in Guinea Pigs.	
4.3.1 Reliability	1	
4.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 20th, 2010
Materials and Methods	Agree with applicant's version.
Results and discussion	3.6 Results of pilot studies. The given results for the pilot study are very incomplete. The necessary information is however given in 4.1. *Materials and methods*. 4.2 Results and discussion. For the record, the following rounding errors in the original report are corrected: • For aqueous 2 % GA the re-challenge severity index at 48 h was 0.1 (0.05 is rounded to 0.1 and not 0.0). • For alkalinized 2 % GA the challenge severity index at 24 h was 0.5 (0.45 is rounded to 0.5 and not 0.4). • For alkalinized 2 % GA the re-challenge severity index at 48 h was 0.1 (0.05 is rounded to 0.1 and not 0.0).
Conclusion	Both aqueous and alkalinized 2 % glutaraldehyde caused sensitisation to guinea pigs in a maximisation test. The risk phrase R43 is warranted: 'May cause sensitisation by skin contact'.
Reliability	1

Section A6.1.5(01) **Annex Point IIA, VI.6.1.5** **IUCLID 5.3/01**	**Skin sensitisation** **Guinea pig maximisation test**
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.5/01-1 **Detailed information including induction/challenge/scoring schedule for skin sensitisation test**

	Day
Induction #1 (intradermal injection)	1
Induction #2 (topical application)	7
Challenge	21
Evaluation #4	22
Evaluation #5	23
Re-Challenge	28
Evaluation #4	30
Evaluation #5	31

Table A6.1.5/01-2 **Incidence of Dermal Response at Challenge/Re-Challenge**

Group	*Material*	*Conc.*	*Hour*	Dermal Scores[a] *0*	*0.5*	*1*	*2*	*3*	*Ed*	*N*	*E*	*P[b]*	*Total No. of Animals*
IA	DNCB	0.1%	24	0	0	3	7	0	10	0	0	100	10
			48	0	0	5	5	0	10	0	0		10
IB	DNCB[C]	0.1%	24	10	0	0	0	0	0	0	0	0	10
			48	10	0	0	0	0	0	0	0		10
Challenge													
IIA	Aqueous 2% glutaraldehyde	10%	24	4	2	11	2	0	0	0	0	68	19
			48	10	2	6	1	0	0	0	0		19
IIB	Aqueous 2% glutaraldehyde[C]	10%	24	10	0	0	0	0	0	0	0	0	10
			48	10	0	0	0	0	0	0	0		10
IIIA	Alkalinized 2% glutaraldehyde	10%	24	7	8	5	0	0	0	0	0	30	20
			48	15	3	2	0	0	0	0	0		20
IIIB	Alkalinized 2% glutaraldehyde[C]	10%	24	10	0	0	0	0	0	0	0	0	10
			48	10	0	0	0	0	0	0	0		10
Re-challenge													
IIA	Aqueous 2% glutaraldehyde	10%	24	7	6	6	0	0	0	0	0	32	19
			48	17	2	0	0	0	0	0	0		19
IIC	Aqueous 2% glutaraldehyde[C]	10%	24	10	0	0	0	0	0	0	0	0	10
			48	10	0	0	0	0	0	0	0		10
IIIA	Alkalinized 2% glutaraldehyde	10%	24	15	4	1	0	0	0	0	0	5	20
			48	18	2	0	0	0	0	0	0		20
IIIC	Alkalinized 2% glutaraldehyde[C]	10%	24	0	0	0	0	0	0	0	0	0	10
			48	0	0	0	0	0	0	0	0		10

[a] Scored using the scoring system presented in Appendix B.
[b] P = positive response; percent of animals with a score of 1 or greater at 24 and/or 48 hours, out of 10 (or 20) animals per group.
[c] Irritation control groups were treated at induction and challenge or re-challenge only.
Ed = Edema; N = Necrosis; E = Eschar.

Table A6.1.5/01-3 Evaluation of the Dermal Response (Appendix B from report)

Description	*Dermal Score*
No reaction	0
Very slight (barely perceptible) erythema, usually non-confluent	0.5
Slight erythema, usually confluent	1
Moderate erythema	2
Severe erythema, with or without edema, necrosis, or eschar formation	3
If edema, necrosis, or eschar formation occurred, they were indicated using the following code: *Edema Ed* *Necrosis N* *Eschar E*	

Section A6.1.5(2) Annex Point IIA, VI.6.1.5 IUCLID 5.3/02	Skin Sensitisation Mouse – Local Lymph Node Assay	
	1 REFERENCE	Official use only
1.1 Reference	[redacted] (1994) Mouse Lymph Node Assay and Mouse IgE Test on Glutaraldehyde, [redacted], Not GLP, Unpublished, 22 July 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	No	
2.2 GLP	No	
2.3 Deviations	Not applicable	
	3 MATERIALS AND METHODS	
3.1 Test material	50% glutaraldehyde in water	
3.1.1 Lot/Batch number	[redacted]	
3.1.2 Specification	Not reported	
3.1.2.1 Description	Aqueous	
3.1.2.2 Purity	[redacted]	
3.1.2.3 Stability	Not Reported	
3.1.2.4 Preparation of test substance for application	The 50% glutaraldehyde in water was diluted to the test concentrations of 0, 5, 10, 25 and 50 %w/v in acetone.	X
3.1.2.5 Pretest performed on irritant effects	No	
3.2 Test Animals		
3.2.1 Species	Mice	
3.2.2 Strain	[redacted]	
3.2.3 Source	[redacted]	
3.2.4 Sex	Female	
3.2.5 Age/weight at study initiation	6-8 weeks old	
3.2.6 Number of animals per group	4/dose	
3.2.7 Control animals	Yes, vehicle controls (acetone), positive control (trimellitic anhydride, TMA), and negative chemical allergen control (2,4-dinitrochlorobenzene, DNCB)	X

Section A6.1.5(2) Annex Point IIA, VI.6.1.5 IUCLID 5.3/02		**Skin Sensitisation** **Mouse – Local Lymph Node Assay**	
3.3	**Administration/ Exposure**		
3.3.1	Induction schedule	*Table A6.1.5/02-1*	
3.3.2	Way of Induction	**LLNA** Animals were dosed on the dorsum of both ears daily for three consecutive days. On the 6th day, mice were injected intravenously with ^{3}H-methyl thymidine. Five hours later, mice were sacrificed. **Mouse IgE** Groups of mice (6) were shaved on flanks, and dosed dermally with 50 uL of test material. Seven days later, the animals were dosed again with the same test material at half the first concentration applied to the dorsum of both ears. Fourteen days later, mice were sacrificed.	
3.3.3	Concentrations used for induction	**LLNA** 25 µl of 0, 5, 10, 25 and 50 %w/v in acetone. **Mouse IgE** 50 µl of 0, 5, 10 and 25 % w/v in acetone, then 25 uL of 0, 2.5, 5, 12.5% w/v in acetone.	
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	Not applicable	
3.3.5	Challenge schedule	**Table A6.1.5/02-1**	
3.3.6	Concentrations used for challenge	Mouse IgE 25 µl of 0, 2.5, 5 and 12.5 % w/v in acetone.	
3.3.7	Rechallenge	There was no re-challenge.	
3.3.8	Scoring schedule	Not Applicable	
3.3.9	Removal of the test substance	Not Applicable	
3.3.10	Positive control substance	Trimellitic anhydride, TMA	
3.4	**Examinations**		
3.4.1	Pilot study	None	
3.5	**Further remarks**	None	
		RESULTS AND DISCUSSION	
3.6	**Results of pilot studies**	Not applicable	
3.7	**Results of test**	Positive	
3.7.1	24h after challenge	Not Applicable	
3.7.2	48h after challenge	Not Applicable	
3.7.3	Other findings	**Tables A6.1.5/02-2, 3**	
3.8	**Overall result**	Test material possesses skin and respiratory tract sensitization potential.	

Section A6.1.5(2) Annex Point IIA, VI.6.1.5 IUCLID 5.3/02	**Skin Sensitisation** **Mouse – Local Lymph Node Assay**	
	4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	**LLNA** Four mice, were tested with each of the dose levels (0, 5, 10, 25, 50%, DCNB as positive control), each group was housed together. Animals were dosed on the dorsum of both ears daily for three consecutive days. On the 6th day, mice were injected with ^{3}H-methyl thymidine in phosphate buffered saline. Five hours later, mice were sacrificed and the draining auricular lymph nodes were removed and pooled for the experimental group. A single cell suspension of lymph node cells was prepared by mechanical disaggregation through a stainless steel gauze. Pooled LNC were washed, and resuspended 12 hours later in TCA prior to transfer into scintillation fluid. Incorporation of 3H-TdR was measured by beta-scintillation counting and expressed as mean disintegrations per minute per node for each test group. The activity of each test group was divided by the activity of the vehicle control group to give a test/control ratio for each concentration tested. **Mouse IgE** Groups of mice (6) were shaved on flanks, and dosed dermally with 50 uL of test material. Seven days later, the animals were dosed again with the same test material at half the first concentration applied to the dorsum of both ears. Fourteen days later, mice were sacrificed, and blood was collected and serum prepared. The concentration of serum IgE (measured using a sandwich ELISA) was compared to the responses of the positive control (trimellitic anhydride) and negative control (DCNB). Results were expressed as serum IgE concentration (ug/mL)	X
4.2 Results and discussion	**LLNA** Each of the test concentrations produced substantial levels of lymph node cell proliferative activity. Increasing concentrations of glutaraldehyde elicited stimulation indices of 15.5, 23.4, 38.7, and 34.9 respectively. The data confirms that glutaraldehyde produces skin sensitization potential, consistent with previous studies. An EC_3 was not calculated. The EC_3 in mice was, however, published in another study (***Ref A6.1.5/04***) as 0.07 to 0.2% determined by linear interpolation. **Mouse IgE** Exposure of mice to glutaraldehyde resulted in a dose-dependent increase in serum IgE relative to the control, elicitating mean values of 1.280 +/- 0.193 ug/mL. A substantial increase in serum IgE levels relative to the historical control is considered indicative of the potential to cause sensitization of the respiratory tract. The data leads to the conclusion that glutaraldehyde exhibits some capacity for sensitization of the respiratory tract.	X
4.3 Conclusion	Glutaraldehyde is considered to be a sensitizer under conditions of the test.	
4.3.1 Reliability	2	
4.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	

Section A6.1.5(2) Annex Point IIA, VI.6.1.5 IUCLID 5.3/02	**Skin Sensitisation** **Mouse – Local Lymph Node Assay**	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 20th, 2010	
Materials and Methods	3.1.2.4. Preparation of test substance for application. The concentrations were actually half of those indicated here: 0, 2.5, 5, 12.5 and 25 %. This is because the percentages given indicate the dilutions starting from the 50 % starting material. 3.2.7. Control animals. The positive and negative controls are those listed for the IgE test. For the LLNA only vehicle control is reported. 4.1. Materials and methods. LLNA: after pooling and washing the cells, they were precipitated with 5 % TCA.	
Results and discussion	Agree with applicant's version. 4.2 Results and discussion. The reference where EC_3 is calculated is an IUCLID entry that cites two studies: Basketter *et al* (2000), Contact Dermatitis 42, 344-348 *Use of the local lymph node assay for the estimation of relative contact allergenic potency* and Basketter *et al* (2003), J. Toxicology 22:4, 187-199 *Biocides: Characterization of the Allergenic Hazard of Methylisothiazolinone*	
Conclusion	Glutaraldehyde has the potential to induce sensitisation on the skin, and the IgE test indicates that it may also induce sensitisation of the respiratory tract.	
Reliability	1	
Acceptability	Acceptable	
Remarks	All concentrations cited should be halved to get the correct values, e.g. 50 % is actually 25 % GA. See comment to 3.1.2.4 above for an explanation.	
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6.1.5/02-1 Detailed information including administration/challenge/sacrifice schedule for local lymph node and Mouse IgE sensitisation tests	
	Day
Induction LLNA	1-3
Sacrifice LLNA	6
Induction Mouse IgE	1
Challenge Mouse IgE	7
Sacrifice Mouse IgE	14

Table A6.1.5/02-2 Activity of 50% Glutaraldehyde in the Local Lymph Node Assay		
Concentration (% w/v)	**Dpm/node x 10^{-2}**	**Stimulation index**
0	2.36	-
5	36.70	15.5
10	55.35	23.4
25	91.45	38.7
50	82.57	34.9

Table A6.1.5/02-3 Activity of 50% Glutaraldehyde in the Mouse IgE Test	
Chemical	**Serum IgE Concentration mean ± SE µg/ml**
AOO	0.262 ± 0.022
DNCB (1% w/v)	0.212 ± 0.042
TMA (25% w/v)	1.991 ± 0.160
Acetone	0.304 ± 0.024
Glutaraldehyde (5% w/v)	0.516 ± 0.038
Glutaraldehyde (10% w/v)	0.640 ± 0.195
Glutaraldehyde (25% w/v)	1.280 ± 0.193
Untreated Mice	Range = 0.115-0.595

AOO- 4:1 acetone:olive oil

DNCB- 2,4-dinitrochlorobenzene

TMA- trimellitic anhydride

Section A6.2(1)
Annex Point IIA6.2
IUCLID 5.0/03

Metabolism studies in mammals
^{14}C-GDA – Study of the Biokinetics in Rats

Official use only

1 REFERENCE

1.1 Reference — (2004) ^{14}C-GDA- Study of the Biokinetics in Rats, , Unpublished, 29 July 2004.

1.2 Data protection — Yes

1.2.1 Data owner — The Dow Chemical Company (Dow)

1.2.2 Companies with letter of access —

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 — The study was performed according to the following guidelines:

- EC Commission Directive 87/302/EEC of November 18, 1987; Part B: Methods for the determination of Toxicity, Toxicokinetics; Official Journal of the European Communities No. L133, p.51-54, 1988
- OECD Guidelines for Testing of Chemicals; Method No. 417 Toxicokinetics, Version dated 4.4.1984.
- US EPA, Health Effects Test Guidelines, OPPTS 870.7485, Metabolism and Pharmacokinetics, August 1998
- Japan/MAFF: Guidelines on the Compiling of Test Results on Toxicity; Tests on In Vivo Fate in Animals, 2001

2.2 GLP — Yes

2.3 Deviations — None

3 MATERIALS AND METHODS

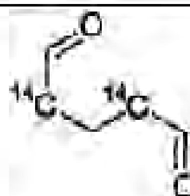
3.1 Test material — Radiolabelled and non-radiolabelled glutaraldehyde were used for this study.

3.1.1 Radiolabelled test materials — The following radiolabelled test material was obtained from the .

- Batch no.
- Code name: ^{14}C-GDA
- Chemical name: Glutaraldehyde-[2,4-^{14}C]
- Concentration of active ingredient in the solution (0.01N sulphuric acid): 0.878mg/g
- Radiochemical purity:
- Specific activity 1.87 MBq/g solution

X

Section A6.2(1)	Metabolism studies in mammals
Annex Point IIA6.2	^{14}C-GDA – Study of the Biokinetics in Rats
IUCLID 5.0/03	



Structure of ^{14}C-GDA

3.1.2	Non-radiolabelled test material	The following non-radiolabelled test material was obtained from [redacted]. [redacted] Batch no. [redacted] • Code name: [redacted] (50% glutaraldehyde) • Chemical name: Glutaraldehyde	
3.1.3	Reference Standards	None	
3.2	**Test Animals**	Male and female [redacted] rats were received from [redacted]. The animals were approximately 9 weeks old at the start of treatment. Weight range 172-400g at commencement of dosing.	
3.2.1	Control animals	No	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Dosing regime	The study design is outlined in Table A6_2(3)-1. Each animal in Groups 1 to 8 was given a single dose *via* oral gavage at 5 or 75 mg/kg bw at a dose volume of 10ml/kg bw.	
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.	
3.3.3	Analysis of dose preparations for radiopurity, homogeneity, and stability	Samples were taken to check the radioactive content, stability and homogeneity. The samples were mixed with scintillation cocktail and then counted for 10 minutes on a liquid scintillation counter (LSC).	X
3.4	**Examinations**	*Non entry field*	
3.4.1	Antemortem Observations	Animals were observed once daily for mortality, moribundity, general health and appearance.	
3.4.2	Bodyweight	Individual bodyweights were recorded at the time of dosing.	
3.4.3	Sample collection for radioanalysis	Antemortem Sample Collection For Radioanalysis The following samples were collected for radio analysis: **Expired Air (Groups 1 and 2)** Expired air and volatiles were trapped to recover volatile organic compounds. In the high dose group the exhaled air was passed through a charcoal filter first before it reached the CO_2 trap. Samples were collected at 6, 12, 24, 48, 72, 96 (females, high dose only) hours. **Urine (Groups 1 and 2)** urine was collected at the following	

intervals 0-6, 6-12, and 12-24 hours postdose, and at 24-hour intervals thereafter up to 7 days after administration of the radioactive dose. The weight of each sample was recorded.

Faeces (Groups 1 and 2) faeces were collected at 0-6, 6-12, and 12-24 hours postdose, and at 24-hour intervals thereafter up to 7 days after administration of the radioactive dose. Faeces were transferred to storage containers and the weight of each sample was recorded.

Bile (Groups 7 and 8). Samples were collected from animals at 3 hour intervals up to 48 hours postdose. The weight of each sample was recorded.

Blood and Plasma (Groups 3 and 4). Blood (approximately 0.1-0.2 mL) was collected *via* the retroorbital sinus at 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 120, 144 and 168 hours postdose. Plasma was harvested at each time point and samples were retained for radioanalysis.

Cage Wash (Groups 1 and 2) Cages were washed. The weight of each sample was recorded.

<u>Terminal Sacrifice And Collection For Radioanalysis</u>

Terminal Sacrifice and Collection Groups 1, 2, 5 and 6 After the last excreta collection, each animal was sacrificed.

The following tissues and organs were collected from all animals at sacrifice, as applicable and weighed:

Heart	Carcass	Adipose tissue
Liver	Muscle	Stomach and contents
Spleen	Kidney	Thyroid gland
Bone	Testes	Adrenal glands
Skin	Brain	Blood and plasma
Lung	Pancreas	Gut and contents
Ovaries	Uterus	Bone marrow

Group 5 males – samples were collected at 1, 17, 36, 64h, 3 animals per timepoint

Group 5 females - samples were collected at 1, 11, 20, 36h, 3 animals per timepoint

Group 6 males and females – samples were collected at 4, 24, 52, 96h, 3 animals per timepoint

<u>Radioanalysis methodology</u>

Aliquots of liquid samples (plasma, urine, CO_2 trap fluid and cage wash) were mixed with scintillation cocktail (Hionic Fluor, Packard) and analyzed for radioactivity without any additional treatment.

The femur was solubilized in 4 N HCI. After addition of scintillation

cocktail radioactivity was measured.

Faeces, contents of gut and stomach were suspended in distilled water. The carcass was homogenized with distilled water using a WARING Blendor. Aliquots of these suspensions were dried by lyophilization in a freeze dryer (LYOVAC GT 3). Aliquots of the remaining powder and of the homogenates of the other tissues were solubilized in SOLUENE (Packard). In order to bleach these samples isopropanol and H_2O_2-solution were added. The samples were left for 24 hours at room temperature. After addition of scintillation cocktail the samples were counted for 10 minutes in a liquid scintillation counter (LSC; Wallac type 1409) and the disintegration rate corrected by the respective background.

4 RESULTS AND DISCUSSION

4.1 Radioanalysis

Absorption and Elimination (Groups 1 and 2)

High Dose - The mean recovery of radioactivity for Group 1 was 93.43% and 94.46% in males and females, respectively. Of the administered dose 60.71% and 61.72% was recovered in faeces, 10.83% and 9.68% in urine and 19.46% and 20.65% in CO_2.

A small amount of radioactivity was retained in tissues and carcass combined at 168 hours postdose, accounting for *ca* 1-2% of the administered radioactivity.

Low dose - The mean recovery of radioactivity for Group 2 was 91.79% and 93.61% in males and females, respectively. Of the administered dose 45.25% and 49.53% was recovered in faeces, 14.04% and 12.78% in urine and 28.70% and 28.63% in CO_2.

A small amount of radioactivity was retained in tissues and carcass combined at 168 hours postdose, accounting for *ca* 1-2% of the administered radioactivity.

Blood/Plasma Level (Groups 3 and 4)

High dose - In rats exposed to a single oral dose of 75 mg/kg bw of ^{14}C-GDA (Group 3), the plasma concentration/time curve showed one peak, which was reached 1 hour post-dosing with a peak level of 14.60 µg Eq/g in males and 23.44 µg Eq/g in females. After having reached peak values, plasma levels declined biphasically to levels of 0.49 µg Eq/g in males and 0.35 µg Eq/g in females at sacrifice after 168 hours. The initial half-life was calculated to be 18.1 hours in males and 12.7 hours in females, respectively. The terminal half-life was 52.7 hours in males and 49.7 hours in females. X

The calculated area under the plasma concentration/time curve (AUC) was 471 µg Eq*h/g in males and 486 µg Eq*h/g in females. These calculations are based on group mean values.

Low dose - In rats exposed to a single oral dose of 5 mg/kg bw of ^{14}C-GDA (Group 4), the plasma concentration/time curve showed one peak, which was reached 4 hours post-dosing with a peak level of 1.84 µg Eq/g in males and 1.87 µg Eq/g in females. After having reached peak values, plasma levels declined biphasically to levels of 0.10 µg Eq/g in males and 0.08 µg Eq/g in females at sacrifice after 168 hours. The initial half-life was calculated to be 24.1 hours in males and 22.1 hours in females, respectively. The terminal half-life

was 48.7 hours in males and 46.5 hours in females.

The calculated area under the plasma concentration/time curve (AUC) was 89 µg Eq*h/g in males and 83 µg Eq*h/g in females. These calculations are based on group mean values.

Increasing the dose by a factor of 15 resulted in an increase of the AUC-values by a factor of 5.3 in males and 5.9 in females. These data give evidence of a decrease of the bioavailability with increasing dose.

Tissue distribution (Groups 5 and 6)

High dose - Male animals were sacrificed 1, 17, 36 and 64 h after administration of the test substance and female animals were sacrificed 1, 11, 20 and 36 h after administration.

At 1 hour after administration to male and female rats highest tissue concentrations (means) were found in the GI tract, thyroid and kidney, being in the ranges of 105.45 - 3121.27 µg Eq/g, 53.56 - 73.98 µg Eq/g and 51.51 - 80.01 µg Eq/g, respectively. Lowest concentrations were measured in brain and adipose tissue of both sexes, being in the ranges of 1.39 - 2.90 µg Eq/g and 1.84- 2.09 µg Eq/g, respectively. Radioactivity concentrations generally declined continuously in organs and tissues during the following 63 h (males) and 35 hours (females) in parallel to the concentration in plasma. Exceptions to this general trend were found in adipose tissue, adrenal glands, ovaries and uterus, where radioactivity concentrations virtually remained constant during the whole experiment. It is noted that these organs had only a very small portion of the total dose and that radioactivity in adipose tissue of both sexes remained at rather low concentrations (below 7 µg Eq/g). At the time point of 1/8 maximum plasma concentration (64 hours post-dosing in males and 36 hours post-dosing in females), concentrations in organs/tissues (excluding GI tract) of both sexes were highest in kidney, thyroid and adrenal glands (males: 12.98 - 27.52 µg Eq/g; females: 38.10 - 48.19 µg Eq/g) and lowest in lung and brain (males: 0.80 - 0.90 µg Eq/g; females: 1.18 - 1.28 µg Eq/g). When comparing these radioactivity concentrations at 64 and 36 hours post dosing with those found after 168 h in the balance experiment, radioactivity concentrations in organs and tissues, including adipose tissue, adrenal glands, ovaries and uterus which remained virtually constant for the first 36-64 hours post-dosing, generally declined in parallel to the concentrations in plasma.

Low dose - Male and female animals were sacrificed 4, 24, 52 and 96h after administration of the test substance.

At 4h after administration to male and female rats highest tissue concentrations (means) were found in the GI tract, pancreas, liver and kidney being in the ranges of 42.03 - 597.64 µg Eq/g, 44.28 - 63.07 µg Eq/g, 34.85 - 52.46 µg Eq/g and 33.61 - 42.89 µg Eq/g, respectively. In both sexes, lowest concentrations were measured in adipose tissue, blood cells, muscles and bone, which were 3.28,

3.53, 3.73 and 3.99 μg Eq/g in males and 1.72, 5.73, 5.00 and 2.75 μg Eq/g in females, respectively. With the exception of adipose tissue, radioactivity concentrations generally declined continuously in organs arid tissues of both sexes during the following 92 hours In parallel to the concentration in plasma. Radioactivity concentrations In adipose tissue of both sexes remained at constant but low levels during the whole experiment (1.72 - 5.50 μg Eq/g). At the time point of 1/8 maximum plasma concentration i.e. 96 hours post-dosing, concentrations in organs/tissues of both sexes were highest in thyroid (14.74 - 18.95 μg Eq/g), adrenal glands (11.61 - 12.68 μg Eq/g) and kidney (5.93 - 10.33 μg Eq/g). At this time point, lowest concentrations in males were measured in brain, blood cells, testes and bone marrow ranging from 1.15 - 1.66 μg Eq/g and in bone, carcass, brain and bone marrow (range: 1.16 - 1.78 μg Eq/g) of females. When comparing these radioactivity concentrations after 96 h with those found after 168h in the balance experiment, radioactivity concentrations in organs and tissues of both sexes generally still declined during the following 72 h in parallel to the concentrations in plasma.

Bile (Groups 7 and 8)

The distribution of radioactivity in bile duct-cannulated animals showed elimination patterns similar to those of non-bile duct animals. Approximately 2.6% (high dose) and 1.8% (low dose) of the administered radioactivity was recovered in bile samples, indicating minimal involvement of the biliary route of excretion for both low and high level doses.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The absorption, distribution, metabolism and elimination of glutaraldehyde were determined in rats after a single oral dose at either 5 or 75 mg/kg. The study design is outlined in Table A6_2(3)-1. For Groups 1 and 2 (excretion/balance) the animals were placed closed metabolism cages to facilitate the collection of excreta samples. For Groups 3-6 (blood/plasma level and tissue distribution) the animals were placed in steel wire mesh cages. For Groups 7 and 8 bile duct cannulated animals were used and were placed into restriction cages after dosing.

5.2 Results and discussion

Balance and excretion - Mean values of the amounts of excreted and residual radioactivity after single oral administration of ^{14}C-GDA to male and female rats at nominal dose levels of 75 and 5mg/kg bw are presented in Table 6_2(3)-2. Mean total recoveries of radioactivity were found to be 93.43 % in males and 94.46 % in females at the high dose level and 91.79% and 93.61% at the low dose level.

X

High dose

Within 72 hours (males) and 96 hours (females) after single oral

administration of 75 mg/kg bw to rats, 19.46 % and 20.65 % of the administered radioactivity were found as CO_2 in exhaled air of males and females, respectively. Most of the $^{14}CO_2$ (about 82 % in males and 85 % in females of total) was already detected within 12 hours post-dosing. In the charcoal filters, through which exhaled air passed before it reached the CO_2 traps, 0.05% (males) and 0.02% (females) of the administered radioactivity were found. Within 48 hours after single oral administration of 75 mg/kg bw to male and female rats 10.03% and 9.09% of the administered radioactivity were found in urine, respectively. Total excretion of radioactivity *via* urine after 168 hours was 10.83 % for males and 9.68 % for females indicating that urinary excretion was virtually complete after 48 h. During the first 48 hours after administration 58.46% and 51.26% of the administered radioactivity were excreted *via* faeces by males and females, respectively. After 168 hours the total amount of radioactivity excreted *via* faeces was found to be 60.71% for males and 61.72% for females. These excretion data from urine, faeces and $^{14}CO_2$ indicate very rapid excretion of the absorbed material.

Together with cage wash, the total amount of excreted radioactivity (including $^{14}CO_2$) was found to be 91.33 % of the administered radioactivity in males and 92.37 % in females reflecting more than 97.7 % of the recovered radioactivity.

168 hours post-dosing, small amounts of remaining radioactivity were found in kidney and liver (0.11-0.15 %), skin (0.43- 0.52 %) and carcass (0.93-0.96 %). Mean concentrations of radioactivity were generally below 17µg Eq/g in all organs and tissues.

Low dose

Within 72 hours after single oral administration of 5 mg/kg bw to male and female rats 28.70% and 28.63% of the administered radioactivity were found as CO_2 in exhaled air, respectively. Most of the $^{14}CO_2$ (about 89% in males and 86% in females of total) was already detected within 12 hours post-dosing.

Within 48 hours after single oral administration of 5 mg/kg bw to male and female rats 13.58% and 12.06% of the administered radioactivity were found in urine, respectively. Total excretion of radioactivity *via* urine after 168 hours was 14.04% for males and 12.78% for females indicating that urinary excretion was virtually complete after 48h. During the first 48 hours after administration, 42.76 % and 48.56 % of the administered radioactivity were excreted *via* faeces by males and females, respectively. After 168 hours the total amount of radioactivity excreted *via* faeces was found to be 45.25 % for males and 49.53 % for females. These data on the time course of excretion of urine, faeces and $^{14}CO_2$ indicate very rapid excretion of the absorbed material.

Together with cage wash, the total amount of excreted radioactivity

(including $^{14}CO_2$) was found to be 88.15% of the administered radioactivity in males and 91.18% in females reflecting more than 95% of the recovered radioactivity.

168 hours after dosing, small amounts of remaining radioactivity were found in kidney (0.08-0.10 %), liver (0.12-0.27 %), skin (0.70 - 1.21 %) and carcass (1.22-1.56 %). Mean concentrations of radioactivity were below 8 µg Eq/g in all organs and tissues.

In comparison to the high dose, the portion of radioactivity found in urine and as $^{14}CO_2$ in exhaled air was about 3-8 % higher at the low dose, indicating a higher bioavailability of GDA at this dose. Accordingly, the portions of radioactivity excreted *via* the faeces were decreased at the low dose as compared to the high dose.

The results of the balance and excretion experiments also show that there was no sex-difference in the excretion pattern at both dose levels.

Plasma and blood levels - The mean plasma concentrations of radioactivity after single oral administration of ^{14}C-GDA to male and female rats at nominal dose levels of 75 and 5 mg/kg bw are presented in Table 6_2(3)-3.

High dose

In rats exposed to a single oral dose of 75 mg/kg bw of ^{14}C-GDA, the plasma concentration/time curve showed one peak, which was reached 1 hour post-dosing with a peak level of 14.60 µg Eq/g in males and 23.44 µg Eq/g in females. After having reached peak values, plasma levels declined biphasically to levels of 0.49 µg Eq/g in males and 0.35 µg Eq/g in females at sacrifice after 168 hours. The initial half-life was calculated to 0.35 µg Eq/g in females at sacrifice after 168 hours. The initial half-life was calculated to be 18.1 hours in males and 12.7 hours in females, respectively. The terminal half-life was 52.7 hours in males and 49.7 hours in females.

The calculated area under the plasma concentration/time curve (AUC) was 471 µg Eq*h/g in males and 486 pg Eq*h/g in females. These calculations are based on group mean values.

Low dose

In rats exposed to a single oral dose of 5 mg/kg bw of ^{14}C-GDA the plasma concentration/time curve showed one peak at 4 hours post-dosing with peak levels of 1.84 µg Eq/g in males and 1.87 µg Eq/g in females. After having reached peak values, plasma levels declined biphasically to levels of 0.10 µg Eq/g in males and 0.08 µg Eq/g in females at sacrifice after 168 hours. The initial half-life was calculated to be 24.1 hours in males and 22.1 hours in females. The terminal half-life was 48.7 hours in males and 46.5 hours in females.

The calculated area under the plasma concentration/time curve

(AUC) was 89 µg Eq*hlg in males and 83 µg Eq*hlg in females. These calculations are based on group mean values.

During the first 24 hours post-dosing, lower concentrations of radioactivity were generally found in blood at both dose levels indicating that major parts of the radioactivity were in plasma and not bound to cellular blood constituents. After 24 hours past-dosing, the ratio of the blood/plasma concentrations increased continuously to values of about 10 at the high dose and to values of 3-4 at the low dose before sacrifice at 168 hour.

A similar time course of radioactivity was found for blood as for plasma in both sexes.

Increasing the dose by a factor of 15 resulted in an increase of the AUC-values by a factor of 5.3 in males and 5.9 in females. These data give evidence of a decrease of the bioavailability with increasing dose.

Tissue distribution - The mean tissue distribution of radioactivity at selected time points after a single oral administration of ^{14}C-GDA to male and female rats at nominal dose levels of 75 and 5 mg/kg bw is presented in Table 6_2(3)-4.

The time points of sacrifice were selected according to the results of the plasma kinetics. The first two time points of sacrifice were selected to be close to the time point of the maximum plasma concentration (MPC) and the time point of half the maximum plasma concentration (1/2 MPC). The third and fourth time points of sacrifice corresponded to the time points of one fourth and one eighth the maximum plasma concentration (1/4 and 1/8 MPC).

High dose

Male animals were sacrificed 1, 17, 36 and 64 h after administration of the test substance and female animals were sacrificed 1, 11, 20 and 36h after administration.

At 1 hour after administration to male and female rats highest tissue concentrations (means) were found in the GI tract, thyroid and kidney, being in the ranges of 105.45 - 3121.27 µg Eq/g, 53.56-73.98 µg Eq/g and 51.51 -80.01 µg Eq/g, respectively. Lowest concentrations were measured in brain and adipose tissue of both sexes, being in the ranges of 1.39 - 2.90 µg Eq/g and 1.84- 2.09 µg Eq/g, respectively. Radioactivity concentrations generally declined continuously in organs and tissues during the following 63 h (males) and 35 hours (females) in parallel to the concentration in plasma. Exceptions to this general trend were found in adipose tissue, adrenal glands, ovaries and uterus, where radioactivity concentrations virtually remained constant during the whole experiment. It is noted that these organs had only a very small portion of the total dose and that radioactivity in adipose tissue of

both sexes remained at rather low concentrations (below 7 µg Eq/g). At the time point of 1/8 maximum plasma concentration (64 hours post-dosing in males and 36 hours post-dosing in females), concentrations in organs/tissues (excluding GI tract) of both sexes were highest in kidney, thyroid and adrenal glands (males: 12.98-27.52 µg Eq/g; females: 38.10-48.19 µg Eq/g) and lowest in lung and brain (males: 0.80-0.90 µg Eq/g; females: 1.18-1.28 µg Eq/g). When comparing these radioactivity concentrations at 64 and 36 hours post dosing with those found after 168 h in the balance experiment, radioactivity concentrations in organs and tissues, including adipose tissue, adrenal glands, ovaries and uterus which remained virtually constant for the first 36-64 hours post-dosing, generally declined in parallel to the concentrations in plasma.

Low dose

Male and female animals were sacrificed 4, 24, 52 and 96 h after administration of the test substance.

At 4 hours after administration to male and female rats highest tissue concentrations (means) were found in the GI tract, pancreas, liver and kidney being in the ranges of 42.03-597.64 µg Eq/g, 44.28 - 63.07 µg Eq/g, 34.85 - 52.46 µg Eq/g and 33.61-42.89 µg Eq/g, respectively. In both sexes, lowest concentrations were measured in adipose tissue, bloodcells, muscles and bone, which were 3.28, 3.53, 3.73 and 3.99 µg Eq/g in males and 1.72, 5.73, 5.00 and 2.75 µg Eq/g in females, respectively. With the exception of adipose tissue, radioactivity concentrations generally declined continuously in organs and tissues of both sexes during the following 92 hours in parallel to the concentration in plasma. Radioactivity concentrations in adipose tissue of both sexes remained at constant but low levels during the whole experiment (1.72-5.50 µg Eq/g). At the time point of 1/8 maximum plasma concentration i.e. 96 hours post-dosing, concentrations in organs/tissues of both sexes were highest in thyroid (14.74-18.95 µg Eq/g), adrenal glands (11.61-12.68 µg Eq/g) and kidney (5.93-10.33 µg Eq/g). At this time point, lowest concentrations in males were measured in brain, bloodcells, testes and bonemarrow ranging from 1.15-1.66 µg Eq/g and in bone, carcass, brain and bonemarrow (range: 1.16-1.78 µg Eq/g) of females. When comparing these radioactivity concentrations after 96h with those found after 168h in the balance experiment, radioactivity concentrations in organs and tissues of both sexes generally still declined during the following 72 h in parallel to the concentrations in plasma.

Excretion *via* bile - The mean biliary excretion of radioactivity after a single oral administration of ^{14}C-GDA to rats at nominal dose levels of 75 and 5 mg/kg bw is presented in Table 6_2(3)-5.

High dose

Within 48 hours after administration of ^{14}C-GDA at a dose level of

75 mg/kg bw, excretion *via* bile was found to be 2.55% and 2.58% of the administered radioactivity in males and females, respectively. Biliary excretion of both sexes was at maximum within the first 6 hours post-dosing and declined gradually thereafter for the next 42 hours.

Low dose

Within 48 hours after administration of ^{14}C-GDA at a dose level of 5 mg/kg bw, excretion *via* bile was found to be 1.76% of the administered radioactivity in males and 1.84% in females. Biliary excretion in males was at maximum in the time interval between 9 and 15 hours after dosing and declined rapidly thereafter for the next 30 hours. In females, biliary excretion was at maximum within the first 9 hours after dosing and continuously declined thereafter for the next 39 hours.

Assuming that the amount of radioactivity excreted *via* urine, bile and CO_2 represents the bioavailable portion of dose, bioavailability was calculated to be approximately 33% at the high dose and about 44% at the low dose. These bioavailability data were corrected by the respective recovery in urine at that time point. These data indicate a decrease of gastrointestinal absorption of ^{14}C-GDA with increasing dose and thus confirm the conclusions drawn from the plasma kinetics.

5.3 Conclusion

After single oral administration, ^{14}C-GDA was rapidly absorbed from the gastrointestinal tract. Absorption was incomplete at both dose levels amounting to about 33% at the high dose and 44% at the low dose indicating decrease of gastrointestinal absorption with increasing dose. After absorption, radioactive material was distributed in all organs and tissues. The excretion of radioactivity was very rapid and occurred mainly *via* the faeces and CO_2. Assuming that the amount of radioactivity excreted *via* urine, bile and CO_2 represents the bioavailable portion of dose, bioavailability was calculated to be approximately 33% at the high dose and about 44% at the low dose. These data indicate a decrease of gastrointestinal absorption of ^{14}C-GDA with increasing dose.

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 23rd, 2010
Materials and Methods	3.1.1 Radiolabelled test materials. The purity and stability are claimed but the

Section A6.2(1) **Annex Point IIA6.2** **IUCLID 5.0/03**	**Metabolism studies in mammals** **^{14}C-GDA – Study of the Biokinetics in Rats**
	analyses are not provided. 3.1.2 Non-radiolabelled test material. The purity and stability are claimed but the analyses are not provided. 3.3.3 Analysis of dose preparations for radiopurity, homogeneity, and stability. It is said that purity, homogeneity and stability were confirmed, but these results are now included in the report. The methodology cited appears not to be included in the study report.
Results and discussion	4.1 Radioanalysis. Absorption and elimination, high dose. • After 168 h, the remaining radioactivity was found in carcass (♂/♀ 0.96/0.93 %), skin (0.52/0.43 %), gut and gut contents (0.22/0.28 %), liver (0.12/0.15 %) and kidney (0.11/0.12 %). • The total radioactivity detected in the body after 168 h was 2.16/2.14 % of the total dose, and 2.31/2.27 % of the recovered dose (♂/♀). 4.1 Radioanalysis. Absorption and elimination, low dose. • After 168 h, the remaining radioactivity was found in carcass (♂/♀ 1.56/1.22 %), skin (1.21/0.70 %), gut and gut contents (0.26/0.11 %), liver (0.27/0.12 %) and kidney (0.10/0.08 %). • The total radioactivity detected in the body after 168 h was 3.63/2.42 % of the total dose, and 3.95/2.59 % of the recovered dose (♂/♀). 4.1 Radioanalysis. Tissue distribution, high dose. • Second paragraph: the tissue concentration for GI tract is given incorrectly; please refer to Table A6_2(1)-4 below, instead of the erroneous text. 4.1 Radioanalysis. Tissue distribution, low dose. • Second paragraph: the tissue concentration for GI tract is given incorrectly; please refer to Table A6_2(1)-4 below, instead of the erroneous text. 5.2 Results and discussion. In the total bioavailable portion, the amounts found in the tissues have to be included. Bioavailability can be calculated as the sum of radioactivity excreted in urine (10.83/9.68 % high dose, 14.04/12.78 % low dose), CO_2 (19.46/20.65 %, 28.70/28.63 %) and bile (2.55/2.58 %, 1.76/1.84 %), adding the amount found in tissues (2.16/2.14 %, 3.63/2.42 %). Correcting for the total recovery (93.43/94.46 %, 91.79/93.61 %), the bioavailability is 37.46/37.11 % at the high dose and 52.43/48.79 % at the low dose.
Conclusion	The absorption, distribution, metabolism and excretion of ^{14}C-GDA were rapid at low and high dose levels after a single oral dose. Absorption was approximately 37 % at the high dose and 51 % at the low dose. After absorption, the radioactive label was distributed in all organs and tissues. Radioactivity generally declined continuously in organs and tissues in parallel to the concentration in plasma, but in the adipose tissue, concentrations remained relatively constant (although low) during the 64 h study period.
Reliability	1
Acceptability	Acceptable
Remarks	Please note that the tabulated numerical results have not been checked in detail by the RMS.

Section A6.2(1) **Metabolism studies in mammals**

Annex Point IIA6.2 **^{14}C-GDA – Study of the Biokinetics in Rats**

IUCLID 5.0/03

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

Table A6_2(1)-1 Metabolism of [^{14}C]-Glutaraldehyde in rats - outline of study design

Group	Target Dose Level			No. of animals		Group description
	(µCi/kg)	(ml/kg)	(mg/kg)	Male	Female	
1		10	75	4	4	Balance/excretion - Single oral dose (high)
2		10	5	4	4	Balance/excretion - Single oral dose (low)
3		10	75	4	4	Blood/plasma level - Single oral dose (high)
4		10	5	4	4	Blood/plasma level - Single oral dose (low)
5		10	75	12	12	Tissue distribution - Single oral dose (high)
6		10	5	12	12	Tissue distribution - Single oral dose (low)
7		10	75	4	4	Excretion *via* bile - Single oral dose (high)
8		10	5	4	4	Excretion *via* bile - Single oral dose (low)

Table A6_2(1)-2 Excretion of [^{14}C]-Glutaraldehyde in rats

Table 1: Mean excretion and retention of radioactivity after single oral administration of ^{14}C-GDA at dose levels of 75 and 5 mg/kg bw to male and female rats, respectively

Results expressed in % of dose administered.

BALANCE / EXCRETION	75 mg/kg bw		5 mg/kg bw	
	Males	Females	Males	Females
Urine 0-6	4.45	4.09	8.24	7.32
Urine 6-12	2.04	2.06	2.24	2.27
Urine 12-24	2.59	1.87	1.86	1.85
Urine 24-48	0.98	0.87	1.14	0.84
Urine 48-72	0.35	0.25	0.29	0.30
Urine 72-96	0.20	0.16	0.11	0.10
Urine 96-120	0.11	0.07	0.05	0.12
Urine 120-144	0.08	0.07	0.02	0.08
Urine 144-168	0.06	0.05	0.02	0.04
Subtotal Urine	10.80	9.69	14.04	12.79
Feces 0-6	0.01	0.01	0.07	0.01
Feces 6-12	0.36	0.07	0.00	7.00
Feces 12-24	31.80	29.90	17.64	22.67
Feces 24-48	25.92	21.28	25.05	18.36
Feces 48-72	1.80	6.26	2.18	0.58
Feces 72-96	0.26	1.18	0.24	0.14
Feces 96-120	0.07	2.14	0.07	0.11
Feces 120-144	0.07	0.43	0.06	0.08
Feces 144-168	0.06	0.16	0.06	0.07
Subtotal Feces	60.71	61.72	45.25	48.63
CO_2 0-6	12.51	13.74	20.98	19.14
CO_2 6-12	3.54	3.87	4.45	5.12
CO_2 12-24	1.41	2.02	3.05	2.74
CO_2 24-48	2.01	0.92	0.23	1.00
CO_2 48-72	0.00	0.13	0.00	0.33
CO_2 72-96	n.d.	0.01	n.d.	n.d.
Subtotal CO_2	19.45	20.86	28.70	20.63
Cage wash	0.28	0.30	0.18	0.24
Bloodcells	0.04	0.05	0.05	0.04
Plasma	0.01	0.01	0.01	0.01
Lung	0.02	0.02	0.03	0.02
Heart	0.01	0.01	0.01	0.01
Spleen	0.01	0.01	0.01	0.01
Kidney	0.11	0.12	0.10	0.09
Adrenals	0.00	0.01	0.00	0.00
Testes/Ovaries	0.02	0.00	0.03	0.00
Uterus	—	0.00	—	0.01
Muscle	0.01	0.01	0.01	0.02
Brain	0.01	0.02	0.02	0.02
Adipose Tissue	0.01	0.01	0.01	0.03
Bone	0.00	0.00	0.00	0.00
Bonemarrow	0.00	0.00	0.00	0.00
Thyroid	0.00	0.00	0.00	0.00
Pancreas	0.01	0.01	0.01	0.01
Stomach cont.	0.00	0.01	0.01	0.00
Stomach	0.03	0.04	0.03	0.02
Gut cont.	0.04	0.07	0.15	0.05
Gut	0.13	0.21	0.11	0.06
Liver	0.12	0.15	0.27	0.12
Skin	0.52	0.43	1.21	0.70
Carcass	0.06	0.93	1.56	1.22
Charcoal filter	0.08	0.02	n.d.	n.d.
Total	93.43	94.40	91.79	83.81

n.d. = not determined

Table A6_2(1)-3 Plasma Distribution and pharmacokinetic parameters of [^{14}C]- Glutaraldehyde in rats

Table 2: Mean plasma concentration of radioactivity after single oral administration of ^{14}C-GDA at dose levels of 75 and 5 mg/kg bw to male and female rats, respectively

Results expressed in µg Eq/g plasma

Time [h]	75 mg/kg bw		5 mg/kg bw	
	Males	Females	Males	Females
0.5	12.67	20.34	1.57	1.44
1	14.60	23.44	1.60	1.73
2	13.58	22.07	1.64	1.80
4	11.58	19.93	1.84	1.87
8	10.61	14.44	1.81	1.84
24	5.02	4.44	0.95	0.90
48	2.41	1.85	0.55	0.50
72	1.59	1.26	0.36	0.33
96	1.15	0.91	0.25	0.22
120	0.83	0.67	0.18	0.16
144	0.63	0.49	0.13	0.12
168	0.49	0.35	0.10	0.08

Table 3: Pharmacokinetic parameters of radioactivity in plasma after single oral administration of ^{14}C-GDA at dose levels of 75 and 5 mg/kg bw to male and female rats, respectively

Sex	Dose [mg/kg bw]	C_{max} [µg Eq/g]	T_{max} [hour]	initial half life [hour]	terminal half life [hour]	AUC [µg Eq *hour/g]
male	75	14.60	1	18.1	52.7	471
	5	1.84	4	24.1	48.7	89
female	75	23.44	1	12.7	49.7	486
	5	1.87	4	22.1	46.5	83

Table A6_2(1)-4 Tissue distribution of [^{14}C]-Glutaraldehyde in rats (high dose)

Table 4: Mean tissue concentration of radioactivity after single oral administration of ^{14}C-GDA at a dose level of 75 mg/kg bw to male and female rats, respectively

Results expressed in µg Eq /g tissue.

	Time after administration (h)							
	Males				Females			
	1 h	17 h	36 h	24 h	1 h	11 h	20 h	36 h
Bloodcells	18.54	4.17	2.72	2.40	19.09	6.32	6.99	4.28
Plasma	16.52	7.21	2.58	1.88	19.88	7.54	6.06	3.26
Lung	6.30	3.28	1.06	0.60	8.03	3.55	1.51	1.26
Heart	8.86	5.62	2.84	2.69	14.69	5.29	5.45	4.17
Spleen	14.37	13.14	8.83	8.48	27.30	13.08	16.12	11.67
Kidney	31.51	52.09	20.33	27.52	80.01	45.81	60.67	38.10
Adrenal glands	24.62	41.60	20.75	19.56	41.44	38.43	47.00	39.93
Testes/Ovaries	2.69	4.02	1.89	2.93	12.98	14.46	12.81	14.22
Uterus	—	—	—	—	12.52	12.80	12.94	12.18
Muscle	4.81	3.92	2.05	2.27	6.56	3.37	3.48	2.29
Brain	1.39	1.67	0.41	0.80	2.90	1.47	1.85	1.19
Adipose tissue	2.09	4.18	8.07	2.30	1.84	3.54	1.73	2.17
Bone	15.74	7.18	1.93	1.51	4.86	5.57	3.56	2.69
Bonemarrow	50.78	34.28	8.70	8.33	18.39	30.95	22.73	15.66
Thyroid	53.56	62.56	31.79	12.89	73.99	44.25	30.35	40.19
Pancreas	31.72	15.23	6.44	4.75	58.48	15.62	8.83	8.55
Stomach cont.	3121.27	1062.89	4.80	3.36	2928.46	1204.30	628.85	18.61
Stomach	1980.35	369.15	21.39	11.71	1457.56	372.51	332.44	42.03
Gut cont.	191.54	624.00	24.48	6.28	272.00	629.89	668.69	85.57
Gut	105.45	91.01	6.56	4.93	125.42	74.86	18.80	17.74
Liver	34.35	23.91	8.86	7.51	57.73	33.71	24.62	11.39
Skin	7.46	8.42	4.89	5.39	9.60	4.40	4.24	4.04
Carcass	7.01	6.78	2.49	1.57	8.77	3.92	4.39	3.57

Table A6_2(1)-5 Tissue distribution of [^{14}C]-Glutaraldehyde in rats (low dose)

Table 5: Mean tissue concentration of radioactivity after single oral administration of ^{14}C-GDA at a dose level of 5 mg/kg bw to male and female rats, respectively

Results expressed in µg Eq /g tissue.

	Time after administration (h)							
	4 h		24 h		52 h		96 h	
	Males	Females	Males	Females	Males	Females	Males	Females
Bloodcells	3.30	5.73	1.58	3.09	1.95	2.83	1.30	2.60
Plasma	14.35	15.64	4.75	7.91	2.82	4.89	1.45	2.27
Lung	10.89	12.94	6.10	8.07	4.23	6.32	2.85	4.27
Heart	7.60	8.07	3.27	5.20	2.75	4.29	1.95	3.06
Spleen	10.88	12.44	7.67	12.44	4.56	7.54	3.03	4.67
Kidney	33.51	42.69	15.16	23.28	11.07	15.44	5.83	10.33
Adrenal glands	22.48	25.87	24.19	31.89	12.01	22.49	11.81	12.68
Testes/Ovaries	4.10	15.55	2.07	11.46	1.76	10.24	1.31	3.64
Uterus	—	15.25	—	14.69	—	10.00	—	5.48
Muscle	3.73	5.00	2.60	2.89	2.16	2.21	1.72	1.87
Brain	4.10	5.62	1.43	3.18	1.24	2.35	1.15	1.73
Adipose tissue	3.29	1.72	4.93	1.95	4.55	2.53	6.60	1.35
Bone	3.90	2.75	2.95	3.83	1.78	1.93	1.70	1.16
Bonemarrow	10.59	19.00	5.17	5.33	4.55	5.39	1.66	1.78
Thymic	15.60	22,94	34.45	53.62	15.20	16.51	14.74	18.95
Pancreas	44.28	63.07	5.92	14.02	5.37	7.06	4.22	4.80
Stomach cont	305.84	597.64	0.71	5.95	0.49	2.90	1.59	0.45
Stomach	110.54	305.62	14.65	14.58	8.74	8.39	3.42	5.62
Gut cont.	401.44	353.43	24.52	22.64	2.39	5.64	1.21	2.07
Gut	42.03	87.03	7.59	12.84	3.34	6.74	1.82	3.68
Liver	52.46	34.85	19,72	16,54	10,84	11,05	5.86	5,86
Skin	7.21	6.48	4.70	4.72	5.10	4.76	4.09	3.26
Carcass	5.47	5.99	2.38	2.84	1.94	2.18	1.76	1.71

Table A6_2(1)-6 Excretion of [^{14}C]-Glutaraldehyde in rats *via* bile

Table 6: Excretion pattern of radioactivity via bile of male and female rats after single oral administration of ^{14}C-GDA at dose levels of 75 and 5 mg/kg bw

Results expressed as % of the radioactivity administered.

Time interval [h]	75 mg/kg bw		5 mg/kg bw	
	Males	Females	Males	Females
0-3	0.40	0.67	0.19	0.31
3-6	0.70	0.8*	0.21	0.34
6-9	0.34	0.38	0.22	0.23
9-12	0.22	0.28	0.31	0.19
12-15	0.15	0.13	0.27	0.15
15-18	0.14	0.08	0.07	0.11
18-21	0.08	0.07	0.07	0.09
21-24	0.14	0.07	0.11	0.07
24-27	0.12	0.04	0.09	0.06
27-30	0.07	0.05	0.07	0.03
30-33	0.05	0.05	0.06	0.03
33-36	0.04	0.00	0.06	0.06
36-39	0.03	0.05	0.03	0.05
39-42	0.03	n.s.	0.02	0.04
42-45	0.03	0.00	0.02	0.03
45-48	0.04	—	0.03	0.02
Total	2.55	2.58	~.75	1.84

n.s. = no sample

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			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted].(2004), Glutaraldehyde: Pharmacokinetics in [redacted] Rats Following Oral Gavage or Dermal Application, [redacted], Unpublished, 16 June 2004	
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]	
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 claims are made in the study report with regards to regulatory compliance, however the study design was equivalent of that detailed in OECD 417.	
2.2	**GLP**	Yes	
2.3	**Deviations**	None reported	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Only non-radiolabelled glutaraldehyde was used for this study.	
3.1.1	Radiolabelled test material	Not used in this study.	
3.1.2	Non radiolabelled test material	[redacted] Supplied as a 50% aqueous solution.	
3.1.3	Reference Standards	None	
3.2	**Test Animals**	Female [redacted] rats were used. Jugular vein cannulated rats were supplied by [redacted] and non-cannulated rats were supplied by [redacted] The animals were 11 weeks old and weighed 155-171 g at the start of the study.	
3.2.1	Control animals	Yes, undosed animals	
3.3	**Administration/ Exposure**	Oral or dermal	
3.3.1	Dosing regime	The study design is outlined in Table A6_2(2)-1, 4 rats per group. Group 1: single oral dose at 5 mg/kg Group 2: single oral dose at 75 mg/kg Group 3: single dermal application of 120 µl of a 0.75% solution Group 4: single dermal application of 120 µl of a 7.5% solution	
3.3.2	Dose preparations	Oral gavage administration: glutaraldehyde was added to distilled water to obtain target doses of 5 and 75 mg glutaraldehyde/kg bw i.e. 0.1% and 1.5% w/w glutaraldehyde solutions. A target dose of 5 g/kg bw was used.	X

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		Dermal application: glutaraldehyde was added to 0.9% physiological saline solution to obtain 0.75% and 7.5% solutions.	
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. Actual values were within 10% of the target values.	X
3.4	**Examinations**	*Non entry field*	
3.4.1	Body weight	Individual body weights were recorded at the time of dosing.	
3.4.2	*In vivo* phase - Sample collection	Blood (*ca* 0.2mL) was collected at 10, 20, 30, 45 minutes and 1, 2, 4, 6, 8 and 12 hours post dose and at terminal sacrifice at 24 h. Glutaraldehyde concentrations were determined using GC-MS.	
3.4.3	*In vitro* phase	The partitioning of glutaraldehyde between whole blood, plasma and plasma protein over an 8h period, with interim measurements at 0, 5, 10 min and 2 h.	
		4 RESULTS AND DISCUSSION	
4.1	***In vivo* experiments**	Oral Pharmacokinetics Orally administered glutaraldehyde was rapidly absorbed from the GI tract, reaching peak blood concentrations (Cmax) by 10-30 minutes post-dosing. Ten minutes after an oral dose of 5 mg/kg mean blood concentrations of glutaraldehyde achieved 0.11 ± 0.01 µg/g blood. Following the high oral dose of 75 mg/kg, maximum blood concentrations of 17.0 µg/g blood were attained by 30 minutes post-dosing. Blood concentrations of glutaraldehyde decreased rapidly after Cmax. By 2 hours post-dosing, none of the low dose (5 mg/kg) animals had quantifiable levels of blood glutaraldehyde. After the peak blood levels at 30 minutes post-dosing with the high dose of 75 mg/kg, low but quantifiable levels of blood glutaraldehyde were measured in all samples from all animals up to 24 hours post-dosing. The initial half-lives of elimination of free glutaraldehyde from blood (t½ approximately 30 minutes at both dose levels) reflects the distribution phase (10 minutes to 1 hour for the low dose and 45 minutes to 2 hours for the high dose) and presumably binding of glutaraldehyde to tissues, including blood components. A terminal half-life of elimination (t½) of 6 hours was calculated from the high dose data from 4 to 24 hours post-dosing. The 15-fold increase in dose from 5 to 75 mg/kg bw produced an AUC(0-1h) that was 168- fold larger and a Cmax that was 152-fold higher suggesting that binding and/or metabolism, of glutaraldehyde was saturated at the high dose. Dermal Pharmacokinetics Only one rat had a quantifiable level of glutaraldehyde in the blood following the low dose of a 0.75%. But, percutaneous absorption was apparent following the dermal application of the high dose of a 7.5% solution of glutaraldehyde. By 20 minutes post-application, glutaraldehyde was quantified in the blood of one rat and in all rat blood samples by 30 minutes post-dosing. Following this high dose, the blood levels of glutaraldehyde remained measurable in most of the animals up to 12 hours post-dosing with peak concentrations about 2 hours post-dosing. By 12 hours post-application,	

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glutaraldehyde levels in whole blood were becoming non-quantifiable in half of the animals. Limited data prevented kinetic analysis following application of the low dose. At the high concentration, a half-life for dermal absorption was estimated as approximately 1 hour. A terminal half-life of elimination of approximately 4 hours was calculated at the high dose.

4.2 *In vitro* experiments

Following the initial phase of this study, an *in vitro* phase was performed to determine if the glutaraldehyde was bound to red blood cells, plasma protein or present in blood as free glutaraldehyde. Freshly obtained whole blood was inoculated with either 250, 2500, or 25000 ng glutaraldehyde/ml and incubated at 37°C. Aliquots of blood were taken, in triplicate, at the times indicated and whole blood, plasma and the ultrafiltration filtrate analyzed for glutaraldehyde. Glutaraldehyde in the filtrate is indicative of free glutaraldehyde present in blood.

Fifteen naïve female rats were anaesthetized with a $C0_2/0_2$ mixture and sacrificed by exsanguination *via* cardiac puncture. The blood was separated into 3 equal volume portions and kept at 37°C in a water bath. For each concentration, approximately 20ml of freshly obtained rat blood was spiked with an appropriate amount of glutaraldehyde in water and allowed to equilibrate for 1-2 minutes with gentle agitation in a 37°C water bath. At the indicated times, aliquots were removed and the acidified internal standard added to the aliquot. At the same time, another aliquot was taken and centrifuged for 5 minutes to obtain plasma. An aliquot of plasma was removed and the acidified internal standard added. The remaining plasma was transferred to a Centricon YM-30 ultrafiltration device [30,000 MW cut-off] (Millipore Co., Billerica, MA) and centrifuged @ 2,000 x g at 37°C for 10 minutes. An aliquot of the filtrate was then removed and the acidified internal standard added. After all the samples were collected they were then analyzed for glutaraldehyde.

At the low concentration, 250 ng/ml, glutaraldehyde was quantifiable in whole blood up to 2 hours after inoculation. Glutaraldehyde was not quantifiable in either plasma or filtrate at any time after inoculation. At 2500 ng/ml, glutaraldehyde was present in whole blood up to 8 hours after fortification. Quantifiable amounts of glutaraldehyde were also detected in plasma 5 and 10 minutes after inoculation. No quantifiable amounts of glutaraldehyde were detected in the protein-free filtrate suggesting rapid binding of glutaraldehyde with blood proteins and/or metabolism. Following inoculation of blood at 25000 ng/ml, glutaraldehyde was detected in all matrices. The highest amounts were found in whole blood. Glutaraldehyde was quantified in whole blood at all times after fortification. Quantifiable levels of glutaraldehyde were also detected in plasma, although at concentrations 8-to 55-fold less than that measured in whole blood. The highest amounts in plasma were obtained immediately after inoculation after which they rapidly dropped to levels of approximately 0.2-0.3 µg/ml. A very small amount of glutaraldehyde was also quantified in the protein-free filtrate, with the highest levels measured at 10 minutes post-inoculation. This suggests that the *in vitro* binding of glutaraldehyde to blood proteins and/or its metabolism is not an instantaneous event. These *in vitro*

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experiments were primarily conducted to determine if free glutaraldehyde would be present after dermal application of glutaraldehyde. These *in vitro* data may be distorted because of the time for preparation of plasma (i.e. 5 minute centrifugation) and an additional 10 minute centrifugation of plasma to prepare the protein-free ultrafiltrate. Additional binding and/or metabolism may occur during these processes which could result in lower amounts of free glutaraldehyde being detected in these samples than was present at the time the aliquot was taken from the incubating blood. Nevertheless, the presence of free glutaraldehyde in blood after dermal exposure was demonstrated.

4.3 Analysis

In the initial *in vivo* phase of this study, whole blood was analyzed for parent glutaraldehyde. Following fortification of blood with glutaraldehyde in the *in vitro* second phase of this study, whole blood and plasma were separated and an aliquot of plasma was filtered as described above to remove proteins. The amount of glutaraldehyde in each of these matrices was determined. A method for the determination of glutaraldehyde in rat blood was developed, based on a previous GC/MS/MS method for malondialdehyde, which utilized glutaraldehyde as an internal standard (Chiesa, et al., 1999). Briefly, the samples were acidified by the addition of acidified water containing a ^{13}C-glutaraldehyde internal standard. The samples were derivatized with pentafluorobenzyl hydroxylamine, extracted with toluene and centrifuged. The toluene was removed and the samples analyzed by gas chromatography/mass spectrometry.

4.4 Statistics

Descriptive statistics were used (i.e. mean ± standard deviation). All calculations were conducted using Microsoft Excel® spreadsheets and databases in full precision mode (15 digits of accuracy). Certain pharmacokinetic parameters were estimated for the *in vivo* blood data i.e., Cmax, AUC and elimination half-lives, using the pharmacokinetic computer modelling program PK Solutions (Montrose, Colorado). The AUCs used for comparisons reflect identical time frames (0-1 hour for the oral dosing). Extrapolation of the low dose to infinity may not be appropriate as the distribution phase is likely not completed before losing the ability to detect glutaraldehyde.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Phase 1: Rats, fitted with indwelling jugular vein cannulae, were administered glutaraldehyde either by oral gavage or by dermal application and blood samples were collected and analysed for glutaraldehyde up to 24h.

For the oral administration a measured volume of dose solution was administered by gavage using a glass disposable syringe with glass plunger and stainless steel feeding needle. Animals were dosed at a target volume of 5 ml dose solution per kg of body weight. The quantity of dose solution actually administered was determined by weighing the syringe prior to and immediately following dosing.

For the dermal application portion of the study, approximately 16-24 hours prior to the application of the test material the animals were anaesthetized with isoflurane. The hair was then removed from the back by clipping with an Oster® small animal electric clipper

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equipped with a size 40 fine cutting blade.

Animals were anaesthetized with isoflurane for dosing. A measured dose of glutaraldehyde was applied topically using a stainless steel feeding needle attached to an all-glass syringe to an approximately 12cm^2 dorsal area. The solution was applied evenly to the skin in a volume of 10μl/cm^2. The syringe and feeding needle used for application of the test materials was weighed prior to and after test material application to determine the actual amount of test material applied. Access to the dose site was restricted by use of a protective appliance described here.

Protective appliances were made from approximately 1.5mm thick Teflon® (4 cm x 5 cm) with a 3 cm x 4 cm cut-out opening and formed into a saddle shape. This frame was positioned intrascapularly and as far anteriorly as possible and attached to the animal using Permabond® Industrial Grade 910 adhesive (National Starch and Chemical Co., Englewood, New Jersey). The application site was semi-occluded by covering with Teflon® Spectra/Mesh® macroporous filter material (Spectrum Laboratories Inc., Rancho Dominguez, California). The covering was attached to the Teflon® frame by Velcro® strips. In addition, the rats were fitted with rodent jackets containing dermal inserts.

Phase 2: *in vitro* experiments were carried out to determine the partitioning of glutaraldehyde between whole blood, plasma and plasma protein over an 8h period. Blood was obtained from 15 naïve female rats.

5.2 Results and discussion

Oral administration - In the oral gavage portion of the study, jugular vein cannulated rats were administered either 5 or 75 mg glutaraldehyde/kg and blood was collected at 10, 20, 30,45 minutes and 1, 2, 4, 6, 8, 12, and 24 hours post-dosing and analyzed for glutaraldehyde. Glutaraldehyde was rapidly absorbed from the GI tract reaching peak blood concentrations (Cmax) by 10-15 minutes post-dosing. The initial half-lives of elimination of free glutaraldehyde from blood (t½ approximately 30 minutes at both dose levels) reflects the distribution phase and presumably binding of glutaraldehyde to tissues, including blood components. Following the initial distribution phase, a terminal half-life of elimination (t½) of 6 hours was calculated for the high dose group. There was no dose proportionality in the AUC or Cmax between the doses. The 15- fold increase in dose from 5 to 75 mg/kg bw produced a 0 to 1 hour AUC that was 168-fold larger and a Cmax that was 152-fold higher suggesting that binding and/or metabolism of glutaraldehyde was saturated at the high dose. X

Dermal administration - In the dermal application portion of the study, jugular vein cannulated rats had 120 μl of either a 7.5 or 0.75 % aqueous solution of glutaraldehyde applied dorsally to an approximately 12 cm^2 area. Blood was collected at 10, 20, 30, 45 minutes and 1, 2, 4, 6, 8, 12, and 24 hours post-dosing and analyzed for glutaraldehyde. Only one rat had a quantifiable level of glutaraldehyde in the blood following the low dose of a 0.75%. Following application of the high dose, detectable amounts of glutaraldehyde were found in blood of all the rats by 30 minutes post-application. The blood levels of glutaraldehyde remained measurable in most of the high dose animals through 12 hours post-dosing with peak concentrations approximately 2 hours post-dosing.

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	By 12 hours post-application, glutaraldehyde levels in whole blood were becoming non-quantifiable in half of the animals. With the limited data obtained following the higher concentration, a terminal half-life of elimination (t½) of approximately 4 hours was calculated at the high dose.
	***In vitro* experiments** - *In vitro* experiments were also conducted to determine if the glutaraldehyde was bound to red blood cells, plasma protein or present in blood as free glutaraldehyde. Freshly obtained whole blood from naive animals was inoculated with 250, 2500 or 25000 ng glutaraldehyde/ml and incubated at 37°C. At 250 ng/ml, glutaraldehyde was quantifiable in whole blood up to 2 hours after inoculation. At this concentration, glutaraldehyde was not quantifiable in either plasma or filtrate at any time after inoculation. At 2500 ng/ml, glutaraldehyde was detectable in whole blood up to 8 hours after inoculation. Quantifiable amounts of glutaraldehyde were also detected in plasma 5 and 10 minutes after inoculation. No quantifiable amounts of glutaraldehyde were detected in the protein-free filtrate suggesting rapid binding of glutaraldehyde with blood proteins. Following inoculation of blood at 25000 ng/ml, glutaraldehyde was detected in all matrices. The highest amounts were detected in whole blood. Quantifiable levels of glutaraldehyde were also detected in plasma, although at concentrations 8- to 55-fold less than that measured in whole blood. The highest amounts in plasma were obtained immediately after inoculation after which they rapidly dropped to levels of approximately 0.2-0.3 µg/ml. A very small amount of glutaraldehyde was quantified in the protein-free filtrate, with highest levels measured 10 minutes post-inoculation.
5.3 **Conclusion**	These data indicate that, although glutaraldehyde is extensively metabolized, as reported in a previous study (Beauchamp *et al.*, 1992), and rapidly excreted, also reported in a previous study (MeKelvey *et al.*, 1992), it appears to be systemically available in the blood of rats. But, systemically available glutaraldehyde is rapidly removed from circulation, either by macromolecular binding or metabolism, following either oral administration or dermal application. In addition, the *in vitro* data suggest that most of the glutaraldehyde in blood is likely associated with blood proteins as only small amounts of free glutaraldehyde were obtained following separation of fortified whole blood into its plasma and protein-free components.
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 26th, 2010
Materials and Methods	3.1.2 Non-radiolabelled test material. Glutaraldehyde purity 50.6 %, water content 47.2 % and impurities 0.3 %.

Section A6.2(2) **Annex Point IIA6.2** **IUCLID 5.0/02**	**Metabolism studies in mammals** **Pharmacokinetic study in rats following oral or dermal application**
	3.3.2 Dose preparation: The applicant appears to have corrected an error in the original report, where it reads "target doses of 5 and 75 mg glutaraldehyde/kg bw i.e. 0.1 % and 1 %..." The version in this Study Summary appears correct. 3.3.3 Analysis of dose preparations for radiopurity, homogeneity, and stability. Actual oral doses were 104-114 % of the target values, and actual dermal doses 95-112 % of the target doses.
Results and discussion	5.2 Results and discussion. • Oral administration: C_{max} was reached in 10 min (low dose) and 30 minutes (high dose) post-dosing. • The C_{max} after oral administration of the 5 mg/kg dose was 0.112 µg/g. Estimating the blood volume to be 7 % of the body volume, it can be concluded that at the time of C_{max} the rat blood contained approximately 7 % × (0.112 µg/g / 5 mg/kg) = 0.16 % of the total dose. • The C_{max} after oral administration of the 75 mg/kg dose was 17.005 µg/g. Estimating the blood volume to be 7 % of the body volume, it can be concluded that at the time of C_{max} the rat blood contained approximately 7 % × (17.005 µg/g / 75 mg/kg) = 1.6 % of the total dose. • The C_{max} after dermal administration of the 58 mg/kg dose was 0.128 µg/g. Estimating the blood volume to be 7 % of the body volume, it can be concluded that at the time of C_{max} the rat blood contained approximately 7 % × (0.128 µg/g / 58 mg/kg) = 0.015 % of the total dose. • *In vitro* experiment: At 25 000 ng/ml, there was an increasing amount of glutaraldehyde in the protein-free filtrate during the first 10 minutes, having decreased to about 5 % of the maximum by 2 h.
Conclusion	Agree with applicant's version.
Reliability	1 (*in vivo* studies) 2 (*in vitro* study)
Acceptability	Acceptable
Remarks	Please note that the numerical results presented in Tables below have not been checked in detail by the RMS. The value of the *in vitro* study is questionable as it concerns *ex vivo* blood, and the description of the fractioning of blood is insufficient. It is difficult to interpret the relevance of the study, but it is concluded to show that a low amount of free glutaraldehyde is present in the blood.
	COMMENTS FROM
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*

Section A6.2(2)	Metabolism studies in mammals
Annex Point IIA6.2	Pharmacokinetic study in rats following oral or dermal application
IUCLID 5.0/02	

Remarks

Table A6_2(2)-1 Dosing Details

Table 1. Concentration of Glutaraldehyde in the Oral and Dermal Dose Solutions and Amount of Dose Solution and Glutaraldehyde Administered or Applied to [redacted] Rats.

Group	Dose Solution Concentration Target (mg/g)	Dose Solution Concentration Actual (mg/g)	Body Weight (kg) Mean	Body Weight (kg) SD	Dose Solution Administered (g) Mean	Dose Solution Administered (g) SD	Glutaraldehyde Administered (mg) Mean	Glutaraldehyde Administered (mg) SD	Glutaraldehyde Administered (mg/kg bw) Mean	Glutaraldehyde Administered (mg/kg bw) SD
5 mg/kg Oral Dose	1.00	1.09	0.167	0.002	0.849	0.020	0.9	0.0	5.5	0.1
75 mg/kg Oral Dose	15.00	16.20	0.168	0.003	0.832	0.023	13.5	0.4	80.2	2.0
0.75% Dermal Application	7.50	6.85	0.162	0.004	0.127	0.005	0.9	0.0	5.4	0.2
7.5% Dermal Application	75.00	70.90	0.162	0.004	0.124	0.007	8.8	0.5	54.4	4.3

Table A6_2(2)-2 Pharmacokinetic Parameters

GLUTARALDEHYDE: PHARMACOKINETICS IN [redacted] RATS FOLLOWING ORAL GAVAGE OR DERMAL APPLICATION

Table 4. Whole Blood Half-life, C_{max}, C_0, and AUC of Glutaraldehyde in Female Rats Following Oral Gavage of 5 or 75 mg/kg bw or Dermal Application of a 0.75 or 7.5% (w/w) Aqueous Solution

Oral Dose	5.0 mg/kg	75 mg/kg
$t_{½ \alpha}$	0.474 hr	0.403 hr
$t_{½ \beta}$	–	5.98 hr
$C_{(0)}$	0.150 µg/g	41.900 µg/g
C_{max}	0.112 µg/g	17.005 µg/g
$AUC_{(0\text{-}1\ hour)}$	0.067 µg-hr/ml	11.28 µg-hr/ml
$AUC_{(0\text{-}infinity)}$	0.091 µg-hr/ml	23.99 µg-hr/ml

$t_{½\alpha}$ estimated from 10 minutes–2 hours (low dose) and 30 minutes–2 hours (high dose).
$t_{½\beta}$ estimated from 6-24 hours (high dose).

Dermal Application	5.9 mg/kg	58 mg/kg
$t_{½\ abs}$	–	1.17 hr
$t_{½ \beta}$	–	4.09 hr
C_{max}	–	0.128 µg/g
$AUC_{(0\text{-}12\ hours)}$	–	0.785 µg-hr/ml

Dermal absorption estimated 20 minutes through 1 hour post-application
Elimination half-life following dermal application estimated from 2 to 12 hours post application.

Table A6_2(2)-3 *In vivo* phase - oral

Table 2. Time Course of Glutaraldehyde in Blood following Oral Administration.

µg Glutaraldehyde/g Blood
5 mg/kg Oral Dose

Time (hour)	Animal Number					
	03A2425	03A2426	03A2427	03A2428	Mean	SD
0	NQ	NQ	NQ	NQ	NQ	–
0.17	0.111	0.112	0.118	0.106	0.112	0.005
0.33	0.095	0.091	0.091	0.086	0.091	0.004
0.5	0.112	0.078	0.078	0.070	0.085	0.019
0.75	0.047	0.047	0.046	0.043	0.046	0.002
1	0.055	0.038	0.027	0.020	0.035	0.015
2	NQ	NQ	NQ	NQ	NQ	–
4	NQ	NQ	NQ	NQ	NQ	–
6	NQ	NQ	NQ	NQ	NQ	–
8	NQ	NQ	NQ	NQ	NQ	–
12	NQ	NQ	NS	NQ	NQ	–
24	NQ	NQ	NS	NQ	NQ	–

µg Glutaraldehyde/g Blood
75 mg/kg Oral Dose

Time (hour)	Animal Number					
	03A2429	03A2430	03A2431	03A2432	Mean	SD
0	NQ	NQ	NQ	NQ	NQ	–
0.17	9.700	13.200	23.600	NS	11.630	9.734
0.33	NS	14.300	31.200	NS	10.294	14.826
0.5	8.520	11.900	27.200	20.400	17.005	8.436
0.75	4.790	8.720	19.800	15.100	12.103	6.662
1	3.530	5.650	13.800	11.000	8.495	4.732
2	0.816	1.260	3.340	2.690	2.027	1.186
4	0.295	0.389	1.270	0.844	0.700	0.450
6	0.190	0.249	0.826	0.271	0.384	0.297
8	0.128	0.180	0.596	0.417	0.330	0.217
12	0.078	0.090	0.307	0.221	0.174	0.110
24	0.038	0.033	0.090	0.076	0.059	0.028

NS = No sample
NQ = Non-quantifiable

Table A6_2(2)-3 *In vivo* phase – dermal

Table 3. Time course of Glutaraldehyde in Blood following Dermal Application.

µg Glutaraldehyde/g Blood

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

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

NS = No sample
NQ = Non-quantifiable

Figure 6_2(2)-1 *In vivo* phase

Figure 1. Time Course of Glutaraldehyde in Whole Blood after Oral Administration of 5 or 75 mg/kg bw to Female Rats.

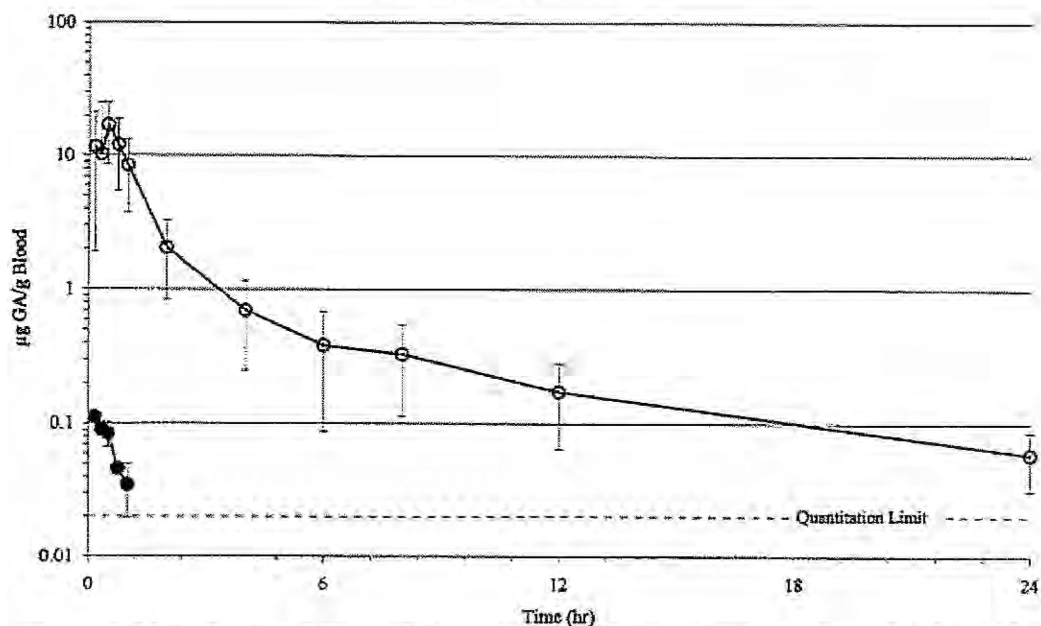
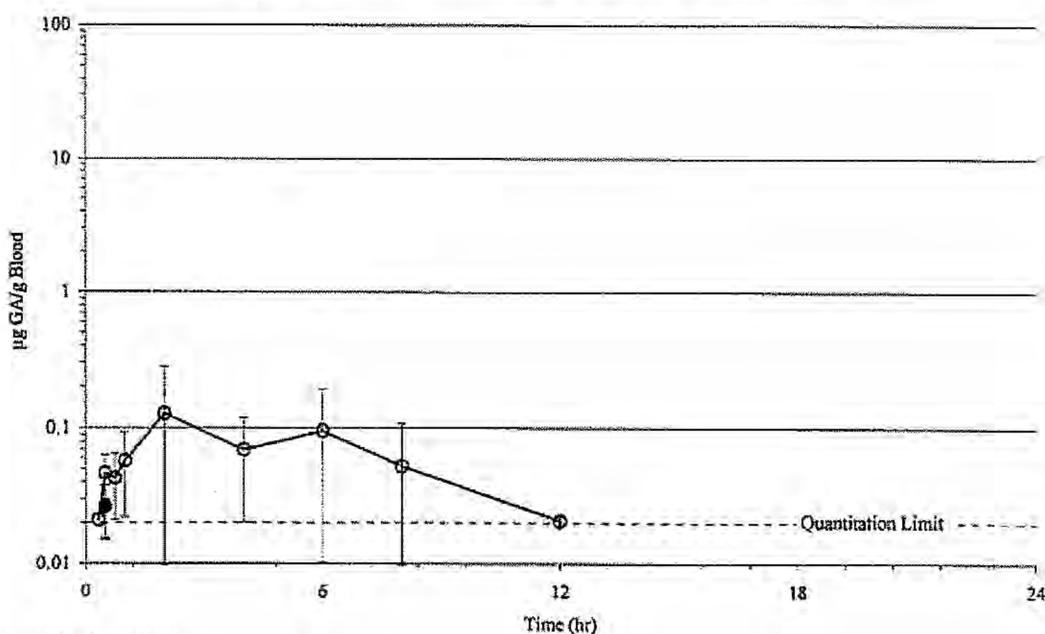


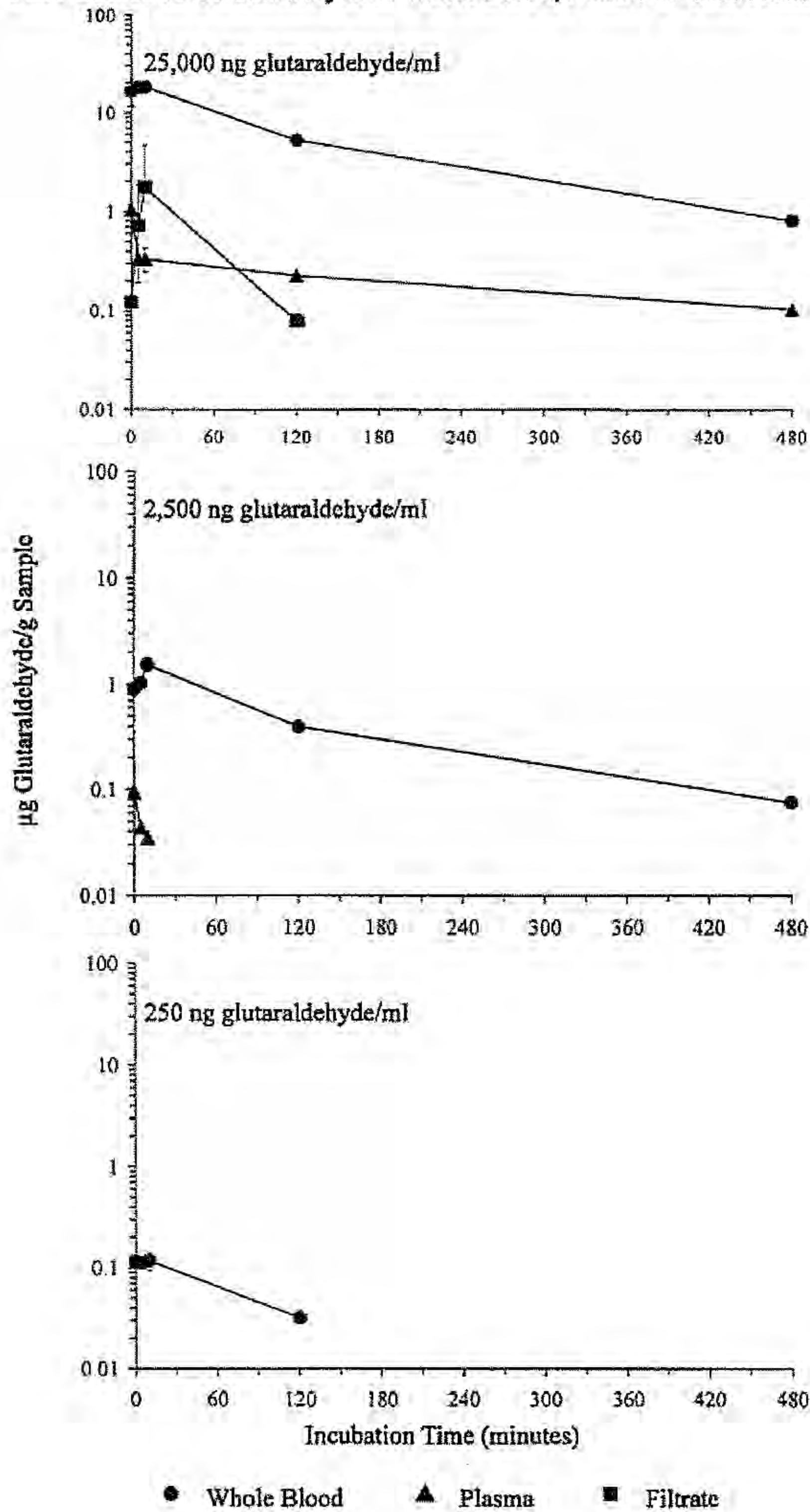
Figure 2. Time Course of Glutaraldehyde in Whole Blood after Dermal Application of a 0.75% or 7.5% Glutaraldehyde Solution to Female Rats



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

Figure 6_2(2)-2 *In vitro* phase

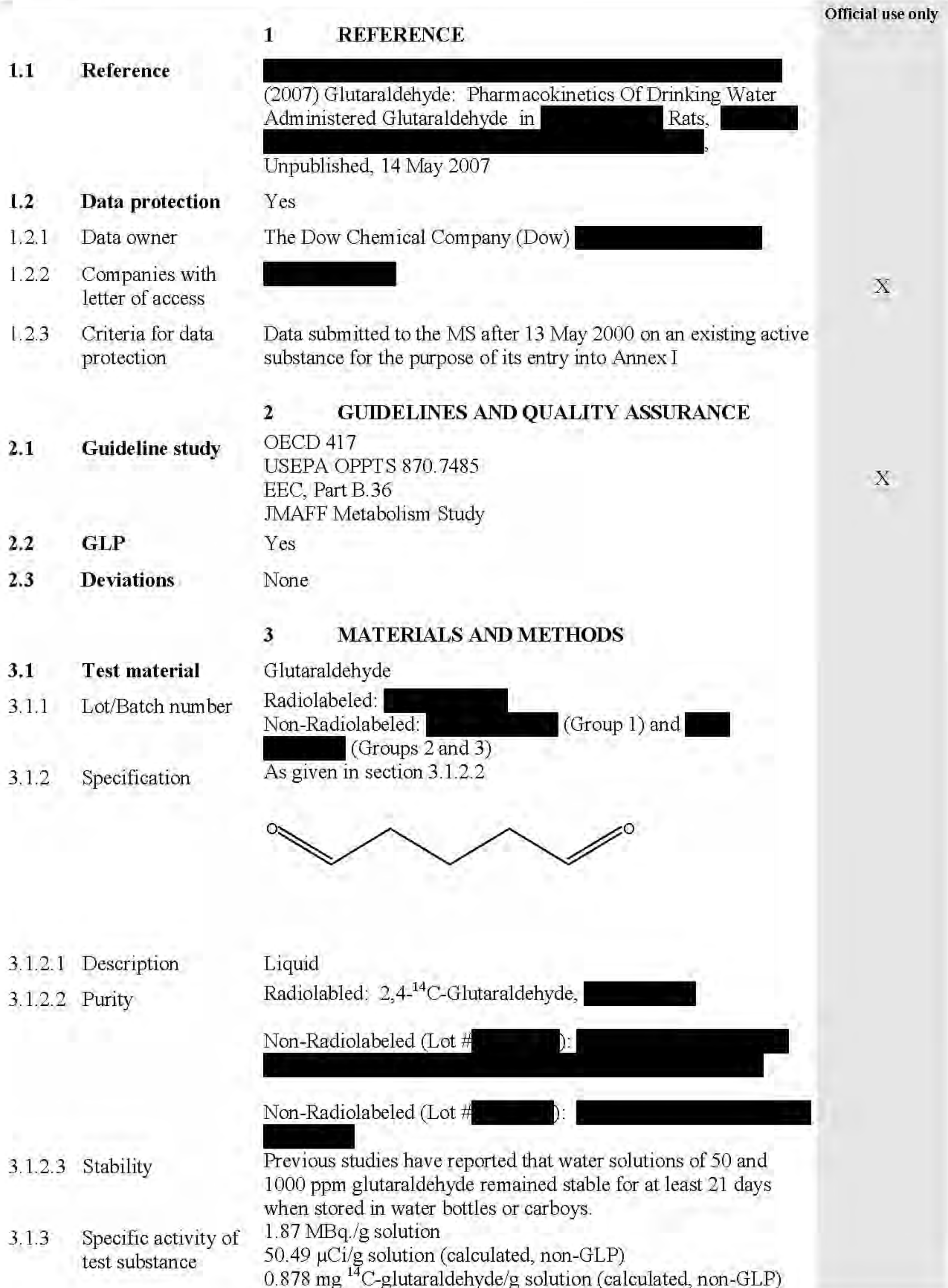
Figure 3. Time Course of Glutaraldehyde in Whole Blood, Plasma or Ultracentifugation Filtrate



Section A6.2(3) **Pharmacokinetics and Metabolism**

Annex Point IIA, VI.6.2

IUCLID 5.0/01

			Official use only
		1 REFERENCE	
1.1	**Reference**	(2007) Glutaraldehyde: Pharmacokinetics Of Drinking Water Administered Glutaraldehyde in Rats, , Unpublished, 14 May 2007	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company (Dow)	
1.2.2	Companies with letter of access		X
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on an existing active substance for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 417 USEPA OPPTS 870.7485 EEC, Part B.36 JMAFF Metabolism Study	X
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde	
3.1.1	Lot/Batch number	Radiolabeled: Non-Radiolabeled: (Group 1) and (Groups 2 and 3)	
3.1.2	Specification	As given in section 3.1.2.2 	
3.1.2.1	Description	Liquid	
3.1.2.2	Purity	Radiolabled: 2,4-^{14}C-Glutaraldehyde, Non-Radiolabeled (Lot #): Non-Radiolabeled (Lot #):	
3.1.2.3	Stability	Previous studies have reported that water solutions of 50 and 1000 ppm glutaraldehyde remained stable for at least 21 days when stored in water bottles or carboys.	
3.1.3	Specific activity of test substance	1.87 MBq./g solution 50.49 µCi/g solution (calculated, non-GLP) 0.878 mg ^{14}C-glutaraldehyde/g solution (calculated, non-GLP)	

Section A6.2(3) **Annex Point IIA, VI.6.2** **IUCLID 5.0/01**		**Pharmacokinetics and Metabolism**	
		5.76 mCi/mmol (calculated, non-GLP)	
3.1.3.1	Radiolabelling	2,4-^{14}C	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	████	
3.2.3	Source	████ ████	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Females weighed 0.178-0.214 kg.	
3.2.6	Number of animals per group	4 rats per dose level	
3.2.7	Control animals	No	X
3.3	**Administration/ Exposure**	Drinking water The rats had free access to ^{14}C-glutaraldehyde-fortified drinking water for 24 hours or until their allotment of fortified water was consumed (whichever came first) beginning at the start of the 12-hour dark cycle.	
3.3.1	Dose Preparations	Appropriate amounts of ^{14}C-labeled and/or non-radiolabeled glutaraldehyde were added to municipal water to obtain the target concentrations of 50 or 1000 ppm. Confirmation of the test material concentration in the drinking water for Group 01 – 1000 ppm, Group 02 – 1000 ppm, and Group 03 – 50 ppm, and Group 03 – 1000 ppm was conducted by high-performance liquid chromatography with ultraviolet detection (HPLC/UV). Radioactivity in the drinking water was quantified by liquid scintillation spectrometry (LSS). ***Group 1*** The measured concentration of glutaraldehyde in the Group 1 – 50 ppm solution was initially determined to be only 51.6% of target concentration (data not shown). The most probable explanation for this low value was the fact that the reference substance used for quantitation was a different lot than the radiolabelled test material used to prepare this dose. Since it has been reported that glutaraldehyde in aqueous solutions exists in multiple isomeric forms it is probable that the glutaraldehyde isomers in the unlabeled reference substance have a difference UV response factor than the aqueous solution of the radiolabelled glutaraldehyde. Therefore reported concentration of test material for the Group 1 – 50 ppm dose was calculated from the radioactivity levels of this solution and the specific activity of the radiotracer. Based on radioactivity the dose for this dose level was 0.050 mg/g (50 ppm).	

Section A6.2(3) **Pharmacokinetics and Metabolism**

Annex Point IIA, VI.6.2

IUCLID 5.0/01

		Group 2 The 50 ppm dose consisted of a mixture of radiolabelled and unlabeled glutaraldehyde. The ratio of the two lots of glutaraldehyde making up the drinking water was approximately 60:40 (radiolabelled:unlabelled) (data not shown). Due to the problem encountered with the Group 1 – 50 ppm drinking water analysis above, this dose solution was not analyzed. The reported concentration of glutaraldehyde in the Group 2 – 50 ppm drinking water was based on nominal concentrations (data not shown). ***Group 3*** The 50 ppm dose consisted of ~10% glutaraldehyde deriving from labeled material. Since the majority of the glutaraldehyde in the dose was the same lot as standards used for quantitation the dose solution was analyzed.
3.3.2	Dose Concentration, Homogeneity and Stability	Confirmation of the test material concentration in the drinking water for Group 01 – 1000 ppm, Group 02 – 1000 ppm, and Group 03 – 50 ppm, and Group 03 – 1000 ppm was conducted by high-performance liquid chromatography with ultraviolet detection (HPLC/UV). Radioactivity in the drinking water of all six dose solutions was quantified by liquid scintillation spectrometry (LSS). LSS analysis of aliquots of the ^{14}C-labeled dosing solution taken from various locations in the solution container were used to confirm the concentration of radioactivity and the homogeneity of the ^{14}C-glutaraldehyde in the dosing solution. For stability see section 3.1.
3.3.3	Exposure period	The rats had free access to ^{14}C-glutaraldehyde-fortified drinking water for 24 hours or until their allotment of fortified water was consumed (whichever came first).
3.4	**Sampling time**	***Plasma and RBC***- Group 1 (50 and 1000 ppm), plasma and red blood cell (RBC) ^{14}C concentrations at 1, 2, 4, 8, 12, 18, 24, 25, 28, 32, 36, and 48 hr-hour post-dosing). All groups at study termination (48 hrs). Selected blood samples underwent chemical analysis. ***Urine/Cage Rinse***- All groups (both doses) 12, 24, 36 (Group 1 50 and 1000 ppm, only) and 48 hrs. ***Feces***- All groups at 24 and 48 hrs ***Expired Volatiles***- 24h (Group1, both doses) ***Expired CO_2***- All groups at 12, 24, 36 and 48 hrs ***Tissues (48 hrs)***- All listed tissues for Groups 1 and 3 (see section 3.8). Group 2 only plasma, RBC, skin and remaining carcass. ***Final Cage Wash***- At termination
3.5	**Samples**	Blood (plasma/RBC), urine (urine + cage rinse), feces, expired volatiles, expired CO_2, tissues (bone marrow, GI tract (including ingesta), kidneys, liver, residual carcass, skin, and spleen.
3.6	**Study Design**	This study consisted of experiments to determine the absorption, distribution, metabolism, and elimination of glutaraldehyde

Section A6.2(3) **Pharmacokinetics and Metabolism**

Annex Point IIA, VI.6.2

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following glutaraldehyde-fortified drinking water administration at concentrations of 50 and 1000 ppm presented to animals for up to 24 hours.

Rats were fitted with indwelling jugular vein cannulae, and plasma- and RBC-^{14}C concentration-time course were evaluated to determine uptake and elimination of glutaraldehyde.

Approximately 0.1-0.2 ml blood were collected at 1, 2, 4, 8, 12, 18, 24, 25, 28, 32, 36, and 48-hour post-dosing and plasma prepared by centrifugation. The RBC were oxidized and the plasma and oxidized RBC analyzed for radioactivity by LSS.

The study continued for 24 hours after the glutaraldehyde-fortified water was removed from the animals (see Discussion for more details). Selected blood samples underwent chemical analysis.

Group 1-50 (Group 01- 50 ppm)

- 50 ppm glutaraldehyde in drinking water
- Four female [redacted] rats.
- Plasma and RBC ^{14}C-concentration-time course was conducted with four rats
- Excreta/tissues were collected and analyzed for radioactivity as described below. Selected urine and feces samples underwent chemical analysis.

Group 1-1000 (Group 01 - 1000 ppm)

- 1000 ppm glutaraldehyde in drinking water
- Four female [redacted] rats.
- Plasma and RBC ^{14}C-concentration-time course was conducted with four rats
- Excreta/tissues were collected and analyzed for radioactivity. Selected urine, and feces samples underwent chemical analysis.

Group 2-50 (Group 02 – 50 ppm)

- 50 ppm glutaraldehyde in drinking water
- Four female [redacted] rats.
- Excreta and select tissues were collected and analyzed for radioactivity.

Group 2-1000 (Group 02 – 1000 ppm)

- 1000 ppm glutaraldehyde in drinking water
- Four female [redacted] rats.
- Excreta and select tissues were collected and analyzed for radioactivity.

Group 3-50 (Group 03 – 50 ppm)

- 50 ppm glutaraldehyde in drinking water
- Four female [redacted] rats.
- Excreta/tissues were collected and analyzed for radioactivity.

Group 3–1000 (Group 03 – 1000 ppm)

- 1000 ppm glutaraldehyde in drinking water
- Four female [redacted] rats.
- Excreta/tissues were collected and analyzed for radioactivity.

3.7 Specimens Collected During In-Life

Animals were placed in Roth cages for the separation and collection of urine and feces. Time course blood samples were collected from the jugular vein cannula.

Section A6.2(3) Pharmacokinetics and Metabolism

Annex Point IIA, VI.6.2

IUCLID 5.0/01

Plasma and RBC
Time-course blood samples, as specified above, were collected from the jugular vein cannulae and placed into capillary tubes to

Urine
All urine voided during the study was collected in dry-ice cooled traps. The urine traps were changed at 12, 24, and 48 hours after the presentation of the ^{14}C-glutaraldehyde-fortified drinking water. The rats in Group 01 (both doses) had an additional urine collection time point at 36 hours. The cages were rinsed with water at the time the traps were changed and the rinse collected. Each urine specimen and urine/cage rinse was weighed, and a weighed aliquot of each sample was analyzed for radioactivity by LSS as described below. Equal volume aliquots of urine samples (per time and dose) from the 0-12-hour and 12-24-hour collection intervals were pooled and stored at –80ºC. These pooled samples were stored at -80 ºC for possible chemical analysis.

Feces
Feces were collected in dry-ice chilled containers at 24 and 48 hours after the presentation of the ^{14}C-glutaraldehyde-fortified drinking water. An aqueous homogenate (~ 25% w/w) was prepared and weighed aliquots of these homogenates were oxidized and quantitated for radioactivity by LSS. In addition, equal volume aliquots of fecal homogenates from each animal were taken from the 0-24-hour collection interval and pooled (per dose). These pooled samples were stored at –80 ºC for possible chemical analysis.

Expired Volatiles
Air was drawn through the cage at approximately 500 ml/minute. Upon exiting the cage, the air was passed through charcoal to trap expired volatiles. The charcoal traps were changed at 24-hour intervals. Radioactivity trapped on the charcoal was desorbed with weighed amounts of toluene. Weighed aliquots of the solvent were analyzed for radioactivity. Because <1% of the administered dose was detected in the charcoal trap during the first interval, the replacement traps were not analyzed for radioactivity. Expired volatiles were only collected for the rats in Group 01 (both doses) but not for the other animals on study..

Expired CO_2
Following the charcoal trap (described above) the expired air was passed through a solution of monoethanolamine:1-methoxy-2-propanol (3:7 v/v) to trap expired CO_2. The CO_2 traps were changed at 12-hour intervals through the 48-hour study period. Weighed aliquots of the trap solution were analyzed for radioactivity.

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3.8 Specimen Collected After Terminal Sacrifice

Tissues

The following tissues were collected at sacrifice for Group 01-50 ppm, Group 01-1000 ppm, Group 03-50 ppm, and Group 03-1000 ppm:

RBC	spleen
plasma	skin
kidneys	residual carcass
liver	gastrointestinal (GI) tract
bone marrow	[including ingesta]

Plasma, RBC, skin, and remaining carcass were the only tissue samples collected from the Group 02 - 50 ppm and Group 02 - 1000 ppm animals at sacrifice.

Final Cage Wash

Following the terminal sacrifice of the animals, a final cage wash was performed. The final cage wash was collected and the weight of the sample was determined. A weighed aliquot of the final cage wash analyzed for radioactivity.

3.9 Collection of Control Samples

Control urine and feces were collected in dry-ice cooled traps from female ██████ rats not presented with ^{14}C-glutaraldehyde-fortified water. The control animals were sacrificed and blood collected by the same procedure as the dosed animals.

3.10 ^{14}C Analysis

Radioactivity was quantified in a liquid scintillation spectrometer (LSS). Counts per minute (cpm) were corrected for quench and converted to disintegrations per minute (dpm). Samples with dpm less than twice the concurrently run background were considered to contain insufficient radioactivity to reliably quantify.

3.11 Profiles

Pooled urine and fecal samples from selected intervals were analyzed and the results will appear in a separate report. X

3.12 Drinking water administration

The rats had free access to ^{14}C-glutaraldehyde-fortified drinking water for 24 hours or until their allotment of fortified water was consumed (whichever came first) beginning at the start of the 12-hour dark cycle. At the end of the first light cycle (at 24 hours), or when the fortified water allotment was consumed, the drinking water was replaced with fresh municipal water without glutaraldehyde fortification.

3.13 Data analyses

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

4 RESULTS AND DISCUSSION

4.1 Administered Dose

Table A6.2/01-1

The dose administered to the animals was determined by the amount of weight loss of fortified-drinking water from the sipper tubes over-time up to 24 hours that it was presented. Inadvertent and random drips of fortified drinking water though out the exposure phase of the study may have contributed to the dose available to the animals and the dose administered to be slightly different. The mean dose administered for the two dose levels were 6.8 ± 1.7, and 87 ± 26 mg/kg bw.

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The 12 animals in the 50 ppm dose level (Group 01, Group 02, and Group 03) had an average of 1.3 ± 0.3 mg glutaraldehyde administered per animal over 24 hours. The majority of the 50 ppm of glutaraldehyde fortified drinking water (85%) was administered to the animals during the dark cycle (0-12 hours).

The 12 animals in the 1000 ppm group (Group 01, Group 02, and Group 03) had an average of 17 ± 5 mg glutaraldehyde administered per animal over 24 hours, with 74% of the glutaraldehyde during the first 12 hours (dark cycle). One of the rats presented with the 1000 ppm glutaraldehyde drinking water (Group 01, [redacted]) ingested only 9.36 mg (1.4 to 2.5 times less than the other animals) in 24 hours. The mean drinking water consumption (volume) was reduced ~60% for the rats of the 1000 ppm group (data not shown), presumably due to a dislike for the taste, smell, and/or irritancy of the glutaraldehyde in the drinking water.

4.2 Disposition

Table A6.2/01-2

Data was consistent amongst the animals in the respective dose groups and were based on the weight loss of fortified water from the sipper tube to determine dose administered. The mean for total radioactivity recovered was 63 ± 5 and 72 ± 10% of the administered dose for the 50 and 1000 ppm drinking water dose groups, respectively. The low recoveries are consistent with the radioactivity recoveries of 61-75% in a rat dermal study. The low recoveries in this study are also consistent with the loss of test material through volatilization via inadvertent and random water drips (see Dose Administered). Approximately 1 ml of drinking water fortified with 50 ppm ^{14}C-gluaraldehyde (0.32 μCi) was transferred to a Petri dish and placed in a metabolism cage that was set up the same as on-study except without the animal. After 18 hours and after the water in the dish had evaporated, 22, 18, and 24% of the radioactivity was recovered in the Petri dish, CO_2 trap, and the FCW, respectively. Thirty-six percent of the radioactivity associated with the glutaraldehyde was uncounted for and would be consistent with loss through volatilization and subsequently not recovered (data not shown).

4.3 Elimination of Radioactivity in Expired Volatiles and CO^2

Table A6.2/01-2

Volatile organic samples were only collected for the animals in Group 01-50 ppm and Group 01-1000 ppm animals. Less than 1% of the administered dose was detected. These data are consistent with [redacted] (2004).

Radioactivity associated with the $^{14}C\text{-}CO_2$ trap (Groups 02 and Group 03) accounted for a mean of 17 ± 4, and 10 ± 2% of the administered dose for the 50 and 1000 ppm animals, respectively. These data are lower than the 29 and 21%, respectively, obtained via oral gavage by [redacted] (2004). The Petri-dish experiment (see Disposition) demonstrated a portion of the volatilized glutaraldehyde was recovered in the CO_2 trap even though the trap was not designed to capture it. Therefore any

X

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inadvertent and random drips (and hence not consumed by the animals) may have resulted in some of the glutaraldehyde recovered as radioactivity in the CO_2 trap, resulting in a slight over estimation of the amount of ^{14}C-CO_2 produced.

The Group 01 animals had a mean concentration of 7 and 3% of the administered dose recovered as ^{14}C-CO_2 for the 50 and 1000 ppm dose groups, respectively. These values are substantially less then the 17 and 10% than seen in Group02 and Group 03, respectively. Based on this these data, the values from Group 01 animals were not included in the CO_2 mean and standard deviation presented. The relatively low recoveries for Group 01 would be consistent with ^{14}C-CO_2 not being captured due to multiple cage openings for the plasma/RBC time-course sample collection.

A probe experiment verified the capacity of the CO_2 trap was not being exceeded when a 3N-NaOH caustic trap contained less than 0.04% of the administered dose when it was installed after the monoethanolamine:1-methoxy-2-propanol (3:7 v/v) CO_2 trap (data not shown). The caustic trap was changed at the same interval as the CO_2 trap; 12, 24, 36, and 48 hours after the start of the study.

Additional efforts were made to determine if the metabolism cage design contributed to ^{14}C-CO_2 recoveries. The Group 03 animals were housed in metabolism cages with ground-glass joint connector (vs. screw-type connectors used for Group 01 and Group 02). The amount of ^{14}C-CO_2 captured for the Group 03 animals was equivalent to the amount of the Group 02 animals. These data indicate the metabolism cage design is not a factor in ^{14}C-CO_2 recoveries. These data, along with the data the CO_2 trap capacity was not being exceeded, indicate the ^{14}C-CO_2 recoveries differences seen via drinking water and oral gavage administration were route dependent.

4.4 Elimination of Radioactivity in Excreta and Distribution to Tissues

Tables A6.2/01-2 and 3

The majority of the dose was recovered in the feces from both dose groups with 23 ± 4 and 40 ± 10% of the administered dose for the 50 and 1000 ppm groups, respectively. These data are lower than the 50 and 62 % of the administered dose, recovered in feces, in a female rat oral gavage study by [redacted] [redacted], 2004, administered 5 and 75 mg/kg bw, respectively. These data are consistent with less efficient absorption from the GI tract via bolus-dose administration.

X

Efforts were made to determine if the overall low radioactivity recoveries were contributed to by the loss of radioactivity from the feces. To verify the completeness of fecal homogeneity, the entire aqueous homogenate from one animal was oxidized and the percentage of administered dose calculated. That value was compared to, and was determined to be equivalent to the original single aliquot value (data not shown). In another experiment, control fecal pellets were spiked with a known amount of ^{14}C-glutaraldehyde. The spiked feces were stored at laboratory temperature for ~90 minutes prior to being stored at 4°C

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overnight. The next day an aqueous homogenate (~ 25% w/w) was prepared and the samples oxidation under the same conditions as study samples. Approximately 97% of the applied radioactivity was recovered. Based on these data there is no evidence there was a loss of radioactivity from the feces.

Urine/rinse accounted for 13 ± 3 and 11 ± 3 % of the administered dose for the 50 and 1000 ppm groups, respectively, with the majority eliminated in the first 24 hours when the glutaraldehyde was consumed. These data are very consistent with an oral gavage study ([redacted], 2004), where 13 and 10% of the oral gavage administered dose was eliminated in female urine for the 5 and 75 mg/kg bw dose groups.

Tissues/carcass accounted for 7-9% of the administered dose for both dose levels. The amount of radioactivity in the GI tract varied substantially. Animals 06A6377 and 06A6378 (Group 03-1000 ppm) ranged from 113 to 8 µg-eq./g. These results are consistent with the test material binding to protein with variations of food intake and the drinking water dose in these non-fasted animals. The carcasses for the Group 02 animals had a higher percentage of administered dose compared to the animals of the same respective dose in Group 01 and Group 03. The higher carcass values would be consistent with tissues included with the remaining carcass that were otherwise individually collected and analyzed for the other groups.

At the 50 ppm drinking water concentration, the highest concentration of radioactivity as µg-eq./g tissue was found in the liver > kidneys > GI tract plus contents> bone marrow > spleen > carcass > skin > plasma > RBC. These respective values are 5-8 times less than was observed at 52 hour post-dosing from a 5 mg/kg single oral gavage study ([redacted], 2004.

A somewhat similar pattern of distribution as seen in the 50 ppm group was observed following the 1000 ppm drinking water administration. The highest concentration, as µg-eq./g tissue of radioactivity, was found in the kidneys > liver > bone marrow > spleen > carcass > plasma > skin > RBC. Whereas the tissues from the 50 ppm dose groups were all less than the tissues from the 5 mg/kg in the oral gavage study, tissues from the 1000 ppm dose group were variable when compared to the 75 mg/kg oral gavage study. The bone marrow, GI tract, kidney, RBC, and spleen values were 1.1-1.8 times less, while the values for the carcass, liver, plasma, and skin were 1.4-1.9 times more than an oral gavage dose group at 36 hours post-dosing ([redacted], 2004.

The final cage wash (FCW) accounted for 4 ± 4, and 7 ± 7% of the administered dose for the 50 and 1000 ppm animals, respectively. These data are lower than the <0.5% than obtained via oral gavage by [redacted] (2004). These higher data would be consistent with inadvertent and random drips of fortified-drinking water from the sipper tube recovered in the

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FCW (see Disposition).

4.5 Time Course Concentration in Plasma and RBC

Table A6.2/01-4
Figure A6.2/01-1

Drinking water-administered glutaraldehyde was rapidly absorbed from the GI tract, with detectable levels in the plasma one hour after the fortified water was presented to the animals for both the 50 and 1000 ppm concentrations. The plasma levels of radioactivity continually increased through the dark cycle (0-12 hours). At the 50 ppm concentration, the highest plasma radioactivity levels were obtained between 8-12 hours after the fortified water was presented to the animals. The elevated levels were maintained near maximum through 24 hours for the two animals with patent canulas, at which time the fortified water was replaced with municipal water. Following the removal of the glutaraldehyde-fortified water, plasma radioactivity slowly dropped through the next 24 hour period until the time of sacrifice.
Similarly, at the 1000 ppm concentration, the highest plasma radioactivity levels (9.0 µg-eq/g plasma) were obtained 24 hours after the fortified water was given to the animals, at which time the fortified water was replaced with municipal water. Following the removal of the glutaraldehyde-fortified water, plasma radioactivity slowly dropped through the next 24 hour period until the time of sacrifice.
Due to variations in the rate and duration of test material intake between animals in each dose level, no pharmacokinetic parameters were conducted on these time-course data.

In general, the RBC radioactivity followed that of plasma but at ~2-3-fold lower concentrations. Similar to plasma, detectable levels were obtained one hour after the fortified water was presented to the animals. The RBC levels of radioactivity continually increased through the dark cycle and then plateaued or slightly increased through the 48 hour experimental period. These data are consistent with glutaraldehyde binding to blood proteins as seen by [redacted] (1990).

4.6 Glutaraldehyde Analysis in Blood

Figure A6.2/01-1

Whole blood was collected during the plasma/RBC time-course and analyzed for parent glutaraldehyde from the Group 01 animals (50 and 1000 ppm). The 1000 ppm animals had reached their mean peak concentration of 0.18 µg glutaraldehyde/g at one hour after being presented with fortified water. This concentration was essentially maintained through four hours at which time it started to decline. By 24 hours after the fortified water had been removed (48 hours after the start of the study) glutaraldehyde was not detected in blood (0.02 µg/g). No glutaraldehyde was detected in any of the time-course blood samples collected from the 50 ppm animals and all radioactivity associated in the blood would be from metabolites. Parent glutaraldehyde concentrations were rapidly eliminated from the blood and were 15- to 250-fold lower than total radioactivity (parent glutaraldehyde plus metabolites). This is consistent with

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	the low-dose gavage and dermal studies by [redacted] (2004) and [redacted] (1989). Parent glutaraldehyde (as the pentafluorobenzyl hydroxylamine derivative) ions in the blood were monitored via gas chromatography with mass selected detection in the negative chemical ionization mode (GC/NCI/MSD) (Appendix A). This analytical technique reduced matrix interferences and achieved a lower quantitation level than HPLC with ultra violet detection (UV).	
4.7 Metabolic Profiles	Metabolic profiles for selected urines and fecal samples from Grp01 animals will be presented in a separate report.	X
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Results and Conclusion	Two groups of 12 female rats were presented with either 50 or 1000 ppm ^{14}C-glutaraldehyde-fortified drinking water for up to 24 hours. After 24 hours, or if the allotment of drinking water was consumed first, the drinking water was changed to municipal water until sacrifice at 48 hours after the start of the study. Glutaraldehyde was rapidly absorbed and eliminated following drinking water administration, attaining measurable levels of radioactivity in blood one hour after the fortified water was presented. Glutaraldehyde–derived radioactivity was quickly eliminated in the feces, urine, and CO_2, while the elimination from the blood was at a slower rate. Parent glutaraldehyde, was present in blood at all time-points in the 1000 ppm dose group, but at concentrations 15- to 250-fold below ^{14}C-levels. No circulating glutaraldehyde was found in the blood of the 50 ppm dose group. Parent glutaraldehyde was rapidly eliminated from the blood, consistent with low-dose oral gavage and dermal studies. Feces, urine, and CO_2 elimination accounted for 23, 13, and 14, and 40, 11, and 8% of the administered dose, for the 50 and 1000 ppm groups, respectively. With the exception of less CO_2 generated via drinking water administration, the urine and fecal data are consistent with oral gavage results, even though the time frames for ^{14}C-glutaraldehyde administration (drinking water vs. bolus dose) and length of excreta collection were different. Drinking water-derived radioactivity was distributed in a fashion similar to that seen following a single dose oral gavage. Total radioactivity recoveries were 63 and 72% of administered dose for the 50 and 1000 ppm dose groups. These low recoveries are consistent with glutaraldehyde volatilized from drinking-water sipper tubes drips, attributed to but not consumed by the rats, and/or not recovered by the system.	X
5.1.1 Reliability	1	
5.1.2 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	

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Materials and Methods

1.2.2 Companies with letter of access. [redacted]

2.1 Guideline study. The JMAFF metabolism study is not mentioned in the original study report.

3.2.7 Control animals. Control urine and faeces were collected from female rats not presented with ^{14}C-glutaraldehyde fortified water.

3.11 Profiles. This refers to Dow study A6.02/05.

Results and discussion

4.2 Disposition. The applicant mentions that the low radioactivity recoveries of 63 ± 5 % (50 ppm group) and 72 ±10 % (1000 ppm group) are consistent with the radioactivity recoveries of 61-75 % in a rat dermal study. It is not appropriate to compare studies that are so different. The low recoveries in this study due to the possible loss of test material through volatilisation via inadvertent and random water drips reduce the accuracy and reliability of the study.

4.3 Elimination of Radioactivity in Expired Volatiles and CO_2. There is a discrepancy between 4.2 and 4.3. In 4.3 it is said that the Petri-dish experiment demonstrated a portion of the volatilised glutaraldehyde was recovered in the CO_2 trap even though the trap was not designed to capture it. Therefore any inadvertent and random drips may have resulted in some of the glutaraldehyde recovered as radioactivity in the CO_2 trap, resulting in a slight overestimation of the amount of ^{14}C-CO_2 produced. In the study, radioactivity associated with the ^{14}C-CO_2 trap accounted for a mean of 17 ± 4 and 10 ± 2 % of the administered dose for the 50 and 1000 ppm animals, respectively. This is less than obtained via oral gavage (29 and 21 %, respectively) (Dow A6.02/01). The study report concludes that ^{14}C-CO_2 recovery differences seen via drinking water and oral gavage administration were route dependent. The RMS concludes that it has not been demonstrated whether the difference is route specific or due to the problems in capturing ^{14}C-CO_2. See also the comment to 5.1 below.

4.4 Elimination of Radioactivity in Excreta and Distribution to Tissues. Please see the corrected percentages in the comment to 5.1 below.

4.7 Metabolic Profiles. This refers to Dow study A6.02/05.

5.1 Results and discussion. The percentages are given erroneously and are corrected as follows: "*Faeces, urine, and CO_2 elimination accounted for 23, 13, and ~~14~~ **17**, and ~~40~~ **39**, 11, and ~~8~~ **10** % of the administered dose, for the 50 and 1000 ppm groups, respectively*." These data are taken from Table A6.2(3)-2 below.

Conclusion

Radioactivity was detected in plasma and red blood cells one hour after administration of glutaraldehyde in drinking water. The radioactivity in plasma did not increase significantly after 8 h, remaining relatively stabile until glutaraldehyde was removed.

At 50 ppm, no glutaraldehyde was detected in blood extracts at any time point. At 1000 ppm, the peak concentration of 0.18 µg/g glutaraldehyde was reached in 1 h. The concentration remained rather constant for 8 h and started to decline after that. At 48 h (24 h after removing glutaraldehyde) no glutaraldehyde was detected in blood extract.

After sacrifice at 48 h, 9 and 7 % of the administered dose were detected in tissues (other than bone marrow, plasma and blood) at 50 and 1000 ppm, respectively.

63 and 72 % of the administered radioactivity were reportedly recovered, but

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	RMS re-calculation gives the recoveries as 66.10 and 74.24 % at 50 and 1000 ppm dose levels, respectively (tissues total + charcoal trap total + CO_2 total + final cage wash + faeces total + urine/rinse total). Radioactivity in adipose tissue was not measured in the study.
Reliability	2 The reliability of the study is reduced by the low recovery of the administered radioactivity, the loss of test material through volatilisation of spilled water, and by the uncertainty of the origin of the radioactivity recovered as CO_2.
Acceptability	Acceptable
Remarks	Please note that the numerical values in the Tables have not been checked in detail by the RMS. Furthermore, this study summary contains large pieces of text directly taken from the study report. The RMS has not checked in detail that no parts were changed as compared to the original study report.
	COMMENTS FROM
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.2(3)-1. Body Weights, Radioactivity, and ^{14}C-Glutaraldehyde Administered to Female [redacted] Rats

		Route Sex Animal													
		Drink_Gp01				Drink_Gp02				Drink_Gp03					
		Female				Female				Female					
Dose	**Data**	**05A7777**	**05A7778**	**05A7779**	**05A7780**	**06A3500**	**06A3501**	**06A3502**	**06A3503**	**06A6372**	**06A6373**	**06A6374**	**06A6375**	**Mean**	**SD**
50 ppm	Body Wt (kg)	0.183	0.178	0.193	0.183	0.207	0.200	0.214	0.201	0.195	0.203	0.193	0.203	0.196	0.011
	μCi Admin	87.9	94.2	107	89.8	38.1	45.8	48.6	39.8	5.78	6.03	6.67	6.09	48.0	38.3
	mg/kg	8.36	9.20	9.63	8.52	5.41	6.74	6.69	5.84	4.95	4.95	5.78	4.99	6.76	1.74
	μCi/kg	481	529	554	490	184	229	227	198	29.7	29.7	34.7	29.9	251	209
	mg 0-12 hr	1.202	1.638	1.489	1.561	0.676	1.350	1.430	0.891	0.793	0.668	0.876	0.855	1.12	0.363
	mg 12-24 hr	0.327	0.000	0.370	0.000	0.446	0.000	0.000	0.282	0.170	0.339	0.237	0.161	0.19	0.16
	mg 0-24 hr	1.529	1.638	1.859	1.561	1.122	1.350	1.430	1.172	0.964	1.006	1.113	1.015	1.31	0.290

		Route Sex Animal													
		Drink_Gp01				Drink_Gp02				Drink_Gp03					
		Female				Female				Female					
Dose	**Data**	**05A7781**	**05A7782**	**05A7783**	**05A7784**	**06A3504**	**06A3505**	**06A3506**	**06A3507**	**06A6376**	**06A6377**	**06A6378**	**06A6379**	**Mean**	**SD**
1000 ppm	Body Wt (kg)	0.179	0.205	0.206	0.192	0.199	0.194	0.207	0.201	0.211	0.197	0.201	0.212	0.200	0.009
	μCi Admin	62.9	55.7	24.8	66.4	28.5	21.3	21.0	30.2	8.16	5.89	5.48	7.87	28.2	22.1
	mg/kg	132	103	45.5	131	87.3	67.0	61.9	92	93.8	72.4	66.3	89.9	86.8	26.4
	μCi/kg	351	272	121	346	143	110	102	151	38.7	29.9	27.3	37.1	144	118
	mg 0-12 hr	16.3	17.2	6.51	17.8	13.0	9.26	7.20	15.2	17.6	6.38	12.1	15.5	12.8	4.45
	mg 12-24 hr	7.40	3.83	2.85	7.29	4.29	3.74	5.61	3.19	2.18	7.90	1.17	3.61	4.42	2.17
	mg 0-24 hr	23.7	21.0	9.36	25.1	17.3	13.0	12.8	18.4	19.8	14.3	13.3	19.1	17.3	4.80

Table A6.2(3)-2. Distribution of Radioactivity Recovered after Drinking Water Administration of 50 ppm or 1000 ppm ^{14}C-Glutaraldehyde to Female [redacted] Rats

Dose	50 ppm

Sum of % Admin			Route Sex Animal													
			Drink_Gp01				Drink_Gp02				Drink_Gp03					
			Female				Female				Female					
Sample Class	Time (hr)	Sample	05A7777	05A7778	05A7779	05A7780	06A3500	06A3501	06A3502	06A3503	06A6372	06A6373	06A6374	06A6375	**Mean**	**SD**
Tissues		Bone Marrow	0.00	0.00	0.00	0.00	NS*	NS	NS	NS	0.00	0.00	0.00	0.00	0.00	0.00
		Carcass	3.24	3.87	3.34	3.57	10.11	5.92	7.33	6.94	3.85	3.78	4.20	4.31	5.04	2.12
		GI Tract	1.40	3.29	1.57	1.39	NS	NS	NS	NS	4.58	2.45	1.53	2.00	2.28	1.14
		Kidneys	0.16	0.20	0.21	0.20	NS	NS	NS	NS	0.23	0.25	0.26	0.29	0.23	0.04
		Liver	1.20	0.85	0.75	1.15	NS	NS	NS	NS	0.90	1.11	1.48	1.02	1.05	0.23
		Plasma	0.00	0.00	0.00	NS	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00
		RBC	0.00	0.00	0.00	NS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Skin	1.30	1.60	1.35	1.69	1.43	2.40	2.85	1.16	1.88	1.98	1.86	1.72	1.77	0.48
		Spleen	0.05	0.05	0.04	0.05	NS	NS	NS	NS	0.04	0.04	0.03	0.04	0.04	0.01
Tissues Total			7.35	9.86	7.27	8.04	11.55	8.32	10.18	8.11	11.48	9.61	9.38	9.38	9.21	1.44
Charcoal Trap	24	Ch. Trap	0.01	0.00	0.01	0.00	NS	NS	NS	NS	NS	NS	NS	NS	0.01	0.00
	48	Ch. Trap	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		
Charcoal Trap Total			0.01	0.00	0.01	0.00	NS	NS	NS	NS	NS	NS	NS	NS	0.01	0.00
CO2	12	CO2	4.59	5.94	3.85	5.41	6.81	7.19	9.79	8.27	17.60	11.50	8.36	11.13	8.37	3.79
	24	CO2	3.27	1.47	1.43	1.33	5.31	2.76	3.86	4.85	3.21	3.46	3.33	2.77	3.09	1.26
	36	CO2	0.57	0.26	0.39	0.20	3.29	0.20	0.54	1.71	3.71	3.07	2.57	3.03	1.63	1.41
	48	CO2	0.12	0.47	0.43	0.13	0.78	0.36	0.43	0.71	0.95	0.56	0.58	0.44	0.50	0.24
CO2 Total			8.55	8.14	6.12	7.07	16.19	10.51	14.63	15.55	25.47	18.59	14.84	17.37	16.64**	4.29**
FCW		FCW	12.53	7.77	11.18	1.98	3.01	1.54	1.50	2.16	1.17	0.87	0.65	0.34	3.72	4.27
Feces	24	Feces	15.21	11.02	5.55	20.61	10.42	18.11	21.02	8.02	6.37	16.14	12.69	19.08	13.69	5.47
	48	Feces	4.96	7.58	17.39	4.33	11.39	6.12	7.30	19.52	10.91	6.95	14.82	3.75	9.58	5.25
Feces Total			20.18	18.60	22.94	24.94	21.81	24.23	28.32	27.54	17.28	23.09	27.51	22.83	23.27	3.50
Urine/Rinse	12	Rinse	0.13	0.45	0.26	0.24	NS	NS	NS	NS	2.30	2.33	2.08	1.83	1.20	1.01
		Urine	4.11	7.39	8.23	10.01	5.39	18.82	9.08	4.71	7.01	5.29	6.16	7.16	7.78	3.90
	12 Total		4.24	7.84	8.49	10.25	5.39	18.82	9.08	4.71	9.31	7.62	8.24	8.99	8.58	3.74
	24	Rinse	0.13	0.11	0.10	0.09	NS	NS	NS	NS	0.29	1.39	0.54	0.42	0.38	0.44
		Urine	3.09	2.06	2.34	2.22	2.77	1.57	2.46	2.37	4.84	4.02	3.68	2.18	2.80	0.94
	24 Total		3.22	2.16	2.44	2.30	2.77	1.57	2.46	2.37	5.13	5.40	4.22	2.60	3.05	1.22
	36	Rinse	0.04	0.03	0.08	0.03	NS	NS	NS	NS	NS	NS	NS	NS	0.05	0.02
		Urine	0.90	0.39	2.71	0.28	NS	NS	NS	NS	NS	NS	NS	NS	1.07	1.13
	36 Total		0.94	0.42	2.79	0.31	NS	NS	NS	NS	NS	NS	NS	NS	1.12	1.15
	48	Rinse	0.01	0.02	0.04	0.05	NS	NS	NS	NS	NS	0.49	0.26	0.21	0.16	0.18
		Urine	0.11	0.17	0.30	0.09	3.56	0.93	0.73	1.90	0.68	1.96	2.00	1.34	1.15	1.05
	48 Total		0.12	0.19	0.33	0.15	3.56	0.93	0.73	1.90	0.68	2.46	2.26	1.56	1.24	1.11
Urine/Rinse Total			8.52	10.62	14.05	13.01	11.72	21.32	12.27	8.97	15.12	15.48	14.73	13.14	13.25	3.40
Grand Total			57.12	55.00	61.56	55.06	64.28	65.92	66.89	62.32	70.52	67.64	67.11	63.07	63.04	5.09

*NS - No Sample

** - Gp01 CO_2 data not included in CO_2 Mean and Standard Deviation.

Table A6.2(3)-2 (Cont'd). Distribution of Radioactivity Recovered after Drinking Water Administration of 50 ppm or 1000 ppm ^{14}C-Glutaraldehyde to Female [redacted] Rats

Dose	1000 ppm

Sum of % Admin			Route Sex Animal													
			Drink_Gp01				Drink_Gp02				Drink_Gp03					
			Female				Female				Female					
Sample Class	Time (hr)	Sample	07A7781	07A7782	07A7783	07A7784	07A3504	07A3505	07A3506	07A3507	07A6376	07A6377	07A6378	07A6379	**Mean**	**SD**
Tissues		Bone Marrow	0.00	0.00	0.00	0.00	NS*	NS	NS	NS	NQ (0.00)	0.00	0.00	0.00	0.00	0.00
		Carcass	2.71	1.69	1.59	1.83	7.23	6.44	4.58	4.80	2.43	2.51	3.28	2.33	3.45	1.89
		GI Tract	0.95	0.86	1.59	1.15	NS	NS	NS	NS	3.75	11.20	1.26	1.39	2.77	3.53
		Kidneys	0.15	0.15	0.13	0.14	NS	NS	NS	NS	0.16	0.24	0.24	0.16	0.17	0.04
		Liver	1.05	0.56	0.55	0.60	NS	NS	NS	NS	0.64	0.69	0.65	0.80	0.69	0.17
		Plasma	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		RBC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Skin	0.89	0.91	0.67	0.92	1.80	0.97	1.69	0.81	1.21	1.25	1.22	1.22	1.13	0.34
		Spleen	0.03	0.02	0.02	0.03	NS	NS	NS	NS	0.03	0.03	0.02	0.02	0.02	0.00
Tissues Total			5.78	4.19	4.54	4.68	9.02	7.42	6.27	5.62	8.23	15.92	6.68	5.94	7.02	3.16
Charcoal Trap	24	Ch. Trap	0.00	0.00	0.00	0.01	NS	NS	NS	NS	NS	NS	NS	NS	0.00	0.00
	48	Ch. Trap	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		
Charcoal Trap Total			0.00	0.00	0.00	0.01	NS	NS	NS	NS	NS	NS	NS	NS	0.00	0.00
CO2	12	CO2	2.03	2.21	1.49	1.79	4.96	6.58	4.44	6.98	7.07	3.64	7.47	4.36	4.42	2.23
	24	CO2	1.41	0.84	0.58	1.16	2.77	3.68	3.44	1.99	1.11	2.78	1.70	1.51	1.91	1.02
	36	CO2	0.37	0.41	0.21	0.48	0.63	1.19	1.07	1.34	1.23	4.36	1.06	1.02	1.11	1.09
	48	CO2	0.09	0.12	0.09	0.10	0.30	0.48	0.34	0.51	0.51	0.85	0.29	0.51	0.35	0.23
CO2 Total			3.90	3.58	2.36	3.53	8.65	11.93	9.30	10.81	9.92	11.63	10.51	7.39	10.02**	1.53**
FCW		FCW	6.98	14.40	24.34	14.38	4.47	4.55	5.81	5.04	1.15	2.18	1.20	1.10	7.13	7.09
Feces	24	Feces	25.26	26.76	11.86	3.92	27.79	23.53	16.39	24.25	0.81	21.63	30.25	24.29	19.73	9.52
	48	Feces	23.85	14.92	11.41	37.65	15.31	14.43	30.55	17.14	13.75	18.52	16.38	23.26	19.77	7.76
Feces Total			49.12	41.68	23.27	41.58	43.10	37.97	46.94	41.40	14.56	40.15	46.63	47.55	39.49	10.34
Urine/Rinse	12	Rinse	0.24	0.44	0.31	0.34	NS	NS	NS	NS	2.22	1.01	2.53	1.88	1.12	0.95
		Urine	3.22	5.63	2.87	4.05	3.35	4.95	4.19	7.12	7.24	2.47	4.79	5.99	4.66	1.59
	12 Total		3.46	6.06	3.18	4.38	3.35	4.95	4.19	7.12	9.46	3.48	7.33	7.87	5.40	2.11
	24	Rinse	0.33	0.14	0.24	0.13	NS	NS	NS	NS	0.61	1.57	0.39	0.49	0.49	0.47
		Urine	3.06	2.22	1.32	2.11	1.99	2.94	4.48	1.92	1.80	6.51	1.50	4.58	2.87	1.56
	24 Total		3.38	2.36	1.56	2.24	1.99	2.94	4.48	1.92	2.41	8.09	1.90	5.06	3.19	1.88
	36	Rinse	0.08	0.11	0.13	0.03	NS	NS	NS	NS	NS	NS	NS	NS	0.09	0.04
		Urine	1.15	1.12	1.06	1.76	NS	NS	NS	NS	NS	NS	NS	NS	1.27	0.33
	36 Total		1.23	1.23	1.19	1.79	NS	NS	NS	NS	NS	NS	NS	NS	1.36	0.29
	48	Rinse	0.02	0.03	0.09	0.01	NS	NS	NS	NS	0.24	0.63	0.33	0.19	0.19	0.21
		Urine	0.15	0.14	0.16	0.19	1.87	2.58	1.76	2.25	1.63	3.32	1.38	1.39	1.40	1.06
	48 Total		0.17	0.17	0.25	0.20	1.87	2.58	1.76	2.25	1.87	3.96	1.71	1.58	1.53	1.17
Urine/Rinse Total			8.25	9.82	6.17	8.61	7.22	10.47	10.43	11.29	13.75	15.53	10.93	14.52	10.58	2.88
Grand Total			74.03	73.68	60.70	72.78	72.47	72.32	78.74	74.16	47.60	85.41	75.95	76.49	72.03	9.52

*NS - No Sample

** - Gp01 CO_2 data not included in CO_2 Mean and Standard Deviation

Table A6.2(3)-3. Distribution of Radioactivity in Tissues Following Drinking Water Administration of 50 or 1000 ppm ^{14}C-Glutaraldehyde to Female [redacted] Rats

Dose	50 ppm

µg eq/g tissue		Route Sex Animal													
		Drink_Gp01				Drink_Gp02				Drink_Gp03					
		Female				Female				Female					
Time	Sample	05A7777	05A7778	05A7779	05A7780	06A3500	06A3501	06A3502	06A3503	06A6372	06A6373	06A6374	06A6375	**Mean**	**SD**
	Bone Marrow	1.832	1.039	2.032	2.055	NS*	NS	NS	NS	0.356	1.085	1.521	1.497	1.427	0.580
	Carcass	0.459	0.598	0.542	0.503	0.686	0.521	0.647	0.537	0.301	0.290	0.385	0.338	0.484	0.132
	GI Tract	0.897	2.465	1.752	0.996	NS	NS	NS	NS	2.845	1.110	0.984	1.031	1.510	0.761
	Kidneys	1.764	2.102	2.853	2.425	NS	NS	NS	NS	1.704	1.771	2.050	1.958	2.078	0.391
	Liver	2.358	2.434	2.301	2.741	NS	NS	NS	NS	1.534	1.810	2.654	1.705	2.192	0.452
	Plasma	0.666	0.813	0.747	NS	0.681	0.596	0.716	0.770	0.439	0.589	0.812	0.436	0.660	0.133
	RBC	0.585	0.553	0.469	NS	0.317	0.180	0.488	0.466	0.166	0.213	0.265	0.170	0.352	0.163
	Skin	0.518	0.674	0.698	0.722	0.366	0.791	0.870	0.349	0.462	0.463	0.521	0.403	0.570	0.175
	Spleen	1.174	1.585	1.281	1.227	NS	NS	NS	NS	0.904	0.821	0.945	0.844	1.098	0.266

Dose	1000 ppm

µg eq/g tissue		Route Sex Animal													
		Drink_Gp01				Drink_Gp02				Drink_Gp03					
		Female				Female				Female					
Time	Sample	07A7781	07A7782	07A7783	07A7784	07A3504	07A3505	07A3506	07A3507	07A6376	07A6377	07A6378	07A6379	**Mean**	**SD**
	Bone Marrow	18.597	13.590	4.675	20.632	NS*	NS	NS	NS	NQ** (18.497)	23.581	13.700	7.021	15.037	6.595
	Carcass	5.870	2.951	1.238	4.128	8.671	6.273	4.150	6.435	3.582	2.909	3.384	3.330	4.410	2.031
	GI Tract	8.564	10.214	5.675	13.473	NS	NS	NS	NS	35.888	113.564	8.642	14.621	26.330	36.486
	Kidneys	23.547	21.401	8.313	26.453	NS	NS	NS	NS	20.981	25.061	22.556	22.357	21.334	5.571
	Liver	34.812	17.435	6.772	22.037	NS	NS	NS	NS	19.589	18.350	13.186	23.781	19.495	8.165
	Plasma	6.695	4.867	1.643	7.218	7.091	6.874	5.185	7.755	5.994	6.829	4.975	7.431	6.046	1.697
	RBC	5.177	2.782	1.266	5.146	3.131	4.336	2.669	4.360	2.741	1.889	1.724	2.659	3.157	1.311
	Skin	6.067	4.427	1.597	6.351	8.123	3.258	6.505	4.218	5.808	4.552	4.260	5.496	5.055	1.710
	Spleen	11.830	8.252	3.203	12.686	NS	NS	NS	NS	9.900	9.754	7.094	9.181	8.987	2.947

* - No Sample

** - Not Quantifiable at or above number in parentheses

Table A6.2(3)-4. Time Course of Radioactivity in Plasma and RBC Following Drinking Water Administration of 50 ppm or 1000 ppm ^{14}C-Glutaraldehyde to Female [redacted] Rats

Dose	50 ppm
Sample	Plasma
Route	Drink_Gp01

µg eq/g tissue	Sex	Animal				
	Female					
Time (hr)	05A7777	05A7778	05A7779	05A7780	**Mean**	**SD**
1	0.285	0.224	0.280	0.391	0.295	0.070
2	0.526	NS*	0.570	0.739	0.612	0.113
4	0.775	NS	1.010	1.227	1.004	0.226
8	1.325	NS	1.332	1.961	1.540	0.365
12	1.419	NS	1.596	1.836	1.617	0.210
18	1.345	NS	1.323	1.518	1.395	0.107
24	1.399	NS	1.486	NS	1.442	0.062
25	1.318	NS	1.478	NS	1.398	0.113
28	NS	NS	1.334	NS	1.334	
32	NS	NS	1.133	NS	1.133	
36	NS	NS	0.898	NS	0.898	
48	0.666	0.813	0.747	NS	0.742	0.074

Dose	50 ppm
Sample	RBC
Route	Drink_Gp01

µg eq/g tissue	Sex	Animal				
	Female					
Time (hr)	05A7777	05A7778	05A7779	05A7780	**Mean**	**SD**
1	0.117	0.104	0.129	NQ** (0.018)	0.092	0.051
2	0.157	NS	0.096	0.087	0.113	0.038
4	0.216	NS	0.283	0.327	0.275	0.055
8	0.386	NS	0.409	0.608	0.468	0.122
12	NS	NS	0.457	0.462	0.459	0.004
18	0.462	NS	0.403	0.460	0.442	0.034
24	0.465	NS	0.541	NS	0.503	0.054
25	NQ (0.012)	NS	0.483	NS	0.247	0.333
28	NS	NS	0.560	NS	0.560	
32	NS	NS	0.768	NS	0.768	
36	NS	NS	0.464	NS	0.464	
48	0.585	0.553	0.469	NS	0.536	0.060

* - No Sample
** - Not Quantifiable at or above number in parentheses

Table A6.2(3)-4(Cont'd). Time Course of Radioactivity in Plasma and RBC Following Drinking Water Administration of 50 ppm or 1000 ppm ^{14}C-Glutaraldehyde to Female [redacted] Rats

Dose	1000 ppm
Sample	Plasma
Route	Drink_Gp01

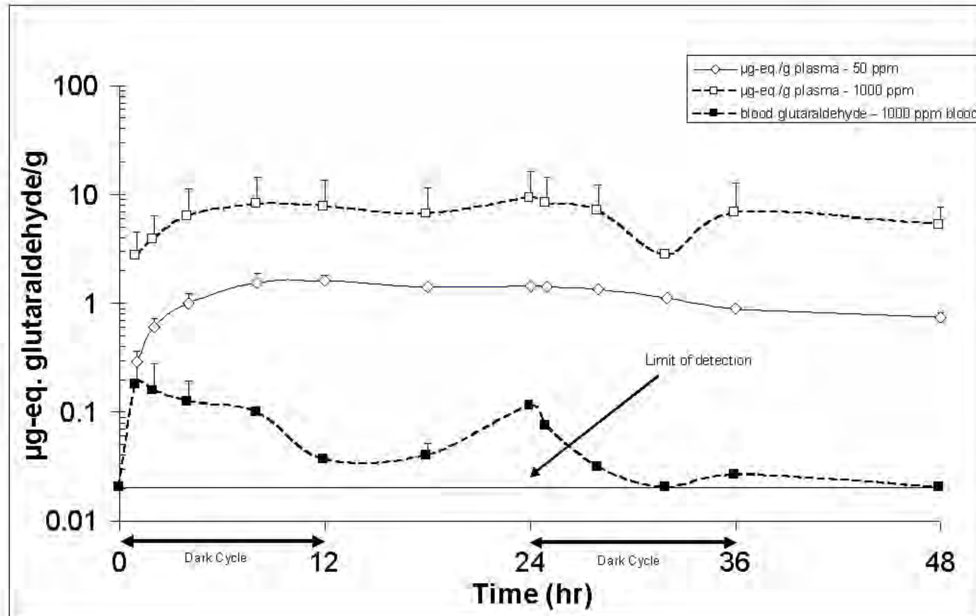
μg eq/g tissue	Sex	Animal				
	Female					
Time (hr)	05A7781	05A7782	05A7783	05A7784	**Mean**	**SD**
1	4.937	1.765	0.734	3.272	2.677	1.832
2	5.024	NS*	0.815	5.527	3.789	2.587
4	7.644	NS	0.806	10.338	6.263	4.914
8	NS	NS	3.321	12.444	7.883	6.452
12	NS	NS	3.309	11.792	7.551	5.998
18	NS	NS	3.013	10.057	6.535	4.981
24	NS	NS	4.000	14.095	9.048	7.138
25	NS	NS	3.809	12.617	8.213	6.229
28	NS	NS	3.204	10.770	6.987	5.350
32	NS	NS	2.750	NS	2.750	
36	NS	NS	2.186	11.061	6.623	6.276
48	6.695	4.867	1.643	7.218	5.106	2.519

Dose	1000 ppm
Sample	RBC
Route	Drink_Gp01

μg eq/g tissue	Sex	Animal				
	Female					
Time (hr)	05A7781	05A7782	05A7783	05A7784	**Mean**	**SD**
1	2.347	0.752	0.350	1.306	1.189	0.866
2	1.252	NS	0.305	2.009	1.189	0.854
4	2.075	NS	0.305	3.033	1.804	1.384
8	NS	NS	0.937	4.023	2.480	2.182
12	NS	NS	0.914	3.136	2.025	1.571
18	NS	NS	1.089	3.830	2.460	1.938
24	NS	NS	1.113	5.666	3.390	3.219
25	NS	NS	1.868	NS	1.868	
28	NS	NS	1.301	3.806	2.553	1.772
32	NS	NS	1.260	NS	1.260	
36	NS	NS	0.915	6.480	3.698	3.935
48	5.177	2.782	1.266	5.146	3.593	1.914

* - No Sample

Figure A6.2(3)-1. Plasma ^{14}C-Concentration-Time Course of Glutaraldehyde-Derived Radioactivity and Glutaraldehyde Concentration Time-Course in Whole Blood, Following Drinking Water Administration of 50 or 1000 ppm, and 1000 ppm, Respectively, to Female [redacted] Rats



Section A6.2(4)	Metabolism studies in mammals	
Annex Point IIA6.2 IUCLID 5.0/04	Species Comparisons of *In Vitro* Skin Penetration Following a Single Application to the Excised Skin of Humans, Rats, Mice, Guinea Pigs and Rabbits	

			Official use only
		1 REFERENCE	
1.1	**Reference**	(1991), Glutaraldehyde: Species Comparisons of *In Vitro* Skin Penetration Following a Single Application to the Excised Skin of Humans, Rats, Mice, Guinea Pigs and Rabbits, , 2 April 1991.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow	
1.2.2	Companies with letter of access		
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, however the study design was compared with OECD 428 (2004) and was found to be comparable, with the following exceptions: • An application to skin of up to 10µl/cm^2 for liquids is recommended, however in this study an application of 250µl to 1.77cm^2 of skin was used. • The temperature of the diffusion chamber (recommended level of 32±1°C) was not reported. • Skin integrity measurements were not reported.	X
2.2	**GLP**	Yes	
2.3	**Deviations**	See Section 2.1	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Radiolabelled and non-radiolabelled glutaraldehyde were used for this study.	
3.1.1	Radiolabelled test materials	The following radiolabelled test material was obtained from • Chemical name: Glutaraldehyde-[1,5-^{14}C] • Concentration of active ingredient in the solution (water): 10mg/mL • Radiochemical purity: • Specific activity 10.50 mCi/mM (0.105mCi/mg)	
3.1.2	Non-radiolabelled test material	The following non-radiolabelled test material was obtained from • Chemical name: Glutaraldehyde	

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- Purity: 51.3% glutaraldehyde

3.1.3	Reference Standards	The radiochemical standard ethanol-1-^{14}C (; ; specific activity 9.5 MCi/mmol) was obtained from .	
3.2	**Test Animals**	Rats – male and female rats , 10-12 weeks old were obtained from Mice – male and female -1 mice, 5-7 weeks old were obtained from Guinea pigs – male and female guinea pigs, 5-7 weeks old were obtained from Rabbits – specific pathogen free male and female rabbits, 10-12 weeks old were obtained from All animals were acclimatised to the laboratory environment for 3 days and examined by a clinical veterinarian prior to entry into the study. Human skin samples – human skin samples, female only, were obtained from women, aged 23-38 years old, undergoing reconstructive mammoplasty. These were supplied by	
3.2.1	Control animals	No	
3.3	**Administration/ Exposure**	Application to excised skin samples.	
3.3.1	Skin preparation	The rats, mice and guinea pigs were given an anaesthetic overdose of Metofane® and the rabbits were injected with T-61 euthanasia solution. The fur was clipped from the dorsal trunk of the animal in the thoracic region. Care was taken to avoid abrading the skin. A section of clipped skin (approximately 6 x 6 cm) was removed and placed in a petri dish containing MEM solution to keep it moist during preparation for the *in vitro* chamber. The human skin was received in MEM medium from the hospital and processed similarly to animal skin. The skin piece was placed on a dissecting board and scraped with a spatula to remove fat and connective tissue. Two 1-inch discs were removed from the animal skin and six 1-inch discs were removed from human skin specimens. Each disc was placed in a petri dish with several drops of MEM to keep it moist before placing the skin sample in the chamber. Discs of skin were prepared by a modification of the method described by Kao et al. (1983) and Holland et al. (1984) for full-thickness excised skin preparation.	X
3.3.2	Dose preparations	The ^{14}C-glutaraldehyde was diluted to the appropriate concentration (0.75% and 7.5% w/w) with deionized Milli-Q® filtered water (water CAS No. 7732-18-5) so that 250 µl would produce a target radioactivity level of at least 5-10 µCi/skin preparation in order to detect the ^{14}C-labelled test chemical which penetrated into media effluents. The higher dose (7.5%) was applied to all species except guinea pig skin. The 0.75% and 7.5% concentrations were normalized across species to the exposed surface area of the *in vitro* cell (1.77 cm^2) in order to compare it with the *in vivo* rat study	

Section A6.2(4) **Annex Point IIA6.2** **IUCLID 5.0/04**	**Metabolism studies in mammals** **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**

		([redacted], 1985). The 7.5% solution was applied over the same surface area (higher concentration). The ^{14}C-ethanol solution was prepared to attain a radioactivity level of 2-4 µCi per skin in 250 µl. This level was chosen because prior experiments have shown that this volume covers the exposed epidermal surface (1.77cm^2) and the level of radioactivity is easily detectable as penetration occurs.
3.3.3	Analysis of dose preparations for radiopurity, homogeneity, and stability	Dosing solutions were analyzed by quantification of radioactivity by liquid scintillation spectrometry.
3.3.4	Dose administration	The skin specimens were placed into the chamber and the dermal surface of the skin preparations were perfused with the minimal essential (organ culture) medium (MEM/d-valine) described by Kao et al. (1983) at a flow rate of 2.5 ml/hr for at least 30 minutes prior to application of test chemical. Glutaraldehyde was applied in a volume of 250 µl of the 0.75% and 7.5% w/w water solution to the exposed epidermal surface (1.77 cm^2) of each skin disc through the openings in the upper plate of the chamber. After dosing, the opening was closed to the environment with a glass plug. Media effluent was voided directly into empty scintillation vials and the effluent was dissolved in Aquasol-2® scintillation cocktail (New England Nuclear) prior to counting for ^{14}C activity in a liquid scintillation spectrometer.
3.4	**Examinations**	*Non entry field*
3.4.1	Sample collection for radioanalysis	Upon termination of the experiment, the section of skin was placed in a petri dish and swabbed using water-wetted, cotton-tipped applicators to remove any remaining test chemical. Each skin disc was placed into a vial and stored frozen until analyzed. Aliquots of the rinsing solution containing the applicators were radioassayed by liquid scintillation spectrometry for inclusion in the calculation of total balance. The skin discs were combusted directly in a Biological Materials Oxidizer to determine radioactivity content [redacted]. After a review of the animal data the skin rinse step was not carried out on the human skin. Also see Section 3.3.4.
		4 **RESULTS AND DISCUSSION**
4.1	**Radioanalysis**	Only minor amounts of the applied ^{14}C dose penetrated human and animal skin over 6 hours of contact following application of a 0.75% and 7.5% aqueous solution. The results for the recovery of the applied ^{14}C in the effluents are summarized in Table 6.2(4)-1 (0.75%) and Table 6.2(4)-2 (7.5%). The average percent of applied ^{14}C dose recovered in the effluent for all animal species was approximately 0.5% for the 0.75% solution and was approximately 0.8% for the 7.5% solution. The lowest percent in effluents was found for rat skin with 0.06% (0.75%) and 0.08% (7.5%) recovered. The next lowest effluent percentage was for the human skin with approximately 0.2% recovered for both solution concentrations. For the lower dose, recovery was generally greater for males than females (mouse, guinea pig, rabbit), but for the higher dose the

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recovery was greater for females (rat, mouse, rabbit).

The overall dose absorbed through the skin for all animal species was 0.006 mg/cm^2 for the 0.75% dose and 0.08 mg/cm^2 for the 7.5% dose. These data are summarized in Table 6.2(4)-3. For human skin, 0.002 mg/cm^2 of the low dose and 0.02 mg/cm^2 for the high dose was absorbed through the skin. Based on the mean dose absorbed values in Table 6.2(4)-3, these results represent approximately a 10-fold larger absorbed amount for a 10-fold increase in dose/concentration.

The amount of test substance which penetrated was presented as cumulative percent absorbed radioactivity and was determined from the sum of counts found in the effluent media divided by the mean dosing solution counts. The penetration rate was determined from interval radioactivity values, which were calculated as the cumulative mg/cm^2 absorbed, and an hourly rate was taken from the linear segment of the curve after plotting these values vs. time at the endpoint of the measurement interval.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Samples of excised skin from rats, mice, guinea pigs, rabbits and humans were evaluated for potential *in vitro* skin penetration of ^{14}C-glutataldehyde. A flow-through skin penetration chamber design was used and aqueous solutions of 0.75% and 7.5% were applied. The results indicated that glutaraldehyde did not penetrate the skin to any substantial degree following application of either a 0.75% or a 7.5% aqueous solution.

5.2 Results and discussion

Only minor amounts of the applied ^{14}C dose penetrated human and animal skin over 6 hours of contact following application of a 0.75% and 7.5% aqueous solution. The results for the recovery of the applied ^{14}C in the effluents are summarized in Table 6.2(4)-1 (0.75%) and Table 6.2(4)-2 (7.5%). The average percent of applied ^{14}C dose recovered in the effluent for all animal species was approximately 0.5% for the 0.75% solution and was approximately 0.8% for the 7.5% solution. The lowest percent in effluents was found for rat skin with 0.06% (0.75%) and 0.08% (7.5%) recovered. The next lowest effluent percentage was for the human skin with approximately 0.2% recovered for both solution concentrations. For the lower dose, recovery was generally greater for males than females (mouse, guinea pig, rabbit), but for the higher dose the recovery was greater for females (rat, mouse, rabbit).

The overall dose absorbed through the skin for all animal species was 0.006 mg/cm^2 for the 0.75% dose and 0.08 mg/cm^2 for the 7.5% dose. These data are summarized in Table 6.2(4)-3. For human skin, 0.002 mg/cm^2 of the low dose and 0.02 mg/cm^2 for the high dose was absorbed through the skin. Based on the mean dose absorbed values in Table 6.2(4)-3, these results represent approximately a 10-fold larger absorbed amount for a 10-fold increase in dose/concentration.

The amount of test substance which penetrated was presented as cumulative percent absorbed radioactivity and was determined from

Section A6.2(4) Annex Point IIA6.2 IUCLID 5.0/04	**Metabolism studies in mammals** **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**
	the sum of counts found in the effluent media divided by the mean dosing solution counts. The penetration rate was determined from interval radioactivity values, which were calculated as the cumulative mg/cm^2 absorbed, and an hourly rate was taken from the linear segment of the curve after plotting these values vs. time at the endpoint of the measurement interval.
5.3 Conclusion	Under these *in vitro* experimental conditions, glutaraldehyde did not penetrate the skin of the animal species to any substantial degree, with an average of 0.49% of the applied ^{14}C dose (0.75% solution) and 0.77% (7.5% solution) being recovered in the effluents. The effluent recovery of applied dose for both concentrations was 0.2% for human skin. A total of 0.006 mg/cm^2 for the low dose and 0.08 mg/cm^2 for the high dose was absorbed through the skin for all animal species tested and 0.002 mg/cm^2 (0.75% solution) and 0.02 mg/cm^2 (7.5% solution) was absorbed through the human skin tested.
5.3.1 Reliability	1
5.3.2 Deficiencies	Yes, the results of the ethanol control have not been reported.

	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 30th, 2010
Materials and Methods	GLP: The audits for the conduct of the study were not performed. The procedures have been generally audited for this *in vitro* technique but not specifically during this study. 2.1 Guideline study. In addition to the differences mentioned, at least the following deviations were noted: – The time of absorption was 6 h instead of 24 h. – The results using a reference material were not reported. 3.3.1 Skin preparation. The references are as follows: – Kao et al., Quantitation of cutaneous toxicity: An in vitro approach using skin organ culture. Toxicol. Appl. Pharmacol. 58:206, 1983 – Holland et al., A multi-sample apparatus for kinetic evaluation of skin penetration in vitro: The influence of viability and metabolic status of the skin. Toxicol. Appl. Pharmacol. 72:64, 1984
Results and discussion	General comment: the study considers as absorbed only the material that completely penetrated the full skin samples to the receptor fluid. The RMS disagrees with this view. The OECD 428 guideline states: "The test substance remaining in the skin should be considered as absorbed unless it can be demonstrated that absorption can be determined from receptor fluid values alone". Therefore the RMS considers the absorbed dose as the radioactivity in

Section A6.2(4) Annex Point IIA6.2 IUCLID 5.0/04	**Metabolism studies in mammals** **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**
	the receptor fluid plus the radioactivity in the skin sample. The radioactivity associated with the skin was as follows (averages of male and female values, except for human skin that was only from females): – Rat: 12/7 % for the solutions containing 0.75/7.5 % GA – Mouse: 7/4 % for the solutions containing 0.75/7.5 % GA – Rabbit: 24/10 % for the solutions containing 0.75/7.5 % GA – Guinea pig: 19 % for the solution containing 0.75 % GA – Human skin: 6.4/4.6 % for the solutions containing 0.75/7.5 % GA The amount found in the receptor fluid in the human skin experiment was 0.16 and 0.20 % for the solutions containing 0.75 and 7.5 % glutaraldehyde, respectively. The sum of absorbed material in the human skin is thus 6.6 % for the 0.75 % solution and 4.8 % for the 7.5 % solution.
Conclusion	The study is of insufficient quality. The corrected results as calculated above can be used as indicative information, but the following problems need to be taken into account: – Application volume of 250 µl onto 1.77 cm^2 is not comparable to the application of up to 10 µl/cm^2. – The time of absorption was 6 h instead of 24 h. – The results using a reference material were not reported. – The temperature of the diffusion chamber was not reported. – Skin integrity was not reported. – Total recovery of the labelled material was low (75-76 % in experiments with human skin)
Reliability	3
Acceptability	Acceptable as supportive information only
Remarks	The study summary contains large pieces of text that were directly copied from the study report. The RMS has not checked in detail that no changes were made in the text.

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

Table A6_2(4)-1 [14C]-Glutaraldehyde material balance for a 0.75% solution

Table 1

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

In Vitro Material Balance Recoveries for 0.75% ^{14}C-Glutaraldehyde
Percent of Applied Dose Recovered

Fraction Recovered	Rat[a]	Mouse[b]	Guinea Pig[b]	Rabbit[a]	Human[c]
A. Males					
Effluents	0.06 ± 0.05	1.73 ± 1.65	0.53 ± 0.69	0.77 ± 0.20	
Unabsorbed Dose	66.14 ± 7.26	74.10 ± 5.36	64.91 ± 6.29	61.55 ± 3.25	
Apparatus Rinse	1.16 ± 0.54	2.27 ± 0.94	1.38 ± 0.58	1.31 ± 0.17	
Skin Combustion	17.42 ± 6.98	7.65 ± 2.49	19.37 ± 6.72	28.02 ± 2.53	
Total Recovery	84.78 ± 1.70	85.76 ± 2.72	86.19 ± 1.14	91.66 ± 3.15	
B. Females					
Effluents	0.05 ± 0.01	0.26 ± 0.04	0.17 ± 0.16	0.34 ± 0.11	0.16 ± 0.14
Unabsorbed Dose	76.42 ± 0.56	79.18 ± 1.34	64.89 ± 6.19	53.51 ± 8.37	66.71 ± 3.44[d]
Apparatus Rinse	1.64 ± 0.47	1.54 ± 0.20	1.38 ± 0.13	0.53 ± 0.29	1.95 ± 1.56
Skin Combustion	6.82 ± 1.73	5.85 ± 0.94	19.52 ± 5.14	20.87 ± 4.29	6.36 ± 2.79
Total Recovery	84.92 ± 1.87	86.83 ± 0.51	85.97 ± 1.16	75.26 ± 8.22	75.17 ± 4.20

[a]Mean of 3 animals.
[b]Mean of 6 males and 3 females.
[c]Mean of 3 humans.
[d]Unabsorbed dose does not include skin rinse.

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Table A6_2(4)-2 [^{14}C]-Glutaraldehyde material balance for a 7.5% solution

Table 2

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

In Vitro Material Balance Recoveries for 7.5% ^{14}C-Glutaraldehyde
Percent of Applied Dose Recovered

Fraction Recovered	Rat[a]	Mouse[b]	Rabbit[c]	Human[d]
A. Males				
Effluents	0.08 ± 0.04	0.39 ± 0.14	0.85 ± 0.71	
Unabsorbed Dose	79.11 ± 0.66	75.56 ± 3.59	70.06 ± 11.01	
Apparatus Rinse	4.32 ± 2.37	3.28 ± 1.69	1.95 ± 0.63	
Skin Combustion	4.75 ± 1.03	2.15 ± 1.02	11.54 ± 11.36	
Total Recovery	88.25 ± 2.37	81.38 ± 0.90	84.40 ± 0.71	
B. Females				
Effluents	0.33 ± 0.10	1.43 ± 1.10	1.55 ± 2.09	0.20 ± 0.08
Unabsorbed Dose	70.90 ± 2.62	77.30 ± 4.31	70.75 ± 8.81	69.18 ± 3.51[e]
Apparatus Rinse	5.36 ± 0.52	2.48 ± 0.65	1.87 ± 0.83	2.07 ± 1.74
Skin Combustion	8.49 ± 2.13	4.98 ± 3.02	8.89 ± 6.03	4.56 ± 1.67
Total Recovery	85.08 ± 1.37	86.19 ± 2.81	83.07 ± 2.27	76.01 ± 3.64

[a]Mean of 3 animals.
[b]Mean of 3 males and 6 females.
[c]Mean of 3 males and 5 females
[d]Mean of 3 humans.
[e]Unabsorbed dose does not include skin rinse.

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