resistance development, the only instances where such reports exist for resistant bacteria stem from applications where Glutaraldehyde is used for cleaning medical equipment (PT 02). In these cases improper uses of the disinfectant on dirty endoscopes (Duarte et al. 2009), use of non-sterile water to rinse disinfected equipment (Griffiths et al 1997) have been implicated.

Griffiths, P. A., J. R. Babb, et al. (1997). "Glutaraldehyde-resistant Mycobacterium chelonae from endoscope washer disinfectors." Journal of Applied Microbiology 82(4): 519-526. x

Glutaraldehyde is used to disinfect flexible and other heat-sensitive endoscopes often with the aid of automated systems. Mycobacterium chelonae is being isolated with increasing frequency from these washer disinfectors and processed endoscopes. This has, on occasions, led to misdiagnosis and iatrogenic infections. Recent reports suggest that disinfecting machines, on a sessional or regular basis, with 2% glutaraldehyde may have selected and therefore encouraged the growth of strains of Myco. chelonae, possibly in biofilm, with decreasing susceptibility to glutaraldehyde. In view of this, the resistance of three strains of Myco. chelonae var. chelonae (the type strain NCTC 916 and two machine isolates) was tested against 2% glutaraldehyde and a wide range of alternative disinfectants. Disinfectants tested were a chlorine releasing agent, sodium dichloroisocyanurate at 1000 ppm and 10 000 ppm av Cl, 0.35% peracetic acid (NuCidex, Johnson & Johnson), 70% industrial methylated spirit (IMS), 1% peroxygen compound ('Virkon', Antec International) and 10% succine dialdehyde ('Gigasept', Sanofi Winthrop). Suspension and carrier tests were carried out in the presence and absence of an organic load. Results showed the type strain, which had not been exposed to the selective pressure of disinfectant usage, to be very sensitive to most disinfectants with the exception of 1% Virkon. The washer disinfector isolates, on the other hand, were extremely resistant to 2% glutaraldehyde and showed greater resistance to 1% Virkon and 1000 ppm NaDCC. Purchasing machines in which the entire fluid pathways, including those for delivering rinse water, are disinfected With an appropriate agent during each cycle are preferred. If this is not possible then sessional cleaning and disinfection at the start of each day and regular maintenance should prevent biofilm formation and contamination with disinfectant-resistant strains of mycobacteria. In addition to machine disinfection, the use of sterile or bacteria-free (filtered <0.45 mu m) water is essential for bronchoscopes and all invasive endoscopes. If there is doubt over the effectiveness of the machine disinfection procedure or eater quality, the channels and surfaces of endoscopes may be rinsed with 70% IMS after automated processing.

Duarte, R. S., M. C. S. Lourenco, et al. (2009). "Epidemic of Postsurgical Infections Caused by Mycobacterium massiliense." Journal of Clinical Microbiology 47(7): 2149-2155.

An epidemic of infections after video-assisted surgery (1,051 possible cases) caused by rapidly growing mycobacteria (RGM) and involving 63 hospitals in the state of Rio de Janeiro, Brazil, occurred between August 2006 and July 2007. One hundred ninety-seven cases were confirmed by positive acid-fast staining and/or culture techniques. Thirty-eight hospitals had cases confirmed by mycobacterial culture, with a total of 148 available isolates

recovered from 146 patients. Most (n = 144; 97.2%) isolates presented a PRA-hsp65 restriction pattern suggestive of Mycobacterium bolletii or Mycobacterium massiliense. Seventy-four of these isolates were further identified by hsp65 or rpoB partial sequencing, confirming the species identification as M. massiliense. Epidemic isolates showed susceptibility to amikacin (MIC at which 90% of the tested isolates are inhibited [MIC(90)], 8 mu g/ml) and clarithromycin (MIC(90), 0.25 mu g/ml) but resistance to ciprofloxacin (MIC(90), > 32 mu g/ml), cefoxitin (MIC(90), 128 mu g/ml), and doxycycline (MIC(90), >= 64 mu g/ml). Representative epidemic M. massiliense isolates that were randomly selected, including at least one isolate from each hospital where confirmed cases were detected, belonged to a single clone, as indicated by the analysis of pulsed-field gel electrophoresis (PFGE) patterns. They also had the same PFGE pattern as that previously observed in two outbreaks that occurred in other Brazilian cities; we designated this clone BRA100. All five BRA100 M. massiliense isolates tested presented consistent tolerance to 2% glutaraldehyde. This is the largest epidemic of postsurgical infections caused by RGM reported in the literature to date in Brazil.

Gregory, A. W., G. B. Schaalje, et al. (1999). "The mycobactericidal efficacy of ortho-phthalaldehyde and the comparative resistances of Mycobacterium bovis, Mycobacterium terrae, and Mycobacterium chelonae." Infection Control and Hospital Epidemiology 20(5): 324-330.

OBJECTIVES: To assess the mycobactericidal efficacy of an agent relatively new to disinfection, ortho-phthalaldehyde (OPA) and to compare the resistances of three Mycobacterium species. Mycobacterium bovis (strain BCG) was compared with Mycobacterium chelonae and Mycobacterium terrae to investigate the feasibility of using either of the latter two species in tuberculocidal testing. M chelonae (a rapid grower) and M terrae (an intermediate grower) both grow faster and are less virulent than M bovis (a slow grower). DESIGN: The quantitative suspension protocol specified by the Environmental Protection Agency (EPA), the Tuberculocidal Activity Test Method (EPA test), was used throughout this study. Standard suspensions of all three species were prepared in a similar manner. Two suspensions of M bovis, created in different laboratories, were used. These were tested against two concentrations of alkaline glutaraldehyde to provide reference data. Two concentrations of OPA were evaluated against all mycobacterial test suspensions. Four replicates of each organism-disinfectant combination were performed. RESULTS: Results were assessed by analysis of variance. M terrae was significantly more resistant to 0.05% OPA than either M bovis or M chelonae. At 0.21% OPA, M terrae was slightly more susceptible than one test suspension of M bovis, but not significantly different from the other. M chelonae was significantly less resistant than the other species at both OPA concentrations. At their respective minimum effective concentration OPA achieved a 6-log(10) reduction of M bovis in nearly one sixth the time required by glutaraldehyde (5.5 minutes vs 32 minutes). CONCLUSIONS: These data, along with other recent studies, lend support to the idea that M terrae may be a suitable test organism for use in the tuberculocidal efficacy testing of disinfectants. They also confirm the relatively rapid tuberculocidal activity of OPA.

5.7.2 Management Avoid continuous dosage to prevent the evolution of tolerant bacteria

Section A5	Effectiveness against target organisms and intended uses	
	strategies	strains.
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	Refer to confidential IIIA Section 5-Appendix 11

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 2012
Materials and methods	The information in this document is based on five key studies and several additional studies provided by the applicant as supportive information. Three studies by [redacted] (2004) were evaluated in connection with PTs 2, 3, 4 and 6. Weaknesses in methodology were identified in these studies but after the applicant supplemented information, they RMS could accept them as key studies. In these studies the effect of glutaraldehyde on specified target organisms was shown in suspension. In addition, two studies by [redacted] (2008) were accepted as key studies. These studies specifically described efficacy in PT 11 and 12 uses and they were performed according to ASTM standard protocols. 5.2.1 Organisms to be controlled Efficacy against [redacted] was studied by [redacted] (1987). The applicant provided a study summary on the study but because of methodological problems it was not accepted. At later stage the applicant provided two more studies ([redacted] 2009) on efficacy against [redacted] as supportive information. There are no study summaries available of these studies and they have not been evaluated as key studies. However, these studies provide probably evidence on efficacy against Legionella, but this should be verified at the product authorization stage. 5.3.1 Effects on target organisms The product showed bactericidal efficacy at [redacted] after a contact time of 24 h against [redacted] Against sporeforming [redacted] concentration needed for bacterical efficacy was [redacted]. Fungicidal activity against moulds was demonstrated after a contact time of 48 h at [redacted] the efficient concentrations were [redacted] if the contact time was 24 h. 5.3.2 Likely concentrations The proposed concentrations seem to be lower than effective cidal concentrations (see point 5.3.1 above) especially in case of moulds and yeasts. 5.7.1 Development of resistance Resistance to other organisms has been specified (see e.g. CDC guideline for Disinfection and Sterilization in Healthcare Facilities, 2008). Resistance is

Section A5	Effectiveness against target organisms and intended uses
	discussed in more detail in connection with PT2. The reference cited ([redacted] 2001) is not a scientific reference but rather a written expert opinion. Abstracts of three other references related to resistance are included in the text. The information of the abstracts should rather be summarised briefly than the whole abstract given in this document.
Conclusion	Glutaraldehyde shows efficacy against studied bacteria, molds and yeasts and can be used as preservative used in open recirculating systems and slimicide in paper pulp industry. Proposed concentrations are fungistatic instead of fungicidic against moulds and yeasts.
Remarks	At the product authorization stage efficacy against [redacted] should be verified in applications where it is claimed. Additional information on efficacy against biofilms is also needed.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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 5.3: Summary table of experimental data on the effectiveness of the active substance against target organisms at different fields of use envisaged, where applicable

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
Bactericide Fungicide against yeast	PT11, PT12	[redacted] Antimicrobial (50% Glutaraldehyde)	<u>Gram negative bacteria</u> [redacted] [redacted] [redacted] [redacted] [redacted] <u>Gram positive bacteria</u> [redacted] [redacted]	Rate of kill test (suspension test in saline solution); Tests have involved planktonic micro-organisms, no slime or biofilm has been tested. The tests have been carried out in a saline phosphate buffer because the usual liquid nutrient media contain proteins that would react with and deactivate glutaraldehyde. This is reason for a flat pattern in the control curves; the bacteria cannot grow and their population remains constant in the absence of the killing agent. Industrial microbes are exposed to adverse conditions and their cell wall is thicker. Therefore the requested biocide killing concentration might be slightly higher than for culture strains which are used in the laboratory tests.	<u>Bacteria</u> T = 37°C pH 7.5 Conc. mg/L (ppm) Glutaraldehyde: 0, 5, 10, 15, 20 Exposure time: 0, 1, 7, 24 hours Initial population: > 10^6 CFU/ml <u>Yeast</u> T = 30°C pH 7.0 Conc. mg/L (ppm) Glutaraldehyde: 0, 75, 100, 125, 150 Exposure time: 0, 1, 3, 5, 24 hours Initial population: > 10^6 CFU/ml	[redacted]	[redacted] (2004a) Key study

			Yeast				
Bactericide Fungicide against yeasts and moulds	PT11, PT12	(50% Glutar-aldehyde)	Bacteria:	The method [Minimum Cidal Concentration (MCC)] is a suspension test in saline Tubes containing cultured cells in 0.1 M buffered saline solution and the biocide to test are incubated. Plates containing a suitable nutrient medium are inoculated with the tube content, incubated and examined for growth (colony formation). The MCC is the smallest concentration of a biocide necessary to kill all the microorganisms within a given period of time, referred to as contact time.	Contact time: 24 h Incubation time: 24 h Incubation T°C: 37 °C Initial population: $5x10^6$ to 10^7 CFU/ml		(2004b) Key study
			Yeasts:		Contact time: 24 h Incubation time: 24-48 h Incubation T°C: 30°C Initial population: $5x10^6$ to 10^7 CFU/ml		
			Fungi:		Contact time: 48 h Incubation time: 8 days Incubation T°C: 30°C Initial population: $5x10^6$ to 10^7 CFU/ml		
Bactericide against sulphate-	PT11, PT12			Suspension test in sterile anaerobic vials containing API RP38	T = 37 °C, pH not reported Contact time 3 and 7		(2004c)

reducing bacteria		(50% Glutar-aldehyde)	[redacted]	media (10 ml) and an iron nail. Untreated control. Concentrations: 40 mg/L (ppm) Glutaraldehyde	hours.	[redacted]	Key study
Bactericide and fungicide	PT11	[redacted] (50% Glutar-aldehyde)	Native populations of bacteria and fungi obtained from cooling water samples.	A stock treatment solution was prepared in sterile water (autoclaved at 121°C for 15 minutes) and re-diluted with sterile water to achieve the desired biocide concentration. **Bacteria**: cooling water was measured for bacterial levels by serial dilution in phosphate buffer, spread plating (100µl) onto tryptic soya agar, TSA, incubation and reading after 1 day. **Fungi**: cooling water was measured for fungal levels by serial dilution in phosphate buffer, spread plating (100µl) onto sabouraud dextrose agar, SDA, incubation and reading after 2 days.	**Bacteria** Conc. mg/L (ppm) Glutaraldehyde: 0, 3.15, 6.25, 12.5, 25, 50, 100 Contact time: 3, 24, 48, 168 h Incubation time: 24 h Incubation T°C: 30 °C Initial population: $5x10^7$ CFU/ml **Fungi** Conc. mg/L (ppm) Glutaraldehyde: 0, 3.15, 6.25, 12.5, 25, 50, 100 Contact time: 3, 24 h Incubation time: 48 h Incubation T°C: 30 °C Initial population: $1.9x10^6$ CFU/ml	[redacted]	[redacted] (2008) Key study
Bactericide against [redacted]	PT11	[redacted] (45% Glutar-aldehyde)	[redacted]	A bacterial suspension of [redacted] was prepared and added to biocide-free cooling	Conc. mg/L (ppm) Glutaraldehyde: 0, 25, 50, 100 pH = 6.7, 8.0 Contact time: 1, 3, 6, 24 h	[redacted]	[redacted] (1987) Supportive information

[redacted]			[redacted]	tower water which had been sterilised by autoclaving. The test was performed at several concentrations and at 2 pH levels. The test samples were then incubated on BCYE plates and the bacterial survival was estimated by determining the CFU/mL.	Incubation time: 3 days Incubation T°C: not reported Initial population: $6x10^5$ CFU/ml at pH 6.7, $4.5x10^6$ CFU/ml at pH 8.0		only
Bactericide against [redacted]	PT11	[redacted] (50% Glutar-aldehyde)	[redacted]	At the 60 min sample points, samples were serially diluted with the hard water containg the interfering substance and then spread plated onto BCYE plates. The resulting plates were incubated and the number of colonies present was counted.	Conc. mg/L (ppm) Glutaraldehyde: 1.5, 3, 6 Contact time: 60 min Incubation time: 7 days Incubation T°C: 36°C Initial population: $1.5x10^7$ CFU/ml	[redacted]	[redacted] (2009) [redacted] Supportive information only
Bactericide against [redacted]	PT11	[redacted] (50% Glutar-aldehyde)	[redacted]	At the 24 hour sample points, samples were serially diluted with the hard water containg the interfering substance and then spread plated onto BCYE plates. The resulting plates were incubated and the number of colonies present was counted.	Conc. mg/L (ppm) Glutaraldehyde: 0.375, 0.75, 1.5 Contact time: 24 h Incubation time: 7 days Incubation T°C: 36°C Initial population: $1.3x10^7$ CFU/ml	[redacted]	[redacted] (2009) [redacted] Supportive information only
Slimicide (bacterial and fungal	PT12	[redacted]	Native populations of bacteria and fungi obtained	A stock treatment solution was prepared in sterile water	**Bacteria** Conc. mg/L (ppm) Glutaraldehyde: 0, 37.5,	[redacted]	[redacted]

slime)		(50% Glutar-aldehyde)	from pulp samples	(autoclaved at 121°C for 15 minutes) and re-diluted with sterile water to achieve the desired biocide concentration. **Bacteria**: the first pulp sample was measured for bacterial levels by serial dilution in phosphate buffer, spread plating (100µl) onto tryptic soya agar, TSA, incubating and reading after 1 day. **Fungi**: the second pulp sample was measured for fungal levels by serial dilution in phosphate buffer, spread plating (100µl) onto sabouraud dextrose agar, SDA, incubating and reading after 2 days.	56.5, 84.5, 126.5, 190, 285 Contact time: 3, 48, 168 h Incubation time: 24 h Incubation T°C: 30 °C Initial population: $2x10^7$ CFU/ml **Fungi** Conc. mg/L (ppm) Glutaraldehyde: 0, 3.15, 6.25, 12.5, 25, 50, 100 Contact time: 3, 24 h Incubation time: 48 h Incubation T°C: 28 °C Initial population: $2x10^6$ CFU/ml	[redacted]	(2008) Key study
Slimicide (bacterial slime)	PT12	[redacted] (50% Glutar-aldehyde)	Contaminated white water from the paper mill	**Step 1** Initial laboratory experiments were performed to determine the potential efficacy of glutaraldehyde in papermaking systems, in accordance with the ASTM Paper Slimicide Test ASTM 600 (7). The biocide was added to aqueous buffered solution contaminated with pulp and rosin followed by inoculation	**Field trials** Concentration: Glutaraldehyde was added at a rate of 24.5 kg/day into the white water system at the 94 635 litres storage tank Intervals of examination: at days 1 to 3, days 6 to 9, and days 53 to 55 after inoculation Incubation time: 48 h Incubation T°C: 37 °C Initial population: 20-50	[redacted]	[redacted] (1990) Supportive information only

				with the test organisms. **Step 2** The field trials were preceded by the determination in the white water of the level of glutaraldehyde needed to control the system. A guideline study was not followed, but a laboratory developed procedure called the "Relative Population Density Test": this procedure is used to determine the ability of a defined level of biocide to reduce the population of microorganisms in a product over a given period of time. The biocide was added to sterile tubes containing re-suspended cell pellets in 0.1 M phosphate buffer at pH 7.0. The percent reduction is determined by comparing initial bacterial counts to bacterial counts after 4 hours of contact with slimicide. **Step 3** For the field trials performed over 69 days, the the biocide was added into the white water o f the paper mill , and bacterial counts were taken by standard pour-plating of serially diluted samples on tryptic soy agar or tryptone glucose extract (TGE) agar.	million/ml		

Slimicide (bacterial and sulphate-reducing bacterial or SRB slime)	PT12	[redacted] (50% Glutaraldehyde)	Contaminated white water from the paper mill	An industrial wadding machine was treated with PIROR® 850 Slimicide for 6 days, additions were made every six hours. Measurement of the bacterial population at various intervals after shock dosing was performed. For enumerating aerobic or facultative bacteria, samples from the trial were evaluated by using a field test kit supplied under the brand name Easicult. These samples were also evaluated in the laboratory to confirm results, using conventional serial dilution plating on nutrient broth for detection of bacteria. For anaerobic growth evaluations, serial dilutions were conducted in sterile anaerobic vials containing API RP38 media and an iron nail, serially diluted and incubated prior to evaluation for the presence of black precipitate that indicates the presence of SRB.	**Bacteria** Conc. mg/L (ppm) Glutaraldehyde: 50 Contact time: every 6 hours over 6 days Incubation time: 48 h Incubation T°C: 37 °C Initial population: in the range of 10^7 CFU/mL **SRB** Conc. mg/L (ppm) Glutaraldehyde: 50 Contact time: every 6 hours over 6 days Incubation time: 10 days Incubation T°C: 37 °C Initial population: in the range of 10^4 to 10^5 CFU/mL	[redacted]	[redacted] (1990) Supportive information only
Slimicide (bacterial slime)	PT12	[redacted] (50% Glutaraldehyde)	Fouled felt and contaminated white water from the paper mill	Isolation of bacteria: 1 g of felt was placed in 9 mL of 0.1 M phosphate buffer at pH 7.0 and vigorously shaken. To culture the bacteria1 mL of this suspension was added to a chemostat containing 250 mL of	**Fouled felt** Conc. mg/L (ppm) Glutaraldehyde: 12.5, 25, 50 Contact time: 4 h Incubation time: not reported Incubation T°C: 37 °C	[redacted]	[redacted] (1990) Supportive

				nutrient broth and 1 L of water at 37°C with slight aeration. After 24 h of growth, aliquots were removed and centrifuged. The pellet was washed twice in 0.1 M phosphate solution. The cell pellet was resuspended in water at pH7.0. Relative Population Density Test (fouled felt): glutaraldehyde was added to sterile tubes containing resuspended cell pellets in 0.1 M phosphate buffer at pH7.0. Aliquots were removed and surviving microorganisms were enumerated by standard pour plating on TGE agar. The percentage reduction was determined by bacterial counts. Microbiocidal assays of white water samples: the samples were diluted with four parts of deionised water and 10 mL aliquots were dispensed into sterile tubes. Glutaraldehyde was serially diluted and added to the tubes. The tubes were incubated, the aliquots removed and surviving microorganisms were enumerated by standard pour plating on TGE agar.	Initial population: approx. $6x10^7$ CFU/mL **White water chest** Conc. mg/L (ppm) Glutaraldehyde: 12.5, 25, 50 Contact time: 4 h Incubation time: not reported Incubation T°C: 25 °C Initial population: 10^7 CFU/mL	[redacted]	information only

References:

[redacted] (2004a), Data on products and processes. [redacted] Rate of kill tests. [redacted]

[redacted] (2004b), Data on products and processes. Minimum cidal concentration tests with [redacted]

[redacted] (2004c), Data on products and processes [redacted]. Biocide efficacy vs. sulfate-reducing bacteria. [redacted]

[redacted] (2008), Determination of the activity of [redacted] as a microbiocide (bactericide and fungicide) in a cooling water sample according to ASTM 645-07 [redacted], unpublished, 2008.

[redacted] (2009), Determination of the bactericidal activity against [redacted], unpublished, 13 July 2009.

[redacted] (2009), Determination of the bactericidal activity against [redacted] unpublished, 13 July 2009.

[redacted] (1987), Efficacy Testing of [redacted] Against [redacted] in Cooling Tower Water, [redacted] 07 October 1987.

[redacted] (2008) Determination of the activity of [redacted] as a slimicide (bacterial and fungal slime) in a paper pulp sample according to ASTM E 1839-07, [redacted] unpublished, 2008.

[redacted] (1990) [redacted] Glutaraldehyde: A New Slimicide for Papermaking, Papermakers Conference April 1990, [redacted].

[redacted] (1990) [redacted] Field Trial at an Industrial Paper Mill in [redacted] October 1990.

Section A6.1.1 _ 01 Acute Toxicity

Annex Point IIA6.1 **Acute Oral Toxicity, Rat, LD_{50}**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994a) Report on the study of the acute oral toxicity 80/265; rat/oral. [redacted] (Unpublished), (original report in German dated 1981), BPD ID A6.01.1_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, but method was comparable to OECD 401	
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	[redacted]	
3.1.2.2	Purity	[redacted]% glutaraldehyde ([redacted]% methanol, [redacted]% water)	
3.1.2.3	Stability	About 12 months	

Section A6.1.1 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Oral Toxicity, Rat, LD_{50}**

3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	[redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	Male / Female
3.2.5	Age/weight at study initiation	Mean weight of the males for each test group at study initiation:

Test group	Mean weight (g)
215 mg/kg bw	250 g
316 mg/kg bw	190 g
464 mg/kg bw	215 g
1470 mg/kg bw	190 g

N = 5

Mean weight of the females for each test group at study initiation:

Test group	Mean weight (g)
215 mg/kg bw	210 g
316 mg/kg bw	160 g
464 mg/kg bw	170 g
1470 mg/kg bw	160 g

N = 5

3.2.6	Number of animals per group	Each test group comprised 5 male and 5 female rats
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	**Oral**
3.3.1	Post-exposure period	14 days
3.3.2	Type	Test substance administrated once by gavage

Section A6.1.1 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Oral Toxicity, Rat, LD_{50}**

3.3.3	Concentration	215, 316, 464 and 1470 mg/ kg bw	
3.3.4	Vehicle	Distilled water	
3.3.5	Concentration in vehicle		

Dose (mg/kg bw)	215	316	464	1470
Concentration in vehicle (w/v)	2.15	3.16	4.64	14.70
Administered volume (ml/kg bw)	10	10	10	10

3.3.6	Total volume applied	See above	
3.3.7	Controls	None	
3.4	**Examinations**	The animals were observed for mortality (twice each workday, once daily at weekends or public holidays), and for clinical symptoms of toxicity (< 15 ′, 15′, 30′, 1 h, 2 h, 4 h and 5 h following treatment and once daily thereafter). Furthermore the rats were weighed prior treatment and thereafter, between day 2 and 4, and on day 7 and 13 post-treatment. At the end of the observation period, the surviving animals were sacrificed for the purpose of necropsy; animals that died during the observations period also were subjected to necropsy.	
3.5	**Method of determination of LD_{50}**	The LD_{50} values were determined by interpolation.	**X**
3.6	**Further remarks**	None	

Section A6.1.1 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Oral Toxicity, Rat, LD_{50}**

4 RESULTS AND DISCUSSION

4.1 Clinical signs

Mortality (cumulative, 14 days):

Applied Dose (mg/kg bw)	Mortality (males)	Mortality (females)	Mortality (both sex)	Mortality (%)	Time of death after dosing
215	1/5	0/5	1/10	10%	Day 2
316	2/5	4/5	6/10	60%	Day 7
464	4/5	5/5	9/10	90%	Day 1
1470	5/5	5/5	10/10	100%	Day 1

Clinical signs of toxicity:

Signs of Toxicity	Test Dose (mg/kg bw)			
	215	316	464	1470
Poor general state	+	+	+	+
Dyspnea	+	+	+	+
Apathy	+	+	+	+
Piloerection	+	+	+	+
Staggering		+	+	+
Trembling			+	+
Exsiccosis		+	+	
Spastic gait		+		+
Tonus with stretching				+
Twitching			+	
Abnormal position			+	

4.2 Pathology

Animals that died during the observation period:

Necropsy revealed acute congestion, lesions in the stomach (e.g. dilatation, thickened wall of the glandular stomach, bloody ulcerations in the forestomach and the glandular stomach, purulent abscesses) and lesions in the intestines (e.g. reddened mucosa of the small intestines, bloody contents in the intestines).

Animals that were sacrificed at the end of the observation period:

Necropsy revealed no abnormalities.

4.3 Other

Body weight:

Section A6.1.1 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Oral Toxicity, Rat, LD_{50}**

Mean weight of the males for each test group:

Test group	Mean weight (g) at test initiation*	Mean weight (g) on day 13**
215 mg/kg bw	250 g	324 g
316 mg/kg bw	190 g	235 g
464 mg/kg bw	215 g	254 g
1470 mg/kg bw	190 g	-***

*, Initial mean weight for N = 5

**, Final mean weight for the survivors

***, No data as all animals were dead

Mean weight of the females for each test group:

Test group	Mean weight (g) at test initiation*	Mean weight (g) on day 13**
215 mg/kg bw	210 g	244 g
316 mg/kg bw	160 g	187 g
464 mg/kg bw	170 g	-***
1470 mg/kg bw	160 g	-***

*, Initial mean weight for N = 5

**, Final mean weight for the survivors

***, No data as all animals were dead

Necropsy:

In animals that died, necropsy revealed acute congestion; the stomach of these animals was dilated and the wall of the glandular stomach was thickened. In both, the forestomach and the glandular stomach, leathery bloody ulcerations were seen, as well as purulent abscesses and purulent fibrinous coatings. The mucosa of the small intestines partly appeared reddened and the intestinal contents were tinged with blood.

In the animals that survived the experiment and that were sacrificed at the end of the post-exposure observation period, necropsy revealed no abnormalities.

4.4 **LD_{50}**

LD_{50} male ca. 316 mg/kg bw

LD_{50} female ca. 285 mg/kg bw

LD_{50} male + female ca.316 mg/kg bw X

Section A6.1.1 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Oral Toxicity, Rat, LD_{50}**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to estimate the acute oral toxicity of glutaraldehyde in rat following a single oral administration of the test substance by gavage.

Test substance: Glutaraldehyde, purity approx. [redacted]

The test method was comparable to the OECD guideline 401; GLP was not compulsory at the time the study was performed.

Five male and five female Sprague-Dawley rats per test group were administered following doses of glutaraldehyde: 215, 316, 464 and 1470 mg/kg bw; the test substance was administered by gavage (single application) as aqueous solution; the application volume was 10 ml/kg. Following treatment, the animals were regularly examined for mortality and clinical signs of toxicity over an observation period of 14 days; body weights were recorded at test starting and during the observation period. All rats that died during the observation period as well as the surviving rats, which were sacrificed at the end of the observation period, were subjected to necropsy.

Section A6.1.1 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Oral Toxicity, Rat, LD_{50}**

5.2 Results and discussion

Mortality (cumulative, 14 days):

Applied Dose (mg/kg bw)	Mortality (%)
215	10%
316	60%
464	90%
1470	100%

Clinical signs of toxicity:

Clinical signs of toxicity were seen in all treated groups and they mainly included a poor general state, dyspnea, apathy, piloerection, staggering, trembling and exsiccosis.

Body weight:

Test group	Males		Females	
	Mean weight (g) at test initiation*	Mean weight (g) on day 13**	Mean weight (g) at test initiation*	Mean weight (g) on day 13**
215 mg/kg bw	250 g	324 g	210 g	244 g
316 mg/kg bw	190 g	235 g	160 g	187 g
464 mg/kg bw	215 g	254 g	170 g	-***
1470 mg/kg bw	190 g	-***	160 g	-***

*, Initial mean weight for N = 5

**, Final mean weight for the survivors

***, No data as all animals were dead

Necropsy:

In animals that died, necropsy revealed acute congestion; the stomach of these animals was dilated and the wall of the glandular stomach was thickened. In both, the forestomach and the glandular stomach, leathery bloody ulcerations were seen, as well as purulent abscesses and purulent fibrinous coatings. The mucosa of the small intestines partly appeared reddened and the intestinal contents were tinged with blood. In the animals that survived the experiment and that were sacrificed at the end of the post-exposure observation period, necropsy revealed no abnormalities.

Determination of the LD50 values (interpolation):

LD_{50} male ca. 316 mg/kg bw

LD_{50} female ca. 285 mg/kg bw

LD_{50} male + female ca.316 mg/kg bw

Section A6.1.1 _ 01	**Acute Toxicity**
Annex Point IIA6.1	**Acute Oral Toxicity, Rat, LD_{50}**

5.3	**Conclusion**	Referring to the test substance as such which contains [redacted]% glutaraldehyde, the determined LD50 for both, males and females, was 316 mg/kg bw. Referring to the 100% active ingredient glutaraldehyde, the LD50 value for both sexes is about 160 mg/kg bw.	X
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	May 5th, 2010
Materials and Methods	Agree with applicant's version. **3.1.2** This refers to Doc IIIA Section A2.
Results and discussion	Agree with the applicant's version with the following changes: **3.5** Only the LD_{50} for females was interpolated, while the LD_{50} value for males was approximated. **4.4** The combined LD_{50} value given in the original study and the study summary is 316 mg/kg bw, but an average of the male and female values could be more accurate. Therefore, LD_{50} combined is (316 + 285) mg/kg bw / 2 = 301 mg/kg bw. Note that at the dose level 316 mg/kg bw, 60 % of the animals died.
Conclusion	Agree with the applicant's version with the following changes: LD_{50} values for the test substance ([redacted] % glutaraldehyde in water): • LD_{50} male 316 mg/kg bw • LD_{50} female 285 mg/kg bw • LD_{50} combined 301 mg/kg bw • The risk phrase R22 "Harmful if swallowed" is warranted. • CLP: Classification in Category 4 for acute toxicity is warranted. LD_{50} values for glutaraldehyde, as calculated from the above: • LD_{50} male 158 mg/kg bw • LD_{50} female 143 mg/kg bw • LD_{50} combined 151 mg/kg bw • The risk phrase R25 "Toxic if swallowed" is warranted. • CLP: Classification in Category 3 for acute toxicity is warranted.
Reliability	2
Acceptability	Acceptable
Remarks	Key study

Section A6.1.1 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Oral Toxicity, Rat, LD_{50}**

	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 A 6.1.2 _ 01 Acute Toxicity

Annex Point IIA6.1 **Acute Dermal Toxicity, Rabbit, LD_{50}**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1995) Acute dermal toxicity study of glutaraldehyde in albino rabbits. [redacted] BPD ID A6.01.2_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EPA Pesticide Assessment Guidelines, Section 81-2	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Clear colorless liquid	
3.1.2.2	Purity	[redacted]% a.i.	
3.1.2.3	Stability	Not specified	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male / Female	
3.2.5	Age/weight at study initiation	The animals were young adults and weighed between 2363 and 2521 g at test initiation.	
3.2.6	Number of animals per group	The test was performed with a single group treated with 2000 mg/kg bw test substance; the group comprised 5 animals per sex.	
3.2.7	Control animals	No	

Section A 6.1.2 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Dermal Toxicity, Rabbit, LD_{50}**

3.3	**Administration/ Exposure**	**Dermal**	
3.3.1	Post-exposure period	14 days	
3.3.2	Area covered	About 23% of the total body surface.	X
3.3.3	Occlusion	Semi-occlusive. Following application of the test substance on the intact clipped skin, the application site was covered with gauze binders, which were again secured by means of non-irritating adhesive tape. Each animal received a collar.	
3.3.4	Vehicle	None	
3.3.5	Concentration in vehicle	The test material was dosed based on density (specific gravity). The dose volume was determined by dividing the dose level (g/kg) by the specific gravity (1.02 g/ml).	
3.3.6	Total volume applied	The individual doses were determined on the basis of the body weight and a dose volume of 1.96 ml/kg bw. The final test concentration was 2000 mg/kg bw.	
3.3.7	Duration of exposure	24 h	
3.3.8	Removal of test substance	After removal of the dressing, the application site was gently cleaned with lukewarm water.	
3.3.9	Controls	None	
3.4	**Examinations**	The animals were observed for mortality (after the first 1, 3 and 4 hours, and twice a day thereafter until day 14), clinical signs of toxicity (after the first 1, 3 and 4 hours, and once a day thereafter until day 14) and local skin changes (after 30 to 60 minutes following removal of the dressing, and daily thereafter until day 14); body weights were recorded on day 0, 7 and 14. At the end of the observation period, the animals were sacrificed for the purpose of necropsy.	
3.5	**Method of determination of LD_{50}**	Not specified	
3.6	**Further remarks**	None	

Section A 6.1.2 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Dermal Toxicity, Rabbit, LD_{50}**

4 RESULTS AND DISCUSSION

4.1 Clinical signs

Mortality (cumulative, 14 days):

No mortality occurred.

Clinical signs of toxicity:

Two males and one female showed mucoid feces on day 1 to 2 of treatment; one female showed wet brown urogenital staining about 4 hours after application. From day 3 of observation, no more signs of toxicity were seen and all animals appeared normal.

Local skin changes:

The treatment resulted in severe erythema, moderate to severe edema and eschar with subsequent exfoliation in all animals. In 6 case, the application sites displayed signs of corrosion. Fissuring of the skin was seen in 9 cases after day 6, and on day 7, yellow staining and desquamation were seen in all animals. These severe signs of skin irritation persisted over the complete period of observation in all animals.

4.2 Pathology

Necropsy revealed thickening and scabbing of the application sites in all animals. No further treatment-related abnormalities were reported.

4.3 Other

Body weight:

Body weights were inconspicuous over the complete period of observation.

4.4 LD_{50}

> 2000 mg/kg bw for both, males and females

Section A 6.1.2 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Dermal Toxicity, Rabbit, LD_{50}**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to estimate the acute dermal toxicity of glutaraldehyde in rabbit following a single application of the test substance on the skin.

Test substance: Glutaraldehyde, [redacted]

The test was conducted according to the EPA Pesticide Assessment Guidelines, Section 81-2 and followed GLP.

Five male and five female New Zealand white rabbits were treated with a single dose of 2000 mg/kg bw glutaraldehyde. The test substance was applied uniformly to the clipped skin of each animal on an area of about 23% of the total body surface, under semi-occlusive conditions. After an exposure period of 24 hours, the dressings were removed and each application site was gently cleaned with lukewarm water. Following application, the animals were regularly observed for mortality, clinical signs of toxicity, local skin changes and body weight gain over an observation period of 14 days. At the end of the observation period, the animals were sacrificed for the purpose of necropsy.

5.2 Results and discussion

The single dermal treatment of the rabbits with 2000 mg/kg bw glutaraldehyde resulted in no mortalities. Three cases of mucoid feces, and one case of wet brown urogenital staining were seen during the first two days following treatment; thereafter, no more signs of toxicity were seen and all animals appeared normal. Considering the application sites, the treatment resulted in severe erythema, moderate to severe edema and eschar with subsequent exfoliation in all animals. In 6 cases, the application sites displayed signs of corrosion. Fissuring of the skin was seen in 9 cases after day 6, and on day 7, yellow staining and desquamation were seen in all animals. These severe signs of skin irritation persisted over the complete period of observation in all animals. Body weights were inconspicuous over the complete period of observation. Necropsy revealed thickening and scabbing of the application sites in all animals; no further treatment-related abnormalities were reported.

5.3 Conclusion

LD_{50} > 2000 mg/kg bw for both males and females

5.3.1 Reliability

1

5.3.2 Deficiencies

None

Section A 6.1.2 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Dermal Toxicity, Rabbit, LD_{50}**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 6th, 2010
Materials and Methods	**3.1.2** This refers to Doc IIIA Section A2.
	3.3.2 Area Covered. Total treated skin area (23 %) was rather large compared to the 10 % recommended in the OECD guideline 402. This is considered not to affect the validity of the results.
	Otherwise agree with applicant's version.
Results and discussion	Agree with applicant's version.
	The acute dermal toxicity was evaluated in a limit test with a single dose of 2000 mg/kg in rabbits. No mortality or signs of systemic tocixity were reported. Treatment caused severe skin irritation.
Conclusion	Agree with applicant's version.
	LD_{50} > 2000 mg/kg bw for both males and females.
Reliability	1
Acceptability	Acceptable
Remarks	Key study
	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 A 6.1.2 _ 02 Acute Toxicity

Annex Point IIA6.1 **Acute Dermal Toxicity, Rat, LD_{50}**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994b) Report on the study of the acute dermal toxicity of "Glutaraldehyde" in the rat. [redacted] (Unpublished), (original report in German dated 1981), BPD ID A6.01.2_02	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No but method was comparable to OECD guideline 402	
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde [redacted]%	
3.1.1	Lot/Batch number	Test substance number: [redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Aqueous solution, [redacted]	
3.1.2.2	Purity	[redacted]% glutaraldehyde ([redacted]% water)	
3.1.2.3	Stability	About 12 months	

Section A 6.1.2 _ 02 **Acute Toxicity**

Annex Point IIA6.1 **Acute Dermal Toxicity, Rat, LD_{50}**

3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male / Female	
3.2.5	Age/weight at study initiation	Mean weight of the males: 219 g Mean weight of the females: 182 g	
3.2.6	Number of animals per group		

Test Dose	Number of males	Number of females	Total
400 mg/kg bw	5	5	10
906 mg/kg bw*	1	-	1
1000 mg/kg bw	4	5	9
2000 mg/kg bw	5	5	10

*, Belongs to the 1000 mg/kg dose group; however, only 960 mg/kg bw was applied by inadvertency.

3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	**Dermal**	
3.3.1	Post-exposure period	14 days	
3.3.2	Area covered	About 50 cm^2	X
3.3.3	Occlusion	Occlusive (the treated area was covered with an inert foil, which was maintained in position with adhesive tape).	X
3.3.4	Vehicle	Water	
3.3.5	Concentration in vehicle	The vehicle was only used for the preparation of the 50% aqueous formulation corresponding to the 400 mg/kg bw dose; the remaining tested doses were applied unchanged.	
3.3.6	Total volume applied	The applied volume was chosen as to result in the wanted concentration.	
3.3.7	Duration of exposure	24 h	
3.3.8	Removal of test substance	The test substance was washed off from the skin with warm water.	
3.3.9	Controls	None	
3.4	**Examinations**	The animals were observed for mortality, clinical signs of toxicity and local changes over an observation period of 14 days. Animals that died during this period were subjected to necropsy; the survivors were sacrificed at the end of the 14 day-period and were also subjected to necropsy.	

Section A 6.1.2 _ 02	Acute Toxicity
Annex Point IIA6.1	Acute Dermal Toxicity, Rat, LD_{50}

3.5	Method of determination of LD_{50}	No data
3.6	Further remarks	None

Section A 6.1.2 _ 02 **Acute Toxicity**

Annex Point IIA6.1 **Acute Dermal Toxicity, Rat, LD_{50}**

4 RESULTS AND DISCUSSION

4.1 Clinical signs

Mortality (cumulative, 14 days):

Applied Dose (mg/kg bw)	Mortality (males)	Mortality (females)	Mortality (both sex)	Mortality (%)	Time of death after dosing
400	0/5	0/5	0/10	0%	-
906	0/1	-	-	0%	-
1000	0/4	0/5	0/9	0%	-
2000	0/5	1/5	1/10	10%	Day 7

Clinical signs of toxicity:

Signs of Toxicity	Test Dose (mg/kg bw)		
	400	906 / 1000	2000
Poor general state		+	+
Dyspnea		+	+
Apathy		+	+
Staggering			+
Excitation	+	+	+
Atony			+
Trembling			+
Ruffle fur			+
Diarrhea		+	

Local changes:

Local changes	Test Dose (mg/kg bw)		
	400	906 / 1000	2000
Severe necrosis		+ (7 d)	
Severe hard necrosis		+ (13 d)	
Severe soft necrosis		+ (24 h)	
Slight soft necrosis	+ (24 h)		+ (24 h)
Very slight to slight spotted necrosis	+ (24 h) + (7 d) + (13 d)	+ (13 d)	+ (24 h)
Severe parchment-like necrosis			+ (7 d)
Parchment-like necrosis		+ (7 d)	
Very slight to slight Parchment-like necrosis	+ (7 d)		
Severe leathery necrosis			+ (13 d)
Slight leathery necrosis	+ (13 d)		
Severe edema			+ (24 h)

Section A 6.1.2 _ 02 **Acute Toxicity**

Annex Point IIA6.1 **Acute Dermal Toxicity, Rat, LD_{50}**

Partly slightly open edema			+ (7 d)
Partly slight edema		+ (7 d)	
Slight edema	+ (24 h)		
Slight cushion-like edema		+ (24 h)	
Slight transverse folds		+ (7 d) + (13 d)	+ (7 d) + (13 d)
Very slight scaling		+ (7 d) + (13 d)	

4.2 Pathology

Animals that died during the observation period:

Necropsy of the one animal that died during the observation period and which was clearly emaciated revealed diarrheal contents in the intestines.

Animals that were sacrificed at the end of the observation period:

Necropsy revealed no abnormalities.

4.3 Other

None

4.4 LD_{50}

> 2000 mg/kg bw for both, males and females

Section A 6.1.2 _ 02 **Acute Toxicity**

Annex Point IIA6.1 **Acute Dermal Toxicity, Rat, LD_{50}**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to estimate the acute dermal toxicity of glutaraldehyde in rat following a single application of the test substance on the skin.

Test substance: Glutaraldehyde, purity approx. [redacted]

The test method was comparable to the OECD guideline 402; GLP was not compulsory at the time the study was performed.

Five male and five female Sprague-Dawley rats per test group were treated with following doses of glutaraldehyde: 400, 1000 and 2000 mg/kg bw. Whereas the highest doses were applied unchanged to the skin, the 400 mg/kg bw dose was tested as [redacted] aqueous formulation. Within the 1000 mg/kg bw group, one male animal accidentally received an application of 906 mg/kg bw instead of 1000 mg/kg bw. The test substance was applied uniformly to the clipped skin of the back and flank of each animal on an area of 50 cm^2. The application site was covered with an inert foil, which was secured in position with adhesive tape. After an exposure period of 24 hours, the bandage was removed and the test substance was washed of from the skin with warm water. Following treatment, the animals were regularly examined for mortality, clinical signs of toxicity and local changes over an observation period of 14 days. All rats that died during the observation period as well as the surviving rats, which were sacrificed at the end of the observation period, were subjected to necropsy.

5.2 Results and discussion

Mortality (cumulative, 14 days):

Only one case on death (female) was observed within the 2000 mg/kg bw group.

Clinical signs of toxicity:

Clinical signs of toxicity were observed in all test groups and included a poor general state, dyspnea, apathy, excitation, staggering, atony, trembling, ruffled fur and diarrhea.

Local changes:

Local changes affecting the application sites were seen at all test doses over the whole observation period: these changes can be summarized as follows:

Local changes	Test Dose (mg/kg bw)		
	400	906 / 1000	2000
Necrosis (severe to slight soft)	+	+	+
Spotted necrosis (very slight to slight)	+	+	+
Parchment-like necrosis	+	+	+
Leathery necrosis	+		+
Edema (severe to slight)	+	+	+
Slight transverse folds		+	+
Scaling		+	

Section A 6.1.2 _ 02	Acute Toxicity	
Annex Point IIA6.1	Acute Dermal Toxicity, Rat, LD_{50}	
5.3 Conclusion	LD_{50} > 2000 mg/kg bw	X
5.3.1 Reliability	**1**	
5.3.2 Deficiencies	One male animal of the 1000 mg/kg bw group was treated per inadvertence with 906 mg/kg test substance instead of 1000 mg/kg bw. This mistake however did not affect the validity of the study.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 10th, 2010
Materials and Methods	**3.1.2** This refers to Doc IIIA Section A2. **3.3.2** The treated area should be reported as % of the total body area. RMS FI estimates that the treated area has been > 10 % of total body area, based on the assumption that a rat weighing 200 g has a body area > 250 cm^2. **3.3.3** According to OECD guideline 402, the test area should be covered with gauze dressing. Inert foil has been used in this study to produce occlusion. Otherwise agree with applicant's version.
Results and discussion	The acute dermal toxicity was evaluated in a test with three doses (400-2000 mg/kg of [redacted] % glutaraldehyde = 200-1000 mg/kg of 100 % glutaraldehyde) in rats. 10 % mortality occurred in the highest dose group. Signs of toxicity were observed in all test groups. Occlusion may have enhanced the absorption of the test substance. Otherwise agree with applicant's version.
Conclusion	LD_{50} > 1000 mg/kg bw for both males and females (for 100 % glutaraldehyde). No risk phrase is warranted based on the test results.
Reliability	2
Acceptability	Acceptable
Remarks	

Section A 6.1.2 _ 02 **Acute Toxicity**

Annex Point IIA6.1 **Acute Dermal Toxicity, Rat, LD_{50}**

	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 _ 01 Acute Toxicity

Annex Point IIA6.1 **Acute Inhalation Toxicity, Rat, LC_{50}**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994a) Acute inhalation toxicity LC50 4 hours (rat) of "Glutaraldehyde, [redacted]", liquid aerosol study ([redacted]). [redacted] (unpublished), (original report in German dated 1982), BPD ID A6.01.3_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, but method was comparable to OECD 403	
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde approx. [redacted]	
3.1.1	Lot/Batch number	No data	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Liquid, weakly yellow	
3.1.2.2	Purity	Approx. [redacted]% glutaraldehyde ([redacted]% water)	
3.1.2.3	Stability	Approx. 12 months	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male / Female	
3.2.5	Age/weight at study initiation	Mean body weight of the males: 298 +/- 35 g Mean body weight of the females: 224 +/- 28 g Age at test initiation: 8 weeks	
3.2.6	Number of animals per group	10 animals/sex/group	
3.2.7	Control animals	Yes, an untreated rat collective served for control (90 rats/sex)	

Section A6.1.3 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Inhalation Toxicity, Rat, LC_{50}**

3.3	**Administration/ Exposure**	**Inhalation**
3.3.1	Post-exposure period	14 days
3.3.2	Concentrations	Nominal concentrations: 0.23, 0.41, 0.53, 0.68 and 0.9 mg/l Analytical concentrations: 0.10, 0.18, 0.28, 0.39 and 0.44 mg/l
3.3.3	Particle size	No data
3.3.4	Type or preparation of particles	-
3.3.5	Type of exposure	Nose/head exposure
3.3.6	Vehicle	Air
3.3.7	Concentration in vehicle	Constant amounts of test substance were supplied to a two-component atomizer by means of a metering pump. By means of compressed air (1.8 bar) a mixture of test substance and air (liquid aerosol) was generated, which was passed into the inhalation system (head-nose inhalation system INA 20, [redacted], V ca. 55 l).
3.3.8	Duration of exposure	4 h
3.3.9	Controls	A control group was mentioned in terms of body weight.
3.4	**Examinations**	The rats were observed over a period of 14 days for mortality (daily), clinical signs of toxicity (each workday) and body weight gain (at test initiation, after 7 days and at the end of the observation period). Animals that died during the observation period were subjected to necropsy and gross pathological examination. The surviving rats were sacrificed at the end of the exposure period by means of CO_2 and were also subjected to necropsy and gross pathological examination.
3.5	**Method of determination of LD_{50}**	Probit Analysis according to Finney DJ, Cambridge University Press, 3rd edition, 1971.
3.6	**Further remarks**	None

Section A6.1.3 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Inhalation Toxicity, Rat, LC_{50}**

4 RESULTS AND DISCUSSION

4.1 Clinical signs

Mortality (cumulative14 days):

Applied Con. (mg/l)	Mortality (males)	Mortality (females)	Mortality (both sex)
0.10	0/10	0/10	0/20 (0%)
0.18	1/10 (d14)*	3/10 (d1-d2)	4/20 (20%)
0.28	1/10 (d1)	3/10 (d2-d7)	4/20 (20%)
0.39	7/10 (4h-d2)	7/10 (4h-d2)	14/20 (70%)
0.44	9/10 (4h-d1)	10/10 (4h-d14)	19/20 (95%)

*, Time of death after dosing (h, hours; d, days)

Clinical signs of toxicity:

During the exposure period vigorous attempts to escape, wiping of the snout, lid closure, and aqueous or red discharge from eyes and noses were observed. During the post-exposure period whooping or gasping respiration with rasping sounds during inspiration was observed. Crusts surrounded the eyes and noses, and a posture to relieve the circulation as well as a poor general state was reported. These symptoms disappeared in the surviving animals within day 5 to 9 post-exposure.

4.2 Pathology

Necropsy of the animals that died during the post-exposure period:

Necropsy revealed acute congestion, pronounced emphysema of the lungs as well as edematization and infarctoid hyperemia.

Necropsy of the animals that were sacrificed:

Necropsy revealed no abnormalities.

4.3 Other

Mean body weights/sex/group:

Test Conc. (mg/l)	Initially		After 7 days**		After 14 days**	
	Males	Females	Males	Females	Males	Females
0*	307 g	230 g	340 g	240 g	367 g	249 g
0.10	289 g	228 g	324 g	238 g	376 g	252 g
0.18	304 g	224 g	319 g	238 g	360 g	246 g
0.28	305 g	222 g	320 g	228 g	343 g	240 g
0.39	287 g	211 g	316 g	211 g	321 g	208 g
0.44	304 g	232 g	285 g	168 g	334 g	-***

*, Control group of 90 animals/sex.

**, The mean body weight refers to the surviving rats.

***, No survivors.

Section A6.1.3 _ 01 Annex Point IIA6.1	Acute Toxicity Acute Inhalation Toxicity, Rat, LC_{50}	
4.4 LC_{50}	4-hour exposure: LC_{50} male: 0.35 mg/l (confidence limits 0.30 – 0.39 mg/l) LC_{50} female: 0.28 mg/l (confidence limits 0.22 – 0.29 mg/l) LC_{50} male + female: 0.28 mg/l < LC_{50} <0.39 mg/l 1-hour exposure (Conversion of the 4-hour values according to Haber´s rule): LC_{50} male: 1.40 mg/l LC_{50} female: 1.10 mg/l LC_{50} male + female: 1.10 mg/l < LC_{50} <1.60 mg/l	X

Section A6.1.3 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Inhalation Toxicity, Rat, LC_{50}**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to estimate the acute inhalation toxicity of glutaraldehyde in rat following exposure to a liquid aerosol of the test substance.

Test substance: Glutaraldehyde approx. [redacted]% solution [redacted] (ca. [redacted]% glutaraldehyde, ca [redacted]% water)

The test method was comparable to the OECD guideline 403; GLP was not compulsory at the time the study was performed.

Ten male and ten female [redacted] rats per dose group were exposed to the test substance as liquid aerosol at following nominal concentrations: 0.23, 0.41, 0.53, 0.68 and 0.9 mg/l; the analytical monitoring of the tested concentrations resulted in following measured values: 0.10, 0.18, 0.28, 0.39 and 0.44 mg/l. The animals were subjected to a head-nose exposure for 4 hours. The exposure period was followed by an observation period of 14 days. During and after exposure, mortality and clinical signs of toxicity were recorded at regular time intervals. The body weight was determined prior test initiation and thereafter, on day 7 and day 14 post-exposure. Rats that died during the experiment were subjected to necropsy; those which survived were sacrificed at the end of the experiment and were also subjected to necropsy. An untreated rat collective served as control. The determination of the LC_{50} values was based on the Probit Analysis.

Section A6.1.3 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Inhalation Toxicity, Rat, LC_{50}**

5.2 Results and discussion

Mortality (cumulative 14 days):

Applied Con. (mg/l)	Mortality (both sex)
0.10	0/20 (0%)
0.18	4/20 (20%)
0.28	4/20 (20%)
0.39	14/20 (70%)
0.44	19/20 (95%)

Clinical signs of toxicity:

During the exposure period vigorous attempts to escape, wiping of the snout, lid closure, and aqueous or red discharge from eyes and noses were observed. During the post-exposure period whooping or gasping respiration with rasping sounds during inspiration was observed. Crusts surrounded the eyes and noses, and a posture to relieve the circulation as well as a poor general state was reported. Theses symptoms disappeared in the surviving animals within day 5 to 9 post-exposure.

Body weight:

After 7 days, the body weights of the treated males were clearly impaired compared to controls; After 14 days this impairment still was observed in the 0.28 and the 0.39 mg/l groups. For the females, the body weight in the 0.39 mg/l group was impaired during the whole observation period; the body weight of the 0.28 mg/l group only was affected after 7 days.

Necropsy:

Necropsy of the animals that died during the experiment revealed acute congestion, pronounced emphysema of the lungs as well as edematization and infarctoid hyperemia. The surviving animals, which were sacrificed at the end of the observation period, showed no pathological abnormalities.

LC_{50} (males and females):

4-hour exposure: $0.28 \text{ mg/l} < LC_{50} < 0.39 \text{ mg/l}$

1-hour exposure: $1.10 \text{ mg/l} < LC_{50} < 1.60 \text{ mg/l}$

5.3 Conclusion

5.3.1 Reliability **1**

5.3.2 Deficiencies Data on characterisation of the aerosol (MMAD and geometrical deviation) were not given in the report.

Section A6.1.3 _ 01 **Acute Toxicity**

Annex Point IIA6.1 **Acute Inhalation Toxicity, Rat, LC_{50}**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 11th, 2010
Materials and Methods	**3.1.2** This refers to Doc IIIA Section A2. Agree with applicant's version.
Results and discussion	4.4 LC_{50}. Conversion to 1-hour exposure without data is not acceptable.
Conclusion	The acute inhalation toxicity was evaluated in a test with five concentrations (analytical) 0,10 – 0,44 mg/l in rats. 4-hour exposure: LC_{50} male: 0.35 mg/l (confidence limits 0.30 – 0.39 mg/l) LC_{50} female: 0.28 mg/l (confidence limits 0.22 – 0.29 mg/l) LC_{50} male + female: 0.28 mg/l < LC_{50} <0.39 mg/l Risk phrase R23 "Toxic by inhalation" is warranted. CLP: Classification in Category 1 for inhalation toxicity is warranted.
Reliability	2
Acceptability	Acceptable
Remarks	Key study Lack of data on the characterisation of the aerosol increases the uncertainty of the result. RMS FI nevertheless considers the study acceptable with a reliability indicator 2.
	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 — Acute Toxicity

Annex Point IIA6.1 — **Acute Inhalation Toxicity, Rat, LC_{50}**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2001) Acute inhalation toxicity LC_{50} 4 hours (rat) of „glutaraldehyde, [redacted]% solution“ (test substance [redacted]), liquid aerosol study [redacted] (Unpublished), (original report in German dated 1985), BPD ID A6.01.3_02	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, but method was comparable to OECD 403	
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed (i.e 1985)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde [redacted]	
3.1.1	Lot/Batch number	Test substance number: [redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Aqueous solution, [redacted]	
3.1.2.2	Purity	[redacted]% glutaraldehyde (ca. [redacted]% methanol, ca. [redacted]% water)	
3.1.2.3	Stability	About 12 months	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male / Female	
3.2.5	Age/weight at study initiation	Mean body weight of the males: 278 +/- 39 g Mean body weight of the females: 205 +/- 50 g Age at test initiation: 8 weeks	
3.2.6	Number of animals per group	10 animals/sex/group	
3.2.7	Control animals	Yes, an untreated rat collective served for control (90 rats/sex)	X

Section A6.1.3 _ 02 **Acute Toxicity**

Annex Point IIA6.1 **Acute Inhalation Toxicity, Rat, LC_{50}**

3.3	**Administration/ Exposure**	**Inhalation**
3.3.1	Post-exposure period	14 days
3.3.2	Concentrations	Nominal concentrations: 0.35, 0.58 and 0.72 mg/l Analytical concentrations: 0.22, 0.31and 0.63 mg/l
3.3.3	Particle size	A particle size analysis was undertaken for the group treated with the highest dose of test substance. The results of this analysis revealed that 100% of the aerosol reached the lung alveoles. As the test substance was applied as finest liquid aerosol (< 2.8 µm) neither the MMAD nor the GSD were calculated.
3.3.4	Type or preparation of particles	Liquid aerosol
3.3.5	Type of exposure	Nose/head exposure
3.3.6	Vehicle	Air
3.3.7	Concentration in vehicle	Constant amounts of test substance were supplied to a two-component atomizer by means of a metering pump. By means of compressed air (1.8 bar) a mixture of test substance and air (liquid aerosol) was generated, which was passed into the inhalation system (head-nose inhalation system INA 20[redacted], V ca. 55 l).
3.3.8	Duration of exposure	4 h
3.3.9	Controls	The control group was mentioned in terms of body weight.
3.4	**Examinations**	The rats were observed over a period of 14 days for mortality (daily), clinical signs of toxicity (each workday) and body weight gain (at test initiation, after 7 days and at the end of the observation period). Animals that died during the observation period were subjected to necropsy and gross pathological examination. The surviving rats were sacrificed at the end of the exposure period by means of CO_2 and were also subjected to necropsy and gross pathological examination.
3.5	**Method of determination of LD_{50}**	The assessment of the dose-effect relation was based on Probit Analysis according to Finney DJ, Cambridge University Press, 3rd edition, 1971. The particle size was determined according to Silverman L. (Particle Size Analysis in Industrial Hygiene, 235-259, 1971)
3.6	**Further remarks**	None

Section A6.1.3 _ 02 **Acute Toxicity**

Annex Point IIA6.1 **Acute Inhalation Toxicity, Rat, LC_{50}**

4 RESULTS AND DISCUSSION

4.1 Clinical signs

Mortality (cumulative14 days):

Applied Con. (mg/l)	Mortality (males)	Mortality (females)	Mortality (both sex)
0.22 mg/l	0/10	0/10	0/20 (0%)
0.31 mg/l	1/10 (d7)*	2/10 (d7)	3/20 (15%)
0.63 mg/l	7/10 (d2)	8/10 (d2)	15/20 (75%)

*, Time of death after dosing (h, hours; d, days)

Clinical signs of toxicity:

During the exposure period a slight nasal discharge, snout wiping, flank respiration and irregular to intermittent respiration were reported. During the post-exposure period, bloody nasal discharge, red crusts surrounding the nose (Sangur positive), whooping or gasping respiration with rasping sounds and a tremulous gait were observed. These symptoms disappeared in the surviving animals within day 5 to 9 post-exposure.

4.2 Pathology

Necropsy of the animals that died during the post-exposure period:

Necropsy revealed general congestion and slightly increased blood content as well as small emphysema-areas in the lung; in three cases of death, pronounced emphysema of the lungs was seen.

Necropsy of the animals that were sacrificed:

Necropsy revealed no abnormalities.

4.3 Other

Mean body weights/sex/group:

Test Conc. (mg/l)	Initially		After 7 days**		After 14 days**	
	Males	Females	Males	Females	Males	Females
0*	307 g	230 g	340 g	240 g	367 g	249 g
0.22 mg/l	297 g	226 g	328 g	234 g	360 g	251 g
0.31 mg/l	289 g	208 g	288 g	219 g	339 g	244 g
0.63 mg/l	249 g	180 g	234 g	195 g	293 g	217 g

*, Control group of 90 animals/sex.

**, The mean body weight refers to the surviving rats.

Section A6.1.3 _ 02	Acute Toxicity	
Annex Point IIA6.1	Acute Inhalation Toxicity, Rat, LC_{50}	
4.4 LC_{50}	4-hour exposure: LC_{50} male: 0.52 mg/l (confidence limits 0.40 – 0.75 mg/l) LC_{50} female: 0.45 mg/l (confidence limits 0.36 – 0.62 mg/l) LC_{50} male + female: 0.48 mg/l (confidence limits 0.41 – 0.59 mg/l) 1-hour exposure (Conversion of the 4-hour values according to Haber's rule): LC_{50} male: 2.10 mg/l LC_{50} female: 1.80 mg/l LC_{50} male + female: 1.90 mg/l	X

Section A6.1.3 _ 02 **Acute Toxicity**

Annex Point IIA6.1 **Acute Inhalation Toxicity, Rat, LC_{50}**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to estimate the acute inhalation toxicity of glutaraldehyde in rat following exposure to a liquid aerosol of the test substance.

Test substance: Glutaraldehyde [redacted]

The test method was comparable to the OECD guideline 403; GLP was not compulsory at the time the study was performed.

Ten male and ten female [redacted] rats per dose group were exposed to the test substance as liquid aerosol at following nominal concentrations: 0.35, 0.58 and 0.72 mg/l; the analytical monitoring of the tested concentrations resulted in following measured values: 0.22, 0.31and 0.63 mg/l. A particle size analysis was undertaken for the group treated with the highest dose of test substance. The animals were subjected to a head-nose exposure for 4 hours. The exposure period was followed by an observation period of 14 days. During and after exposure, mortality and clinical signs of toxicity were recorded at regular time intervals. The body weight was determined prior test initiation and thereafter, on day 7 and day 14 post-exposure. Rats that died during the experiment were subjected to necropsy; those which survived were sacrificed at the end of the experiment and were also subjected to necropsy. An untreated rat collective served as control. The determination of the LC_{50} values was based on the Probit Analysis.

Section A6.1.3 _ 02 **Acute Toxicity**

Annex Point IIA6.1 **Acute Inhalation Toxicity, Rat, LC_{50}**

5.2 Results and discussion

Particle size:

The results of this analysis revealed that 100% of the aerosol reached the lung alveoles. As the test substance was applied as finest liquid aerosol (< 2.8 µm) neither the MMAD nor the GSD were calculated.

Mortality (cumulative 14 days):

Applied Con. (mg/l)	Mortality (both sex)
0.22 mg/l	0/20 (0%)
0.31 mg/l	3/20 (15%)
0.63 mg/l	15/20 (75%)

Clinical signs of toxicity:

During the exposure period a slight nasal discharge, snout wiping, flank respiration and irregular to intermittent respiration were reported. During the post-exposure period, bloody nasal discharge, red crusts surrounding the nose (Sangur positive), whooping or gasping respiration with rasping sounds and a tremulous gait were observed. These symptoms disappeared in the surviving animals within day 5 to 9 post-exposure.

Body weight:

The body weight gain of the male rats of the 0.31 and the 0.63 mg/l groups was impaired in a dose-dependent manner. In contrast, the body weight gain of the females was not impaired.

Necropsy:

Whereas necropsy of the surviving animals, which were sacrificed at the end of the observation period, revealed no abnormalities, animals that died during the observation period showed general congestion and slightly increased blood content as well as small emphysema-areas in the lung; in three cases of death, pronounced emphysema of the lungs was seen.

LC_{50} (males and females, based analytical values):

4-hour exposure: 0.48 mg/l (confidence limits 0.41 – 0.59 mg/l)

1-hour exposure: 1.90 mg/l

5.3 Conclusion

5.3.1 Reliability **1**

5.3.2 Deficiencies None

Section A6.1.3 _ 02 **Acute Toxicity**

Annex Point IIA6.1 **Acute Inhalation Toxicity, Rat, LC_{50}**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 12th, 2010
Materials and Methods	**3.1.2** This refers to Doc IIIA Section A2. **3.2.7** There were no control animals: these are not mentioned in the original report and they are not required either. Otherwise agree with applicant's version.
Results and discussion	4.4 LC_{50}. Conversion to 1-hour exposure without data is not acceptable.
Conclusion	Agree with applicant's version. The acute inhalation toxicity was evaluated in a test with three concentrations 0,22 – 0,63 mg/l in rats. LC_{50} male: 0.52 mg/l (confidence limits 0.40 – 0.75 mg/l) LC_{50} female: 0.45 mg/l (confidence limits 0.36 – 0.62 mg/l) LC_{50} male + female: 0.48 mg/l (confidence limits 0.41 – 0.59 mg/l) Risk phrase R23 "Toxic by inhalation" is warranted. CLP: Classification in Category 1 for inhalation toxicity is warranted.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.3 _ 03 Acute Toxicity

Annex Point IIA6.1.3 **Inhalation Hazard Test, Rat**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994b) Study of the acute inhalation toxicity in rats in the inhalation hazard test. [redacted] (Unpublished), (original report in German dated 1981), BPD ID A6.01.3_03	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline was mentioned; the study was conducted according to an accepted BASF Test method.	
2.2	**GLP**	No; in fact, the original study was conducted in 1981, at that time GLP was not compulsory.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde [redacted]	
3.1.1	Lot/Batch number	Test substance number: [redacted] (see remark 1 in 3.6)	
3.1.2	Specification	As given in section 2	x
3.1.2.1	Description	Aqueous solution [redacted]	
3.1.2.2	Purity	[redacted]% glutaraldehyde (ca [redacted]% water)	
3.1.2.3	Stability	About 12 months	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male/Female	
3.2.5	Age/weight at study initiation	Mean weight of the males: 180 - 250 g Mean weight of the females: 180 - 250 g Age at test initiation: 7 to 10 weeks	
3.2.6	Number of animals per group	A total of 12 rats was used for the experiment	x
3.2.7	Control animals	None	

Section A6.1.3 _ 03 Acute Toxicity

Annex Point IIA6.1.3 Inhalation Hazard Test, Rat

3.3	**Administration/ Exposure**	
3.3.1	Post-exposure period	14 days
3.3.2	Concentrations	The animals were exposed to a mixture of air and the volatile component at a temperature of 20 °C. The mean concentration of test substance calculated was 15 mg/l (for details see remark 2 in 3.6).
3.3.3	Particle size	Not relevant
3.3.4	Type or preparation of particles	Preparation of the air/test substance mixture: The test substance was filled in a fritted glass bottle to a 5 cm layer and the net weight was determined. A stream of compressed air (200 l/h) was supplied to the glass bottle, which has been placed in a water bath (20 +/-1 °C). The mixture of air and test substance was then conducted to 6 glass tubes, each containing one animal. The emerging mixtures were exhausted. The test substance consumed was determined by reweighing the fritted glass bottles.
3.3.5	Type of exposure	Whole body exposure in glass inhalation chambers (one rat per chamber).
3.3.6	Vehicle	Air
3.3.7	Duration of exposure	1, 3 and 7 hours
3.3.8	Controls	None
3.4	**Examinations**	The rats were observed over a period of 14days for mortality and clinical signs of toxicity. At the end of the observation period, the rats were sacrificed by means of CO_2 and were subjected to necropsy and gross pathological examination. Animals that died during the test also were subjected to necropsy.
3.5	**Method of determination of LC_{50}**	Not relevant
3.6	**Further remarks**	Remark1: The same test substance (80/265) was used within a dermal toxicity study reported by [redacted] (1994) and within an inhalation study reported by [redacted] (1994). Within these two studies, the test substance was described with more details than in present study, and the data referring to purity, composition and stability were taken from there. Remark 2: The test substance concentration was assessed and following mean concentrations were reported: After 1 hour: ca. 16 mg/l (ca. 3800 ppm). After 3 hours: ca. 17 mg/l (ca. 4100 ppm). After 7 hours: ca. 13 mg/l (ca. 3300 ppm).

Section A6.1.3 _ 03 **Acute Toxicity**

Annex Point IIA6.1.3 **Inhalation Hazard Test, Rat**

4 RESULTS AND DISCUSSION

4.1 Clinical signs

Mortality:

Exposure	1 hour	3 hours	7 hours
Mortality (number of deaths/total number of exposed rats)	0/12	1/12	6/6

Clinical signs of toxicity:

A series of symptoms indicative of toxicity were seen, which included lid closure, snout wiping, salivation, lacrimation, aqueous to reddish discharge from the nose as well as sticky eyes and dyspnea.

4.2 Pathology

Necropsy of the rats that died during the experiment revealed acute dilation of the heart (atrium) and acute congestion, whereas slight to moderate acute emphysema, often accompanied by edema, was seen in the lungs.

Necropsy of the animals that were sacrificed at the end of the observation period revealed no abnormalities.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate to inhalative toxicity of glutaraldehyde in rats by means of the inhalation hazard test.

Test substance: Glutaraldehyde [redacted], aqueous solution [redacted], composition: [redacted]% glutaraldehyde, [redacted] water.

No guideline was mentioned; the study was conducted according to an accepted BASF Test method. GLP was not compulsory at the time the study was conducted.

Male and female [redacted] rats were purchased from [redacted]. At study initiation, both males and females weighed between 180 and 250 g and were about 7 to 10 weeks old.

The test substance was filled in a fritted glass bottle to a 5 cm layer and the net weight was determined. A stream of compressed air (200 l/h) was supplied to the glass bottle, which has been placed in a water bath (20 +/-1 °C). The mixture of air and test substance was then conducted to 6 glass tubes, each containing one animal. The emerging mixtures were exhausted. The test substance consumed was determined by reweighing the fritted glass bottles. The exposure times were 1, 3 and 7 hours depending on lethality. Following the treatment, the animals were subjected to an observation period of 14 days; during this period the animals were daily checked for mortality and clinical signs of toxicity. Animals that died were subjected to necropsy; animals that surviving were sacrificed at the end of the observation period and were also subjected to necropsy.

Section A6.1.3 _ 03		**Acute Toxicity**
Annex Point IIA6.1.3		**Inhalation Hazard Test, Rat**
5.2	**Results and discussion**	Mean concentrations of test substance: A mean test concentration of 15 mg/l was calculated. Mortality: One of the 12 animals died after 3 hours of treatment. After 7 hours of treatment 6 of 6 animals died. Clinical signs of toxicity: Lid closure, snout wiping, salivation, lacrimation, aqueous to reddish discharge from the nose as well as sticky eyes and dyspnea were observed. Necropsy:Necropsy of the animals that died revealed acute dilation of the heart (atrium) and acute congestion, whereas slight to moderate acute emphysema, often accompanied by edema, was seen in the lungs. Necropsy of the animals that were sacrificed at the end of the observation period revealed no abnormalities.
5.3	**Conclusion**	The exposure of rats at a temperature of 20 °C to air supplemented with volatile glutaraldehyde at a concentration of 15 mg/l resulted in 100% mortality
5.3.1	Reliability	**1**
5.3.2	Deficiencies	The study was conducted according to an accepted BASF Test method. GLP was not compulsory at the time the study was conducted.

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	May 12th, 2010
Materials and Methods	**3.1.2** This refers to Doc IIIA Section A2. **3.2.6** Reporting is poor, and depending on the interpretation, it can be concluded that the total number of animals used was 6, 12, 18 or 30.
Results and discussion	In general agree with applicant's version, but see conclusions below.
Conclusion	The whole body exposure of rats at a temperature of 20 °C to air supplemented with volatile glutaraldehyde at a calculated concentration of 15 mg/l resulted in 100 % mortality. The study has major deficiencies, including the following: • There are no details of the nature of vapour or the consistency of the exposure. • It cannot be concluded whether all the animals received the same amount of test substance. • Reporting is poor. Conclusion: The study used a high dose of glutaraldehyde and all animals died. The results are consistent with other studies of better quality and lower doses. The overall usability of the study is poor.
Reliability	3
Acceptability	Not acceptable
Remarks	

Section A6.1.3 _ 03	**Acute Toxicity**
Annex Point IIA6.1.3	**Inhalation Hazard Test, Rat**
	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 A6.1.4 _ 01 Acute Dermal Irritation

Annex Point IIA6.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994a) Study on the irritation to the intact dorsal skin of the albino rabbit (short-term test). [redacted] (Unpublished), (original German report dated 22 Dec 1981), BPD ID A6.01.4_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, but the study was based on the acknowledged Draize test	
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde approx. [redacted]% solution	
3.1.1	Lot/Batch number	Test substance no: [redacted]	
3.1.2	Specification	As given in section 2	x
3.1.2.1	Description	Liquid	
3.1.2.2	Purity	[redacted]% glutaraldehyde ([redacted]% water)	
3.1.2.3	Stability	About 12 months	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male/Female	
3.2.5	Age/weight at study initiation	The weight for the males ranged from 2.53 to 3.06 kg (Mean = 2.80 kg) The female weighed 3.17 kg	
3.2.6	Number of animals per group	3 male and one female rabbits were used for the test.	
3.2.7	Control animals	Untreated skin areas of the same animals served for control.	
3.3	**Administration/ Exposure**	**Dermal**	

Section A6.1.4 _ 01 Acute Dermal Irritation

Annex Point IIA6.4

3.3.1	Application		
3.3.1.1	Preparation of test substance	The 2.5 x 2.5 cm test patch was dipped into the undiluted test substance; about 0.5 ml test substance was so absorbed by the patch.	
3.3.1.2	Test site and Preparation of Test Site	The application site (2.5 x 2.5 cm) was located on the upper third of the back. The fur had been clipped at least 15 hours prior application.	
3.3.2	Occlusion	Occlusive	X
3.3.3	Vehicle	None	
3.3.4	Concentration in vehicle	None	
3.3.5	Total volume applied	About 0.5 ml	
3.3.6	Removal of test substance	With Lutrol followed by Lutrol:water (1:1)	
3.3.7	Duration of exposure	4 hours	
3.3.8	Post-exposure period	8 days	
3.3.9	Controls	Untreated skin areas served for control	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Dermal examination	Yes	

Section A6.1.4 _ 01 Acute Dermal Irritation

Annex Point IIA6.4

3.4.2.1 Scoring system

The assessment of the dermal findings was based on Draize JH (Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. In Dermal Toxicity, 46-59, 1959. Austin, Tex.: Association of Food and Drug Officials of the United States, Texas, State Department of Health). The evaluation of erythema and edema was based on following numerical scoring:

Evaluation of erythema and edema	
0	None
1	Questionable
2	Slight
3	Distinct
4	Very distinct

Following signs were used:

Explanation of signs	
SO	Irritation index could not be read because of staining due to the color of the test substance
ED	Edema
ED:e	Edema extending beyond the area of exposure
ER	Erythema
ER:e	Erythema extending beyond the area of exposure
SN	Spotted necrotic-like skin areas
N	Necrosis
N:su	Superficial necrosis
N:p	Parchment-like necrosis
N:l	Leathery necrosis
+	Findings confirmed by gross-pathology.

3.4.2.2 Examination time points

After 15 to 30 minutes following removal of the patch, and after 24 h, 48 h and 8 days following initiation of the application.

X

3.4.3 Other examinations

All animals were sacrificed at the end of the observation period and were subjected to necropsy. In case of clinical signs of necrosis, this was confirmed by gross-pathological examination of the incised skin.

3.5 Further remarks

None

4 RESULTS AND DISCUSSION

Section A6.1.4 _ 01 Acute Dermal Irritation

Annex Point IIA6.4

4.1 Average score

4.1.1 Erythema

After 4 hours of exposure				
Readings	Animal	Erythema	Edema	Signs
24 h	1	*	3	SO/ Ed:e
	2	*	3	SO/ Ed:e
	3	2	2	Er:e/Ed:e/SN
	4	2	2	Er:e/Ed:e
48 h	1	*	2	SO/ Ed:e
	2	*	3	SO/ Ed:e/SN
	3	2	1	Er:e/Ed:e
	4	2	1	Er:e/Ed:e
8 d	1	4	2	N:su/Ed:e/+
	2	4	2	N:p/Ed:e/+
	3	4	2	N:p/Ed:e/+
	4	4	2	N:l/Ed:e/+

*, According to the author, no reading was possible because of the staining due to the color of the test substance.

4.1.2 Edema — See table above

4.2 Reversibility — No reversibility of the effects was noticeable.

4.3 Other examinations — The clinical signs of necrosis were confirmed by gross-pathological examination of the incised skin (see + sign in table above)

4.4 Overall result — The findings indicate that the test substance was corrosive to the skin of rabbit.

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.1.4 _ 01 Acute Dermal Irritation

Annex Point IIA6.4

5.1	**Materials and methods**	The aim of the present study was to look for the irritating potential of glutaraldehyde to the intact skin of rabbit within a short term- test. Test substance: Glutaraldehyde approx. [redacted] solution The test was conducted according to Draize JH (Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. In Dermal Toxicity, 46-59, 1959. Austin, Tex.: Association of Food and Drug Officials of the United States, Texas, State Department of Health), without GLP. Three male and one female [redacted] rabbits with a mean weight of 2.80 kg for the males and 3.17 kg for the female were used for the test. Approximatively 15 hours prior application, the fur of the rabbits was clipped; the chosen application site (2.5 x 2.5 cm) was located on the upper third of the back. For test application, a patch (2.5 x 2.5 cm) was dipped into the unchanged test substance and was placed on the application site; the application volume of the test substance was about 0.5 ml. The application was performed under occlusive conditions. The exposure period was 4 hours. Untreated skin areas of the test animals served for control. Following application, the test substance was removed from the skin by washing with Lutrol, followed by Lutrol: water (1:1). Readings of the skin were performed after 15 to 30 minutes following removal of the test patch and thereafter, after 24 h, 48 h and 8 days following test initiation. The assessment of the findings was based on Draize JH (1959). At the end of the observation period of 8 days, the animals were sacrificed for the purpose of necropsy. Clinical signs of necrosis were confirmed by gross-pathological examination of the incised skin.
5.2	**Results and discussion**	<u>Summarized findings after 4 hours of exposure:</u> At reading time point 24 h, erythema could not be assessed in two cases because of the staining of the skin due to the color of the test substance; in the remaining two animals (one male, one female), slight erythema was visible. Edema was seen in all cases and ranged from slight (one male and one female) to distinct (two males). Erythema and edema were extended beyond the application areas; in one male spotted necrotic-like skin areas were seen. At reading time point 48 h, erythema was as described above; edema was still seen in all animals and ranged from questionable to distinct. Erythema and edema still were extended beyond the application areas and the spotted necrotic-like skin areas of the one male still were visible. At reading time point 8 d, all animals displayed very distinct erythema and slight edema; edema was extended beyond the application area. One male showed superficial necrosis whereas the two others displayed parchment-like necrosis of the skin; the female showed leathery necrosis. For all animals, the onset of necrosis was confirmed by gross pathology.
5.3	**Conclusion**	The findings reported above indicate that the test substance glutaraldehyde was corrosive to the skin of rabbit.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	No

Section A6.1.4 _ 01 Acute Dermal Irritation

Annex Point IIA6.4

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 15th, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2. 3.3.2 Occlusion is not mentioned in the study report. 3.4.2.2 The readings at 15-30 min after removing the patch are not included in the study report (also valid for 5.1). Otherwise agree with applicant's version.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version: Glutaraldehyde was corrosive to the skin of rabbit. Risk phrase R34 "Causes burns" is warranted.
Reliability	1
Acceptability	Acceptable
Remarks	Key study. 1.1 Reference. Apparently the given Report number is not correct, and refers to the test substance number (80/265). This same Report number refers to several studies.
	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 A6.1.4 _ 02 Acute Dermal Irritation

Annex Point IIA6.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994b) Study on the irritation to the intact dorsal skin of the albino rabbit (short-term test). [redacted] (Unpublished), (original German report dated 22 Dec 1981), BPD ID A6.01.4_02	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, but the study was based on the acknowledged Draize test	
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde approx. [redacted]% solution	
3.1.1	Lot/Batch number	Test substance no: [redacted]	
3.1.2	Specification	As given in section 2	x
3.1.2.1	Description	Liquid	
3.1.2.2	Purity	[redacted]% glutaraldehyde (ca [redacted]% water)	
3.1.2.3	Stability	About 12 months	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Animal 1: about 2.23 kg Animal 2: about 2.17 kg	
3.2.6	Number of animals per group	2 female rabbits were used for the test.	
3.2.7	Control animals	Untreated skin areas of the same animals served for control.	
3.3	**Administration/ Exposure**	**Dermal**	

Section A6.1.4 _ 02 Acute Dermal Irritation

Annex Point IIA6.4

3.3.1	Application		
3.3.1.1	Preparation of test substance	The 2.5 x 2.5 cm test patch was dipped into the undiluted test substance; about 0.5 ml test substance was so absorbed by the patch.	
3.3.1.2	Test site and Preparation of Test Site	The application site (2.5 x 2.5 cm) was located on the upper third of the back. The fur had been clipped at least 15 hours prior application.	
3.3.2	Occlusion	Occlusive	X
3.3.3	Vehicle	None	
3.3.4	Concentration in vehicle	None	
3.3.5	Total volume applied	About 0.5 ml	
3.3.6	Removal of test substance	With Lutrol followed by Lutrol:water (1:1)	
3.3.7	Duration of exposure	3 minutes and one hour	
3.3.8	Post-exposure period	8 days	
3.3.9	Controls	Untreated skin areas of the same animals served for control.	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Dermal examination	Yes	

Section A6.1.4 _ 02 Acute Dermal Irritation

Annex Point IIA6.4

3.4.2.1 Scoring system

The assessment of the dermal findings was based on Draize JH (Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. In Dermal Toxicity, 46-59, 1959. Austin, Tex.: Association of Food and Drug Officials of the United States, Texas, State Department of Health). The evaluation of erythema and edema was based on following numerical scoring:

Evaluation of erythema and edema	
0	None
1	Questionable
2	Slight
3	Distinct
4	Very distinct

Following signs were used:

Explanation of signs	
SO	Irritation index could not be read because of staining due to the color of the test substance
ED	Edema
ED:e	Edema extending beyond the area of exposure
ER	Erythema
ER:e	Erythema extending beyond the area of exposure
S	Scaling
S:s	Severe scaling

3.4.2.2 Examination time points

After 15 to 30 minutes following removal of the patch, and after 24 h, 48 h and 8 days following initiation of the application.

3.4.3 Other examinations

All animals were sacrificed at the end of the observation period and were subjected to necropsy. In case of clinical signs of necrosis, this was confirmed by gross-pathological examination of the incised skin.

3.5 Further remarks

None

4 RESULTS AND DISCUSSION

Section A6.1.4 _ 02 Acute Dermal Irritation

Annex Point IIA6.4

4.1 Average score

4.1.1 Erythema

After 3 minutes of exposure				
Readings	Animal	Erythema	Edema	Signs
24 h	1	1	0	
	2	1	0	
48 h	1	0	0	
	2	1	0	
8 d	1	0	0	
	2	0	0	S
After 1 hour of exposure				
Readings	Animal	Erythema	Edema	Signs
24 h	1	*	2	SO
	2	*	2	SO
48 h	1	2	2	ER:e / ED:e
	2	*	2	SO / ED:e
8 d	1	2	1	ER:e / S:s
	2	2	1	S:s / ED:e

*, According to the author, no reading was possible because of the staining due to the color of the test substance.

4.1.2 Edema — See table above

4.2 Reversibility — Following exposure for 3 minutes, all effects disappeared within 8 days. Following exposure for 1 hour, no clear reversibility of the effects was noticeable. X

4.3 Other examinations — None

4.4 Overall result — The findings indicate that the test substance was irritating to the skin of rabbit.

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.1.4 _ 02 Acute Dermal Irritation

Annex Point IIA6.4

5.1	**Materials and methods**	The aim of the present study was to look for the irritating potential of glutaraldehyde to the intact skin of rabbit within a short term- test. Test substance: Glutaraldehyde approx. [redacted]% solution The test was conducted according to Draize JH (Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. In Dermal Toxicity, 46-59, 1959. Austin, Tex.: Association of Food and Drug Officials of the United States, Texas, State Department of Health), without GLP. Two [redacted] female rabbits with a mean weight of 2.20 kg were used for the test. Approximatively 15 hours prior application, the fur of the rabbits was clipped; the chosen application site (2.5 x 2.5 cm) was located on the upper third of the back. For test application, a patch (2.5 x 2.5 cm) was dipped into the unchanged test substance and was placed on the application site; the application volume of the test substance was about 0.5 ml. The application was performed under occlusive conditions. Two exposure periods were considered: 3 minutes and one hour. Untreated skin areas of the test animals served for control. Following application, the test substance was removed from the skin by washing with Lutrol, followed by Lutrol: water (1:1). Readings of the skin were performed after 15 to 30 minutes following removal of the test patch and thereafter, after 24 h, 48 h and 8 days following test initiation. The assessment of the findings was based on Draize JH (1959). At the end of the observation period of 8 days, the animals were sacrificed for the purpose of necropsy.	X
5.2	**Results and discussion**	<u>Summarized findings after 3 minutes of exposure:</u> At reading time point 24 h both animals showed questionable erythema whereas no signs of edema were seen. After 48 hours, questionable erythema only remained visible in one case. Excepted for scaling seen in one animal, no more effects were seen after 8 days. <u>Summarized findings after 1 hour of exposure:</u> At reading time point 24 h, erythema could not be assessed. According to the author, this was because of the staining of the skin due to the color of the test substance. Slight edema was seen in both animals. After 48 hours, slight erythema, extended beyond the application area, became visible in one case, whereas for the second animal, assessment of erythema still was not possible. Slight edema still was seen but was now extended beyond the application area. After 8 days, slight erythema was reported for both animals; in one case erythema was extended beyond the application area; edema became questionable. Both animals showed severe scaling.	
5.3	**Conclusion**	The findings reported above indicate that the test substance glutaraldehyde was irritating to the skin of rabbit.	
5.3.1	Reliability	**1**	
5.3.2	Deficiencies	No	

Section A6.1.4 _ 02 Acute Dermal Irritation

Annex Point IIA6.4

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 15th, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2. 3.3.2 Occlusion is not mentioned in the study report. 3.4.2.2 The readings at 15-30 min after removing the patch are not included in the study report (also valid for 5.1). Otherwise agree with applicant's version.
Results and discussion	Following exposure for 3 minutes, all effects did not disappear within 8 days, but there was scaling on day 8.
Conclusion	The methodology used is different from the current guidelines. The conclusion can be made that an exposure of 1 h causes irritation that is not reversible in 8 days.
Reliability	2
Acceptability	Acceptable
Remarks	1.1 Reference. Apparently the given Report number is not correct, and refers to the test substance number ([redacted]). This same Report number refers to several studies.
	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 A6.1.4 _ 03 Acute Eye Irritation

Annex Point IIA6.1.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1994c) Report on the study of the irritation to the eye of white rabbits based on Draize. [redacted] (Unpublished), (original German report dated 22 Dec 1981). BPD ID A6.01.4_03	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, but the study was based on the acknowledged Draize test	
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde [redacted]%	
3.1.1	Lot/Batch number	Test substance number: [redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Aqueous solution [redacted]	
3.1.2.2	Purity	[redacted]% glutaraldehyde ([redacted]% water)	
3.1.2.3	Stability	12 months	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Mean weight of the males: 2.75 kg Mean weight of the females: 2.74 kg	
3.2.6	Number of animals per group	6 (3 per sex)	
3.2.7	Control animals	The untreated eye of each test animal served for control.	

Section A6.1.4 _ 03 Acute Eye Irritation

Annex Point IIA6.1.4

3.3	**Administration/ Exposure**	**Instillation**
3.3.1	Preparation of test substance	The test substance was applied unchanged.
3.3.2	Amount of active substance instilled	0.1 ml of the test substance was applied into the conjunctival sac of the right eyelid.
3.3.3	Exposure period	The test substance was not washed out.
3.3.4	Post-exposure period	8 days

Section A6.1.4 _ 03 Acute Eye Irritation

Annex Point IIA6.1.4

3.4	**Examinations**	
3.4.1	Ophthalmoscopic examination	The tested eyes were examined for signs of irritation.

Section A6.1.4 _ 03 Acute Eye Irritation

Annex Point IIA6.1.4

3.4.1.1 Scoring system

The assessment of the findings in the eye was based on Draize JH (Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. In Dermal Toxicity, 46-59, 1959). The scale for scoring was as follows:

Eye		G	Definition of the grading (G)
Cornea	Opacity	0	None
		1	Scattered or diffuse, iris clearly visible
		2	Easily discernible translucent areas, details of iris slightly obscured
		3	Opalescent areas, no details of iris visible, size of pupil barely discernible
		4	Total, iris invisible
	Area involved	1	> 0, <= 0.25 of the area
		2	> 0.25, < 0.50 of the area
		3	> 0.50, < 0.75 of the area
		4	> 0.75, <= 1 of the area
Conjunctiva	Redness	0	Normal vessels
		1	Injected vessels (above normal)
		2	Diffuse, deeper crimson red, individual vessels not easily discernible
		3	Diffuse beefy red
	Chemosis	0	No swelling
		1	Any swelling above normal (including nictitating membrane)
		2	Obvious swelling with partial eversion of the lids
		3	Swelling with half closed lids
		4	Swelling with half to completely closed lids
	Discharge	0	None
		1	Any amount different from normal
		2	Discharge with moistening of lids and adjacent hairs
		3	Discharge with moistening of lids, adjacent hairs and a considerable area around the eye
Iris	0	Normal	
	1	Folds above normal, swelling, circum-corneal injection, still reacting to light	
	2	No reaction to light, hemorrhage, destruction	

Maximal score for cornea: cornea grading x area grading x 5 = 80

Maximal score for conjunctivae: (Redness + Chemosis + Discharge) x 2 = 20

Maximal score for iris: Value x 5 = 10

Section A6.1.4 _ 03 Acute Eye Irritation

Annex Point IIA6.1.4

3.4.1.2	Examination time points	1, 24, 48 and 72 hours after application, and 8 days after application
3.4.2	Other investigations	None
3.5	**Further remarks**	None

4 RESULTS AND DISCUSSION

4.1 Clinical signs No effects reported

4.2 Average score

4.2.1 Cornea

Scoring of the findings in the cornea (mean score of 6 animals):

Cornea		
Readings	Opacity	Area involved
24 h	1	4
48 h	2	4
72 h	3	4
8 d	3.5	4

4.2.2 Iris

Scoring of the findings in the Iris (mean score of 6 animals):

Iris	
Readings	Value
24 h	1
48 h	1
72 h	1
8 d	1

4.2.3 Conjunctiva

4.2.3.1 Redness

Scoring of redness in the conjunctivae (mean score of 6 animals):

Conjunctiva	
Readings	Redness
24 h	2
48 h	2
72 h	2.5
8 d	2.3

4.2.3.2 Chemosis

Scoring of chemosis in the conjunctivae (mean score of 6 animals):

Conjunctiva	
Readings	Chemosis
24 h	2
48 h	2
72 h	3
8 d	3

Section A6.1.4 _ 03 Acute Eye Irritation

Annex Point IIA6.1.4

4.2.3.3 Discharge

Scoring of discharge in the conjunctivae (mean score of 6 animals):

Conjunctiva	
Readings	Discharge
24 h	1
48 h	1
72 h	2
8 d	2

4.3 Reversibility

No

4.4 Other

Further signs:

Readings	Signs
24 h	Two cases of contracted pupils
48 h	One case of small retractions in the eyelid; Three cases of contracted pupils
72 h	Suppuration in all animals; Three cases of small retractions in the eyelid
8 d	Suppuration in all animals; Small retractions in the eyelid in all animals Loss of hair in the margin of the eyelid In three cases, the irritation index could not be read because of corneal opacity

4.5 Overall result

The mean irritation index at each reading time point was as follows:

Readings	Mean Irritation Index
24 h	39
48 h	56
72 h	80
8 d	90

A primary irritation index of 58 was determined. The test substance was highly irritating to the eye of rabbits.

Section A6.1.4 _ 03 Acute Eye Irritation

Annex Point IIA6.1.4

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to look for the irritating potential of glutaraldehyde to eye of rabbit.

Test substance: Glutaraldehyde approx. [redacted]% solution

The test was conducted according to Draize JH (Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. In Dermal Toxicity, 46-59, 1959. Austin, Tex.: Association of Food and Drug Officials of the United States, Texas, State Department of Health), without GLP.

A volume of 0.1 ml of the unchanged test substance was instilled into the right eye of each of 6 [redacted] rabbits of both sex. The test substance was not washed out and the animals were scored for corneal changes, iris effects and conjunctival reaction, after 1, 24, 48 and 72 hours and after 8 days following test substance application. The untreated eye of each rabbit served for control. The assessment of the findings was based on the scoring system of Draize JH (1959).

5.2 Results and discussion

The main findings can be summarized as follows:

Reading of the cornea revealed increasing opacity, ranging from scattered or diffuse (24 h) to nearly complete (8 d); the involved area was > 0.75, <= 1.

Reading of the iris revealed folds above normal, swelling and circum-corneal injection over the whole observation period. The iris was still reacting to light.

Reading of the conjunctiva revealed redness (diffuse, deeper crimson red, with individual vessels, which were not easily discernible); swelling (Obvious swelling with partial eversion of the lids during the first 48 hours, and swelling with half-closed lids seen until day 8) and eye discharge, which was above normal during the first 48 hours and resulted in moistening of lids and adjacent hairs within the following days (until day 8).

Additional effects included contraction of pupils, small retractions in the eyelid, suppuration and loss of hair in the margin of the eyelid. These effects increased with time and no reversibility of the findings in the eyes was evident. A primary irritation index of 58 was determined.

5.3 Conclusion

The test substance was highly irritating to the eye of rabbits.

5.3.1 Reliability **1**

5.3.2 Deficiencies No

Section A6.1.4 _ 03 Acute Eye Irritation

Annex Point IIA6.1.4

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 16th, 2010
Materials and Methods	Agree with applicant's version.
	3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version:
	The test substance was highly irritating to the eye of rabbits. The risk phrase R41 is warranted: Risk of serious damage to eyes. CLP: Classification in Category 1 for irreversible effects on the eye is warranted.
Reliability	2 (due to insufficient documentation)
Acceptability	Acceptable
Remarks	1.1 Reference. Apparently the given Report number is not correct, and refers to the test substance number ([redacted]). This same Report number refers to several studies.
	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 A6.1.4 _ 04 Respiratory sensory irritation in mice

Annex Point IIA6.1.5

			Official use only
		1 REFERENCE	
1.1	**Reference**	Werley MS, Burleigh-Flayer HD, Ballantyne B (1995) Respiratory peripheral sensory irritation and hypersensitivity studies with glutaraldehyde vapor. Toxicol. Ind. Health 11(5): 489-501 (Published), BPD ID A6.01.4 _ 04	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde 51%	
3.1.1	Lot/Batch number	No data provided	
3.1.2	Specification	As given in section 2	x
3.1.2.1	Description	Aqueous solution	
3.1.2.2	Purity	[redacted]% (shown analytically)	
3.1.2.3	Stability	No data provided	
3.1.2.4	Preparation of test substance for application	Vapor of glutaraldehyde was generated dynamically by passing purified pressurized air from a cylinder at a constant rate through a gas washing bottle containing the test substance. The vapor from the generation bottle was then conducted through a further gas washing bottle containing a fritted disc for removal of any aerosols. The bottles were joined to one side of a T-tube. Diluting air was introduced through the other side to get the wanted glutaraldehyde vapor concentrations. Absence of aerosols was confirmed by visual observation of a light beam (Tyndall effect), and samples were taken for particle size analysis (TSI Aerodynamic Particle Sizer).	

Section A6.1.4 _ 04 Respiratory sensory irritation in mice

Annex Point IIA6.1.5

3.2	**Test Animals**	
3.2.1	Species	Mice
3.2.2	Strain	[redacted]
3.2.3	Source	[redacted]
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	45 to 55 days old weighing between 23 and 26 g
3.2.6	Number of animals per group	4 animals/ group
3.3	**Administration/ Exposure**	
3.3.1	Test conduct	For the purpose of the PSI test, groups of 4 mice were exposed to 1.60, 3.99, 4.65, 5.60, 7.47, 17.7 and 36.7 ppm glutaraldehyde vapor; the animals were head-only exposed for 30 minutes in plethysmographs. The respiration rate (RR) was measured by recording pressure changes due to breathing movements, using a pressure transducer. The pressure signals were amplified and displayed on a writing oscilloscope/chart recorder, and analog signals were digitalized for computer recording every 15 seconds of RR according to Burleigh-Flayer et al. (Burleigh-Flayer H, Schaper M, Thompson R, Alarie Y (1988) Computerization of pulmonary function studies in laboratory animals. Toxicologist 8: 142). Prior to the 30 minutes glutaraldehyde vapor exposure, the mice were subjected to a 10 minutes acclimatization period followed by a 10 minutes period where they were head-only exposed to air for determination of the baseline respiration rate (RR). After the 30 minutes of exposure to glutaraldehyde vapor, the mice again were subjected to a 10 minutes period of post-exposure recovery with air. From the changes in RR as a function of glutaraldehyde vapor concentration, the RD_{50} (concentration resulting in 50% decrease in RR) was calculated. Temperature: 21.6 – 24.1 °C Relative humidity: 41 - 57%
3.4	**Examinations**	
3.4.1	Peripheral sensory irritation potential (PSI)	Treatment-related changes in respiratory rate were measured; from the changes in RR as a function of glutaraldehyde vapor concentration, the RD_{50} (concentration resulting in 50% decrease in RR) was calculated, which served for determination of the vapor test concentration to be used for induction exposure within the main test. The background is, that the concentration to be used for induction must be a concentration known to be irritant, and the respiratory sensitizing effect occurs at concentrations within the range inducing PSI. The mice were furthermore observed for symptoms of toxicity and body weight changes over an observation period of 7 days following exposure.

Section A6.1.4 _ 04 Respiratory sensory irritation in mice

Annex Point IIA6.1.5

3.5 Further remarks

Monitoring of the glutaraldehyde vapor concentrations:

The glutaraldehyde vapor concentration during exposure was monitored periodically. The number of sampling during the PSI test ranged from 1 (at 1.60 ppm) to 3 samples of 2 to 12 minutes duration, depending on the test concentration. Glutaraldehyde was measured by spectrophotometry.

4 RESULTS AND DISCUSSION

4.1 Analytical monitoring

The measured concentrations of glutaraldehyde vapor during the preliminary PSI test were as follows:

1.60, 3.99 +/- 0.95, 4.65 +/- 0.68, 5.60 +/- 0.15, 7.47 +/- 0.84, 17.70 +/- 4.92, 36.7 +/- 4.47 ppm

4.2 Results of PSI test

Symptoms of toxicity and body weight:

No symptoms of toxicity were seen. One animal of the 1.60 ppm group showed corneal opacity in the left eye, which persisted for 7 days and was considered to be due to corneal dehydration. Excepted for the 4.65 ppm group where no change was measured, all groups gained body weight over the experimental period.

Mean maximum decrease in respiratory rate (RR) in the mice exposed to different concentrations of glutaraldehyde vapor for 30 minutes:

See figures 4.2.1 and 4.2.2.

Glutaraldehyde concentrations (measured means +/-SD)	Mean maximum decrease in respiratory rate
1.60 ppm	26.4%
3.99 +/- 0.95 ppm	30.2%
4.65 +/- 0.68 ppm	41.5%
5.60 +/- 0.15 ppm	39.6%
7.47 +/- 0.84 ppm	41.1%
17.70 +/- 4.92 ppm	57.1%
36.70 +/- 4.47 ppm	59.0%

The respiratory rate decreased in all glutaraldehyde treated groups almost immediately following exposure starting; a plateau was reached within 5 to 10 minutes, as shown in the figure below (figure 4.2.1). Figure 4.2.2 shows that the decrease in respiratory rate was related to prolongation of the expiratory phase of the respiratory cycle; this resulted in a notching indicative of peripheral sensory irritation of the respiratory tract. A clear effect-concentration relationship was evident. During the post-exposure period, the respiratory rate increased again but without reaching control values. The RD_{50} was calculated to be 13.86 ppm (95% confidence limits: 9.86 – 23.58 ppm).

4.3 Overall result

Glutaraldehyde was found to be a moderately potent peripheral sensory irritant in mice.

Section A6.1.4 _ 04 Respiratory sensory irritation in mice

Annex Point IIA6.1.5

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

A peripheral sensory irritation (PSI) study was conducted with male [redacted] mice for determination of the respiratory irritating potential of glutaraldehyde.

Test substance: Glutaraldehyde [redacted]% aqueous solution

Groups of 4 mice (45 - 55 days old, 23 - 26 g) each were head-only exposed for 30 minutes to 1.60, 3.99, 4.65, 5.60, 7.47, 17.7 and 36.7 ppm glutaraldehyde vapor (in plethysmographs). The respiration rate (RR) was measured using a pressure transducer; the pressure signals were converted into computerized data according to Burleigh-Flayer et al. (1988). Prior to glutaraldehyde vapor exposure, the mice were subjected to a 10 minutes exposure to air for determination of the baseline respiration rate (RR). The 30 minutes exposure to glutaraldehyde vapour was followed by a 10 minutes post-exposure recovery period with air. From the changes in RR as a function of glutaraldehyde vapor concentration, the RD_{50} was calculated. The mice also were observed for symptoms of toxicity and body weight changes.

Generation of the glutaraldehyde vapor: The test vapor was generated dynamically by passing purified pressurized air from a cylinder at a constant rate through two gas washing bottles containing the test material. Absence of aerosols was confirmed by visual observation of a light beam (Tyndall effect), and samples were taken for particle size analysis (TSI Aerodynamic Particle Sizer). The glutaraldehyde vapor concentration was monitored periodically during the both, the preliminary and the main test.

5.2 Results and discussion

The respiratory rate decreased in all glutaraldehyde treated groups almost immediately following exposure starting; a plateau was reached within 5 to 10 minutes. The decrease in respiratory rate was related to prolongation of the expiratory phase of the respiratory cycle; this resulted in a notching indicative of peripheral sensory irritation of the respiratory tract. A clear effect-concentration relationship was evident. During the post-exposure period, the respiratory rate increased again but without reaching control values. The RD_{50} was calculated to be 13.86 ppm (95% confidence limits: 9.86 – 23.58 ppm). Excepted for one case of corneal opacity (seen at 1.60 ppm), no symptoms of toxicity were seen. Excepted for the 4.65 ppm group where no change was measured , all groups gained body weight over the experimental period.

5.3 Conclusion

Glutaraldehyde was found to be a moderately potent peripheral sensory irritant in mice.

5.3.1 Reliability

2

5.3.2 Deficiencies

The authors made an attempt to investigate the respiratory sensitizing potential of glutaraldehyde in male [redacted] guinea pigs. The data reported within the present summary refer to a preliminary respiratory sensory irritation test (PSI) conducted with male [redacted] mice for determination of the test concentration to be used for induction within the main sensitization test.

X

Section A6.1.4 _ 04 Respiratory sensory irritation in mice

Annex Point IIA6.1.5

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 19th, 2010
Materials and Methods	Agree with applicant's version. 3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. Glutaraldehyde was described as a moderately potent peripheral sensory irritant in mice.
Reliability	2
Acceptability	Acceptable
Remarks	5.3.2. Deficiences. The statement is correct but appears to be misplaced as this is not a deficiency. The RMS has included Figure RMS below, indicating the results on a logarithmic scale. This allows an approximate derivation of RD10, albeit with considerable uncertainties. The derived RD10 value is approximately 0.4 ppm (400 ppb).
	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 A6.1.4 _ 04 Respiratory sensory irritation in mice

Annex Point IIA6.1.5

Figure 4.2.1 Preliminary peripheral sensory irritation test: Respiratory rate in mice exposed to glutaraldehyde vapor

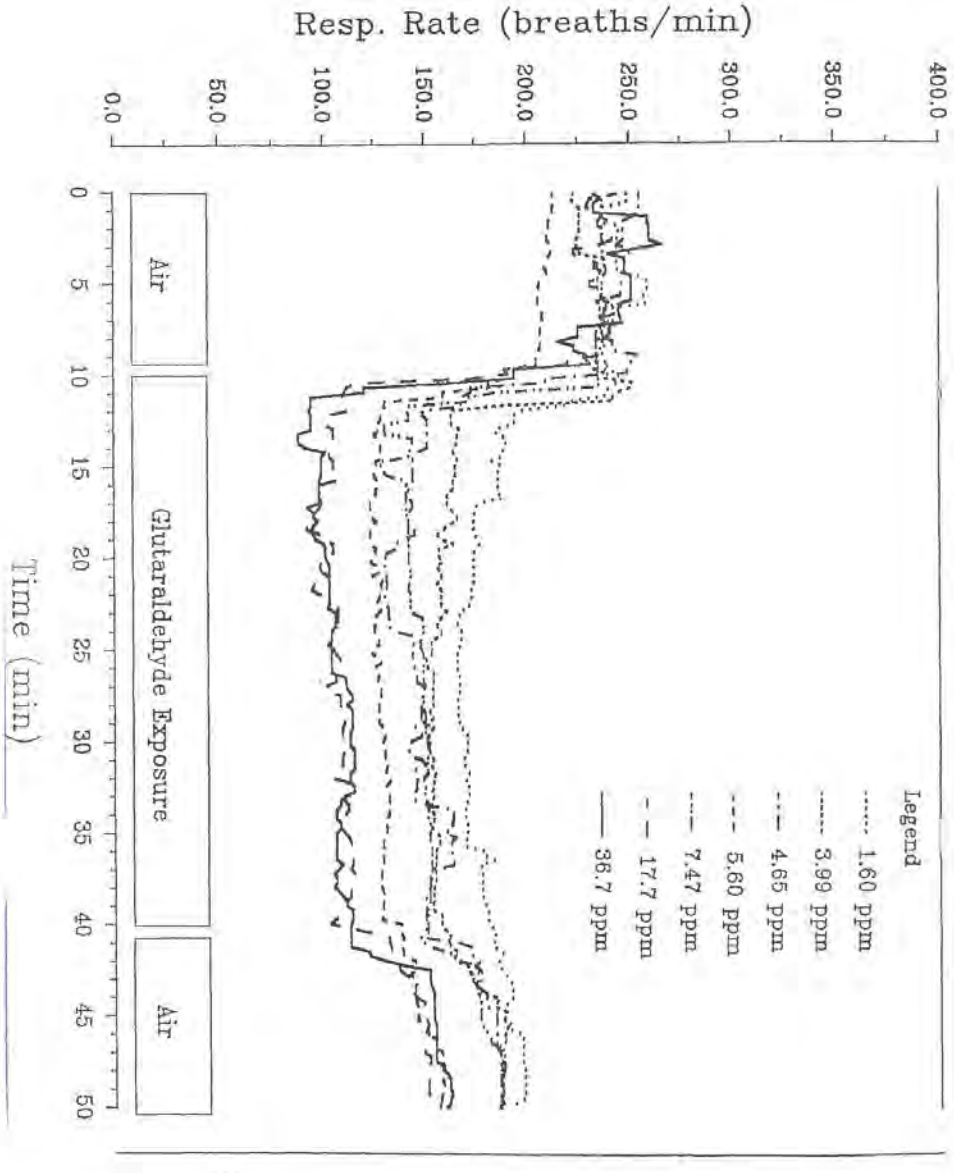
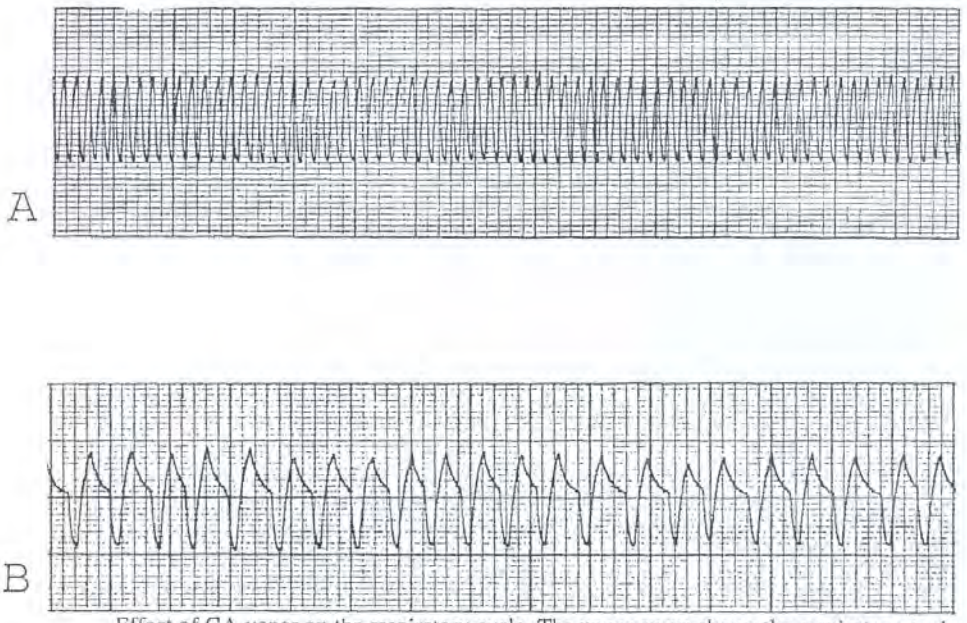


Figure 4.2.2 Preliminary peripheral sensory irritation test: Respiratory cycle of mice exposed to glutaraldehyde vapor

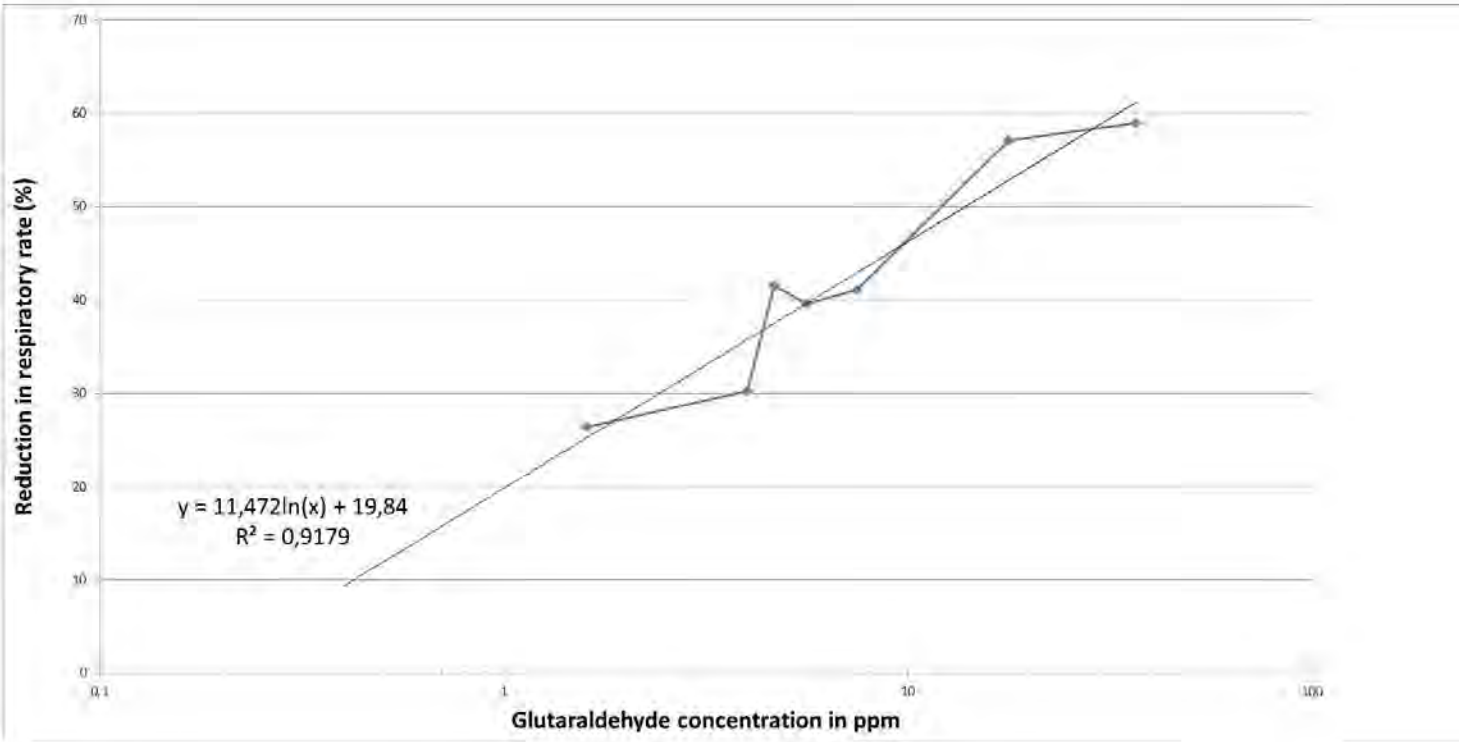
Section A6.1.4 _ 04 Respiratory sensory irritation in mice

Annex Point IIA6.1.5



Effect of GA vapor on the respiratory cycle. The upper curve shows the respiratory cycles for a mouse during the 10-min air preexposure period. The lower chart shows a change in the rate and shape of the respiratory cycle during exposure of the same mouse to GA vapor. The expiratory phase of the respiratory cycle is prolonged due to notching.

Figure RMS. Reduction in respiratory rate as a function of glutaraldehyde concentration.



Section A6.1.5 _ 01 Skin sensitization

Annex Point IIA6.1.5 Open epicutaneous test with Guinea Pig

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1975) Bericht über die Prüfung von Methoxidihydropyran im Vergleich zu Methoxidihydropyran, roh und [redacted] auf etwaige hautsensibilisierende Wirkung. [redacted] (Unpublished), (report in German), BPD ID A6.01.5_01	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, However, the study was performed under standardized conditions according to an acceptable BASF test.	
2.2	**GLP**	No, No, GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] (Glutardialdehyde, [redacted]% aqueous solution)	
3.1.1	Lot/Batch number	No data	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Liquid	
3.1.2.2	Purity	About [redacted]% glutaraldehyde (ca. [redacted]% water)	X
3.1.2.3	Stability	-	
3.1.2.4	Preparation of test substance for application	a) <u>for induction:</u> Test substance was used unchanged b) <u>for challenge:</u> Test substance was used unchanged	
3.1.2.5	Pre-test performed on irritant effects	No	

Section A6.1.5 _ 01 **Skin sensitization**

Annex Point IIA6.1.5 **Open epicutaneous test with Guinea Pig**

3.2	**Test Animals**	
3.2.1	Species	Guinea pigs
3.2.2	Strain	[redacted]
3.2.3	Source	Not specified
3.2.4	Sex	Females
3.2.5	Age/weight at study initiation	No data
3.2.6	Number of animals per group	10
3.2.7	Control animals	Yes (3)
3.3	**Administration/ Exposure**	State study type: Non-Adjuvant
3.3.1	Induction schedule	Within the fore region of each flank of each animal, a skin area of about 25 cm2 was clipped. After 4 hours, the application site for induction, which was chosen to be on the left flank, was degreased using ether. The test substance was then applied unchanged on this area using a piece of cotton wool. The application was cross-shaped and was repeated 3 times. The application was repeated once daily, on five consecutive days per week, over two weeks (total of 10 applications).
3.3.2	Way of Induction	Topical Open
3.3.3	Concentrations used for induction	The test substance was applied unchanged to the skin.
3.3.4	Concentration Freund's Complete Adjuvant (FCA)	Not relevant
3.3.5	Challenge schedule	Challenge was performed after a period of 11 days following induction. For this purpose, both flanks of each animal were clipped. After 4 hours, the right flank of each animal was degreased using ether and the unchanged test substance was applied to the skin (single application). The control animals were treated similarly.
3.3.6	Concentrations used for challenge	The test substance was applied unchanged to the skin.
3.3.7	Rechallenge	No
3.3.8	Scoring schedule	After 12 hours following challenge.
3.3.9	Removal of the test substance	None
3.3.10	Positive control substance	None

Section A6.1.5 _ 01 **Skin sensitization**

Annex Point IIA6.1.5 **Open epicutaneous test with Guinea Pig**

3.4 Examinations The animals were examined for skin reaction.

3.5 Further remarks None

4 RESULTS AND DISCUSSION

4.1 Results of pilot studies None

4.2 Results of test

4.2.1 Induction

Animals/sex	Treatment	Application site	Result
10 / females	Test substance applied unchanged, 10 applications over 2 weeks)	Left flank, clipped 25 cm2 area within the fore region	All animals showed thick bloody scabs

4.2.2 Challenge

Animals/sex	Treatment	Application site	Result after 12 h
10 induced females	Test substance applied unchanged, Single application	Right flank, clipped	All animals showed slight spot-like redness of the skin and brownish residues of the test substance.
Control, 3 untreated females			None of the control animal showed skin reaction. Brownish test substance residues on the skin were seen.

X

4.3 Overall result All 10 treated animals showed a slight spot-like redness of the skin, and brownish residues of the test substance. The control animals showed no skin reactions. These results indicate that the test substance was sensitizing to guinea pig in the open epicutaneous test.

Section A6.1.5 _ 01 **Skin sensitization**

Annex Point IIA6.1.5 **Open epicutaneous test with Guinea Pig**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present test was to look for the sensitizing potential of glutaraldehyde using the open epicutaneous test with guinea pig.

Test substance: [redacted] (glutardialdehyde [redacted] aqueous solution); composition: ca. [redacted]% glutardialdehyde [redacted] [redacted]% water.

No guideline, but the study was based on an acknowledged standardized test method and was conducted according to an acceptable BASF test. No GLP, as GLP was not compulsory at the time the study was performed.

For induction, the unchanged test substance was applied to the shaved left flank (area 25 cm^2) of 10 [redacted] Guinea pigs. Application was performed once daily, for 5 consecutive days, over 2 weeks (total of 10 applications). The shaved right flank remained untreated.

Following an 11-day recovery period, both flanks were shaved once again and the unchanged test substance was applied, this time to the right flank of each treated animal (single application). Three control animals, which had not been previously subjected to induction, were treated similarly. Skin reactions were recorded after 12 hours.

5.2 Results and discussion

Induction:

Following induction the skin of all 10 treated animals showed thick bloody scabs.

Challenge:

Whereas the control animals showed no skin reaction, slight spot-like redness of the skin was reported for all treated animals. Both, the treated and control animals displayed brownish residues of the test substance on the skin.

X

5.3 Conclusion

Glutaraldehyde was sensitizing to guinea pig in the open epicutaneous test.

5.3.1 Reliability

2

5.3.2 Deficiencies

The study was conducted prior to the implementation of the actual guidelines and GLP was not compulsory at the time the study was performed. However, the study was based on an acknowledged standardized test method and was conducted according to an acceptable BASF test; basic data were given.

Section A6.1.5 _ 01 Skin sensitization

Annex Point IIA6.1.5 **Open epicutaneous test with Guinea Pig**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 19th, 2010
Materials and Methods	Agree with applicant's version. 3.1.2 This refers to Doc IIIA Section A2. 3.1.2.2 The product used in this study ([redacted], which is higher than [redacted] reported for the Annex I application (in Doc IIB).
Results and discussion	4.2.2. Challenge. The wording is different in the translation of the study report: the effect was not "slight spot-like redness of the skin" but "spotted erythema" or erythema/distinct/spotted as given in the tabulated results. 5.2 Results and discussion. See comment for 4.2.2 above. The effect [redacted] on the results has not been stipulated. The test provides information on the sensitising properties of [redacted] and can be considered indicative for glutaraldehyde.
Conclusion	Agree with applicant's version. The test substance was sensitizing to guinea pig in the open epicutaneous test. Risk phrase **R43 "May cause sensitization by skin contact"** is warranted.
Reliability	2
Acceptability	Acceptable
Remarks	1.1 Reference. A translation was subsequently provided, entitled "Report on the study of methoxy-dihydropyrane compated with crude methoxy-dihydropyrane and [redacted] for possible sensitization", dated November 28th, 2007. Key study
	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 A6.1.5 _ 02 Skin sensitisation

Annex Point IIA6.1.5 **Mouse local lymph node assay and cytokine response following sensitisation**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Ulrich P, Grenet O, Bluemel J, Vohr HW, Wiemann C, Grundler O, Suter W (2001) Cytokine expression profiles during murine contact allergy: T helper 2 cytokines are expressed irrespective of the type of contact allergen. Arch. Toxicol. 75: 470-479 (Published), BPD ID A6.01.5_02_a Ulrich P, Streich J, Suter W (2001) Intralaboratory validation of alternative endpoints in the murine local lymph node assay for the identification of contact allergic potential: primary ear skin irritation and ear-draining lymph node hyperplasia induced by topical chemicals. Arch. Toxicol. 74(12): 733-744 (Published), BPD ID A6.01.5_02_b Ulrich P, Homey B, Vohr HW (1998) A modified murine local lymph node assay for the differentiation of contact photoallergy from phototoxicity by analysis of cytokine expression in skin-draining lymph node cells. Toxicology 125(2-3): 149-168 (Published), BPD ID A6.01.5_02_c	X
1.2	**Data protection**	No	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, the study was conducted according to Ulrich P et al. (Arch. Toxicol. 74: 733-744, 2001)	X
2.2	**GLP**	Not specified	
2.3	**Deviations**	Yes/No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde [redacted]	
3.1.1	Lot/Batch number	No data	X
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	None provided	X
3.1.2.2	Purity	Not specified	X
3.1.2.3	Stability	No data	X
3.1.2.4	Preparation of test substance for application	a) <u>For induction:</u> 1% in vehicle b) <u>For challenge:</u> 0.5% in vehicle	
3.1.2.5	Pre-test performed	No	

Section A6.1.5 _ 02	Skin sensitisation	
Annex Point IIA6.1.5	Mouse local lymph node assay and cytokine response following sensitisation	
	on irritant effects	
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	[redacted]
3.2.3	Source	[redacted] X
3.2.4	Sex	Female
3.2.5	Age/weight at study initiation	6-8 weeks old Weight not specified X
3.2.6	Number of animals per group	6 mice per group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Treatment	Groups of six mice received 25µl of a 1% test substance preparation in the vehicle on the dorsum of both ears on 3 consecutive days. Auricular lymph nodes draining the ear tissue were excised 24 hrs after the last exposure. Control animals were exposed to the vehicle (see 3.3.5). For comparison of induction and challenge responses, Groups of six mice received 50 µl of a 1% test substance preparation in the vehicle on the shaved back on 3 consecutive days (induction phase). Twelve days after the last induction, these mice were challenged with 25µl test substance solution (0.5% in vehicle) on the dorsum of both ears for a further 3 days (challenge phase treatment). Lymph nodes were excised 24 hrs after the last challenge treatment. Control animals were exposed to the vehicle.
3.3.2	Immune responses of mice	Following induction and challenge conducted as described above, the ear weights and the lymph node weights were determined. Group means and standard deviations were calculated and indices were determined for the treated groups relative to the vehicle control with an index set to 1.

Section A6.1.5 _ 02	Skin sensitisation	
Annex Point IIA6.1.5	**Mouse local lymph node assay and cytokine response following sensitisation**	
3.3.3	Quantification of cytokines in lymph node cells culture supernatants	For the determination of individual lymph node cell counts, single cell suspensions from lymph node pairs of individual animals were prepared by sterile mechanical tissue disaggregation. Cell numbers were determined in an electronic cell counter gating on a particle size above 4.88 µm. Lymph node cells were taken into culture at a density of 10E+6 cells/ml and restimulated with anti-CD3 monoclonal antibody. For restimulation with anti-CD3 antibody 24-well plates were coated with a 5 µg/ml dilution of the antibody; incubation was conducted with 1 ml cell suspension per well at 37 °C, 7% CO_2 and saturated humidity for 24 hours. After pelleting the cells, supernatants were harvested at were stored at –30 °C until cytokine analysis; the analysis for cytokine content by based on an enzyme-linked immunosorbant assay. The cytokine concentrations in culture supernatants were determined by means of the OPTEIA kits of BD Pharmingen according to the manufacturer´s protocols. The results were the mean value of triplicate ELISA measurements with a coefficient of variation of less than 15%. The supernatants of different test groups were tested on one cytokine-specific ELISA plate, allowing best comparability. The ELISA results were checked for validity by means of parallel restimulation assays prepared with the same cell suspensions.

Section A6.1.5 _ 02	Skin sensitisation
Annex Point IIA6.1.5	**Mouse local lymph node assay and cytokine response following sensitisation**

3.3.4	Cytokine secretion assay	Lymph node cells in culture were restimulated with the anti-CD3 monoclonal antibody as described above. They were then subjected to the cytokine secretion assay according to a manufacturer protocol obtained from Miltenyi Biotech, Bergisch Gladbach, Germany. The assay is based on an IL-4 catch reagent consisting of an anti-IL-4 monoclonal antibody (mAb) coupled to an anti-CD45 monoclonal antibody, which allows binding of the construct to the surface of lymphocytes, and an anti-IL-4 detection monoclonal antibody. The lymph node cells were washed and resuspended in 80 µl buffer per 10^7 cells; 20 µl of the IL-4 catch reagent were added and the mixture was allowed to incubate for 5 minutes on ice. The antibody-labelled cells were then incubated at 37°C for 45 minutes at a density of 10^6 cells per ml. The cells were then again incubated, this time with anti-IL-4 mAb-PE (PE, phycoerythrin, fluorescent dye conjugate) and anti-CD4 mAb-FITC (fluorescein-5-isothiocyanate, fluorescent dye conjugate). The cells were subjected to fluorescence-activated cell sorting analysis (FACS) and fluorescence microscopy. The remaining cells were subjected to incubation with anti-PE microbeads and positive lymphoid cells were enriched with an octo-MACS system for further FACS analysis and fluorescence microscopy. By means of a magnetic activated cell separation system (MACS), b-lymphocyte $B220^+$ cells were depleted, with or without subsequent removal of the remaining I-A positive cells from the total lymph node cells. The MACS system was developed by Miltenyi S et al. (High gradient magnetic cell separation with MACS. Cytometry 11(2):231-238, 1990) and the principle of the system was described in a summarized form as follows: cells stained sequentially with biotinylated antibodies, fluorochrome-conjugated avidin, and superparamagnetic biotinylated-microparticles (about 100 nm diameter) are separated on high gradient magnetic (HGM) columns. Unlabelled cells pass through the column, while labelled cells are retained. The retained cells can be easily eluted. More than 10^9 cells can be processed in about 15 min. Enrichment rates of more than 100-fold and depletion rates of several 1,000-fold can be achieved. The simultaneous tagging of cells with fluorochromes and very small, invisible magnetic beads makes this system an ideal complement to flow cytometry. Light scatter and fluorescent parameters of the cells are not changed by the bound particles. Magnetically separated cells can be analysed by fluorescence microscopy or flow cytometry or sorted by fluorescence-activated cell sorting without further treatment. Magnetic tagging and separation does not affect cell viability and proliferation. In present case, the lymph node cells were stained with anti-B220 microbeads for 20 minutes at 6 to 8 °C; the cell suspensions were then washed and passed through a steel wool column placed in a strong magnetic field. The purity of the unstained flow-through was checked by staining with fluorescent antibodies and subsequent flow cytometry.
3.3.5	Cytokine gene expression	For determination of cytokine gene expression, the total RNA was extracted from lymph nodes and was quantified by absorbance at ß = 260 nm. RNA was stored at –80°C until being subjected to real-time polymerase chain reaction (PCR) for gene expression analysis.
3.3.6	Vehicle	DAE433 (40% (v/v) N,N-dimethylacetamide, 30% acetone, 30% ethanol)

Section A6.1.5 _ 02	Skin sensitisation	
Annex Point IIA6.1.5	**Mouse local lymph node assay and cytokine response following sensitisation**	
3.3.7	Positive control substance	OXA, oxazolone DNFB, dinitrofluorobenzene DNCB, dinitrochlorobenzene
3.3.8	Statistics	Statistical assessment was based on following methods: One-way analysis of variance, multiple comparison analysis based on the methods of Tuckey (Hayter 1989), Sidak (1967) and Dunnett's test (1964).
3.4	**Further remarks**	The study mainly focused on the cytokine response pattern following sensitisation (induction) of BALB/c mice with different chemicals including glutaraldehyde, and elicitation (challenge) of contact allergy in sensitised animals. The data reported within the present summary refer to glutaraldehyde.

Section A6.1.5 _ 02 **Skin sensitisation**

Annex Point IIA6.1.5 **Mouse local lymph node assay and cytokine response following sensitisation**

4 RESULTS AND DISCUSSION

4.1 Immune responses of mice

Ear weights:

Induction	Challenge	Ear weight		
		Mean in mg	Standard deviation	Indice
Vehicle	Vehicle	8.57	0.48	1.00
Vehicle	0.5% DNFB	21.49**	3.19	2.51
0.5% DNFB	0.5% DNFB	21.22**	2.49	2.48
Vehicle	0.5% DNCB	9.92**	0.64	1.16
0.5% DNCB	0.5% DNCB	11.75 **##	0.57	1.37
Vehicle	0.5% GA	9.39**	0.51	1.09
1% GA	0.5% GA	10.46**#	1.07	1.22
Vehicle	0.5% OXA	10.91**	1.27	1.27
0.5% OXA	0.5% OXA	13.15**##	0.86	1.53

Lymph node weights:

Induction	Challenge	Lymph node weight		
		Mean in mg	Standard deviation	Indice
Vehicle	Vehicle	4.7	0.38	1.00
Vehicle	0.5% DNFB	15.60**	2.51	3.27
0.5% DNFB	0.5% DNFB	22.85**##	2.90	4.79
Vehicle	0.5% DNCB	15.94 mg**	5.19	3.34
0.5% DNCB	0.5% DNCB	21.87 mg**#	3.88	4.59
Vehicle	0.5% GA	7.37 mg**	0.87	1.55
1% GA	0.5% GA	12.91 mg**#	1.55	2.71
Vehicle	0.5% OXA	18.63**	2.54	3.91
0.5% OXA	0.5% OXA	21.13**#	2.34	4.43

**, $p<0.1\%$ relative to the corresponding vehicle control; #, $0.1<p<1\%$ relative to the corresponding induction control groups (DNFB and DNCB); ##, $p<0.1\%$ relative to the corresponding induction control groups (DNFB and DNCB).

Glutaraldehyde-treatment resulted in a significant increase in ear weight coupled in comparison to the induction control groups, coupled with significant increase in lymph node weight. These effects are indicative of contact allergy in the skin.

X

4.2 Quantification of cytokines in lymph node cells culture supernatants

Glutaraldehyde treatment resulted in an increased amount of the cytokine IL-4, reaching about 6000 pg/ml, which was in the range of oxazolone.

Section A6.1.5 _ 02	Skin sensitisation	
Annex Point IIA6.1.5	**Mouse local lymph node assay and cytokine response following sensitisation**	
4.3	**Cytokine secretion assay**	By means of the MACS system, The $CD4^+$ T cells could be identified as the main cell type responsible for IL-4 release.
4.4	**Cytokine gene expression**	RT-PCR measurements of cytokine gene expression for lymph node cells isolated from glutaraldehyde-treated mice revealed a marked up-regulation of the IL-4 gene expression following the challenge, whereas IFNß gene expression was comparable to control. In fact, gene expression for IL-4 expressed as ratio [IL-4/rbActin x 10^4] reached about 5.5 and was in the range of DNFB (ca. 4.5) and oxazolone (ca. 3.8); control value was < 1.00. For IFNß, gene expression in lymph node cells from glutaraldehyde-treated mice following challenge, expressed as as ratio [IFNß/rbActin x 10^4] was within control range (< 1.00 versus 1.2 for control; DNFB: ca. 2.00 and oxazolone: ca. 3.6).

Section A6.1.5 _ 02 **Skin sensitisation**

Annex Point IIA6.1.5 **Mouse local lymph node assay and cytokine response following sensitisation**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study mainly focused on the cytokine response pattern following sensitisation (induction) of BALB/c mice with different chemicals including glutaraldehyde, and elicitation (challenge) of contact allergy in sensitised animals. The data reported within the present summary refer to glutaraldehyde.

Test substance: Glutaraldehyde [redacted], no further data given

Female [redacted] mice (6-8 weeks old) were obtained [redacted] and were induced and challenged with the test substance dissolved in DAE433 (40% (v/v) N,N-dimethylacetamide, 30% acetone, 30% ethanol). Induction was conducted with 1% glutaraldehyde in DAE433 whereas challenge was done with 0.5% glutaraldehyde in DAE433.

Six mice received 25µl of a 1% test substance preparation in the vehicle on the dorsum of both ears on 3 consecutive days. Auricular lymph nodes draining the ear tissue were excised 24 hrs after the last exposure. Control animals were exposed to the vehicle. For comparison of induction and challenge responses, Groups of six mice received 50 µl of a 1% test substance preparation in the vehicle on the shaved back on 3 consecutive days (induction phase). Twelve days after the last induction, these mice were challenged with 25µl test substance solution (0.5% in vehicle) on the dorsum of both ears for a further 3 days (challenge phase treatment). Lymph nodes were excised 24 hrs after the last challenge treatment. Control animals were exposed to the vehicle. The ear and the lymph node weights were determined; group means and standard deviations were calculated and indices were determined for the treated groups relative to the vehicle control with an index set to 1. Cytokines amount in lymph node cells culture supernatants was quantified by means of the ELISA assay. For the identification of the main cell type responsible of cytokine production a cytokine secretion assay was conducted, which was based on the magnetic activated cell separation system (MACS) as developed by Miltenyi S et al. (High gradient magnetic cell separation with MACS. Cytometry 11(2):231-238, 1990) coupled with staining with fluorescent antibodies and subsequent flow cytometry. For determination of cytokine gene expression, the total RNA was extracted from lymph nodes and was quantified by absorbance at ß = 260 nm. RNA was then subjected to real-time polymerase chain reaction (PCR) for gene expression analysis.

Positive controls were oxazolone (OXA), dinitrofluorobenzene (DNFB) and dinitrochlorobenzene (DNCB)

Statistical assessment was mainly based on the one-way analysis of variance, multiple comparison analysis based on the methods of Tuckey (Hayter 1989), Sidak (1967) and Dunnett's test (1964).

Section A6.1.5 _ 02		**Skin sensitisation**
Annex Point IIA6.1.5		**Mouse local lymph node assay and cytokine response following sensitisation**
5.2	**Results and discussion**	Glutaraldehyde treatment resulted in a significant increase in ear weight in comparison to the induction control groups, coupled with significant increase in lymph node weight. Glutaraldehyde treatment resulted in an increased amount of the cytokine IL-4, reaching about 6000 pg/ml, which was in the range of the positive substance oxazolone. By means of the MACS system, The $CD4^+$ T cells could be identified as the main cell type responsible for IL-4 release. RT-PCR measurements of cytokine gene expression for lymph node cells isolated from glutaraldehyde-treated mice revealed a marked up-regulation of the IL-4 gene expression following the challenge.
5.3	**Conclusion**	From a sensitizing point of view, glutaraldehyde was assessed in this assay as a moderately active contact allergen. Considering the cytokine response pattern to sensitisation, in contrast to interferon IFNß (T helper 1 cell cytokine) which remained unaffected (neither up- nor down-regulation), interleukin IL-4 (T helper 2 cell cytokine) was found to be upregulated during challenge. The authors concluded that skin sensitisers are able to elicit cytokine response patterns and that Th 2cytokines measurement after challenge may by considered as a stable marker of secondary contact allergy. On the other hand, particular affinity of cytokine response patterns for either dermal or respiratory sensitisers was not evident (study data not reported here).
5.3.1	Reliability	**2**
5.3.2	Deficiencies	Scientifically acceptable data.

Section A6.1.5 _ 02	**Skin sensitisation**
Annex Point IIA6.1.5	**Mouse local lymph node assay and cytokine response following sensitisation**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 21st, 2010
Materials and Methods	2.1 Guideline study. There were no guidelines. 3.1.1; 3.1.2.1; 3.1.2.2; 3.1.2.3 There is [redacted] 3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	4.1 Immune responses in mice. The statistical significance for the lymph node weights (induction 1 % GA; challenge 0.5 % GA) is marked as *0.1 % < P < 1 %*, while the correct value *is P < 0.1 %*.
Conclusion	The response was clearly positive, but no regulatory conclusions on sensitisation potential can be made. This can be considered as supportive data. IL-4 is up-regulated by GA, while interferon-β is not. This suggests that the sensitisation process is mediated by Th2 cells and not Th1 cells. The study has only limited value in risk assessment.
Reliability	2
Acceptability	Acceptable as supportive data
Remarks	1.1 Reference. The present study summary only summarises the reference A6.01.5_02_a. The other two studies could be cited elsewhere, but should not be given here.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

				Official use only
		1 REFERENCE		
1.1	**Reference**	[redacted] (2004), Report on ^{14}C-GDA - Study of the biokinetics in rats. [redacted] (Unpublished), BPD ID A6.02_01		
1.2	**Data protection**	Yes		
1.2.1	Data owner	BASF AG		
1.2.2	Companies with letter of access	[redacted]		
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]		
		2 GUIDELINES AND QUALITY ASSURANCE		
2.1	**Guideline study**	Yes, OECD 417 (1984)		
2.2	**GLP**	Yes		
2.3	**Deviations**	No		
		3 MATERIALS AND METHODS		
3.1	**Test material**	Glutaraldehyde-[2,4-^{14}C]	[redacted]% glutaraldehyde)	
3.1.1	Lot/Batch number	[redacted]	[redacted]	
3.1.2	Specification	As given in section 2	As given in section 2	X
3.1.2.1	Description	Clear solution in 0.01N sulfuric acid	Clear colorless liquid	
3.1.2.2	Concentration of active ingredient in the solution	0.878 mg/g	–	
3.1.2.3	Purity	[redacted]%	[redacted]% (w/w; [redacted])	X
3.1.2.4	Stability	Verified and confirmed analytically	Verified and confirmed analytically	X
3.1.2.5	Radiolabelling	^{14}C	–	
3.2	**Test Animals**			
3.2.1	Species	Rat		
3.2.2	Strain	[redacted]		
3.2.3	Source	[redacted]		
3.2.4	Sex	Male/Female		
3.2.5	Age/weight at study initiation	At least 9 weeks old at test start.		X

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

3.2.6	Number of animals per group	(1)-4 males and 4 females per experiment and test dose were used for the experiments referring to balance and excretion, to blood and plasma and to the excretion via bile. (2)-12 males and 12 females per experiment and test dose were used for the experiments referring to tissue distribution.	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	**Oral (gavage)**	
3.3.1	Test doses	5 and 75 mg/kg bw (selection based on the results of chronic toxicity study with rat)	X
3.3.2	Test item	Respective amounts of non-labelled test substance were mixed with an aliquot of the stock solution of the labelled test substance in 0.01 N sulphuric acid. Tap water was added to the mixture until getting the wanted final volume that corresponded to the required specific activity.	
3.3.3	Volume applied	1ml of test item preparation/100 g bw.	
3.3.4	Specific activity of test substance	1.87 Mbq/g solution, corresponding to 2.13 MBq/ mg active ingredient	X

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

3.3.5	Experimental schedule	(1)-Balance and excretion: One experiment per test dose was conducted. Following dosage of the animals, excreta were collected after 6, 12 and 24 hours, and thereafter at 24h-intervals for up to 168 hours. CO_2 in exhaled air was determined and checked for radioactivity. After 168 hours, the animals were sacrificed and following tissues were checked for radioactivity contents: liver, kidney, heart, lung, brain, gonads, spleen, pancreas, adrenals, thyroid, stomach, stomach contents, gut, gut contents, blood cells and plasma, uterus, muscle tissue, skin, bone, bone marrow, fat, remaining carcass. For balance estimates the cage wash was also checked for radioactivity. (2)-Blood/plasma level of radioactivity: One experiment per test dose was conducted. Following dosage, blood samples (100 – 200 µl) were collected from the treated animals at following time points after treatment: 30 min., 1, 2, 4, 8, 24, 48, 72, 96, 120, 144 and 168 hours. Whole blood and plasma samples were checked for total radioactivity. (3)-Tissue distribution: Dosing was selected on the basis of the results obtained within the balance and excretion investigation. Following 3 experiments were conducted: (1) 12 males received 75 mg/kg bw test substance, 3 animals were sacrificed after 1, 17, 36 and 64 hours. (2) 12 females received 75 mg/kg by test substance, 3 animals were sacrificed after 1, 11, 20 and 36 hours. (3) 12 males and 12 females received 5 mg/kg bw test substance, 3 animals per sex were sacrificed after 4, 24, 52 and 96 hours. Following sacrifice, radioactivity was measured in the following solubilized organs: liver, kidney, heart, lung, brain, gonads, spleen, pancreas, adrenals, thyroid, stomach, stomach contents, gut, gut contents, blood cells and plasma, uterus, muscle tissue, skin, bone, bone marrow, fat, remaining carcass. (4) Excretion via bile: The bile duct of each treated animal was cannulated and bile was collected at time intervals of 3 hours for up to 48 hours. One experiment per test dose was conducted.

4 RESULTS AND DISCUSSION

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

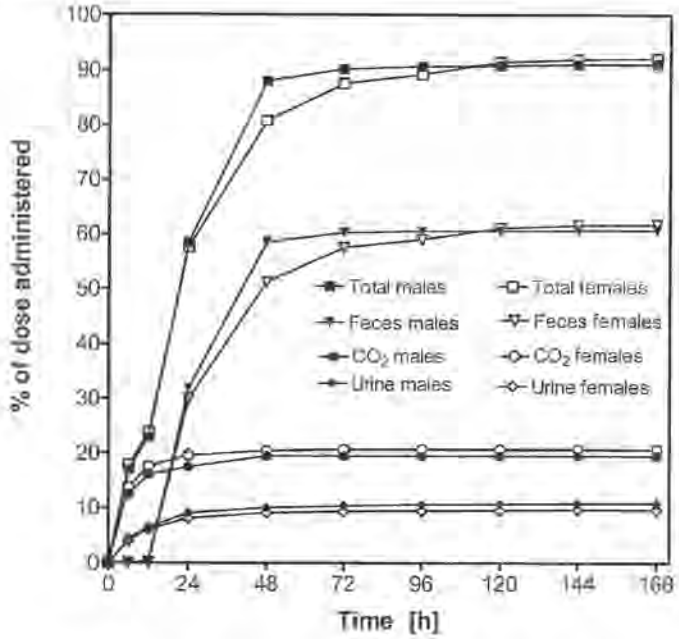
Annex Point IIA6.2

4.1 Balance and excretion at a dosage of 75 mg/kg bw

See table 1 (end of the document)

75 mg/kg bw, main findings:

The cumulative radioactivity excretion at a test dose of 75 mg/kg bw is illustrated in following figure.



Mean total radioactivity recovery in males and females respectively was 3.43% and 94.46%. Recovery of radioactivity in exhaled air ($^{14}CO_2$) reached 19.46% in males within 72 hours (therefrom 82% already detected within the first 12 hours following dosage) and 20.65% in females within 96 hours (therefrom 85% already detected within the first 12 hours following dosage). Radioactivity recovery in urine almost was complete after 48 hours with values of 10.03% and 9.09% respectively for the males and females; after 168 hours recovery was 10.83% for males and 9.68% for females. During the first 48 hours following dosage, radioactivity recovery in feces was 58.46% for males and 51.26% for females; after 168 hours recovery was 60.71% for males and 61.72% for females. After 168 hours, remaining radioactivity in organs/tissues was about 0.11 to 0.15% in kidney and liver, 0.43 to 0.52% in skin and 0.93 to 0.96% in the carcass; the mean radioactivity concentrations in all organs /tissues generally were < 17 µg Eq/g.

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

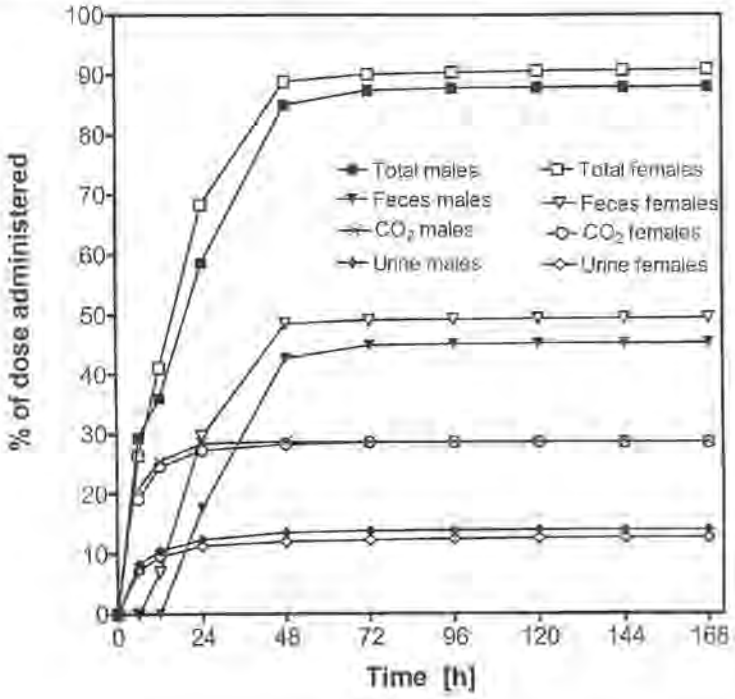
Annex Point IIA6.2

4.2 Balance and excretion at a dosage of 5 mg/kg bw

See table1

5 mg/kg bw, main findings:

The cumulative radioactivity excretion at a test dose of 5 mg/kg bw is illustrated in following figure.



Mean total radioactivity recovery in males and females respectively was 1.79% and 93.61%. Recovery of radioactivity in exhaled air (i..e. as $^{14}CO_2$) reached 28.70% in males (therefrom 89% already detected within the first 12 hours following dosage) and 28.63% in females within 72 hours (therefrom 6% already detected within the first 12 hours following dosage). Radioactivity recovery in urine almost was complete after 48 hours with values of 13.58% and 12.06% respectively for the males and females; after 168 hours recovery was 14.04% for males and 12.78% for females. During the first 48 hours following dosage, radioactivity recovery in feces was 42.76% for males and 48.56% for females; after 168 hours recovery was 45.25% for males and 49.53% for females. After 168 hours, remaining radioactivity in organs/tissues was about 0.08 to 0.10% in kidney, 0.12 to 0.27% in liver, 0.70 to 1.21% in skin and 1.22 to 1.56% in the carcass; the mean concentrations of radioactivity in all organs /tissues generally were < 8 µg Eq/g.

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

4.3 Balance and excretion, summary

For both tested doses, the radioactivity recovery data reported above as well as the figures indicate a very rapid excretion of the absorbed test substance via expired air, feces and urine. Furthermore, no sex-related differences are seen. Comparing the high and low tested doses, it appears that at the low dose, excretion via the expired air and via the urine was higher than at the high dose (see table 2), indicating an increased bioavailability of the test substance at a dosage of 5 m/kg bw. As a result therefrom, radioactivity recovery in the feces at 5 mg/kg bw was lower than at 75 mg/kg bw.

Table 2

Way of excretion*	75 mg/kg bw		5 mg/kg bw	
	Males	Females	Males	Females
Expired air ($^{14}CO_2$)	19.46%	20.65%***	28.70%	28.63%
Urine	10.83%	9.68%	14.04%	12.78%
Feces	60.71%	61.72%	45.25%	49.53%

*, Expired air after 72 h; urine and feces after 168 h; ***, after 96 h

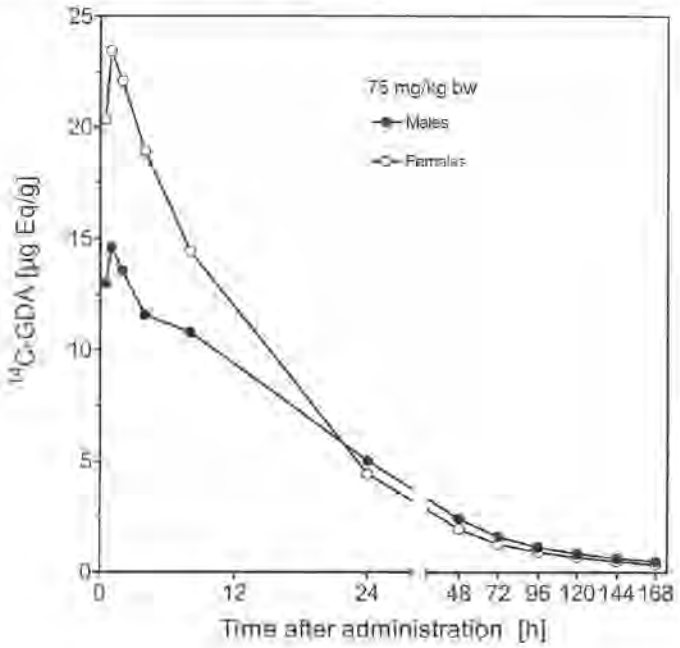
Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

4.4 Blood/plasma level of radioactivity at a dosage of 75 mg/kg bw

75 mg/kg bw, main findings:

The mean plasma radioactivity concentration at a test dose of 75 mg/kg bw is illustrated in following figure.



The figure above shows that a peak in the plasma concentration/time curve was reached after 1 hour following dosage, with a value of 14.60 and 23.44 µg Eq/g respectively for males and females. Each peak was followed by a biphasic decrease until reaching a level of 0.49 µg Eq/g for males and 0.35 µg Eq/g for females after 168 hours. The initial and final half-life was as follows:

Males, initial half-life = 18.1 h

Females, initial half-life = 12.7 h

Males, final half-life = 52.7 h

Females, final half-life =49.7 h

The mean calculated areas under the plasma concentration/time curve (AUC) was 471 µg Eq*h/g and 486 µg Eq*h/g respectively for males and females.

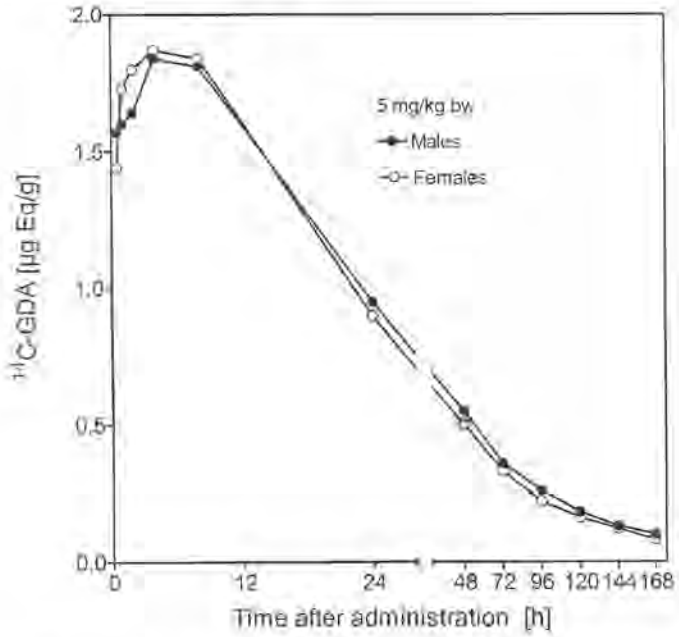
Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

4.5 Blood/plasma level of radioactivity at a dosage of 5 mg/kg bw

5 mg/kg bw, main findings:

The mean plasma radioactivity concentration at a test dose of 5 mg/kg bw is illustrated in following figure.



The figure above shows that a peak in the plasma concentration/time curve was reached after 4 hours following dosage, with a value of 1.84 and 1.87 µg Eq/g respectively for males and females. Each peak was followed by a biphasic decrease until reaching a level of 0.10 µg Eq/g for males and 0.08 µg Eq/g for females after 168 hours. The initial and final half-life was as follows:

Males, initial half-life = 24.1 h

Females, initial half-life = 22.1 h

Males, final half-life = 48.7 h

Females, final half-life =46.5 h

The mean calculated areas under the plasma concentration/time curve (AUC) was 89 µg Eq*h/g and 83 µg Eq*h/g respectively for males and females.

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

4.6 Blood/plasma level of radioactivity, summary

The pharmacokinetic parameters of radioactivity in the plasma for both tested doses can be summarized as follows:

Table 3

Sex	Dose	Cmax	Tmax	Initial half-life	Final half.life	AUC
Males	75 mg/kg	14.6 µg Eq/g	1	18.1 h	52.7 h	471 µg Eq*h/g
	5 mg/kg	1.84 µg Eq/g	4	24.1 h	48.7 h	89 µg Eq*h/g
Females	75 mg/kg	23.44 µg Eq/g	1	12.7 h	49.7 h	486 µg Eq*h/g
	5 mg/kg	1.87 µg Eq/g	4	22.1 h	46.5 h	83 µg Eq*h/g

The data indicated that during the first 24 hours following dosage, the major parts of radioactivity rather were in the plasma than bound to the cellular blood components. Thereafter, the blood/plasma concentration ratio increased continuously until time point 168 h. The course was similar for both sexes. Considering the AUC, the values obtained at 75 mg/kg were about 15 times higher than those reported for 5 mg/kg bw, indicating that the bioavailability of the test substance decreased with increasing test dose.

X

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

4.7 Tissue distribution at a dosage of 75 mg/kg bw

Following table summarized the findings referring to the mean tissue concentration of radioactivity at a test dose of 75 mg/kg bw.

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	64 h	1 h	11 h	20 h	36 h
Bloodcells	16.54	4.17	2.72	2.40	10.09	5.32	6.99	4.28
Plasma	10.62	7.21	3.55	1.55	18.68	7.54	6.08	3.26
Lung	5.38	3.28	1.88	0.80	8.03	3.55	1.81	1.28
Liver	8.90	5.53	2.84	2.80	14.56	5.29	5.45	6.17
Spleen	14.37	13.14	6.83	6.48	27.20	13.06	15.12	11.57
Kidney	51.51	52.09	20.33	27.52	60.01	46.81	50.67	38.10
Adrenal glands	24.62	41.65	25.75	19.85	41.44	38.43	47.00	39.66
Testes/Ovaries	2.69	4.02	1.50	2.03	12.09	14.45	12.91	14.72
Uterus	—	—	—	—	12.52	12.80	12.94	13.16
Muscle	4.61	3.92	2.05	2.27	8.55	3.37	3.48	2.29
Brain	1.36	1.57	0.41	0.90	2.90	1.47	1.85	1.18
Adipose tissue	2.10	4.16	6.07	2.20	1.84	3.84	1.73	2.17
Bone	16.74	7.16	1.93	1.51	4.90	5.57	3.50	2.38
Bonemarrow	30.70	34.20	9.70	8.23	18.39	30.95	22.73	16.08
Thyroid	53.86	62.56	31.79	12.90	73.98	44.35	30.35	48.19
Pancreas	31.72	15.23	6.44	4.76	56.48	15.82	9.93	9.88
Stomach cont.	3121.27	1062.69	4.08	2.36	2029.46	1204.30	828.85	16.51
Stomach	1980.35	389.15	21.99	11.71	1457.58	372.51	332.44	42.07
Gut cont.	191.84	624.99	24.48	6.22	272.00	629.69	658.68	85.07
Gut	105.45	61.01	6.56	4.53	123.42	74.95	46.50	17.74
Liver	34.35	28.21	3.88	7.51	57.73	33.21	24.53	11.39
Skin	7.46	6.42	4.83	5.19	8.96	4.40	4.74	4.04
Carcass	7.01	5.78	3.49	1.57	6.77	3.92	4.39	3.17

X

The time points sacrifice were chosen on the basis of the results of the plasma kinetics. Therefore, the first two time points of sacrifice respectively were close to the time points of maximum plasma concentration and of half the MPC, whereas the remaining two time points of sacrifice corresponded respectively to ¼ and 1/8 of the MPC.

The values of the table above show that for both sexes, the highest radioactivity concentrations in tissue after 1 hour were seen in the gastro-intestinal tract, the thyroid and the kidney; the lowest concentrations were found in brain and adipose tissue. A decrease in tissue concentration, which ran parallel to the decrease in plasma concentration, was observed over 63 hours for the males and 35 hours for the females. This was true for all organs/tissues excepted for the adipose tissue, the adrenals, the ovaries and the uterus; these organs/tissues showed nearly constant concentrations of radioactivity over the whole experimental period. After 64 hours for the males and 36 hours for the females (corresponding to 1/8 of MPC) and excluding the gastro-intestinal tract, following organs showed the highest concentrations of radioactivity: kidney, thyroid and adrenals; the lowest concentrations were seen in lung and brain.

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

4.8 Tissue distribution at a dosage of 5 mg/kg bw

Following table summarized the findings referring to the mean tissue concentration of radioactivity at a test dose of 5 mg/kg bw.

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
Blood cells	3.58	5.73	1.56	3.09	1.95	2.85	1.50	2.60
Plasma	14.85	16.64	4.75	7.91	2.89	4.89	1.45	2.27
Lung	10.89	12.94	5.10	8.97	4.23	6.82	2.65	4.27
Heart	7.60	8.07	3.27	5.26	2.75	4.26	1.95	3.00
Spleen	10.88	12.44	7.87	12.44	4.50	7.54	3.03	4.87
Kidney	33.61	42.69	15.15	23.28	11.07	15.44	9.93	15.32
Adrenal glands	22.43	26.87	24.19	31.89	12.01	22.49	11.61	12.68
Testes/Ovaries	4.16	15.55	2.07	11.46	1.75	10.24	1.31	9.64
Uterus	—	15.25	—	14.09	—	10.00	—	5.48
Muscle	3.73	5.00	2.00	2.89	2.15	2.21	1.72	1.87
Brain	4.19	5.62	1.48	3.18	1.24	2.35	1.15	1.73
Adipose tissue	3.28	1.77	4.63	1.95	4.55	2.54	5.50	1.95
Bone	2.69	2.75	2.98	3.63	1.76	1.93	1.78	1.16
Bone marrow	10.59	15.00	5.17	9.33	4.80	6.30	1.56	1.78
Thyroid	18.60	22.94	34.46	50.82	16.20	18.51	14.74	18.95
Pancreas	44.28	63.07	8.93	14.02	5.37	7.05	4.22	4.80
Stomach cont.	305.64	597.64	0.71	3.96	0.49	2.80	1.89	0.45
Stomach	110.54	305.62	14.85	14.56	6.74	5.39	3.42	5.02
Gut cont.	401.44	363.43	34.02	22.64	2.39	5.84	1.21	2.07
Gut	42.03	67.03	7.59	12.84	3.34	6.74	1.82	3.68
Liver	52.46	34.85	19.72	15.54	10.04	11.08	5.69	5.58
Skin	7.21	6.88	5.79	4.72	5.10	4.76	4.00	3.25
Carcass	5.47	5.95	2.33	2.84	1.64	2.18	1.76	1.71

The time points sacrifice were chosen on the basis of the results of the plasma kinetics. Therefore, the first two time points of sacrifice respectively were close to the time points of maximum plasma concentration and of half the MPC, whereas the remaining two time points of sacrifice corresponded respectively to ¼ and 1/8 of the MPC.

The values of the table above show that for both sexes, the highest radioactivity concentrations in tissue after 4 hours were seen in the gastro-intestinal tract, the pancreas, the liver and the kidney; the lowest concentrations were found in adipose tissue, blood cells, muscles and bone. A decrease in tissue concentration, which ran parallel to the decrease in plasma concentration, was observed over 92 hours for both males and females. This was true for all organs/tissues excepted for the adipose tissue, which showed nearly constant low concentrations of radioactivity over the whole experimental period. After 96 hours for both males and females (corresponding to 1/8 of MPC) following organs showed the highest concentrations of radioactivity: thyroid, adrenals and kidney. In males, the lowest concentrations reported after 96 hours referred to brain, blood cells, testes and bone marrow, whereas in females, the lowest concentrations were found in bone, carcass, brain and bone marrow.

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

4.9 Excretion via bile for both test doses

The respective radioactivity excretion pattern via the bile, for both test doses are summarized in following table.

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	0.42	0.67	0.15	0.31
3-6	0.73	0.81	0.21	0.34
6-9	0.34	0.38	0.20	0.23
9-12	0.22	0.28	0.31	0.19
12-15	0.15	0.13	0.21	0.16
15-18	0.14	0.09	0.07	0.11
18-21	0.08	0.07	0.01	0.00
21-24	0.14	0.07	0.11	0.07
24-27	0.12	0.04	0.00	0.00
27-30	0.07	0.05	0.07	0.00
30-33	0.06	0.03	0.05	0.06
33-36	0.04	0.03	0.05	0.06
36-39	0.03	0.05	0.03	0.05
39-42	0.03	n.a.	0.03	0.04
42-45	0.03	0.00	0.02	0.03
45-48	0.02	–	0.02	0.02
Total	2.55	2.58	1.76	1.84

n.a. = no sample

After 48 hours following dosage with 75 mg/kg bw test substance, total biliary excretion was about 2.55 and 2.58% of the administered radioactivity respectively for the males and females. The maximum excretion was reached within the first 6 hours following dosage, for both sexes; thereafter the biliary excretion declined continuously.

Following dosage with 5 mg/kg bw test substance, total biliary excretion after 48 hours was about 1.76 and 1.84% of the administered radioactivity respectively for the males and females. The maximum excretion was reached within the period ranging from 9 to 15 hours for males; thereafter biliary excretion declined continuously. For the females, the maximum excretion was reached within the first 9 hours following dosage; thereafter the biliary excretion declined continuously.

Section A6.2 _ 01 **In vivo, toxicokinetics of ^{14}C-GDA in rats**

Annex Point IIA6.2

4.10 Conclusion

Following single oral administration, the radioactivity was rapidly absorbed from the gastro-intestinal tract with the absorption being incomplete at both tested dose levels. In fact, as shown in following table, the bioavailable part of the radioactive dose, which corresponded to the part excreted via urine, bile and expired air (CO_2), was about 33% of the total amount at a test dose of 75 mg/kg bw, and about 44% of the total amount at 5 mg/kg bw. This indicates that the gastro-intestinal absorption decreased with increasing test dose. Following absorption, the radioactivity was distributed in all organs/tissues. The excretion was very rapid and mainly occurred via the feces and the expired air (CO_2).

Table 7: Comparison of excretion pattern after administration of ^{14}C-GDA at different dose levels and application sites

Results expressed as % of the radioactivity administered.

Dose [mg/kg bw] Application site Application mode	75 oral single	5 oral single
Males		
Urine 0-48	10.02	13.58
Feces 0-48	66.46	42.76
CO_2 0-48	15.47	28.70
Subtotal	87.95	85.04
Bile 0-48	2.55	1.76
Females		
Urine 0-48	9.09	17.03
Feces 0-48	61.26	40.56
CO_2 0-48	20.50	28.30
Subtotal	80.85	86.92
Bile 0-48	2.58	1.84

4.11 Remark

The stability, homogeneity and correctness of the concentrations of ^{14}C-glutaraldehyde in tap water were analytically verified and confirmed.

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

5.1 Materials and methods

The aim of present study was to investigate the absorption, distribution, excretion and biokinetic pathways of glutaraldehyde in [redacted] rat following single oral dosage and using ^{14}C-labelled test substance.

Test substances: (1) Glutaraldehyde-[2,4-^{14}C], [redacted], specific activity1.87 Mbq/g solution (2.13 MBq/ mg a.i.); (2) [redacted] glutaraldehyde), batch No [redacted]

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

The test substance was administered orally to male and female rats by single gavage; the tested doses were 75 and 5 mg/kg bw. The experimental design of the study was as follows:

1)-Balance and excretion: 4 males and 4 females were used per experiment and test dose. One experiment per test dose was conducted. Following dosage of the animals, excreta were collected after 6, 12 and 24 hours, and thereafter at 24h-intervals for up to 168 hours. CO_2 in exhaled air was determined and checked for radioactivity. After 168 hours, the animals were sacrificed and following tissues were checked for radioactivity contents: liver, kidney, heart, lung, brain, gonads, spleen, pancreas, adrenals, thyroid, stomach, stomach contents, gut, gut contents, blood cells and plasma, uterus, muscle tissue, skin, bone, bone marrow, fat, remaining carcass. For balance estimates the cage wash was also checked for radioactivity.

(2)-Blood/plasma level of radioactivity: 4 males and 4 females were used per experiment and test dose. One experiment per test dose was conducted. Following dosage, blood samples (100 – 200 µl) were collected from the treated animals at following time points after treatment: 30 min., 1, 2, 4, 8, 24, 48, 72, 96, 120, 144 and 168 hours. Whole blood and plasma samples were checked for total radioactivity.

(3)-Tissue distribution: 12 males and 12 females were used per experiment and test dose. Dosing was selected on the basis of the results obtained within the balance and excretion investigation. Following 3 experiments were conducted: (1) 12 males received 75 mg/kg bw test substance, 3 animals were sacrificed after 1, 17, 36 and 64 hours. (2) 12 females received 75 mg/kg by test substance, 3 animals were sacrificed after 1, 11, 20 and 36 hours. (3) 12 males and 12 females received 5 mg/kg bw test substance, 3 animals per sex were sacrificed after 4, 24, 52 and 96 hours. Following sacrifice, radioactivity was measured in the following solubilized organs: liver, kidney, heart, lung, brain, gonads, spleen, pancreas, adrenals, thyroid, stomach, stomach contents, gut, gut contents, blood cells and plasma, uterus, muscle tissue, skin, bone, bone marrow, fat, remaining carcass.

(4) Excretion via bile: 4 males and 4 females were used per experiment and test dose. The bile duct of each treated animal was cannulated and bile was collected at time intervals of 3 hours for up to 48 hours. One experiment per test dose was conducted.

The stability, homogeneity and correctness of the concentrations of ^{14}C-glutaraldehyde in tap water were analytically verified and confirmed.

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

5.2 Results and discussion

Balance and excretion: At 75 mg/kg, mean total radioactivity recovery was 93.43% in males and 94.46% in females. Recovery in exhaled air ($^{14}CO_2$) reached 19.46% in males (82% detected within the first 12 h following dosage) and 20.65% in females (85% detected within the first 12 h). Recovery in urine almost was complete after 48 h with 10.03% for males and 9.09% for females. Recovery in feces was 58.46% for males and 51.26% for females (48 h), and 60.71% for males and 61.72% for females (168 h). After 168 h, radioactivity in organs/tissues was 0.11 - 0.15% in kidney and liver, 0.43 - 0.52% in skin and 0.93 - 0.96% in carcass; the radioactivity concentrations in all organs /tissues were < 17 µg Eq/g. At 5 mg/kg bw, mean total radioactivity recovery was 91.79% in males and 93.61% in females. Recovery in exhaled air reached 28.70% in males (89% detected within the first 12 h) and 28.63% in females (86% detected within the first 12 h). Recovery in urine almost was complete after 48 h with 13.58% for males and 12.06% for females. During the first 48 h following dosage, recovery in feces was 42.76% for males and 48.56% for females; after 168 h recovery was 45.25% for males and 49.53% for females. After 168 h, radioactivity in organs/tissues was 0.08 - 0.10% in kidney, 0.12 - 0.27% in liver, 0.70 - 1.21% in skin and 1.22 - 1.56% in carcass; the concentrations of radioactivity in all organs /tissues were < 8 µg Eq/g.

Blood/plasma level of radioactivity:

Sex	Dose	Cmax (µg Eq/g)	Tmax	Initial half life	Final half.life	AUC (µg Eq*h/g)
Males	75 mg/kg	14.6	1	18.1 h	52.7 h	471
	5 mg/kg	1.84	4	24.1 h	48.7 h	89
Females	75 mg/kg	23.44	1	12.7 h	49.7 h	486
	5 mg/kg	1.87	4	22.1 h	46.5 h	83

During the first 24 h after dosage, the major parts of radioactivity were in the plasma; thereafter, the blood/plasma concentration ratio increased continuously. The AUC values obtained at 75 mg/kg were about 15 times higher than those for 5 mg/kg bw, indicating that the bioavailability of the test substance decreased with increasing dose.

Tissue distribution: At 75 mg/kg (both sexes), highest radioactivity concentrations in tissue after 1 h were seen in GIT, thyroid and kidney. A decrease running parallel to that of plasma concentration was seen over 63 h for males and 35 h for females (all organs/tissues excepted adipose tissue, adrenals and ovaries and uterus). After 64 h for the males and 36 h for the females and excluding the GIT, kidney, thyroid and adrenals showed the highest concentrations. At 5 mg/kg (both sexes), the highest tissue concentrations after 4 h were seen in the GIT, pancreas, liver and kidney. A decrease running parallel to that of plasma concentration was seen over 92 h (both sexes; all organs/tissues excepted adipose tissue). After 96 h, thyroid, adrenals and kidney had the highest concentrations.

Biliary excretion: At 75 mg/kg and after 48 h, total biliary excretion was 2.55 and 2.58% for males and females. The maximum excretion was reached the first 6 h after dosage; thereafter excretion declined. At 5 mg/kg, biliary excretion after 48 h was 1.76 and 1.84% for males and females. The maximum excretion was reached from 9-15 h for males; thereafter excretion declined. For females, the maximum excretion was reached in the first 9 h after dosage; thereafter excretion declined.

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

5.3	**Conclusion**	Following single oral administration in rats, the radioactivity was rapidly absorbed from the gastro-intestinal tract. However, absorption was incomplete at both tested dose levels (5 and 75 mg/kg bw). The bioavailable part of the radioactive dose, which corresponded to the part excreted via urine, bile and expired air (CO_2), was about 33% of the total amount at a test dose of 75 mg/kg bw, and about 44% of the total amount at 5 mg/kg bw. This indicates that the gastro-intestinal absorption decreased with increasing test dose. Following absorption, the radioactivity was distributed in all organs/tissues. The excretion was very rapid and mainly occurred via the feces and the expired air (CO_2). These findings were confirmed by the plasma kinetic data.	X
5.3.1	Reliability	**1**	
5.3.2	Deficiencies	No	

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 23rd, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2. 3.1.2.3 Purity. The purity of both the labelled and non-labelled material is claimed but the analyses are not provided. 3.1.2.4 Stability. The stability of both the labelled and non-labelled material is claimed but the analyses are not provided. 3.3.4 Specific activity of test substance. The values given refer to the labelled active substance before preparing the dose solution.
Results and discussion	4.1 Balance and excretion at a dosage of 75 mg/kg bw. • Spelling error: "Mean total radioactivity recovery in males and females respectively was **9**3.43 % and 94.46 %." • 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 4.2 Balance and excretion at a dosage of 5 mg/kg bw. • Spelling error: "Mean total radioactivity recovery in males and females respectively was **9**1.79 % and 93.61 %." • Spelling error on line 5 after the figure: "(therefrom **8**6 % already detected within the first 12 hours following dosage)" • 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 (♂/♀). 4.6 Blood/plasma level of radioactivity, summary. Spelling mistake in the last paragraph: "Considering the AUC, the values obtained at 75 mg/kg were ~~about 15~~ **5.3-5.9** times higher". 4.7 Tissue distribution at a dosage of 75 mg/kg bw. MPC = maximum plasma concentration. 5.3 Conclusion. 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), CO2 (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.

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

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

Section A6.2 _ 01 In vivo, toxicokinetics of ^{14}C-GDA in rats

Annex Point IIA6.2

Table 1: Mean excretion and retention of radioactivity after single oral administration o ^{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.

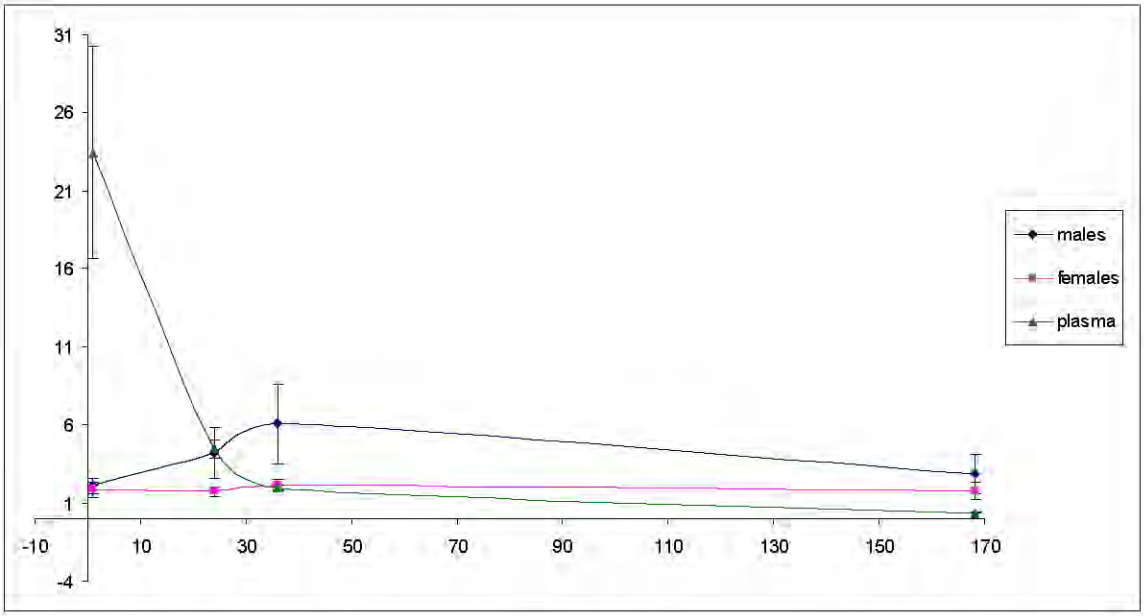
BALANCE / EXCRETION	75 mg/kg bw		5 mg/kg bw	
	Males	Females	Males	Females
Urine 0-6	4.45	4.09	8.34	7.32
Urine 6-12	2.04	2.06	2.24	2.27
Urine 12-24	2.58	1.97	1.86	1.83
Urine 24-48	0.96	0.97	1.14	0.64
Urine 48-72	0.35	0.25	0.29	0.30
Urine 72-96	0.20	0.15	0.11	0.19
Urine 96-120	0.11	0.07	0.05	0.12
Urine 120-144	0.09	0.07	0.02	0.08
Urine 144-168	0.06	0.05	0.02	0.04
Subtotal Urine	10.83	9.68	14.04	12.78
Feces 0-6	0.01	0.01	0.07	0.01
Feces 6-12	0.38	0.07	0.00	7.00
Feces 12-24	31.55	29.90	17.64	22.67
Feces 24-48	26.52	21.28	25.05	18.88
Feces 48-72	1.80	6.26	2.18	0.59
Feces 72-96	0.26	1.48	0.24	0.14
Feces 96-120	0.07	2.14	0.07	0.11
Feces 120-144	0.07	0.43	0.06	0.06
Feces 144-168	0.06	0.16	0.06	0.07
Subtotal Feces	60.71	61.72	45.25	49.53
CO_2 0-6	12.51	13.74	20.98	19.14
CO_2 6-12	3.54	3.82	4.45	5.42
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	19.46	20.65	28.70	28.63
Cage wash	0.28	0.30	0.16	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	0.08
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	0.02
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	0.18	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	0.96	0.93	1.56	1.22
Charcoal filter	0.05	0.02	n.d.	n.d.
Total	93.43	94.46	91.79	93.61

n.d. = not determined

BASF communication, December 3rd, 2008

A RSS has been prepared of the biokinetics study by [redacted] (see attached) who attempted to identify metabolites of glutaraldehyde following single oral administration. Unfortunately, except for one metabolite which was identified as glutaric acid, the identification of the other metabolites failed. This was due to the fact that they had reacted with endogenous molecules from which they could not be separated.

Concerns were raised by the RMS that glutaraldehyde may have the potential to accumulate in fatty tissue. During the toxicokinetics study A6.02_01, the tissue concentration of radioactivity was determined (see tables 4, p. 36 and 9, p. 40 for details). The concentration of radioactivity in adipose tissue remains relatively constant up to 64 h post dosing but parallels the decreasing plasma levels at the 168 h sampling time. This is shown in the following diagram and table (values are in µg Eq/g tissue):



Time [h]	males	SD	females	SD	plasma	SD
1	2,09	0,46	1,84	0,48	23,44	6,81
24	4,18	1,63	1,73	0,32	4,44	0,56
36	6,07	2,55	2,17	0,35	1,95	0,21
168	2,84	1,29	1,78	0,56	0,35	0,06

The retention of radioactivity was negligible in all tissues. Only about 0.01% of the administered dose were retained in adipose tissue whereas the highest retention was found for the kidney (0.12%), the liver (0.15%) followed by the gut (0.21%) and the skin (0.43%) (see table 9 of the study report for details). Additional evidence that glutaraldehyde does not likely bioaccumulate comes from phys-chem and ecotoxicological data. Glutaraldehyde has a high water solubility (50.5 g/100 g of water, A3.05_01), independent of pH or temperature. The water/n-octanol partition coefficient (log P_{ow}, A3.09_01) is between -0.41 and -0.8, depending on the pH which indicates that the substance is not likely to be found in the organic phase, i.e. does not likely bioaccumulate. The less favorable, calculated log P_{ow} of -0.18 was used to calculate the bioconcentration factor (BCF). The EPISUITE program calculated a BCF value of between 1 and 100 which indicates that glutaraldehyde does not significantly accumulate in organisms.

We have previously shown that liver enzymes are not induced by glutaraldehyde (see attached statement dated May 7, 2008). The study by [redacted]. (see attached RSS) failed to identify most of the predicted metabolites because of the high reactivity of glutaraldehyde towards endogenous macromolecules as evidenced by covalently linked high molecular weight compounds identified during the study. As bioaccumulation of glutaraldehyde does not seem likely, the elucidation of the metabolic pathway is not technically possible and liver enzymes are not induced, a repeated dose toxicokinetics study is not warranted.

Section A6.2 _ 02 _ a Toxicokinetics following oral gavage (in vivo test)

Annex Point IIA6.2

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

Section A6.2 _ 02 _ a Toxicokinetics following oral gavage (in vivo test)

Annex Point IIA6.2

		Weight: 155 – 171 g	
3.2.6	Number of animals per group	4 animals/group	
3.2.7	Control animals	Yes (not treated with glutaraldehyde)	
3.3	**Administration/ Exposure**	Oral (gavage)	
3.3.1	Doses of test substance	5 mg/kg bw and 75 mg/kg bw	
3.3.2	Administration of the test substance	A defined amount of test substance was added to distilled water to obtain the target doses of 5 and 75 mg/kg bw; this corresponded to respectively 0.1% and 1% [w/w] glutaraldehyde solutions. Animals received a target volume of 5 ml dose solution/kg bw using a glass disposable syringe with glass plunger and stainless steel feeding needle. The selection of the test doses was based on previous toxicity data (chronic toxicity study).	X
3.3.3	Sampling time	10, 20, 30 and 45 minutes, 1, 2, 4, 6, 8, 12 hours after oral administration of the test substance.	X
3.3.4	Samples	0.2 ml blood collected at each sampling time point	
3.3.5	Samples analysis	The whole blood samples were analyzed for the parent compound glutaraldehyde using a method based on a previous gas chromatographic–mass spectrometric analysis method (GC/MS) developed for malondialdehyde and which used glutaraldehyde as an internal standard (Chiesa LM et al., Archiv für Lebensmittelhygiene, 50:41-43, 1999). The main steps of the method can be summarized as follows: (1)-The samples were acidified by addition of acidified water containing a ^{13}C-glutaraldehyde internal standard, (2)-The vials were capped, chilled and vortexed, (3)-The samples were derivatized with pentafluorobenzyl hydroxylamine, vortexed, extracted with toluene and centrifuged (10 min., 2350 x g), (4)- Toluene was removed, (5)-The samples were subjected to GC/MS.	
3.3.6	Statistics and estimation of pharmacokinetic parameters	Statistical assessment was based on the calculation of mean and standard deviation. Estimation of pharmacokinetic parameters (Cmax, AUC, elimination half-lives) was based on the pharmacokinetic computer modelling program PK Solutions (Montrose, Colorado).	
3.3.7	Remark	The whole study was conducted in two phases, an *in vivo* and an *in vitro* phase. The *in vivo* phase consisted of two experiments; within one experiment, the test substance was administered to the cannulated rats by oral gavage whereas in the second experiment, the cannulated rats were subjected to dermal application of the test substance. We here only report the data referring to the *in vivo* oral administration experiment.	

4 RESULTS AND DISCUSSION

Section A6.2 _ 02 _ a Toxicokinetics following oral gavage (in vivo test)

Annex Point IIA6.2

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

	5 mg/kg bw, Gavage	75 mg/kg bw, Gavage
Target concentration of the dose solution	1.00 mg/g	15.00 mg/g
Actual concentration of the dose solution	1.09 mg/g	16.20 mg/g
Body weight (mean +/-SD)	0.167 kg +/- 0.002	0.168 kg +/- 0.003
Dose solution administered (mean +/- SD)	0.849 g +/- 0.020	0.832 g +/- 0.023
Glutaraldehyde administered (mean +/- SD; mg)	0.9 mg +/- 0.0	13.5 mg +/- 0.4
Glutaraldehyde administered (mean +/- SD; mg/kg bw)	5.5 mg/kg bw +/- 0.1	80.2 mg/kg bw +/- 2.0

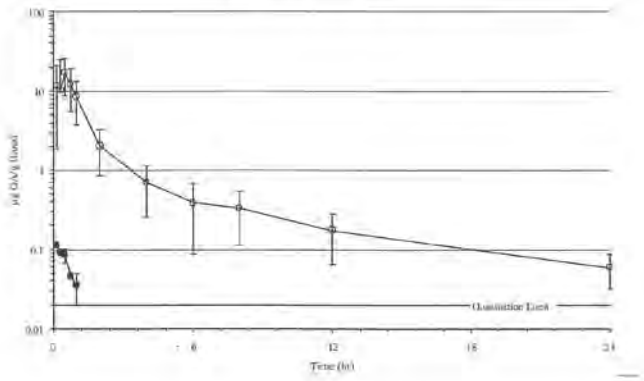
The actual doses administered orally were about 104 to 114% of the target amounts applied.

Section A6.2 _ 02 _ a Toxicokinetics following oral gavage (in vivo test)

Annex Point IIA6.2

4.2 Glutaraldehyde time course in blood

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



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

Section A6.2 _ 02 _ a Toxicokinetics following oral gavage (in vivo test)

Annex Point IIA6.2

4.3 Toxicokinetics following oral administration

Glutaraldehyde was rapidly absorbed from the gastro-intestinal tract and peak blood concentrations were reaching after 10 to 30 minutes following treatment. In fact, at the lowest tested dose of 5 mg/kg bw, the maximum mean concentration of glutaraldehyde in blood was reached after ca. 10 minutes (mean = 0.112 µg/g blood +/- 0.005). This was followed by a rapid decline in concentration, reaching a mean value of 0.035 µg/g +/- 0.015 at time point 1 hour; thereafter, glutaraldehyde in blood was no more quantifiable (all 4 animals). At a test dose of 75 mg/kg bw, a maximum mean concentration of glutaraldehyde in blood of 17 µg/g +/- 8.44 was reached after 30 minutes. This was followed by a rapid decline in concentration. However, at this test dose, low concentrations of glutaraldehyde still were quantifiable in all animals at time point 24 hours, with values ranging from 0.033 to 0.090 µg/g (mean = 0.059 µg/g +/- 0.028).

Following parameters could be calculated (PK Solutions computer modelling software):

Oral dose	5 mg/kg bw	75 mg/kg bw
T ½ a	0.474 h	0.403 h
T ½ ß	-	5.98 h
C(0)	0.150 µg/g	41.900 µg/g
Cmax	0.112 µg/g	17.005 µg/g
AUC (0-1h)	0.067 µg-h/ml	11.28 µg-h/ml
AUC (0-infinity)	0.091 µg-h/ml	23.99 µg-h/ml

T ½ a estimated from 10 minutes – 2 hours (low dose) and from 30 minutes – 2 hours (high dose).

T ½ ß estimated from 6 to 24 hours (high dose)

The initial half-lives of elimination of free glutaraldehyde from blood (0.474 and 0.403 h, corresponding to ca. 30 minutes) reflects the distribution phase and presumably binding of glutaraldehyde to tissues, including blood components. A terminal half-life of ca. 6 hours was calculated for the highest test dose, from the data between 4 and 24 hours post treatment. The AUC (0-1h) and the C max calculated at 75 mg/kg bw were respectively about 168 and 152-fold increased compared to the data referring to 5 mg/kg bw; these findings leaded to the suggestion that at the higher test dose, binding and/or metabolism of glutaraldehyde was saturated.

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.2 _ 02 _ a Toxicokinetics following oral gavage (in vivo test)

Annex Point IIA6.2

5.1	**Materials and methods**	The aim of the present study was to provide toxicokinetic data on glutaraldehyde in blood. The whole study was conducted in two phases, an in vivo and an in vitro phase. The in vivo phase consisted of two experiments; within one experiment, the test substance was administered to rats by oral gavage whereas in the second experiment, the rats were subjected to dermal application of the test substance. The present summary only refers to the in vivo dermal application experiment. Test substance: Glutaraldehyde ([redacted], purity [redacted]% glutaraldehyde, [redacted]% water, [redacted]% impurities ([redacted]) No guideline was given, however the study was guideline-like and was conducted in accordance with GLP. [redacted] female [redacted] rats were purchased from [redacted]. The test substance was administered to each animal by gavage. Two doses were tested: 5 and 75 mg/kg bw; the application volume was 5 ml dose solution/kg bw. Each test group comprised 4 animals. Blood samples (0.2 ml) were collected over the whole treatment period at following time points: 10, 20, 30 and 45 minutes, 1, 2, 4, 6, 8, 12 and 24 hours after initiation of skin contact. After 24 hours, the rats were sacrificed. Sampling analysis for glutaraldehyde was based on gas chromatography and mass spectrometry.	X
5.2	**Results and discussion**	Glutaraldehyde was rapidly absorbed from the gastro-intestinal tract and peak blood concentrations were reaching after 10 to 30 minutes following treatment. In fact, at the lowest tested dose of 5 mg/kg bw, the maximum mean concentration of glutaraldehyde in blood was reached after ca. 10 minutes (mean = 0.112 µg/g blood +/- 0.005). This was followed by a rapid decline in concentration, reaching a mean value of 0.035 µg/g +/- 0.015 at time point 1 hour; thereafter, glutaraldehyde in blood was no more quantifiable (all 4 animals). At a test dose of 75 mg/kg bw, a maximum mean concentration of glutaraldehyde in blood of 17 µg/g +/- 8.44 was reached after 30 minutes. This was followed by a rapid decline in concentration. However, at this test dose, low concentrations of glutaraldehyde still were quantifiable in all animals at time point 24 hours, with values ranging from 0.033 to 0.090 µg/g (mean = 0.059 µg/g +/- 0.028). The initial half-lives of elimination of free glutaraldehyde from blood (0.474 and 0.403 h, corresponding to ca. 30 minutes) reflects the distribution phase and presumably binding of glutaraldehyde to tissues, including blood components. A terminal half-life of ca. 6 hours was calculated for the highest test dose, from the data between 4 and 24 hours post treatment. The AUC (0-1h) and the C max calculated at 75 mg/kg bw (11.28 µg-h/ml and 17.005 µg/g) were respectively about 168 and 152-fold increased compared to the data referring to 5 mg/kg bw (0.067 µg-h/ml and 0.112 µg/g); these findings leaded to the suggestion that at the higher test dose, binding and/or metabolism of glutaraldehyde was saturated.	
5.3	**Conclusion**	In rats, following oral administration, glutaraldehyde was found to be systemically available in the blood, but also was rapidly removed from there, either by macromolecular binding or by metabolism.	

Section A6.2 _ 02 _ a Toxicokinetics following oral gavage (in vivo test)

Annex Point IIA6.2

5.3.1	Reliability	**1**
5.3.2	Deficiencies	No guideline was mentioned; however, the study was well conducted and well documented and followed GLP.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 25th, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2. 3.3.2 Administration of the test substance. This gives the same information as in the study report, but the RMS concludes that the percentages must have been 0.1 and 1.5 % to achieve 5 and 75 mg/kg bw. 3.3.3 Sampling time. Another sample was taken at terminal sacrifice (24 h) 5.1 Materials and methods. First paragraph: "The present summary only refers to the *in vivo* ~~dermal~~ **oral** application experiment."
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. Binding and/or metabolism of glutaraldehyde was found to decrease with increasing dose.
Reliability	1
Acceptability	Acceptable
Remarks	Please note that the tabulated numerical results have not been checked in detail by the RMS.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	