FOREWORD INTRODUCTION

# *GLYOXAL*

## *CAS N°: 107-22-2*

**SIDS INITIAL ASSESSMENT PROFILE**

| | |
|---|---|
| **CAS No.** | 107-22-2 |
| **Chemical Name** | Glyoxal |
| **Structural Formula** | HC(=O)-C(=O)H |

**RECOMMENDATIONS**

**Human Health:** The chemical is a candidate for further work.

**Environment:** The chemical is currently of low priority for further work.

**SUMMARY CONCLUSIONS OF THE SIAR**

**Human Health**

The acute toxicity of glyoxal ranged from low to harmful in animal experiments performed with different species, depending on the active ingredient concentration of the tested product. Glyoxal 40% has a moderate toxicity by the oral route, a low toxicity by the dermal route and a moderate toxicity by inhalation. Glyoxal causes slight to definite skin irritations depending on the exposure duration. Irritations up to necrotic changes have been described on the rabbit eye. It acts as a sensitizer to the skin of guinea pigs and humans.

In a subacute inhalation study on rats for 29 days, a 40% glyoxal aerosol concentration of 10 and 2 mg/m$^3$ results in a minimum squamous metaplasia of the epiglottal epithelium in the larynx. A NOEL of 0.4 mg/m$^3$ is given for local effects and of > 10 mg/m$^3$ for the systemic toxicity. In a 28 day oral study in rats, a NOEL of 100 mg/kg bw/d (40% glyoxal) was determined. A dose related decrease of the water, food consumption and body weight were noted at 300 mg/kg and 1000 mg/kg. Variations of some haematological and blood parameters occurred at these doses. No macrocopic and microscopic pathological findings were seen that were considered to be compound related. In a 90d feeding study in rats, glyoxal in daily doses of ca. 30 to 250 mg/kg bw/d is tolerated without clinical, macroscopic and histopathological changes. A temporary reduced body weight gain and an increase of the relative liver and kidney weights, without any histopathological correlation, have been observed only in the males of the highest dose group. The NOEL is ca. 125 mg/kg bw/d related to 40% glyoxal. In 90-d drinking water studies with male rats with daily doses of ca. 140, 290 and 370 mg/kg bw/d, a decreased food and water intake as well as retarded body weight gain was found in the highest dose group. The glyoxalase activities in the liver, kidney and erythrocytes are increased, while the aspartate aminotransferase activity, the alanine aminotransferase and lactate dehydrogenase activities as well as the albumin and total protein value are also determined in the low dose group. The LOEL lies at 107 mg/kg bw/d related to pure glyoxal. Overall, a NOEL of 100 mg/kg bw/d related to 40% glyoxal (40 mg/kg bw/d related to active ingredient) can be retained for repeated dose toxicity.

Glyoxal is shown to be mutagenic in *in vitro* genotoxicity studies in prokaryotes and eukaryotes. *In vivo*, glyoxal is proven to be negative in the micronucleus test on the mouse after oral administration. On Drosophilia melanogaster, glyoxal is proven to be negative in the sex-linked

recessive-lethal test, in the dominant-lethal test and in the studies on the reciprocal translocation and on the loss of sex chromosomes. Chromosome aberrations in the duodenum, testes and spleen are described in an older, only insufficiently documented study after subcutaneous administration to rats. After oral administration to the rat, a significant increase of the unscheduled DNA synthesis is found in the pyloric mucosa, but not in primary hepatocytes, as well as an increase of DNA single-strand breaks in the liver and in the pyloric mucosa. These findings indicate that glyoxal reacts at the point of entry (the stomach) and immediately downstream (the liver), but not in more remote organs.

No dose-dependant effects were found on reproductive organs in repeated dose studies up to a dose of approx. 300 mg/kg bw/d (related to the active ingredient). Furthermore, a NOAEL of 125 mg/kg bw/d (related to the active ingredient) could be derived for prenatal development toxicity and of 25 mg/kg bw/d for maternal toxicity.

No carcinogenic effect is detected in mice after dermal application of glyoxal over the entire life span. Glyoxal possesses no tumor initiating effect after the dermal administration to mice After oral administration, glyoxal exhibits local tumor promoting properties in the mucosa of the forestomach of the rat (tissue not existing in other species or man). In a liver promotion model on the rat, no indications were found for a promoting effect of glyoxal through systemic action. Finally, glyoxal is a metabolite of ethylene glycol and there are two negative carcinogenic studies on ethylene glycol (rats and mice).

**Environment**

Glyoxal is not volatile and is not expected to accumulate in biota or soil/sediment. It is clearly readily biodegradable.

In short-term tests with fish, daphnids and algae the following results were found: *Pimephales promelas*: 96 h-LC50 = 215 mg/l; *Daphnia magna*: 48h-EC50 = 404 mg/l; *Scenedesmus subspicatus*: 96h-EC50 > 500 mg/l. With an assessment factor of 1000 a PNECaqua of 215 µg/l can be calculated from the LC50 for fish (The results refer to the 40% aqueous solution). For the active ingredient, the results are *Pimephales promelas*: 96 h-LC50 = 86 mg/l; *Daphnia magna*: 48h-EC50 = 161 mg/l; *Scenedesmus subspicatus*: 96h-EC50 > 200 mg/l; PNEC = 86 µg/l).

**Exposure**

The worldwide production volume of glyoxal is estimated to be approx. 120000 to 170000 t/a. Glyoxal is commercialized as a 40% aqueous solution. Glyoxal is mainly used as a chemical intermediate and also for a small part as an active ingredient in disinfectant products in preparation with other components (formaldehyde, glutaraldehyde, quaternary ammonium).

**NATURE OF FURTHER WORK RECOMMENDED**

**Human health**: Taking into account the skin irritation, the skin sensitising properties and the genotoxic potential and, based on the use pattern of glyoxal, a detailed risk assessment would be necessary. Especially the risks based on the exposure from open uses (e.g. as a disinfectant) should be evaluated.

**Environment**: No further work is necessary

## SIDS SUMMARY

| CAS-NO.: 107-22-2 | | | PROTOCOL | RESULTS |
|---|---|---|---|---|
| PHYSICAL CHEMICAL | | | | |
| 2.1 | Melting Point | 40% aq. sol. | NA | -14°C |
| | | Anhydrous * | NA | 15°C |
| 2.2 | Boiling Point | 40% aq. sol. | NA | 104° C (at 101.3 kPa) |
| | | Anhydrous * | NA | 50.4°C (at 101.3 kPa) |
| 2.3 | Density | 40% aq. sol. | NA | 1270 $kg/m^3$ |
| | | Anhydrous * | NA | 1140 $kg/m^3$ |
| 2.4 | Vapour Pressure | Anhydrous * | NA | < 0.01Pa at 20°C |
| | | 40% aq.sol | NA | 1800 Pa at 20°C |
| 2.5 | Partition Coefficient (Log Pow) | Anhydrous * | calculated | - 1.65 |
| 2.6 A | Water solubility | | NA | miscible at 20°C |
| ENVIRONMENTAL FATE / BIODEGRADATION | | | | |
| 3.1.1 | Photodegradation | | calc. (Atkinson) | In air $T_{1/2}$ = 15.2 hour |
| 3.3 | Transport and Distribution | | calculated | In air % |
| | | | (fugacity | In water 100 % |
| | | | level 1 type) | In sediment % |
| | | | | In soil % |
| | | | | In biota % |
| | | Koc | HPLC (40% aqueous solution) | Koc = 2.1 l/kg |
| 3.5 | Biodegradation | | OECD 301 (several tests) | readily biodegradable |
| | | | OECD 303 | 82% elimination after 6 days |

* for information only, anhydrous glyoxal is not stable and is not marketed

| CAS-NO.:107-22-2 | | SPECIES | PROTOCOL | RESULTS |
|---|---|---|---|---|
| ECOTOXICOLOGY | | | | |
| 4.1 | acute/prolonged toxicity to fish | Pimephales promelas | US-EPA | $LC_{50}$ (96 hr) = 215 mg/l (i.e. 86 mg/l related to active substance) |
| 4.2 | acute/prolonged toxicity to aquatic invertebrates (daphnia) | Daphnia magna | 79/831/EEC, C.2 | $EC_{50}$ (48 hr) = 404 mg/l (i.e. 162 mg/l related to active substance) |
| 4.3 | toxicity to aquatic plants e. g. algae | Scenedesmus subspicatus | UBA | $EC_{50}$ (96 hr) > 500 mg/l<br>EC20 (96 hr) = 345 mg/l (i.e. >200 resp 138 mg/l related to active substance) |
| 4.4 | toxicity to microorganisms | Pseudomonas putida | DIN 38412 | $EC_{50}$ (16 hr) > 102 mg/l<br>EC10 (16 hr) = 57 mg/l (i.e. 41 resp 23 mg/l related to active substance) |
| TOXICOLOGY | | | | |
| 5.1.1 | acute oral toxicity | rat | NA (several tests) | $LD_{50}$ = 640-8979 mg/kg |
| | acute dermal toxicity | Rat, rabbit, guinea pig | NA (several tests) | $LD_{50}$ > 2000 mg/kg |
| 5.1.2 | acute inhalation toxicity | rat | NA | $LC_{50\ (4h)}$ > 2.4 mg/l |
| | Skin irritation | Rabbit | OECD 404 | negative |
| | Eye irritation | Rabbit | OECD 405 | positive |
| | Sensitisation | Guinea pig | NA | positive |
| 5.4 | repeated dose toxicity | rat | OECD 412 (inhalation) | NOEL (29d) = 0.4 mg/l (40% aqueous solution) |
| | | Rat | NA | NOEL (90d) = 125 mg/kg bw/d (40% aqueous solution) |
| | | Rat | OECD 407 | NOEL (28d) = 100 mg/kg bw/d (40%aqueous solution) |
| 5.5 | genetic toxicity in vitro | | | |
| A. | bacterial test (gen mutation) | Prokaryotes & eukaryotes | many | + (with metabolic activation)<br>+ (without metabolic activation) |
| | Genetic toxicity in vivo | | Micronucleus test | negative |
| | | Drosophilia melanogaster | Many | negative |
| 5.8 | toxicity to reproduction | | | NOAEL = mg/kg (rep. tox. parental, rats) |
| 5.9 | developmental toxicity / teratogenicity | rat | OECD 414 | NOAEL = 25 mg/kg (maternal toxicity)<br>NOAEL = 125 mg/kg (foetal data) (active ingredient) |
| 5.10 | Carcinogenicity | Mouse | dermal application | negative |
| 5.11 | experience with human exposure | | | sensitizing |

# SIDS INITIAL ASSESSMENT REPORT

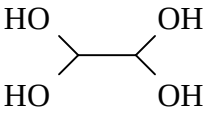
## 1. GENERAL SUBSTANCE INFORMATION

### Identity

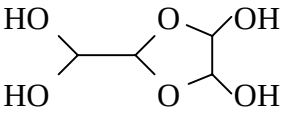
| | |
|---|---|
| Chemical name: | Glyoxal |
| CAS-Nr.: | 107-22-2 |
| EINECS Nr.: | 203-474-9 |
| Synonyms | 1,2-Ethanedione<br>Ethandial |
| Empirical Formula: | C2H2O2 |
| Structural Formula: | HC(=O)-C(=O)H |

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

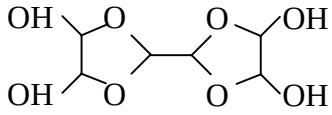
In aqueous solution, the hydrated monomer (ethane bis-gemdiol) (I) is the main form of glyoxal in aqueous solution. However this gemdiol tends to polymerise to acetals-semiacetals, the presence of which depends on both the pH and the concentration of the solution. The main oligomeric forms are the dioxolane dimer (II) and the bis(dioxolane) trimer (III).




(I)




(II)




(III)

If highly concentrated, oligomeric forms precipitate (polyglyoxal). 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 content amounts to as little as 11% of glyoxal, the dimer and trimer forms being dominant.

(Mattioda & Blanc, 1989, Chastrette et al., 1983, Fratzke et al., 1986, Slonim et al., 1990).

In the environment, at low concentrations, it can be assumed that only the monomer is present.

Impurities:

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:

- traces of formic acid, acetic acid, glyoxylic acid and glycolic acid: total acid impurities: ca. 1500 ppm
- formaldehyde: < 200 ppm

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

- traces of organic acids: ca. 1000-2000 ppm
- 1,2-ethanediol: 15000 ppm
- hydroxyacetaldehyde: 5000 ppm

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

**Physico-chemical properties**

Anhydrous glyoxal is a liquid at ambient temperature; it crystallises at 15 °C in the form of yellow prismatic crystals and boils at 50.4 °C, giving off green vapours with a pungent odour. It has a vapour pressure of approx. 0.1 Pa and is miscible in water. Glyoxal is commercialised as a 40% aqueous solution. The 40% aqueous solution has a melting point of –14°C, a boiling point of 104 °C and a vapour pressure of 1800 Pa at 20 °C, the latter reflecting mainly the properties of water. The octanol-water partition coefficient cannot be experimentally determined due to its reaction with octanol. The calculated LogKow value for anhydrous glyoxal is -1.65. For ethane bis-gemdiol, the dimer as well as the trimer, the calculated values are respectively -1.29, -1.45 and -2.55.

## 2. GENERAL INFORMATION ON EXPOSURE

The worldwide production volume is estimated to approx. 120000 to 170000 t/a

The bifunctionality of glyoxal is used to cross-link functionalized macromolecules such as cellulose, polyacrylamides, polyvinyl alcohol, keratin and other polycondensates. For example, glyoxal is used as a cross-linking agent for imparting wet strength to coated paper. With cellulose, unstable hemiacetals are obtained in the cold, which irreversibly form acetals when heated in the presence of acid catalysts.

Glyoxal is also the 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 for imparting crease resistance to textiles, ion exchange resins, and cross-linking agents. Glyoxal bisulfite is used as a resist agent in printing with reactive dyes and as a levelling agent in dyeing polyamide with acid dyes.

The reducing properties of glyoxal are used in the photographic industry and in glassmaking for the production of silvered glass mirrors.

Glyoxal has bactericidal properties comparable with those of glutaraldehyde and is used as a bactericide in preparation with other components (formaldehyde, glutaraldehyde, quaternary ammonium).

The use pattern of glyoxal, by order of importance is presented in table 2.1. As the number of producers is very low, precise figures can not be presented in this document due to confidentiality.

**Table 2.1**: Use pattern of glyoxal

| **Description** | **Industry category** | **Use category** |
|---|---|---|
| chemical intermediate for pharmaceuticals and dyestuff etc. | IC = 3: Chemical industry: chemicals used in synthesis | UC = 33: chemical intermediates |
| cross-linking agent for textiles | IC = 13: textile processing agent | UC = 43: process regulators |
| manufacture of reactant resins in the textile industry | IC = 3: Chemical industry: chemicals used in synthesis | UC = 33: chemical intermediates |
| in coating bath for offset and special papers (as such or in form of reactant resins) | IC = 12: pulp, paper and board industry | UC = 2: adhesive, binding agents |
| anti-lump treatment of cellulose ethers | | |
| component of adhesives and coatings | | |
| H2S scavenger in crude oil and gas industry (deodorising agent) | IC = 9: Mineral and fuel industry | UC = 43: process regulators |
| cleaning agent and biocide for household and hospital disinfection | IC = 5: personal/domestic | UC = 39: biocides, non-agricultural |
| reducing agent in photographic industry | IC = 10: Photographic industry | UC = 44: reducing agents |

## 3. ENVIRONMENT

### 3.1 Environmental behaviour and occurrence

**Degradation**

Hydrolysis

Anhydrous glyoxal immediately reacts with water to form ethane bis-gemdiol, which is stable in water. Polymerisation to acetals-semiacetals is possible, depending on concentration and pH.

Biodegradation

Results on biodegradation are available, showing that glyoxal is clearly readily biodegradable:

- 90 % biodegradation after 28 days according to OECD guideline 301 D (not clear whether the 10-day-window criterion was met) (Gerike & Gode, 1990)
- 95 % biodegradation after 28 days according to OECD guideline 301 E (10-day-window criterion was clearly met) (Hoechst AG, 1991)

- 65% biodegradation after 14 days according to OECD guideline 301 C (CITI, 1992)

Furthermore, as expected, high removal rates (> 90%) have been determined in several tests on inherent biodegradability (Zahn-Wellens-tests).

In a Coupled-Units-Test (OECD Guideline 303A). A mean elimination of 82% was reached after 6 days of operation (BASF AG, 1996b).

Results from biodegradation simulation tests in surface water and soil are not available and have to be estimated based on the above described screening test and the partition behaviour of glyoxal (CEC, 1996):

| **compartment / medium** | **biodegradation rate** |
|---|---|
| activated sludge (WWTP) | $k_{WWTP} = 1\ h^{-1}$ |
| surface water | $k_{sw} = 0.047\ d^{-1}$ |
| sediment | $k_{sed} = 0.0023\ d^{-1}$ * |
| soil | $k_{soil} = 0.023\ d^{-1}$ * |

* Values taking into account the partition behaviour in these compartments (cf. below)

Photooxidation

As glyoxal is commercialized in a 40% aqueous solution, and given the low Henry's law constant (see below), the reaction with the photochemically produced hydroxyl radicals is probably not an important fate process. Based upon atmospheric concentrations of $5 \cdot 10^5$ ·OH/$cm^3$ the atmospheric half-life of anhydrous glyoxal has been estimated to be 15.2 hours.

**Distribution and accumulation**

The Henry's law constant of $H < 33.7 \cdot 10^{-6}$ Pa.$m^3$/mol at 15-45 °C for the hydrated form suggests that glyoxal is not volatile from aqueous solutions.

An experimental Koc according to the HPLC method was determined to be 2.1 l/kg with a 40% aqueous solution (BASF AG, 1996a). This value indicates that the compound is mobile in soil and that it has a low potential for accumulation in soil. No relevant partitioning to soil, sediment, suspended matter or sewage sludge is expected.

No experimental results on bioaccumulation are available. The estimated logPow of < 0 indicates a low potential for bioaccumulation.

According to a level I fugacity model, the hydrosphere is the main target compartment of glyoxal:

| | |
|---|---|
| Air | 0 % |
| Water | 100.00 % |
| Sediment | 0 % |
| Soil | 0 % |
| Biota | 0 % |

Assuming even emission to air, soil and water, with the above estimated half-lives, a level III fugacity model gives the following results:

| | |
|---|---|
| Air | 0.1 % |

| | |
|---|---|
| Water | 45.6 % |
| Sediment | 0.1 % |
| Soil | 54.2 % |

These results would tend to indicate that, if released to air, the substance rapidly partitions to soil and water, and that if released to soil or water, the substance will mostly remain in these compartments and degradation prevents partitioning from one compartment to the other.

Based on the use pattern of glyoxal and the physical chemical properties, the substance will mainly be released to water and it can be assumed that partitioning to other compartments is low.

Elimination in WWTPs

Based on the above cited physical chemical properties (log H < -4.5; logPow <0), as well as the biodegradation rate of 1 $h^{-1}$ in STP, the elimination through biodegradation and distribution can be estimated with the model SIMPLETREAT:

| | |
|---|---|
| % to air | 0 |
| % to water | 13 |
| % to sludge | 0 |
| % degraded | 87 |
| % removal | 87 |

These estimations are coherent with the results from a STP simulation test according to the Coupled-Units-Test (OECD Guideline 303A). A mean elimination of 82% was reached after 6 days of operation (BASF AG, 1996b).

**Monitoring data**

Surface water concentrations have been measured in Japan in 1980. In one study, 18 samples from 5 rivers were analysed. The concentrations ranged from 1.07 to 2.6 µg/l. In another study, glyoxal was detected in 20 out of 33 samples with concentrations of 1 to 6 µg/l.

Glyoxal has a low tendency to partition to the sediment. Nevertheless, sediment concentrations have been measured in Japan in 1980. In one study, 10 samples from 2 rivers were analysed. The concentrations ranged from 1.12 to 12.6 mg/kg dw. In another study, glyoxal was detected in 29 out of 33 samples at concentrations of 0.06 to 2.8 mg/kg dw.

## 3.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

### 3.2.1 Aquatic compartment (incl. sediment)

**Available effect data**

In the following, the most relevant results from acute toxicity tests with aquatic organisms are presented:

vertebrates:

| | | |
|---|---|---|
| *Leuciscus idus* | 96h-LC50 | 460-680 mg/l |

(static; nominal concentrations related to 40% aqueous solution) (Hoechst AG, 1989a)

*Pimephales promelas* 96h-LC50 215 mg/l
(static; nominal concentrations, not clear whether related to aqueous solution or active ingredient) (Conway et al., 1983)

*Rhombus maximus* 96h-LC50 > 500 mg/l
(static; measured concentrations, not clear whether related to aqueous solution or active ingredient) (Hoechst AG, 1990)

invertebrates:

*Daphnia magna* 48h-EC50 404 mg/l
(effect: immobilisation; static, nominal concentration related to 40% aqueous solution) (BASF AG, 1988)

Another result of 24h-LC50 = 430 mg/l could not be validated because of insufficient documentation (Société Française Hoechst, 1979).

plants:

| | | |
|---|---|---|
| *Scenedesmus subspicatus* | 72h-EbC50 | > 250 mg/l |
| (nominal concentrations related to 40% aqueous solution) | 72h-EμC50 | > 250 mg/l |
| (Société Française Hoechst, 1993) | | |

| | | |
|---|---|---|
| *Scenedesmus subspicatus* | 96h-EbC50 | > 500 mg/l |
| (nominal concentrations related to 40% aqueous solution) | 96h-EbC20 | > 343 mg/l |
| (BASF AG, 1988) | | |

microorganisms:

| | | |
|---|---|---|
| *Pseudomonas putida* | 16h-EC50 | 133.7 mg/l |
| (endpoint: respiration) | 16h-EC10 | 45.9 mg/l |
| (not clear whether related to aqueous solution or active ingredient, Hoechst AG, 1989b) | | |

| | | |
|---|---|---|
| *Pseudomonas putida* | 16h-EC50 | 102 mg/l |
| (endpoint: respiration) | 16h-EC10 | 57 mg/l |
| (related to 40% aqueous solution, BASF AG, 1996c) | | |

Furthermore, the elimination potential of an OECD Confirmatory Test system was not affected up to a concentration of 500 mg/l.

For the active ingredient, the results are *Pimephales promelas*: 96 h-LC50 = 86 mg/l; *Daphnia magna*: 48h-EC50 = 161 mg/l; *Scenedesmus subspicatus*: 96h-EC50 > 200 mg/l; PNEC = 86 µg/l).

**Determination of $PNEC_{microorganisms}$**

The EC10 from the single species test can be used directly as a PNEC. This is confirmed by the result with the OECD Confirmatory Test. In the absence of specific information, it is assumed that all results are related to the 40% aqueous solution.

Therefore: **$PNEC_{microorganisms}$ = 45.9 mg/l (related to the 40% aqueous solution)**
**$PNEC_{microorganisms}$ = 18.4 mg/l (related to the active ingredient)**

**Determination of $PNEC_{aqua}$**

As only acute studies are available for species out of 3 trophic levels, an assessment factor of 1000 can be used to derive a PNEC. In the absence of confirmation, it is assumed that all results are related to the 40% aqueous solution.

Therefore: **$PNEC_{aqua}$ = 215 000 / 1000 = 215 µg/l (related to the 40% aqueous solution)**
**$PNEC_{aqua}$ = 86 µg/l (related to the active ingredient)**

**Sediment**

No experimental results with benthic organisms are available. As glyoxal has a low tendency to partition to the sediment, there is no need for performing an assessment for this compartment.

### 3.2.2 Terrestrial compartment

No data are available.

### 3.2.3 Atmosphere

No data are available.

### 3.2.4 Non compartment specific exposure relevant to the food chain (secondary poisoning)

As glyoxal has a low potential for bioaccumulation (logPow < 0), an assessment of secondary poisoning is not necessary.

## 4. HUMAN HEALTH

The following sections are largely based on the toxicological evaluation performed by BG Chemie, within its Programme for the prevention of health hazards caused by industrial substances (BG Chemie, 1997).

### 4.1 Toxicokinetics and metabolism

*In vitro* glyoxal is presumably metabolized to oxalic acid via glycolic and glyoxylic acids in the rat liver and by haemoglobin (Kun, 1952 ; Francoeur & Denstedt, 1954 ; Hills and Berry, 1967).

It was reported that with glutathione, glyoxal is converted *in vitro* to S-glycoloyl-glutathione by means of glyoxalase I (no further details ; Racker, 1952).

The mode of action of glyoxal is likely to be related to the chemically reactive nature of glyoxal as it contains two stronly electrophylic groups. Glyoxal forms stable adducts with guanosine by reaction with the N-1 as well as with the exocyclic nitrogen of guanine (see section on genotoxicity *in vitro*).

### 4.2 Acute toxicity

The acute toxicity of Glyoxal ranged from low to harmful in animal experiments performed with different species, depending on the active ingredient concentration of the tested product.

The $LD_{50}$ and $LC_{50}$ values specified in the literature are listed in table 4.1. The studies were generally performed with the 30 % or 40 % marketed aqueous solutions. But some studies have been performed with glyoxal 20 %. Thereby, it was often unclear, if the values where based on the examined commercial solutions or the active ingredient.

With 40 % glyoxal the toxicity is moderate and $LD_{50}$ values are of 640 to 8979 mg/kg weight specified for the rat after oral administration. The oral $LD_{50}$ values for the mouse was 4064 mg/kg. After dermal application, the LD50 values for the 40 % glyoxal solution were > 2000 mg/kg body weight in the rat, 12700 mg/kg body weight in the rabbit and > 5000 mg/kg body weight in the guinea pig and so, the toxicity is low. $LC_{50}$ values of 2440 and > 1300 mg/$m^3$ were reported in the rat after 4-hour inhalation to 40 % glyoxal. The product was thus shown to be harmful after inhalation. In inhalation hazard tests on rats, all the applied animals survived 7- or 8-hour exposures to 30 % or 40 % glyoxal to rats and mice, respectively.

After oral and intraperitoneal administration, the rat, mouse, guinea pig, rabbit and cat showed the following intoxication symptoms : 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, atonia and abnormal posture (crouching posture, high-legged posture) as well as lying on the abdomen or side (BASF, 1954; 1963a, 1963b, 1985a). 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 non findings (BASF, 1954). 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 (BASF, 1954, 1963b, 1985a).

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 (Hoechst, 1984b). The autopsy of the rats, that died within 9 days after terminating the inhalative exposure to 40 % glyoxal, showed hyperaemia as well as a foamy secretion in the lung (Hoechst, 1984b). With the exposure to a glyoxal vapour-saturated atmosphere, only irregular breathing was observed (Hoechst, 1984 d, e). 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 (BASF, 1963a; Hoechst, 1984b).

Conclusion:

In tests on acute toxicity, glyoxal has shown to be of low toxicity to harmful in animal experiments performed with different species depending on the active ingredient concentration of the tested product. Glyoxal 40% has a moderate toxicity by oral route, a low toxicity by dermal route and a moderate toxicity by inhalation. The main effects are gastric irritation and kidney damage after oral administration and respiratory tract irritation from aerosol inhalation.

**Table 4.1. Acute toxicity of glyoxal after, oral, dermal, inhalative and intraperitoneal administration**

| Species, strain, sex* | Route of administration | Dosage or concentration (mg/kg body weight or mg/m$^3$)** | Effect | Observation period | Reference |
|---|---|---|---|---|---|
| **20 % Glyoxal** | | | | | |
| Rat, Sprague-Dawley, male, female | Oral | 1 680 | $LD_{50}$ ; diarrhoea, weakness ; macroscopic findings : haemorrhagic lung, liver, heart ; inflamed gastrointestinal tract | No data | NTP (1988) |
| **30 % Glyoxal** | | | | | |
| Rat, Carworth-Farms, male | Oral*** | 7.46 ml/kg body weight (according to the authors : 2.200 mg pure active ingredient/kg body weight) | $LD_{50}$ ; at 8 ml/kg macroscopic findings : congestion of lungs, gastrointestinal tract and adrenal glands, patchy livers, pale kidneys | 14 days | Smyth et al., 1962 |
| Mouse | Oral | Ca. 5.0 ml/kg body weight | $LD_{50}$ ; apathy, reeling, dyspnoea | 7 days | BASF, 1963a |
| Rat | Oral | Ca. 4700 mg/kg body weight | $LD_{50}$ ; disturbances of balance, apathy | No data | BASF, 1954 |
| Mouse | Oral | Ca. 3300 mg/kg body weight | LD50 ; disturbances of balance, apathy | no data | BASF, 1954 |
| Rabbit | Oral | Ca. 1700 mg/kg body weight | Lethal within 8 days, proteinuria, erythrocytes and leukocytes in sediment ; macroscopic findings : intestinal inflammation, kidney swelling | no data | BASF, 1954 |

**Table 4.1 (cont.) Acute toxicity of glyoxal after, oral, dermal, inhalative and intraperitoneal administration**

| Species, strain, sex* | Route of administration | Dosage or concentration (mg/kg body weight or mg/m$^3$)** | Effect | Observation period | Reference |
|---|---|---|---|---|---|
| Cat | Oral | 1700 – 3300 mg/kg body weight | Mortality at 1700 mg/kg body weight 1/1, at 3000 mg/kg body weight 1/2 ; protein, erythrocytes and leukocytes found in urine within 5 days ; macroscopic findings : gastritis, entiritis, follicular hyperplasia of the spleen, kidney swelling | no data | BASF, 1954 |
| Rabbit, male | Dermal (24-hour application) | > 20 ml/kg body weight | $LD_{50}$ ; mortality : ¼ | 14 days | Smyth et al., 1962 |
| Rabbit | i.v. | 50 mg/kg body weight twice within 2 hours | Survived ; temporary great excitation, accelerated breathing | no data | BASF, 1954 |
| Mouse | i.p. | Ca. 0.75 ml/kg body weight | $LD_{50}$ ; apathy, reeling , dyspnoea | 7 days | BASF, 1963a |
| Mouse, male | i.p. | Ca. 200 | $LD_{50}$ | 10 days | Doull et al., 1964 |
| Mouse, female | i.p. | 7 mmol/kg body weight (corresp. to 406 mg/kg body weight) | $LD_{50}$ | 30 days | Ashwood-Smith et al., 1967 |
| Rat, Albino, female/male | Inhalative*** (8 hours) | Atmosphere at room temperature | Inhalation hazard test Mortality : 0/6 | 14 days | Smyth et al., 1962 |

**Table 4.1 (cont.) Acute toxicity of glyoxal after, oral, dermal, inhalative and intraperitoneal administration**

| Species, strain, sex* | Route of administration | Dosage or concentration (mg/kg body weight or mg/m$^3$)** | Effect | Observation period | Reference |
|---|---|---|---|---|---|
| Rat | Inhalative (8 hours) | Atmosphere saturated 20° C | Inhalation hazard test : no findings during autopsy | No data | BASF, 1963a |
| **40 % Glyoxal** | | | | | |
| Rat, Wistar, male, Female, Male, Female | Oral | 3 300<br>3 660<br>2 960 | $LD_{50}$ decreased spontaneous activity, weakened reflexes, increased respiratory rate, lying on the abdomen or side ; macroscopic findings : reddened stomach mucosa, patchy liver, dark discoloured adrenal glands, increased pulmonary hyperaemia in the deceased animals | 14 days | Hoechst, 1984a |
| Rat, Wistar, Female | Oral | 0.5 - 0.6 ml/kg body weight (corresp. to 640-770 mg/kg body weight) | $LD_{50}$ ; mortality at 0.5 ml/kg 0/20 ; 0.7 ml/kg 10/10 ; comatose state, piloerection, chromodacryorrhoea | 14 days | Société Française Hoechst, 1980 |
| Rat, Harlan-Wistar, Male | oral | 3.08 ml/kg body weight (corresp. to 3 912 mg/kg body weight) | $LD_{50}$ piloerection, inertia, dyspnoea ; macroscopic findings : petechial haemorrhage in the lung and liver, patchy spleen, congestion in the kidney and adrenal gland | No data | American Cyanamid Corporation, 1974 |

**Table 4.1 (cont.) Acute toxicity of glyoxal after, oral, dermal, inhalative and intraperitoneal administration**

| Species, strain, sex* | Route of administration | Dosage or concentration (mg/kg body weight or mg/m$^3$)** | Effect | Observation period | Reference |
|---|---|---|---|---|---|
| Rat,<br>male<br>female | Oral | 7.07 ml/kg body weight (corresp. to 8,979 mg/kg body weight)<br>6.16 ml/kg body weight (corresp. to 7,823 mg/kg body weight) | $LD_{50}$ ; no clinical symptoms ; macroscopic findings : congestion of the abdominal viscera, intestinal haemorrhage | No data | Union Carbide Corporation, 1965 |
| Rat,<br>Wistar,<br>male, female | Oral | > 2 000 < 5 000 | $LD_{50}$ ; reeling, apathy, dyspnoea, piloerection, poor general condition, tremor ; macroscopic findings : redding of the mucosa of the glandular stomach, distended vessels in the deceased animals | 14 days | BASF, 1985a |
| Rat,<br>Wistar,<br>Male, female | Oral | > 5 000 | $LD_{50}$ ; ataxia, hypersensitivity to external stimuli | 14 days | Société Française Hoechst, 1982 |
| Mouse | Oral | Ca. 3.2 ml/kg body weight (corresp. to 4064 mg/kg body weight | $LD_{50}$ ; apathy, reeling, dyspnoea | 7 days | BASF, 1963b |
| Rabbit | Oral | Ca. 2.5 ml/kg body weight (corresp. to 3175 mg/kg body weight) | $LD_{50}$ | 8 days | BASF, 1963b |
| Rat,<br>Wistar,<br>male, female | Dermal<br>(24 hour application, semi-occlusive) | > 2 000 | $LD_{50}$ ;<br>Macroscopic findings : none | 14 days | BASF, 1985b |

**Table 4.1 (cont.) Acute toxicity of glyoxal after, oral, dermal, inhalative and intraperitoneal administration**

| Species, strain, sex* | Route of administration | Dosage or concentration (mg/kg body weight or mg/m$^3$)** | Effect | Observation period | Reference |
|---|---|---|---|---|---|
| Rabbit | Dermal (occlusive) | 10 ml/kg body weight (corresp. to 12,700 mg/kg body weight | $LD_{50}$ ;skin necroses, congestion and haemorrhage of the lung, congestion of the liver and kidneys | No data | Union Carbide Corporation, 1965 |
| Rat, female | i.p. | 0.49 ml/kg body weight (corresp. to 622 mg/kg body weight | $LD_{50}$ | No data | Union Carbide Corporation, 1965 |
| Mouse | i.p. | Ca 0.5 ml/kg ; body weight (corresp. to 635 mg/kg body weight) | LD50 ; apathy, reeling, dyspnoea ; slight irritations in the injection area | 7 days | BASF, 1963b |
| Rat, Wistar, male, female<br>male<br>female | Inhalative (aerosol, 4 hours) | 2 440<br>2 470<br>2 410 | LC50 (4 hours ; irregular breathing, nasal secretion, partly, closed eyes, ruffled fur, dizziness, lying on the abdomen ; macroscopic findings : dark-red lung | 14 days | Hoechst, 1984b |
| Rat, Wistar, male, female | Inhalative (7 hours) | Atmosphere enriched at 20°C | Inhalation hazard test ; mortality 0/10 ; irregular breathing rate ; no findings during autopsy | 14 days | Hoechst, 1984d, e |
| Rat | Inhalative (8 hours) | Atmosphere enriched at 20°C | Inhalation hazard test ; mortality 0/12 ; no findings during autopsy | No data | BASF, 1963b |
| **80 % Active ingredient** | | | | | |
| Rat, Wistar male, female | Inhalative (dust, 4 hours) | > 1 300 | $LC_{50}$ (4 hours); irregular breathing, irritations;<br>Macroscopic findings: none | No data | Hoechst, 1984c |

**Table 4.1 (cont.) Acute toxicity of glyoxal after, oral, dermal, inhalative and intraperitoneal administration**

| Species, strain, sex* | Route of administration | Dosage or concentration (mg/kg body weight or mg/m$^3$)** | Effect | Observation period | Reference |
|---|---|---|---|---|---|
| **No data on the applied glyoxal** | | | | | |
| Rabbit | Dermal | 6600 | $LD_{50}$ | No data | Fasset, 1962 |
| Guinea pig | Dermal | 5000-10000 | $LD_{50}$ | No data | Fasset, 1962 |
| Rat | i.p. | < 100 mg/hg body weight | $LD_{50}$ | No data | Fasset, 1962 |
| Rat, Sprague-Dawley | Inhalative (7 hours) | Atmosphere enriched at 20 °C | Inhalation hazard test ; mortality 0/12 ; no finding during autopsy | 14 days | BASF, 1979 |

i.p. Intraperitoneal application
i.v. Intravenous application
s.c. Subcutaneous application
* Insofar as specified
** The values are based on the respectively given glyoxal solutions
*** 29.2 % Glyoxal was used

## 4.3 Irritation

### Skin irritation

In one patch test according to OECD guideline no.404, glyoxal (40 % aqueous solution) caused no irritation to rabbit skin after a 4-hour exposure as well as during the 72-hour observation period (BASF, 1985 c).

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 hours. In addition, the rabbit ear was exposed for 20 hours. 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-hour treatment period. For an application period of 1 and 5 minutes, respectively, no or slight erythemas with a yellowing of the skin could be observed 24 hours 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-hour 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-hour exposure to the rabbit ear led to erythema and inflammation as well as to minor skin defects 24 hours 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 (BASF, 1956, 1963, a, b, 1970). Thus, slight to pronounced irritation could be seen, depending on the application period.

White adult rabbits (2 kg) obtained 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 $4^{th}$ day. These changes were less pronounced on the $9^{th}$ day, and a regeneration of the epidermis was observed on the $18^{th}$ day (Ito, 1963).

The application of 10 µl glyoxal (29,2 % aqueous solution) to the depilated abdominal skin of the rabbit caused a slight irritation (minor hyperaemia, irritation index 2 of 10 (Smyth et al., 1962)).

Within the framework of an acute toxicity study (see Sect. 4.2), the single dermal administration (occlusive) of 1.57 ml 40 % aqueous glyoxal solution (corresponding to 798 mg glyoxal/kg body weight) to the shaven skin (dorsal, dorsolateral) of 5 Wistar rats/sex for an exposure period of 24 hours caused erythemas in all of the animals (BASF, 1985b).

In conclusion, after a four hour exposure patch test on rabbits (OECD guideline 404), glyoxal 40% caused no irritation. But taking into account studies by BASF where the duration of exposure is longer (up to 20 hours), glyoxal is clearly irritating to skin.

### Eye irritation

In a study according to OECD guideline no. 405, 0.1 ml glyoxal (40 % aqueous solution) caused a definite reddening and chemosis of the conjunctiva of the rabbit eye (White Viennese, 1 male, 2 females) one hour after the instillation. After 24 hours, 2 animals exhibited a slight to definite

chemosis of the conjunctiva. A slight conjunctival reddening and chemosis were still observed 72 hours after the administration. The symptoms completely subsided after 8 days (BASF, 1985c).

In another study on the irritating effect of glyoxal to the eye according to OECD guideline no. 405, glyoxal was likewise proven to be irritating (no further details (IFREB, 1982).

In earlier studies on the rabbit eye, the instillation of 50 µl 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 (BASF, 1956, 1963a,b).

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 50 µl 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 (BASF, 1970).

In conclusion, the key study is the study by BASF (1985c) which conducted according to OECD guideline 405, showing that Glyoxal 40% is an eye irritant.

### 4.4 Sensitisation

The testing of a skin-sensitising effect of pure glyoxal occurred on 20 female Pirbroght-White guinea pigs in the maximisation test according to Magnusson and Kligman. For the induction, the animals were intradermally administered 0.1 ml of a 20 % aqueous glyoxal solution into the shoulder area and one week later, were epicutaneously administered (occlusively) ca. 0.3 g of a 40 % glyoxal solution into the intradermal administration locally (occlusively) on the shaven flank with 0.15 g of a 10 % aqueous glyoxal solution. 24 hours after the application, the intradermal induction caused formation of clearly dilated erythemas and oedemas as well as necrotic skin changes.

One guinea pig died after the intradermal induction. The percutaneous induction led to necrotic skin changes and to formation of clearly defined erythemas and, in addition, one animal a slight oedema. 7 of 19 test animals exhibited slight erythemas. In the control groups no findings were observed. Thus, glyoxal showed a sensitising effect on the skin of guinea pigs (BASF, 1987).

Another maximisation test with glyoxal according to Magnusson and Kligman was conducted on female Dunkin-Hartley guinea pigs. The intradermal and dermal induction took place with 10 % and the dermal challenge with 5 % aqueous solution. 86 % of the guinea pigs (no data on the animal number) showed a positive reaction which was evaluated to be very strong (Foussereau et al., 1992).

In the Bühler test on guinea pigs, 40 % glyoxal was likewise proven to be sensitising (no further detail (American Cyanamid Company, 1988). In the murine Local Lymph Node essay (LLNA) , the positive results observed with Glyoxal confirmed that Glyoxal can be considerd as a sensitiser. (Basketter and al, 1994)

The sensitive nature of glyoxal was furthermore confirmed in several experiences with humans (see section 4.11).

In conclusion, the positive results in the Magnusson and Kligman test (2 studies), the Bühler test on guinea pigs and the LLNA test confirmed that Glyoxal can be considered as a sensitising substance.

### 4.5 Repeated dose toxicity

Glyoxal (40 %) was administered with the diet to 10 Harlan-Wistar rat/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 hereby reached for the male animals and doses of 32, 63.2, 127 and 271 mg glyoxal body weight/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 body weight gain was noted only during the first 2 weeks of the study. During the subsequent study period, however, the body weight 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 was not significant. Substance-related macroscopic or histo-pathological organ changes (13 different organs) were not observed in any dose groups. The authors derived a no effect level of ca. 125 mg/kg bw/day (active ingredient). Haematological and clinical-chemical examinations were not performed (Union Carbide Company, 1966).

In a 28-day study on 6 rats each per dose and control group as well as sex (strain Crl CD(SD)BR), glyoxal (40 % aqueous solution) was administered in doses of 100, 300 and 1000 mg/kg bw/d via the drinking water according to OECD guideline n°407. The concentrations in drinking water were geared weekly to the body weight and the drinking water consumption. 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 body weight retardation coincided with a 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 the final autopsy, substance-related macroscopic or histopathological organ changes were not observed in any dose group. Based on these findings and specically on the dose related decrease of the water, food consumption and body weight, a no toxic effect level of 100 mg 40 % glyoxal/kg body weight/day was established (Société Française Hoechst, 1987).

The subchronic toxicity of glyoxal (purity 98.7 %) was studied on male Sprague-Dawley rats (average initial weight 110 to 130 g, age 5 weeks) with oral administration 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 4.2.

**Table 4.2.** 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 in drinking water (mg/l) | Average substance intake (mg/kg body weight and day) | | |
| 2 000 | 188 | 135 | 107 |
| 4 000 | 407 | 239 | 234 |
| 6 000 | 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 the food-and water consumption as well as a corresponding body weight 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 weigths 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 were affected in the liver, kidney or erythrocytes. Substance-related macroscopic or histopathological organ changes were no 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 groupe (lowest observed effect level 107 mg/kg bw/d) (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 6 000 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 day group corresponded to that in the study described above. In the 180-day group, it was 298 mg/kg bw/d. The body weight 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 dehydeogenase 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 obsered macroscopically in 2 of the treated animals which, however, were assessed by the authors no 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 (Ueno et al., 1991a).

10 male and female Fischer-344 rats each obtained 0, 1000, 2000, 4000, 8000 and 16000 mg glyoxal/l drinking water for ca. 90 days (no data on drinking water consumption). Depending on the concentration, there was a reduce food and water intake and the body weight gain was correspondingly retarded. All male and female rats of the high concentration group had to be killed on the 12$^{th}$ day in a cachectic state. Other symptoms were not observed. Haematological and clinical-chemical examinations were not performed. The histopathological examination showed minor findings in all groups such as haemorrhages in the mesenteric lymph nodes (male and female

rats) and mild to moderate hyperplasia of the mandibular lymph nodes (male rats) as well as atrophic and degenerative changes of the submandibular salivary glands and slight to minimum changes in the kidneys (male rats) in the 8000 and 16000 mg/l-groups. Moreover, hypospermia in the epididymis with atypical cells and slight degenerative changes of the germ epithelium in the testes occurred in the male animals in the highest concentration group. For the female rats of the high dose group, atrophy of the thymus was observed. These histopathological findings were possibly not substance-related, but rather were attributed to cachexia of the animals caused by the reduced water intake (NTP, 1991). A no effect level cannot be derived, because the feed and drinking water intake was reduced down to the lowest tested concentration of 1000 mg/l. It is unclear whether the reduced consumption of drinking water and food indicates toxic effects or more likely a palatability effect.

In the same way, groups of 10 male and female B6C3F1 mice were given glyoxal in the drinking water at doses of 0, 1000, 2000, 4000, 8000 and 16000 mg/l (no data on drinking water consumption). No deaths occurred, but a concentration-dependent reduction of the food and water intake resulted here, too. Consequently, the body weight gain was delayed and the organ weights reduced. Other symptoms did not appear.

Haematological and clinical-chemical tests were not conducted. In the male mice of all dose groups histological changes in the submanibular salivary glands (secretion depletion) were observed which were possibly evaluated to be substance-related, whereby the authors interpret the toxicological relevance to be unclear (NTP, 1991). A no effect level cannot be derived, because the food and drinking water intake was reduced down to the lowest tested concentration of 1000 mg/l. It is unclear whether the reduced consumption of drinking water and food indicates toxic effects or more likely a palatability effect.

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 body weight/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 subtance-related macroscopic or histopathological organ changes were observed (18 different organs). The authors derived a no effect level of ca. 115 mg/kg body weight/day (Union Carbide Company, 1966).

For testing the subacute inhalation toxicity according to OECD guideline no. 412, groups of 5 male and female Wistar rats each (average initial weight 193 and 171 g, respectively) inhaled nominal concentrations of 0, 0.4, 0.2 and 10 mg glyoxal (40 % aqueous solution)/$m^3$ as an aerosol for 6 hours daily, 5 times per week over a period of 29 days (nose only, a total of 20 exposures). The analytically controlled concentrations amounted to 0.6 (± 0.2), 2.3 (± 0.8) and 8.9 (± 1.9) mg/$m^3$, 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 minimum saquamous metaplasia of the epiglottal epithelium in the larynx that was accompanied by a minimum submucous lymphoid cell infiltration. No substance-related changes could be noted histopathologically in the rats of the 0.4 mg/$m^3$ group. Thus, the no observed effect

level for local effects was 0.4 mg glyoxal/$m^3$ air. With regard to the systemic toxicity, the no observed effect level was given at > 10 mg/$m^3$ (Hoechst, 1995).

In table 4.2 bis a summary of these repeated dose toxicity studies is presented

No dermal repeated dose toxicity studies have been performed.

In conclusion, by oral route the key study is the study conducted by Société Française Hoechst (1987) according to OECD guideline 407 on rats. A no effect level of 100 mg/kg bw/d was established for glyoxal 40% (i.e. 40 mg/kg bw/d related to the active ingredient). This value is supported by the studies by Ueno (1991a, b) on rats which obtained a LOEL of 107 mg/kg bw/d related to pure glyoxal, as well as by the study from Union Carbide (1966) on dogs which derived a NOEL of 115 mg/kg bw/d related to pure glyoxal. In a subacute inhalation study on rats for 29 days, a NOEL of 0.4 mg/$m^3$ was derived for local effects and of > 10 mg/$m^3$ for the systemic toxicity (40%glyoxal). No dermal repeated toxicity studies have been performed.

**Tableau 4.2bis - Repeated dose Toxicity Studies**

| Test Substance | Route of Administration Species | Exposure Period | Doses | NOAEL | NOAEL related to active substance | Référence |
|---|---|---|---|---|---|---|
| Glyoxal (40 %) | Oral (diet)<br>Rat | 90 days | Male : 32.7, 63.2, 132 and 253 mg/kg bw/d<br>Female : 32, 63.2, 127 and 271 mg/kg bw/d | 125 mg/kg bw/d | 50 mg/kg bw/d | Union Carbide (1966) |
| Glyoxal (40 %) | Oral (drinking water)<br>Rat | 28 days | 100, 300, 1000 mg/kg bw/d | 100 mg/kg bw /d | 40 mg/kg bw /d | Société Française Hoechst (1987) |
| Glyoxal (100 %) | Oral (drinking water)<br>Rat | 30, 60, 90 days | 2000, 4000, 6000 mg/l | LOAEL =<br>107 mg/kg bw/d | LOAEL =<br>107 mg/kg bw/d | Ueno et al (1991a) |
| Glyoxal (no data) | Oral (drinking water)<br>Rat - Mice | 90 days | 0, 1000, 2000, 4000,<br>8000, 16000 mg/l | Not derived | Not derived | NTP (1991) |
| Glyoxal (100 %) | Oral (diet)<br>Dogs | 90 days | 31, 65, 115 mg/kg bw/d | 115 mg/kg bw/d | 115 mg/kg bw/d | Union Carbide (1966) |
| Glyoxal (40 %) | Inhalation<br>Rats | 29 days | 0, 0.4, 2, 10 mg/m$^3$ | NOEL :<br>Local effects<br>0.4 mg/m$^3$<br>Systemic effects<br>> 10 mg/m$^3$ | NOEL :<br>Local effects<br>0.1 mg/m$^3$<br>Systemic effects<br>> 4 mg/m$^3$ | Hoechst (1995) |

## 4.6 Mutagenicity

In vitro

The results of the mutagenicity tests with glyoxal *in vitro* are summarized in table 4.3 and 4.4.

Glyoxal consistently led to positive results in the *Salmonella*/microsome test on strains TA 100, TA 102, TA 104 as well as TA 2638, whereby the mutagenic effect was weakened by addition of a metabolic activation system. Single positive results were also reported on the strains TA 98 and TA 1535 (see Table 3). The mutagenic effect of glyoxal on *Salmonella typhirmurium* strain TA 100 was suppressed by sodium sulfite (Suwa et al., 1982). A negative result was obtained with Escherichia coli in the standard-plate-incorporation test (Hoechst, 1984f), while a positive result was found in the preincubation test (Kato et al., 1989).

In the L-arabinose-resistance test (ARA test) on the *Salmonella typhirmurium* strains BA 9 as well as BA 13, a mutagenic effect of glyoxal was determined without metabolic activation.

An impairment of the DNA-repair was ascertained in the SOS umu test on *Salmonella typhimurium* (TA 1535/pSK1002) and in the SOS chromo test on *Escherichia coli* (PQ37) (Ono et al., 1991a,b; von der Hude et al., 1988). The DNA-repair tests on *Escherichia coli* strain K-12 as well as on *Bacillus sbtilis* H17/M45 were positive with as well as without metabolic activation (Hellmer & Bolcsfoldi, 1992a; Matsui et al., 1989). In a DNA-repair test as a host-mediated assay with *Escherichia coli* K-12 and NMRI mice (oral administration of glyoxal and intravenous injection of the bacteria suspension), a positive result was observed neither with blood samples nor with the homogenates of the liver, lung, kidney or testes (Hellmer & Bolcsfoldi, 1992b).

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 (Zimmermann and Mohr, 1992).

In the *in vitro* tests on mammalian cells, a genomutagenic, chromosome-damaging as well as DNA-damaging effect were likewise determined in several test systems and by considering various genetic end points (see Table 4). Glyoxal led to a thymidine-kinase decrease in mouse lymphoma cells (Wangenheim & Bolcsfoldi, 1988) and caused an increase of the glycine-adenosine-thymidine-prototrophic revertant count in ovarian cells of the Chinese hamster (Taylor & Wu, 1980; Taylor et al., 1983). In contrast, glyoxal was proven to be negative in the HPRT test on lung fibroblasts as well as on ovarian cells of the Chinese hamster. Furthermore, glyoxal caused an increased chromosome aberration rate in lung fibroblasts as well as ovarian cells of the Chinese hamster (Henkel, 1986; Nishi et al., 1989). Glyoxal caused an increase of the sister-chromatid exhange rat in ovarian cells of the Chinese hamster and in human lymphocytes (Tucker et al., 1989; American Cyanamid Company, 1982) and caused unscheduled DNA synthesis (UDS) in TC-SV40 cells of the Syrian hamster (Cornago et al., 1989). It also caused DNA-strand breaks in mouse lymphoma cells as well as in primary rat hepatocytes (Garberg et al., 1988; Ueno et al., 1991c). An induction of cross-links by glyoxal, however, could not be detected on primary rat hepatocytes (Ueno et al., 1991c). Increased endoreduplication was detected in ovarian cells of the Chinese hamster (Tucker et al., 1989).

**Studies on DNA-adduct formation**

Through spectral-analytical studies, it was proven that, *in vitro*, glyoxal reacts with nucleic acids, nucleotides and their bases in high concentrations, while only guanine or guanylic acid were specifically altered with low concentrations. The reaction took place within 30 minutes at pH 8.3 to 9.8 and at room temperature. Native calf thymus DNA was not changed by glyoxal, while a transformation of ca. 72 % was noted with heat-denatured DNA (Nakaya et al., 1968).

It was shown on plasmid pCoIIR215 of *Escherichia coli* strain K12 HB101 as well as with guanosine that glyoxal forms stabile 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 (Broude & Budowski, 1971; Kasai et al., 1984, 1986; Lilley, 1986).

Corresponding results were also found in studies on DNA of the mouse, yeast and calf thymus (Birnboim & Mitchel, 1978; Brooks & Klamert, 1968; Shapiro & Hachmann, 1966; Shapiro et al., 1970, 1986).

**Table 4.3** ***In vitro*** **genotoxicity tests with glyoxal on bacteria**

| **Test system** | **Tested concentration range [1] (µg/plate)** | **Metabolic activation system** | **Result [2]** | | **Reference** |
|---|---|---|---|---|---|
| | | | **With Metab.activ.** | **Without Metab.activ.** | |
| **Gene mutations**<br>***Salmonella*/microsome test as standard-plate incorporation test** | | | | | |
| *Salmonella typhimurium* TA 98, TA 100 | Ca. 10-500<br>(30 % glyoxal)<br>toxicity tested | S9-mix from phenobarbital-induced rat liver | Positive<br>(TA 100) | Positive<br>(TA 100) | Bjeldanes & Chew, 1979 |
| *Salmonella typhimurium* TA 100 | Up to 600 | No data on the applied metabolic system | Positive | Positive | Garst et al., 1983 |
| *Salmonella typhimurium* TA 100 | Ca. 116-929<br>(ca. 2-16 µmol/plate)<br>(30 % glyoxal) | _ | Not tested | Positive | Dorado et al., 1992 |
| *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538 | 10-10 000<br>toxicity tested | S9-mix from Aroclor 1254-induced rat liver | Positive<br>(TA 100) | Positive<br>(TA 100) | Niemand et al., 1983 |
| *Salmonella typhimurium* TA 102, TA 2638 | 1 000 | _ | Not tested | Positive<br>(TA 102, TA 2638) | Levin et al., 1982 |

**Table 4.3 (cont.)** ***In vitro*** **genotoxicity tests with glyoxal on bacteria**

| Test system | Tested concentration range [1] (µg/plate) | Metabolic activation system | Result [2] | | Reference |
|---|---|---|---|---|---|
| | | | With Metab.activ. | Without Metab.activ. | |
| *Salmonella typhimurium*<br>TA 98, TA 100, TA 1535, TA 1537, TA 1538 | 4-5 000<br>(40 % glyoxal)<br>toxicity tested | S9-mix from Aroclor 1254-induced rat liver | Positive<br>(TA 100, TA 1535) | Positive<br>(TA 100) | Hoechst, 1984 f |
| *Salmonella typhimurium*<br>TA 102 | 4-5 000<br>toxicity tested | S9-mix from Aroclor 1254-induced rat liver | Positive | Positive | Hoechst, 1988 |
| *Salmonella typhimurium*<br>TA 98, TA 100, TA 1535, TA 1537, | 4-127<br>3.15-100 000 nl/plate)<br>(40 % glyoxal) | S9-mix from Aroclor 1254-induced rat liver | Positive<br>(TA 100) | Positive<br>(TA 100) | Oesch, 1979 |
| *Salmonella typhimurium*<br>TA 7006, TA 98 | 20-100 µg/plate | No data | Positive | Positive | Muramata-Kamiya et al. (1997a) |
| *Escherichia coli*<br>WP2uvrA | 4-5 000<br>(40 % glyoxal)<br>toxicity tested | S9-mix from Aroclor 1254-induced rat liver | Negative | Negative | Hoechst, 1984 f |
| *Escherichia coli*<br>W 3110 (F-) | 0-400 µg glyoxal/ml solution | No data | Positive | Positive | Muramata-Kamiya et al. (1997b) |

**Table 4.3. *In vitro* genotoxicity tests with glyoxal on bacteria**

| Test system | Tested concentration range [1] (µg/plate) | Metabolic activation system | Result [2] | | Reference |
|---|---|---|---|---|---|
| | | | With Metab.activ. | Without Metab.activ. | |
| ***Salmonella*/micrososome tests as preincubation test** | | | | | |
| *Salmonella typhimurium* TA 100 | 40*<br>(40 % glyoxal) | S9-mix from PCB-induced rat liver | positive | positive | Yamaguchi & Nakagawa, 1983 |
| * In total, 5 concentrations tested ; only the concentration with the highest potential was specified | | | | | |
| *Salmonella typhimurium* TA 104 | Up to ca. 100<br>Toxicity tested | _ | Not tested | Positive | Marnett et al., 1985 |
| *Salmonella typhimurium* TA 100, TA 104 | 50-100 | _ | Not tested | Positive*<br>(TA 100, TA 1004) | Ueno et al., 1991b |
| * Addition of glutathione reduced the mutagenic effect | | | | | |
| *Salmonella typhimurium* TA 100, TA 102, TA 104 | 30-120 | S9-mix from phenobarbital- and 5,6-benzoflavone-induced rat liver | Positive<br>(TA 100, TA 102, TA 104) | Positive<br>(TA 100, TA 102, TA 104) | Sayato et al., 1987 |
| *Salmonella typhimurium* TA 100 | 58-325<br>(1-5.6 mM) | _ | Not tested | Positive* | Suwa et al., 1982 |
| * Weakening of the mutagenic effect after addition of 8 mM sodium sulfite | | | | | |
| *Salmonella typhimurium* TA 100, TA 102, TA 104 | 5-500<br>(99 % glyoxal) | S9-mix from Aroclor 1254-induced rat liver | Positive<br>(TA 100, TA 102, TA 104) | Positive<br>(TA 100, TA 102, TA 104) | Shane et al., 1988 |

**Table 4.3 (cont.)** ***In vitro*** **genetoxicity tests with glyoxal on bacteria**

| **Test system** | **Tested concentration range [1] (µg/plate)** | **Metabolic activation system** | **Result [2]** | | **Reference** |
|---|---|---|---|---|---|
| | | | **With Metab.activ.** | **Without Metab.activ.** | |
| *Salmonella typhimurium* TA 98, TA 100, TA 102 | 14.5-580 (0.25-10 µmol/plate) toxicity tested | S9-mix from Aroclor 1254-induced rat liver | Positive (TA 100, TA 102) | Positive (TA 100, TA 102) | Aeschbacher et al., 1989 |
| *Salmonella typhimurium* TA 98**,** TA 100 | No data | S9-mix from KC-500-induced rat liver | Weakly positive (TA 100) | Positive (TA 100) | Sasaki & Endo, 1978 |
| *Salmonella typhimurium* TA 98**,** TA 100, TA 104 | No data | No data on the applied metabolic system | Positive (TA 98, TA 100, TA 104) | Positive (TA 98, TA 100, TA 104) | Kato et al., 1989 |
| *Escherichia coli* WP2uvrA/pKM101 | No data | No data on the applied metabolic system | positive | positive | Kato et al., 1989 |
| **Further gene mutation tests on bacteria** | | | | | |
| L-arabinose-resistance test (ARA test), *Salmonella typhimurium* BA9, BA13 (preincubation test) | 238-1 190 nmol/ml (corresponding to 13.8-69.1 µg/ml) | _ | Not tested | Positive (BA9, BA13) | Ruiz-Rubio et al., 1985 |
| L-arabinose-resistance test (ARA test), *Salmonella typhimurium* BA13 (preincubation test) | Up to 238 nmol/ml (corresponding to 13.8 µg/ml) | _ | Not tested | positive | Ariza et al., 1988 |

**Table 4.3. *In vitro* genetoxicity tests with glyoxal on bacteria**

| Test system | Tested concentration range [1] (µg/plate) | Metabolic activation system | Result [2] | | Reference |
|---|---|---|---|---|---|
| | | | With Metab.activ. | Without Metab.activ. | |
| **DNA damage** | | | | | |
| umu test, *Salmonella typhimurium* TA1535/pSK1002 | 100 µg/ml | S9-mix from phenobarbital- and 5,6-benzoflavone-induced rat liver | positive | positive | Ono et al., 1991a, b |
| SOS chromo test, *Escherichia* coli PQ37 | 0.1-0.6 mM (corresponding to 5.8-34.8 µg/ml) | _ | Not tested | positive | Von der Hude et al., 1988 |
| Rec assay, *Bacillus subtilis* H17 (arg, trp, recE*), M45 (arg, trp, recE) | 8.18-30.3 µg/ml | S9-mix (no further details) | positive | positive | Matsui et al., 1989 |
| DNA-repair test, *Escherichia coli* K12 343/636, K12 343/591 (preincubation test) | Up to 15.9 mmol/l (corresponding to 923 µg/ml) | S9-mix from Aroclor 1254-induced rat liver | positive | positive | Hellmér & Bolcsfoldi, 1992a |
| DNA-repair test, host : male NMRI mice, test system : *Escherichia coli* K12 343/636, K12 343/591 (applied intravenously) | 570 and 1 700 mg/kg body weight oral | *In vivo* metabolism | Negative* | _ | Hellmér & Bolcsfoldi, 1992a |
| * Blood as well as homogenates of liver, lung, kidneys and testes were examined ; sacrifice of the animals 2 hours after injection | | | | | |

1) Insofar as not specified, theare no data found in the publications on the cytotoxic effects as well as on the applied glyoxal
2) If data were given publications that a positive result was determined only for certain strains, these were cited

**Table 4.4. *In vitro* genotoxicity tests with mammalian cells**

| Test system | Tested concentration range [1] (µg/ml) | Metabolic activation system | Result [2] | | Reference |
|---|---|---|---|---|---|
| | | | With Metab.activ. | Without Metab.activ. | |
| **Gene mutations** | | | | | |
| CHO-S/HPRT test, ovarian cells of the Chinese hamster | No data | No data | negative | | Taylor et al., 1983 |
| V79/HPRT test, V79 cells of the Chinese hamster | 0.1-1.5 mg/container (40 % glyoxal) toxicity tested | S9-mix from Aroclor 1254-induced rat liver | negative | negative | Société Française Hoechst, 1986a |
| CHO AUXB1/GAT, reversion to glycine-adenosine-thymidine-prototrophy, glycine-adenosine-thymidine-auxotrophic ovarian cells of the Chinese hamster | No data | No data | positive | | Taylor & Wu, 1980<br>Taylor et al., 1983 |
| Mouse lymphoma test, L5178TK$^{+/-}$ cells | 27.8-61.5<br>(4.79 x $10^{-4}$ – 10.6 x $10^{-4}$ mol/l) | - | No tested | positive | Wangenheim & Bolcsfoldi, 1988 |
| Co S-7-<br>Plasmid pMy 189 | 0-150 µg glyoxal/100 µl plasmid solution | No data | Cytoxicity and mutations frequancy increased | Muramata-kimaya (1997) | |
| **Chromosome damage** | | | | | |
| Chromosome aberration, ovarian cells of the Chinese hamster | 50-500<br>toxicity tested | S9-mix from Aroclor 1254-induced rat liver | positive | positive | Henkel, 1986 |
| Chromosome aberration, V79 cells of the Chinese hamster | 100-400<br>(40 % glyoxal) | _ | Not tested | positive | Nishi et al., 1989 |

**Table 4.4 (cont.)** ***In vitro*** **genotoxicity tests with mammalian cells**

| Test system | Tested concentration range [1] (µg/ml) | Metabolic activation system | Result [2] | | Reference |
|---|---|---|---|---|---|
| | | | With Metab.activ. | Without Metab.activ. | |
| **DNA damage** | | | | | |
| USD test, TC-SV40 cells of the Syrian hamster | 5 x $10^{-5}$ M | _ | Not tested | positive | Cornago et al., 1989 |
| SCE test, ovarian cells of the Chinese hamster (CHO AUXB1) | 1.2-92.9 (20-1 600 µM) | _ | Not tested | Positive* | Tucker et al., 1989 |
| * Weakening of the mutagenic effect after addition of 1 mM sodium bisulfite | | | | | |
| SCE test, human lymphocytes | 23.3-162.2 (400-2 800 µM) | _ | Not tested | positive | Tucker et al., 1989 |
| SCE test, ovarian cells of the Chinese hamster (CHO) | No data | No data | positive | | American Cyanamid Company, 1982 |
| DNA-single-strand breaks, mouse lymphoma cells ($L5178Y/TK^{+/-}$), alkaline unwinding | 27-214 (0.461 x $10^{-3}$- 3.69 x $10^{-3}$ mol/l) | _ | Not tested | positive | Garberg et al., 1988 |
| DNA-single-strand breaks, alkaline elution, rat hepatocytes | 100-600 | Primary rat hepatocytes | positive | _ | Ueno et al., 1991 c |
| Cross-links, alkaline elution, rat hepatocytes | 100-600 | Primary rat hepatocytes | negative | _ | Ueno et al., 1991 c |

**Table 4.4 (cont.)** ***In vitro*** **genotoxicity tests with mammalian cells**

| **Test system** | **Tested concentration range [1] (µg/ml)** | **Metabolic activation system** | **Result [2]** | | **Reference** |
|---|---|---|---|---|---|
| | | | **With Metab.activ.** | **Without Metab.activ.** | |
| **Genome damage** | | | | | |
| Endoreduplication, ovarian cells of the Chinese hamster (CHO AUXB1) | 1.2-92.9<br>(10-1 600 µM) | _ | Not tested | positive | Tucker et al., 1989 |
| * Weakening of the mutagenic effect after additiion of 1 mM sodium bisulfite | | | | | |

**Table 4.5. Mutagenicity tests with glyoxal *in vivo***

| Test system | Dose/treatment scheme | Toxicity | Result | Reference |
|---|---|---|---|---|
| **Gene mutations** | | | | |
| *Drosophila melanogaster* Sex-linked recessive-lethal test | 0.73 mg/ml intrabdominal injection | No data | Lethality 0.30 % compared to 0.08 % for the controls | Barnett & Munoz, 1969 |
| *Drosophila melanogaster* Sex-linked recessive-lethal test | 40 µg/animal intraabdominal injection | No data | Negative | Barnett & Munoz, 1989 |
| **Chromosome damage** | | | | |
| Micronocleus test, mouse (Swiss), 5 animals/sex and preparation time ; 1 000 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 | MTD tested | Negative | Société Française Hoechst, 1986b |
| Chromosome abberation test, rat ; duodenum, testes, spleen, liver and pancreas were examined | 0.5 and 1 ml subcutaneous, 10 % glyoxal, sacrifice 12 h, 23 h and 36 h after administration | Toxic dosage : 1 ml | Positive* | Thomas, 1958 |
| * Positive in duodenum, testes and spleen. The autors also reported mitosis disturbances. The study is documented only insufficiently | | | | |
| *Drosophila melanogaster*, dominant-lethal test | 40 µg/animal intraabdominal injection | No data | negative | Barnett & Munoz, 1989 |
| *Drosophila melanogaster*, reciprocal translocation | 40 µg/animal intraabdominal injection | No data | negative | Barnett & Munoz, 1989 |

**Table 4.5 (cont.) Mutagenicity tests with glyoxal *in vivo***

| Test system | Dose/treatment scheme | Toxicity | Result | Reference |
|---|---|---|---|---|
| **DNA damage** | | | | |
| UDS test, male Wistar rats, primary hepatocytes, autoradiographic UDS test | 100, 500, 1 000 mg/kg body weight oral, 40 % glyoxal, sacrifice 2h and 16 h after administration | No toxicity up to 1 000 mg/kg body weight | negative | CCR, 1992 |
| UDS test, male F344-rats, pyloric mucaso, autoradiographic UDS test | 120, 240, 360 and 400 mg/kg body weight oral, sacrifice 2 h after administration | Higest dose corresponds to ½ of the $LD_{50}$ value | positive | Furihata et al., 1985; Furihata & Matsushima, 1989 |
| DNA single-strand breaks, alkaline elution, liver DNA, rat (Sprague-Dawley) | 120, 240, 360 and 400 mg/kg body weight oral, sacrifice 2 h after administration | No data | Positive* | Ueno et al., 1991 c |
| * Dose-dependant ; weakly positive in spleen tisuue ; negative in tissue of the kidney, bone marrow, pancreas and lung | | | | |
| DNA single-strand breaks, alkaline elution, liver DNA of pyloric mucosa, rat (F344) | 5, 50, 500 and 550 mg/kg body weight oral, sacrifice 2 h after administration | No data | Positive* | Furihata et al., 1988; Furihata & Matsushima, 1989 |
| * Dose-dependant | | | | |
| **Genome damage** | | | | |
| *Drosophila melanogaster*, Loss ofthe X- or Y-chromosome | 40 µg/annimal, intraabdominal injection | No data | negative | Barnett & Munoz, 1989 |

In vivo

The results of the *in vivo* mutagenicity studies with glyoxal are presented in table 4.5.

Glyoxal showed no clastogenic effect in the micronucleus test after oral administration to the mouse (Société Française Hoechst, 1986b). An increased chromosome aberration rate in the tissue of the duodenum, spleen and testes of the rat after subcutaneous administration was reported in an older study (Thomas, 1958). In the UDS test with oral application to rats, an unscheduled DNA synthesis could be detected in the pyloric mucosa (Furihata et al., 1985; Furihata & Matsushima, 1989) but not in hepatocytes (CCR, 1992). Glyoxal caused DNA-strand breaks in the liver as well as in the pyloric mucosa after oral application to rats (Ueno et al., 1991c; Furihata et al., 1988, 1989; Furihata & Matsushima, 1989).

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 intraabdominal 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 ofter the intraabdominal injection of a glyoxal solution, nonetheless without detailed information on the applied dose (Barnett & Munoz, 1969, 1989).

**Conclusion:**

Glyoxal is shown to be mutagenic in *in vitro* genotoxicity studies in prokariotes and eukariotes. *In vivo* Glyoxal is proven to be negative in the micronucleus test on the mouse after oral administration. On Drosophila melanogaster, glyoxal is also proven to be 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. Chromosomes aberrations in the duodenum, testes and spleen are described in an older, only insufficienttly documented study after subcutaneous administration to rats. After oral administration to the rat , a significant increase of the unsheduled DNA synthesis is found in the pyloric mucosa, but not in primary hepatocytes, as well as an increase of DNA single-strand breaks in the liver and in the pyloric mucosa. These findings indicate that glyoxal reacts at the point of entry ( the stomachs ) and immediatly downstream ( the liver ), but not in more remote organs.

## 4.7 Carcinogenicity

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. A control group of the same size (treated with 25 µl deionized water) was also assayed. Skin tumours were not found in any of the treated mice. A fibrosarcoma in one of the mice treated with European Glyoxal 40 was judged by the authors not to be substance-related. The conclusions is based on the control data, available at the testing institute, according to which fibrosarcomas occur spontaneously in the male C3H mice. An increase of the mortality rate was not observed. The mean survival time for the treated mice, with 580 and 594 days, was longer than that for the controls (488 days). One treated animal showed skin irritations with necroses (American Cyanamid Company, 1982).

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-tetra-decanoylphorbol-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 papillomas. 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 papillomas and 31 squamous cell carcinomas). Thus, glyoxal was shown not to be a tumour initiator in this (Miyakawa et al., 1991).

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 8$^{th}$ to 40$^{th}$ 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 8$^{th}$ to 40$^{th}$ week. For the rats pretreated with MNNG and NaCl, glyoxal caused a significant increase of adenocarcinomas in the glandular stomach (12/28 animals, ca. 43 %), that were mainly localized in the pylorus region. A hyperplasia of the mucosa was also found there. 5 tumours (5/30 animals, ca. 17 %) were observed for the sole 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 (Takahashi et al., 1989). The results are presented in the following table.

**Table 4.6** 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 groups** | | |
|---|---|---|---|
| | **Initiation plus glyoxal** | **Initiation without glyoxal** | **Without initiation plus glyoxal** |
| Total examined | 28 | 30 | 8 |
| Adenocarcinomas<br>total<br>in fundus<br>in pylorus | <br>12 (43 %)*<br>1 (4 %)<br>11 (39 %)* | <br>5 (17 %)<br>1 (3 %)<br>4 (13 %) | <br>0<br>0<br>0 |
| Hyperplasias<br>in fundus<br>in pylorus | <br>4 (14 %)*<br>17 (61 %)** | <br>1 (3 %)<br>8 (27 %) | <br>0<br>0 |

* $p > 0.05$

** $p > 0.01$

According to a 2-step concept, 12 male F344 rats (6 weeks old at the study begin) were first injected intraperitoneally with a single dose of 200 mg diethylnitrosamine/kg bw as the initiator. Starting 2 weeks later, they obtained 5.000 ppm glyoxal with the diet (corresponding to ca. 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 of the total of 94 tested compounds. In these studies, glyoxal did not cause any increase of the number of foci, but rather a significant reduction (Hasegawa & Ito, 1992).

3 glyoxal samples (40 %) of various origin were investigated *in vitro* in the cell transformation test with C3H/10T½CL8 cells (embryonal 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 (American Cyanamid Company, 1980a, b, c).

In conclusion, no carcinogenic effect is detected in mice after dermal application of glyoxal over the entire life span. Glyoxal possesses no tumor initiating effect after the dermal administration to mice. After oral administration, glyoxal exhibits local tumor promoting properties in the mucosa of the forestomach of the rat ( tissues not existing in other species or man ). In the liver, promotion model on the rat, no indications were found for a promoting effect of glyoxal through systemic action. Glyoxal is a metabolite of ethylene glycol and there are two negative carcinogenic studies on ethylene glycol ( rat and mice ).

## 4.8 Toxicity for reproduction

### Toxicity to reproductive organs

In a 90-day toxicity study with male and female rats, no substance-related macroscopic or histopathological organ changes were observed up to a dose of approx. 250 mg/kg bw/d (related to the active ingredient) (Union Carbide Company, 1966). In a 90-day toxicity study with male rats a dose-dependant 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 (107, 234 & 315 mg/kg bw/day related to the active ingredient). Substance related macroscopic or histopathological organ changes were not found. Similarly, no decrease of the absolute weight of the testes were found in rats exposed to a dose of 298 mg/kg bw/d for 180 days (Ueno et al., 1991a). In a further 90-day study with dogs, no substance-related macroscopic or histopathological organ changes were observed (Union Carbide Company, 1966)

In a 90-day study with rats, hypospermia in the epididymis with atypical cells and slight degenerative changes of the germ epithelium in the testes occurred in the male animals in the highest concentration group (16000 mg glyoxal/l drinkink water). These histopathological findings were possibly not substance-related, but rather were attributed to cachexia of the animals caused by the reduced water intake. A no effect level cannot be derived, because the feed and drinking water intake was reduced down to the lowest tested concentration of 1000 mg/l. It is unclear whether the reduced consumption of drinking water and food indicates toxic effects or more likely a palatability effect (NTP, 1991).

### Developmental toxicity

A test on developmental toxicity according to OECD guideline 414 was performmed recently (BASF & Clariant, 2001). The test substance was administered to female Wistar rats as a solution in distilled water at doses of 5; 25 and 125 mg/kg bw/d (related to the active ingredient) on day 6 through day 19 post coitum (p.c.). Maternal toxicity was observed at 125 mg/kg bw/d i.e. transient and sporadically occurring salivation immediately after gavaging, significantly reduced food consumption, significantly lower corrected body weight gain. No substance related effects were observed on gestational parameters or fetuses. At 25 and 5 mg/kg bw/d, no substance related effects were observed on dams, gestational parameters or fetuses. Based on these results, the NOAEL for maternal toxicity is 25 mg/kg bw/d, while it is 125 mg/kg bw/d for prenatal developmental toxicity.

Two other toxicity studies have been conducted on rats and rabbits by NTP (1994, 1993) with glyoxal trimeric dihydrate, which is the oligomeric form shown as compound III in chapter I and would be in equilibrium with the monomer in water.

Glyoxal trimeric dihydrate (100%) was administred by gavage in water to rats on gestational days 6 through 15 at levels 0, 50, 150 or 300 mg/kg/day. All animals were killed on day 20. No maternal lethality occured in this study. Maternal toxicity was manifested as only a slight reduction in maternal body weight and food cosumption at 300 mg/kg/day during the treatment period. Thus, NOAEL for maternal toxicity was 300 mg/kg/day. No developmental toxicity was observed at doses as high as 300 mg/kg/day. This study established a NOAEL for developmental toxicity in the presence of mild maternal toxicity of > or = 300 mg/kg/day.

In the study on rabbits, glyoxal trimeric dihydrate (100%) was administered by gavage in water to New Zealand rabbits on gestation days 6 through 19 at levels of 0 and 50 mg/kg/day.All the animals were killed on day 30. Maternal toxicity was manifested only as a transient reduction in body weight and food consumption at 50 mg/kg/day during the treatment period. No clearcut evidence of developmental toxicity was observed at 50 mg/kg/day in the presence of mild maternal toxicity. This study suggests a NOAEL for maternal effects of < 50 mg/kg/day and a NOAEL of > or = 50 mg/kg/day for developmental toxicity.

In conclusion, no dose-dependant effects were found on reproductive organs in repeated dose studies up to a dose of approx. 250 mg/kg bw/d (related to the active ingredient). Furthermore, a NOAEL of 125 mg/kg bw/d could be derived for prenatal development toxicity and of 25 mg/kg bw/d for maternal toxicity.

### 4.9 Immunotoxicity

The effect of glyoxal on the antibody synthesis was evaluated on inbred CBA-mice. The animals were immunized with ovalbumin and a conjugate of dinitrophenol and ovalbumin in Freund's adjuvant, which was administered intraperitoneally. Glyoxal at doses of 100 µg/kg body weight 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 complementarity 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 occured primarily during the late phase of the antibody formation after 10 and 20 days. The IgG-bab-and Fc-concentrations were reduced with simultaneous administration of glyoxal by respectively 10 % after 5 days, 45 % after 10 days and ca. 40 % after 20 days. A statistical evaluation of the results was not undertaken (no further details; Torosyan, 1979).

The possible inductive effect of various aldehydes, including glyoxal, on the erythrophagocytosis by human bone marrow macrophages was tested. The incubation of erythrocytes, previously treated with 58 mg glyoxal/l, with human serum did not lead to any increase of the phagocytosis rate compared to that of untreated erythrocytes. Malondialdehyde was used as the positive control (Hebbel & Miller, 1988)

### 4.10 Other effects

The single intravenous administration of a 30 % glyoxal solution/kg body weight 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 (no further details ; Union Carbide Corporation, 1955).

Glyoxal intravenously administered in a dose of 19.5 mg/kg body weight induced a decrease in blood pressure in dogs anaesthetised with barbiturate (no further details ; Wingard et al., 1955).

A glucose tolerance test was conducted with rabbits on the $5^{th}$ and $10^{th}$ 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. Compared to the untreated control animals (mean value maximum of 83 mg), the average glucose content was clearly increased up to 154 mg. The histopathological examination showed a considerable granular degeneration of the liver, a moderate granular degeneration of the cordial renal tubuli and a considerable granular degeneration as well as atrophy and fibrotic changes of the islets of Langerhans in the pancreas (Ito, 1963).

The influence of glyoxal on protein synthesis was studied *in vivo* based on the dose dependent decrease of the total protein content in serum obsered in rats in a subchronic study with administration of glyoxal in the drinking water (see Sect. 7.5). 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 body weight orally. 4 hours after the glyoxal administration, the animals were injected i.p. with 2.4 MBqL [4,5-$^{3}$H]-leucine/kg body weight. 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 (Ueno et al., 1991a).

Glyoxal as of a concentration of 29.0 µg/ml inhibited *in vitro* the DNA-and protein synthesis in human fibroblasts (Klamerth, 1968).

In vitro, glyoxal inhibited the rabbit liver aldolase activity as of a concentration of 87.1 µg/ml and the muscle aldolase activity as of 174.1 µg/ml (Spolter et al., 1965).

Glyoxal in concentration of $1.5 \times 10^{-3}$, $2.7 \times 10^{-3}$ and $1.4 \times 10^{-3}$ M inhibited *in vitro* the cell respiration of brain, kidney and heart sections (Kun, 1952).

Glyoxal (95 % to 98 %) was tested *in vitro* for its cytotoxic effect on TC-SV40/INO cells of the golden hamster. The $LC_{50}$ value was 0.58 mmol/l medium after a 2-hour incubation at 37° C (Cornago et al., 1990).

Under anaerobic conditions in vitro, Glyoxal (95 % to 98 %) in concentrations of $5 \times 10^{-6}$ and $5 \times 10^{-5}$ mol/l acted non-radiosensitizing on TC-SV40/INO cells of the golden hamster, while a moderate radiosensitizing effect was detectable through concentrations of $5 \times 10^{-5}$ mol/l under hypoxic conditions. The non-protein-bound sufhydryl groups were reduced only by 10% glyoxal, so that an interaction between them and Glyoxal was condiered to be little probable for the radiosensitizing effect (Cornago et al., 1990).

The cytotoxicity of Glyoxal was examined on Malpighi cell cultures of the human skin. The $LC_{50}$ value was 120 µg/ml after a 24-hour exposure (no further details, Hoh et al., 1987).

Glyoxal in concentration of 5 mM (corresponding to 0.29 µg/ml) acted cytotoxic to V79 cells of the Chinese hamster after a 6-hour incubation at 37° C in an air and nitrogen atmosphere. The concentration of 0.5 mM (corresponding to 0.029 µg/ml) was not cytotoxic under these conditions (Raleigh & Liu, 1983).

The non-oxidative inactivation of the α-proteinase inhibitor (α-PI) by Glyoxal and other carbonyl compounds found in cigarette smoke was investigated in an in *vitro* study. Proteinase inhibitors inhibitors the proteolytic enymes such as elastase and trypsin. The cited enzymes could cause lung emphysemas throug inhibition of α-PI. The tests were conducted on α-PI as well as on unfractionated human plasma. With incubation of α-PI with 11.6 mg glyoxal/l for 2-hours, a reduction of the elastase and trypsin inhibitory activity of α-PI of ca. 25% and 30% was observed, respectively. After a 2-hour incubation with unfractionated human plasma and ca. 29 mg glyoxal/ml plasma, an about 30 % reduction of the elastase inhibitory activity of α-PI was determined, and with incubation with ca. 26 mg glyoxal/ml plasma, an about 35 % reduction of the trypsin inhibitory activity of α-PI (Gan & Ansar, 1986).

With regard to a possible antitumour activity of aldehydes *in vitro* on Yoshida ascites hepatoma cells, Glyoxal was tested for its effect on oxygen consumption, the aerobic and anaerobic incorporation of radio-labelled DL-leucine in protein as well as the anaerobic glycolysis. The incubation took place for 60 minutes at 38° C. On the average, 5 mM glyoxal decreased the oxygen consumption by 13 %, the aerobic incorporation of DL-leucine in protein by 90 %, the anaerobic incorporation of DL-leucine in protein by 80 % and the anaerobic glycolyxis by 14 % (Guidotti et al., 1965).

In vitro treatment of murine thymocytes and fibroblasts with glyoxal induced extensive tyrosine phosphorylation of multiple proteins, which was drastically inhibited by the addition of OPB-9195, an inhibitor of the carbonyl reaction with proteins. Glyoxal induced cross-linking of a number of cellular proteins, including glycosylphosphatidylinositol (GPI)-anchored cell surface Thy-1. Treatment of cells with glyoxal promptly induced activation of non-receptor protein-tyrosine kinasec c-Src, which was partially inhibited by OPB-9195. It is suggested from this results that carbonyl amine reaction quickly activates c-Src, possibly through cross-linkage of GPI-anchored proteins or putative specific receptors (Akhand et al., 1999)

### 4.11 Experience in humans

In the maximization test, a positive skin reaction appeared in 24 of 24 patients. The induction occurred with a 10 % glyoxal solution. In the studies, Glyoxal possessed an extremely strong skin-sensitizing effect (Kligman, 1966).

Dry eczema, preceeded by itching, developed on the back of the hand, index and middle finger of the left hand of a 27-year-old female worker (left-hander), who wrapped smooth fibre glass pipes in canvas using an aqueous polyvinyl resin as an adhesive. A positive reaction to polyvinyl resin was found in a standard-patch test. The components of the polyvinyl resin were vinyl acetate, glyoxal, formaldehyde and ammonium persulfate. Other patch tests with 1 % to 10 % glyoxal solutions resulted in positive reactions depending on the concentration. The threshold value lay betwenn a 0.1 % and 1 % solution. 10 control persons reacted negatively. The authors suppose that the skin

wounds caused by the glass fibres promoted the development of a glyoxal-contact sensitization (Hindson & Lawlor, 1982).

3 employees of a clinic handled disinfectants which, among others, contained formaldehyde, glutaraldehyde and glyoxal. After an average of 3 weeks of occupational exposure, a hand eczema developed which was proceeded by itching and dryness of the skin. For one of the concerned employees, the dermatosis also affected the legs and the feet. Patch tests with the disinfectants in concentrations of 0.5 % and 1 % yielded positive results. Glyoxal was proven to be positive in a 1 % solution. The “allergy index” (no elucidation) was 6 % for the disinfectant and 3 % for glyoxal (Calliès et al., 1985).

Between 1965 and 1990, 65 cases of allergies to formaldehyde, glutaraldehyde and glyoxal were observed among the employees of a skin clinic. Patch tests produced positive results for 41 persons. Among them, 6 individuals reacted positively only to glyoxal, 4 to glyoxal and formaldehyde, 8 to glyoxal and glutaraldehyde and 2 to all 3 compounds. Cross reactions thus apparently existed for 12 persons (Foussereau et al., 1992).

5 volunteers were painted with a 5 % and 30 % aqueous glyoxal solution, respectively, 5 to 7 times onto their skin. 3 persons developed severe vesicular and hemorrhagic reactions, and one individual, who had already been exposed previously, showed focal eczemas on the application site. All cases resulted in an irregular discoloration of the skin as well as an irritation on the application site (Goldman et al., 1960).

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).

At last, a 44 years old dental nurse presented two positive reactions after two patch test sessions with glyoxal (10%) (Kanarva et al., 2000).

## 5. CONCLUSION / RECOMMENDATION

Environment

Glyoxal is not volatile and is not expected to accumulate in biota or soil/sediment. It is clearly readily biodegradable.

In short-term tests with fish, daphnids and algae the following results were found: *Pimephales promelas*: 96 h-LC50 = 215 mg/l; *Daphnia magna*: 48h-EC50 = 404 mg/l; *Scenedesmus subspicatus*: 72h-EC50 > 500 mg/l. With an assessment factor of 1000 a PNECaqua of 215 µg/l can be calculated from the LC50 for fish (the results refer to the 40% aqueous solution). For the active ingredient, the results are *Pimephales promelas*: 96 h-LC50 = 86 mg/l; *Daphnia magna*: 48h-EC50 = 161 mg/l; *Scenedesmus subspicatus*: 72h-EC50 > 200 mg/l; PNEC = 86 µg/l).

Human Health

In tests on acute toxicity, glyoxal has shown to be of low toxicity to harmfulin animal experiments performed with different species depending on the active ingredient concentration of the tested product. Glyoxal 40% has a moderate toxicity by oral route , a low toxicity by dermal route and a moderate toxicity by inhalation. Glyoxal causes slight to definite skin irritations depending on the

exposure duration. Irritations up to necrotic changes have been described on the rabbit eye. It acts sensitizing to the skin of guinea pigs as well as of humans.

In a subacute inhalation study on rats for 29 days, a 40% glyoxal aerosol concentration of 10 and 2 $mg/m^3$ results in a minimum squamous metaplasia of the epiglottal epithelium in the larynx. A NOEL of 0.4 $mg/m^3$ is given for local effects and of > 10 $mg/m^3$ for the systemic toxicity. In a 28 day oral study in rats, a NOEL of 100 mg/kg bw/d (40% glyoxal) was determined.A dose related decrease of the water , food consumption and body weight were noted at 300 mg/kg and 1000 mg/kg . Variations of some haematological and blood parameters occurred at these doses. No macrocopic and microscopic pathological findings were seen that were considered to be compound related. In a 90d feeding study in rats, glyoxal in daily doses of ca. 30 to 250 mg/kg bw/d is tolerated without clinical, macroscopic and histopathological changes. A temporary reduced body weight gain and an increase of the relative liver and kidney weights, without any histopathological correlation, have been observed only in the males of the highest dose group. The NOEL is ca. 125 mg/kg bw/d related to 40% glyoxal. In 90-d drinking water studies with male rats with daily doses of ca. 140, 290 and 370 mg/kg bw/d, a decreased food and water intake as well as retarded body weight gain was found in the highest dose group. The glyoxalase activities in the liver, kidney and erythrocytes are increased, while the aspartate aminotransferase activity, the alanine aminotransferase and lactate dehydrogenase activities as well as the albumin and total protein value are also determined in the low dose group. The LOEL lies at 107 mg/kg bw/d related to pure glyoxal. Overall, a NOEL of 100 mg/kg bw/d related to 40% glyoxal (40 mg/kg bw/d related to active ingredient) can be retained for repeated dose toxicity.

Glyoxal is shown to be mutagenic in *in vitro* genotoxicity studies in prokaryotes and eukaryotes. In vivo, glyoxal is proven to be negative in the micronucleus test on the mouse after oral administration. On Drosophilia melanogaster, glyoxal is proven to be negative in the sex-linked recessive-lethal test, in the dominant-lethal test and in the studies on the reciprocal translocation and on the loss of sex chromosomes. Chromosome aberrations in the duodenum, testes and spleen are described in an older, only insufficiently documented study after subcutaneous administration to rats. After oral administration to the rat, a significant increase of the unscheduled DNA synthesis is found in the pyloric mucosa, but not in primary hepatocytes, as well as an increase of DNA single-strand breaks in the liver and in the pyloric mucosa. . These findings indicate that glyoxal reacts at the point of entry (the stomach ) and immediately downstream (the liver), but not in more remote organs.

No dose-dependant effects were found on reproductive organs in repeated dose studies up to a dose of approx. 300 mg/kg bw/d (related to the active ingredient). Furthermore, a NOAEL of 125 mg/kg bw/d (related to the active ingredient) could be derived for prenatal development toxicity and of 25 mg/kg bw/d for maternal toxicity.

No carcinogenic effect is detected in mice after dermal application of glyoxal over the entire life span. Glyoxal possesses no tumor initiating effect after the dermal administration to mice After oral administration, glyoxal exhibits local tumor promoting properties in the mucosa of the forestomach of the rat (tissue not existing in species or man). In a liver promotion model on the rat, no indications were found for a promoting effect of glyoxal through systemic action.At last, glyoxal is a metabolite of ethylene glycol and there are two negative carcinogenic studies on the ethylene glycol (rats and mice).

Recommendation

Human health:

Taking account the skin irritation , the skin sensitising properties and the genotoxic potential and based on the use pattern of glyoxal, a detailed risk assessment would be necessary. Especially the risks based on the exposure from open uses (e.g. as a desinfectant) should be evaluated.

Environment:

No further work is necessary

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