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

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

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

3.5.3 Other examinations

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3.5.4 Statistics

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

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

3.6 Further remarks

Neoplasm incidences from die NTP historical control database, which is updated yearly, were included in die NTP report.

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs

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

No treatment-related clinical symptoms were seen in mice.

4.1.2 Mortality

The main survival data can be summarized as follows:

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

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

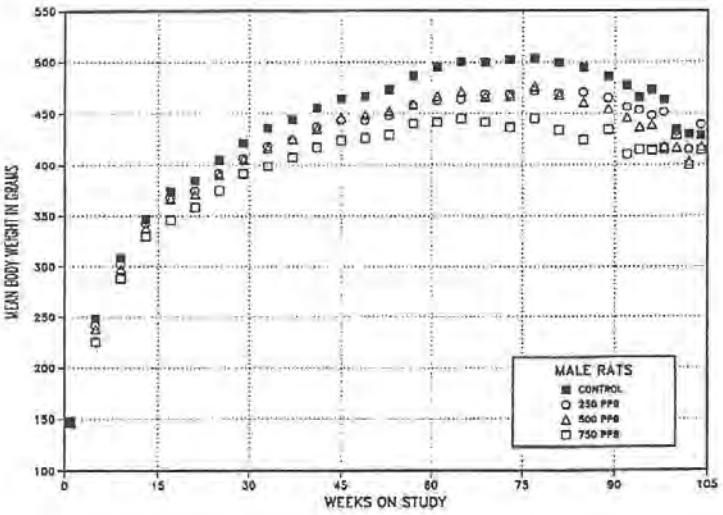
The table above shows that survival of female rats treated with 500 and 7500 ppb test substance was decreased compared to controls; survival of the treated males was similar to control. For the mice, survival in all test groups and for both sexes was similar to controls.

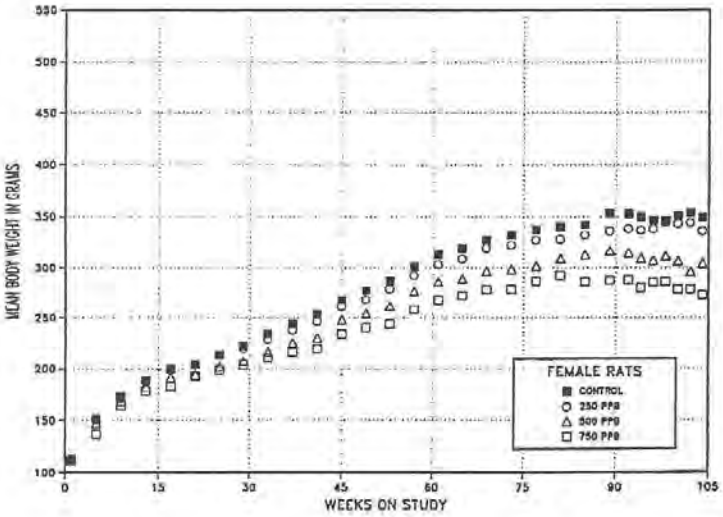
Section A6.7 _ 03 **Carcinogenicity**

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

4.2 Body weight gain, rats

Growth curve for rats exposed to glutaraldehyde for 2 years:





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

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

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

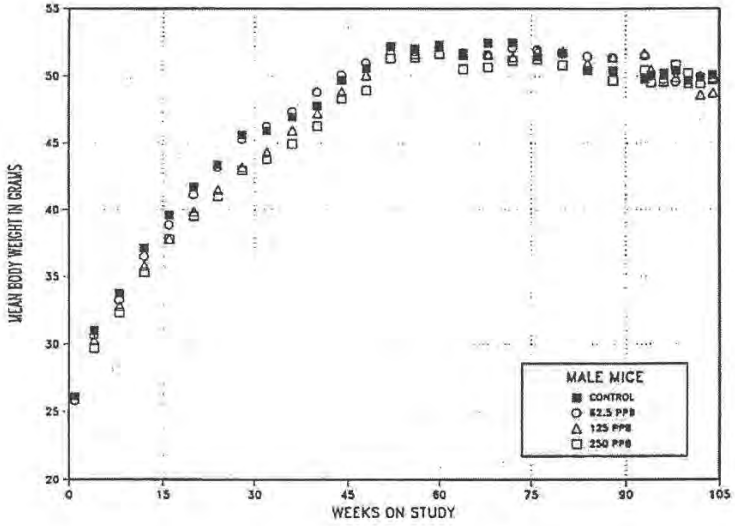
The mean body weights of all treated males and of the females of the 500 and 750 ppb groups were below control values; this effect was considered to be test substance-related.

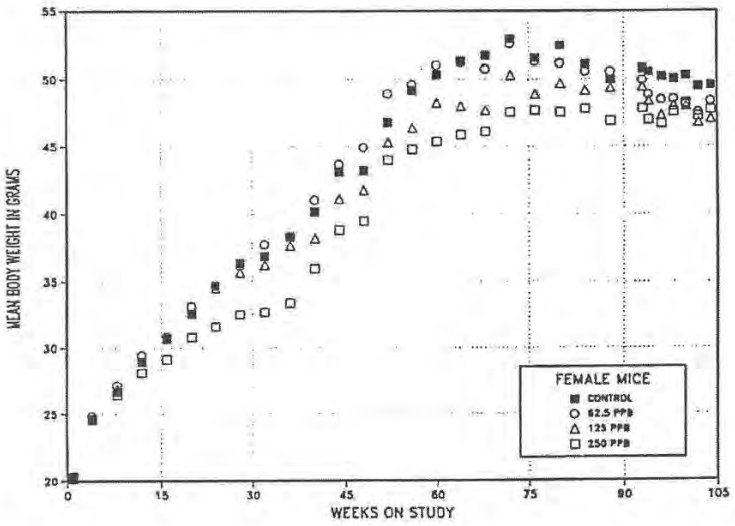
Section A6.7 _ 03 **Carcinogenicity**

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

4.3 Body weight gain, mice

Growth curve for mice exposed to glutaraldehyde for 2 years:





Section A6.7 _ 03 **Carcinogenicity**

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

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

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

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

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

4.4 Sacrifice and pathology

4.4.1 Rats

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

Section A6.7 _ 03 **Carcinogenicity**

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

Main lesions in the nose of glutaraldehyde-treated rats:

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

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

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

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

Main lesions in the lung of glutaraldehyde-treated rats:

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

Main lesions in the thyroid of glutaraldehyde-treated rats:

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

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

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

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

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

Main lesions in the kidney of glutaraldehyde-treated rats:

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

4.4.2 Mice

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

Main lesions in the nose of glutaraldehyde-treated mice:

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

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

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

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

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

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

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

An increased incidence in hyperplasia of the pituitary gland (pars distalis), which was classified as minimal to mild, was reported for the females of the 250 ppb group (28 cases versus 19 in control). The females showed no increased incidence in adenoma of the pituitary gland, and the males were free of treatment-related effects affecting the pituitary gland. Therefore, the increased incidence in hyperplasia of the pituitary gland seen in the 250 ppb females was considered as an incidental, not treatment-related finding.

X

Main lesions in the liver of glutaraldehyde-treated mice:

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

4.5 Other

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the carcinogenic potential of glutaraldehyde when administered via inhalation over a period of two years to rats and mice. Particular attention was given (1) to the nasal passage as target for treatment-related lesions, and (2) to neoplastic changes induced by the tested substance.

Test substance: Glutaraldehyde ca. [redacted] % relative to the reference standard was reported for the test substance from [redacted] relative to the reference standard was reported for the test substance of [redacted]. To ensure stability, the bulk chemical used in present studies was stored under N_2 headspace at ca. 0°C in 1-gallon amber glass bottles. The stability of the bulk material was monitored during the 2-years study by gas chromatography with flame ionization detection and by ultraviolet/visible spectroscopy. No degradation of the bulk chemical was detected.

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

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

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

Section A6.7 _ 03 **Carcinogenicity**

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

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 23rd, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	4.1.1 Clinical signs. Additionally, 8 male and 5 female rats in the high dose group had breathing difficulties and were euthanized on weeks 13-21. 4.4.2 Mice. In the table, inflammation is marked as occurring in the squamous epithelium of the females. The inflammation was actually within the epithelium and lamina propria, not just on the squamous epithelium.
Conclusion	No evidence of carcinogenicity was observed over two years of inhalation exposure of rats and mice. The study is not suitable for determining a true NOAEL for chronic toxicity because of the lack of some critical data like haematology and clinical chemistry. The effects described below may be secondary to local irritant effects. <u>Rats:</u> LO(A)EL: 250 ppb, based on significantly increased incidences of hyperplasia (♂, ♀) and inflammation (♂, ♀) of the squamous epithelium. Furthermore, trends of increasing incidence without statistical significance at this dose level were seen for hyperplasia (♀) and inflammation (♀) of the respiratory epithelium, and for hyaline degeneration of the olfactory epithelium (♂). NO(A)EL: < 250 ppb <u>Mice:</u> LO(A)EL: 62.5 ppb, based on significantly increased incidence of hyaline degeneration of the respiratory epithelium (♀) and few cases of turbinate necrosis (♀) which is not a common spontaneous lesion. The latter was seen at all dose levels in females (0, 3, 1, 4) and at 250 ppb in males (0, 0, 0, 2). NO(A)EL: < 62.5 ppb
Reliability	2
Acceptability	Acceptable
Remarks	An almost identical study summary of the same study is presented for chronic toxicity (A6.5_04), but the study actually concerns only carcinogenicity. 2.2 GLP. There is no GLP certificate, but the study is reported to be in compliance with GLP. Please note that the tabulated numerical results copied to the study summary have not been checked in detail by the RMS.
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*

Section A6.7 _ 03 Carcinogenicity

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

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

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

NTP Study: van Birgelen APJM (1999) NTP technical report on the toxicology and carcinogenesis of glutaraldehyde (CAS No. 111-30-8) administered in F344/N rats and B6C3F1 mice (inhalation studies). US Department of Health and Human Services, Public Health Service, National Institutes of Health NIH, NTP TR No: 490, NIH Publication No: 99-3980 (Published), BPD ID A6.5_04_a

Parameter	OECD TG 451 Requirements	Fulfilled/Not fulfilled	Deviations	
Test substance	Must be well characterized (purity & impurities, relevant physical-chemical properties, stability data)	Yes	-	
Test animals	TG recommends testing of a compound of unknown activity on two animal species	Yes	-	
	Rat and mouse preferred	Yes (both tested)	-	
	Preference should be given to strains with a low incidence of spontaneous tumours	The used strains were F344 rats and B6C3F$_1$ mice, both are well-characterized in terms tumour incidence	-	
	Healthy animals	Yes (parasite evaluation and gross examination for disease prior test initiation)	-	
	A period of acclimatization to environmental conditions, and in case of animals from outside sources, an adequate period of quarantine must be warranted	Yes (quarantine period of 18 days for rats, 14 days form mice)	-	
	Both sexes must be considered	Yes	-	
	Dosing of the rodents should begin preferably before the animals are 6 weeks old.	Yes (animals were ca. 6 to 7 weeks old at test initiation, i.e.)	-	
	Adequate randomization procedures needed for properties allocation of animals to test and	Yes	-	

	control groups			
	Low initial BW variation must be warranted	Yes	-	
	50/sex/group when no interim sacrifice planned	Yes	-	
	Inclusion of a high dose satellite group for the evaluation of pathology other than neoplasia with 20/animal/sex & a satellite group with 10 animals/sex	No, however not compulsory.	-	
Housing and environment	No specification given on housing/cage	Individual housing, one animal/cage	-	
	Cages must be capable of regular and easy cleaning.	Yes (stainless-steel wire-bottom cages were used and changed weekly)	-	
	Bedding should be sterilized.	Partly (bedding used was obtained [redacted]; it can be assumed that OECD requirement about sterility was fulfilled) Bedding was changed daily, and removed during exposures.		

Table Comparison of the NTP test conduct with OECD TG413, continues

Parameter	OECD TG 451 Requirements	Fulfilled/Not fulfilled	Deviations	
Housing and environment	The use of disinfectants and pesticides should be avoided, particularly where they may come in contact with the animal.	No information here about the provided in the report as such, but assumable.	-	
	Animals should be housed in quiet, well-ventilated rooms, with controlled lighting, temperature and humidity.	Yes (for details see below)	-	
	Temperature 22±2°C	Yes (22-25.5°C)	-	
	Rel.Hum. 30-70 %	Yes (55±15%)	-	
	Dynamic air change in exposure room of 12-15/hr	Yes (15±3 changes/hr)		
	12 hrs light/12 hrs night	Yes	-	
Diet	The diet should meet all the nutritional requirements of the species tested, must be free of impurities, replaced at leas weekly	Yes (detail on ingredients, nutrient composition, vitamins and minerals provided in the report as table)	-	
	The diet must be replaced at least weekly	Yes (weekly)	-	
	3 types of diet are generally used: conventional (standard), synthetic, and various open formula diets. The first two are the most widely used in carcinogenicity bioassays.	Open formula diet in pellet (NIH-07, accepted by OECD TG)	-	
	Periodic analysis of diet may be carried out for unintentional contaminants, including carcinogens. Results from such analyses should be retained and	Yes (detail on contaminants and contaminant levels provided in the report as table)	-	

	included in the final report.			
Water	Drinking water ad libitum	Yes	-	
	The presence of potential contaminants in the water offered to the animals must be considered. Data available on the components in the water should be ascertained.	Partly	Details on the supply and watering system were provided. The water was described as softened tap water from municipal supply; no further information provided.	
Test conditions	At least three dose levels should be tested & control group & vehicle control if necessary	Yes Rats: 0, 250, 500 and 700 ppb Mice: 0, 62.5, 125 and 250 ppb	-	
	Selection of concentration levels must be justified, preferably on the results of a sub chronic study.	Yes (13 week NTP study 1993)	-	

Table Comparison of the NTP test conduct with OECD TG413, continues

Parameter	OECD TG 451 Requirements	Fulfilled/Not fulfilled	Deviations	
Test conditions	The highest dose level should elicit signs of minimal toxicity without substantial altering the normal life span.	Yes	-	
	The lowest dose should not interfere with normal growth, development, and longevity and it must not be toxic. It should no be <10% of the high dose.	Yes	-	
	The intermediate dose(s) should be establish in a mid-range between the high and low doses.	Yes	-	
	A concurrent control group which is identical in every respect to the exposed groups, expected for exposure to the test substance, should be used.	Yes	-	
	Proposed/recommended: either daily exposure of 6 hrs after equilibration of chamber concentrations, 5 days/week, with 22-24 hours of exposure per day, 7 days/week.	Yes (exposure of 6 hrs +25 minutes (T_{90}), 5 days/week	Remark: build up and decay rates for the test concentrations in the chambers were taken into account.	
	Whole body exposure accepted.	Yes	-	
	Fasting during exposure (feed & water)	Yes	-	
Test system	A dynamic inhalation system with suitable analytical concentration check system.	Yes	-	
	Monitoring of test concentration	Yes (chamber GA concentration monitored by	-	

		online gas chromatography)		
	Concentrations maintained stable during exposure	Yes (in order to maintain uniform exposure concentrations, chamber air circulation was increased by means of a recirculation system, which was added to each exposure chamber)	Remark: To overcome the adsorption of the vapour once it entered the exposure chambers, recirculation system were added to increase the air velocity through the chambers; this did not affect the normal air exchange rate. The stainless-steel inhalation exposure chamber was Hazleton H-2000® from Harford Systems Division of Lab Products, Inc., Aberdeen, MD, allowing maintenance of uniform vapour concentrations in the chamber by means of catch pans.	

Table Comparison of the NTP test conduct with OECD TG413, continues

Parameter	OECD TG 451 Requirements	Fulfilled/Not fulfilled	Deviations	
Test system	Air flow monitoring	Yes		
	Particle size distribution analysis.	Not relevant as vapour tested.	A small particle detector was used to check that GA was present in the exposure chamber as vapour and not as aerosol, both in presence and absence of test animals. No particle counts above the minimum resolvable level of ca. 200 particles/cm^3 was detected.	
Duration of the study	Generally, the termination of the study should be at 18 months for mice and hamsters and 24 months for rats; however, for certain strains of animals with greater longevity and/or low spontaneous tumour rate, termination should be at 24 months for mice and hamsters and at 30 months for rats.	For both species, the test duration was 104 weeks, i.e. 26 months, which is above the range recommended by the OECD TG. This however did not affect the quality of the study and findings.	-	
Acceptability criteria for negative test	No more than 10 per cent on any group is lost due to autolysis, cannibalism, or management problems.	Yes (only one case of accidental death reported for mice)	-	

	Survival in each group is no less than 50 per cent at 18 months for mice and at 24 months for rats.	Partly fulfilled (mice); however the reported survival percentage refer to 104 weeks, (i.e. 26 months)	Rat♀: 52% in control, 62% at 250 ppb, 30% at 500 ppb and 28% at 750 ppb. Rat♂: 24% in control, 74% at 62.5 ppb, 70% at 125 ppb and 64% at 250 ppb. Mice♀: 68% in control, 74% at 62.5 ppb, 80% at 125 ppb and 76% at 250 ppb. Mice♂: 62% in control, 54% at 62.5 ppb, 80% at 125 ppb and 76% at 250 ppb.	
Examination	Sacrifice in extremis of moribund animals, necropsy of animals sacrificed prior test ending	Yes		
	Clinical signs and mortality should be recorded for all animals. Special attention must be paid to tumour development.	Yes		

Table Comparison of the NTP test conduct with OECD TG413, continues

Parameter	OECD TG 451 Requirements	Fulfilled/Not fulfilled	Deviations	
Examination	Body weights should be recorded individually for all animals once a week during the first 13 weeks of the test period and at least once every 4 weeks thereafter.	Partly fulfilled; the selected time points were different from those recommended by the TG. BW was recorded at test initiation and thereafter, every 4 weeks from week 5 to week 89, and every 2 weeks from week 92 (rats) and 93 (mice) until test ending. This, however had no negative influence on the quality and validity of the results.	-	
	Food intake should be determined.	No	-	
	If clinical observations suggest a deterioration in health of the animals during the study, a differential blood count of the affected animals should be performed.	No, however not relevant.	-	
	At 12 months, 18 months and prior to sacrifice, a blood smear is obtained for all animals. A differential blood count is performed on samples on those animals in the highest dosage group and the controls.	No, however not relevant as detailed haematology was performed in a sub-chronic inhalation study conducted with the same strains, under same conditions, with dose levels similar to those tested in present case. In fact, at the end of the 13-	-	

		week experimental period, no biologically relevant/treatment-related changes in haematology were seen.		
	Clinical chemistry not required by the TG	Yes (not considered)	-	
	Urinanalysis not required by the TG	Yes (not considered)	-	

Table Comparison of the NTP test conduct with OECD TG413, continues

Parameter	OECD TG 451 Requirements	Fulfilled/Not fulfilled	Deviations	
Pathology	Gross necropsy should be carried out under the guidance of trained laboratory animal pathologist.	Yes (evaluations were completed by the study laboratory pathologist. The slides, individual animal data records, and pathology data/tables were further evaluated by an independent quality assessment laboratory. The quality assessment report and the reviewed slides were submitted to die NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell et al. (1986)	-	
	Full gross necropsy	Yes	-	

	required			
	Weighing of organs not required.	Yes (not considered)	-	
	All organs and tissues of all animals should be preserved for microscopic examination.	Yes (complete histopathology was performed on all rats and mice, for gross lesions, tissue masses and a wide range of organs and tissues)	-	
	Full and detailed histopathological examinations	Yes (well-described and reported)	-	
	Historical control to be taken into account in addition to the study own control data.	Yes: Neoplasm incidences from the NTP historical control database, which is updated yearly, were included in the NTP report)	-	
Data evaluation	Data summaries in tabular form	Yes (Appendix A)	-	
	Data statistical evaluation	Yes	-	

Table Comparison of the NTP test conduct with OECD TG413, continues

Parameter	OECD TG 451 Requirements	Fulfilled/Not fulfilled	Deviations	
Data reporting	Test report should include description of test conditions (e.g. exposure apparatus), exposure data (e.g., air flow rate, nominal and actual test concentrations), animal data.	Yes	-	
Conclusion: Test conduct and data reporting are almost in accordance with the requirements of the OECD guideline 451 (1981). Few requirements were not or only partly fulfilled; this, however did not affect the quality and validity of the results reported. Thus, the present study can be seen as acceptable for assessment. The chronic toxicity aspect only was considered up to a certain point: no data on food and water consumption, haematology, clinical-chemistry, urinalysis and organs weights were reported. On the other hand, mortality and body weights data and especially non-neoplastic findings were well documented /described and support the findings of the subchronic inhalation study (see A6.4.3). Thus the toxicity data of the present carcinogenicity study were entered in the BPD dossier as supplementary information (see A6.5_04).				

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1991), Report on the study of the prenatal toxicity of glutaraldehyde in rabbits after oral administration (gavage). [redacted] (Unpublished), BPD ID A6.08.1_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 active substance (a.s.) for first entry to Annex I authorisation.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 414 (1981)	
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	Fluid and colorless	
3.1.2.2	Purity	[redacted]% in water	
3.1.2.3	Stability	The purity, the homogeneity and the stability of the test substance, were checked.	
3.2	**Test Animals**		
3.2.1	Species	[redacted] rabbit	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: About 26 to 27 weeks old at study start Mean weight: About 2.596 kg /animal	
3.2.6	Number of animals per group	15 females /group	
3.2.7	Control animals	Yes	

3.2.8	Mating period	The rabbits were fertilized by means of artificial insemination, using pooled ejaculate from male [redacted] rabbits of the same breed as the females. The day of insemination was designated as day 0 (study start) and the following day as day 1 post-insemination (p.i.).
3.3	**Administration/ Exposure**	
3.3.1	Duration of exposure	From day 7 to day 19 p.i. (corresponding to the period of organogenesis)
3.3.2	Post-exposure period	From day 20 to day 29 p.i. (10 days post exposure)
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	The test doses used within the present study were chosen on the basis of the results of following range-finding studies: (1)-[redacted] (1991), Report on the range-finding study of the prenatal toxicity of glutaraldehyde in rabbits after oral administration (drinking water). [redacted] (unpublished) (2)-[redacted] (1991), Report on the range-finding study of the prenatal toxicity of glutaraldehyde in rabbits after oral administration (gavage). [redacted] (unpublished) The test doses were as follows:

Test group	Dose (mg/kg bw/day)	Concentration (mg/100 ml)***	Volume (ml/kg bw)
0	0*	0	10
1	5	99	10
2	15	298	10
3	45	895	10

*, Control animals treated with doubly distilled water.
***, Taking into account the active ingredient content of 50.3%

3.3.5	Vehicle	Aqueous solution
3.3.6	Concentration in vehicle	See 3.3.4
3.3.7	Total volume applied	See 3.3.4
3.3.8	Controls	Control animals were treated with doubly distilled water.
3.4	**Examinations**	
3.4.1	Clinical signs of toxicity and mortality	Yes, the animals were checked daily, at least once for clinical symptoms and twice for mortality on working days. On Saturdays, Sundays or public holidays, the animals were only checked once a day for mortality. In case of presence of clinical signs of toxicity, the animals were checked several times a day.

3.4.2	Body weight	Yes, body weight was determined on day 0, 2, 4, 7, 9, 11, 14, 16, 19, 21, 23, 25 and 29 p.i.; body weight change was calculated on the basis of he values obtained.
3.4.3	Food consumption	Yes, the food consumption was determined daily during the entire study period.
3.4.4	Necropsy	On day 29 p.i., all surviving females were sacrificed for the purpose of necropsy; they were examined macroscopically. The fetuses were dissected from the uterus and subjected to further examinations. Dams showing signs of abortion also were sacrificed and examined as above.
3.4.5	Net maternal body weight change	Following sacrifice, the net maternal body weight change (i.e. the corrected body weight gain) was calculated from the terminal body weight by subtraction of (1) the uterus weight and (2) the body weight measured on day 7 p.i.
3.4.6	Examination of uterine content	Gravid uterine weight Number of corpora lutea Number and distribution of implantations sites classified as live fetuses and dead implantations. Dead implantations comprised early resorptions, late resorptions and dead fetuses. The conception rate (CR) as well as the pre- and post- implantation losses (Pre-I, Post-I) were calculated according to following formulas: $CR = \frac{\text{Number of pregnant animals}}{\text{Number of fertilized animals}} \times 100$ $\text{Pre-I} = \frac{\text{N*(corpora lutea) - N (implantations)}}{\text{N (implantations)}} \times 100$ $\text{Post-I} = \frac{\text{N(implantations) - N (live fetuses)}}{\text{N (implantations)}} \times 100$ *, N = number of.
3.4.7	Examination of foetuses	

3.4.7.1	General	The fetuses were extracted from the uterus and were examined for following parameters: Fetal weight, external abnormalities, viability, placentae, umbilical cords, fetal membranes, and fetal liquids. Individual placental weights were recorded.
3.4.7.2	Skelet	The skeletons of the fetuses fixed in ethyl alcohol were stained according to the method of Dawson (Stain Technol. 1: 123, 1926)
3.4.7.3	Soft tissue	For the purpose of soft tissue examination, the fetuses were sacrificed and their abdomen and thorax were opened for in situ examination of the organs prior removal. Heart and kidney were sectioned for internal examination. The sex of the fetuses was determined by internal examination of the gonads. In case of abnormalities (e.g. hydrocephalus, microphthalmia or cleft palate), the head was separated from the remaining body and was fixed in Bouin's solution for further procession and assessment according to Wilson's method (Wilson and Warkany, Teratology: principles and techniques. The university of Chicago Press, Chicago and London, 1965).
3.5	**Statistical evaluation**	Food and water consumption, body weight and body weight change, corrected maternal body weight gain, gravid uterine weight, weight of the fetuses, weight of the placentae, corpora lutea, implantations, pre- and post implantation losses, resorptions and live fetuses, were assessed by means of Dunnett's Test. Conception rate, maternal mortality and all fetal findings were assessed by means of Fisher's Exact Test.
3.6	**Further remarks**	The purity, homogeneity and stability of the test substance were checked ([redacted]). The test substance solutions (glutaraldehyde in doubly distilled water) also were checked for stability. Reanalysis was performed at the end of the study period. Samples of the test substance preparations were verified for concentrations. Food as well as drinking water also was subjected to analyses according to the EPA guideline (Fed. Reg. Vol. 44, No.91, 1979) and to the German Drinking Water Regulation (Trinkwasserverordnung, Bundesgesetztblatt, 1990).

4 RESULTS AND DISCUSSION

4.1 Females excluded from data evaluation and/or calculation

Following females were partly or totally excluded from data evaluation/calculation because of non pregnancy:

- 1 female of the 5 mg/kg bw/day group
- 1 female of the 15 mg/kg bw/day group
- 5 females of the 45 mg/kg bw/day group (these animals died intercurrently).

4.2 Clinical signs of toxicity and mortality

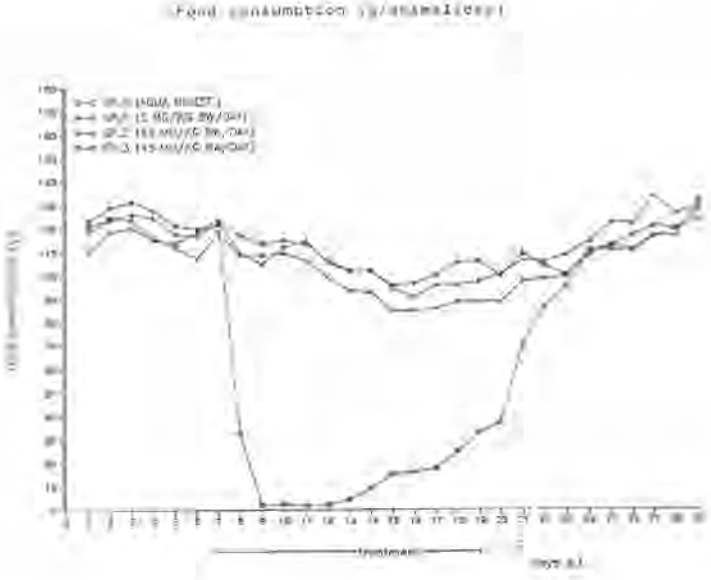
Mortality was seen in the 45 mg/kg bw /day group, where 3 dams died on day 9 p.i., and 2 further on day 11 p.i. Almost all surviving dams of the 45 mg/kg bw/day group showed no defecation during one or more days of the treatment and the post-treatment period. This effect was related to the decrease in food consumption reported for the same days/periods of days. Moreover, 9 surviving dams of the 45 mg/kg bw/day also suffered from soft feces and/or diarrhea (6 cases). Blood was seen between day 14 and 18 p.i. in the bedding of 3 dams of this group.

The remaining test groups were inconspicuous.

4.3 Food consumption

Food consumption in the 45 mg/kg bw/day group was severely reduced during the treatment period (day 7 to day 19 p.i.) and thereafter during the post- treatment period from day 19 to day 21 p.i. Food consumption thereafter turned back to or even exceeded control values. The impairment in food consumption observed at 45 mg/kg bw/day was considered to be treatment-related.

Whereas food consumption in the 15 mg/kg bw/day group was inconspicuous, a slight impairment was seen at 5 mg/kg bw/day, which however rather was incidental and without any biological significance than treatment-related.

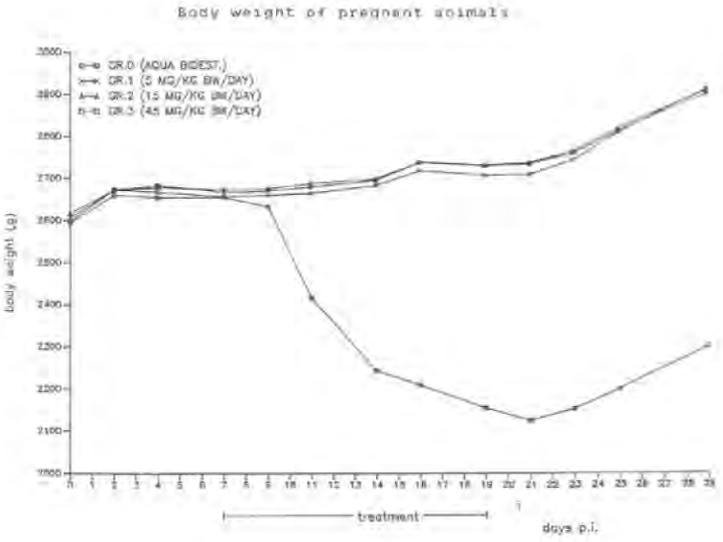


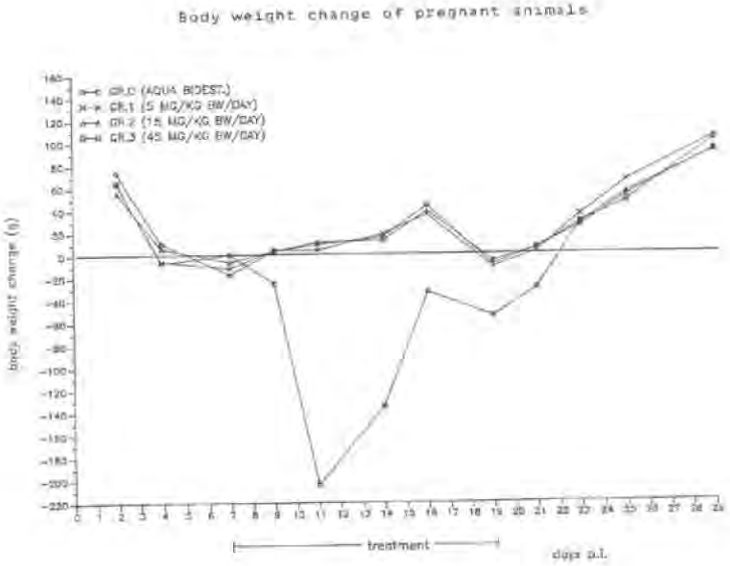
4.4 Maternal body weight data

The mean body weight of the 45 mg/kg bw/day females was statistically significantly reduced from day 11 to day 29 p.i. (about 22% below control value at study termination). A severe loss in body weight in this group was observed during the treatment period (from day 7 to day 19 p.i.) and thereafter, from day 19 to day 21 p.i. From day 21 to day 29 p.i., the females

of the 45 mg/kg bw/day group gained weight again. The impairments in body weight clearly were related to the reduced food consumption of the animals and were therefore considered to be treatment-related.

Body weight and body weight change in the remaining test groups (5 and 15 mg/kg bw/day) were inconspicuous compared to control.





4.5 Dams at study termination

Gravid uterine weight (GUW):

Test doses				
GUW (g)	0 (N=15)	5 mg/kg bw/day (N=14)	15 mg/kg bw/day (N=14)	45 mg/kg bw/day (N=10)
	337.9 +/- 81.49	383.4 +/- 78.91	325.3 +/- 108.80	24.4** +/- 51.69

**, $p<0.01$

The mean gravid uterus weight in the 45 mg/kg bw/day group was found to be statistically significantly reduced compared to control, reaching about 7%

of the control value. This effect was considered to be treatment-related and furthermore was in line with the increased number of resorptions and postimplantation loss seen in the 45 mg/kg bw/day group. For the remaining groups (control, 5, 15 mg/kg bw/day), the gravid uterine weight was quite similar and/or within the range of biological variation.

Net maternal body weight change from day 7 p.i:

	Test doses			
NBW (g)	0 (N=15)	5 mg/kg bw/day (N=14)	15 mg/kg bw/day (N=14)	45 mg/kg bw/day (N=10)
	-101.6 +/- 61.60	-127.0 +/- 42.17	-88.6 +/- 82.79	-324.4** +/- 163.71

**, $p<0.01$

Whereas both, the 5 and the 15 mg/kg bw/day group were inconspicuous, the net maternal body weight change in the 45 mg/kg bw/day group significantly was reduced compared to control.

Necropsy:

Necropsy of the animals that died intercurrently revealed signs of irritation within the gastro-intestinal tract, including diffuse reddening of the fundus, thickening of the wall of the fundus and of the pylorus due to edema, and ulceration and/or distention of the cecum or the colon. No such signs were seen in the 45 mg/kg bw/day survivors that were sacrificed at the end of the study.

Reproduction data:

The main reproduction data were summarized in table A6_8-1.

A conception rate of about 93% was reported for respectively the 5 and the 15 mg/kg bw/day group, whereas conception rate reached 100% in control and in the 45 mg/kg bw/day group. Moreover, all considered parameters (conception rate, number of corpora lutea, number of implantation sites, pre- and post implantation losses, resorptions and live fetuses) were inconspicuous for the 5 and the 15 mg/kg bw/day groups; in fact, differences here were incidental and within the normal range of deviations known for the used test animal. In contrast, in the 45 mg/kg bw/day group, 9 of 15 animals had no viable fetuses at all but only early resorptions; only one female gave 4 alive fetuses on the scheduled date. Therefore, a severely increased resorption rate was reported for this test group, and as a consequence the post-implantation loss also was clearly increased (94.3%).

4.6 Teratogenic / embryotoxic effects

Fetal external findings:

Fetal sex-distribution, placental weight and fetal weight in the 5 and the 15 mg/kg bw/day groups were quite similar to control and/or within the range of biological variation. For the 45 mg/kg bw/day group, due to the limited number of live fetuses, no clear assessment of the parameters mentioned above was possible. In fact, for this group, the placental weight was the lowest compared to control (mean of 4.1 g versus 4.9 g for control, and 4.6 g for respectively the 5 and the 15 mg/kg bw/day group); the fetal weight also was the lowest (mean of 26.9 g versus 41.7 g for control, 40.2 g for the 5 mg/kg bw/day group, and 41.3 g for the 15 mg/kg bw/day group) and furthermore was outside the historical control range (29.9 – 53.2 g, based on 188 control litters). The examination of the fetuses revealed neither malformations nor variations or unclassified abnomalities in the treated groups; in contrast, a single case of cheiloschisis (malformation) and a single

X

case of pseudoankylosis (variation) were reported for the control group.

Soft tissues:

One case of hydrocephaly was reported for the control group. Three cases of agenesia of the gallbladder (2 fetuses of the 5 mg/kg bw/day group, one fetus of the 15 mg/kg bw/day group) further were reported. A case of truncus arteriosus communis also was seen in the 15 mg/kg bw/day group. Cases of separated origin of carotids, which was a very common finding for the strain used, as well as cases of hearts with traces of interventricular foramen/septum membranaceum were reported for all the groups. The following table summarizes the fetal incidence (%) of these two types of soft tissue variation:

Soft tissue variation	0 mg/kg bw/day (control)	5 mg/kg bw/day	15 mg/kg bw/day	45 mg/kg bw/day
Fetuses evaluated (N)	89	101	83	4
Separated origin of carotids	37%	32%	39%	50%
Hearts with traces of interventricular foramen/septum membranaceum	21%	24%	25%	50%

In addition, 3 cases of focal liver necrosis and one case of autolysis (dead fetus) were reported as unclassified observations for the 5 mg/kg bw/day group.findings. None of all these findings was treatment-related.

Skeletal findings:

Malformations of skull, vertebral column and sternum were reported for the control group (4 cases) and the 15 mg/kg bw /day group (one case); in contrast, no such skeletal malformations were seen in the 5 and the 45 mg/kg bw/day groups. Variations such as e.g. accessory or shortened ribs were seen in single fetuses of all groups including the control; signs of retardations (e.g. incomplete or missing ossification of skull bones) also occurred in all groups. All the findings occurred without dose-relationship and/or were without any biologically relevance, and/or within the range of historical control data known for the rabbit strain used.

4.7 Other effects

Test substance:

The homogeneity and stability of the test substance was confirmed. The reanalysis revealed a content in active ingredient >= [redacted]%. The stability of the test substance solutions over a period of 4 days was confirmed, but the correctness of the prepared concentrations showed some discrepancies. In fact, within the first analyses on samples taken at the study start, the actual values were significantly increased compared to the nominal values (up to 20%). In contrast, the analyses conducted at the end of the study period revealed values that were about 20% below the nominal values (intermediate and high dose), whereas additional analyses conducted on frozen samples revealed concentrations which were about 10% above the nominal values (intermediate and high dose).

The following table summarizes the mean values obtained during the different concentration control analyses:

Target concentration (mg/100 ml)	Actual concentration (mg/100 ml) (Analyses: Apr. 2 - 4, 1990)	Actual concentration (mg/100 ml) (Reanalyses: July 5 - 10, 1990)	Actual concentration (mg/100 ml) (Analyses: May 17 - 21, 1990)	Actual concentration (mg/100 ml) (Reanalyses: July 6 - 10, 1990)
0 0	112 (113%) 112 (113%)	105 (106%) 102 (103%)	102 (103%) 102 (103%)	104 (105%) 106 (107%)
296 296	357 (120%) 357 (120%)	337 (114%) 333 (112%)	240 (81%) 240 (81%)	325 (110%) 332 (112%)
885 885	1,050 (117%) 1,050 (117%)	1,006 (112%) 1,019 (115%)	748 (83%) 748 (83%)	999 (112%) 970 (106%)

Food and water:

Food and water were found to be suitable.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate glutaraldehyde for its prenatal toxicity in Himalayan rabbit.

Test substance: Glutaraldehyde, [redacted]% in water

The test was conducted according to OECD guideline 414 (1981), with GLP.

Following artificial insemination, the fertilized rabbits (15/test group) were treated by gavage from day 7 to day 19 post-insemination (p.i.) with glutaraldehyde at doses of 0, 5, 15 and 45 mg/kg bw/day. The animals were observed for clinical symptoms, mortality, body weight and body weight change, and for food consumption. On day 29 p.i. all females were sacrificed and assessed by gross pathology. The gravid uterine weight as well as the net maternal body weight change was determined. A series of reproduction parameters including conception rate, number of corpora lutea, number of implantation sites, pre- and post implantation losses, resorptions and live fetuses were examined. The fetuses were dissected from the uterus, sexed weighed and further investigated for any external, soft tissue and/or skeletal findings.

The test substance was analyzed for purity, homogeneity and stability. Test substance preparations were examined for stability and homogeneity and the correctness of the test concentrations was verified. The homogeneity and stability of the test substance were confirmed. The reanalysis revealed a content in active ingredient >= [redacted]%. The stability of the test substance solutions over a period of 4 days was confirmed.

5.2	**Results and discussion**	Maternal toxicity: Treatment-related signs of maternal toxicity were observed at the highest tested dose of 45 mg/kg bw/day; the remaining test doses were inconspicuous. In fact, 5 cases of mortality (day 9 and day 11 p.i.) occurred in the 45 mg/kg bw /day group. The surviving dams of this group showed symptoms of toxicity including absence of defecation (during one or more days), soft feces and/or diarrhea; blood was seen between day 14 and 18 p.i. in the bedding of 3 dams of this group. Food consumption in the 45 mg/kg bw/day group was severely reduced during the treatment period (day 7 to day 19 p.i.) and during the post-treatment period from day 19 to day 21 p.i.; food consumption thereafter turned back to or even exceeded control values. The mean body weight of the 45 mg/kg bw/day females was statistically significantly reduced from day 11 to day 29 p.i. (ca. 22% below control value at study termination); a severe loss in body weight was observed during the treatment period (from day 7 to day 19 p.i.) and thereafter from day 19 to day 21 p.i.; from day 21 to day 29 p.i., the females of the 45 mg/kg bw/day group gained weight again. Necropsy of the animals that died intercurrently revealed signs of irritation within the gastro-intestinal tract; no such signs were seen in the 45 mg/kg bw/day survivors that were sacrificed at the end of the study. The mean gravid uterus weight in the 45 mg/kg bw/day group was found to be statistically significantly reduced (ca.7% of control value); the net maternal body weight change in the 45 mg/kg bw/day group also was significantly reduced compared to control. Considering the reproduction data, the conception rate was inconspicuous (100%). Nine of 15 animals had no viable fetuses at all but only early resorptions; only one female gave 4 alive fetuses on the scheduled date. Therefore, a severely increased resorption rate was reported for this test group, and as a consequence the post-implantation loss also was clearly increased (94.3%). Embryo-/fetotoxicity, teratogenicity: Neither embryo/fetotoxic nor teratogenic effects were seen at 5 and 15 mg/kg bw/day. In the 45 mg/kg bw/day group, the findings referring to the fetuses were indicative of an increased embryotoxicity/-lethality (only one female gave 4 alive fetuses, resorptions and post-implantation losses were significantly increased compared to the remaining groups, reduction in mean placental and fetal weights), but did not indicate teratogenicity. The decreased number of fetuses, which was available for examination, however must be taken into account.
5.3	**Conclusion**	
5.3.1	LO(A)EL maternal toxic effects	45 mg/kg bw/day
5.3.2	NO(A)EL maternal toxic effects	15 mg/kg bw/day
5.3.3	LO(A)EL embryo/ fetotoxic effects	45 mg/kg bw/day
5.3.4	NO(A)EL embryo/ fetotoxic effects	15 mg/kg bw/day

5.3.5	NO(A)EL teratogenic effects	Not evaluable.
5.3.6	Reliability	**2**
5.3.7	Deficiencies	(1) The correctness of the prepared concentrations showed some discrepancies. In fact, within the first analyses on samples taken at the study start, the actual values were significantly increased compared to the nominal values (up to 20%). In contrast, the analyses at the end of the study period revealed values about 20% below the nominal values (intermediate and high dose), whereas additional analyses conducted on frozen samples revealed concentrations, which were about 10% above the nominal values (intermediate and high dose). These discrepancies however do not affect the validity of the study. (2) Only 4 fetuses were available in the 45 mg/kg bw/day group for the evaluation of the teratogenic potential of the test substance. Because of this low number of fetuses, no clear assessment of teratogenicity was possible.

Section A6.8.1 _ 01 **Teratogenicity Study**

Annex Point IIA6.8.1 **Himalayan Rabbit**

	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 1st, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	4.6 Teratogenic/embryotoxic effects. There was a slightly increased incidence of total foetal skeletal variations (12, 14, 18, 25 %). The 25 % indicates one affected foetus out of the 4 foetuses that survived in the high dose group, and can be disregarded in any analyses. The 18 % at the mid-dose group is mainly due to the findings of sternebrae of irregular shape (6 %, n=5, historical control range 0-8.6 %) and rudimentary cervical ribs (6 %, n=5, historical control range 0-4.5 %). This finding is concluded incidental.
Conclusion	Glutaraldehyde was not teratogenic to [redacted] rabbits. There were no significant differences between the control group, low-dose group and mid-dose group with regard to any of the parameters studied. Severe embryotoxicity resulted at a concentration that was also lethal to 5/15 of the dams. LOAEL (maternal): 45 mg/kg bw/day NOAEL (maternal): 15 mg/kg bw/day LOAEL (embryotoxicity): 45 mg/kg bw/day NOAEL (embryotoxicity and teratogenicity): 15 mg/kg bw/day
Reliability	2
Acceptability	Acceptable
Remarks	The study would not satisfy the requirements of the current OECD 414 guideline with regard to the animals used, and because of the high toxicity at the highest dose level and the lack of toxic effects at the mid-dose level. The requirements are however satisfied of the guideline that was valid at the time of the test (OECD 414 guideline, 1981). The selection of the dose levels (5, 15 and 45 mg/kg bw/day) is poor because of the high toxicity at the high dose level (maternal mortality 5/15, only 4 viable foetuses) and no signs of toxicity at the mid-dose level. The rationale for the dose spacing was based on the two dose levels in the range finding study, where the lower dose level caused no toxicity and was used as the low dose in the main study (5 mg/kg bw/day), and the higher dose was 25 mg/kg bw/day and caused maternal toxicity (gastritis, reduced food consumption). The RMS considers this problem to be due to the very steep dose-mortality curve, and considers that the high dose and mid-dose are sufficiently close to each other for the acceptability of the study. The deficiencies do not compromise the essential results of the study. Please note that the tabulated results in the Tables 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*

Section A6.8.1 _ 01 **Teratogenicity Study**

Annex Point IIA6.8.1 **Himalayan Rabbit**

Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_8-1. Summary of reproduction data

Reproduction parameters		0 mg/kg bw/day (control)	5 mg/kg bw/day	15 mg/kg bw/day	45 mg/kg bw/day
Mated females (N)		15	15	15	15
Pregnant females (%)		100%	93%	93%	100%
Abortions (N)		0	0	0	0
Premature births (N)		0	0	0	0
Dams with viable fetuses (N)		100%	93%	93%	7%
Dams with resorptions (%)		0%	0%	0%	60%
Female mortality, N (%)		0 (0%)	0 (0%)	0 (0%)	**5* (33%)**
Pregnant at Ceasarian section (%)		100%	93%	93%	**10* (67%)**
Mean corpora lutea (total corpora lutea)		8.5 +/- 1.2 (128)	8.7 +/- 1.5 (122)	8.1 +/- 1.1 (113)	7.6 +/- 1.7 (76)
Mean implantation sites (total implantation sites)		6.9 +/- 1.6 (103)	7.6 +/- 1.8 (107)	6.6 +/- 2.1 (92)	7.2 +/- 1.2 (72)
Mean pre-implantation loss (%)		19.2% +/- 17.7	12.1% +/- 13.7	19.0% +/-21.8	4.0% +/- 9.1
Mean post-implantation loss (%)		13.6% +/- 14.6	7.4 +/- 13.9	12.0 +/- 19.1	**94.3%**** +/- **18.1**
Mean total resorptions (%)		13.6% +/- 14.6 Early: 7.6% +/- 10.9 Late: 6.0% +/- 9.5	6.2 +/- 10.1 Early: 4.2% +/- 7.2 Late: 2.0% +/- 5.2	12.0 +/- 19.1 Early: 4.3% +/- 9.7 Late: 7.7% +/- 11.9	**94.3%**** +/- **18.1** Early: **91.4%**** +/- **27.1** Late: 2.9% +/- 9.0
Dead fetuses (N)		0	1	0	0
Live fetuses	Total (N)	89	100	83	**4**
	Mean (N)	5.9 +/- 1.7	7.1 +/- 2.1	5.9 +/- 2.4	4.0 +/- 0.0
	Mean (%)	86.4% +/- 14.6	92.6% +/- 13.9	88.0% +/- 19.1	57.1 +/- 0.0
	Females (%)	60.7%	48%	56.6%	100%
	Males (%)	39.3%	52%	43.4%	0.0%

*, $p<0.05$; **, $p<0.01$

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1987), Report on the study of the prenatal toxicity of glutaraldehyde in rats after oral administration (drinking water). [redacted] (Unpublished), BPD ID A6.08.1_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 active substance (a.s.) for first entry to Annex I authorisation.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 414 (1981)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde	
3.1.1	Lot/Batch number	[redacted]	X
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Fluid and colorless	
3.1.2.2	Purity	[redacted]% in water	
3.1.2.3	Stability	The purity, the homogeneity and the stability of the test substance, were checked by the analytical laboratories of [redacted] prior study start.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: about 68 to 77 days old at study start (corresponding to day 0, detection of sperms in the vaginal smear) Mean weight: About 230 g /animal	
3.2.6	Number of animals per	25 females /group	

	group	
3.2.7	Control animals	Yes
3.2.8	Mating period	Three to four untreated females were mated with one untreated fertile male rat of the same breed. Mating took place from ca. 16.00 hours to ca. 7.30 hours on the following day; the mating period was therefore about 15 to 16 hours.
3.3	**Administration/ Exposure**	
3.3.1	Duration of exposure	From day 6 post coitum (p.c.) to day 16 p.c.
3.3.2	Post exposure period	From day 17 p.c. to day 20 p.c. (4 days post exposure)
		Oral
3.3.3	Type	Drinking water
3.3.4	Concentration	The test doses used within the present study were chosen on the basis of the results of following range-finding studies: (1)- [redacted] (1991a), Report on the range-finding study of the prenatal toxicity of glutaraldehyde in rats after oral administration (drinking water). [redacted] (unpublished) (2)- [redacted] (1991b), Report on the range-finding study of the prenatal toxicity of glutaraldehyde in rabbits after oral administration (gavage). [redacted] (unpublished)

Test group	Test concentration (ppm)*	Concentration of test solution (mg/100 ml**)
0	0	0
1	50	9.9
2	250	49.7
3	750	149.1

*, mg glutaraldehyde/kg of doubly distilled water; **, taking into account the active ingredient content of [redacted]%

3.3.5	Vehicle	Test substance offered in the drinking water
3.3.6	Concentration in vehicle	–
3.3.7	Total volume applied	–
3.3.8	Controls	Controls received doubly distilled water in the drinking water
3.4	**Examinations**	

3.4.1	Clinical signs of toxicity and mortality	Yes, the animals were checked daily, at least once for clinical symptoms and twice for mortality on working days. On Saturdays, Sundays or public holidays, the animals were only checked once a day.
3.4.2	Body weight	Yes (weighing on day 0, 1, 3, 6, 8, 10, 13, 15, 17 and 20 p.c.)
3.4.3	Food consumption	Yes (excepted for day 0 p.c., determination on the same days as body weight) Only pregnant dams were considered for calculation.
3.4.4	Water consumption	Yes (excepted for day 0 p.c., determination on the same days as body weight and additionally on day 16 p.c.) Only pregnant dams were considered for calculation.
3.4.5	Intake of test substance	The intake of test substance (IT, in mg/kg bw/day) was calculated according to following formula: $IT_x = \frac{WC \times D}{BWx}$ D = dose in ppm WC = mean daily water consumption on day x + y, y = 1, 2 or 3; in g BWx = body weight on day x; in g Only pregnant dams were considered for calculation.
3.4.6	Necropsy	On day 20 p.c. all dams were sacrificed for the purpose of necropsy. The sacrificed dams were subjected to gross pathology and the uterus and ovaries were removed. Only pregnant dams, which were sacrificed at the end of the study period, were considered for the evaluations of following parameters: gravid uterine weight, mean net maternal body weight gain, reproduction data.
3.4.7	Net maternal body weight change	Following sacrifice, the corrected body weight gain (i.e. the net maternal body weight change) was calculated from the terminal body weight by subtraction of (1) the uterus weight and (2) the body weight measured on day 6 p.c.
3.4.8	Examination of uterine content	Gravid uterine weight Number of corpora lutea Number and distribution of implantations sites classified as live fetuses and dead implantations. Dead implantations comprised early resorptions, late resorptions and dead fetuses.

The conception rate (CR) as well as the pre- and post- implantation losses (Pre-I,Post-I) were calculated according to following formulas:

$$CR = \frac{\text{Number of pregnant animals}}{\text{Number of fertilized animals}} \times 100$$

$$\text{Pre-I} = \frac{\text{N*(corpora lutea) - N (implantations)}}{\text{N (implantations)}} \times 100$$

$$\text{Post-I} = \frac{\text{N(implantations) - N (live fetuses)}}{\text{N (implantations)}} \times 100$$

*, N = number of.

3.4.9 Examination of foetuses

3.4.9.1 General

The fetuses were extracted from the uterus and were examined for following parameters:

Fetal weight, sex (measurement of the anogenital distance, later confirmed by internal examination), external abnormalities, viability, placentae, umbilical cords, fetal membranes, and fetal liquids. Individual placental weights were recorded.

One half of the fetuses per dam were placed in ethyl alcohol whereas the other half was fixed in Bouin´s solution for further evaluation.

3.4.9.2 Skelet

The skeletons of the fetuses fixed in ethyl alcohol were stained according to the method of Dawson (Stain Technol. 1: 123, 1926) for examination under a stereomicroscope.

3.4.9.3 Soft tissue

The fetuses fixed in Bouin´s solution were examined for effects in the organs according to the method of Barrow and Taylor (J. Morph. 127: 291-306, 1969)

3.5 Statistical evaluation

Food and water consumption, body weight and body weight change, corrected maternal body weight gain, gravid uterine weight, weight of the fetuses, weight of the placentae, corpora lutea, implantations, pre- and post implantation losses, resorptions and live fetuses, were assessed by means of Dunnett´s Test.

Conception rate, maternal mortality and all fetal findings were assessed by means of Fisher´s Exact Test.

3.6 Further remarks

The purity, homogeneity and stability of the test substance were checked by the analytical laboratories of [redacted], prior study start. The test substance solutions (glutaraldehyde in doubly distilled water) also were checked for stability prior study start. Reanalysis was performed at the end of the study period. Samples of the test substance preparations were verified for concentrations twice during the study period.

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

4 RESULTS AND DISCUSSION

4.1 Females excluded from data evaluation and/or calculation

Following females were partly or totally excluded from data evaluation/calculation because of non pregnancy:

- 5 females of the control group
- 3 females of the 50 ppm group
- 2 females of the 250 ppm group
- 2 females of the 750 ppm group

X

4.2 Clinical signs of toxicity and mortality

Neither treatment-related signs of toxicity nor mortality were observed.

4.3 Maternal body weight data

Mean maternal body weight during gestation (grams):

Day (p.c.)	Test concentrations			
	0 ppm (N = 20)	50 ppm (N = 22)	250 ppm (N = 23)	750 ppm (N = 23)
0	231.6 +/- 16.34	227.9 +/- 12.94	229.3 +/- 13.00	229.1 +/- 12.39
6	264.8 +/- 19.81	260.2 +/- 12.33	257.7 +/- 15.99	262.3 +/- 12.58
15	316.5 +/- 23.10	311.0 +/- 17.44	307.1 +/- 17.82	314.5 +/- 15.56
20	384.6 +/- 30.66	381.9 +/- 27.16	384.6 +/- 27.23	391.2 +/- 23.85

N, number of dams considered

Mean body weight change between day 0 and day 20 p.c. (grams):

0 ppm (N = 20)	50 ppm (N = 22)	250 ppm (N = 23)	750 ppm (N = 23)
152.9 +/- 24.16	154.0 +/- 22.07	155.4 +/- 20.01	162.1 +/- 15.26

The maternal body weights and body weight changes were quite similar for the treated and the control group. The values were within the range of biological variation and differences between the groups were without biological relevance.

4.4 Food consumption

Mean maternal food consumption during gestation (g/animal/day):

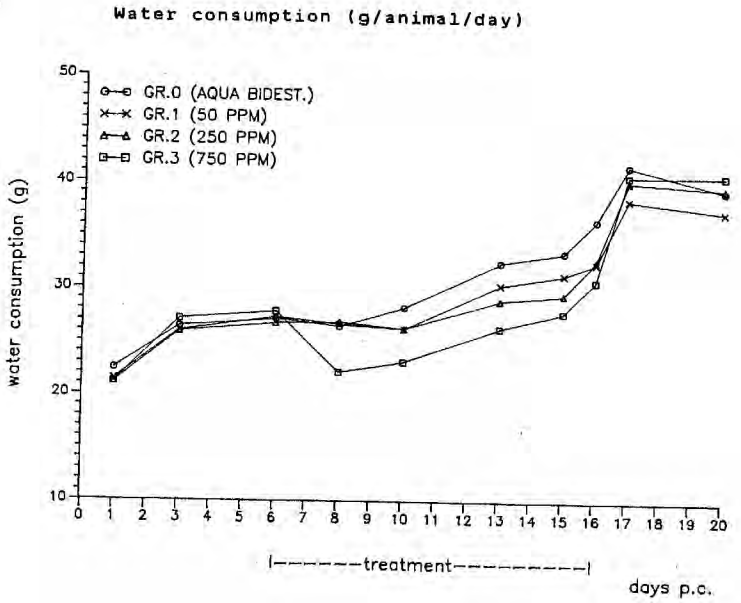
Day (p.c.)	Test concentrations			
	0 ppm (N = 20)	50 ppm (N = 22)	250 ppm (N = 23)	750 ppm (N = 23)
0 - 6	22.6 +/- 2.4	22.2 +/- 2.2	21.7 +/- 1.9	22.6 +/- 2.3
6 - 15	25.9 +/- 0.9	25.4 +/- 0.8	24.7 +/- 1.0	25.7 +/- 1.4
15 - 20	28.9 +/- 0.2	28.6 +/- 0.2	28.6 +/- 0.8	29.8 +/- 0.5
0 - 20	25.5 +/- 2.8	25.0 +/- 2.8	24.6 +/- 2.9	25.6 +/- 3.1

The maternal food consumption was quite similar for the treated and the control group. The values were within the range of biological variation and differences between the groups showed no treatment-relationship.

4.5 Water consumption

Mean maternal water consumption during gestation (g/animal/day):

Day (p.c.)	Test concentrations			
	0 ppm (N = 20)	50 ppm (N = 22)	250 ppm (N = 23)	750 ppm (N = 23)
0 - 6	25.3 +/- 2.5	24.9 +/- 3.1	24.6 +/- 3.0	25.3 +/- 3.7
6 - 15	31.4 +/- 4.1	29.4 +/- 2.8	28.8 +/- 2.6	**26.0 +/- 3.5**
15 - 20	40.5 +/- 1.6	37.9 +/- 0.8	39.9 +/- 0.4	40.8 +/- 0.1
0 - 20	31.4 +/- 6.3	29.8 +/- 5.3	29.8 +/- 6.1	28.8 +/- 7.0



Water consumption was inconspicuous in the 50 ppm group whereas a slight decrease in water consumption (up to 12%) was reported for the 250 ppm group from day 10 to day 15 p.c. In the 750 ppm group, water consumption was clearly reduced to about 19% below control during the period ranging from day 6 to day 16 p.c.

4.6 Test substance intake

The approximate test substance intake (mg/kg bw/day) was as follows:

Period	50 ppm	250 ppm	750 ppm
Day 6 to day 16 p.c.	5.2	25.7	68.0

4.7 Dams at study termination

Necropsy:

Necropsy revealed no treatment-related abnormalities.

Gravid uterine weight (GUW):

	Test concentrations			
GUW (g)	0 ppm (N=20)	50 ppm (N=22)	250 ppm (N=23)	750 ppm (N=23)
	74.9 +/- 25.13	77.8 +/- 16.32	82.2 +/- 16.14	80.4 +/- 14.73

The mean gravid uterine weight was quite similar for all treated and the control groups.

Net maternal body weight change from day 6 p.c.:

	Test concentrations			
NBW (g)	0 ppm (N=20)	50 ppm (N=22)	250 ppm (N=23)	750 ppm (N=23)
	44.9 +/- 8.53	43.9 +/- 8.45	44.8 +/- 7.23	48.5 +/- 9.32

No significant differences between treated and control groups were seen.

Reproduction data:

A conception rate of 92% respectively for the 250 and 750 ppm groups was reported, versus 80% for control. Moreover, all considered parameters (conception rate, number of corpora lutea, number of implantation sites, pre- and post implantation losses, resorptions and live fetuses) were inconspicuous, indicating that there were no adverse treatment-related effects on these parameters. Occasionally occurring differences were incidental or within the normal range of deviation.

4.8 Teratogenic / embryotoxic effects

Fetal external findings:

No treatment-related effects on sex ratio, placental weight, fetal weight were reported. No treatment-related external malformations were seen. In fact, a single case of malformation (aglossotomia) was reported for a fetus of the 750 ppm group; this type of malformation is known to occur in the historical control at a low frequency and was therefore considered to be a spontaneous finding. One case of fused placenta was reported for one fetus of the 50 ppm group.

Soft tissues:

Organ examination revealed no treatment-related effects. In fact, one case of organ malformation (situs inversus) was reported for one control fetus. Variations were seen, including dilated renal pelvis and hydroureter, which showed no dose-response relationship and were within the normal range of biological variation for the used strain.

X

Skeletal findings:

Malformations of the skull, the sternum (e.g. dislocated ossification centers), the vertebral column and/or the ribs were reported for 9 fetuses of the control group, 8 fetuses of the 50 ppm group, 2 fetuses of the 250 ppm group and 9 fetuses of the 750 ppm group. The only statistically significant difference was the lower number of fetuses affected of the 250 ppm group. Variations concerning the ribs (e.g. shortened 13^{th} ribs or accessory 14^{th} ribs), the sternum and the vertebral column). Signs of retardations (e.g. incomplete or missing ossification of vertebral bodies) were seen in all groups including control. A statistically significantly increased number of 50 ppm fetuses with incomplete ossification of the sternebrae, as well as an increased litter incidence of fetuses with incomplete ossification of the sternebrae were reported, which indeed were considered to be of spontaneous nature.

4.9 Other

The homogeneity and stability of the test substance was confirmed. The reanalysis revealed a content in active ingredient [redacted]%. The stability of the test substance solutions over a period of 4 days as well as the correctness of the prepared concentrations was confirmed.

Food and water were found to be suitable.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate glutaraldehyde for its prenatal toxicity in [redacted] rats.

Test substance: Glutaraldehyde, [redacted], fluid and colorless, purity [redacted]% in water

The test was conducted according to OECD guideline 414 (1981), with GLP.

The dams (25/test group) were treated from day 6 to day 16 post coitum (p.c.) with glutaraldehyde at concentrations of 0, 50, 250 and 750 ppm in drinking water. The animals were observed for clinical symptoms, mortality, body weight and body weight change, and for food and water consumption. The daily intake of test substance was determined. On day 20 p.c. all females were sacrificed and assessed by gross pathology. The gravid uterine weight as well as the net maternal body weight change was determined. A series of reproduction parameters including conception rate, number of corpora lutea, number of implantation sites, pre- and post implantation losses, resorptions and live fetuses were examined. The fetuses were dissected from the uterus, sexed weighed and further investigated for any external, soft tissue and/or skeletal findings.

The test substance was analyzed for purity, homogeneity and stability. Test substance preparations were examined for stability and homogeneity and the correctness of the test concentrations was verified twice during the study period.

5.2 Results and discussion

The homogeneity and stability of the test substance was confirmed. The reanalysis revealed a content in active ingredient >= [redacted]%. The stability of the test substance solutions over a period of 4 days as well as the correctness of the prepared concentrations was confirmed.

The approximate test substance intake (mg/kg bw/day) was as follows:

Period	50 ppm	250 ppm	750 ppm
Day 6 to day 16 p.c.	5.2	25.7	68.0

Maternal toxicity:

At the highest tested concentration of 750 ppm, water consumption was clearly reduced to about 19% below control during the period ranging from day 6 to day 16 p.c. A slight decrease in water consumption (up to 12%) also was reported for the 250 ppm group. As all other considered parameters were inconspicuous, the reduction in water consumption was considered to be due to the bad tasting or smell of the drinking test solution, This finding could therefore be seen as a physiological effect rather than a toxicological one.

Embryo-/fetotoxicity, teratogenicity:

No indication for an embryo-/fetotoxic or teratogenic potential of glutaraldehyde in rat was seen.

5.3 Conclusion

Section A6.8.1 _ 02 Annex Point IIA6.8.1		Teratogenicity Study Rodent (Wistar Rat)
5.3.1	NOEL maternal toxic effects	50 ppm (corresponding to about 5 mg/kg bw/day)
5.3.2	NO(A)EL embryotoxic / teratogenic effects	750 ppm (corresponding to about 68 mg/kg bw/day)
5.3.3	Reliability	**1**
5.3.4	Deficiencies	No

Section A6.8.1 _ 02	**Teratogenicity Study**
Annex Point IIA6.8.1	**Rodent (Wistar Rat)**

	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 2nd, 2010
Materials and Methods	3.1.1 Lot/Batch number. [redacted] 3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	4.1 Females excluded from data evaluation and/or calculation. Additionally, one female in the control group was excluded because of having no viable foetuses. 4.8 Teratogenic/embryotoxic effects. The following details should be added/corrected: • An unclear sentence under *Skeletal findings* is corrected as follows: *Variations concerneding the ribs (e.g. shortened 13th ribs or accessory 14th ribs), the sternum and the vertebral column).* • Total skeletal retardations. The foetal incidence was slightly increased in all dose groups, but without statistical significance or dose relation (37, 52, 45, 46 %). The historical mean value is 41 %. • Sternebrae not ossified. There was an increase in foetal incidence (7, 12, 12, 10 %) and litter incidence (32, 55, 48, 52 %). There was no statistical significance or dose relation. The foetal incidences are within historical control range (4.1-14.7 %), and the litter incidence slightly above the control range (28-50 %). • Sternebrae incompletely ossified or reduced in size. There was an increase in foetal incidence (11, 25, 19, 20 %) and litter incidence (42, 73, 48, 83 %). Statistical significance was reached for foetal incidence in the low dose group, and for litter incidence in the high dose group, but the values are within the historical control range (8.6-32 % and 40-90 %). • Note that the findings above are selected as possible indications of teratogenic effects. For several other effects the dose relation is reversed, i.e. the highest incidences are in the control group. All findings listed in these bullet points are considered incidental.
Conclusion	Glutaraldehyde was not teratogenic or embryotoxic when tested at concentrations up to 35 mg GA/kg bw/day, equivalent to 68 mg test substance/kg bw/day (see Remarks below). NOAEL for maternal toxicity, embryotoxicity and teratogenicity is the highest dose used, 35 mg GA/kg bw/day. LOAEL was not established for maternal or foetal effects.
Reliability	2 The value of the study would be higher if maternal toxicity were reached. This could have been achieved by using other dosing methods, as it is known that GA causes aversion when administered in the drinking water.
Acceptability	Acceptable

Section A6.8.1 _ 02 Annex Point IIA6.8.1	Teratogenicity Study Rodent (Wistar Rat)
Remarks	1.1 Reference. The study is from the year 1991, not 1987. **Test substance intake.** RMS concludes that the dose levels reported in the study report concern the test substance and not GA, despite the fact that GA concentrations in drinking water are given as well. The corrected concentrations are given only in the RMS Conclusion above. An explanation follows since this might otherwise be controversial: 1. High dose group received 750 ppm GA in water, containing 149.1 mg/100 ml (Study report, p. 9). 2. The high dose group consumed approximately 25 g water per day (equal to 25 ml). 3. An estimation of the high dose group GA intake per day can be calculated as follows: 25 ml × 149.1 /100 mg/ml = 37 mg. 4. The calculation above is approximated, and the GA intake brought forward is calculated using 51 % of the reported 68 mg/kg bw/day = 35 mg/kg bw/day. Please note that the tabulated results in the tables 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 A6.8.1 _ 03 **Teratogenicity Study**

Annex Point IIA6.8.1 **Himalayan Rabbit – Range finding study 1**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1991a), Report on the range-finding study of the prenatal toxicity of glutaraldehyde in rabbits after oral administration (drinking water). [redacted] (Unpublished), BPD ID A6.08.1_03	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new active substance (a.s.) for first entry to Annex I authorisation.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 414 (1981)	X
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	
3.1.2.1	Description	Fluid and colorless	
3.1.2.2	Purity	[redacted]% in water	
3.1.2.3	Stability	The purity, the homogeneity and the stability of the test substance, were checked.	
3.2	**Test Animals**		
3.2.1	Species	Himalayan rabbit	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: About 36 to 37 weeks old at study start Mean weight: About 2.612 kg /animal	
3.2.6	Number of animals per group	6 females /group	

3.2.7	Control animals	Yes
3.2.8	Mating period	The rabbits were fertilized by means of artificial insemination, using pooled ejaculate from male Himalayan rabbits of the same breed as the females. The day of insemination was designated as day 0 (study start) and the following day as day 1 post-insemination (p.i.).
3.3	**Administration/ Exposure**	
3.3.1	Duration of exposure	From day 7 to day 20 p.i. (corresponding to the period of organogenesis)
3.3.2	Post-exposure period	None, the animals were sacrificed on day 20 p.i.
		Oral
3.3.3	Type	Test substance administered in the drinking water
3.3.4	Concentration	The test doses were as follows:

Test group	Test concentration (ppm)	Concentration*** (mg/100 ml)
0	0*	0
1	100	19.9
2	500	99.4

*, Control animals treated with doubly distilled water.

***, taking into account the active ingredient content of [redacted]%

3.3.5	Vehicle	Aqueous solution
3.3.6	Controls	Control animals were treated with doubly distilled water.
3.4	**Examinations**	
3.4.1	Clinical signs of toxicity and mortality	Yes, the animals were checked daily, at least once for clinical symptoms and twice for mortality on working days. On Saturdays, Sundays or public holidays, the animals were only checked once a day for mortality. In case of presence of clinical signs of toxicity, the animals were checked several times a day.
3.4.2	Body weight	Yes, body weight was determined on day 0, 2, 4, 7, 9, 11, 14, 16, 19 and 20 p.i.; body weight change was calculated on the basis of he values obtained.
3.4.3	Food consumption	Yes, the food consumption was determined daily during the entire study period, excepted for day 0 and day 20 p.i.
3.4.4	Water consumption	Yes, the water consumption was determined daily during the entire study period, excepted for day 0 p.i.

3.4.5 Test substance intake

The intake of test substance (IT, in mg/kg bw/day) was calculated according to following formula:

$$IT_x = \frac{WC \times D}{BWx}$$

D = dose in ppm
WC = mean daily water consumption on day x + y, y = 1, 2 or 3; in g
BWx = body weight on day x; in g

3.4.6 Necropsy

On day 20 p.i., all females were sacrificed for the purpose of necropsy; they were examined macroscopically. The fetuses were dissected from the uterus and subjected to further examinations. Dams showing signs of abortion also were sacrificed and examined as above.

3.4.7 Net maternal body weight change

Following sacrifice, the net maternal body weight change (i.e. the corrected body weight gain) was calculated from the terminal body weight by subtraction of (1) the uterus weight and (2) the body weight measured on day 7 p.i.

3.4.8 Clinical chemistry

On day of sacrifice, blood samples were collected from the ear vein of the animals for the determination of following parameters: sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol.

3.4.9 Organ weights

Liver and kidney were weighed.

3.4.10 Examination of uterine content

Gravid uterine weight

Number of corpora lutea

Number and distribution of implantations sites classified as live fetuses and dead implantations. Dead implantations comprised early resorptions and late resorptions.

The conception rate (CR) as well as the pre- and post- implantation losses (Pre-I, Post-I) were calculated according to following formulas:

$$CR = \frac{\text{Number of pregnant animals}}{\text{Number of fertilized animals}} \times 100$$

$$\text{Pre-I} = \frac{\text{N*(corpora lutea) - N (implantations)}}{\text{N (implantations)}} \times 100$$

$$\text{Post-I} = \frac{\text{N(implantations) - N (live fetuses)}}{\text{N (implantations)}} \times 100$$

*, N = number of.

3.4.11 Examination of foetuses

3.4.11.1	General	The fetuses were extracted from the uterus and were examined for following parameters: Fetal weight, external abnormalities, viability, placentae, umbilical cords, fetal membranes, and fetal liquids. Individual placental weights were recorded.
3.5	**Statistical evaluation**	Food and water consumption, body weight and body weight change, corrected maternal body weight gain, gravid uterine weight, weight of the fetuses, weight of the placentae, corpora lutea, implantations, pre- and post implantation losses, resorptions and live fetuses, were assessed by means of Dunnett's Test. Conception rate, maternal mortality and all fetal findings were assessed by means of Fisher's Exact Test. For the clinical chemical parameters, means and standard deviations were assessed. Further assessment was based on the Kruskal-Wallis-h-test and if necessary on the Mann-Whitney-U-test.
3.6	**Further remarks**	The purity, homogeneity and stability of the test substance were checked ([redacted]). The test substance solutions (glutaraldehyde in doubly distilled water) also were checked for stability. Reanalysis was performed at the end of the study period. Samples of the test substance preparations were verified for concentrations. Food as well as drinking water also was subjected to analyses according to the EPA guideline (Fed. Reg. Vol. 44, No.91, 1979) and to the German Drinking Water Regulation (Trinkwasserverordnung, Bundesgesetztblatt, 1990).

4 RESULTS AND DISCUSSION

4.1 Females excluded from data evaluation and/or calculation

Only one female of the 500 ppm group was excluded from the diverse evaluations/calculations because of non pregnancy.

4.2 Clinical signs of toxicity and mortality

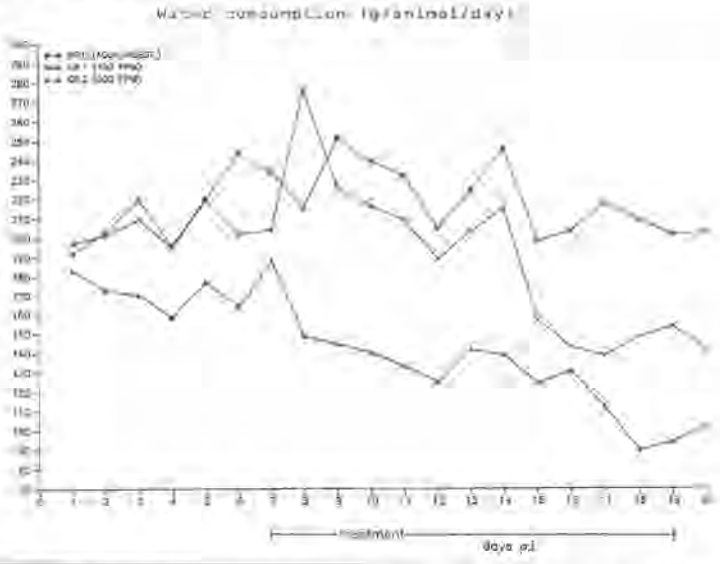
Neither mortality nor clinical symptoms indicative of toxicity were reported.

4.3 Food consumption

Food consumption of the dams was clearly reduced during the treatment period (day 7 to day 20 p.i.) in both, the 100 and the 500 ppm group (respectively about 17% and 21% below control value). The impairment in food consumption was considered as possibly treatment-related.

4.4 Water consumption

Water consumption of the dams was clearly reduced in both, the 100 and the 500 ppm group when compared to control (respectively up to 36 and 57% below control). The impairment in water consumption was considered to be treatment-related.



4.5 Maternal body weight data

Body weight and body weight change in both, the 100 and the 500 ppm groups, were quite similar to control and therefore no treatment-related effect of these parameters was evident.

4.6 Intake of test substance

<u>The approximate test substance intake (mg/kg bw/day) was as follows:</u>

Period	100 ppm	500 ppm
Day 7 to day 19 p.i.	7.1	23.4

4.7 Clinical-chemical parameters

All considered clinical-chemical parameters were inconspicuous.

4.8 Dams at study termination

<u>Gravid uterine weight (GUW):</u>

The gravid uterine weight for both treated groups was inconspicuous

		compared to control. Net maternal body weight change from day 7 p.i: Net maternal body weight change for both treated groups was inconspicuous compared to control. Necropsy: Necropsy revealed no treatment-related abnormalities. Organ weights: No statistically significant changes in organ weight were reported for the liver and kidney of the treated animals compared to control. Reproduction data: The conception rate varied between 83% (100 ppm group) and 100% (control and 100 ppm group). Conception rate, number of corpora lutea, number of implantation sites, pre implantation losses, resorptions and live fetuses were inconspicuous. The post-implantation loss in the 500 ppm group was increased (25.7% versus 22.8% for the control group) and further was outside the historical control range; therefore this effect was considered to possibly be treatment-related.	
4.9	**Teratogenic / embryotoxic effects**	Neither the placental weight nor the fetal weight showed effects that could be assessed as treatment-related. External examination of the fetuses revealed a single case of anasarca in the control group, as well as a single case of gastroschisis in the 500 ppm group. These findings were considered to be incidental.	X
4.10	**Other effects**	Test substance: The homogeneity and stability of the test substance was confirmed. The stability of the test substance solutions over a period of 4 days at room temperature as well as the correctness of the prepared concentrations was confirmed. Food and water: Food and water were found to be suitable.	

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.8.1 _ 03 **Teratogenicity Study**

Annex Point IIA6.8.1 **Himalayan Rabbit – Range finding study 1**

5.1 Materials and methods

The present range-finding study was conducted prior to the main teratogenicity study (report No: 40R0599/89026) in order to define the dose level inducing overt maternal toxicity in pregnant rabbits, however without exceeding 10% mortality. Furthermore the administration of the test substance via the drinking water was here tested as way of treatment in comparison to a further range-finding study where the test substance was administered by gavage.

Test substance: Glutaraldehyde, batch No: 89/599, purity 50.3% in water

The test was conducted according to OECD guideline 414 (1981), with GLP.

Following artificial insemination, the fertilized rabbits (6/test group) were treated from day 7 to day 20 post-insemination (p.i.) with glutaraldehyde at concentrations of 0, 100 and 500 ppm in the drinking water. The animals were observed for clinical symptoms, mortality, body weight and body weight change, food and water consumption. On day 20 p.i., blood samples were collected for the assessment of a series of clinical-chemical parameters; thereafter all females were sacrificed and assessed by gross pathology. The liver and kidney were retained for organ weight assessment. The gravid uterine weight as well as the net maternal body weight change was determined. A series of reproduction parameters including conception rate, number of corpora lutea, number of implantation sites, pre- and post implantation losses, resorptions and live fetuses were examined. The fetuses were dissected from the uterus and were investigated for external abnormalities.

The test substance was analyzed for purity, homogeneity and stability. Test substance preparations were examined for stability and homogeneity and the correctness of the test concentrations was verified. The homogeneity and stability of the test substance were confirmed. The stability of the test substance solutions over a period of 4 days as well as the correctness of the test doses was confirmed.

5.2 Results and discussion

Intake of test substance (mg/kg bw/day):

Period	100 ppm	500 ppm
Day 7 to day 19 p.i.	7.1	23.4

Maternal toxicity:

No maternal mortality was observed, but treatment-related effects indicative of maternal toxicity were seen at both tested doses. At 100 ppm, these effects consisted of a reduction in food and water consumption (respectively 17% and 36% below control values). In the 500 ppm group, the effects included a reduction in food consumption (21% below control), a severe reduction in water consumption (57% below control) and an increase in post implantation loss (25.7% versus 22.8% for the control group).

Embryo-/fetotoxicity, teratogenicity:

Because of the early sacrifice of the dams, only a limited examination of the fetuses was possible. Taking this aspect into account, neither embryo/fetotoxic nor teratogenic treatment-related effects were seen.

5.3 Conclusion

As a consequence of the severe reduction in water consumption observed under the present test conditions (administration of the test substance in the drinking water), the test substance intake quite sure was decreased and not uniform. For this reason, a further range-finding study was undertaken, where the test substance was administered to the pregnant rabbits by gavage.

5.3.1 Reliability **1**

Section A6.8.1 _ 03	**Teratogenicity Study**
Annex Point IIA6.8.1	**Himalayan Rabbit – Range finding study 1**

5.3.2 Deficiencies None

	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 3rd, 2010
Materials and Methods	2.1 Guideline study. It is misleading to state that OECD guideline 414 was followed. This is a range-finding study with fewer animals, shorter time periods and less investigations as compared with the full guideline study. 3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	4.9 Teratogenic/embryotoxic effects. The placental weights all viable foetuses were significantly reduced in both dose groups (3.2, 2.1, 2.4 g).
Conclusion	Except for decreased placental weights, no signs of embryotoxic or teratogenic effects were seen. The severely reduced water consumption is taken as an indication that drinking water should not be the preferred method of test substance administration.
Reliability	1
Acceptability	Acceptable
Remarks	The reduction in placental weight was not confirmed in the main 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 A6.8.1 _ 04 **Teratogenicity Study**

Annex Point IIA6.8.1 **Himalayan Rabbit – Range finding study 2**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1991b), Report on the range-finding study of the prenatal toxicity of glutaraldehyde in rabbits after oral administration (gavage). [redacted] (Unpublished), BPD ID A6.08.1_04	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new active substance (a.s.) for first entry to Annex I authorisation.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 414 (1981)	X
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	Fluid and colorless	
3.1.2.2	Purity	[redacted]% in water	
3.1.2.3	Stability	The purity, the homogeneity and the stability of the test substance, were checked.	
3.2	**Test Animals**		
3.2.1	Species	Himalayan rabbit	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: About 36 to 38 weeks old at study start Mean weight: About 2.590 kg /animal	
3.2.6	Number of animals per group	6 females /group	

3.2.7	Control animals	Yes
3.2.8	Mating period	The rabbits were fertilized by means of artificial insemination, using pooled ejaculate from male Himalayan rabbits of the same breed as the females. The day of insemination was designated as day 0 (study start) and the following day as day 1 post-insemination (p.i.).
3.3	**Administration/ Exposure**	
3.3.1	Duration of exposure	From day 7 to day 19 p.i. (corresponding to the period of organogenesis)
3.3.2	Post-exposure period	None, the animals were sacrificed on day 20 p.i.
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	The test doses were as follows:

Test group	Dose (mg/kg bw/day)	Concentration (mg/100 ml)***	Volume (ml/kg bw)
0	0*	0	10
1	5	99	10
2	25	497	10

*, Control animals treated with doubly distilled water.
***, Taking into account the active ingredient content of [redacted]%

3.3.5	Vehicle	Aqueous solution
3.3.6	Concentration in vehicle	See 3.3.4
3.3.7	Total volume applied	See 3.3.4
3.3.8	Controls	Control animals were treated with doubly distilled water.
3.4	**Examinations**	
3.4.1	Clinical signs of toxicity and mortality	Yes, the animals were checked daily, at least once for clinical symptoms and twice for mortality on working days. On Saturdays, Sundays or public holidays, the animals were only checked once a day for mortality. In case of presence of clinical signs of toxicity, the animals were checked several times a day.
3.4.2	Body weight	Yes, body weight was determined on day 0, 2, 4, 7, 9, 11, 14, 16, 19 and 20 p.i.; body weight change was calculated on the basis of he values obtained.
3.4.3	Food consumption	Yes, the food consumption was determined daily during the entire study period, excepted for day 0 and day 20 p.i.
3.4.4	Necropsy	On day 20 p.i., all females were sacrificed for the purpose of necropsy; they were examined macroscopically. The fetuses were dissected from the uterus and subjected to further examinations. Dams showing signs of abortion also were sacrificed and examined as above.

3.4.5	Net maternal body weight change	Following sacrifice, the net maternal body weight change (i.e. the corrected body weight gain) was calculated from the terminal body weight by subtraction of (1) the uterus weight and (2) the body weight measured on day 7 p.i.
3.4.6	Clinical chemistry	On day of sacrifice, blood samples were collected from the ear vein of the animals for the determination of following parameters: sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol.
3.4.7	Organ weights	Liver and kidney were weighed.
3.4.8	Examination of uterine content	Gravid uterine weight Number of corpora lutea Number and distribution of implantations sites classified as live fetuses and dead implantations. Dead implantations comprised early resorptions and late resorptions. The conception rate (CR) as well as the pre- and post- implantation losses (Pre-I, Post-I) were calculated according to following formulas: $CR = \frac{\text{Number of pregnant animals}}{\text{Number of fertilized animals}} \times 100$ $\text{Pre-I} = \frac{\text{N*(corpora lutea)} - \text{N (implantations)}}{\text{N (implantations)}} \times 100$ $\text{Post-I} = \frac{\text{N(implantations)} - \text{N (live fetuses)}}{\text{N (implantations)}} \times 100$ *, N = number of.
3.4.9	Examination of foetuses	
3.4.9.1	General	The fetuses were extracted from the uterus and were examined for following parameters: Fetal weight, external abnormalities, viability, placentae, umbilical cords, fetal membranes, and fetal liquids. Individual placental weights were recorded.
3.5	**Statistical evaluation**	Food and water consumption, body weight and body weight change, corrected maternal body weight gain, gravid uterine weight, weight of the fetuses, weight of the placentae, corpora lutea, implantations, pre- and post implantation losses, resorptions and live fetuses, were assessed by means of Dunnett's Test. Conception rate, maternal mortality and all fetal findings were assessed by means of Fisher's Exact Test. For the clinical chemical parameters, means and standard deviations were assessed. Further assessment was based on the Kruskal-Wallis-h-test and if necessary on the Mann-Whitney-U-test.

3.6	**Further remarks**	The purity, homogeneity and stability of the test substance were checked ([redacted]). The test substance solutions (glutaraldehyde in doubly distilled water) also were checked for stability. Reanalysis was performed at the end of the study period. Samples of the test substance preparations were verified for concentrations. Food as well as drinking water also was subjected to analyses according to the EPA guideline (Fed. Reg. Vol. 44, No.91, 1979) and to the German Drinking Water Regulation (Trinkwasserverordnung, Bundesgesetztblatt, 1990).

4 RESULTS AND DISCUSSION

4.1 Females excluded from data evaluation and/or calculation

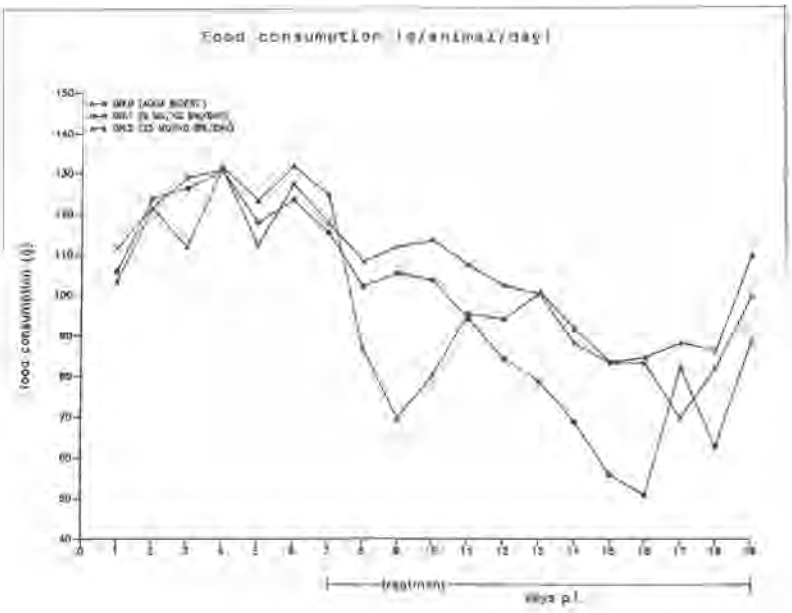
Following females were partly or totally excluded from data evaluation/calculation because of non pregnancy:

- 1 female of the control group
- 1 female of the 5 mg/kg bw/day group

4.2 Clinical signs of toxicity and mortality

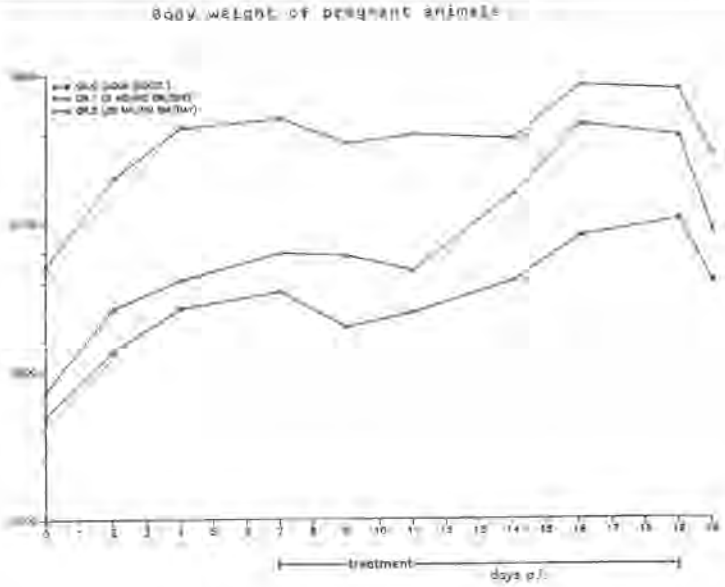
Neither mortality nor clinical symptoms indicative of toxicity were reported.

4.3 Food consumption



Food consumption of the dams was clearly reduced during the first half of the treatment period (day 7 to day 10 p.i.) in the 25 mg/kg bw/day group (up to 34% below control value). Thereafter, food consumption in this group turned back to or even exceeded control value. Neverless, the impairment in food consumption was considered to be treatment-related. For the 5 mg/kg bw/day group, food consumption was statistically significantly increased between day 7 and day 19 p.i..; however, this effect was without any biological relevance and was not considered to be treatment-related.

4.4 Maternal body weight data



The body weight and body weight changes of the treated dams were similar to control; in fact, the observed variations were within the normal range of biological variation and/or without any biological relevance.

4.5 Clinical-chemical parameters

In the 25 mg/kg bw/day group, following parameters differed from control values: total protein and albumin were both clearly decreased whereas calcium and glucose were slightly reduced compared to control. These effects probably are related to the reduced food consumption (see 4.3), and can be seen as treatment-related.

4.6 Dams at study termination

Gravid uterine weight (GUW):

The gravid uterine weight for both treated groups was inconspicuous compared to control.

Net maternal body weight change from day 7 p.i:

Net maternal body weight change for both treated groups was inconspicuous compared to control.

Necropsy:

Necropsy revealed a lesion within the pyloric region of the stomach of one control animal. Histopathological examination identified this lesion as acute focal erosive gastritis. Two dams of the 25 mg/kg bw/day group showed subacute microfocal gastritis in the fundus region of the stomach; one of these animals furthermore showed acute focal erosive gastritis of the pyloric region of the stomach. A relationship of these findings to the treatment however is questionable.

Organ weights:

No statistically significant changes in organ weight were reported for the liver and kidney of the treated animals compared to control.

Reproduction data:

The conception rate varied between 83% (control and 5 mg/kg bw/day) and 100% (25 mg/kg bw/day). The post-implantation loss for the 5 mg/kg bw/day group was outside the historical control range (40.3% versus 4.9 - 23.1%); the pre-implantation loss had a value of 27.5% and was nearby the upper limit of historical control for this reproduction parameter (4.9 – 28.5%). This was due to the fact that one dam of the 5 mg/kg bw/day group had no viable

fetuses but only resorptions. These effects however were incidental. All other reproduction parameters were inconspicuous.

4.7 Teratogenic / embryotoxic effects

Neither the placental weight nor the fetal weight showed effects that could be assessed as treatment-related. External examination of the fetuses revealed a single case of gastroschisis in the control group. No treatment-related findings could be reported for fetuses of the treated groups.

4.8 Other effects

Test substance:

The homogeneity and stability of the test substance was confirmed. The stability of the test substance solutions over a period of 4 days at room temperature as well as the correctness of the prepared concentrations was confirmed.

Food and water:

Food and water were found to be suitable.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The present range-finding study was conducted prior to the main teratogenicity study ([redacted]) in order to define the dose level inducing overt maternal toxicity in pregnant rabbits, however without exceeding 10% mortality. Furthermore the administration of the test substance by gavage was here tested as way of treatment in comparison to a previous range-finding study ([redacted]) where the test substance was administered to the pregnant rabbits in the drinking water.

Test substance: Glutaraldehyde, batch No: [redacted]% in water

The test was conducted according to OECD guideline 414 (1981), with GLP.

Following artificial insemination, the fertilized rabbits (6/test group) were treated by gavage from day 7 to day 20 post-insemination (p.i.) with glutaraldehyde at following doses: 0, 5, 25 mg/kg bw/day. The animals were observed for clinical symptoms, mortality, body weight and body weight change, and for food consumption. On day 20 p.i., blood samples were collected for the assessment of a series of clinical-chemical parameters; thereafter all females were sacrificed and assessed by gross pathology. The liver and kidney were retained for organ weight assessment. The gravid uterine weight as well as the net maternal body weight change was determined. A series of reproduction parameters including conception rate, number of corpora lutea, number of implantation sites, pre- and post implantation losses, resorptions and live fetuses were examined. The fetuses were dissected from the uterus and were investigated for external abnormalities.

The test substance was analyzed for purity, homogeneity and stability. Test substance preparations were examined for stability and homogeneity and the correctness of the test concentrations was verified. The homogeneity and stability of the test substance were confirmed. The stability of the test substance solutions over a period of 4 days as well as the correctness of the test doses was confirmed.

Section A6.8.1 _ 04		**Teratogenicity Study**
Annex Point IIA6.8.1		**Himalayan Rabbit – Range finding study 2**
5.2	**Results and discussion**	Maternal toxicity: Neither maternal mortality nor clinical symptoms of toxicity were seen. Findings indicative of maternal toxicity were restricted to the higher tested dose of 25 mg/kg bw/day and mainly included reduced food consumption (first half of the treatment period; up to 34% below control value), and decreased total protein, albumin, calcium and glucose contents in blood; necropsy revealed two cases of subacute microfocal gastritis in the fundus region of the stomach, a clear relationship to the treatment however is questionable as one control dam showed similar lesions. Embryo-/fetotoxicity, teratogenicity: Because of the early sacrifice of the dams, only a limited examination of the fetuses was possible. Taking this aspect into account, neither embryo/fetotoxic nor teratogenic treatment-related effects were seen.
5.3	**Conclusion**	On the basis of the results of the present study and of those of the range finding study report [redacted], (1) gavage as way of treatment and (2) dosages of 0, 5, 15 and 45 mg/kg bw/day, were selected for the conduct of the main teratogenicity study with rabbits ([redacted]).
5.3.1	Reliability	**1**
5.3.2	Deficiencies	None

Section A6.8.1 _ 04 **Teratogenicity Study**

Annex Point IIA6.8.1 **Himalayan Rabbit – Range finding study 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 3rd, 2010
Materials and Methods	2.1 Guideline study. It is misleading to state that OECD guideline 414 was followed. This is a range-finding study with fewer animals, shorter time periods and less investigations as compared with the full guideline study. 3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	4.6 Dams at study termination. The following additions/corrections should be made: • The gravid uterus weights were clearly reduced in both dose groups, although without statistical significance (102.5, 63, 84.9 g). • Necropsy: it is stated that "*Two dams of the 25 mg/kg bw/day group showed subacute microfocal gastritis in the fundus region of the stomach; one of these animals furthermore showed acute focal erosive gastritis of the pyloric region of the stomach. A relationship of these findings to the treatment however is questionable.*" The statements are otherwise correct, but it seems doubtful to question the relationship to treatment, as the effects are well in line with other studies. RMS concludes that these are adverse effects resulting from the test substance administration.
Conclusion	No signs of embryotoxic or teratogenic effects were seen, except for the increased numbers of pre- and postimplantation losses at the low dose group which are considered incidental. The high dose group dams showed signs of toxicity in 1) clinical-chemical parameters that may be connected with the reduced food consumption during the first days of dosing, and 2) gastritis in 2/6 of the high dose animals.
Reliability	1
Acceptability	Acceptable
Remarks	The reduction in gravid uterine weight was not confirmed in the main study and is considered incidental.
	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 A6.8.1 _ 05	**Teratogenicity Study**		
Annex Point IIA6.8.1	**Rodent (Wistar Rat) – Range finding study 1**		
			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1991c), Report on the range-finding study of the prenatal toxicity of glutaraldehyde in rats after oral administration (drinking water). [redacted] (unpublished), BPD ID A6.08.1_05	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new active substance (a.s.) for first entry to Annex I authorisation.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 414 (1981)	X
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	Fluid and colorless	
3.1.2.2	Purity	[redacted]% in water	
3.1.2.3	Stability	The purity, the homogeneity and the stability of the test substance, were checked.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: About 68 days old at study start Mean weight: About 222 g /animal	
3.2.6	Number of animals per	10 females /group	

	group	
3.2.7	Control animals	Yes
3.2.8	Mating period	One to four untreated females were mated with one untreated fertile male rat of the same breed. Mating took place from ca. 16.00 hours to ca. 7.30 hours on the following day; the mating period was therefore about 15 to 16 hours.
3.3	**Administration/ Exposure**	
3.3.1	Duration of exposure	From day 6 post coitum (p.c.) to day 16 p.c.
3.3.2	Post-exposure period	None, the animals were sacrificed on day 16 p.c.
		Oral
3.3.3	Type	Test substance administered in the drinking water
3.3.4	Concentration	The test doses were as follows:

Test group	Test concentration (ppm)	Concentration*** (mg/100 ml)
0	0*	0
1	100	19.9
2	500	99.4

*, Control animals treated with doubly distilled water.

***, Taking into account the active ingredient content of [redacted]%

3.3.5	Vehicle	Aqueous solution
3.3.6	Controls	Control animals were treated with doubly distilled water.
3.4	**Examinations**	
3.4.1	Clinical signs of toxicity and mortality	Yes, the animals were checked daily, at least once for clinical symptoms and twice for mortality on working days. On Saturdays, Sundays or public holidays, the animals were only checked once a day for mortality. In case of presence of clinical signs of toxicity, the animals were checked several times a day.
3.4.2	Body weight	Yes (weighing on day 0, 1, 3, 6, 8, 10, 13, 15 and 16 p.c.) Body weight change was calculated on the basis of he values obtained.
3.4.3	Food consumption	Yes (excepted for day 0 and 16 p.c., determination on the same days as body weight)
3.4.4	Water consumption	Yes (excepted for day 0 p.c., determination on the same days as body weight)

3.4.5	Test substance intake	The intake of test substance (IT, in mg/kg bw/day) was calculated according to following formula: $IT_x = \frac{WC \times D}{BWx}$ D = dose in ppm WC = mean daily water consumption on day x + y, y = 1, 2 or 3; in g BWx = body weight on day x; in g
3.4.6	Necropsy	On day 16 p.c. following blood sampling, all females were sacrificed for the purpose of necropsy; they were examined macroscopically. The fetuses were dissected from the uterus and subjected to further examinations.
3.4.7	Net maternal body weight change	Following sacrifice, the net maternal body weight change (i.e. the corrected body weight gain) was calculated from the terminal body weight by subtraction of (1) the uterus weight and (2) the body weight measured on day 6 p.c.
3.4.8	Clinical chemistry	Blood samples collected from the animals prior sacrifice were used for the determination of following parameters: sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol.
3.4.9	Organ weights	Liver and kidney were weighed.
3.4.10	Examination of uterine content	Gravid uterine weight Number of corpora lutea Number and distribution of implantations sites classified as live fetuses and dead implantations. Dead implantations comprised early resorptions and late resorptions. The conception rate (CR) as well as the pre- and post- implantation losses (Pre-I, Post-I) were calculated according to following formulas: $CR = \frac{\text{Number of pregnant animals}}{\text{Number of fertilized animals}} \times 100$ $\text{Pre-I} = \frac{\text{N*(corpora lutea) - N (implantations)}}{\text{N (implantations)}} \times 100$ $\text{Post-I} = \frac{\text{N(implantations) - N (live fetuses)}}{\text{N (implantations)}} \times 100$ *, N = number of.
3.4.11	Examination of foetuses	

3.4.11.1	General	The fetuses were extracted from the uterus and were examined for following parameters: Fetal weight, external abnormalities, viability, placentae, umbilical cords, fetal membranes, and fetal liquids. Individual placental weights were recorded.
3.5	**Statistical evaluation**	Food and water consumption, body weight and body weight change, corrected maternal body weight gain, gravid uterine weight, weight of the fetuses, weight of the placentae, corpora lutea, implantations, pre- and post implantation losses, resorptions and live fetuses, were assessed by means of Dunnett's Test. Conception rate, maternal mortality and all fetal findings were assessed by means of Fisher's Exact Test. For the clinical chemical parameters, means and standard deviations were assessed. Further assessment was based on the analysis of variance (ANOVA) and Dunnett's Test.
3.6	**Further remarks**	The purity, homogeneity and stability of the test substance were checked ([redacted]). The test substance solutions (glutaraldehyde in doubly distilled water) also were checked for stability. Reanalysis was performed at the end of the study period. Samples of the test substance preparations were verified for concentrations. Food as well as drinking water also was subjected to analyses according to the EPA guideline (Fed. Reg. Vol. 44, No.91, 1979) and to the German Drinking Water Regulation (Trinkwasserverordnung, Bundesgesetztblatt, 1990).

4 RESULTS AND DISCUSSION

4.1 Females excluded from data evaluation and/or calculation

None of the females was excluded from the diverse evaluations/calculations because of non-pregnancy.

4.2 Clinical signs of toxicity and mortality

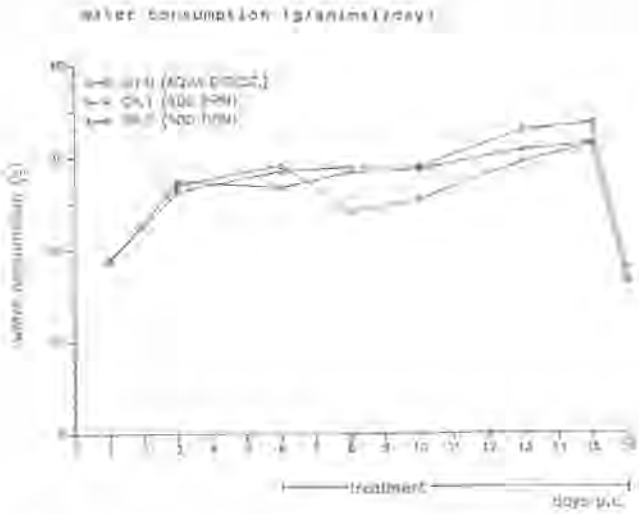
Neither mortality nor clinical symptoms indicative of toxicity were reported.

4.3 Food consumption

Food consumption of the dams was slightly reduced at the beginning of the treatment period (day 6 to day 8 p.c.) in the 500 ppm group (about 8% below control value). The impairment in food consumption was considered to be treatment-related.

4.4 Water consumption

Water consumption of the dams was reduced in the 500 ppm group when compared to control (about 17% below control, day 6 to 13 p.c.). The impairment in water consumption was considered to be treatment-related.



4.5 Maternal body weight data

Body weight and body weight change in both, the 100 and the 500 ppm groups, were similar to control and therefore no treatment-related effect of these parameters was evident.

4.6 Intake of test substance

<u>The approximate test substance intake (mg/kg bw/day) was as follows:</u>

Period	100 ppm	500 ppm
Day 6 to day 15 p.c.	11.0	51.0

4.7 Clinical-chemical parameters

All considered clinical-chemical parameters were inconspicuous.

4.8 Dams at study termination

<u>Gravid uterine weight (GUW):</u>

The gravid uterine weight for both treated groups was inconspicuous

		compared to control. Net maternal body weight change from day 7 p.i: Net maternal body weight change for both treated groups was inconspicuous compared to control. Necropsy: Necropsy revealed a pale-red focus (diameter of ca. 0.5 mm) in the glandular stomach of two animals of the 500 ppm group, which probably was treatment-related. Organ weights: A statistically significant decrease in relative liver weight was reported for the 100 ppm group; this finding was not considered to be treatment-related. Reproduction data: The conception rate was 100% in all groups. Conception rate, number of corpora lutea, number of implantation sites, pre and post implantation losses, resorptions and live fetuses were inconspicuous. Differences between the groups were incidental and/or within the normal range of biological variation for the test animals used.
4.9	**Teratogenic / embryotoxic effects**	Neither the placental weight nor the fetal weight showed effects that could be assessed as treatment-related. External examination of the fetuses revealed a single case of blood coagulum surrounding the placenta of two fetuses from one litter, in the control group. No further abnormalities could be reported.
4.10	**Other effects**	Test substance: The homogeneity and stability of the test substance was confirmed. The stability of the test substance solutions over a period of 4 days at room temperature as well as the correctness of the prepared concentrations was confirmed. Food and water: Food and water were found to be suitable.

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.8.1 _ 05	**Teratogenicity Study**	
Annex Point IIA6.8.1	**Rodent (Wistar Rat) – Range finding study 1**	
5.1	**Materials and methods**	The present range-finding study was conducted prior to the main teratogenicity study ([redacted]) in order to define the dose level inducing overt maternal toxicity in pregnant rats, however without exceeding 10% mortality. Furthermore the administration of the test substance via the drinking water was here tested as way of treatment in comparison to a further range-finding study ([redacted]) where the test substance was administered by gavage. Test substance: Glutaraldehyde, batch No [redacted] % in water The test was conducted according to OECD guideline 414 (1981), with GLP. The dams (10/test group) were treated from day 6 to day 16 post coitum (p.c.) with glutaraldehyde at concentrations of 0, 100 and 500 ppm in drinking water. The animals were observed for clinical symptoms, mortality, body weight and body weight change, and for food and water consumption. The daily intake of test substance was determined. On day 16 p.c. blood samples were collected from all females, which then were sacrificed for the purpose of necropsy. The blood samples served for the assessment of a series of clinical-chemical parameters. The liver and kidney were retained for organ weight assessment. The gravid uterine weight as well as the net maternal body weight change was determined. A series of reproduction parameters including conception rate, number of corpora lutea, number of implantation sites, pre- and post implantation losses, resorptions and live fetuses were examined. The fetuses were dissected from the uterus and were investigated for external abnormalities. The test substance was analyzed for purity, homogeneity and stability. Test substance preparations were examined for stability and homogeneity and the correctness of the test concentrations was verified. The homogeneity and stability of the test substance were confirmed. The stability of the test substance solutions over a period of 4 days as well as the correctness of the test doses was confirmed.
5.2	**Results and discussion**	Intake of test substance (mg/kg bw/day): Period | 100 ppm | 500 ppm Day 6 to day 16 p.i. | 11 | 51 Maternal toxicity: No maternal mortality was observed, but treatment-related effects indicative of maternal toxicity were seen at the highest tested dose of 500 ppm. These effects consisted of a reduction in food and water consumption (respectively 8% and 17% below control values), furthermore, two females of the 500 ppm showed foci in their glandular stomach. Embryo-/fetotoxicity, teratogenicity: Because of the early sacrifice of the dams, only a limited examination of the fetuses was possible. Taking this aspect into account, neither embryo/fetotoxic nor teratogenic treatment-related effects were seen.
5.3	**Conclusion**	On the basis of the results of the present study and of those of the range finding study report [redacted], (1) treatment via the drinking water and (2) test concentrations of 0, 50, 250 and 750 ppm, were selected for the conduct of the main teratogenicity study with rats (report [redacted]).
5.3.1	Reliability	**1**
5.3.2	Deficiencies	None

Section A6.8.1 _ 05	**Teratogenicity Study**
Annex Point IIA6.8.1	**Rodent (Wistar Rat) – Range finding study 1**

	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 3rd, 2010
Materials and Methods	2.1 Guideline study. It is misleading to state that OECD guideline 414 was followed. This is a range-finding study with fewer animals, shorter time periods and less investigations as compared with the full guideline study. 3.1.2 This refers to Doc IIIA Section A2.
Results and discussion	Agree with applicant's version.
Conclusion	No signs of embryotoxic or teratogenic effects were seen. In the high dose group dams, the pale-red focus in the glandular stomach of two animals is considered as a sign of maternal toxicity. Additionally, food and water consumption were reduced in the high dose group. Judging by water consumption, GA concentrations and animal weights, the concentrations given concern the test substance and not GA. Therefore the GA intake at the dose levels were as follows: 100 ppm: 5.6 mg GA/kg bw/day 500 ppm: 26 mg GA/kg bw/day
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 A6.8.1 _ 06	**Teratogenicity Study**		
Annex Point IIA6.8.1	**Rodent (Wistar Rat) – Range finding study 2**		
			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1991d), Report on the range-finding study of the prenatal toxicity of glutaraldehyde in rats after oral administration (gavage). [redacted] (Unpublished), BPD ID A6.08.1_06	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new active substance (a.s.) for first entry to Annex I authorisation.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 414 (1981)	X
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	Fluid and colorless	
3.1.2.2	Purity	[redacted]% in water	
3.1.2.3	Stability	The purity, the homogeneity and the stability of the test substance, were checked.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: About 62 to 101 days old Mean weight: About 210 g/animal	
3.2.6	Number of animals per group	10 females /group	
3.2.7	Control animals	Yes	

3.2.8	Mating period	Four untreated females were mated with one untreated fertile male rat of the same breed. Mating took place from ca. 16.00 hours to ca. 7.30 hours on the following day; the mating period was therefore about 15 to 16 hours.
3.3	**Administration/ Exposure**	
3.3.1	Duration of exposure	From day 6 to day 15 p.c.
3.3.2	Post-exposure period	None, the animals were sacrificed on day 16 p.c.
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	The test doses were as follows:

Test group	Dose (mg/kg bw/day)	Concentration (mg/100 ml)***	Volume (ml/kg bw)
0	0*	0	10
1	10	100	10
2	50	500	10

*,
Control animals treated with doubly distilled water.
***. Taking into account the active ingredient content of [redacted]%

X

3.3.5	Vehicle	Aqueous solution
3.3.6	Concentration in vehicle	See 3.3.4
3.3.7	Total volume applied	See 3.3.4
3.3.8	Controls	Control animals were treated with doubly distilled water.
3.4	**Examinations**	
3.4.1	Clinical signs of toxicity and mortality	Yes, the animals were checked daily, at least once for clinical symptoms and twice for mortality on working days. On Saturdays, Sundays or public holidays, the animals were only checked once a day for mortality. In case of presence of clinical signs of toxicity, the animals were checked several times a day.
3.4.2	Body weight	Yes, body weight was determined on day 0, 1, 3, 6, 8, 10, 13, 15 and 16 p.c.; body weight change was calculated on the basis of he values obtained.
3.4.3	Food consumption	Yes (excepted for day 0 and 16 p.c., determination on the same days as body weight)
3.4.4	Necropsy	On day 16 p.c., following blood sampling, all females were sacrificed for the purpose of necropsy; they were examined macroscopically. The fetuses were dissected from the uterus and subjected to further examinations.
3.4.5	Net maternal body weight change	Following sacrifice, the net maternal body weight change (i.e. the corrected body weight gain) was calculated from the terminal body weight by subtraction of (1) the uterus weight and (2) the body weight measured on day 6 p.c.

3.4.6	Clinical chemistry	The blood samples served for the determination of following parameters: sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol.
3.4.7	Organ weights	Liver and kidney were weighed.
3.4.8	Examination of uterine content	Gravid uterine weight Number of corpora lutea Number and distribution of implantations sites classified as live fetuses and dead implantations. Dead implantations comprised early resorptions and late resorptions. The conception rate (CR) as well as the pre- and post- implantation losses (Pre-I, Post-I) were calculated according to following formulas: $CR = \frac{\text{Number of pregnant animals}}{\text{Number of fertilized animals}} \times 100$ $\text{Pre-I} = \frac{\text{N*(corpora lutea) - N (implantations)}}{\text{N (implantations)}} \times 100$ $\text{Post-I} = \frac{\text{N(implantations) - N (live fetuses)}}{\text{N (implantations)}} \times 100$ *, N = number of.
3.4.9	Examination of foetuses	
3.4.9.1	General	The fetuses were extracted from the uterus and were examined for following parameters: Fetal weight, external abnormalities, viability, placentae, umbilical cords, fetal membranes, and fetal liquids. Individual placental weights were recorded.
3.5	**Statistical evaluation**	Food consumption, body weight and body weight change, corrected maternal body weight gain, gravid uterine weight, weight of the fetuses, weight of the placentae, corpora lutea, implantations, pre- and post implantation losses, resorptions and live fetuses, were assessed by means of Dunnett's Test. Conception rate, maternal mortality and all fetal findings were assessed by means of Fisher's Exact Test. For the clinical chemical parameters, means and standard deviations were assessed. Further assessment was based on the analysis of variance (ANOVA) and Dunnett's Test.

3.6	**Further remarks**	The purity, homogeneity and stability of the test substance were checked ([redacted]). The test substance solutions (glutaraldehyde in doubly distilled water) also were checked for stability. Reanalysis was performed at the end of the study period. Samples of the test substance preparations were verified for concentrations. Food as well as drinking water also was subjected to analyses according to the EPA guideline (Fed. Reg. Vol. 44, No.91, 1979) and to the German Drinking Water Regulation (Trinkwasserverordnung, Bundesgesetztblatt, 1990).

4 RESULTS AND DISCUSSION

4.1 Females excluded from data evaluation and/or calculation

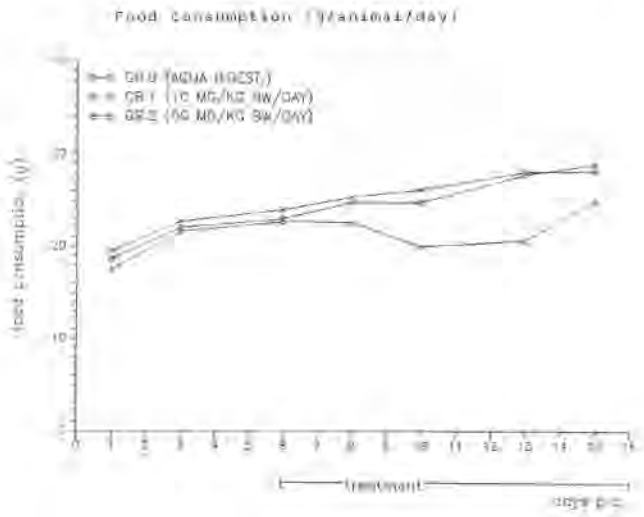
Following females were partly or totally excluded from data evaluation/calculation because of non pregnancy:

- 1 female of the control group (non pregnancy)
- 1 female of the 50 mg/kg bw/day group (no viable fetuses)

4.2 Clinical signs of toxicity and mortality

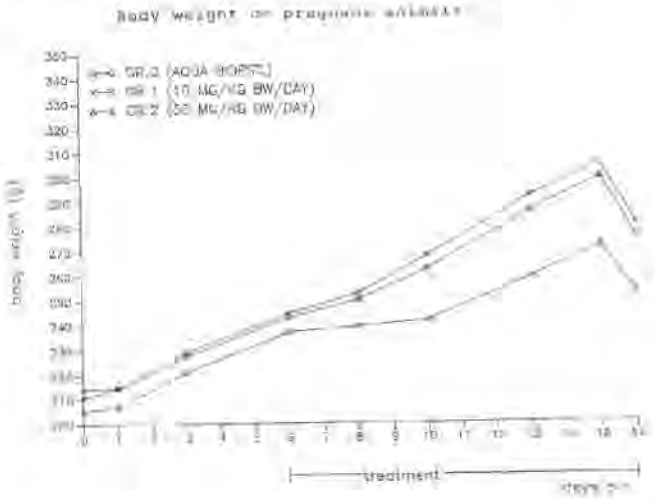
No mortality was reported, but following clinical symptoms indicative of toxicity were reported for the females treated with 50 mg/kg bw: reduced nutritional state, labored breathing, piloerection and one case of vaginal hemorrhage (day 15 and 16 p.c., no viable fetuses). In the 10 mg/kg bw group, some animals refused gavage, probably because of some irritating effect of the test substance on the gastro-intestinal tract; therefore treatment could not be regularly performed in this group. X

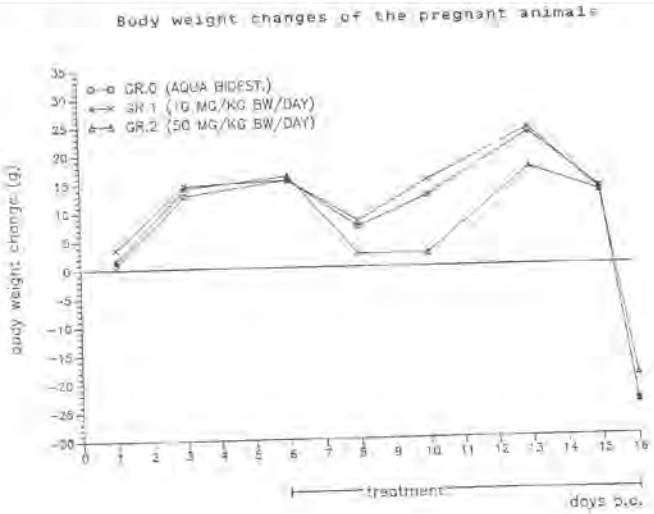
4.3 Food consumption



Food consumption of the dams treated with 50 mg/kg bw was clearly reduced (ca. 17% below control) during the treatment period (day 6 to day 15 p.c.).

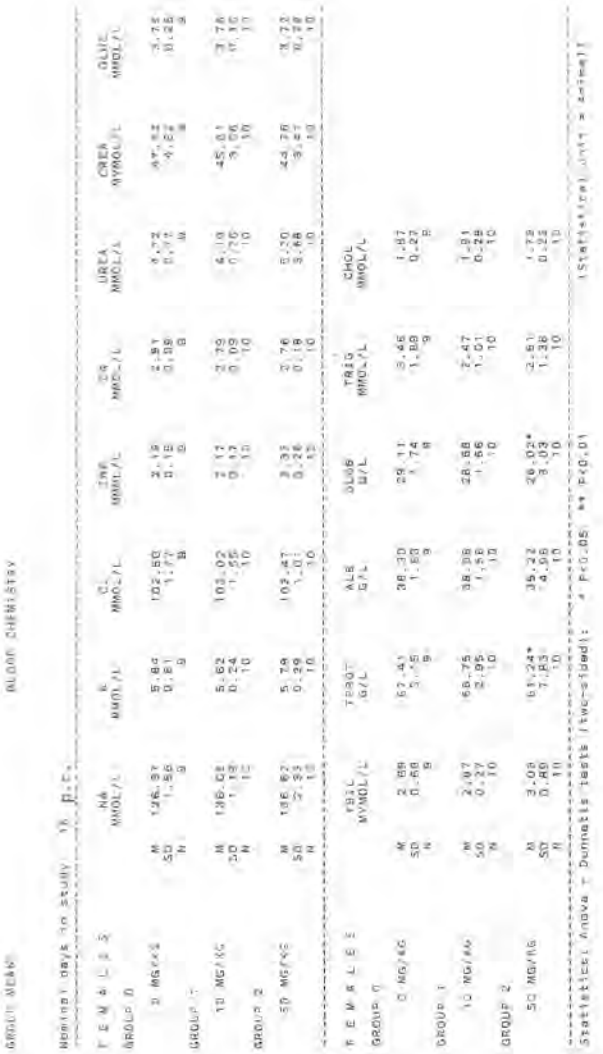
4.4 Maternal body weight data





In the 50 mg/kg bw group, mean body weights (especially on day 13 p.c.) and mean body weight change were reduced compared to control (statistically significantly reduced on day 8 – 10 p.c.).

4.5 Clinical-chemical parameters



In the 50 mg/kg bw group, a statistically significant decrease in total protein and globulins was reported. The decrease was considered to be treatment-related and probably was due to the reduction in food consumption observed within the same group.

4.6 Dams at study termination

Gravid uterine weight (GUW):

The gravid uterine weight for both treated groups was inconspicuous compared to control.

Net maternal body weight change from day 6 p.c:

Test concentrations			
NBW (g)	0 ppm (N=9)	10 mg/kg bw (N=10)	50 mg/kg bw (N=10)
	10.6 +/- 9.69	12.1 +/- 7.97	**-5.2 +/- 29.04**

Net maternal body weight change for the 50 mg/kg bw group was clearly decreased compared to control.

Necropsy:

Necropsy revealed thickening of the margo plicatus in the forestomach in

all animals of the 50 mg/kg bw group, and in one rat of the 10 mg/kg bw group. Furthermore, hemorrhagic mucosal lesions in the glandular stomach were seen in three rats of the 50 mg/kg bw group; these lesions were attributed to the irritating effect of the test substance.

Organ weights:

An increase in relative kidney weight war reported for the 50 mg/kg bw group; however this effect was considered to be a consequence of the decrease in body weight whereas the absolute kidney weight remained unchanged.

Reproduction data:

The conception rate varied between 90% (control) and 100% (10 and 50 mg/kg bw/day). Excepted for post implantation loss and late resorptions in the 50 mg/kg bw group, all reproduction parameters were inconspicuous and differences between the groups were incidental and/or within the normal range of biological variation for the test animals used. Due to an increase in number of late resorptions (mean 10.8% +/- 31.44 versus 0.0% +/- 0.00 in control), a slightly increased post implantation loss was observed at 50 mg/kg bw (13.9% +/- 31.26 versus 4.3 +/- 7.04).

4.7 Teratogenic / embryotoxic effects

Neither the placental weight nor the fetal weight showed effects that could be assessed as treatment-related. None of the fetuses showed external abnormalities.

4.8 Other effects

Test substance:

The homogeneity and stability of the test substance was confirmed. The stability of the test substance solutions over a period of 4 days at room temperature as well as the correctness of the prepared concentrations was confirmed.

Food and water:

Food and water were found to be suitable.

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.8.1 _ 06		**Teratogenicity Study**
Annex Point IIA6.8.1		**Rodent (Wistar Rat) – Range finding study 2**
5.1	**Materials and methods**	The present range-finding study was conducted prior to the main teratogenicity study (report [redacted]) in order to define the dose level inducing overt maternal toxicity in pregnant rats, however without exceeding 10% mortality. Furthermore the administration of the test substance by gavage was here tested as way of treatment in comparison to a further range-finding study (report [redacted]) where the test substance was administered in the drinking water. Test substance: Glutaraldehyde, batch No: [redacted]% in water The test was conducted according to OECD guideline 414 (1981), with GLP. The dams (10/group) were treated by gavage from day 6 to day 15 post coitum (p.c.) with glutaraldehyde at doses of 0, 10 and 50 mg/kg bw/day. The animals were observed for clinical symptoms, mortality, body weight and body weight change, and for food consumption. On day 16 p.c. blood samples were collected from all females, which then were sacrificed for the purpose of necropsy. The blood samples served for the assessment of a series of clinical-chemical parameters. The liver and kidney were retained for organ weight assessment. The gravid uterine weight as well as the net maternal body weight change was determined. A series of reproduction parameters including conception rate, number of corpora lutea, number of implantation sites, pre- and post implantation losses, resorptions and live fetuses were examined. The fetuses were dissected from the uterus and were investigated for external abnormalities. The test substance was analyzed for purity, homogeneity and stability. Test substance preparations were examined for stability and homogeneity and the correctness of the test concentrations was verified. The homogeneity and stability of the test substance were confirmed. The stability of the test substance solutions over a period of 4 days as well as the correctness of the test doses was confirmed.

5.2	**Results and discussion**	Maternal toxicity: Treatment-related symptoms of toxicity mainly were seen in the 50 mg/kg bw group. For this group reduced nutritional state, labored breathing, piloerection and one case of vaginal hemorrhage (day 15 and 16 p.c., no viable fetuses) were reported. Furthermore food consumption was clearly reduced (ca. 17% below control) during the treatment period and mean body weights as well as mean body weight change were reduced compared to control. Considering the clinical chemical parameters, a statistically significant decrease in total protein and globulins was reported, which probably was due to the reduction in food consumption. The net maternal body weight change for the 50 mg/kg bw group was clearly decreased compared to control. Necropsy revealed thickening of the margo plicatus in the forestomach of all animals of this 50 mg/kg bw group, as well as three cases of hemorrhagic mucosal lesions in the glandular stomach, which were attributed to the irritating effect of the test substance. An increase in relative kidney weight war reported, which however was considered to be a consequence of the decrease in body weight whereas the absolute kidney weight remained unchanged. Considering the reproduction parameters, excepted for post implantation loss and late resorptions, all other parameters were inconspicuous and differences between the groups were incidental and/or within the normal range of biological variation for the test animals used. Due to an increase in number of late resorptions (mean 10.8% +/- 31.44 versus 0.0% +/- 0.00 in control), a slightly increased post implantation loss was observed (13.9% +/- 31.26 versus 4.3 +/- 7.04). For the 10 mg/kg bw group, necropsy revealed a single case of thickening of the margo plicatus in the forestomach. Moreover, the authors reported that some animals refused gavage, probably because of some irritating effect of the test substance on the gastro-intestinal tract; therefore treatment could not be regularly performed in this group. Embryo-/fetotoxicity, teratogenicity: Because of the early sacrifice of the dams, only a limited examination of the fetuses was possible. Taking this aspect into account, neither embryo/fetotoxic nor teratogenic treatment-related effects were seen.
5.3	**Conclusion**	On the basis of the results of the present study and of those of the range finding study report [redacted], (1) treatment via the drinking water and (2) test concentrations of 0, 50, 250 and 750 ppm, were selected for the conduct of the main teratogenicity study with rats (report [redacted]).
5.3.1	Reliability	**1**
5.3.2	Deficiencies	None

Section A6.8.1 _ 06	**Teratogenicity Study**
Annex Point IIA6.8.1	**Rodent (Wistar Rat) – Range finding study 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 3rd, 2010
Materials and Methods	2.1 Guideline study. It is misleading to state that OECD guideline 414 was followed. This is a range-finding study with fewer animals, shorter time periods and less investigations as compared with the full guideline study. 3.1.2 This refers to Doc IIIA Section A2. 3.3.4 Concentration. The numbers in the column "Concentration" refer to the test substance ([redacted] % GA), not glutaraldehyde.
Results and discussion	4.2 Clinical signs of toxicity and mortality. The animals refusing gavage were in the high dose group. 5.2 Results and discussion. Same comment as for 4.2 above.
Conclusion	No signs of teratogenicity were seen. Embryotoxic effects were seen at the highest dose level, which caused maternal toxicity. These effects consisted of increased incidences of postimplantation losses and late resorptions, and were seen at the high dose level only. There were many signs of maternal toxicity at the highest dose level: • Labored breathing, piloerection and one case of vaginal hemorrhage (no viable fetuses); gavage refusal • Reduced food consumption and body weight • Decrease in total protein and globulins in the blood • Thickening of margo plicatus in the forestomach, hemorrhagic mucosal lesions in the glandular stomach In the mid-dose group dams, there was a single case of thickening of the margo plicatus in the forestomach. Due to the same effect occurring in the high dose group, this is considered treatment related. It is not evident whether the concentrations give the GA dose or the test substance dose, containing [redacted] % GA. Judging by consistency with the other range finding studies in this dossier by the same authors, they are concluded to give doses of the test substance. Therefore the doses are as follows: • 10 mg/kg bw/day: 5.1 mg GA/kg bw/day • 50 mg/kg bw/day: 25.5 mg GA/kg bw/day
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*

Section A6.8.1 _ 06	**Teratogenicity Study**
Annex Point IIA6.8.1	**Rodent (Wistar Rat) – Range finding study 2**

Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2001) [redacted]% Glutaraldehyde) – Two-generation reproduction toxicity study in Wistar rats – Continuous administration in the drinking water. [redacted] (Unpublished), BPD ID A6.08.2_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 active substance (a.s.) for first entry to Annex I authorisation.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 416 (Draft 1998, quite similar to the update version of 2001)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Determination of food consumption: food consumption for the F0 and F1 dams was not determined, as required by the guideline, between day 14 and 21 pp since during this period the pups will start consumption of solid food; therefore there was no point in such a measurement. Determination water consumption: water consumption for the F0 and F1 dams was not determined, as required by the guideline, from day 15 pp onwards, since during this period the pups will start consumption of water; therefore there will be no point in such a measurement.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]% Glutaraldehyde)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Colorless-clear liquid	
3.1.2.2	Purity	[redacted]% (analysis performed by [redacted])	
3.1.2.3	Stability	The stability of the test substance in drinking water over a period of 14 days at room temperature had been proven prior to starting the experiment. The stability of the test substance was proven by reanalysis ([redacted])	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male/Female	

3.2.5	Age/weight at study initiation	The animals of the F0 generation were about 34 +/- 1 day old at test starting. Mean body weight for the males: 100.5 (89.3 - 116.6) g Mean body weight for the females: 89 (77.6 – 99.6) g
3.2.6	Number of animals per group	108 males and 108 females were used for the study and were distributed into 4 test groups (27 animals/sex/group).
3.2.7	Mating	Each male was paired with one female of the same group; pairing happened overnight over a period of maximum 2 weeks. For this purpose, the female was placed into the cage of the male from ca. 4.00 p.m. to 7.00 – 9.00 a.m. of the following day. Mating was carried out with the same partner in each case. After each mating, a vaginal smear was taken from the female and checked for presence of sperm. If sperm was present, the female was considered as fertilized and the day was designated as day 0. The following day was then day 1 post-coitum (p.c.).
3.2.8	Duration of mating	2 weeks
3.2.9	Deviations from standard protocol	No
3.2.10	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Animal assignment to dosage groups	F0 generation parental animals:

Test group	Test Concentration referring to the test substance as such (i.e. [redacted])	Number of animals per group	
		Males	Females
00	0 ppm	27	27
01	100 ppm	27	27
02	500 ppm	27	27
03	2000 ppm	27	27

F1 generation parental animals:

Test group	Test concentration referring to the test substance as such (i.e. [redacted])	Number of animals per group	
		Males	Females
10	0 ppm	27	27
11	100 ppm	27	27
12	500 ppm	27	27
13	2000 ppm	27	27

3.3.2	Duration of exposure before mating	At least 76 days

3.3.3	Duration of exposure in general P, F1, F2 males, females	Parental animals (F0 generation): About 20 weeks, including the premating exposure period of about 10 - 12 weeks. F1 parental generation: About 17 weeks. **Oral**
3.3.4	Type	Drinking water
3.3.5	Concentration	The nominal test concentrations were 100, 500 and 2000 ppm [redacted]
3.3.6	Controls	The control animals received drinking water only (i.e. without tests substance)
3.4	**Examinations**	
3.4.1	Clinical signs	The animals were checked daily for mortality (dead and moribund animals) and clinical symptoms of toxicity. Particular attention was given to the nesting, littering and lactation behaviour of the dams, but only special findings were documented.
3.4.2	Body weight	Parental animals: generally, body weight was determined once weekly until the end of the study, and at the time of necropsy. F0 and F1 fertilized females and females with litter: body weight was determined on the day of sperm evidence in the vaginal smear and thereafter on days 7, 14 and 20 of gestation, one day after of parturition, and on days 7, 14 and 21 post-parturition. Females without positive evidence of sperms: body weight was not determined during the mating interval. Females without litter: body weight was not determined during the lactation phase.
3.4.3	Food consumption	F0 and F1 parental animals: food consumption was determined once weekly (over 7 days) during the period prior mating. Pregnant females: food consumption was determined for days 0-7, 7-14, 14-20 post coitum (pc). Lactating females: food consumption was determined for days 1-4, 4-7, 7-14 post parturition (pp). F0 and F1 dams between day 14 and 21 pp: food consumption was not determined for the F0 and F1 dams between day 14 and 21 pp, since during this period the pups will start consumption of solid food; therefore there will be no point in such a measurement. Females during mating period, females without positive evidence of sperms, females without litter: food consumption was not determined respectively during mating period, gestation period or lactation phase.

3.4.4	Water consumption	F0 and F1 parental animals: water consumption was determined once weekly (over 3 days) during the period prior mating. Pregnant females: water consumption was determined for days 0-1, 6-7, 13-14, 19-20 post coitum (pc). Lactating females: water consumption was determined for days 1-2, 4-5, 7-8, 14-15 post parturition (pp). F0 and F1 dams from day 15 pp onwards: water consumption was not determined for the F0 and F1 dams from day 15 pp onwards, since during this period the pups will start consumption of water; therefore there will be no point in such a measurement. Females during mating period, females without positive evidence of sperms, females without litter: water consumption was not determined respectively during mating period, gestation period or lactation phase.
3.4.5	Intake of the test substance	The intake of test substance (IT, in mg/kg bw/day() was calculated according to following formula: $IT_x = \frac{WC_x \times D}{BWy}$ D = dose in ppm WCx = daily water consumption on day x; in g BWy = body weight on day y (i.e. last weighing before day x); in g
3.4.6	Oestrus cycle	The oestrus cycle was evaluated daily for length and normality for all f0 and F1 parental females, over a period of at least 3 weeks prior mating and through mating period until mating was definitively evidenced. A last evaluation was done at necropsy.
3.4.7	Male reproduction parameters	Mating and fertility indices were calculated according to following formulas: Male mating index (%) = $\frac{\text{N (males with confirmed mating)}}{\text{N (males placed with females)}} \times 100$ Male fertility index (%) = $\frac{\text{N (males with proved fertility)}}{\text{N (males placed with females)}} \times 100$ Remark: N = number of Males were defined as "with confirmed mating" by the presence of vaginal sperm in the female, or by the production of a litter, or by the presence of fetuses in the uterus. Males were defined as "with proved fertility" by female giving birth to a litter or having pups or fetuses in the uterus.

3.4.8 Sperm parameters

Following necropsy and organ weighing, the right testis and cauda epididymis were taken from the F0 and F1 males of all test groups for the evaluation of following parameters:

Parameter	Test group	Unit	Evaluation method
Sperm motility	All groups	%	Microscopy
Sperm morphology	Control and 2000 ppm groups	%	Microscopy following vital staining (eosin)
Sperm head count (cauda epididymis)	Control and 2000 ppm groups	Sperm heads x 10^6/g cauda epididymis	Microscopy (MAKLER chamber following homogenization
Sperm head count (testis)	Control and 2000 ppm groups	Sperm heads x 10^6/g testis	Microscopy (MAKLER chamber following homogenization

3.4.9	Female reproduction and delivery data	Mating, fertility and gestation indices were calculated according to following formulas: Female mating index (%) = N (females mated) **x** 100 / N (females placed with males) Female fertility index (%) = N (pregnant females) **x** 100 / N (mated females) Gestation index (%) = N (females with live pups on day of birth) **x** 100 / N (pregnant females) Remark: Females were defined as mated when vaginal sperm was evidenced, or when they gave birth to a litter, or had fetuses in the uterus. Females were defined as pregnant when they gave birth to a litter or had pups of fetuses in the uterus. The live birth index was calculated for the F1 and F2 litters according to following formula: Live birth index (%) = N (liveborn pups at birth) **x** 100 / N (total pups born) The postimplantation loss was calculated according to following formula: Postimplantation loss (%) = N (implantations) – N (pups delivered)**x**100 / N (implantations)

3.4.10 Offspring

The pups (F1 and F2 litters) were examined on their day of birth for the determination of the total number of pups and the number of liveborn and stillborn pups (pups died on day of birth prior the first examination). Thereafter the pups were checked twice daily on workdays (once a day on week ends and public holidays) for mortality (i.e. dead and moribund pups) and the mortality (number and percentage) was determined for the day of birth (i.e. day 0) and for the periods: day 1 - day 4, day 5 - day 7, day 8 - day 14 and day 15 - day 21 of lactation. Pups that died accidentally and had to be sacrificed because of maternal death were not considered for calculation. The number of surviving pups was determined for day 0, day 4, day 7, day 14 and day 21 and served for the calculation of the viability index and the lactation index, according to following formulas:

$$\text{Viability index (\%)} = \frac{\text{N (live pups on day 4 after birth)}}{\text{N (total live pups on day of birth)}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{N (live pups on day 21 after birth)}}{\text{N (live pups on day 4 after birth)}} \times 100$$

Remark:
Day 4 after birth preceded standardization of the litters.
Day 21 after birth followed standardization of the litters.

The sex of the pups was determined on day 0 and day 21 (measurement of the anogenital distance, which is known to be greater in male pups than in females), and the sex ratio was calculated according to following formula:

$$\text{Sex ratio} = \frac{\text{N (live male } \textbf{or} \text{ female pups on day 0/ 21)}}{\text{N (live male } \textbf{and} \text{ female pups on day 0/21)}} \times 100$$

The pups were weighed on day 1, 4, 7, 14 and 21 after birth, and they were examined daily for clinical symptoms or gross morphological abnormalities.

Within necropsy of sacrificed pups (F1 and F2 generations), the brain, spleen and thymus were weighed. The determination of the relative organ weight was based on the pup body weight on day 21 after birth. The bodies of the sacrificed pups were examined for external abnormalities and the organs also were subjected to gross pathology; skeletal staining according to the modified Dawson's method and/or further processing of the head according to Wilson's method was done in case of abnormal findings. Stillborn pups as well as pups that died during weaning also were subjected to necropsy.

All female pups selected for the parental F1 generation (27/group) were evaluated daily for vaginal opening indicative of sexual maturation, starting from day 27 after birth. The selected male pups also were evaluated for sexual maturation by examination for preputial separation starting from day 40 after birth.

3.4.11	Organ weights P and F1	The parental F0 and F1 animals were sacrificed for the purpose of necropsy. Animals that died also were subjected to necropsy as soon as possible after death. Following organs were weighed: Whole body, liver, kidneys, adrenals, testes, epididymes (total and caudal), prostate, seminal vesicles (with coagulating glands and their fluid), ovaries, uterus (with cervix uteri and oviducts), spleen, brain and pituitary.
3.4.12	Histopathology F0 and F1	Following tissues/organs were sampled and fixed (4% formaldehyde or Bouin's solution: Vagina, cervix uteri, uterus, oviducts, ovaries, left testicle, left epididymis, seminal vesicles, coagulating glands, prostate, pituitary, liver, kidneys, spleen, brain, adrenals and all gross lesions. Following tissues/organs were subjected to light microscopical examination: All gross lesions were examined. All tissues/organ samples were examined in the control and the 2000 ppm group. In the 100 and 500 ppm groups, all tissue/organ samples of animals suspected of impaired fertility were examined. Particular attention was given to correlate gross lesions with microscopical findings. DOFC: The ovaries of each animal were subjected to a differential ovarian follicle count (DOFC).
3.5	**Statistics**	The statistical assessment of the different data obtained within the present study was based on following methods, depending on the parameters considered: Dunnett test Fisher's exact test Wilcoxon test Kruskall-Wallis test.
3.6	**Further remarks**	Food and drinking water also were subjected to analysis. Food was analysed according to the EPA guideline (Fed. Reg. Vol. 44, No. 91, 1979); water was analysed according to the German Drinking Water Regulation (Trinkwasserverordnung, Bundesgesetztblatt, 1990).
		4 RESULTS AND DISCUSSION
4.1	**Effects**	

4.1.1	F0 parent males	No mortality was observed. Neither treatment-related clinical symptoms nor disturbances of the general behaviour were observed. The mean body weights and body weight changes for the treated F0 males were within the control range, with occasional isolated in- or decreases, which were statistically significant but without any biological significance. Food consumption for the F0 males of all treated groups was quite similar to that observed in the control group. In fact, a statistically significant but transient decrease in food consumption was observed at 2000 ppm during the first week of treatment only. This decrease was of no biological relevance as the food uptake thereafter reached or even exceeded that of control animals. Water consumption was statistically significantly reduced in the F0 males of the 2000 ppm group (about 22% below control during the premating period). For the F0 males of the 500 ppm group, water consumption was slightly reduced compared to control (about 6% below control during the premating period). Water consumption of the 100 ppm F0 males was similar to control and within the normal variation range for the used rat strain.
4.1.2	Male reproduction parameters for the F0 males	Excepted for one male of the control group and one male of the100 ppm group, mating was confirmed for all males of the parental F0 generation in all test groups. Thus the mating index for all groups varied between 96 and 100%, independently of treatment or not. The fertility index of the males varied between 92 and 100% independently of treatment or not.
4.1.3	Sperm parameters	For all considered parameters, data were quite similar for the treated and the control groups, indicating that there was no treatment-related effect on these parameters.

4.1.4	F0 parent females	F0 females: no treatment-related mortality was observed. In fact one control female was found dead on the first day of mating; necropsy revealed an incidental occurring malignant lymphoma that had infiltrated several organs. Neither treatment-related clinical symptoms nor disturbances of the general behaviour were observed. The mean body weights and body weight changes for the treated F0 females were within the control range, with occasional isolated in- or decreases, which were statistically significant but without any biological significance (this was also true for the post weaning period). Food consumption for the F0 females of all treated groups was quite similar to that observed in the control group. In fact, a statistically significant but transient decrease in food consumption was observed at 500 and 2000 ppm during the first week of treatment only. This decrease was of no biological relevance as the food uptake thereafter reached or even exceeded that of control animals. Water consumption was statistically significantly reduced in the F0 females of the 2000 ppm group (about 25% below control during the premating period). For the F0 females of the 500 ppm group, water consumption was slightly reduced compared to control (about 10% below control during the premating period). Water consumption of the 100 ppm F0 females was similar to control and within the normal variation range for the used rat strain. F0 females during gestation: no treatment-related clinical symptoms were seen. The mean body weights and body weight changes for the treated F0 females were within the control range, with occasional isolated in- or decreases, which were statistically significant but without any biological significance. During gestation, food consumption for the F0 females of all treated groups was similar to that observed in the control group. Water consumption was statistically significantly reduced in the F0 females of the 2000 ppm group during gestation (about 29% below control). Water consumption for the 500 ppm F0 females during gestation was slightly reduced compared to control (about 9% below control). Water consumption of the 100 ppm F0 females was similar to control and within the normal variation range for the used rat strain. F0 females during lactation: no treatment-related clinical symptoms were seen. The mean body weight and body weight gain of the 2000 ppm F0 females during the lactation period was impaired compared to control. In fact, at the end of the lactation period (i.e.day 21 of lactation), the mean body weight of the 2000 ppm females was about 227 g versus ca. 262 g for control females, and therefore it was about 13% below control. The mean body weight change over the whole lactation period (from day 1 to day 21) was about 8 g for the 2000 ppm females versus 28 g for the control females, corresponding to 72% below control. Food consumption for the 2000 ppm F0 females during lactation was found to be statistically significantly reduced between day 4 to day 7; the overall food consumption from day 1 to day 14 post parturition was about 7% below control. Water consumption was statistically significantly reduced in the F0 females of the 2000 ppm group during lactation (about 21% below control).. During the lactation phase, water consumption of the 500 ppm F0 females was similar to control. Water consumption of the 100 ppm F0 females was similar to control and within the normal variation range for the used rat strain.	X

4.1.5	Oestrus cycle	The mean cycle from oestrus to oestrus for the F0 female of the treated groups varied between 5.5 +/- 1.41 and 6.3 + 1.85 days, versus 5.3 +/- 1.43 days for the control F0 females. The cycles were generally regular and in a few cases of all groups including control, an unusually prolonged oestrus cycle duration (>= 9 days) was observed. This effect was considered to be spontaneous in nature.	X
4.1.6	Female reproduction and delivery data	The female mating index for the F0 females ranged between 96 and 100% for all groups including the control. The mean duration of the sperm detection period was about 2.3 and 3 days and showed to treatment-relationship. Because of the non-pregnancy of respectively one control female and one 500 ppm female, the fertility index was about 96% for each of the control and 500 ppm group. In each of the 100 ppm and the 2000 ppm group, all females became pregnant, resulting in a fertility index of 100%for each of these two groups. Moreover, the percentages reported above were within the range of historical control values.	
		The mean gestation period was quite similar in all groups and ranged from 21.8 to 22.1 days. As all pregnant females had live F1 pups in their litters, the gestation index for all groups was 100%. The mean number of implantation sites was quite similar for all groups, ranging from 259 to 302 per group. Postimplantation loss ranged from 3.5 to 5.4% and showed no treatment-relationship. This indicates that no embryo-/fetolethality resulted from the treatment. . The mean number of F2 pups delivered per dam was quite similar for all groups, ranging from10.8 to 11.6. The number of stillborn pups was about 2 -3 per group and was comparable for all groups. The total number of liveborn pups per group ranged between 247 (control) and 290 (500 ppm group); the number of live pups per litter was about 10.1 to 11.2 and was therefore within the same range for all groups. The live birth index for all groups varied between 98 and 100%.	X

4.1.7 Test substance intake, F0 parents

The nominal test concentrations were 100, 500 and 2000 ppm [redacted]. The corresponding mean approximate test substance intake (mg/kg bw/day) was as follows:

Animals	Mean intake of [redacted] (mg/kg bw/day)		
	100 ppm	500 ppm	2000 ppm
F0 males	9.4	44.8	152
F0 females (Premating period)	12.1	57.4	191.2
F0 females (F1litter):			
- Gestation period	12	53.9	167.3
- Lactation period	17.1	84.5	286.6

Animals	Mean intake of Glutaraldehyde (mg/kg bw/day)		
	50 ppm	250 ppm	1000 ppm
F0 males	4.7	22.4	76
F0 females (Premating period)	6.1	28.7	95.6
F0 females (F1litter):			
- Gestation period	6	27	83.7
- Lactation period	8.6	42.3	143.3

4.1.8	F1 offspring, viability, sex ratio, body weight and symptoms of toxicity	Pup viability, day 0 to day 4 after birth: The mean number of delivered F1 pups per dam as well as the rate of liveborn and stillborn pups was not affected by the treatment. During the first 4 days following birth, 11 cases of pup mortality were reported for the 2000 ppm group, versus 2 cases in the control group. Five cases were reported for the 100 ppm group and 2 cases for the 500 ppm group. The viability index for the 2000 ppm group was therefore about 95% versus 98% for the control group. In fact, the reduced pup viability observed at 2000 ppm was mainly related to one dam only, which lost 9 of 14 pups because of bad nursing. Moreover, the viability index of 95% reported for the 2000 ppm group still was within the range of historical control data. Pup viability, day 4 to day 21after birth: The pup mortality in each group for this period was indicated by the lactation index. The lactation index ranged from of 97% (control) to 100% (500 ppm group) and no treatment-related differences were evident. Sex ratio: On day 0, the sex distribution (%males, %females) was as follows: 52.2% males and 47.8% females for control 49.6% males and 50.4% females for the 100 ppm group 47.9% males and 52.1% females for the 500 ppm group 47.0% males and 53.0% females for the 2000 ppm group The sex distribution and sex ratio of the live F1 pups of the day of birth therefore were quite similar for all groups. On day 21, the sex distribution (%males, %females) was as follows: 51.6% males and 48.4% females for control 51.8% males and 48.2% females for the 100 ppm group 48.0% males and 52.0% females for the 500 ppm group 47.8% males and 52.2% females for the 2000 ppm group The sex distribution and sex ratio of the live F1 pups of day 21after birth also were quite similar for all groups. Body weight: The mean body weight of the F1 pups (males + females) of the 2000 ppm group on day 21 were statistically significantly reduced compared to control. In fact, the mean body weight of the 2000 ppm F1 pups was about 15% below control. In contrast, the differences in mean body weight seen between control and each of the 100- and the 500 ppm group were negligible as they were in the range of biological variation. Clinical symptoms of toxicity: The F1 pups showed no treatment-related clinical symptoms of toxicity.

4.1.9	F1 offspring, organ weight, pathology, sexual maturation	Organ weights: Statistically significant decreases in absolute organ weights were reported for the thymus and the spleen of the F1 pups from the treated groups when compared to those of the control group. In fact, the mean thymus weight in the pups of the 100, the 500 and the 2000 ppm group respectively was about 10%, 12% and 19% below control value. The mean spleen weight was 16% and 31% below control value respectively for the pups of the 500 and the 2000 ppm group. Considering the relative pup organ weight, statistically significant differences were reported for the brain and the spleen of the 500 and the 2000 ppm groups when compared to control. In fact, the mean relative brain weight was increased about 7% over control value for the pups of the 500 ppm group, and about 19% over control value for those of the 2000 ppm group. The mean relative spleen weight for the pups of the 500 ppm and the 2000 ppm group respectively was about 11% and 17% below control value. Pathology: The macroscopical examination of stillborn pups, pups that died intercurrently, culled or surplus pups of the F1 litter revealed no treatment-related abnormalities. In fact, for all groups (i.e. including control) spontaneous findings were seen, which were without any dose-relationship and/or were known to occur at similar or higher incidences within historical control. One pup of the 2000 ppm group was reported to show a malpositioned tail and was subjected to further skeletal examination; this anomaly was related to several misshapen sacral vertebrae. Sexual maturation: Vaginal opening in the selected F1 female pups occurred within day 30 and 41 post parturition. Preputial separation in the selected F1 male pups occurred between day 42 and 50 post parturition. Differences between treated and control group were not statistical significant and further were within the range of biological variation.
4.1.10	F1 males	No unscheduled mortality was observed. Neither treatment-related clinical symptoms nor disturbances of the general behaviour were observed. The mean body weight of the F1 males of the 2000 ppm group was statistically significantly lowered throughout the study period (about 8% below the mean body weight of control males) and at the end of this period, body weight gain for the 2000 ppm F1 males was about 7% below control value. Food consumption for the F1 males of the 2000 ppm group was impaired compared to control (about 7% below control value); in contrast, food uptake in the 100 and 500 ppm was similar to of the control F1 males. Water consumption was statistically significantly reduced in the F1 males of the 2000 ppm group (about 28% below control during the premating period). Water consumption of the 500 ppm F1 males also was reduced to about 14% below control; for the F1 males of the 100 ppm, water consumption was quite similar to that of controls and was within the normal biological variation.
4.1.11	Male reproduction parameters for the F1 males	Excepted for one male of the 100 ppm group mating was confirmed for all males of the parental F1 generation in all test groups. Thus the mating index for all groups varied between 96 and 100%, independently of treatment or not. The fertility index of the males varied between 93 and 100% independently of treatment or not.
4.1.12	Sperm parameters	For all considered parameters, data were quite similar for the treated and the control groups, indicating that there was no treatment-related effect on these parameters.

4.1.13 F1 females	F1 females: no unscheduled mortality was observed. Neither treatment-related clinical symptoms nor disturbances of the general behaviour were observed. The mean body weight of the F1 females of the 2000 ppm group was about 4% below control value during the premating period. During the premating period, food consumption for the F1 females of the 2000 ppm group was impaired compared to control (about 8% below control value); in contrast, food uptake in the 100 and 500 ppm was similar to of the control F1 females. During the premating period, water consumption was statistically significantly reduced in the F1 females of the 2000 ppm group to about 33% below control; the F1 females of the 500 ppm groups also showed reduced water consumption (about 14% below control). Water consumption of the 100 ppm F1 females was quite similar to that of controls and was within the normal biological variation. F1 females during gestation: no treatment-related clinical symptoms were observed during gestation. The mean body weight of the F1 females of the 2000 ppm group was about 5% below control value during this period. Food consumption for the F1 females of the 2000 ppm group was impaired compared to control and was about 7% below control value; in contrast, food uptake in the 100 and 500 ppm was similar to of the control F1 females. A clear reduction in water consumption during the gestation period (day 0 to 20 post coitum) was reported for both, the 2000 ppm- and the 500 ppm F1 females. In fact, the 2000 ppm females consumed about 35% less water than the control whereas the 500 ppm F1 females consumed about 18% less than control. F1 females during lactation: no treatment-related clinical symptoms were observed during lactation. The mean body weight of the F1 females of the 2000 ppm group was about 12% below control value during the this period. The mean body weight gain for the 2000 ppm females during lactation was about 64% below that body weight gain of the corresponding control females when calculated for the period day 1 to day 21 post parturition. Food consumption for the F1 females of the 2000 ppm group was impaired during the lactation period from day 1 to day 14 post parturition and was about 10% below control value; in contrast, food uptake in the 100 and 500 ppm was similar to of the control F1 females. During lactation, the water consumption of the 2000 ppm F1 females was statistically reduced to about 26% below control.
4.1.14 Oestrus cycle	The mean cycle from oestrus to oestrus for the F1 females of all group (i.e. control and treated) was generally regular and varied between 4.4 and 4.8 days.

4.1.15 Female reproduction and delivery data

The female mating index for the F1 females ranged between 96% (100 ppm group) and 100% (all remaining groups including control). In fact, one F1 female of the 100 ppm group showed neither sperm in the vaginal smear nor indications of successful implantation. The mean duration of the sperm detection period was about 2.2 and 2.9 days and showed to treatment-relationship; these values were within the normal biological range for the used rat strain. Because of the non-pregnancy of respectively one female of the 100 and one of the 2000 ppm group, the fertility index was about 96% (100 ppm and 2000 ppm) and 100% (control and 500 ppm group). The percentages reported above were within the range of historical control values. The mean gestation period was quite similar in all groups and ranged from 21.8 to 22.1 days. Four pregnant F1 females of the 2000 ppm group did not deliver (implantation sites were seen at necropsy), resulting in a gestation index of 85% for this group. For all other groups, the gestation index was 100%. The mean number of implantation sites was quite similar for all groups, ranging from 256 to 285 per group. The mean postimplantation loss was about 3.7% in control, 2.5% in the 100 ppm group, 15.3% in the 500 ppm group and 2.8% in the 2000 ppm group. Because of its isolated occurrence and the lack of dose-response relationship, the increase in resorption rate observed at 500 ppm was considered to be incidental. The number of stillborn pups was about 0 to 2 per group. The total number of liveborn pups per group ranged between 242 (2000 ppm) and 261 (100 ppm group); the number of live pups per litter was about 9.7 to 11 and was therefore within the same range for all groups. The live birth index for all groups varied between 97 and 100%.

X

4.1.16 Test substance intake, F1 parents

The nominal test concentrations were 100, 500 and 2000 ppm [redacted]. The corresponding mean approximate test substance intake (mg/kg bw/day) was as follows:

Animals	Mean intake of [redacted] (mg/kg bw/day)		
	100 ppm	500 ppm	2000 ppm
F1 males	12.8	60.4	213.1
F1 females (Premating period)	15.0	69.7	239.1
F1 females (F2 litter):			
- Gestation period	13.4	56.4	188.8
- Lactation period	18.6	92.9	304.7

4.1.17	F2 offspring, viability, sex ratio, body weight and symptoms of toxicity	Pup viability, day 0 to day 4 after birth: The mean number of delivered F2 pups per dam as well as the rate of liveborn and stillborn pups was not affected by the treatment. During the first 4 days following birth, 9 cases of pup mortality were reported for the 2000 ppm group, versus 6 cases in the control group. 8 cases were reported for the 100 ppm group and 2 cases for the 500 ppm group. The viability index of the F2 pups for the 2000 ppm group was therefore about 96% versus 97% for the control group. Moreover, the viability index of 96% reported for the 2000 ppm group still was within the range of historical control data. Pup viability, day 4 to day 21after birth: The pup mortality in each group for this period was indicated by the lactation index. The lactation index was 99% in control, 98% in the 100 ppm group, 100% in the 500 ppm group and 97% in the 2000 ppm group; no treatment-related differences were evident. Sex ratio: On day 0, the sex distribution (%males, %females) was as follows: 55.2% males and 44.8% females for control 45% males and 55% females for the 100 ppm group 50.4% males and 49.6% females for the 500 ppm group 46.6% males and 53.4% females for the 2000 ppm group The sex distribution and sex ratio of the live F2 pups of the day of birth therefore were quite similar for all groups. On day 21, the sex distribution (%males, %females) was as follows: 52.7% males and 47.3% females for control 49.2% males and 50.8% females for the 100 ppm group 48.9% males and 51.1% females for the 500 ppm group 49.2% males and 50.8% females for the 2000 ppm group The sex distribution and sex ratio of the live F2 pups of day 21after birth also were quite similar for all groups. Body weight: The mean body weight of the F2 pups (males + females) of the 2000 ppm group was statistically significantly reduced compared to control from day 14 post parturition upwards. In fact, the mean body weight of the 2000 ppm F2 pups was about 11% below control on day 21. The mean body weight gain at 2000 ppm was statistically impaired from day 7 to day 14 post parturition. In fact, from day 4 to day 21, the mean body weight gain of the 2000 ppm F2 pups was about 14% below control. The changes in body weight observed for the 2000 ppm F2 pups were considered to be treatment-related. The body weight of the F2 pups in the 100 and the 500 ppm groups were inconspicuous. Clinical symptoms of toxicity: The F2 pups showed no treatment-related clinical symptoms of toxicity.

4.1.18	F2 offspring, organ weight, pathology	Organ weights: A statistically significant decrease in absolute organ weight was reported for the spleen of the 2000 ppm F2 pups when compared to those of the control group. In fact, the mean spleen weight for the 2000 ppm F2 pups was about 15% below control value. Considering the relative pup organ weight, a statistically significant difference was reported for the brain only, which showed an increase in relative weight for the 2000 ppm F2 pups compared to control. In fact, the mean relative brain weight was increased about 14% over control value. The changes in absolute and relative organ weights reported above were related to the significant delays in mean body weight gains, which were reported for the 2000 ppm F2 pups. Pathology: The macroscopical examination of stillborn pups, pups that died intercurrently, culled or surplus pups of the F1 litter revealed no treatment-related abnormalities. In fact, for all groups (i.e. including control) spontaneous findings were seen, which were without any dose-relationship and/or were known to occur at similar or higher incidences within historical control. One pup of the 500 ppm group was reported to show an anophthalmia and was subjected to further examinations according to Wilson 's method; the bilateral anophthalmia was confirmed.
4.2	**Pathology of parental animals (F0 generation)**	Organ weights: The mean absolute organ weights for the treated F0 animals were not significantly different from those of the control animals. The relative kidney and spleen weight of the 2000 ppm group were statistically significantly increased compared to control. In fact, the mean relative kidney weight of the 2000 ppm male and female F0 rats respectively was 5.2% and 6.5% above control value whereas the mean relative spleen weight was 11.3% above control for the F0 females only. Gross pathology: Sacrificed animals: Animals of all groups which were sacrificed at the end of the experiment showed gross lesions, e.g. in the liver (focal constriction), the kidneys (cyst or pelvic dilatation), the testes (reduced size) or the ovaries (cyst). These lesions however occurred isolated and there was to indication of dose-effect relationship. Necropsy of a control female that died on day 76 of treatment: gross pathology revealed a series of lesions including among other presence of a mass in the spleen, enlarged lymph nodes, enlarged adrenal glands, acinar pattern in the liver and foci in the lungs. Histopathology revealed a malignant lymphoma. Necropsy of females which were not pregnant and of the corresponding mating partners: Two control females, one female of the 100 ppm group and one female of the 500 ppm did not become pregnant. Excepted for an area of diffuse sparse hair reported for the 100 ppm female, gross pathology of these females revealed no abnormalities. Excepted for a reduction in size of the testes and epididymides of one control male, gross pathology of the corresponding males revealed no abnormalities.

4.3 Histopathology of parental animals (F0 generation)

Most gross lesions described above could be confirmed/supplemented histopathologically. Moreover, histopathology revealed no adverse treatment-related effects. Considering the reproductive organs of the males and females, which were sacrificed at study termination, only incidental findings were reported, including e.g. dilated uterus horn (4 control females, one 100 ppm female, one 500 ppm female and seven 2000 ppm females), focal tubular atrophy in the left testicle (one male of the 2000 ppm group) and focal epithelial vacuolization in the left epididymis (2 control and one 2000 ppm males). Moreover, no histopathological effects were reported for the vagina, the cervix uteri and the oviducts of the females, and the seminal vesicles and coagulating glands of the males.

DOFC:

As no significant differences were seen in the F1 parental females, no differential ovarian follicle count was needed for the control and the 2000 ppm F0 females. The differential ovarian follicle count performed on the four females, which did not become pregnant, resulted in following values:

F0 female	DOFC				
	Primordial follicles (PF)	Growing follicles (GF)	PF + GF	Antral follicles	Corpora lutea
Female 1 of the control group	88	28	116	4	21
Female 2 of the control group	96	35	131	5	16
Female 1 of the 100 ppm group	176	48	224	6	20
Female 1 of the 500 ppm group	173	33	206	8	15

These values indicate that there was no treatment-related adverse effect on the follicle incidence and distribution.

4.4	**Pathology of parental animals (F1 generation)**	Organ weights: The mean terminal body weight of the 2000 ppm males was significantly reduced to about 8.3% below control value. Following organs showed changes in mean absolute weights compared to control: the testes of the 2000 ppm males (reduction of 6.8% below control), the prostate of the 2000 ppm males (reduction of 10.3% below control), the brain of the 100 ppm and the 2000 ppm males (respectively increase of 3.7% and 2.5% above control). The mean terminal body weight of the 2000 ppm females also was reduced to about 8.25 below control value. An increase in absolute weight was reported for the ovaries of the 100 ppm females only (10.4% above control); no further significant changes in absolute organ weights for the females were seen. Considering the mean relative organ weights, following changes were reported for the males of the 2000 ppm group: increase in relative kidney weight (8.6% above control), increase in relative spleen weight (10.5% above control), increase in relative brain weight (11.9% above control). For the females of the 2000 ppm group, increases in relative kidney, brain and pituitary weights of respectively 8.9%, 10.6% and 20% above control were reported. Gross pathology: Sacrificed animals: Gross treatment-related lesions were reported for the glandular stomach and consisted of small erosions and ulcers within the mucosa. These lesions occurred with a higher incidence in the females of the 2000 ppm group compared to control and to the remained treated groups (i.e., 17 cases versus respectively 6 cases in the control group, 4 cases in the 100 ppm group and 3 cases in the 500 ppm group). In males, no such increase in incidence of lesions in the glandular stomach was seen (one case in the control group, 0 cases in the 100 ppm group, 3 cases in the 500 ppm group and 4 cases in the 2000 ppm group); therefore and in contrast to the females, no association of these lesions to the treatment could be done for the males. Further gross lesions were reported, which occurred isolated with no indication of dose-effect relationship. Necropsy of females which were not pregnant and of the corresponding mating partners: Two females of the 100 ppm group and one female of the 2000 ppm did not become pregnant. Gross pathology of the two 100 ppm females revealed no abnormalities. In contrast, gross pathology of the 2000 ppm female revealed black erosion/ulcer in the mucosa of the glandular stomach. Gross pathology of the corresponding males revealed no abnormalities.

4.5 Histopathology of parental animals (F1 generation)

Most gross lesions could be confirmed histopathologically. In some cases however, no such confirmation could be reached. This for example was true for the erosion/ulcer in the mucosa of the glandular stomach in following animals: one male and one female of the control group, one 100 ppm female, one 500 ppm female, one 2000 ppm male and two 2000 ppm females. For these animals, no reasonable histological confirmation of the lesions in the glandular stomach was obtained. Considering the reproductive organs, only incidental findings were reported for both males and females, including e.g. dilated uterus horn (9 control females, one 100 ppm female and 11 females of the 2000 ppm group) and focal/diffuse tubular atrophy in the left testicle (one control male, one 2000 ppm male). No histopathological effects were seen in the vagina, the cervix uteri and the oviducts of the females, and the seminal vesicles and coagulating glands of the males.

Treatment-related findings in the mucosa/submucosa of the glandular stomach: In the females, focal erosions in the mucosa were reported for 5 controls, two 100 ppm females, two 500 ppm females and 14 females of the 2000 ppm group. Slight to moderate inflammatory edema in the submucosa was reported for 3 control females, 3 females of the 100 ppm group, 2 females of the 500 ppm group and 10 females of the 2000 ppm group. Focal erosions were not always associated with inflammatory edema and vice versa. The number of females displaying either focal erosion or inflammatory edema or both together was: 5 control females, 3 females of the 100 ppm group, 2 females of the 500 ppm group and 15 females of the 2000 ppm group.

DOFC: The DOFC was performed on all parental F1 females of each of the control and the 2000 ppm group, and resulted in following values: X

F1 parental females (N=27)	DOFC (mean values)				
	Primordial follicles (PF)	Growing follicles (GF)	PF + GF	Antral follicles	Corpora lutea
Control group	157	35	192	4.7	15.7
2000 ppm group	175	34	209	4.3	15.4

These values indicate that there was no treatment-related adverse effect on the follicle incidence and distribution.

Considering the 3 females that did not become pregnant (2 of the 100 ppm and one of the 2000 ppm group), The differential ovarian follicle count resulted in following values:

F1 female	DOFC				
	Primordial follicles (PF)	Growing follicles (GF)	PF + GF	Antral follicles	Corpora lutea
Female 1 of the 100 ppm group	83	37	120	5	29
Female 2 of the 100 ppm group	171	36	207	5	31
Female 1 of the 2000 ppm group	181	50	231	2	16

These values indicate that there was no treatment-related adverse effect on the follicle incidence and distribution.

4.6	**Other**	The stability of the test substance in water up to 14 days at room temperature was proven. Since the test substance preparations were stored less than 7 days, and no longer than 24 hrs in a drinking water bowl, stability was assured. The concentration control analyses confirmed the nominal concentrations (recovery: 94.4-102%). The food analysis revealed that food was suitable, with a number of microorganisms that did not exceed 10^5/g food. Water was also found to be suitable according to the considered guideline.	X

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The aim of the present study was to look for the effects of glutaraldehyde on the reproduction of Wistar rats, which were treated continuously with the test substance in drinking water within a two-generation study. Test substance: [redacted]% Glutaraldehyde), batch No: [redacted]% (analysis performed by [redacted]), stability in drinking water over a period of 14 days at room temperature confirmed. The study was conducted according to OECD Guideline 416 (1983), with GLP. [redacted] was administered to groups of 27 male and 27 female Wistar rats (F0 parental generation) in the drinking water at concentrations of 0, 100, 500 and 2000 ppm. The age of the F0 Generation animals was 36 +/- 1 day. At least 70 days after the beginning of treatment, the animals were paired to produce the F1 litter. Rats of the F1 litter which were selected to create the F1 parental generation (27 males and 27 females per group) received treatment similar to that of their own parents from weaning until production of the F2 generation. At the end of the experimental period, the F1 adult animals and the F2 weanlings were sacrificed. Parents and pups were examined for mortality and clinical symptoms of toxicity. Parents were checked for their mating and reproductive performances. Water and food consumption was determined regularly over the premating period, and for the females over the gestation and over the lactation period; body weight also was determined regularly The parental males (F0 and F1) were examined for a series of reproductive parameters such as the mating and the fertility indices; sperm parameters (e.g. sperm motility and morphology) also were considered. The parental F0 and F1 females were examined for their estrus cycle and a series of reproductive and delivery parameters (e.g. mating and fertility indices, gestation period). The F1 and F2 pups were checked for viability, sex ratio, body weight and symptoms of toxicity; at necropsy, organ weights, pathology and sexual maturation (F1) were considered. The parental F0 and F1 animals were subjected to gross pathology and extensive histopathological examination with special attention to the organs of the reproductive system; terminal and organ weights also were assessed. For the females, a differential ovarian follicle count (DOFC) was done. The test substance was analyzed for purity, homogeneity and stability. Test substance preparations were examined for stability and homogeneity. Concentrations were verified during the study period.	

5.2 Results and discussion

Test substance intake:

Animals	Mean intake of Protectol GDA (mg/kg bw/day)		
	100 ppm	500 ppm	2000 ppm
F0 males	9.4	44.8	152
F0 females	12.1	57.4	191.2
F0 females, gestation period	12	53.9	167.3
F0 females, lactation period	17.1	84.5	286.6
F1 males	12.8	60.4	213.1
F1 females	15.0	69.7	239.1
F1 females, gestation period	13.4	56.4	188.8
F1 females, lactation period	18.6	92.9	304.7

Following treatment-related effects were reported:

Systemic toxicity:

Treatment-related effects indicative of systemic toxicity were seen at 2000 ppm and affected parental males and females of the F0 and the F1 generation. These effects consisted of decreased food and water consumption, decreased body weights and impaired body weight gain and were observed within the premating period (both sexes) and during the gestation and lactation phase (females). From a pathological point of view, the 2000 ppm F1 females showed treatment-related lesions in the glandular stomach. At 500 ppm, a slight decrease in water consumption was observed, which was neither accompanied by a decrease in food consumption nor by changes affecting the body weights of the animals. Therefore, the slightly reduced water consumption here was not considered to be relevant/treatment-related. No signs of systemic toxicity were seen at 100 ppm.

Reproductive performance and fertility:

All considered reproductive parameters (e.g. mating and fertility indices, estrus cycle, sperm parameters, sexual organ weight and sexual organ pathology) were inconspicuous for all tested concentrations of [redacted], indicating no treatment-related effect of the tested substance on reproductive performance and fertility.

Developmental toxicity:

The F1 and F2 pups resulting from the 2000 ppm group showed symptoms indicative of developmental toxicity including reduction in body weight and impairments in body weight changes as well as decreases in organ weights, the latter being seen as direct consequence of the delays in pup body weight gain. No such effects were reported for the F1 and F2 pups of the 100 and the 500 ppm groups.

5.3 Conclusion

5.3.1 Remark

Taking into account the mean intake of test substance (i.e. [redacted] containing [redacted]% glutaraldehyde) in parental males and females of the F0 and the F1 generation during premating, the mean doses of test substance administered were calculated to be as follows:

Nominal concentrations	Corresponding mean dose of [redacted]
100 ppm	Approxim. 12 mg/kg bw/day
500 ppm	Approxim. 58 mg/kg bw/day
2000 ppm	Approxim. 199 mg/kg bw/day

5.3.2	Reproductive performance and fertility, Parent F0 and F1 (males and females), NO(A)EL	2000 ppm
5.3.3	Systemic toxicity, Parent F0 and F1 (males and females)	
5.3.3.1	LO(A)EL	2000 ppm
5.3.3.2	NO(A)EL	500 ppm
5.3.3.3	NOEL	100 ppm
5.3.4	Developmental toxicity, F1 and F2 pups, NO(A)EL	500 ppm
5.3.5	Reliability	**1**
5.3.6	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 4th, 2010
Materials and Methods	3.1.2 This refers to Doc IIIA Section A2.

Results and discussion	4.1.4 F0 parent females. • Food consumption was somewhat affected during the first 3 weeks of premating and during gestation and lactation: during all these periods, the high dose group females had the smallest food consumption. For the mid-dose group, the only difference could be seen during the first 2-3 weeks of premating where food consumption was marginally reduced. • During lactation: one high dose dam did not nurse the pups properly and the pups died. 4.1.5 Oestrus cycle. There was a possibility of dose related increase in the mean F0 oestrus cycle lengths (5.3, 5.6, 5.5, 6.3) without statistical significance. 4.1.6 Female reproduction and delivery data. • For clarity, a correction of a spelling mistake on lines 2-4: "*The mean duration of the sperm detection period was about 2.3 and 3 days and showed **no** ~~to~~ treatment-relationship*". • The mean number of implantation sites correlated with dose (259, 280, 301, 302). • Correction to latter paragraph (middle): "*The mean number of **F1** ~~F2~~ pups delivered per dam was quite similar for all groups, ranging from **10.2** ~~10.8~~ to **11.2** ~~11.6~~.*" It is not clear where the erroneous numbers are taken from, as the figures in the original text do not correspond with either F1 or F2 pups. • Numbers of stillborn pups are given incorrectly. The correct numbers are 3, 3, 0, 5 (F1 pups) and 7, 0, 3, 1 (F2 pups). 4.1.13 F1 females. Food consumption was reduced not only in the high dose group, but also in the mid-dose group especially on day 20 p.c. 4.1.15 Female reproduction and delivery data. • The numbers of implantation sites were apparently dose related (285, 276, 256, 261). • The number of stillborn pups is given incorrectly. The correct numbers are 7, 0, 3, 1. • The gestation index of 85 % (and the four F1 females that did not deliver) concerns the 500 ppm group, while it was 100 % for the other groups (including the high dose group). 4.4 Pathology of parental animals (F1 generation). The RMS concludes that the frequencies of erosions/ulcers in the glandular stomach of the high dose group (♀ 6, 4, 3, 17 and ♂ 1, 0, 3, 4) are treatment related. 4.5 Histopathology of parental animals (F1 generation). The high dose group male with diffuse tubular atrophy in testicle had a concurrent aspermia in the left epididymis, and had no cells of spermatogenesis. This animal was however not the mating partner of a non-pregnant female. 4.6 Other. The recovery range in the control analyses was 92.4 – 102 %.
Conclusion	There was no evidence of effects on any reproductive parameters at doses below maternal toxicity.
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*

Section A6.8.2 _ 01 Annex Point IIA6.8.2	Multigeneration Reproduction Toxicity Study Two-generation reproduction toxicity study, Wistar Rat
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 A6.12.1 _ 01
Annex Point VI.6.9.1

Medical surveillance data on manufacturing plant personnel

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (2007) Monitoring of manufacturing plant personnel. [redacted] (Unpublished), BPD ID A6.12.1_01

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	Glutaraldehyde [redacted]%
3.2	**Persons exposed**	
3.2.1	Sex	Not stated
3.2.2	Age/weight	Not stated
3.2.3	Known Diseases	Not stated
3.2.4	Number of persons	Not stated
3.2.5	Other information	None
3.3	**Exposure**	
3.3.1	Reason of exposure	Occupational
3.3.2	Frequency of exposure	Not stated
3.3.3	Overall time period of exposure	15 years
3.3.4	Duration of single exposure	Not stated
3.3.5	Exposure concentration/dose	Measurements of glutaraldehyde concentrations at the workplace are conducted regularly. The obtained values did not exceed the threshold limit value of 0.21 mg/m^3 of glutaraldehyde stated by the DFG (Deutsche Forschnungsgemeinschaft, MAK- und BAT-Werte-Liste 2006).
3.3.6	Other information	None
3.4	**Examinations**	Cases of adverse health effects due to exposure to glutaraldehyde
3.5	**Treatment**	None
3.6	**Remarks**	None

4 RESULTS

4.1	**Clinical Signs**	None
4.2	**Results of examinations**	No cases of adverse health effects related to glutaraldehyde exposure were reported within a period of 15 years.

Section A6.12.1 _ 01 Annex Point VI.6.9.1	Medical surveillance data on manufacturing plant personnel

4.3	**Effectivity of medical treatment**	Not relevant
4.4	**Outcome**	Not relevant
4.5	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Report of registered cases within a period of 15 years with occupational exposure in production plant workers. A validated method in accordance with NIOSH method No. 2532 with personal air sampling which makes use of a silica gel cartridge coated with 2,4,-dinitrophenylhydrazine was applied. Following desorption, glutaraldehyde was quantitatively identified by HPLC with a relative limit of detection of 0.0023 mg/m^3 (0.0006 ppm).
5.2	**Results and discussion**	No cases of adverse health effects related to glutaraldehyde exposure were reported within a period of 15 years. This is in accordance with regular exposure measurements that have been conducted over the past 20 years which show that glutaraldehyde concentrations in the air did not exceed the threshold limit value of 0.21 mg/m^3 (0.05 ppm) suggested by the MAK-comission (Deutsche Forschnungsgemeinschaft, MAK- und BAT-Werte-Liste 2006). The spectrum of workplaces where air samples were drawn covers production, filling, sampling, the laboratory for analytics, as well as production sites for subsequent processing of glutaraldehyde. Representative data of the past five years (24 Mar 2002 - 21 Nov 2006) revealed that in 28 of 57 measurements (8-hour shift median values), the concentration of glutaraldehyde in the air was below the limit of quantification. In a further 17 cases, the concentration of glutaraldehyde was below the limit of detection. In 12 cases, concentrations of glutaraldehyde were between < 0.0023 mg/m^3 – 0.0085 mg/m^3 (< 0.0006 ppm – 0.0021 ppm) with a median of 0.008 mg/m^3 (0.002 ppm). An additional five measurements are available for the production site. The highest concentration of glutaraldehyde in the air was determined as 0.032 mg/m^3 (0.008 ppm).
5.3	**Conclusion**	The highest concentration measured at the manufacturing plant, namely 0.032 mg/m^3 (0.008 ppm), is a factor of 6 below the threshold limit value suggested by the DFG. In addition, this value is a factor of 12 below the official German threshold limit value of 0.42 mg/m^3 (0.1 ppm) which was in force until the end of 2005. It seems plausible that the reason for the absence of reports on adverse effects caused by glutaraldehyde over a period of 15 years is due to this high margin of safety. Because the threshold limit value was observed at all times, German legislation (GefStoffV) does not require medical surveillance on plant personnel.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	February 14th, 2011
Materials and Methods	Agree with applicant's version.

Section A6.12.1 _ 01 **Annex Point VI.6.9.1**	**Medical surveillance data on manufacturing plant personnel**
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Remarks	The information is given in a statement document and cannot be verified by the RMS.
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*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*
Remarks	

Section A6.12.2 _ 01
Annex Point VI.6.9.2

Case report, endoscopy nurse developing symptoms suggestive of occupational asthma

Official use only

1 REFERENCE

1.1	**Reference**	Stenton SC, Beach JR, Dennis JH, Keaney NP, Hendrick DJ (1994) Glutaraldehyde, asthma and work - cautionary tale. Occup. Med. 44: 95-98 (Published), BPD ID A6.12.2_01

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	Glutaraldehyde (not further specified)
3.2	**Persons exposed**	
3.2.1	Sex	Female
3.2.2	Age/weight	46 years
3.2.3	Known Diseases	Past history of pulmonary embolism
3.2.4	Number of persons	1
3.2.5	Other information	None
3.3	**Exposure**	Inhalation
3.3.1	Reason of exposure	Occupational
3.3.2	Frequency of exposure	Recurrent.
3.3.3	Overall time period of exposure	About seven years.
3.3.4	Duration of single exposure	No data
3.3.5	Exposure concentration/dose	No data
3.3.6	Other information	None
3.4	**Examinations**	Series of double-blind inhalation challenges with activated glutaraldehyde vapour at exposure levels of 0.01 – 0.32 ppm
3.5	**Treatment**	Beclomethasone and terbutaline.
3.6	**Remarks**	None

4 RESULTS

4.1	**Clinical Signs**	Symptoms of breathlessness, wheeze, chest tightness and cough at the workplace; the 0.032 ppm and the first 0.32 challenges were accompanied by asthmatic symptoms.
4.2	**Results of examinations**	Double-blind inhalation challenges with carefully controlled exposures to glutaraldehyde (up to 0.32 ppm for 10 min) gave rise to no obvious asthmatic reactions.

Section A6.12.2 _ 01 Annex Point VI.6.9.2		Case report, endoscopy nurse developing symptoms suggestive of occupational asthma
4.3	Effectivity of medical treatment	Not stated
4.4	Outcome	Recovered
4.5	Other	None
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	Materials and methods	Case report of a 46-year-old endoscopy nurse developing symptoms suggestive of occupational asthma after seven years of exposure to glutaraldehyde. Inhalation challenges with activated glutaraldehyde vapour at exposure levels of 0.01 – 0.32 ppm were performed.
5.2	Results and discussion	An initial inhalation challenge test at the endoscopy suite caused a very dramatic immediate fall in FEV1 (1-s forced expiratory volume) from 3.6 to 1.5 litres. A series of double-blind inhalation challenges with activated glutaraldehyde vapour at exposure levels of 0.01-0.32 ppm. The results suggested marked dual asthmatic reactions following challenges with 0.032 ppm glutaraldehyde; however, similar 'reactions' were observed on control days. These were associated with an increase in airway responsiveness to methacholine, with the dose causing a 20 % fall in the 1-s forced expiratory volume (PD20FEV1) falling from > 6400 micrograms to 135 micrograms. The interpretation of these results was potentially confounded by an intercurrent respiratory tract infection and by technically poor FEV1 recordings, so the challenge series was repeated three weeks later. The second series of double-blind inhalation challenges with carefully controlled exposures to glutaraldehyde (up to 0.32 ppm for 10 min) gave rise to no obvious asthmatic reactions, in marked contrast to the results of the unblinded workplace challenge. There was a slight increase in airway responsiveness, with the PD20FEV1 falling from > 6400 micrograms to 1850 micrograms.
5.3	Conclusion	These results illustrate the potential for misdiagnosis of occupational asthma when unblinded challenge tests are used.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 8th, 2011

Section A6.12.2 _ 01 **Annex Point VI.6.9.2**	**Case report, endoscopy nurse developing symptoms suggestive of occupational asthma**
Materials and Methods	Agree with applicant's version.
Results and discussion	Agree with applicant's version.
Conclusion	The initial assumption of glutaraldehyde clearly being the cause of occupational asthma was questioned. Glutaraldehyde was not excluded from being the causative agent, but it was shown that there is no clear evidence of it. This is a single case study that demonstrates the possible inaccuracy of the initial diagnosis.
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*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*
Remarks	

Section A6.12.2 _ 02
Annex Point VI.6.9.2

Case report, upper respiratory tract irritation, skin rash and recurrent epistaxis in a hospital employee

Official use only

1 REFERENCE

1.1	**Reference**	Wiggins P, McCurdy SA, Zeidenberg W (1989) Epistaxis due to glutaraldehyde exposure. J. Occup. Med. 31: 854 -856 (Published), BPD ID A6.12.2_02

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	Glutaraldehyde (not further specified)
3.2	**Persons exposed**	1
3.2.1	Sex	Female
3.2.2	Age/weight	38 years
3.2.3	Known Diseases	None
3.2.4	Number of persons	1
3.2.5	Other information	None
3.3	**Exposure**	Inhalation and dermal.
3.3.1	Reason of exposure	Occupational
3.3.2	Frequency of exposure	Recurrent
3.3.3	Overall time period of exposure	About 4 months
3.3.4	Duration of single exposure	No data
3.3.5	Exposure concentration/dose	Not stated
3.3.6	Other information	None
3.4	**Examinations**	Physical examination, clinical chemistry tests.
3.5	**Treatment**	No data
3.6	**Remarks**	None

4 RESULTS

4.1	**Clinical Signs**	Upper respiratory tract irritation, skin rash and recurrent epistaxis.
4.2	**Results of examinations**	Physical examination and clinical laboratory tests normal at the time of the clinic visit.
4.3	**Effectivity of medical treatment**	Not stated
4.4	**Outcome**	Recovered
4.5	**Other**	None