EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

Glyoxal

Adopted by the SCCP
during the 4th plenary of 21 June 2005

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1. BACKGROUND

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) stated in its opinion of 25 September 2001 that substances classified pursuant to Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances as carcinogenic (except substances only carcinogenic by inhalation), mutagenic or toxic for reproduction, of category 1 or 2, and substances with similar potential, must not be intentionally added to cosmetic products, and that substances classified pursuant to Directive 67/548/EEC as carcinogenic, mutagenic or toxic for reproduction, of category 3, and substances with similar potential, must not be intentionally added to cosmetic products unless it can be demonstrated that their levels do not pose a threat to the health of the consumer.

Council Directive 2003/15/EEC amended Directive 76/768/EEC introducing Article 4b. It states that "*the use in cosmetic products of substances classified as carcinogenic, mutagenic or toxic for reproduction, of category 1, 2 and 3, under Annex I to Directive 67/548/EEC shall be prohibited. To that end the Commission shall adopt the necessary measures in accordance with the procedure referred to in Article 10(2). A substance classified in category 3 may be used in cosmetics if the substance has been evaluated by the SCCNFP and found acceptable for use in cosmetic products*."

Glyoxal is classified as a category 3 mutagen. The substance is not regulated in an annex to the Cosmetics Directive nor has it been evaluated by the SCCNFP before.

The European Commission received a submission from the European Federation for Cosmetic Ingredients with data indicating that a content of up to 100 ppm Glyoxal in a finished cosmetic product is safe.

2. TERMS OF REFERENCE

The SCCP is requested to answer the following questions:

1. *On the basis of provided data the SCCP is asked to assess the risk to consumers when Glyoxal is present up to 100 ppm in cosmetic products.*

2. *Does the SCCP recommend any further restrictions with regard to its presence in cosmetic products?*

3. OPINION

The present Opinion is primarily based on materials submitted by The European Federation for Cosmetic Ingredients (referred to by numbers)(Ref.: 1) and the IPCS Concise International Assessment Document 57 Glyoxal (Cicads 57: 2004). References are referred to by the name of the authors. References marked with "u" have not been available to the rapporteur.

3.1. Chemical and Physical Specifications

Glyoxal with a different content of active ingredient was investigated in the reported studies. Most of the studies were performed using glyoxal as 40% aqueous solution.

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Glyoxal (INCI name)

3.1.1.2. Chemical names

Ethandial (IUPAC), Biformal, Biformyl, Diformyl, 1,2-Ethandial, Ethanedione, Glyoxalaldehyde, Glyoxal aldehyde, Oxal, Oxalaldehyde, Oxaldehyde, Odix

3.1.1.3. Trade names and abbreviations

Aerotex Glyoxal 40, Daicel GY 60, Glyfix CS 50, Glyoxal 40, Glyoxal P, Gohsezal P, Odix, Parez 802, Permafresh 144, Protectol GL

3.1.1.4. CAS / EINECS number

CAS : 107-22-2
EINECS : 203-474-9

3.1.1.5. Structural formula

Glyoxal can undertake rotational isomerization between the planar *cis* and *trans* conformations, with *trans*-glyoxal being the more stable isomer (Bulat & Toro-Labbé, 2002):

trans-glyoxal *cis*-glyoxal

3.1.1.6.	Empirical formula

Formula : $C_2H_2O_2$

3.1.2.	Physical form

Anhydrous glyoxal is a liquid at ambient temperature; it crystallizes at 15 °C in the form of yellow prismatic crystals.

3.1.3.	Molecular weight

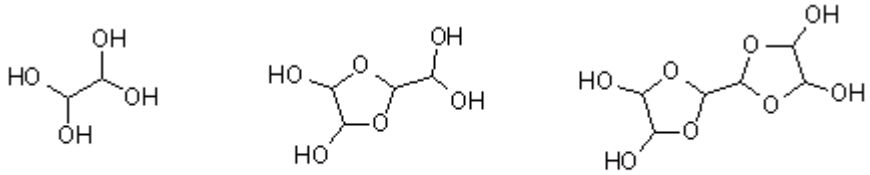
Molecular weight : 58.04

3.1.4.	Purity, composition, and substance codes

Anhydrous Glyoxal can only be produced in the laboratory and does not exist in a stable form. Glyoxal is commonly supplied in the form of aqueous solution at 40% (w/w) (expressed in CHOCHO). Less concentrated forms have been formerly commercialized (essentially at 30% w/w). Very small quantities of an 80% powder are produced (less than 0.1 % of the marketed quantities).

The hydrated monomer (ethane bis-gemdiol) is the main form of glyoxal in aqueous solution. However this gemdiol tends to polymerize to acetals and semiacetals. The polymerisation depends on both the pH and the concentration of the solution. The main oligomeric forms are the dioxolane dimer and the bis(dioxolane) trimer. The equilibrium between monomer and dimer and trimer depends largely on the glyoxal concentration in the aqueous solution:

- in a 5% solution, 39% of glyoxal is present in the monomer form;
- in a 40% solution, the monomer (I) content amounts to as little as 11% of glyoxal, the dimer (II) and trimer (III) forms being dominant.



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Ref.: 1

3.1.5.	Impurities / accompanying contaminants

The nature of the impurities depends on the synthesis route used. If the process used is the oxidation of acetaldehyde with nitric acid diluted in an aqueous medium, the main impurities are the following:

- <200 ppm formaldehyde
- formic acid, acetic acid, glyoxalin acid and glycolic acid - in total approximately 1500 ppm

If the process used is the oxidation of 1,2-ethanediol with oxygen in the presence of water, glyoxal is mainly contaminated with:

- 5000 ppm hydroxyacetaldehyde
- 1500 ppm 1,2-ethanediol
- approximately 1000-2000 ppm organic acids

Former production processes yielded glyoxal with acid contents of up to 2.1% total acids and 1000 ppm of formaldehyde.

Ref.: 1

3.1.6. Solubility

Very soluble in water (600 g/l), miscible in water in all ratios (40% aqueous solution), soluble in ethanol and ethyl ether

3.1.7. Partition coefficient (Log P_{ow})

- 0.85

3.1.8. Additional physical and chemical specifications

Organoleptic properties	:	Colourless, deliquescent powder (pure substance) colourless liquid (40% solution)
Melting point	:	15°C (pure substance), -10°C (40% solution)
Boiling point	:	50.4°C (pure substance), 105°C (40% solution)
Flash point	:	/
Vapour pressure	:	293.3 hPa at 20°C (pure substance), < 10^{-4} kPa (40% solution)
Density	:	1.14 g/cm^3 at 20°C (pure substance), 1.27 g/cm^3 (40% solution)
Viscosity	:	/
pH	:	2.1 – 2.7 (20 °C, 40% solution)
Refractive index	:	/
Conversion factor	:	1mg/m^3 = 0.42 ml/ m^3 (ppm) at 25°C, 1013 hPa 1 ml/m³ (ppm) = 2.37 mg/m³ at 25°C, 1013 hPa
Stability	:	40% aqueous solution of glyoxal is stable at room temperature at least for 6 months, when stored in dark (content of dimer, trimer and other molecules is not described).

3.2. Function and uses

Glyoxal is marketed mainly as a 40% aqueous solution. Glyoxal is used as starting point for the production of a number of other compounds. The dual functionality and the ability of glyoxal to form heterocyclic compounds are used in the production of resins and for cross-linking functionalized macromolecules such as cellulose, polyacrylamides, polyvinyl alcohol, keratin and other polycondensates. With cellulose, unstable hemiacetals are obtained in the cold, which irreversibly form acetals when heated in the presence of acid catalysts.

In Annex I of the Cosmetic Directive several cosmetic products using hydroxyl–ethyl cellulose R-types are listed (e.g., creams, emulsions, lotions, gels and oils for skin, face masks, tinted bases, different powders (make-up, after bath, hygienic), hair-care products (tints, bleaches, cleansing and conditioning products), shaving products, sunbathing, tanning etc.). The maximum glyoxal level is 100 ppm in the cosmetic product. In finished cosmetic products, glyoxal is present only as residual from polymerising reactions.

Ref.: 1

3.3. Toxicological Evaluation

Glyoxal is present in a huge range of different fermented food and beverage products and as a consequence, the main way of exposure of the general population to glyoxal is probably via intake of water and food containing glyoxal.

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline	:	/
Species/strain	:	Rat/Wistar
Group size	:	5 animals per sex and dose
Test substance	:	Glyoxal (40% aqueous solution)
Batch no	:	No data
Dose level	:	2000 and 5000 mg/kg bw (corresponding to pure active ingredient)
Route	:	Oral (gavage)
Exposure	:	Single application
Observation period	:	14 days
GLP	:	Not certified by QUA but carried out in a GLP laboratory

The method is not mentioned in the report but comparable to OECD guideline 402 (fixed dose method)

The test substance was orally administered to male and female Wistar rats. Two groups of each 5 fastened rats per sex received 10 ml/kg bw of an aqueous solution of the test substance at concentrations of 20% and 50% to provide dose levels of 2000 and 5000 mg/kg bw in respect to the pure active ingredient. The animals were observed for treatment-related effects on the day of dosing and for a subsequent 14-day observation period. Mortality, signs of toxicity and body weight were recorded. At study termination gross necropsy was performed on all animals.

Results
None (0/10) of the low dose animals and all (10/10) of the high dose animals died. Deaths occurred within 2 days after dosing. Transient diarrhoea occurred on the day of administration in males and females at 2000 mg/kg bw, while the animals at 5000 mg/kg bw showed several unspecific signs of intoxication until death. The surviving animals gained weight. Necropsy of the animals which died during the course of the study showed signs of irritation of the stomach and discoloured livers with broadened lobes. Those that were sacrificed at the end of the 14 days observation period did not reveal any gross pathologic alterations in the tissues and organs examined.
The acute oral toxicity of glyoxal was >2000 mg/kg bw and <5000 mg/kg bw for male and female Wistar rats in respect to the pure active ingredient, respectively.

Ref.: 26

3.3.1.2. Acute dermal toxicity

Rats

Guideline	:	/
Species/strain	:	Rat/Wistar
Group size	:	5 animals per sex and dose
Test substance	:	Glyoxal (40% aqueous solution)
Batch no	:	Tank B 401
Dose level	:	2000 mg/kg bw (corresponding to 800 mg/kg bw pure active ingredient)
Route	:	Dermal (semi-occlusive)
Exposure	:	24 hours
Observation period	:	14 days
GLP	:	Not certified by QUA but carried out in a GLP laboratory

The method used is not mentioned in the report but comparable to OECD guideline 402 (fixed dose method)

5 male and 5 female Wistar rats were administered glyoxal (40%) to the clipped epidermis (dorsal and dorso-lateral) parts of the trunk under semi-occlusive dressing. After a 24-h exposure period, the dressing was removed and the application site was rinsed with warm water. The animals were observed for 14 days for clinical symptoms and body weight was recorded. Skin findings were scored 30-60 min after removal of the dressing and at least once weekly during the following observation period. At study termination gross necropsy was performed on all animals.

Results
No deaths or clinical signs of toxicity and no pathological findings were seen. Temporary body weight loss was observed during the first few days, but was reversible within 7 days and the animals gained weight. Erythema was noted only for one day after application in both sexes. No abnormalities were detected by gross pathology.

The acute dermal toxicity of glyoxal 40% was >2000 mg/kg bw for male and female Wistar rats (corresponding to >800 mg/kg bw pure active ingredient).

Ref.: 25

Rabbits

Guideline	:	/
Species/strain	:	Rabbit/New Zealand White
Group size	:	5 animals per sex and dose
Test substance	:	Glyoxal as Parez 802 (Ethanedial, no information on content)
Batch no	:	8-528 (yellow liquid)
Dose level	:	2000 mg/kg bw
Route	:	Dermal (occlusive)
Exposure	:	24 hours
Observation period	:	14 days
GLP	:	In compliance

The method used is not mentioned in the report but comparable to OECD guideline 402 (fixed dose method)

Two grams of the undiluted test substance were applied onto the abraded skin of each of 5 male and 5 female New Zealand White rabbits. The test substance was held in contact with the skin for 24 hours under occlusive conditions. Scores according to Draize were performed at 2, 4, 24 and 72 h after application and then daily. Body weights were recorded and surviving animals were sacrificed at the end of a 14-day observation period.

Results
No deaths occurred. Slight erythema and oedema (score = 1) was visible at the 2 h and 4 h observation period. Gross pathology revealed only unspecific signs commonly noted in rabbits.
The acute dermal toxicity of glyoxal was >2000 mg/kg bw for male and female New Zealand White rabbits.

Ref.: 133

3.3.1.3. Acute inhalation toxicity

Guideline	:	OECD guideline 403, EEC Directive 79-831, Annex V
Species/strain	:	Rat/Wistar
Group size	:	5 animals per sex and dose
Test substance	:	Glyoxal 40 N (40.1% aqueous solution)
Batch no	:	No data
Concentration	:	2200, 2600, 2700 mg/m³ (corresponding to pure active ingredient)
Route	:	Inhalation (aerosol)
Exposure	:	4 hours
Observation period	:	14 days
GLP	:	In compliance

Three groups of 5 male and 5 female Wistar rats were exposed to a glyoxal aerosol at analytically verified concentrations of 2200, 2600 and 2700 mg/m³. The exposure period was for 4 hours using a snout-only exposure system. Thereafter, the animals were observed for 14 days. The rats were observed continuously for signs of intoxication during exposure and at least twice daily throughout the observation period and weighed at regular intervals. At the end of the 14 days observation period, all surviving animals were sacrificed and subjected to a detailed macroscopic examination.

Results
Mortalities occurred within 9 days after inhalation. The incidence of mortality was in total 2/10 at 2200 mg/m³, 5/10 at 2600 mg/m³ and 10/10 at 2700 mg/m³. Signs of toxicity were recorded in form of irregular breathing, nasal secretion, partly, closed eyes, ruffled fur, dizziness, lying on the abdomen; macroscopic findings. Necropsy of the animals which died during the course of the study showed dark-red lungs. Those that were sacrificed at the end of the 14 days observation period did not reveal any gross pathologic alterations in the tissues and organs examined.

Under the condition of this study the inhalation LC_{50} for Glyoxal was determined to be 2440 mg/m³ for both sexes, 2470 mg/m³ for male and 2410 mg/m³ for female Wistar rats in respect to the pure active ingredient.

Ref.: 71

3.3.1.4. Other acute toxicity studies

After oral and intraperitoneal administration, the rat, mouse, guinea pig, rabbit and cat showed intoxication symptoms in form of decreased spontaneous activity, apathy, decreased respiratory rate, dyspnoea, ruffled fur, tremor, diarrhoea, disturbances of equilibrium, paresis, reduced or missing reflex reactions (wrighting, pain and corneal reflex), increased startle reflex, abdominal walls and flanks that were drawn in, atony and abnormal posture (crouching posture, high-legged posture) as well as lying on the abdomen or side.

Ref.: 11u, 12u, 26

In cats and rabbits, protein in the urine as well as erythrocytes, leukocytes, hyaline and granulated cylinders in sediment were determined after a single oral administration. A reversible increase of the blood urea occurred in the surviving rabbits. The liver function test (bromosulphthalein retention) showed no findings.

Cited in ref.: 14, 45, Cicads 57: 2004

After oral administration, the autopsy of the animals, which died prematurely, and at the end of the observation period, showed an irritation of the gastrointestinal tract (forestomach bleeding and congestion in the gastrointestinal tract), lungs, kidneys and adrenal glands. After intraperitoneal administration, the autopsy revealed a slight irritation at the application site.

Ref.: 11u

The symptoms after inhalation of 40% glyoxal in aerosol form (fog or dust) for the rat included dyspnoea, partly closed eyes, sneezing, blood-coloured lacrimation, piloerection, flanks that were drawn in, lying on the abdomen. The autopsy of the rats, that died within 9 days after terminating the inhalation exposure to 40% glyoxal, showed hyperaemia as well as a foamy secretion in the lung.

Ref.: 66

With the exposure to a glyoxal vapour-saturated atmosphere, only irregular breathing was observed.

Ref.: 69u, 70u

After inhalative uptake of gaseous glyoxal as well as glyoxal aerosol, no substance-related macroscopic organ changes were found during autopsy of the rats that survived at the end of the exposure period and 14-day observation period.

Ref.: 11u, 66

General results

The acute oral toxicity of glyoxal is low, the LD_{50} value for the rat ranged between >2000 to <5000 mg/kg bw in respect to the pure active ingredient. After dermal application, the LD_{50} values for the 40% glyoxal solution were >2000 mg/kg bw in the rat (corresponding to >800 mg/kg bw active ingredient) and for glyoxal of unspecified content >2000 mg/kg bw in the rabbit. After inhalation of a glyoxal aerosol the LC_{50} value was 2440 mg/m^3 for both sexes in respect to the pure active ingredient.

In the other studies, which are also described in Table 3.3.1, the toxicity of glyoxal is moderate and LD_{50} values are of 640 to 8979 mg/kg bw specified for the rat after oral administration. The oral LD_{50} value for the mouse was 4064 mg/kg bw.

After dermal application, the LD_{50} values for the 40% glyoxal solution were 12700 mg/kg bw in the rabbit and > 5000 mg/kg bw in the guinea pig. A LC_{50} value of >1300 mg/m^3 was additionally reported in the rat after 4-h inhalation to 40 % glyoxal. In inhalation hazard tests on rodents, all the treated animals survived 7- or 8-h exposures to 30% or 40% glyoxal (rats and mice, respectively).

Glyoxal 40% has a low to moderate toxicity by oral route and a low toxicity by dermal route and by inhalation. The main effects are gastric irritation and kidney damage after acute oral administration and respiratory tract irritation from aerosol inhalation.

Table 3.3.1 *Acute toxicity of glyoxal after, oral, dermal, inhalation and intraperitoneal administration*

Species, strain	Route	LD_{50}/ LC_{50} (mg/kg bw or mg/m³)** (sex*)	Comment/Effects	Observation Period	Ref.
20% glyoxal					
Rat, Sprague-Dawley	Oral	1680 (males, females)	diarrhoea, weakness; macroscopic findings : hemorrhagic lung, liver, heart ; inflamed gastrointestinal tract	No data	99u
30 % Glyoxal					
Rat, Carworth-Farms	Oral***	7.46 ml/kg bw (according to the authors : 2.200 mg pure active ingredient/kg bw)(males)	at 8 ml/kg macroscopic findings : congestion of lungs, gastrointestinal tract and adrenal glands, patchy livers, pale kidneys	14 days	116u
Mouse	Oral	Ca. 5.0 ml/kg bw	apathy, reeling, dyspnoea	7 days	11u
Rat	Oral	Ca. 4700 mg/kg bw	disturbances of balance, apathy	No data	10u

Species, strain	Route	LD_{50}/ LC_{50} (mg/kg bw or mg/m³)** (sex*)	Comment/Effects	Observation Period	Ref.
Mouse	Oral	Ca. 3300 mg/kg bw	disturbances of balance, apathy	No data	10u
Rabbit	Oral	Ca. 1700 mg/kg bw	lethal within 8 days, proteinuria, erythrocytes and leukocytes in sediment; macroscopic findings: intestinal inflammation, kidney swelling	No data	10u
Cat	Oral	1700 – 3300 mg/kg bw	mortality at 1700 mg/kg bw 1/1, at 3000 mg/kg bw 1/2; protein, erythrocytes and leukocytes found in urine within 5 days; macroscopic findings: gastritis, enteritis, follicular hyperplasia of the spleen, kidney swelling	No data	10u
Rabbit, male	Dermal , (24 h application)	> 20 ml/kg bw	mortality: ¼	14 days	116u
Mouse	i.p.	Ca. 0.75 ml/kg bw	apathy, reeling, dyspnoea	No data	11u
Mouse	i.p.	Ca. 200 (male)		10 days	35u
Mouse	i.p.	7 mmol/kg bw (corresp. to 406 mg/kg bw)(females)		30 days	5u
Rat, albino,	Inhalation *** (8 h)	-	inhalation hazard test : Mortality: 0/6(male/female)	14 days	116u
Rat	Inhalation (8 h)	-	inhalation hazard test at atmosphere saturated 20°; no findings at necropsy	No data	11u
40% Glyoxal					
Rat, Wistar	Oral	3 300 (both sexes) 3 660 in males; 2 960 in females	decreased spontaneous activity, increased respiratory rate, macroscopic findings : reddened stomach mucosa, patchy liver, dark discoloured adrenal glands, increased pulmonary hyperaemia in the deceased animals	14 days	68u
Rat, Wistar	Oral	0.5 - 0.6 ml/kg bw (corresp. to 640-770 mg/kg bw, females)	mortality at 0.5 ml/kg 0/20 ; 0.7 ml/kg 10/10; comatose state, piloerection, chromodacryorrhoea	14 days	117u
Rat, Harlan-Wistar	Oral	3.08 ml/kg bw (corresp. to 3 912 mg/kg bw, males)	-	No data	2u
Rat	Oral	7.07 ml/kg bw (corresp. To 8.98 mg/kg bw, males) 6.16 ml/kg bw (corresp. To 7.82 mg/kg bw, females)	no clinical symptoms ; macroscopic findings : congestion of the abdominal viscera, intestinal haemorrhage	No data	157u
Rat, Wistar	Oral	> 2 000 <5000 (males/females, corresponding to pure active ingredient)	apathy, dyspnoea, poor general condition, tremor; macroscopic findings: redding of the mucosa of the glandular stomach, distended vessels in the deceased animals	14 days	26
Rat, Wistar	Oral	> 5000 (males/females)	ataxia, hypersensitivity to external stimuli	14 days	117u

Species, strain	Route	LD_{50}/ LC_{50} (mg/kg bw or mg/m³)** (sex*)	Comment/Effects	Observation Period	Ref.
Mouse	Oral	Ca. 3.2 ml/kg bw (corresp.to 4064 mg/kg bw	apathy, reeling, dyspnoea	7 days	12u
Rabbit	Oral	Ca. 2.5 ml/kg bw (corresp. to 3175 mg/kg bw)	-	8 days	12u
Rat,Wistar	Dermal (24 h application, semi-occlusive)	> 2000 (males and females, corresp. to > 800 mg/kg bw pure active ingredient)	no macroscopic findings	14 days	25
Rabbit, New Zealand White	Dermal (24 h application, occlusive)	> 2000 (males and females)	no macroscopic findings	14 days	133
Rabbit	Dermal (occlusive)	10 ml/kg bw (corresp. To 12,700 mg/kg bw	skin necroses, congestion and haemorrhage of the lung, congestion of the liver and kidneys	No data	157u
Rat	i.p.	0.49 ml/kg bw (corresp. to 622 mg/kg bw)(females)	-	No data	157u
Mouse	i.p.	Ca 0.5 ml/kg ; bw (corresp. to 635 mg/kg bw)	apathy, reeling, dyspnoea ; slight irritations at the injection site	7 days	12u
Rat, Wistar	Inhalation (aerosol, 4 h)	2 440 (both sexes) 2 470 in males 2 410 in females (all conc. Corresp. to the pure active ingredient)	irregular breathing, nasal secretion, partly, closed eyes, ruffled fur, dizziness, lying on the abdomen; macroscopic findings: dark-red lungs	14 days	71
Rat, Wistar,	Inhalative (7 h)	-	inhalation hazard test with an atmosphere enriched at 20°C; mortality 0/10 (males and females); irregular breathing rate ; no findings at necropsy	14 days	69u
Rat	Inhalative (8 h)	-	inhalation hazard test with an atmosphere enriched at 20°C; mortality 0/10; no findings at necropsy	No data	13u
80% Glyoxal					
Rat, Wistar male, female	Inhalative (dust, 4 h)	> 1300	irregular breathing, irritations; no macroscopic findings	No data	70u
Glyoxal of unknown content					
Rabbit	Dermal	6600	-	No data	37u
Guinea pig	Dermal	5000-10000	-	No data	37u
Rat	i.p.	>100 mg/kg bw	-	No data	37u

i.p. = intraperitoneal application, s.c. = subcutaneous application, bw = body weight, h = hour(s), * = sex, if specified, ** = the values are based on the respectively given glyoxal solutions, *** = 29.2 % glyoxal was used

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline	:	OECD guideline 404
Species/strain	:	Rabbit/White Vienna
Group size	:	3 female animals
Test substance	:	Glyoxal 40%
Batch no	:	No data
Concentration	:	Unchanged test substance
Route	:	Dermal application (semi-occlusive)
Exposure	:	4 hours
Observation period	:	14 days
GLP	:	Not certified by QUA but carried out in a GLP laboratory

0.5 ml of the unchanged test substance was applied for 4 hours onto the intact skin of each of 3 female White Vienna rabbits under semi-occlusive conditions. After the end of the 4-hour application period, the application patch was removed and the application site was washed with water/Lutrol (1:1). The animals were observed for 72 hours; the skin sites were scored at 30-60 minutes after removal of the patch, and at 24, 48 and 72 hours after the beginning of the application.

Results
No mortalities occurred. No signs of irritation were observed and the scores for erythema and oedema were 0 for each animal at each time point.
Under the condition of this study glyoxal was shown to be not irritating to the intact skin of White Vienna rabbits.

Ref.: 22

The acute skin irritation was examined in earlier studies on the shaven back skin of white rabbits in the patch test. Glyoxal was used as a 30% or 40% aqueous solution. The treatment times for the substance-covered cotton patches (ca. 2.5 cm x 2.5 cm; no further details on the applied quantity) were 1, 5, 15 minutes and 20 h. In addition, the rabbit ear was exposed for 20 h. After an application period of 1, 5 and 15 minutes, the treated skin was first washed with undiluted polyethylene glycol 400 and then with a 50% aqueous polyethylene glycol 400 solution. The skin was not washed after the 20-h treatment period. For an application period of 1 and 5 minutes, respectively, no or slight erythema with a yellowing of the skin could be observed 24 h after the exposure depending on the treatment time. With a treatment period of 15 minutes, a mild oedema was also noted. After 8 days, a yellowing and scaling of the skin at the application site was observed. The 20-h exposure caused a slight, in some cases also a strong, erythema and oedema formation. After 8 days, a scaling of the skin, scab formation and a superficial necrosis were observed. The 20-h exposure to the rabbit ear led to erythema and inflammation as well as to minor skin defects 24 h after the application (no further details). After 8 days, scab formation and a slight necrosis were noted. No significant differences could be found between a 30% and 40% aqueous solution of pure glyoxal and a 40% aqueous solution of raw glyoxal. Thus, slight to pronounced irritation could be seen, depending on the application period.

Ref.: 11u, 12u

White adult rabbits (2 kg) received a 40% glyoxal solution onto the shaven back skin (5 cm x 7 cm; no details on the administration period). From the third day a strong reddened inflammation, followed by a necrosis with tissue demarcation were observed. The changes almost completely disappeared 30 days later. The histopathological examination showed severe necrotic skin changes on the 4th day. These changes were less pronounced on the 9th day, and a regeneration of the epidermis was observed on the 18th day (Ito, 1963).

A further old study showed that the application of 10 µl glyoxal (29.2% aqueous solution) to the depilated abdominal skin of the rabbit caused a slight irritation as indicated by minor hyperaemia and an irritation index of 2 on a scale of 10.

Ref.: 116u

Within the framework of an acute dermal toxicity study, the single occlusive administration of 1.57 ml 40% aqueous glyoxal solution (corresponding to 798 mg glyoxal/kg bw) to the shaven skin (dorsal, dorso-lateral) of 5 Wistar rats/sex for an exposure period of 24 h caused erythema in all of the animals.

Ref.: 25

General results
The recent study performed according to OECD guideline 404 showed that glyoxal (40% aqueous solution) caused no irritation after a 4 h exposure patch test on rabbits under semi-occlusive condition. However, in studies carried out under more severe conditions such as exposure up to 24 hours and occlusive conditions, glyoxal showed an irritation potential.

3.3.2.2. Mucous membrane irritation

Guideline	:	OECD guideline 405
Species/strain	:	Rabbit/White Vienna
Group size	:	1 male and 2 females
Test substance	:	Glyoxal 40%
Batch no	:	No data
Concentration	:	Unchanged test substance
Route	:	Instillation in the conjunctival sac of the right eye
Observation period	:	8 days
GLP	:	Not certified by QUA but carried out in a GLP laboratory

0.1 ml of the unchanged test substance (40% glyoxal solution) was instilled into one eye of each of 3 White Vienna rabbits (1 male, 2 females). The test substance was not washed out. Observation period was 8 days and the readings were performed at 1, 24, 48, 72 hours and 8 days. The untreated eye served as control.

Results
Slight to moderate conjunctival erythema and chemosis was observed 1, 24 and 72 h after instillation. Mean scores (OECD grading) were 0.0, 0.0, 1.6, and 0.8 for corneal opacity (max. = 0), iritis (max. = 0), conjunctival redness (max. = 2) and conjunctival swelling (chemosis; max. = 2), respectively. The symptoms completely subsided after 8 days.

Glyoxal 40% was shown to be slightly irritating to the eyes of 3 Vienna White rabbits. However, according to current EU classification requirements, a test substance is considered an eye irritant, if the mean score for conjunctival redness and chemosis is equal to or greater than 2.5 and 2, respectively, in at least two rabbits.

Ref.: 23

In a study conducted according to OECD guideline 405, glyoxal (40%) led to a reversible reddening and chemosis of the conjunctiva within 8 days and thus showed an irritating effect. This confirmed older studies reporting that glyoxal caused irritations and even necrotic changes in the rabbit eye (see details in BUA, 1997).
In earlier studies on the rabbit eye, the instillation of 0.05 ml glyoxal (30% or 40% aqueous solution) into the conjunctival sac caused a slight to strong reddening, mild oedemas, an inflammation as well as a hazy clouding of the cornea depending on the concentration. These changes completely healed within 1 or 2 weeks.

Ref.: 11u, 12u

In a comparative study between pure glyoxal (40% aqueous solution) and raw glyoxal (40% aqueous solution; no data on the impurities) on the rabbit eye, the instillation of 0.05 ml into the conjunctival sac caused a clear reddening and very strong inflammation on the conjunctiva. After installation of pure glyoxal, a temporary, hazy corneal clouding developed, while a milky clouding of the cornea and scarification on the upper eyelid resulted after instillation of raw glyoxal. Slight reddening and inflammation (pure product) and reddening, corneal clouding and scarification (raw product) were still observed 8 days after the administration.

Ref.: Cicads 57:2004

General results
The key study for irritation on mucous membranes was the study performed according to OECD guideline 405 under GLP conditions. Although these results were indicative for some irritative potential, this would not lead to classification. However, other and mostly older studies showed that glyoxal 40% has to be considered as an irritant to the eye.

3.3.3. Skin sensitisation

Animal studies

Maximization (Magnusson and Kligman) Test

Guideline	:	Based on OECD guideline 406 (Maximization test according to Magnusson and Kligman, 1969)
Species/strain	:	Guinea pig/Pirbright white
Group size	:	10 female animals in the control, 20 female animals in the test groups
Test substance	:	Glyoxal 40%
Batch no	:	No data
Route	:	Intradermal induction and two percutaneous challenges
GLP	:	In compliance

Glyoxal 40% was tested for its sensitizing potential in 20 female Pirbright-White guinea pigs in the maximization test according to Magnusson and Kligman.

For induction the animals were intradermally injected with 0.1 ml of a 20% solution into the shoulder region followed by occlusive epicutaneous application of ca. 300 mg of a 40% solution one week after intradermal injection.
The challenge was done by occlusive epicutaneous application of 150 mg of a 10% solution at days 19 and 26 after intradermal induction (1st and 2nd challenge) on the shaven flank.

Results
One guinea pig died after the intradermal induction for unknown reasons. The intradermal induction of the test substance formulation in Freund's adjuvant/distilled water (1:1) caused necrotic skin changes and oedema.

After the first challenge 1/19 animals with a slight erythema and 6/19 animals with distinct erythema (one of which additionally had a slight oedema) were recorded. Thus, a positive response was observed in 7/19 animals in total. After the second challenge, a positive skin response occurred in 11/19 animals in total, where 7/19 animals showed a slight erythema and 4/19 animals had a distinct erythema (one of which additionally had a slight oedema). No skin reactions were observed in the control group at any time point.
Glyoxal was shown to have a sensitizing effect on the skin of guinea pigs in the Maximization test.

Ref.: 24

Buehler Test

Guideline	:	Based on OECD guideline 406(Method according to Buehler, 1965)
Species/strain	:	Guinea pig/Hartley
Group size	:	10 animals in the control (5 males and 5 females), 15 animals in the test groups (8 males and 7 females)
Test substance	:	Glyoxal 40%
Batch no	:	No data
Route	:	Occlusive epicutaneous induction (30%) and challenge (3%)
GLP	:	In compliance

The sensitising property of the test substance was evaluated in a closed-patch repeated insult dermal sensitisation study using male and female albino Hartley guinea pigs. Three groups of 15 animals received induction exposures at concentrations of 1.25%, 5% and 20% (6-hour occluded patch, 3 times per week for a total of 9 exposures). Challenge was performed 2 weeks after the last induction by an occlusive 6-hour patch. Each of the animals of the 3 induction groups received 5 different concentrations of the test substance (0.01%, 0.03%, 0.1%, 0.3%, 1.0%). One week later, re-challenge was performed with each animal receiving 3 different concentrations of the test substance (0.3%, 1.0% and 3%). Positive control group and 2 irritation control groups were included (positive control: 5 animals/sex, induction with 0.5% 1-chloro-2,4-dinitrobenzene (DNCB), challenge with 0.3% DNCB, no re-challenge; irritation control group 1: 5 animals/sex, 0.01%, 0.03%, 0.3% and 1% of the test substance and 0.3% DNCB at challenge; irritation control group 2: 5 animals/sex, 0.3%, 1.0% and 3% of the test substance at re-challenge). Evaluations were made at 24 and 48 h (after challenge and after re-challenge).

Results

One animal in each of the groups induced with the test substance died for unknown reasons. Concentration-dependent primary irritation and cumulative irritation was evident in all groups treated with the test substance. Challenge concentrations of 1% and 3% produced some responses in all groups. Incidence and degree of response showed a trend to dose-response to the concentrations of induction, challenge and re-challenge. All positive control animals exhibited dermal sensitisation.

It was demonstrated that glyoxal exhibited a potential to induce dermal sensitisation in Guinea pigs in the Buehler test under the conditions used.

Ref.: 131

General results
The positive results in the Magnusson and Kligman tests as well as the Buehler test in Guinea pigs revealed that glyoxal can be assessed as a sensitising substance.

Human studies

Maximization test

Guideline	:	Maximization test according to Kligman, 1966
Subjects	:	Human volunteers
Group size	:	24 men
Test substance	:	Glyoxal
Batch no	:	No data
Route	:	Induction: occlusive patch. 1.0 ml of 10% solution; challenge: occlusive patch of 2% solution
GLP	:	Not in compliance

Glyoxal was tested in 24 volunteers for its sensitising effect. Since glyoxal was considered irritating to the skin, no pre-treatment with sodium lauryl sulphate (SLS) was performed. The induction phase consisted of five 48-hour occlusive applications of a patch soaked with 1 ml of a 10% solution (no data on vehicle) followed by a rest period of 24 hours. For the challenge procedure one 48-hour occlusive application of a 2% solution (no data on vehicle) was performed.

Results
Slight irritation was observed after the induction procedure. After challenge skin reactions indicative for sensitization was observed in 24/24 volunteers. The skin reactions were graded as 5 on a scale ranging from 1 – 5 and were classified as extreme.
Glyoxal showed a sensitising potential in human volunteers in the Maximization procedure.

Ref.: 84u

Repeated insult patch test (RIPT)

Guideline	:	Repeated insult patch test (according to Shelanski and Shelanski, 1953)
Subjects	:	Human volunteers
Group size	:	24 men, 31 women
Test substance	:	Glyoxal
Batch no	:	No data (white powder)
Route	:	Patch with neat material and occlusive epicutaneous application for induction and challenge
GLP	:	Not in compliance

The sensitising activity of the test substance was evaluated with 55 volunteers (24 men, 31 women). The solid test substance was applied under occlusive conditions. For induction, the subjects were applied 15 patches for 24-hour separated by 24-hour rest periods, 3 times per week for a total of 15 applications. The challenge patch was applied after a 14-day period without any contact to the test substance for 24 hours under occlusive conditions. Application sites were examined immediately after removal of the patch and were re-examined 24 and 48 h later for delayed reaction.

Results
No signs of sensitisation, fatiguing, or primary irritation was observed in any of the 55 subjects. Under the chosen test conditions of the repeated insult patch test glyoxal exhibited no sensitizing potential in human volunteers.

Ref.: 143

Of 14 workers who had contact with 40% glyoxal, 9 exhibited a contact dermatitis with localizations mainly on the lower arms and the fingers. Patch tests with a 20% glyoxal solution produced a positive reaction in 7 of 9 workers. The glucose tolerance test was negative for all 14 persons (Ito, 1963).

Overall results
Glyoxal exhibits a sensitising potential.

3.3.4. Dermal / percutaneous absorption

There are limited qualitative and no quantitative data on the absorption and distribution of glyoxal in humans and experimental animals. Acute and subacute inhalation exposure resulted in local effects on eyes and respiratory organs, the extent of systemic absorption being unclear. After acute and chronic oral administration, there is evidence of systemic absorption, with distribution to erythrocytes, liver, lung, kidney, pancreas, and adrenal glands (BUA, 1997, Ueno et al., 1991a). There is some qualitative evidence that glyoxal is absorbed after dermal exposure. Granular and vacuole degeneration in liver, kidney, and pancreas have been observed along with a distinct increase in blood glucose levels following dermal application (Ito, 1963). Further, data on skin sensitization provide supportive qualitative evidence that glyoxal is absorbed across the skin.

In normal human urine, a glyoxal concentration of 132 µmol/litre was found by HPLC analysis (Espinosa-Mansilla et al., 1998). However, this could either be produced endogenously or stem from an exogenous source, such as food intake.

There is no percutaneous absorption study *in vitro* available.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

Subacute oral (drinking water) toxicity study in rats

Guideline	:	OECD 407 (1981)
Species/strain	:	Rat/ Crl CD(SD)BR)
Group size	:	6 animals per sex and dose
Test substance	:	Glyoxal 40
Batch no	:	No data
Dose level	:	100, 300, 1000 mg/kg bw (corresponding to 40, 120, 400 mg/kg bw pure active ingredient)
Route	:	Oral (drinking water)
Exposure period	:	28-days
GLP	:	In compliance

The subacute toxicity was investigated in a 28-day study using 6 rats per group and sex. Glyoxal (40% aqueous solution) was administered in doses of 0, 100, 300 and 1000 mg/kg bw via the drinking water. The concentrations in drinking water were adjusted weekly to the body weight and the drinking water consumption. Clinical examinations covering clinical signs, mortality, body weight, food and water consumption and haematological, clinico-chemical and urinalysis were performed in all animals. At termination, all animals were sacrificed and macroscopically examined, organs were weighed and histopathology was performed.

Results
No deaths occurred during the test period. The body weight gain was not retarded in the low dose group, only slightly retarded in the intermediate dose group and was significantly retarded in the high dose group. The reduced body weight gain coincided with decreased food consumption. A dose dependent decrease of the water consumption was noted for the male animals from the lowest dose group and for the female animals from the intermediate dose group. A slight increase of the erythrocyte count in the male rats of the high dose groups was evaluated as secondary effect of the reduced water consumption. The effect on the various organ weights in the high dose group was attributed to the reduced body weight. Moreover, in none of the dose groups a substance-related effect on haematological and biochemical parameters and of the urinary status was seen. During necropsy no substance-related macroscopic findings were recorded and histopathology revealed no findings in any organ at any dose group.
Based on these findings and mainly on the dose related decrease of the water and food consumption and body weight gain, a NOEL of 100 mg/kg bw/day was established for glyoxal 40% (corresponding to about 40 mg/kg bw pure active ingredient).

Ref.: 149

Subacute inhalation toxicity study in rats

Guideline	:	OECD 412 (1981), Directive 92/69/EEC (1992)
Species/strain	:	Rat/Wistar (SPF)
Group size	:	5 animals per sex and dose
Test substance	:	Glyoxal 40
Batch no	:	No data
Dose level	:	0, 0.4, 2.0 and 10 mg/m³ (nominal concentrations)
Route	:	Inhalation
Exposure period	:	29-days
GLP	:	In compliance

The subacute inhalation toxicity was investigated in groups of 5 male and female Wistar rats each (average initial weight 193 and 171 g, respectively) inhaled nominal concentrations of 0, 0.4, 2.0 and 10 mg/m³ as an aerosol for 6 hours daily, 5 times per week over a period of 29 days (nose only, a total of 20 exposures). Behaviour and state of health were observed daily in all groups. Body weights and food consumption were recorded twice weekly and water consumption once a week. Haematological examinations, clinical chemistry and urinalysis were carried out at the end of the study. The rats were necropsied one day after the last exposure and all animals were investigated by gross pathology. Organs weights were determined and several organs were processed for histopathology and were microscopically examined.

Results
The analytically controlled concentrations amounted to 0.6 (± 0.2), 2.3 (± 0.8) and 8.9 (± 1.9) mg/m³, and the mean aerodynamic mass diameter was 0.8 to 1.2 µm with a mean geometric standard deviation of 1.5 to 1.7. The exposure was tolerated by all groups without any visible signs of toxicity. There were no differences in body weight gain, food and water intake compared to the controls as well as regarding haematological and clinical-chemical findings and results of the urinalysis. The autopsy at the end of the study showed no substance-related differences compared to the controls. Concerning histopathology, the animals of the intermediate and high concentration groups showed a minimal squamous metaplasia of the epiglottal epithelium in the larynx that was accompanied by a minimal submucous lymphoid cell infiltration. No substance-related changes could be noted histopathologically in the rats of the 0.4 mg/m³ group.
The no observed effect level for local effects was 0.4 mg/m³air pure active ingredient. Since no signs of systemic intoxication were recorded the no observed effect level was >10 mg/m³ pure active ingredient.

Ref.: 73

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Subchronic oral toxicity study in rats

Glyoxal (40%) was administered with the diet to 10 Harlan-Wistar rats/group in a 90-day study. Based on the food consumption, doses of 32.7, 63.2, 132 and 253 mg glyoxal (based on the pure ingredient)/kg bw/day were reached for the male animals and doses of 32, 63.2, 127 and 271 mg glyoxal bw/day for the female animals. Data on the stability of glyoxal in the diet are missing. No substance-related deaths occurred. An effect on the food consumption could not be observed. For the male animals of the highest dose group, a significant retardation of the bw gain was

noted only during the first 2 weeks of the study. During the subsequent study period, however, the bw was in the range of the controls. Moreover, a significant increase of the relative liver and kidney weight was determined for the male animals in the highest dose group. The organ weight changes in both intermediate dose groups were not significant. Substance related macroscopic or histopathological organ changes (13 different organs) were not observed in any dose groups. The authors derived a no effect level of about 127/132 mg/kg bw/day for females/males (pure active ingredient). Haematological and clinical-chemical examinations were not performed.

Ref.: 142

In a further study the toxicity after repeated oral administration of glyoxal (purity 98.7%) was studied on male Sprague-Dawley rats (average initial weight 110 to 130 g, age 5 weeks) via the drinking water. 5 animals were used per group. The respective exposure period as well as the attained glyoxal doses are presented in Table 3.3.2

Table 3.3.2*. Study design for testing the subchronic toxicity of glyoxal administered in the drinking water to male rats*

Administration period (days)	**30**	**60**	**90**
Glyoxal concentration (mg/ml)	**Average substance intake (mg/kg bw) corresponding to pure active ingredient**		
2	188	135	107
4	407	239	234
6	451	344	315

Clinical-chemical studies were performed at the end of each administration period. In addition, the activities of glyoxalase I and II, the glutathione content and the formation of 2-thiobarbituric acid reactive substances were measured in the liver, kidney and erythrocytes. The liver, kidneys, spleen, heart, testes and brain were weighed and the liver, kidneys, spleen, stomach, thymus and mesenteric lymph nodes examined histopathologically. For the 90-day exposure period, a dose-dependent and, in the intermediate and high dose group, significant decrease of food and water consumption as well as a corresponding bw retardation were observed. In the low dose group, only the water consumption was significantly reduced. A dose-dependent decrease of the absolute weight of the examined organs, excluding the weights of the testes and brain, was seen in the animals of all dose groups and exposure periods. In the upper dose group, the relative kidney weights after 90 days exceeded those of the controls. In the intermediate and high dose groups, the clinical-chemical examination showed decreased activities of alanine and aspartate aminotransferase as well as lactate dehydrogenase and reduced albumin and total protein values. In the lowest dose group, a decreased alanine aminotransferase activity and a reduced total protein value were determined. Only after a 30-day exposure a significant increase of the activity of glyoxalase I and II was measured in the liver and in the erythrocytes in the animals of the intermediate and high dose groups as well as the glyoxalase I activity in the kidneys in the animals of the high dose group. In contrast, no substance-related effect on the enzymatic activity of glyoxalase I and II was detectable for the longer exposure periods. Neither the glutathione level nor the synthesis of 2-thiobarbituric acid-active substances was affected in the liver, kidney or erythrocytes. Substance-related macroscopic or histopathological organ changes were not found. According to the authors, a no observed adverse effect level could not be determined due to the reduced serum protein levels in the lowest dose group (lowest observed effect level of 107 mg/kg bw pure active ingredient).

Ref.: Ueno et al., 1991a

In another experiment of this research group with approximately the same study design as described above, the male Sprague-Dawley rats (5 to 7 animals/group) obtained glyoxal (100%) in a concentration of 6000 mg/l for 90 or 180 days in the drinking water. In addition to one control group fed the *diet ad libitum*, a control group was led which obtained the same amount of diet as the treated group (pair-fed control group). The daily substance intake in the 90 days group corresponded to that in the study described above. In the 180-day group, it was 298 mg/kg bw/d. The bw retardation after administration of glyoxal for 180 days was greater than that in the pari-fed control group. With the exception of those of the brain and testes, the absolute weights of the weighed organs were below those of the controls. The relative weights of the liver, kidneys and heart were increased compared to the pair-fed control group. Slightly reduced activities of alanine and aspartate aminotransferase as well as lactacte dehydrogenase were determined after 180 days. The total protein content in the serum was significantly below that of both control groups. After 180 days, haemorrhage and polyps in the forestomach were observed macroscopically in 2 of the treated animals which, however, were assessed by the authors not to be treatment-related. A slight swelling of the papillary epithelial cells in the kidneys as well as a papillary interstitial oedema and congestion of the lymph nodes in this area were observed after 90 and 180 days in 4 animals of the glyoxal group. Electron microscopic examinations of the liver and kidneys showed no findings.

Ref.: Ueno et al., 1991a

Fischer 344 rats (10 per dose group and sex) were exposed daily to drinking-water containing 0, 1000, 2000, 4000, 8000 or 16 000 mg glyoxal/litre for 90 days to establish dose ranges for a chronic study. All animals of the highest dose group were sacrificed prematurely on day 12 in a moribund state. Decreased dose-related body and organ weights as well as decreased food and water consumptions were observed at the lowest dosage. For chronic exposure, the maximum tolerated dose for rats was estimated in the range of 500-2000 mg/litre for males as the more sensitive sex (decrease of water consumption up to 28%) and 1000-4000 mg/litre for females (decrease of water consumption up to 46%) (NTP, 1991a).

Subchronic oral (drinking water) toxicity study in mice

In a similar study in B6C3F1 mice (10 per sex per dose group) exposed daily to drinking-water containing the same doses (0, 1000, 2000, 4000, 8000, or 16 000 mg glyoxal/litre for 90 days), all animals survived. The salient features observed were decreased body weight (decrease of 7-30% from 4000 to 16 000 mg/litre) and selected organ weights, decreased food and water consumption, and, in the male mice of all dose groups, possible chemical-related salivary gland changes (secretory depletion of submandibular gland). It was felt that the decreased water consumption (dose-dependently about 10-50%) was due to unsatisfactory palatability of the dosed water, subsequently leading to lower daily dosages and decreased feed consumption (up to 24%). From this preliminary study, recommended doses for further studies with long-term exposure were estimated to be in the range of 500-2000 mg/litre for males as the more sensitive sex (decrease of water consumption up to 12%) and 1000-4000 mg/litre for females (decrease of water consumption up to 27%) (NTP, 1991b).

Subchronic oral toxicity study in dogs

In another 90-day study, glyoxal was administered in the diet to 3 Beagle dogs each per dose and control group (no data on sex). The doses were 31, 65 and 115 mg (based on pure glyoxal/kg bw/day. All the animals survived. No substance-related effect on the body weight as well as on the relative or absolute weight of the liver and kidney was ascertained. A substance-related effect on haematological or clinical-chemical parameters of the blood (haematocrit, erythrocyte and leukocyte count, haemoglobin and urea nitrogen levels, alkaline phosphatase activity, bromosulphthalein retention) did not occur in any dose group. In addition, no substance-related macroscopic or histopathological organ changes were observed (18 different organs). The authors derived a no effect level of ca. 115 mg/kg bw/day pure active ingredient.

Ref.: 149

General results

The key study for repeated oral toxicity is considered the 28 days drinking water study in rats since this study was performed according to a valid and internationally accepted testing guideline as well as under GLP conditions. A no effect level of 100 mg/kg bw was established for glyoxal 40% (i.e. 40 mg/kg bw related to the active ingredient). This value is supported by the published drinking water studies of Ueno et al. (1991a) (Reference: 123) carried out in rats, which obtained a LOEL of 107 mg/kg bw related to pure glyoxal. Moreover, also the 90-day study in dogs resulted in a NOEL of 115 mg/kg bw related to pure glyoxal. In a subacute inhalation study on rats for 29 days, a NOEL of 0.4 mg/m³ was derived for local effects and of > 10 mg/m³ for the systemic toxicity (40% glyoxal). An overview of NOAEL values from repeated dose toxicity studies is given in Table 3.3.3.

Table 3.3.3. *Overview of NOAEL values from repeated dose toxicity studies*

Exposure period Route/Species	**Dosage**	**NOAEL**	**Ref.**
28 days Oral (drinking water) Rat	0, 100, 300, 1000 mg/kg bw (Glyoxal 40%)	100 mg/kg bw (40 mg/kg bw related to active ingredient)	142
30, 60, 90 days Oral (drinking water) Rat	0, 2000, 4000, 6000 mg/l (Glyoxal 100%)	LOAEL 107 mg/kg bw (related to active ingredient)	Ueno et al., 1991a
90 days Oral (drinking water) Rat and mice	0, 1000, 2000, 4000, 8000, 16000 mg/l (Glyoxal, unknown content)	Not derived	45
90 days Oral (diet) Rat	0, 32.7, 63.2, 132, 253 mg kg for males; 0; 32, 63.2, 127, 271 mg for females	127/132 mg/kg bw for females/males (related to active ingredient)	142
90 days Oral (diet) Dog	0, 31, 65, 115 mg/kg bw	115 mg/kg bw (related to active ingredient)	142
29 days Inhalation Rat	0, 0.4, 2.0, 10 mg/m³	NOEL Local effects: 0.4 mg/m³ Systemic effects:>10 mg/m³ (pure active ingredient)	73

3.3.5.3.	Chronic (> 12 months) toxicity

No data submitted

3.3.6.	Mutagenicity / Genotoxicity

In vitro

Bacterial gene mutation assay

Guideline	:	OECD 471 (1997), EEC Directive 2000/32, B.13/B.14 (2000)
Test system	:	Salmonella typhimurium, TA98, TA100, TA 102, TA1535, TA1537
Replicates	:	Triplicate plates, two independent assays
Test substance	:	Protectol GL (about 40% Glyoxal in water)
Batch No.	:	B 61 (produced August 27, 2001)
Concentrations	:	52 – 13,000 µg/plate with and without metabolic activation
GLP	:	In compliance

Protectol GL (about 40% aqueous glyoxal) was tested for mutagenicity in the reverse mutation assay on bacteria both, with and without metabolic activation (S9 mix from the liver of Aroclor 1254 induced male Sprague-Dawley rats) in the standard plate test (SPT). The Salmonella typhimurium strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 were exposed to the test substance (dissolved in water) at concentrations ranging from 52 µg/plate to 13,000 µg/plate (with and without S9 mix) in the first experiment. In the second experiment concentrations ranged from 1,000 to 5000 µg/plate with and without S9 mix. For control purposes solvent (water) and positive controls (without S9 mix: N-methyl-N-nitro-N-nitrosoguanidine for strains TA 98, TA 1535 (5 µg/plate); 4-nitro-o-phenylendiamine for strain TA 98 (10 µg/plate); 9-aminoacridine for strain TA 1537 (100 µg/plate) and mitomycin C for strain TA 102 (0.25 µg/plate); with S9 mix: 2-amino-anthracene for strains TA 98, TA 100, TA 1535 and TA 1537 (2.5 µg/plate) and strain TA 102 (3.0 µg/plate)).

Results
No precipitation was noted. A bacteriotoxic effect was observed depending on the strain and test conditions from about 4,000 – 6,500 µg/plate. A slight increase in the number of revertants was observed in the strains TA 100 and TA 102 both with and without addition of the metabolizing system. The sensitivity and validity of the test system used was demonstrated by the expected induction of a significantly increased number of revertants with the positive controls.
In conclusion, Protectol GL is weakly mutagenic under the experimental conditions reported.
Ref.: 27

Mammalian cell gene mutation assay

Guideline	:	OECD Guideline 476 (1984)
Test system	:	HPRT gene mutation test with V79 cells (Chinese hamster cell line)
Replicates	:	Two independent assays
Test substance	:	Glyoxal 40 N (40% aqueous solution)
Batch No.	:	B40C/25.11.85

Concentrations	:	0.1 – 1.5 mg/plate with and without metabolic activation
GLP	:	In compliance

The potential mutagenic effect of glyoxal 40 N in mammalian cells was examined by assaying the induction of 8-azaguanine resistant mutants in Chinese hamster V79 cells. In a preliminary cytotoxicity assay, the test substance was applied at concentrations between 0.1 – 80.0 mg/plate with and without metabolic activation. Thereafter, two independent main assays were conducted using concentration ranges of 0.1 – 1.5 mg/plate. (with and without S9 mix). S9 mix was obtained from the liver of rats induced with Aroclor 1254. Test substance was dissolved in distilled water, which was also used as solvent control, whereas the positive control substances N-methyl-N-nitroso-N-nitroguanidine (4.4 µg/plate) and dimethylnitrosamine (5 mg/plate) were diluted in DMSO. After expression time of six days, the cells were trypsinized and seeded for cloning efficiency and for mutant cell selection. 24 hours after seeding, 8-azaguanine was added and cells were exposed 10±1 days. Thereafter, the clones were fixed and stained with Giemsa.

Results
In the presence of S9 mix, a dose-dependent increase in mutation rate was observed at concentrations between 0.7 and 1.0 mg/plate. However, this increase did not reach the threshold defined for a positive test. Thus, the test substance was judged to be not mutagenic in this assay. Doses of 1.0 mg/plate and more were cytotoxic in the presence/absence of S9 mix. The sensitivity and validity of the test system used was demonstrated by the expected induction of a significantly increased number of revertants with the positive controls.

In conclusion, glyoxal revealed no mutagenic potential in the V79/HPRT assay under the selected condition.

Ref.: 139

Guideline	:	/
Test system	:	HPRT gene mutation test with CHO cells (Chinese hamster cell line)
Replicates	:	Two independent assays
Test substance	:	Glyoxal 40 LF (40% aqueous solution)
Batch No.	:	No data
Concentrations	:	37.5 – 600 µg/ml with and without metabolic activation
GLP	:	In compliance

The method used was according to O'Neill, J.P. et al. (1977), Mutat., Res., 45, 91-101

A further gene mutation test in mammalian cells with glyoxal was carried out using Chinese hamster ovary cells. The potential mutagenic was examined by assaying the induction of 6-thioguanine resistant mutants. Based on a preliminary cytotoxicity assay, where concentrations between 0.03 – 1,000 µg/ml were investigated with and without metabolic activation, two independent main assays were conducted using concentration ranges of 37.5 – 600 mg/ml (with and without S9 mix, 5 mg/plate). Test substance was dissolved in distilled water, which was also used as solvent control. Ethylmethane sulfonate (200 µg/ml, without S9 mix) and dimethylnitrosamine (100 µg/ml, with metabolic activation) were used as positive control substances. After expression time of 7 days, the cells were trypsinized and seeded for cloning efficiency and for mutant cell selection. After a incubation of 7 days, the colonies were fixed and stained with Giemsa to determine cloning efficiency and mutation frequency.

Results
In the presence of the metabolic activation system a weak increase in the mutation frequency was only observed at the highest concentration of 600 µg/ml. However, this increase was not statistically significant. In contrast, no increase was observed at any concentration without S9 mix. The cytotoxicity test resulted in no cell survival at 1000 µg/ml and at 333 µg/ml, only 26% and 69% relative survival resulted with and without metabolic activation, respectively. The sensitivity and validity of the test system used was demonstrated by the expected induction of a significantly increased number of revertants with the positive controls.
The authors of the study concluded that glyoxal should be considered as suspect mutagen, due to the slight increase at 600 µg/ml.

Ref.: 127, 137, 146

Guidelines	:	OECD 476 (1998), US EPA OPPTS 870.5300 (1998)
Test system	:	HPRT gene mutation test with L5178Y cells (mouse lymphoma cell line)
Replicates	:	Three independent assays
Test Substance	:	Protectol GL (40% Glyoxal in water)
Batch No.	:	Lot 1 and2 (no further information)
Concentrations	:	25-375 µg/ml without and 25-250 µg/ml with metabolic activation
GLP	:	In compliance

Glyoxal was tested for the possible induction of forward mutations in the HPRT test (6-thioguanine resistance) with L5178Y cells using a fluctuation method. A cytotoxicity range-finding assay was performed, followed by three independent experiments conducted in the absence of metabolic activation and two independent assays in the presence of metabolic activation by an Aroclor 1254 induced rat liver post-mitochondrial fraction (S9 mix). All concentrations were expressed in terms of active ingredient.

The cytotoxicity tests comprised 10 concentrations between 3.13 – 1,600 µg/ml. 200 µg/ml was the highest concentration, where cells survived with a relative survival of 41% and 8% in the absence and presence of S9 mix, respectively.

In the mutation assay, cells were incubated in duplicate both +/- S9 for 3 hours. Thereafter, cells were washed and re-suspended for plating and expression growth. For plating for survival the cells were diluted to 8 cells/ml, plated onto 2 x 96 wells and incubated 10 - 11 days. Wells containing visible clones were counted. For expression growth cultures were grown for 7 d and plating was performed as described. Plating for 6-thioguanine (6-TG) resistance included growth in the presence of 6-TG for a period of 14 days and subsequent counting of visible clones.

Negative controls comprised treatment with the solvent purified water diluted in culture medium. The positive controls were treated with 4-nitroquinoline-1-oxide (0.1 and 0.15 mg/l) and benz(a)pyrene (2.0 and 3.0 µg/ml) dissolved in DMSO without and with metabolic activation system, respectively.

Results
Based on the observed cytotoxicity in the range finding assays, eight concentrations were chosen for the first test ranging from 25 – 400 µg/ml with/without S9 mix. However, 400 µg/ml was rejected from analysis due to excessive cytotoxicity. The highest concentration analyzed was 350 µg/ml without S9 mix and 200 µg/ml with S9 mix, showing 17% and 80% relative survival, respectively. In the second test, concentrations between 100 – 400 µg/ml without S9 mix and 25 – 400 µg/ml with S9 mix were investigated. Again due to severe cytotoxicity the highest concentration analyzed was 300 µg/ml without S9 mix and 250 µg/ml with S9 mix, which yielded 12% and 10% relative survival, respectively. A third confirmatory test was performed in the absence of S9 mix only and with concentrations ranging from 100 – 400 µg/ml. Seven days after treatment, all concentrations were selected to determine viability and 6-thioguanine resistance. However, 400 µg/ml was rejected due to severe cytotoxicity and the highest concentration analyzed was 375 µg/ml with a relative survival of 42%. In the presence of S9 mix, no statistically significant increases in the mutant frequency were recorded at any concentration analyzed in test 1 and 2. In the absence of S9 mix, no statistically significant increases in the mutant frequency were observed in the first test at any dose level, but a weak linear trend was recorded. Statistically significant elevations were noted at 250 and 300 µg/ml in test 2 and at 275, 325 and 375 µg/ml in test 3.

Under the conditions of the study, Glyoxal showed some evidence of mutagenic activity at the hprt locus (6-thioguanine resistance) in L5178Y mouse lymphoma cells in the absence of metabolic activation but not in the presence of S9 mix, when investigated up to its limit of cytotoxicity.

Ref.: 21

Chromosome aberration test in cultured Chinese hamster ovary (CHO) cells

Guideline	:	OECD 473 (1997), ICH Tripartite Harmonized Guideline on Genotoxicity, (1995), US EPA OPPTS 870.5375 (1998)
Test system	:	Chinese hamster ovary (CHO) cells
Replicates	:	Duplicate culture in a single experiment
Test substance	:	Protectol GL (about 40% glyoxal in water)
Batch No.	:	B 61 (no further information)
Concentrations	:	25.5 - 580 µg/ml with and without metabolic activation (doses related to active ingredient)
GLP	:	In compliance

Glyoxal was assessed for its potential to induce structural chromosome aberrations in Chinese hamster ovary (CHO) cells in vitro. Glyoxal was tested in the presence and absence of metabolic activation (S9 mix prepared from Aroclor 1254 induced rat liver). The test article was dissolved in purified water for injection. Duplicate cultures of CHO cells were exposed to the test substance for 3 hours at concentrations of 25.5, 190 and 580 µg/ml in the non-activation assay and for 3 hours at concentrations of 190, 371, and 580 µg/ml in the presence of metabolic activation. The highest concentration of 580 µg/ml corresponds to 10 nM of the active ingredient. For both assays, cells were harvested at 20 hours after treatment had commenced. Approximately 2 hours prior to harvest, cultures were exposed to colchicine. Following harvest, cells were fixed on slides, stand and examined for chromosomal aberrations. 100 metaphases from each duplicate culture were analyzed. 4-Nitroquinoline-1-oxide (NQO, 0.0625 – 0.25 µg/ml for the non-activation set) and cyclophosphamide (CPA, 3.15 – 12.5 µg/ml requiring

activation) served as positive control substances. A solvent control (purified water) was also included in the test.

Results
Treatment of glyoxal in the absence of S9 mix resulted in increased number of cells with structural aberrations at the highest concentration of 580 mg/ml, only, while in the presence of S9 mix the increase occurred at 371 and 580 µg/ml. The validity and sensitivity of the test system was shown since the vehicle control led to no findings but the positive control substances (NQO and CPA) led to statistically significantly increases in the proportion of cells with structural aberrations.
Under the conditions of the assay described, glyoxal did induce an increase in structural chromosome aberrations in CHO cells in the presence and absence of metabolic activation system.

Ref.: 18

This result corresponds to results obtained either in V79 cells from Chinese hamster or CHO cells, were glyoxal caused an increased chromosome aberration rate in lung fibroblasts as well as in the ovarian cells.

Ref.: 151; Nishi et al., 1989

UDS test in primary rat hepatocytes in vitro

Guideline	:	/
Test system	:	Primary rat hepatocytes obtained from male F-344 rats
Replicates	:	Triplicate culture in a single experiment
Test substance	:	Glyoxal 40 LF (about 40% glyoxal in water)
Batch No.	:	No information provided
Concentrations	:	0.03 – 3.0 mg/ml without metabolic activation
GLP	:	In compliance

The method was according to Williams GM (1977). Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures, Cancer Res., 37, 1845-1851
The ability of glyoxal to interact with DNA was investigated *in vitro* in primary hepatocyte cultures originated from the liver of an untreated male F-344 rat. A preliminary cytotoxicity test was performed on viable hepatocytes and concentration ranged from 10 – 1000 mg/ml in triplicate cultures. Distilled water served as vehicle and was concurrently evaluated. Due to the deaths of all cells in culture, the cytotoxicity test was repeated and concentrations of 0.03, 0.1, 0.3, 1.0 and 3.0 mg/ml were selected for the main study. For the assay, a rat was perfused, the liver excised and combed to yield a sufficient number of hepatocytes with a viability of 95%. A $5x10^5$ dilution was made for seeding and the cells were treated in triplicates with tritiated thymidine together with 20 µl of the respective glyoxal concentrations, the vehicle distilled water and 2-acetoamidofluorene (2AAF) as positive control in a concentration of $1x10^{-4}$ M. After treatment the cells were incubated for 18 – 20 hours and then swelled and fixed. They were dipped in NTB nuclear track emulsion and stored at approximately 4 °C. Seven days later, the slides were prepared and stained with H&E. Unscheduled DNA synthesis (UDS) was quantified by net nuclear increase of black silver grains for 20 cells/slide.

Results

The autoradiographic analysis of DNA repair in the primary hepatocyte cultures showed a statistically significant increase in net grain counts at each concentration. However, there was no dose-dependency but the validity criteria were fulfilled since each concentration led at least to a five fold increase above the respective control groups. The highest concentration of 3.0 mg/ml was clearly cytotoxic. The sensitivity was demonstrated since 2AAF led to a clear increase in the net grain count.

Under the test condition chosen, Glyoxal led to an increase in unscheduled DNA repair in exposed primary rat hepatocytes.

Ref.: 135

Sister chromatid exchange (SCE) assay in Chinese hamster ovary (CHO) cells

Guideline	:	/
Test system	:	Chinese hamster ovary (CHO) cells
Replicates	:	Duplicate cultures
Test substance	:	Glyoxal 40 LF (about 40% glyoxal in water)
Batch No.	:	/
Concentrations	:	10, 50, 100, 200, 250 mg/l (without S9 mix); 10, 25, 50, 75, 100mg/l (with S9 mix)
GLP	:	In compliance

Glyoxal was examined for effects on sister chromatid exchanges by staining the cells and evaluation of the metaphases. The test was performed with and without metabolic activation (S9 mix from the liver of Aroclor 1254 induced rats). The test substance was dissolved in distilled water. Prior to the main study the cytotoxicity was evaluated in concentrations ranging from 0.33 - 1000 µg/ml. Based on the observed toxicity, concentrations of 10, 50, 200 and 250 µg/ml were selected for the non-activated series and concentrations of 10, 25, 50, 75 and 100 µg/ml for the metabolically activated cultures. Duplicate cultures of CHO cells were exposed to the test substance concentrations for 5 hours. Subsequently, cells were exposed to 5´-bromodeoxyuridine (BrdU) for 27 hours in total. After 25 hours exposure, colcemid was added for an additional 2 hours. 24 hours after end of treatment cells were harvested. The solvent distilled water was used as negative control, while ethylmethane sulphonate (EMS; 10^{-3} M) was used as a positive control without metabolic activation and N-nitrosodimethylamine (DMN; $5x10^{-4}$ M) with metabolic activation. No information is given with respect to analysis of the test substance and test substance/solvent mixture.

Results
A statistically significant increase in SCE/cells was observed at the highest concentration without metabolic activation. A slight increase in SCE frequency was noted with each increase in concentration but only the increase at 200 µg/ml was statistically significant without metabolic activation. Concentrations with S9 mix led also to a significant elevation of SCE's at 50, 75 and 100 µg/ml.
Glyoxal induced a concentration related increase in SCEs in CHO cells with and without metabolic activation.

Ref.: 136

Depending on the dose, glyoxal (40%) in applied concentrations of 1.5 to 12 µl/ml (corresponding to 1.9-15.2 µg/ml) induced mitotic recombinations in *Saccharomyces cerevisiae*

at incubation temperatures of 28°C or with cold-shock treatment. Combined with 14.39 mg propionitrile/ml, glyoxal concentrations of 1.12 to 6.67 µl/ml (corresponding to 1.4 to 8.5 µg/ml) caused, aside from mitotic recombinations, chromosome losses in a small number of colonies.

Ref.: Zimmermann and Mohr, 1992

Glyoxal led to a thymidine-kinase decrease in mouse lymphoma cells and caused an increase of the glycine-adenosinethymidine-prototrophic revertant count in ovarian cells of the Chinese hamster.

Ref.: 160, Wangenheim and Bolcsfoldi, 1988

Glyoxal caused an increase of the sister-chromatid exchange rat in ovarian cells of the Chinese hamster and in human lymphocytes and caused unscheduled DNA synthesis (UDS) in TC-SV40 cells of the Syrian hamster

Ref.: Cornago et al, 1989; Ueno et al., 1991c

Further effects on DNA were observed since glyoxal caused DNA-strand breaks in mouse lymphoma cells as well as in primary rat hepatocytes.

Ref.: Garberg et al., 1988; Ueno et al., 1991c

However, the same working group demonstrated that an induction of cross-links by glyoxal, could not be detected in primary rat hepatocytes but increased endoreduplication was detected in ovarian cells of the Chinese hamster

Ref.: Tucker et al., 1989; Ueno et al., 1991c

Studies on DNA-adduct formation

It was demonstrated using plasmid pCoIIR215 of *Escherichia coli* strain K12 HB101 as well as with guanosine that glyoxal forms stable adducts with guanosine by reaction with the N-1- as well as with the exocyclic nitrogen of guanine. The adduct formation was irreversible at a pH value of 5 to 7.

Ref.: 80, 81, 87

Corresponding results were also found in *in vitro* studies on DNA of the mouse, yeast and calf thymus

Ref: 115

Further mutagenicity/genotoxicity studies were performed and published in the literature. They are cited shortly in the following sections and the most relevant information is provided in the overview table 3.3.4.
For more information please refer to the overview references: 14, 45, 46, Cicads 57,2004.

Table 3.3.4. *Mutagenicity tests in vitro*

Test system/species	Test conditions	Results/ Remarks	Ref.

Test system/species	Test conditions	Results/ Remarks	Ref.
Gene mutation in bacteria			
S. typhimurium TA98, TA100, TA102 TA1535, TA1537	+ S9: 52 – 13,000 µg/plate (S9 mix from the liver of Aroclor 1254 induced male Sprague-Dawley) - S9: 52 – 13,000 µg/plate	Positive in TA100, TA102 Positive in TA100, TA102	27
S. typhimurium TA98, TA100, TA102 TA1535, TA1537	+ S9: 100 – 10,000 µg/plate (S9 mix as above) - S9: 100 – 10,000 µg/plate	Negative Positive in TA100,	132
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538 *Escherichia coli* WP2uvr	+ S9: up to 10,000 µg/plate (S9 mix as above) - S9: up to 10,000 µg/plate (5 different bathes of 40% glyoxal tested)	Positive in TA100, (all) batches), in TA98 and Wp2uvr (some batches) Positive in TA100, (all) batches), in TA98 and Wp2uvr (some batches)	145
S. typhimurium TA98, TA100, TA1535, TA1537	+ S9: 4 – 12,700 µg/plate (S9 mix as above) - S9: 4 – 12,700 µg/plate (3.15 – 100.000 nl/plate, Glyoxal 40%)	Positive in TA 100 and TA1335(marginal) Positive in TA100	16
S. typhimurium TA98, TA100, TA1535, TA1537, TA 1538	+ S9: 33 – 10,000 µg/plate (S9 mix as above) - S9: 33 – 10,000 µg/plate (Glyoxal as white powder solved in DMSO)	Negative Negative	152
S. typhimurium TA98, TA100, TA102 TA1535, TA1537	+ S9: ca. 10 – 500 µg/plate (S9 mix from the liver of phenobarbital induced rats) - S9: ca. 10 – 500 µg/plate	Positive in TA100 Positive in TA100	28
S. typhimurium TA100	+ S9: Up to 500 µg/plate - S9: Up to 500 µg/plate	Positive Positive	44u
S. typhimurium TA100	- S9: ca. 116 – 929 µg/plate (ca. 2 – 16 µmol/plate; 30% Glyoxal)	Positive	34u
S. typhimurium TA98, TA100, TA1535, TA1537, TA 1538	+ S9: 10 – 10,000 µg/plate (S9 mix from the liver of Aroclor 1254 induced rats) - S9: 10 – 10,000 µg/plate	Positive in TA100 Positive in TA100	97u
S. typhimurium TA102, TA2638	- S9: 1,000 µg/plate	Positive in TA102, TA2638	86u
S. typhimurium TA98, TA100, TA1535, TA1537, TA 1538 *Escherichia coli* WP2uvr	+ S9: 4 – 5,000 µg/plate (S9 mix as above) - S9: 4 – 5,000 µg/plate (40% Glyoxal tested)	Positive in TA100, TA1535 Positive in TA100	72, 141, 147
S. typhimurium TA102	+ S9: 4 – 5,000 µg/plate ((S9 mix as above) - S9: 4 – 5,000 µg/plate	Positive Positive	74, 79, 148
S. typhimurium TA7006, TA98	+ S9: 20 - 100 µg/plate - S9: 20 - 100 µg/plate	Positive Positive	93
Escherichia coli W3110 (F-)	+ S9: 0 – 400 µg/plate - S9: : 0 - 400 µg/plate	Positive Positive	94u
S. typhimurium TA100	+ S9: 40 µg/plate (S9 mix from the liver of PCB induced rats) - S9: 40 µg/plate (in total 5 concentrations but only the highest was specified)	Positive Positive	Yamaguchi et al., 1994
S. typhimurium TA104	- S9: up to ca. 100 µg/plate	Positive	89
S. typhimurium TA100, TA104	- S9: 50 –100 µg/plate	Positive in TA100, TA104 (addition of glutathione reduced the effect)	155u

Test system/species	Test conditions	Results/ Remarks	Ref.
S. typhimurium TA100, TA102, TA104	+ S9: 30 - 120 µg/plate (S9 mix from the liver of phenobarbital and 5,6 benzoflavone induced rats) - S9: 30 - 120 µg/plate	Positive in TA100, TA102, TA104 Positive in TA100, TA102, TA104	110
S. typhimurium TA100	-S9: 58 – 325 µg/plate (1 – 5.6 mM)	Positive	118u
S. typhimurium TA100, TA102, TA104	+ S9: 5 – 500 µg/plate (S9 mix from the liver of Aroclor 1254 induced rats) - S9: 5 - 500 µg/plate (99% Glyoxal)	Positive in TA100, TA102, TA104	114
S. typhimurium TA98, TA100, TA102	+ S9: 14.5 - 580 µg/plate (S9 mix as above) - S9: 14.5 - 580 µg/plate (0.25 – 10 µM/plate)	Positive in TA100, TA102 Positive in TA100, TA102	3
S. typhimurium TA98, TA100	+ S9: No data (S9 mix from the liver of KC-500 induced rats) - S9: No data	Weakly positive in TA100 Positive in TA100	109
S. typhimurium TA98, TA100, TA104 *Escherichia coli* WP2uvrA/pKM101	+ S9: No data (no data on the metabolic system) - S9: No data	Positive in TA98, TA100, TA104, WP2uvrA/pKM101 Positive in TA98, TA100, TA104, WP2uvrA/pKM101	82
Further gene mutation tests in bacteria			
L-arabinose-resistance test (ARA test) *S. typhimurium* BA9, BA13	- S9: 238 – 1,190 nmol/ml (corresponding to 13.8 – 69.1 µg/ml)	Positive in BA9, BA13	Ruiz-Rubio et al., 1985
L-arabinose-resistance test (ARA test) *S. typhimurium* BA13	- S9: up to 238 nmol/ml (corresponding to 13.8 µg/ml)	Positive	Ariza et al., 1988
DNA damage in bacteria			
Umu test *S. typhimurium* TA1535/pSK1002	+ S9: 100 µg/plate (S9 mix from the liver of phenobarbital and 5,6 benzoflavone induced rats) - S9: 100 µg/plate	Positive Positive	104u, 105u
SOS chromo test *Escherichia coli* PQ37	- S9: 0.1 – 0.6 mM (corresponding to 5.8 – 34.8 µg/ml)	Positive	von der Hude et al., 1988
Rec assay *Bacillus subtilis* H17 (arg, trp, recE) M45 (arg, trp, recE)	+ S9: 8.18 – 30.3 µg/plate (S9 mix without further details) - S9: 8.18 – 30.3 µg/plate	Positive Positive	Matsui et al., 1989
DNA –repair test *Escherichia coli* K12 343/636 K12 343/591	+ S9: up to 15.9 mmol/l (corresponding to 923 µg/ml) (S9 mix from the liver of Aroclor 1254 induced rats) - S9: : up to 15.9 mmol/l (corresp.to 923 µg/ml)	Positive Positive	Helmer and Bolcsfoldi, 1992a
DNA –repair test Host: male NMRI mice Test system: *Escherichia coli* K12 343/636 K12 343/591 (applied intravenously)	In vivo metabolism: 570 and 1,700 mg/kg bw oral (blood and homogenates of liver, lung, kidneys, and testes; sacrifice of mice 2 h after injection)	Negative	Helmer and Bolcsfoldi, 1992b
Gene mutations in mammalian cells			
CHO/HPRT test Ovarian cells from Chinese	+ S9 mix: 37.5, 75, 150, 300, 600 µg/ml	Weakly positive at 600 µg/ml only	127, 137,

Test system/species	Test conditions	Results/ Remarks	Ref.
hamster	- S9 mix: 37.5, 75, 150, 300, 600 µg/ml (40% Glyoxal tested)	Negative	146
V79/HPRT test. V79 cells from Chinese hamster	+ S9: 0.1 – 1.5 mg/plate (40% glyoxal tested) (S9 mix from the liver of Aroclor 1254 induced rats) - S9: 0.1 – 1.5 mg/plate (40% Glyoxal tested)	Negative Negative	139
Mouse lymphoma test, L5178TK+/- cells	+S9: 25-250 µg/ml (S9 mix from liver of Aroclor 1254 induced rats) - S9: 25-375 µg/ml (40% Glyoxal tested)	Negative Positive	21
Mouse lymphoma test, L5178TK+/- cells	-S9: 27.8-61.5 µg/ml (4.79 x 10-4 – 10.6 x 10-4 mol/l)	Positive	160, Wang-enheim and Bolcsfoldi, 1988
Co S-7- Plasmid pMy 189	+S9: 0-150 µg glyoxal/100 µl plasmid solution (no data on metabolic activation)	Cytotoxicity and mutation frequency increased	95u
Chromosomal damage in mammalian cells			
Chromosome aberration, Ovarian cells from Chinese hamster	+ S9:190.1 – 580 µg/ml (S9 mix from the liver of Aroclor 1254 induced rats) - S9: 25.51 - 580 µg/ml (40% Glyoxal) (doses related to active ingredient)	Positive Positive	18
Chromosome aberration, Ovarian cells from Chinese hamster	+ S9:50 – 500 µg/ml (S9 mix from the liver of Aroclor 1254 induced rats) - S9: 50 – 500 µg/ml	Positive Positive	151
Chromosome aberration, V79 cells from Chinese hamster	- S9: 100 – 400 µg/ml (40% Glyoxal)	Positive	Nishi et al., 1989
DNA damage in mammalian cells			
UDS test, Primary hepatocytes F-344 rats Autoradiographic analysis	-S9 mix: 0.03 – 3.0 mg/ml (Glyoxal 40%)	Positive	135
UDS test, TC-SV40 cells Syrian hamster	- S9: ca. 2.9 mg/l (0.05mM)	Positive	Cornago et al, 1989
SCE test, ovarian cells of the Chinese hamster (CHO AUXB1)	- S9: 1.2-92.9 µg/ml (20-1600 µM)	Positive (addition of 1 mM sodium bisulfite weakened the effect)	Tucker et al., 1989
SCE test, Human lymphocytes	- S9: 23.3 – 162.2 µg/ml (400 – 2,800 µM)	Positive	Tucker et al., 1989
SCE test, Ovarian cells from Chinese hamster (CHO-K1-BH4-7182) cells	+S9:10, 25, 50, 75, 100 mg/l (S9 mix from the liver of Aroclor 1254 induced rats) -S9: 10, 50, 100, 200, 250 mg/l(40% Glyoxal)	Positive Positive	136
DNA-single-strand breaks, mouse lymphoma cells (L5178Y/TK+/-), alkaline unwinding	- S9: 27-214 µg/ml (0.461 x 10-3- 3.69 x 10-3 mol/l)	Positive	Garberg et al., 1988
DNA-single-strand breaks, alkaline elution, rat hepatocytes	100 – 600 µg/ml (activation by primary rat hepatocytes)	Positive	Ueno et al., 1991c
Cross links, alkaline	100 – 600 µg/ml (activation by primary rat	Negative	Ueno et

Test system/species	Test conditions	Results/ Remarks	Ref.
elution, rat hepatocytes	hepatocytes)		al., 1991c
Endoreduplication, ovarian cells of the Chinese hamster (CHO AUXB1)	- S9: 1.2-92.9 µg/ml (10-1600 µM)	Positive	Tucker et al., 1989

General results - in vitro mutagenicity/genotoxicity

Glyoxal has been tested in various bacterial and mammalian test systems *in vitro*. The different studies were performed either according to current test guidelines, both under GLP conditions or without or according to various scientifically sound but in some cases not validated test systems. In most of the studies it was shown that glyoxal induced gene mutations in bacterial test systems in specific strains (with and without metabolic activation). However, there are also studies with negative results in the same strains. Positive as well as negative results were also obtained for induction of DNA damage in bacteria. Gene mutations, chromosomal damage and DNA damage were also observed in mammalian test assays. There is indication that glyoxal can induce single strand breaks in DNA of mammalian cells under certain test conditions, but within the same study no indication of DNA cross-links were detected. It can be concluded that glyoxal poses a mutagenic/genotoxic potential *in vitro*.

In vivo

Mouse Micronucleus Assay

Guidelines	:	OECD 474 (1997), ICH Tripartite Harmonized Guideline on Genotoxicity (1995), US EPA OPPTS 8705395 (1998)
Species/strain	:	Mouse/CD-1
Group size	:	6 male animals per group
Test Substance	:	Protectol GL (40% glyoxal in water)
Batch No.	:	Lot 1 and2 (no further information)
Dose level	:	56.25, 112.5, 225 mg/kg bw (related to active ingredient)
Route	:	i.p.
Exposure period	:	Once daily on two consecutive days
GLP	:	In compliance

The ability of glyoxal to cause chromosomal damage *in vivo* was investigated in the mouse bone marrow micronucleus test. The choice of dose levels was based on an initial range-finding study in which glyoxal, formulated in water for injection was administered via intraperitoneal injection (i.p.). The test substance was administered to groups of 3 male and 3 female CD-1 mice once daily on 2 consecutive days in doses ranged between 127 – 2540 mg/kg bw. Doses above 320 mg/kg bw were lethal and evoked severe clinical findings. There was no difference between the males and females. Therefore, dose levels of 56.25, 112.5, 225 mg/kg bw (calculated for the active ingredient) were selected and six male mice were chosen for the main study. A vehicle control (water for injection) and a positive control (Cyclophosphamide, 40 mg/kg bw, i.p. on the second day) were also tested. Following dosing the animals were examined regularly and any mortality or clinical signs of reaction to the test compound were recorded. The mice were killed 24 hours after the second administration. Bone marrow for micronuclei examination was prepared 24 hours after test substance or vehicle application. After staining of the preparations

1000 polychromatic erythrocytes (PCE) were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 1000 polychromatic erythrocytes were also determined. Counting continued (but of PCE only) until at least 2000 PCE had been observed. The ratio of poly-(PCEs) to normochromatic (NCEs) erythrocytes was determined to assess inhibition of erythropoesis.

Results
Clinical signs of lethargy were noted in all mice at the highest dose level of 225 mg/kg bw. Mice treated with glyoxal showed frequencies of micronucleated PCE, which were similar or lower than the values for the control and fell also in the range of the historical control data. The respective values for the mean ratio of PCE/NCE were 0.95; 0.98; 0.89; 0.71 for vehicle control, 56.25, 112.5 and 225 mg/kg bw, respectively. Thus, a slight decrease in PCE:NCE ratio was observed at the high dose level. This was assessed as indication of slight bone marrow toxicity. The sensitivity of the mice was demonstrated since the positive control exhibited increased numbers of micronucleated polychromatic erythrocytes.
Glyoxal did not induce micronuclei in the polychromatic erythrocytes of bone marrow of mice treated up to 225 mg/kg bw, a dose shown to cause clinical signs and bone marrow toxicity.
Ref.: 19

This result is in line with the results of previous studies where glyoxal showed also no clastogenic effect in the micronucleus test after oral administration to the mouse (Ref.: 32, 140) or after single or double i.p. injections at higher doses (see table 3.3.5.) (Ref.: 134).

Unscheduled DNA assay in rat liver

Guideline	:	OECD 486 (1997), UK-EMS (1993), US EPA OPPTS 870.5550 (1998)
Species/strain	:	Rat/Han Wistar
Group size	:	16 male animals per dose
Test Substance	:	Protectol GL (40% Glyoxal in water)
Batch No.	:	Lot 1 and2 (no further information)
Dose level	:	1000 and 2000 mg/kg bw (related to pure active ingredient)
Route	:	Oral (gavage)
Exposure period	:	2 – 4 hours and 12 – 14 hours
GLP	:	In compliance

Glyoxal was assessed for its potential to induce DNA-damage and -repair in the *in vivo/in vitro* UDS test using rat hepatocytes. The choice of dose levels was based on an initial range-finding study in which glyoxal was administered by gavage to 3 male rats once at 2540 mg/kg bw and 3 female rats received once 1270 or 2540 mg/kg bw. During the 2 days post observation period severe clinical findings including mortality occurred but there were no differences between the male and female rats. Therefore, in the main study the test substance was administered orally (by gavage) at dose levels of 1000 and 2000 mg/kg bw (related to active ingredient) for a period of 2 – 4 or 12 – 14 hours to groups of male Han Wistar rats. For each treatment group as well as for the control groups 8 animals were used. After the treatment periods, the animals were sacrificed and liver perfusion was carried out. From each animal at least six primary hepatocytes cultures were established and exposed for 4 hours to ^{3}H-thymidine, which is incorporated into the DNA, if UDS occurs. Following the ^{3}H -thymidine exposure period, the cells were washed and mounted on cover slips, coated and stored in darkness for 14 days at -20 °C. Thereafter, the slides were developed at room temperature, fixed and stained with haemalum/eosin Y. The net

nuclear grain counts were determined by counting three slides per animal and 100 cells per slide. Appropriate reference mutagens (2-acetylaminofluorene (2-AAF) at 75 mg/kg bw mg/kg bw for the 12 – 14 hour preparation interval and dimethylnitrosamine (DMN) at 10 mg/kg bw for the 2 – 4 hour preparation interval) were used as positive controls.

Results
One animal was found dead (12 – 14 hour experiment), which was surprising and not consistent with the results of the range-finding part. Since no further information was provided, it remained unclear whether this was caused by glyoxal or – more likely – a consequence of improper gavage. No other clinical signs were observed in any other main study animal. Treatment with 1000 or 2000 mg/kg bw of glyoxal did not produce a group mean net grain count (NNG) value greater than -0.8 nor were more than 4.0% cells found in repair at either dose or interval. The sensitivity of the assay was demonstrated since the positive control substances (2-AAF and DMN) clearly increased the NNG and more than 25.4 or more than 50% cells were in repair, respectively.
Glyoxal did not induce unscheduled DNA synthesis under the experimental conditions.

Ref.: 20

Unscheduled DNA assay in the pyloric mucosa of the rat stomach

Guidelines	:	/
Species/strain	:	Rat/Fischer 344
Group size	:	3 – 4 male animals per test substance group, 7 male rats in the control
Test Substance	:	Glyoxal 40% aqueous solution
Batch No.	:	No data
Dose level	:	5, 50, 500, 550 mg/kg bw (probably corresponding to pure active ingredient)
Route	:	Oral (gavage)
Exposure period	:	2 hours
GLP	:	Not in compliance

The potential of glyoxal as 40% aqueous solution to induce DNA damage in the pyloric mucosa of the stomach was additionally examined in Fischer rats. Groups of 3 – 4 restricted fed male rats received a single oral application by gavage at dose levels of 5, 50, 500 and 550 mg/kg bw in an application volume of 1.0 ml. Vehicle controls received the same volume of distilled water. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG; 1-100 mg/kg), a known rat glandular stomach carcinogen was used as positive control. 2-Acetylaminofluorene (2-AAF; 200-400 mg/kg), a non-carcinogen in the rat stomach was used as negative control. The stomach was removed 2 h later and the pyloric mucosa was processed and the effect on DNA was investigated by the alkaline elution technique.

Results
The test substance induced dose-dependently DNA damage detected by a significant increase in the elution rate (5- to 12-fold of vehicle control) at dose levels of 50, 500 and 550 mg/kg bw. At the dose of 5 mg/kg bw no DNA damage was detected. In the positive control (MNNG) groups, a 11- to 24-fold increase in the elution rate was recorded. No increase in the elution rate was observed in negative control (2-AAF) groups.

Glyoxal induced DNA damage in the pyloric mucosa of the rat stomach after oral application of dose levels in the range of 50 – 550 mg/kg bw. No damage was noted at 5 mg/kg bw, indicating that this dose level can be considered as a "NOAEL" for local DNA damage.

Ref.: 40, 41

Another group examined the potential of glyoxal to induce UDS in male Fischer 344 rats. Groups of 5 rats received a single oral application of the test substance (content not specified) in distilled water at dose levels of 0, 120, 240, 300, 360, 400 mg/kg in an application volume of 0.5 ml. A homogenate of the pyloric mucosa of the stomach was prepared at 2, 5 and 16 h after dosing and assayed unscheduled DNA synthesis. Significant and dose-related induction of UDS was apparent in the pyloric mucosa of the stomach within 2 h after dosing. The increase in DNA synthesis was slightly at a dose of 360 mg/kg and significantly at 400 mg/kg bw, while no increase was observed at 120 and 240 mg/kg bw.

Ref.: 39, 42

DNA damage investigated by alkaline elution in vivo in rats

Guidelines	:	No guideline available for this screening study
Species/strain	:	Rat/Sprague-Dawley
Group size	:	Male rats (no further information)
Test Substance	:	Glyoxal (content not specified)
Batch No.	:	No data
Dose level	:	200, 500, 1000 mg/kg bw
Route	:	Oral (gavage)
Exposure period	:	1 – 24 hours
GLP	:	Not in compliance

The potential of glyoxal to induce DNA damage in form of single strand breaks was investigated in Sprague-Dawley rats by the alkaline elution technique. Groups of fasted male rats received a single oral application by gavage at dose levels of 200, 500 and 1000 mg/kg bw in an application volume of 1.0 ml. After 1 – 24 hours of exposure the animals were killed and the livers were perfused. Thereafter, the tissues were removed and processed further to obtain a nuclear pellet for the alkaline elution assay. Also the kidney, spleen, pancreas and lung were minced and processed further to obtain the respective nuclear pellet for the assay.

Results
DNA lesions were detected in the liver within 2 hours at all dose levels and reached a maximum at 9 hours after exposure, returning nearly to control levels 24 hours after exposure at any dose level. The alkaline elution of DNA from other tissues following oral exposure of rats to 1000 mg/kg bw showed no biologically relevant DNA lesions. Glyoxal induced DNA single strand breaks in the liver with a LED of 200 mg/kg. Hardly any DNA lesions could be detected in the kidney, spleen, pancreas or lung.

Ref.: Ueno et al., 1991c

In one test on determining sex-linked recessive-lethal mutations, dominant-lethal mutations, reciprocal translocations and on the loss of sex chromosomes *on Drosophila melanogaster*, the intra-abdominal injection of Glyoxal did not indicate any mutagenic effect. In an older abstract, however, the same authors reported about a slight increase of the rate of sex-linked recessive-

lethal mutations after the intra-abdominal injection of a Glyoxal solution, nonetheless without detailed information on the applied dose

Ref.: 6u, 7

General results - mutagenicity/genotoxicity in vivo

In vivo glyoxal was negative in the micronucleus test on mice after oral administration. On *Drosophila melanogaster*, glyoxal is also negative in the sex-linked recessive –lethal test, in the dominant test and in the studies on the reciprocal translocation and on the loss of sex chromosomes. After oral administration to the rat, a significant increase of the unscheduled DNA synthesis is found in the pyloric mucosa of the stomach. Glyoxal induced DNA single strand breaks in the liver with a LED of 200 mg/kg. Hardly any DNA lesions could be detected in the kidney, spleen, pancreas or lung.

Glyoxal at dose level of 50 mg/kg bw and above induced DNA damage in the pyloric mucosa of the rat stomach, but no damage could be noted at 5 mg/kg bw, indicating that this dose level can be considered as a "NOEL" for local DNA damage.

Further *in vivo* mutagenicity/genotoxicity studies were performed and published in the literature. They are cited shortly in the following sections and the most relevant information is provided in the overview table 3.3.5.

Table 3.3.5 Mutagenicity tests *in vivo*

Test system/species	Test conditions	Results/ Remarks	Ref.
Gene mutations			
Drosophila melanogaster Sex-linked recessive-lethal test	0.73 mg/ml intraabdominal injection	Lethality 0.30 % compared to 0.08 % for the controls	6u
Drosophila melanogaster Sex-linked recessive-lethal test	40 µg/animal intraabdominal injection	Negative (no data on toxicity provided)	7
Chromosomal damage			
Micronucleus test, Mouse (CD-1), 6 male animals/group; 1 000 polychromatic erythrocytes (bone marrow) /animal examined	56.25, 112.5, 225 mg/kg bw, i.p., once daily on 2 consecutive days, 40 % glyoxal, sacrifice 24 h after administration (doses related to active ingredient)	Negative (MTD tested)	19
Micronucleus test, mouse (Swiss), 5 animals/sex and preparation time; 1000 polychromatic erythrocytes (bone marrow) /animal examined	1 000 mg/kg body weight oral, 40 % glyoxal, sacrifice 24 h, 48 h and 72 h after administration	Negative (MTD tested)	32, 140
Micronucleus test, Mouse (CD-1), 4 animals/sex and preparation time; 1000 polychromatic erythrocytes (bone marrow) /animal examined	400 mg/kg bw, i.p., single dose or each one on 2 consecutive days, 40 % glyoxal, sacrifice 24 h and 48 h h after administration	Negative (MTD tested)	134
Drosophila melanogaster,	40 µg/animal intraabdominal injection	Negative	7

Test system/species	Test conditions	Results/ Remarks	Ref.
dominant-lethal test		(no data on toxicity provided)	
Drosophila melanogaster, reciprocal translocation	40 µg/animal intraabdominal injection	Negative (no data on toxicity provided)	7
DNA damage			
UDS test, male Wistar rats, primary hepatocytes, autoradiographic UDS test (net grain count)	1000 and 2000 mg/kg bw, oral 40% Glyoxal, sacrifice 2- 4 or 12 – 14 h after administration (doses related to active ingredient)	Negative (MTD tested)	20
UDS test, male Wistar rats, primary hepatocytes, autoradiographic UDS test (net grain count)	100, 500, 1 000 mg/kg body weight oral, 40 % glyoxal, sacrifice 2h and 16 h after administration	Negative (No toxicity up to 1 000 mg/kg body weight)	31
UDS test, male F344-rats, pyloric mucosa, autoradiographic UDS test	120, 240, 360 and 400 mg/kg bw, single oral (gavage) application, sacrifice 2, 5 and 16 h after administration	Positive at 360 and 400 mg/kg bw (Highest dose corresponds to ½ of the LD50 value) Negative at 120, 240 mg/kg bw	39, 42
DNA single-strand breaks, alkaline elution, liver DNA, rat (Sprague-Dawley)	200, 500, 1000 mg/kg bw, single oral (gavage) application, sacrifice 2 h after administration	Positive in the liver (dose-dependently). Negative in the kidney, spleen, pancreas, and lung (no data on toxicity provided)	Ueno et al., 1991c
DNA single-strand breaks, alkaline elution, DNA of pyloric mucosa, rat (F344)	5, 50, 500 and 550 mg/kg bw, single oral (gavage) application, sacrifice 2 h after administration	Positive at 50 – 550 mg/kg bw(dose-dependent, but no data on toxicity provided) Negative at 5 mg/kg bw	40, 41
Genome damage			
Drosophila melanogaster, Loss of the X- or Y-chrom.	40 µg/animal, intraabdominal injection	Negative (no data on toxicity provided)	7

3.3.7. Carcinogenicity

After exposure of Sprague-Dawley rats to dosages of 6000 mg glyoxal/litre drinking-water for up to 180 days, there were no neoplastic changes found upon gross and histopathological examination of liver, kidneys, spleen, stomach, thymus, and mesenteric lymph nodes (Ueno et al., 1991a).

Carcinogenicity study in mice (dermal application)

Guideline : /
Species/strain : Mice/C3H/HeJ
Group size : 40 male mice per group
Test substances : Aerotex® Glyoxal 40 and European Glyoxal 40 (40% aqueous solutions)
Batch no. : BRRC No. 41-429, identification no. R9516-44
Dose level : 25 µl of 1:8 dilutions in deionized water (i.e. 5% test substance concentration)
Route : Dermal (on the clipped fur on the back)
Exposure period : 3 times a week for life time (about 18 months)
GLP : In compliance

40 male C3H/HeJ mice each were treated with 25 µl of a 1:8 dilution of American glyoxal 40 in deionized water onto the back skin three times weekly during their lifespan (ca. 18 months). A second group of 40 male C3H/HeJ mice was treated in the same manner with 25 µl of a corresponding 1:8 dilution of European glyoxal 40. The selected concentration corresponds to a concentration of about 5% or a dose level of about 125 mg/kg (calculated with a body weight of 25 g and a density of 1 g/ml). A control group of the same size (treated with 25 µl deionized water) was also investigated. The dosing concentration was determined previously in a two weeks study. This concentration was shown to be non-irritating and non toxic within this period. Each week the fur was clipped from the back of each mouse and the mice were transferred to clean cages. The test material was applied by means of Eppendorf automatic pipette. The mice were observed daily for mortality, clinical findings and skin reactions and were additionally carefully examined for lesions of the skin once per months. Following the death of each mouse, a necropsy was performed. All body cavities were examined and all suspected internal tumours were fixed for histopathological examination. The dorsal skin of all mice (with and without lesions) were also fixed, stained and processed for histopathology.

Results
The mean survival time of the mice of both glyoxal treated groups was statistically greater (580 and 594 days) than the survival of the control group (488 days). The American batch caused skin irritation resulting in inflammation and necrosis of some mice but no skin or subcutaneous neoplasms were found. A fibrosarcoma in one of the mouse treated with European glyoxal 40 was judged by the authors not to be substance-related. The conclusion is based on the control data, available at the testing institute, according to which fibrosarcomas occur spontaneously in the male C3H mice.

No skin tumours were found in any mice treated for life time with 25 µl of a 1:8 dilution, corresponding to 5% or a dose level of about 125 mg/kg bw of an American or European batch of glyoxal. Also no subcutaneous tumours were found with the exception of a spontaneous fibrosarcoma in the European batch treated group. Mortality was not affected in any of the glyoxal treated groups.

It is concluded that glyoxal showed no tumorigenic potential in this dermal long-term study in mice.

Ref.: 30, 129

Tumour initiation study in mice

In addition, the possible tumour-initiating effect of glyoxal (37% to 43%) was examined on the skin. Groups of 20 female CD-1 mice each (age at the beginning of the study: 7 weeks) were administered 0.1 ml glyoxal onto the mechanically shaven back skin twice weekly for a period of 5 weeks (initiation phase) and, following a 1-week pause, were then applied 12-O-tetradecanoylphorbol-13-acetate (TPA) as a promoter for 47 weeks. 7,12-dimethylbenzo(a)-anthracene (DMBA) was used as the positive control and dimethylsulfoxide (DMSO) as the negative control. The total initiation dose was 500 µmol glyoxal/mouse (corresponding to 30 mg/mouse). All animals survived the entire 53-week test period. In the glyoxal-treated group, 2 of 20 mice showed papilloma. No tumours were found in the DMSO group. In the positive control group (DMBA/TPA), all 20 mice had a total of 134 skin tumours (99 of which were papilloma and 31 squamous cell carcinomas). Thus, glyoxal was not shown to be a tumour initiator in this study.

Ref.: Miyakawa et al., 1991

Tumour initiation/promotion study in rats

A tumour promoting effect of glyoxal in the stomach was detected in a 2-stage model in male Wistar rats. In the initiation phase, 2 groups of 30 male, 7-week old Wistar rats each received N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in the drinking water (concentration 100 mg/l) for 8 weeks and simultaneously a diet containing 10% sodium chloride. From the 8th to 40th week, the first group was administered drinking water with 0.5% glyoxal and the second group, drinking water without any additive. A third group (10 animals) received a diet and drinking water without additives in the first 8 weeks and then received drinking water with 0.5% glyoxal from the 8th to 40th week. For the rats, pre-treated with MNNG and NaCl, glyoxal caused a significant increase of adenocarcinomas in the glandular stomach (12/28 animals, ca. 43%) mainly localized in the pylorus region. A hyperplasia of the mucosa was also found there. For comparison, 5 tumours (5/30 animals, ca. 17%) were observed for initiation treatment without subsequent administration of 0.5% glyoxal in the drinking water. According to the authors, the results infer that glyoxal possesses local tumour promoting properties on the glandular stomach of the rat. The results are presented in the table 3.3.6.

Table 3.3.6. *Findings about hyperplasias and adenocarcinomas in the glandular stomach of the Wistar rat after oral administration of Glyoxal in an initiation/promotion study*

	Number of affected animals in the treatment group		
	Initiation plus glyoxal	**Initiation without glyoxal**	**Without initiation plus glyoxal**
Total examined	28	30	8
Adenocarcinomas			
total	12 (43 %)*	5 (17 %)	0
in fundus	1 (4 %)	1 (3 %)	0
in pylorus	11 (39 %)*	4 (13 %)	0
Hyperplasias			
in fundus	4 (14 %)*	1 (3 %)	0
in pylorus	17 (61 %)**	8 (27 %)	0

* $p > 0.05$ ** $p > 0.01$

Ref.: Takahashi et al., 1989

Short term liver carcinogenicity screening assay in rats

According to a 2-step concept, 12 male F344 rats (6 weeks old) were first injected intraperitoneally with a single dose of 200 mg diethylnitrosamine/kg bw as the initiator. Starting 2 weeks later, they obtained 5000 ppm glyoxal with the diet (corresponding to 333 mg/kg bw/d) for 6 weeks. The rats underwent a 2/3 hepatectomy after a feeding time of one week. At the end of the study after a total of 8 weeks, the GST-P-(placental glutathione-S-transferase) positive foci in the liver were evaluated. A significant increase of the number of these foci, compared to the controls (rats treated only with diethylnitrosoamine), ought to infer a carcinogenic potential. In these studies, glyoxal did not cause any increase of the number of foci, but rather a significant reduction (Hasegawa and Ito, 1992).

Cell transformation assays in vitro

Three glyoxal samples (40%) of various origin were investigated *in vitro* in the cell transformation test with C3H/10T½CL8 cells (embryonic mouse cell-line). The concentrations amounted to 0.0013-0.0098 µl/ml (Group A), 0.0025-0.195 µl/ml (Group B) and 0.0049-0.0039 µl/ml (Group C). The highest concentrations were each in the cytotoxic range. None of the 3 glyoxal samples caused cell transformations.

Ref.: 128, 144

General results
No carcinogenic effect is detected in mice after dermal application of glyoxal over the entire life span. Glyoxal possesses no tumour initiating effect after the dermal administration to mice. Oral administration of glyoxal after initiation with MNNG was associated with increased incidences of adenocarcinomas and hyperplasias in both pylorus and fundus of rat stomach. In the liver promotion model of the rat, no indications were found for a promoting effect of glyoxal through systemic action.

3.3.8.	Reproductive toxicity

3.3.8.1.	Effects on fertility

There were no studies available on the effects of glyoxal on fertility.

3.3.8.2.	Developmental toxicity

In developmental toxicity studies with Sprague-Dawley rats, glyoxal trimeric dihydrate was administered by gavage on gestation days 6-15 at doses of 0, 200, 800, 1200, 1600, or 2000 mg/kg body weight per day in the preliminary study and 50, 150, or 300 mg/kg body weight per day in the main study (NTP, 1991c, 1994a,b). In the initial range-finding study, maternal toxicity (decreased weight gain) was observed at 200 mg glyoxal dihydrate/kg body weight per day (corresponding to 123 mg glyoxal/kg body weight per day), with clinical signs of toxicity and decreased gravid uterine weight at 800 mg glyoxal dihydrate/kg body weight per day and maternal deaths at 1200 mg/kg body weight per day and above (NTP, 1991c). No maternal toxicity was observed, however, in the second study at the top dose of 300 mg/kg body weight per day (corresponding to 185 mg glyoxal/kg body weight per day). No embryotoxicity was

observed at 200 mg glyoxal dihydrate/kg body weight per day in the preliminary study or at the highest dose in the main study.

Developmental toxicity range-finding studies in New Zealand White rabbits administered glyoxal by gavage yielded a NOEL of 200 mg glyoxal trimeric dihydrate/kg body weight per day, corresponding to 123 mg glyoxal/kg body weight per day (NTP, 1991d), and a LOEL of 400 mg glyoxal dihydrate/kg body weight per day, corresponding to 247 mg glyoxal/kg body weight per day (NTP, 1992), for both maternal toxicity and embryotoxicity. Maternal signs of systemic toxicity and decreases of weight parameters were accompanied by reduced fetal weight (NTP, 1992). The application of doses in the range of 200 mg glyoxal dihydrate/kg body weight per day was discussed as problematic due to the corrosive nature of the substance, leading to damage of gastric mucosa of pregnant rabbits (unpublished observations cited in NTP, 1993). In a subsequent study with a single dose level of 50 mg glyoxal dihydrate/kg body weight per day, corresponding to 31 mg glyoxal/kg body weight per day, there was no maternal mortality or persistent signs of toxicity, although minimal reductions in body weight gain and food consumption were noted. Glyoxal exposure did not significantly alter post-implantation loss and had no effect on foetal body weight or the incidence of external, visceral, or skeletal malformations. The authors gave a NOAEL for developmental toxicity for rabbits of 50 mg glyoxal dihydrate/kg body weight per day, corresponding to 31 mg glyoxal/kg body weight per day (NTP, 1993).

Guidelines	:	OECD 414 (Draft 1999), Commission Directive 87/302/EEC (1988), US EPA OPPTS 870.3700 (1988)
Species/strain	:	Wistar rat, Chbb:THOM (SPF)
Group size	:	25 mated female rats
Test substance	:	Glyoxal 40%
Batch no	:	B 61 (produced March 1999)
Dose level	:	0, 5, 25, 125 mg/kg bw corresponding to pure active ingredient
Route	:	oral (gavage)
Exposure period	:	day 6 – day 19 post coitum
GLP	:	In compliance

Material and methods
25 rats per dose were used. On day 0 (detection of sperm) animals were 9-11 weeks old. Mean bw was 228.9 - 233.4 g. Animals were singly housed. Glyoxal 40% was given orally by gavage at a volume of 10 ml/kg once a day from day 6-19 post coitum (pc) at 5, 25, and 125 mg glyoxal (pure active ingredient)/kg bw. Control animals received the solvent, distilled water.

The test substance was analytically characterized and the solutions were analyzed at start and end of the study. Dams: food consumption, bw on days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20 p.c. Corrected bw gain was calculated (bw d 20-(uterus weight and body weight on d 16)). Animals were examined at least daily for clinical symptoms. Mortality was checked twice a day or once on weekend and holidays.

The dams were sacrificed on day 20 pc. Necropsy included gross pathology assessment and removal of uterus and ovaries with subsequent examination and recording of: uterus weight; no. of corpora lutea; no. and classification of implantation sites, as live foetuses, dead implantations (early, late resorptions; dead foetuses). Calculation of conception rate and pre- and postimplantation losses. Foetuses: were weighed and sexed, macroscopically examined

(viability, condition of placenta, foetal membranes, umbilical cords, placental weight). Approx. 50% of all foetuses were subjected to soft tissue examinations after fixation in Bouin's solution, the other 50% of foetuses was examined for skeletal changes.

Statistical evaluation of data included Fishers exact test for conception rate, mortality of the dams, and all foetal findings, and Dunnett's test for all other data including water consumption and bw gain.

Results
No mortality was seen in any of the groups of dams. Several animals (12/25 rats) of the high dose group showed transient salivation immediately after dosing which persisted for some minutes. After cessation of treatment on day 19 pc salivation did no longer occur. Food intake of the high dose animals was significantly reduced (-13%) on several days. Over the treatment period (day 6-19), food intake was ca. 7% below that of controls. Bw of treated animals was slightly, but not significantly reduced, whereas corrected bw gain (net maternal bw change) was significantly reduced in the high dose group. Thus, an enhanced uterus weight compensated a more pronounced maternal effect on bw in these animals. Uterus weight was highest in the animals receiving 125 mg/kg bw/d due to an increased number of foetuses. No substance-related findings were seen during necropsy.

19-24 rats/group became pregnant. Conception rate was between 76-96%. Differences were without biological relevance. No substance-related relevant differences were seen with regard to conception rate, mean number of corpora lutea, implantation sites, or calculated pre- and postimplantation losses, number of resorptions and viable foetuses.

Sex distribution, placental weight and weight of foetuses were similar to control values. External malformations were seen in all groups. Incidence was low and similar to historical control data. Moreover, no dose-relation was seen. No external variation was seen. Two cases of soft tissue malformation were seen in two control animals; soft tissue variations were seen in all groups including the control, as were malformations and variations of the foetal skeletons. However, the scattered occurrence, low incidence as compared with controls and historical control data, did not show a clear relation to dosing and were of no biological relevance.
Thus, glyoxal had no effect on foetal morphology at doses up to 125 mg/kg bw given to pregnant rats.

In summary, findings after oral administration of glyoxal up to and including 125 mg/kg bw/d to pregnant rats were as follows:

- Overt signs of maternal toxicity were seen at the highest dose (125 mg/kg bw/d) only in terms of reduced bw gain and reduced food intake. Animals receiving 5 or 25 mg/kg bw/d did not show signs of toxicity
- No influence on the gestational parameters were seen at any of the doses tested - no signs of prenatal developmental toxicity were seen
- No signs of teratogenicity were seen.

Oral administration of glyoxal to pregnant Wistar rats from implantation to one day prior to the expected day of parturition resulted in overt maternal toxicity at 125 mg/kg bw, but not at 5 and 25 mg/kg bw.

No substance-related influence on gestational parameters was seen. No signs of prenatal developmental toxicity, especially no signs of teratogenicity, were seen at any dose level including the highest dose tested at 125 mg/kg bw. In this study, the NOAEL for maternal toxicity is 25 mg/kg bw; the NOAEL for prenatal developmental toxicity is 125 mg/kg bw, each correspond to the pure active ingredient, respectively.

Ref.: 17

Overall results
No dose-dependent effects on reproductive organs were observed in repeated dose toxicity studies lasting for a sufficient period of 90 days up to a dose of approx. 250 mg/kg bw (related to pure active ingredient).

A prenatal developmental toxicity study performed according to current testing guidelines and under GLP conditions showed a NOAEL of 125 mg/kg bw for prenatal developmental toxicity. This was the highest investigated dose level. The NOAEL for maternal toxicity was achieved at 25 mg/kg bw in this study related to pure active ingredient.

3.3.9. Toxicokinetics

Glyoxal is endogenously produced during normal cellular metabolism by a multitude of enzyme-independent pathways, such as the spontaneous reaction of amino groups in proteins with reducing sugars (Maillard reaction), sugar autoxidation, DNA oxidation, peroxidation of polyunsaturated fatty acids, and UV photodamage, and in conditions of oxidative stress and depletion of GSH (Kasper & Funk, 2001; Ulrich & Cerami, 2001; Kasai, 2002; Thornalley, 2002; Wondrak et al., 2002) (see Figure 3.3.1). Furthermore, glyoxal is a product of the metabolism and microsomal oxidation of compounds such as glycolaldehyde, ethylene glycol, and *beta*-hydroxy-substituted *N*-nitrosamines and possibly contributes to the toxic, genotoxic, and tumorigenic action of these substances (Loeppky & Goelzer, 2002; Loeppky et al., 2002).

In biological materials, less than 10% of the glyoxal present is in unbound forms in aqueous solution (free glyoxal and hydrates), as most of the reactive carbonyl groups are reversibly bound to cysteinyl, lysyl, and arginyl residues of proteins (Thornalley, 1995).

The endogenous concentrations of glyoxal in human tissues and body fluids, as with other *alpha*-oxoaldehydes, are limited by the high catalytic efficiency of the glyoxalase system (Thornalley, 1995) as well as by the rapid reaction of glyoxal with proteins (Sady et al., 2000).

During certain pathological conditions (e.g., diabetes mellitus, uraemia), elevated concentrations of glyoxal have been measured. The concentration of glyoxal in blood samples from normal human subjects (n = 19) was 0.21 ± 0.14 µmol/kg (Thornalley et al., 1996). For blood plasma, a value of approximately 0.1 µmol/litre was estimated for normal healthy subjects, which can double in diabetics (Thornalley, 1998; Thornalley et al., 2000).

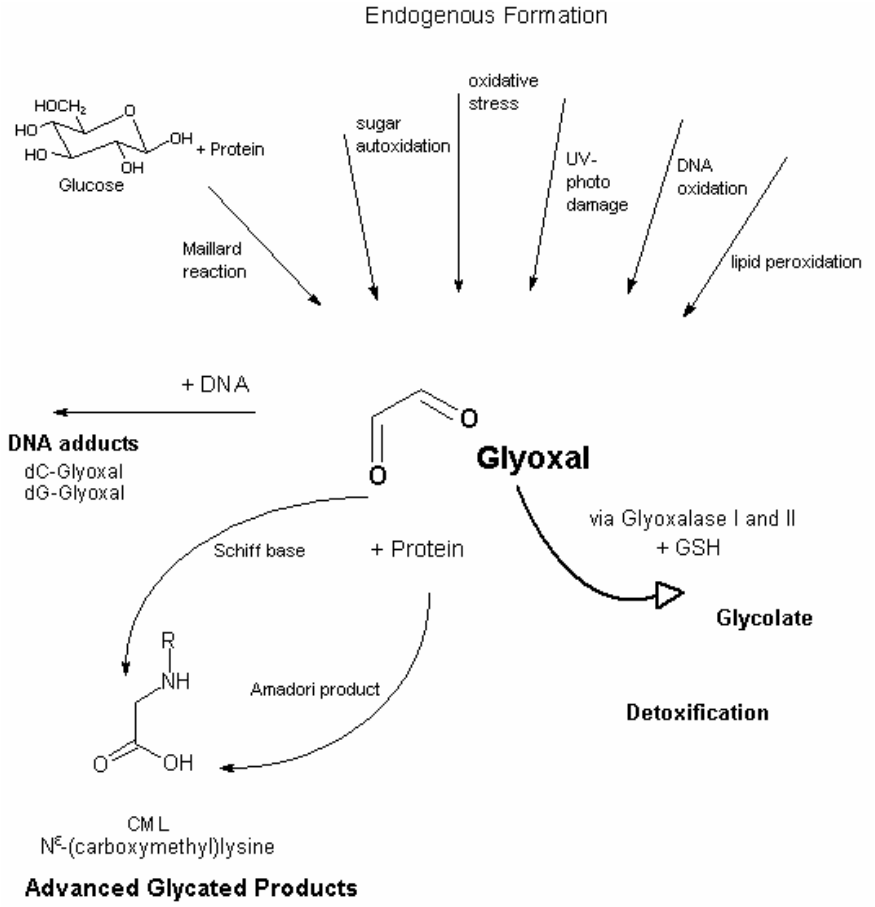

Fig. 3.3.1. *Scheme of formation, detoxification, and protein and DNA adduct formation of glyoxal (*Cicads 57: 2004).

The cytosolic GSH-dependent glyoxalase system is the major pathway for the detoxification of glyoxal (see Figure 3.3.1). Glyoxal reacts non-enzymatically with GSH with formation of a hemithioacetal, which is subsequently converted to *S*-glycolylglutathione by glyoxalase I.
Glyoxalase II catalyses the hydrolysis of *S*-glycolylglutathione to glycolate, re-forming the GSH from the first reaction. The activity of glyoxalase I *in situ* is approximately proportional to the cytosolic concentration of GSH. When GSH is severely depleted (e.g., under conditions of oxidative stress), however, 2-oxoaldehyde dehydrogenase and aldose reductase may also metabolize glyoxal. Imbalances in intracellular redox systems may impair these detoxification mechanisms, resulting in higher levels of glyoxal (Thornalley, 1995, 1998; Abordo et al., 1999; Miyata et al., 1999, 2001). A further GSH-independent route of detoxification via glyoxalase III exists. Glyoxalase III is reported to be the most abundant glyoxalase in *Escherichia coli* (MacLean et al., 1998; Okada-Matsumoto & Fridovich, 2000).

The glyoxalase I concentration in human tissues and blood cells is about 0.2 µg/g protein. In human tissues, the specific activity is highest in pancreas, lung, kidney, and brain and lowest in adipose tissue and liver. Specific activities in foetal tissues are about 3 times higher than in corresponding adult tissues. Human glyoxalase I was found to exhibit genetic polymorphism, with three phenotypes resulting from a diallelic gene. The frequency of the GLO allele in various populations on average ranges from 0.046 to 0.853 (Thornalley, 1993).
Exposure to glyoxal induced the activity of glyoxalase I in *Salmonella typhimurium* strains TA 100 and TA 104 (0.1 mg glyoxal/ml) (Ueno et al., 1991b) as well as in erythrocytes, liver, and

kidney of male Sprague-Dawley rats (4000 or 6000 mg glyoxal/litre drinking-water for 30 days, no increased activity for longer exposure periods) (Ueno et al., 1991a).

3.3.10. Photo-induced toxicity

/

3.3.11. Human data

/

3.3.12. Special investigations

Glyoxal content in food

Glyoxal is a substance frequently detected in fermented food and beverages. This is mainly due to microbial activity as well as non-enzymatic browning reactions such as caramelization and Maillard reactions of saccharides (Hollnagel & Kroh, 2002). Accordingly, Barros et al. (1999) found glyoxal present in different brands of beer and wine on sale in Portugal. Sampling three different brands of white wine, they detected glyoxal at concentrations of 6.2, 8.7, and 26 µmol/litre (about 360, 464, and 1509 µg/litre). De Revel & Bertrand (1993) evaluated a range of French wines and detected glyoxal in one white wine (mean of 125 µg/litre), red wines (151-368 µg/litre), and five sherry wines (lowest level of glyoxal in a Seco with 435 µg/litre and highest level in an Olorosso with 1556 µg/litre). Palamand et al. (1970) detected glyoxal levels ranging from about 230 to 1000 µg/litre in eight different beers.

Nagao et al. (1986) found glyoxal in soybean paste (4.2 mg/kg), soy sauce (4.9 mg/litre), toast (0.5 mg/kg), and bread (0.3 mg/kg). Markianova et al. (1971) reported glyoxal levels in bread ranging from 0.07 to 0.31 mg/kg, depending on the yeast type employed. However, Roiter & Borovikova (1972) showed that using amylase in the baking process led to glyoxal levels of up to 1.4 mg/kg in the bread crust and of up to 1.6 mg/kg in the bread crumbs. Plant materials used for brewing (rice — about 14 mg/kg; barley — about 3 mg/kg; malt — about 7 mg/kg) might contain glyoxal as well (Palamand et al., 1970). Yamaguchi et al. (1994) detected glyoxal in fermented food such as yoghurt (about 0.63-0.92 mg/kg). Due to heat-induced autoxidation, edible oils might contain glyoxal, as was shown for sardine oil, containing up to 6.5 mg/kg (Hirayama et al., 1984). Glyoxal is used as an additive to cigarettes by Phillip Morris and has been reported to be present in tobacco smoke.

Human exposure

The main route of exposure of the general population to glyoxal is probably via intake of water and food containing glyoxal. Glyoxal is present in a broad range of different food products. However, due to the lack of quantitative data on the presence of glyoxal in food products such as meat, dairy products, or fish, an exact value cannot be given. The general population might also be exposed to glyoxal via cigarettes or residential log fire smoke or vehicle exhaust containing glyoxal.

An exposure scenario has been compiled as a hypothesized worst case. A food/drink intake of 10 mg/day has been calculated from foods with a known glyoxal content. About three cups of brewed coffee per day (>400 µg glyoxal), toast (>50 µg glyoxal), a stir-fried meal containing rice (>4 mg glyoxal), oil (>500 µg glyoxal), soy sauce (>200 µg glyoxal), a pint of beer (500 µg glyoxal), one yoghurt (>130 µg glyoxal), and one glass of sherry (>30 µg glyoxal) leads to an intake of about 6 mg/day. A further intake of 3-4 mg glyoxal/day might come from other fermented products (dairy products or vegetables), from other popular roasted or fried products (meat, fish, mushrooms, sausages), or from additional bakery products (Cicads 57,2004).

Assuming a daily intake of 20 m^3 air containing about 4 µg glyoxal/m^3 (Borrego et al., 2000), a daily consumption of 2 litres of water containing 9 µg glyoxal/litre (median for ozone-treated drinking-water; IPCS, 2000), and an estimated daily intake of 10 mg glyoxal via food, an intake of about 160 µg of glyoxal per kg body weight (using 64 kg as the value for body weight) per day can be calculated. This intake is almost totally from food.

Glyoxal has been reported as being present in some household cleaners up to a concentration of 4% (product databanks, Switzerland, Denmark, and Germany; R. Hertel, personal communication, 2003). People can therefore be exposed to glyoxal during its use as a household cleaner.

Examples of exposure from non-cosmetic products

General population: An exposure scenario has been compiled as a hypothesized worst case. Using the daily intake of, maximally, 10 mg glyoxal via food, an estimated intake of 0.16 mg glyoxal/kg body weight per day can be calculated. This is slightly less than the tolerable intake of about 0.2 mg/kg body weight per day for lifetime oral exposure to glyoxal (based on a NOAEL of 100 mg/kg bw./day and an uncertainty factor of 100 and a factor of 5 for less-than-lifetime exposure) (Cicads 57, 2004). (The above NOAEL is based on reduced body weight gain in a 28 day rat study (149). It is not clear from the report if the actual glyoxal dose was 40 mg/kg bw./day). A LOAEL of 107 mg/kg bw./day was obtained in a 90 day rat study based on reduced serum protein levels (Ueno et al., 1991a))

A nurse or hospital cleaner or consumer using disinfectant: A typical brand of disinfectant (7.5 g in 100 g = 7.5% glyoxal) is used at a dilution of 1% for disinfection and cleaning of surfaces (i.e., 0.075% glyoxal). Using a rounded-up 0.1% glyoxal solution and a calculation derived from a model gives an uptake of about 4µg/kg bw/day, assuming a body weight of 64 kg. This is much (50 times) less than the tolerable intake of about 0.2 mg/kg body weight per day for lifetime oral exposure. However, using a worst-case exposure to 4% glyoxal and the same assumptions as above would give an uptake of about 0.15 mg/kg body weight, which is approximately the same as the tolerable intake of about 0.2 mg/kg body weight per day for lifetime oral exposure (Cicads 57, 2004).

Immunotoxicity

The effect of glyoxal on the antibody synthesis was evaluated in inbred CBA-mice. The animals were immunized with ovalbumine and a conjugate of dinitrophenol and ovalbumine in Freund's

adjuvant, which was administered intraperitoneally. Glyoxal at doses of 100 µg/kg bw was administered to the animals 2 days before, simultaneously and 5 days after the immunization. The serum samples were processed 5, 10 and 20 days after the administration and, among others, the immunoglobulin G (IgG) level as well as the concentration of the Fab-fragments (with the antigen binding site) and of the Fc-fragments (binding site for complementarily proteins) were determined as the parameter for the antigen-antibody-binding affinity in the serum. Damage to the antibody synthesis with the simultaneous injection was thereby greater than with the administration 2 days before and 5 days after the immunization. The impairment of the antibody synthesis by glyoxal occurred primarily during the late phase of the antibody formation after 10 and 20 days. The IgG-fab- and Fc-concentrations were reduced with simultaneous administration of glyoxal by 10 % after 5 days, 45 % after 10 days and ca. 40 % after 20 days, respectively. A statistical evaluation of the results was not undertaken (no further details).

Cited in ref.: 45

Pharmacological effects

The single intravenous administration of a 30 % glyoxal solution (dose not available) was lethal in the dog within 5 minutes after initial respiratory failure and subsequent cardiac failure. Sublethal glyoxal doses exhibited parasympathomimetic, slightly spasmolytic as well as strong respiratory analeptic effects and caused a stimulation of the central nervous system as well as forced diuresis. It was supposed that these effects were not directly in relation with the induction of an increased histamine release, but rather that glyoxal possibly has a direct effect on the medulla oblongata and the parasympathomimetic receptor cells of the vegetative nervous system.

Ref.: 157u

A glucose tolerance test was conducted with rabbits on the 5th and 10th day after dermal application of 40 % glyoxal. After the intravenous injection of 2.5 ml of a 20 % glucose solution, the glucose level in the blood was determined according to Hagedorn-Jensen. The average glucose content was clearly increased up to 154 mg compared to the untreated control animals (mean value maximum of 83 mg). The histopathological examination showed a considerable granular degeneration of the liver, a moderate granular degeneration of the renal tubuli and a considerable granular degeneration as well as atrophy and fibrotic changes of the islets of Langerhans in the pancreas (Ito, 1963).

Biochemical investigations

The influence of glyoxal on protein synthesis was studied *in vivo* based on the dose dependent decrease of the total protein content in serum observed in rats in a subchronic study with administration of glyoxal in the drinking water (see section 3.1.2). For this, male Sprague-Dawley rats (weight 110-120 g, 4 animals/group) were administered once 0 and 150 mg glyoxal/kg bw intravenously or 0 and 1.000 mg/kg bw orally. 4 hours after the glyoxal administration, the animals were injected i.p. with 2.4 MBqL [4,5-^{3}H]-leucine/kg bw. The incorporation of the radio-labelled leucine in the liver, kidney and spleen was determined 2 hours later. A strong decrease of the leucine incorporation was observed in the liver and spleen after oral glyoxal application and in the liver after intravenous injection.

Ref.: Ueno et al., 1991a

Glyoxal, which reacts with amino groups of proteins, with nucleotides, and with lipids, is considered an important intermediate in the formation of advanced glycation end-product (AGE). AGE modification alters protein function and inactivates enzymes, resulting in disturbance of cellular metabolism, impaired proteolysis, and inhibition of cell proliferation and protein synthesis (Gan & Ansari, 1986; Ueno et al., 1991a,b; Kasper et al., 1999; Witowski et al., 2000; Bulteau et al., 2001; Kasper & Funk, 2001; Murata-Kamiya & Kamiya, 2001). The extent of AGE modification increases with the increasing life span of proteins. Consequently, AGEs are especially associated with long-lived proteins, such as collagens, lens crystalline, and neurofilaments, but also have been identified in shorter-lived proteins, including haemoglobin, plasma proteins, lipoproteins, and intracellular proteins. AGEs have a role in the pathogenesis or progression of many pathological conditions — e.g., diabetes, Alzheimer's disease and other neurodegenerative diseases, chronic inflammatory diseases, arthritis, atherosclerosis, vascular damage, cataract formation and skin changes during ageing, pulmonary fibrosis, and renal failure — as well as in peritoneal dialysis complications (Miyata et al., 1999, 2000; Thornalley et al., 1999; Cerami & Ulrich, 2001; Ulrich & Cerami, 2001; Thornalley, 2002). Although glyoxal is known to be an intermediate in the formation of AGEs, these effects have not specifically been shown for glyoxal.

Endogenous Glyoxal

Glyoxal is endogenously produced during normal cellular metabolism by a multitude of enzyme independent pathways, such as the spontaneous reaction of amino groups in proteins with reducing sugars (Maillard reaction), sugar auto-oxidation, DNA oxidation, peroxidation of polyunsaturated fatty acids, UV-photodamage and in conditions of oxidative stress and depletion of GSH. Furthermore, glyoxal is a product of the metabolism and microsomal oxidation of compounds such as glycoaldehyde, ethylene glycol and β-hydroxy-substituted N-nitrosamines (Abordo et al., 1999; Kasai et al., 1998; Thornalley et al., 2000).

In biological materials, less than 10% of the glyoxal concentration is present as unbound form in aqueous solution (free glyoxal, monohydrate, dehydrate) as most of the reactive carbonyl groups are reversibly bound to cysteinyl, lysyl and arginyl residues of proteins (Thornalley, 1995).

3.3.13. Safety evaluation (including calculation of the MoS)

3.3.13.1 Effects of concern

Glyoxal is directly genotoxic *in vitro* in bacterial and mammalian cells, inducing, for example, DNA adducts, mutations, chromosomal aberrations, DNA repair, sister chromatid exchanges, and DNA single strand breaks. *In vivo*, a genotoxic activity of glyoxal was detected at the site of application in the pyloric mucosa of rats by demonstration of unscheduled DNA synthesis and DNA single strand breaks. After oral application, DNA strand breaks were further observed in rat liver. The Lowest Effective Dose (LED) inducing unscheduled DNA synthesis in the pyloric mucosa of the rat stomach after gavage was 50 mg/kg bw. This effect could be due to a direct effect not mediated by systemic absorption. LED for DNA single strand break in the liver after gavage was 200 mg/kg bw. No DNA damage was found in the kidney, spleen, pancreas, or lung even at 1000 mg/kg bw (Ueno et al., 1991c)

No carcinogenic effect is detected in mice after dermal application of glyoxal over the entire life span. Glyoxal possesses no tumour initiating effect after the dermal administration to mice. Oral administration of glyoxal after initiation with MNNG was associated with increased incidences of adenocarcinomas and hyperplasias in both pylorus and fundus of rat stomach.

A 28-day study in which glyoxal was administered to rats in drinking-water resulted in a no-observed-adverse-effect level (NOAEL) of 100 mg glyoxal/kg body weight per day (It is not clear from the report if the actual glyoxal dose was calculated as 40% glyoxal. Thus, the NOAEL could be 40 mg/kg bw/day). The 90-day feeding of glyoxal to rats resulted in a NOAEL of 127 mg/kg bw/day (dosage corresponding to 100% glyoxal). Effects stated at higher dosages in the two studies above were retardation of body weight gain. In a study examining more sensitive end-points (serum clinical biochemistry), the lowest tested dosage of 107 mg/kg bw/day (99% glyoxal) corresponded to the lowest-observed-adverse-effect level (LOAEL) for a 90-day exposure of rats via drinking-water.

No dose-dependent effects on reproductive organs were observed in repeated dose toxicity studies lasting for a sufficient period of 90 days up to a dose of approx. 250 mg/kg bw (related to pure active ingredient).

A prenatal developmental toxicity study performed according to current testing guidelines and under GLP conditions showed a NOAEL of 125 mg/kg bw for prenatal developmental toxicity. This was the highest investigated dose level. The NOAEL for maternal toxicity was achieved at 25 mg/kg bw in this study related to pure active ingredient.

Glyoxal is irritating to mucous membranes and acts as a skin sensitising agent in humans and experimental animals.

3.3.13.2 EU Classification

Glyoxal is listed on Annex I of Directive 67/458/EEC in the EU chemical legislation (since 26th – 28th ATP; Annex I Index# 605-016-007) with a classification as:
Xn; Muta. Cat. 3; R68 Possible risk of irreversible effects. Concentration limit for labelling 1%

Xi; R43 May cause sensitization by skin contact. Concentration limit for labelling 1%

Xn; R36/38 Irritating to eyes and skin. Concentration limit for labelling 10%

3.3.13.3. Use of Extrapolation Factor for *in vivo* genotoxicity

For the calculation of the extrapolation factor for glyoxal, it is considered that the glyoxal content of cosmetics is at a maximum of 100 ppm (i.e. 0.01%).

The SCCNFP calculated a **global daily exposure value** for all cosmetic products that one person may daily apply on the skin. In a worst-case scenario, considering that consumer would use a set of cosmetic products containing the same ingredient, the SCCNFP-value of **17.79 g/day** will have to be used in the calculation of the MoS [SCCNFP/0321/00]. This will correspond to a maximum glyoxal exposure of 1.8 mg/day.

Since data for *in vivo* or *in vitro* dermal absorption are missing, 100% absorption has to be used for the calculations according to the requirements of the SCCP as laid down in the document SCCNFP/0321/00.

No quantitative or semi-quantitative hazard characterisation methods are currently in use for regulatory purposes of mutagens. Mutagens demonstrated to be carcinogenic are in general regulated on the basis of their carcinogenicity since the carcinogenic effect is considered to be more critical than germ cell mutagenesis. In the case of mutagens where no carcinogenicity studies are available, no safe level of exposure can be assessed. However, the recent publication of Sanner and Dybing (2005) demonstrating a linear relationship between lowest effective dose (LED) for *in vivo* genotoxicity and the carcinogen dose descriptor T25 in cases of mutagens with carcinogenic activity identified in IARC Monographs, may offer a pragmatic approach for a semi-quantitative risk assessment.

Using the above approach, a linear relationship between LED and T25, and an extrapolation factor of 25,000 would correspond to a lifetime cancer risk of 10^{-5}.

Maximum amount of ingredient applied (I)	mg	=	1.8
Typical body weight of human	kg	=	60
Maximum absorption through the skin (A)	%	=	100
Systemic exposure (mg)	IxA/100	=	1.8
Systemic exposure dose (SED) (mg/kg bw)	IxA/100 x 60 kg	=	0.03
LED unscheduled DNA assay liver	LED mg/kg bw	=	200

Extrapolation factor = 25,000	LED / 25000	=	0.008 mg/kg bw

The calculated systemic exposure dose (SED) is 4 times higher than the dose corresponding to a life time cancer risk of 10^{-5} (0.03/0.008 = ± 4). In the case of glyoxal, the dermal absorption is probably significantly less than 100% as used in the calculation above. Moreover, only in one *in vivo* experiment it was possible to demonstrate that DNA damage had occurred in an organ not in direct contact with glyoxal. In addition, due to the metabolism and high reactivity of glyoxal the systemic concentration of glyoxal is likely to be rather low. Under these conditions, the cancer risk may be considerably less than anticipated from the above semi-quantitative calculation.

Thus, on the basis of the above reasoning the use of 100 ppm glyoxal in cosmetic products may be considered safe even though SED divided by the dose corresponding to the life time cancer risk of 10^{-5} (LED/extrapolation factor) is greater than 1.

3.3.13.4. Evaluation of the safety of glyoxal in cosmetics

The presence of up to 100 ppm of glyoxal in cosmetics will result in a maximum skin dose of 1.8 mg/day. Since data on dermal absorption are lacking, 100% absorption is used in the considerations. Thus, the systemic dose is considered to be ([1.8 mg/day]/60 kg) 0.03 mg/kg bw/day.

The evaluation of the safety of glyoxal is based on three elements.

Glyoxal is endogenously produced during normal cellular metabolism. The concentration of glyoxal in blood plasma from normal healthy subjects is estimated to be approximately 0.1 µmol/l or 0.006 mg/l. Since less than 10% is present in unbound form, it is unlikely that glyoxal from cosmetics will significantly enhance this value.

A worst case food/drink intake of glyoxal is calculated to be 10 mg/day (0.17 mg/kg bw/d), and the tolerable intake is given as 0.2 mg/kg bw/d for lifetime oral exposure. The worst case systemic dose from cosmetics represents less than 20% of the oral exposure.

As discussed above in Section 3.3.13.3 any risk of a possible carcinogenic effect of 100 ppm glyoxal is considered to be negligible.

3.3.14. Discussion

Anhydrous glyoxal has a melting point of about 15 °C. However, it is generally available as an aqueous solution (typically containing 30-50% glyoxal) in which hydrated oligomers are present. Glyoxal is used as a chemical intermediate in the production of pharmaceuticals and dyestuffs, as a cross-linking agent in the production of a range of different polymers, as a biocide, and as a disinfecting agent. Releases to the environment are primarily emissions to ambient air and water.

The main routes of occupational exposure to glyoxal during use as a disinfectant are via inhalation of aerosol and dermal absorption. The general population is exposed mainly through ingestion of glyoxal-containing food, glyoxal-containing cosmetics, but could be exposed through polluted air in urban regions and through traces of glyoxal in drinking-water.

Glyoxal is endogenously produced during normal cellular metabolism by a multitude of enzyme-independent pathways. Glyoxal is also a product of the metabolism and microsomal oxidation of other compounds, such as glycolaldehyde, ethylene glycol, and *beta*-hydroxy-substituted *N*-nitrosamines. The concentration of glyoxal in human blood plasma has been reported to be 0.1-1 µmol/litre, with higher levels reported for patients with diabetes or renal failure. In biological materials, less than 10% of the glyoxal present is in unbound forms in aqueous solution (free glyoxal and hydrates), as most of the reactive carbonyl groups are reversibly bound to cysteinyl, lysyl, and arginyl residues of proteins.

The acute toxicity of glyoxal in experimental animals is low to moderate. In rats, for 40% glyoxal, the LC_{50} for a single 4-h inhalation of aerosol is 2440 mg/m^3, the oral LD_{50} value ranges from 3000 to 9000 mg/kg body weight (with higher sensitivity in females), and dermal LD_{50} values are >2000 mg/kg body weight. After inhalation exposure, local irritations of the eyes and respiratory organs as well as hyperaemia and foamy secretion in the lungs predominate. After

oral exposure to glyoxal, macroscopic observations include irritations of the gastrointestinal tract and congestion in the gastrointestinal tract, lung, kidney, and adrenal glands. In the prominent target organs, pancreas and kidney, the toxic action of glyoxal leads to severe degenerative changes resembling those induced during diabetes.

In animal studies, 30% and 40% aqueous glyoxal caused slight to definite skin irritations, depending on the application time. Glyoxal is irritating to mucous membranes and acts as a skin sensitising agent in humans and experimental animals.

A 28-day study in which glyoxal was administered to rats in drinking-water resulted in a no-observed-adverse-effect level (NOAEL) of 100 mg glyoxal/kg body weight per day (It is not clear from the report if the actual glyoxal dose was calculated as 40% glyoxal, Thus, the NOAEL could be 40 mg/kg bw/day). The 90-day feeding of glyoxal to rats resulted in a NOAEL of 127 mg/kg bw/day (dosage corresponding to 100% glyoxal). Effects stated at higher dosages in the two studies above were retardation of body weight gain. In a study examining more sensitive end-points (serum clinical biochemistry), the lowest tested dosage of 107 mg/kg bw/day (99% glyoxal) corresponded to the lowest-observed-adverse-effect level (LOAEL) for a 90-day exposure of rats via drinking-water.

No dose-dependent effects on reproductive organs were observed in repeated dose toxicity studies lasting for a sufficient period of 90 days up to a dose of approx. 250 mg/kg bw (related to pure active ingredient).

A prenatal developmental toxicity study performed according to current testing guidelines and under GLP conditions showed a NOAEL of 125 mg/kg bw for prenatal developmental toxicity. This was the highest investigated dose level. The NOAEL for maternal toxicity was achieved at 25 mg/kg bw in this study related to pure active ingredient.

Glyoxal is directly genotoxic *in vitro* in bacterial and mammalian cells, inducing, for example, DNA adducts, mutations, chromosomal aberrations, DNA repair, sister chromatid exchanges, and DNA single strand breaks. *In vivo*, a genotoxic activity of glyoxal was established at the site of application in the pyloric mucosa of rats by demonstration of unscheduled DNA synthesis and DNA single strand breaks. After oral application, DNA strand breaks were further observed in rat liver.

No carcinogenic effect is detected in mice after dermal application of glyoxal over the entire life span. Glyoxal possesses no tumour initiating effect after the dermal administration to mice. Oral administration of glyoxal after initiation with MNNG was associated with increased incidences of adenocarcinomas and hyperplasias in both pylorus and fundus of rat stomach.

In a risk assessment for the general population, an exposure scenario has been compiled as a hypothesized worst case. Using the daily intake of, maximally, 10 mg glyoxal via food, an estimated intake of 0.17 mg glyoxal/kg body weight per day can be calculated. This is similar to the tolerable intake of about 0.2 mg/kg body weight per day for lifetime oral exposure to glyoxal. The worst case systemic dose from cosmetics represents less then 20% of the above calculated oral exposure.

4. CONCLUSION

In response to the questions asked, the SCCP is of the opinion that:

* On the basis of provided data any risk to consumers when glyoxal is present up to 100 ppm in cosmetic products is considered to be negligible.

* The SCCP does not recommend any further restrictions with regard to its presence in cosmetic products.

5. MINORITY OPINION

Not applicable

6. REFERENCES

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