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	< 0.01 Pa 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 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 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) + (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) 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
                             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).
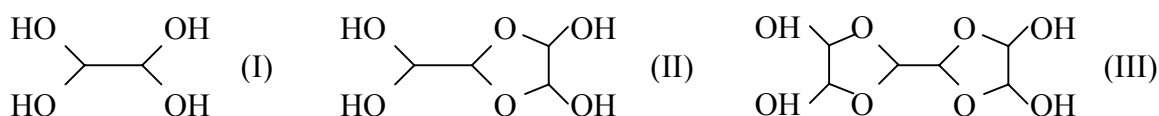
If highly concentrated, oligomeric forms precipitate (polyglyoxal). The equilibrium between monomer and dimer and trimer depends largely on the glyoxal concentration in the aqueous solution:
- in a 5% solution, 39% of glyoxal is present in the monomer form;
- in a 40% solution, the monomer content amounts 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)	$k_{WWTP} = 1 \text{ h}^{-1}$
surface water	$k_{sw} = 0.047 \text{ d}^{-1}$
sediment	$k_{sed} = 0.0023 \text{ d}^{-1}$ *
soil	$k_{soil} = 0.023 \text{ d}^{-1}$ *

  * 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 $5 \cdot 10^5$ $\cdot$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/m³)**	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 3 660 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, male female	Oral	7.07 ml/kg body weight (corresp. to 8,979 mg/kg body weight) 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, Wistar, 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, Wistar, 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, Wistar, male, female	Dermal (24 hour application, semi-occlusive)	> 2 000	LD_{50} ; 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 male female	Inhalative (aerosol, 4 hours)	2 440 2 470 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; 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$^{\text{th}}$ day. These changes were less pronounced on the 9$^{\text{th}}$ day, and a regeneration of the epidermis was observed on the 18$^{\text{th}}$ 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 specially 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 12th 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 ($\pm$ 0.2), 2.3 ($\pm$ 0.8) and 8.9 ($\pm$ 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) Rat	90 days	Male : 32.7, 63.2, 132 and 253 mg/kg bw/d 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) 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) Rat	30, 60, 90 days	2000, 4000, 6000 mg/l	LOAEL = 107 mg/kg bw/d	LOAEL = 107 mg/kg bw/d	Ueno et al (1991a)
Glyoxal (no data)	Oral (drinking water) Rat - Mice	90 days	0, 1000, 2000, 4000, 8000, 16000 mg/l	Not derived	Not derived	NTP (1991)
Glyoxal (100 %)	Oral (diet) 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 Rats	29 days	0, 0.4, 2, 10 mg/m^3	NOEL : Local effects 0.4 mg/m^3 Systemic effects > 10 mg/m^3	NOEL : Local effects 0.1 mg/m^3 Systemic effects > 4 mg/m^3	Hoechst (1995)

4.6 Mutagenicity

<u>In vitro</u>

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					
Salmonella/**microsome test as standard-plate incorporation test**					
Salmonella typhimurium TA 98, TA 100	Ca. 10-500 (30 % glyoxal) toxicity tested	S9-mix from phenobarbital-induced rat liver	Positive (TA 100)	Positive (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 (ca. 2-16 µmol/plate) (30 % glyoxal)	–	Not tested	Positive	Dorado et al., 1992
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538	10-10 000 toxicity tested	S9-mix from Aroclor 1254-induced rat liver	Positive (TA 100)	Positive (TA 100)	Niemand et al., 1983
Salmonella typhimurium TA 102, TA 2638	1 000	–	Not tested	Positive (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 TA 98, TA 100, TA 1535, TA 1537, TA 1538	4-5 000 (40 % glyoxal) toxicity tested	S9-mix from Aroclor 1254-induced rat liver	Positive (TA 100, TA 1535)	Positive (TA 100)	Hoechst, 1984 f
Salmonella typhimurium TA 102	4-5 000 toxicity tested	S9-mix from Aroclor 1254-induced rat liver	Positive	Positive	Hoechst, 1988
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537,	4-127 3.15-100 000 nl/plate) (40 % glyoxal)	S9-mix from Aroclor 1254-induced rat liver	Positive (TA 100)	Positive (TA 100)	Oesch, 1979
Salmonella typhimurium TA 7006, TA 98	20-100 µg/plate	No data	Positive	Positive	Muramata-Kamiya et al. (1997a)
Escherichia coli WP2uvrA	4-5 000 (40 % glyoxal) toxicity tested	S9-mix from Aroclor 1254-induced rat liver	Negative	Negative	Hoechst, 1984 f
Escherichia coli 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* (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 Toxicity tested	–	Not tested	Positive	Marnett et al., 1985
Salmonella typhimurium TA 100, TA 104	50-100	–	Not tested	Positive* (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 (TA 100, TA 102, TA 104)	Positive (TA 100, TA 102, TA 104)	Sayato et al., 1987
Salmonella typhimurium TA 100	58-325 (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 (99 % glyoxal)	S9-mix from Aroclor 1254-induced rat liver	Positive (TA 100, TA 102, TA 104)	Positive (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 Taylor et al., 1983
Mouse lymphoma test, L5178TK$^{+/-}$ cells	27.8-61.5 (4.79 x 10^{-4} – 10.6 x 10^{-4} mol/l)	-	No tested	positive	Wangenheim & Bolcsfoldi, 1988
Co S-7- 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 toxicity tested	S9-mix from Aroclor 1254-induced rat liver	positive	positive	Henkel, 1986
Chromosome aberration, V79 cells of the Chinese hamster	100-400 (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	5×10^{-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 (0.461×10^{-3}- 3.69×10^{-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 (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

<u>In vivo</u>

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 8th to 40th week, the first group was administered drinking water with 0.5 % glyoxal and the second group, drinking water without any additive. A third group (10 animals) received a diet and drinking water without additives in the first 8 weeks and then received drinking water with 0.5 % glyoxal from the 8th to 40th week. For the rats 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 total in fundus in pylorus	12 (43 %)* 1 (4 %) 11 (39 %)*	5 (17 %) 1 (3 %) 4 (13 %)	0 0 0
Hyperplasias in fundus in pylorus	4 (14 %)* 17 (61 %)**	1 (3 %) 8 (27 %)	0 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 performed 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 5th and 10th day after dermal application of 40 % glyoxal. After the intravenous injection of 2.5 ml of a 20 % glucose solution, the glucose level in the blood was determined according to Hagedorn-Jensen. 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 $[4,5-^3H]$-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 1.5×10^{-3}, 2.7×10^{-3} and 1.4×10^{-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 5×10^{-6} and 5×10^{-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 5×10^{-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

6.   REFERENCES

Aeschbacher, H.U., Wolleb, U., Löliger, J., Spadone, J.C., Liardon, R. (1989) Contribution of coffee aroma constituents to the mutagenicity of coffee. Food Chem. Toxicol. 27, pp 227-232.

Akhand, A.A., Kato, M., Susuki, H., Liu, W., Du, J., Hamaguchi, M., Miyata, T., Kurokawa, K., Nakashima, I. (1999). Carbonyl compounds cross-link cellular proteins and activate protein-tyrosine p60c-Src. Journal of Cellular Biochemistry 72, 1-7.

American Cyanamid Company (1974): Unpublished report no 74-7

American Cyanamid Company (1980a): Unpublished report 029-626-292-8 by EG&G Mason Research Institute

American Cyanamid Company (1980b): Unpublished report 029-626-293-8 by EG&G Mason Research Institute

American Cyanamid Company (1980c): Unpublished report 029-626-321-8 by EG&G Mason Research Institute

American Cyanamid Company (1982) Unpublished report 45-508 by Bushy Run Research Center

American Cyanamid Company (1982): Project No. CT 096-82

American Cyanamid Company (1988): New Jersey, Projekt No. 5529/85

Anonym (1969): Toxicol. Drugs Chem., 291; cited in: RTECS (1991)

Ariza, R.R., Dorado, G., Barbancho, M., Pueyo, C. (1988) Study of the causes of direct-acting mutagenicity in coffee and tea using the ara test in Salmonella typhimurium. Mutat. Res. 201, pp 89-96

Ashwood-Smyth, M.J., Robinson, D.M., Barnes, J.H., Bridges, B.A. (1967) Radiosensitization of bacterial and mammalian cells by substituted glyoxals. Nature 216, 137-139

Auerbach et al. (1977): Mutat. Res. 39, 317-362

Barnett, B.M. & Munoz, E.R. (1969) Mutation test with glyoxal in drosophilia melanogaster males. Drosophila Inf. Serv. 44, p 119

Barnett, B.M. & Munoz, E.R. (1989) Effect of glyoxal pretreatment on radiation-induced genetic damage in Drosophilia melanogaster. Mutat. Res. 212, pp 173-179

BASF AG (1954) unpublished report (III/622)

BASF AG (1956): Unpublished report Nr. III/622

BASF AG (1963a) Department of toxicology, unpublished report (XIII/257) from 10.10.63

BASF AG (1963b), Department of toxicology, unpublished report (XIII/258) from 10.10.63

BASF AG (1970) Unpublished report Nr. XX/162-163

BASF AG (1979) Unpublished report Nr. 77/99

BASF AG (1979): Unpublished report Nr. 78/552 (HOE 89.0200)

BASF AG (1985a): Unpublished report Nr. 85/16 (HOE 89.0199)

BASF AG (1985b) Department of toxicology, unpublished report (85/248) from 17.10.85

BASF AG (1985c): Unpublished report Nr. 85/16 (HOE 89.0243)

BASF AG (1987): Unpublished report Nr. 86/342 (HOE 89.0215)

BASF AG (1988) Unpublished study: Determination of acute effects of Glyoxal on the waterflea Daphnia magna Straus of 05.02.88 (1/0002/2/88-0002/88)

BASF AG (1988): Unpublished report 2/0002/88/t96 from 02.09.1088

BASF AG (1988): Unpublished study of the department of analytics (BRU 88.157)

BASF AG (1995) Safety data sheet: Glyoxal Pure Sol. App. 40%, 31.01.1995

BASF AG (1995) Unpublished study 95/26 from Dec. 1995

BASF AG (1996) Determination of the biodegradability or eliminability in the activated sludge simulation test, unpublished study, 06/03-16/04/1996, project number 96/0054/30/1

BASF AG (1996) Determination of the sorption coefficient by the HPLC screening method, Laboratory for ecological analysis, unpublished study, 13-19/04/1997, project number 96/0054/94/1

BASF AG (1996) Inhibitory effect on the cell multiplication of Pseudomonas putida, Labor für Ökologie, unpublished study, 15/16.04.1996, project number 96/0054/70/1

BASF AG (1996) Laboratory for ecological testing, unpublished study, 22/02 - 12/03/1996, report number 96/0054/21/1

BASF AG (1996) Respiration inhibition test with activated sludge, Labor Ökologie, unpublished study, 14.02.96, project number 96/0054/08/1

BASF AG & CLARIANT SA (2001) Glyoxal 40% - Prenatal Developmental Toxicity Study in Wistar Rats. Unpublished report BASF No 30R0146/99011 of January 2001

Basketter, D.A., Scholes, E.W., Kimber, I. (1994) The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximisation test. Fd. Chem. Toxic. 32(6), pp. 543-547.

Betterton, E.A. & Hoffmann, M.R. (1988) Henry's law constants of some environmentally important aldehydes. Environ. Sci. Technol. 22, pp 1415-1418

Betterton, E.A., Olson, T.M., Hoffmann, M.R. (1986) Symposium on acid rain: I. Sources and atmospheric processes presented before the division of Petroleum Chemistry, Inc., Americal Chemical Society, Dicarbonyls as S(IV) reservoirs. Prep.-Am. Chem. Soc. Div. Pet. Chem. 31, pp 573-576

BG Chemie (1997) Toxicological Evaluation, Glyoxal, Vol. 12. Potential hazards of Existing Chemicals, Springer-Verlag, Berlin, Heidelberg, New York

Bjeldanes, L.F. & Chew, H. (1979) Mutagenicity of 1,2-dicorbonyl compounds: maltol, kojic acid, diacetyl and related substances. Mutat. Res. 67, pp. 367-371

Broude, N.E. & Budowsky, E.I. (1971) The reaction of glyoxal with nucleic acid components. III. Kinetics of the reaction with monomers. Biochim. Biophys. Acta, 254, pp 380-388

Calliès, F.X., Taieb, M., Dômont, A., Cottentot, F., Proteau, J. (1985) A propos de trois cas cliniques d'eczéma de contact à un dialdéhyde contenu dans un produit désinfectant d'utilisation courante en hôpital. Arch. Mal. Prof., 2, pp 109-110

Carpenter, C.P. & Smyth, H.F. (1946) Chemical burns of the rabbit cornea. Am. J. Ophthalmol. 29, pp. 1363-1372

CCR (Cytotest Cell Research, Rossdorf) (1992) Unpublished report CCR Project 230602, sponsored by Berufsgenossenschaft der chemischen Industrie.

CEC (Commission of the European Communities), 1996, Technical Guidance Document in support of Directive 93/67/EEC on risk assessment of notified new substances and Regulation (EC) N° 1488/94 on risk assessment of existing substances (Parts I, II, III and IV)

Chastrette F., Bracoud C., Chastrette M., Mattioda G., Christidis Y. (1983), Bull. Soc. Chim. Fr., N° 1-2, pp 33-40

Chemicals Inspection & Testing Institute Japan (1992), Biodegradation and Bioaccumulation data of existing chemical based on the CSCL Japan, October 1992

Clariant (2000) Fiche de données de sécurité, Glyoxal 40, 13.04.2000

Conway, R.A., Waggy, G.T., Spiegen, M.H., Berglund, R.L. (1983) Environmental fate and effects of ethylene oxide. Environ. Sci. Technol. 17, 107-112

Cornago, M.P., Lopez Zumel, M.C., Alvarez, M.V., Izquierdo, M.C. (1990) Glyoxylic compounds as radiosensitizers of hypoxic cells. Biochem. Med. Metabol. Biol., 43, pp 253-262

Cornago, P., Lopez Zumel, M.C., Santos, L., Pintado, M. (1989) Semiconservative and unscheduled DNA synthesis on mammalian cells and its modification by glyoxylic compounds. Biochemie, 71,

pp 1205-1210

Dorado, L., Ruiz Montoya, M., Rodriguez Mellado, J.M. (1992) A contribution to the study of the structure-mutagenicity relationships for alpha-dicarbonyl compounds using the Ames test. Mutat. Res., 269, pp 301-306

Doull, J., Plzak, V., Oldfield, D.G. (1964) Pharmacological or toxicological compounds as protective or therapeutic agents against radiation injury in experimental animals. III. Dose-mortality relationships for several radioprotective agents. in: The University of Chicago USAF Radiation Laboratory, Quarterly Progress Report No. 51, Nat. Techn. Inf. Serv. (NTIS) AD 438-895, pp 75-98

Environment Agency Japan (1996) Environmental Health and Safety Division: Chemicals in the Environment. Report on EnvironmentalSurvey and Wildlife Monitoring of Chemicals in F.Y. 1994 (May, 1996).

Fassett (1962): Industrial Hygiene and Toxicology, 2nd ed., 2, 1980-1984

Foussereau, J., Cavelier, C., Zissu, D. (1992) L'allergie de contact professionnelle aux antiseptiques aldéhydés en milieu hospitalier. Arch. Mal. Prof., 53 (5), pp 325-338

Francoeur, M. & Denstedt, O.F. (1954) Metabolism of mammalian erythrocytes. VI. Heme as a catalyst for the oxidation of glyoxal in the erythrocyte. Can. J. Biochem. Physiol. 32, pp 655-662

Fratzke A. R. & Reilly P.J. (1986), Int. J. Chem. Kinet., Vol 18, pp 775-789

Furihata, C. & Matsushima, T. (1989) Prediction of possible carcinogens, tumor-promotors and anti-tumor promotors in the glandular stomach. Environ. Mol. Mutagen., 14 (15), 63, Ref. 178

Furihata, C., Hatta, A., Sato, Y., Matsushima, T. (1989) Alkaline elution of DNA from stomach pyloric mucosa of rats treated with glyoxal. Mutat. Res. 213, pp 227-231

Furihata, C., Sato, Y., Matsushima, T. (1988) Alkaline elution of DNA from stomach pyloric mucosa of rats treated with MNNG and glyoxal. Mutat. Res. 203, p 371

Furihata, C., Yoshida, S., Matsushima, T. (1985) Potential initiating and promoting activities of diacetyl and glyoxal in rat stomack mucosa. Jpn. J. Cancer Res. (Gann) 76, pp 809-814

Gan, J.C. & Ansari, G.A.S. (1986) Non-oxidative inactivation of plasma alpha1-proteinase inhibitor by carbonyl compounds found in cigarette smoke. Res. Commun. Subst. Abuse, 7, pp 59-69

Garberg, P., Akerblom, E.L., Bolcsfoldi, G. (1988) Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution. Mutat. Res. 203, pp 155-176

Garst, J., Stapleton, P., Johnston, J. (1983) Mutagenicity of alpha-hydroxy ketones may involve superoxide anion radical. in: Greenwald, R.A., Cohen, G. (eds.) Oxy radicals and their scavenger systems, volume II: cellular and medical aspects, pp 125-130. Proc. Int. Conf. Superoxide Dismutase, 3rd ed. Elsevier Science Publishing Co

Gerike, P. & Gode, P. (1990) The biodegradability and inhibitory threshold concentration of some disinfectants. Chemosphere 21, pp 799-812

Goldman, L., Barkoff, J., Blaney, D., Nakai, T., Suskind, R. (1960) Investigative studies with the skin coloring agents dihydroxyacetone and glyoxal. J. Invest. Dermatol. 35, pp 161-164

Guidotti, G.G., Loreti, L., Ciaranfi, E. (1965) Studies on the anti-tumor activity of aliphatic aldehydes - I. The mecanism of inhibition of amino acid incorporation into protein of Yoshida ascites hepatoma cells. Eur. J. Cancer, 1, pp 23-32

Hasegawa, R. & Ito, N. (1992) Liver medium-term bioassay in rats for screening of carcinogens and modifying factors in hepatocarcinogenesis. Food Chem. Toxicol., 30 (11), pp 979-992

Hebbel, R.P. & Miller, W.J. (1988) Unique promotion of erythrophagocytosis by malondialdehyde. Am. J. Hematol., 29, pp 222-225

Hellmér, L. & Bolcsfoldi, G. (1992a) An evaluation of the E. coli K-12 uvr/recA DNA repair host-mediated assay. I. In vitro sensitivity of the bacteria to 61 compounds. Mutat. Res., 272, pp 145-160

Hellmér, L. & Bolcsfoldi, G. (1992b) An evaluation of the E. coli K-12 uvr/recA DNA repair host-mediated assay. II. In vivo results for 36 compounds tested in the mouse. Mutat. Res., 272, pp 161-173

Henkel (1986): unpublished report by NOTOX 0367/EC 124 (HOE 87.0447)

Hindson, C. & Lawlor, F. (1982) Allergy to glyoxal in a polyvinyl resin emulsion. Contact Dermatitis 8, 213

Hoechst AG (1929): Unpublished report Nr 29.0013

Hoechst AG (1984a): Unpublished report (84.0195)

Hoechst AG (1984b): Unpublished report (84.0378)

Hoechst AG (1984d): Unpublished report (84.0443, July 1984)

Hoechst AG (1984e): Unpublished report (84.0450)

Hoechst AG (1984c): Unpublished report (84.0693)

Hoechst AG (1984f): Unpublished report No 84.0205 (April 24, 1984)

Hoechst AG (1984): Unpublished study (W 84-007)

Hoechst AG (1984): Unpublished study (W 84-087)

Hoechst AG (1984): Unpublished study (W 84-105)

Hoechst AG (1988): Unpublished report No 88.0271 (March 10, 1988)

Hoechst AG (1989) Unpublished report (V 89-074)

Hoechst AG (1989): Unpublished study (89.0222)

Hoechst AG (1990) Unpublished study (90.0486, Glyoxal 40)

Hoechst AG (1990): Safety Data Sheet (06.1990)

Hoechst AG (1991): Analytisches Labor Oekochemie / Analytik E (1977/91 B, 29.06.1991)

Hoechst AG (1995) Unpublished report No 94.1056

Hoh, A., Maier, K., Dreher, R.M. (1987) Multilayered keratinocyte culture used for in vitro toxicology. Mol. Toxicol. 1, pp 537-546

IFREB (1982) unpublished report Nr 206311

Ito, K. (1963) Glyoxal as a cause of occupational disease. Bull. Pharmacol. Res. Inst., 44, pp 8-15

Kasai, H., Hayami, H., Yamaizumi, Z., Saito, H., Nishimura, S. (1984) Detection and identification of mutagens and carcinogens as their adducts with guanosine derivatives. Mucleic Acids Res. 12, pp 2127-2136

Kato, F., Araki, A., Nozaki, K., Matsushima, T. (1989) Mutagenicity of aldehydes and diketones. Mutat. Res. 216, pp 366-367

Kawata, K., Ozaki, K., Mukai, H. (1980) Gas chromatographic determination of micro amount of glyoxal in water and sediment. Bunseki Kagaku, 29 (8): pp 517-522

Klamerth, O.L. (1968) Influence of glyoxal on cell function. Biochim. Biophys. Acta, 155, pp 271-279

Kligman, A.M. (1966) The identification of contact allergens by human assay. III. The maximization test: a procedure for screening and rating contact dermatitis. J. Invest. Dermatol. 47 (5), pp 393-409

Kun, E. (1952) A study on the metabolism of glyoxal in vitro. J. Bil. Chem. 194, pp 603-611

Levin, D.E., Hollstein, M., Christman, M.F., Schwiers, E.A., Ames, B.N. (1982) A new Salmonella tester strain (TA 102) with A-T base pairs at the site of mutation detects oxidative mutagens. Proc. Natl. Acad. Sci. 79, pp 7445-7449

Lilley, D.M.J. (1986) Cyclic adduct formation at structural perturbations in supercoiled DNA molecules. IARC Sci. Publ. 70, pp 83-99

Marnett, L.J., Hurd, H.K., Hollstein, M.C., Levin, D.E., Esterbauer, H., Ames, B.N. (1985) Naturally occurring carbonyl compounds are mutagens in Salmonella tester Strain TA 104. Mutat. Res. 148, pp 25-34

Matsui, S., Yamamoto, R., Yamada, H. (1989) The bacillus subtilis/microsome rec-assay for the detection of DNA damaging substances which may occur in chlorinated and ozonated waters. Water Sci. Technol. 21, pp 875-887

Mattioda & Blanc (1989): Ullmann's Encyclopedia of Industrial Chemistry, 5th ed, Verlag Chemie, Weinheim, New York, Vol A12: 491 - 494.

Mattioda G. & Blanc A. (1989) in Ullmann's Encyclopedia of Industrial Chemistry, Vol. A 12, pp 491-494

Miyakawa, Y., Nishi, Y., Kato, K., Sato, H., Takahashi, M., Hayashi, Y. (1991) Initiating activity of eight pyrolysates of carbohydrates in a two-stage mouse skin tumorigenesis model. Carcinogenesis, 12 (7), 1169-1173

Murata-Kamiya, N, Kaji, H., Kasai, H. (1997a) Types of mutations induced by glyoxal, a major oxidative DNA-damage product, in Salmonella typhimurium. Mutation Research 377, pp 13-16.

Murata-Kamiya, N., Kamiya, H., Kaji, H., Kasai, H. (1997b). Mutational specificity of glyoxal, a product of DNA oxidation, in the lacI gene of wild-type Escherichia coli W3110. Mutation Research, 377, pp 255-262.

Murata-Kamiya, N., Kamiya, H., Kaji, H., Kasai, H. (1997c). Glyoxal, a major product of DNA oxidation, induces mutations at G:C sites on a shuttle vector plasmid replicated in mammalian cells. Nucleic Acids Research, 25(10), pp 1897-1902.

Nakaya, K., Takenaka, O., Horinishi, H., Shibata, K. (1968) Reactions of glyoxal with nucleic acids, nucleotides and their component bases. Biochem. Biophys. Acta 101, pp 23-31

Niemand, J.G., den Drijver, L., Pretorius, D.J., Holzapfel, C.W., van der Linde, H.J. (1983) A study of the mutagenicity of irradiated sugar solutions: implications for the radiation preservation of subtropical fruits. J. Agric. Food Chem., 31, pp 1016-1020

Nishi, Y., Miyakawa, Y., Kato, K. (1989) Chromosome aberrations induced by pyrolysates of carbohydrates in Chinese hamster V79 cells. Mutat. Res. 227, pp 117-123

NTP (1988): Executive summary of data glyoxal. Report No.N01-ES-5-5097

NTP (1991), as cited in: BUA (1996) Glyoxal, BUA Report 187, GDCh-Advisory Committee on Existing Chemicls of Environmental Relevance (BUA), S. Hirzel, Stuttgart, Germany.

NTP (1993) Final report of the developmental toxicity of glyoxal trimeric dihydrate (CAS nr 4405-13-4) in New Zealand White (NZW) rabbits. TER92121. (NTIS PB94-104064).

NTP (1994) Final report of the developmental toxicity of glyoxal trimeric dihydrate (CAS nr 4405-13-4) in Sprague-Dawley (CD) rats. TER92116. (NTIS PB94-151974).

Ono, Y., Somiya, I., Kawamura, M. (1991a) Genotoxicity of by-products in the chemical oxidation processes. Suishitsu Odaku Kenkyu, 14, pp 633-641

Ono, Y., Somiya, I., Kawamura, M. (1991b) The evaluation of genotoxicity using DNA repairing test for chemicals produced in chlorination and ozonation processes. Water Sci. Technol., 23, pp 329-338

Plum, C.N., Sanhueza, E., Atkinson, R., Carter, W.P.L., Pitts, J.N. (1983) OH radical rate constants and photolysis rates of alpha-dicarbonyls. Environ. Sci. Technol. 18, pp 981-984

Raleigh, J.A. & Liu, S.F. (1983) Reductive fragmentation of 2-nitroimidazoles in the presence of nitroreductases - glyoxal formation from misonidazole. Biochem. Pharmacol. 32, pp 1444-1446

Ruiz-Rubio, M., Alejandre-Duran, E., Pueyo, C. (1985) Oxidative mutagens specific for A-T base pairs induce forward mutations to L-arabinose resistance in Salmonella typhimurium. Mutat. Res. 147, pp 153-163

Sasaki, Y. & Endo, R. (1978) Mutagenicity of aldehydes in Salmonella. Mutat. Res. 54, 251-252

Sayato, Y., Nakamuro, K., Ueno, H. (1987) Mutagenicity of products formed by ozonation of naphthoresorcinol in aqueous solutions. Mutat. Res. 189, pp 217-222

Shane, B.S., Troxclair, A.M., McMillin, D.J., Henry, C.B. (1988) Comparative mutagenicity of nine brands of coffee to Salmonella typhimurium TA100, TA102 and TA104. Environ. Mol. Mutagen. 11, pp 195-206

Shapiro, R. & Hachmann, J. (1966) The reaction of guanine derivatives with 1,2-dicarbonyl compounds. Biochemistry, 5, pp 2799-2807

Shapiro, R., Sodum, R.S., Everett, D.W., Kundu, S.K. (1986) Reactions of nucleosides with glyoxal and acrolein. in: Singer, B., Bartsch, H. (eds.) The role of cyclic nucleic acid adducts in carcinogenesis and mutagenesis. IARC Sci. Publ. 70, pp 165-173

Slonim I.Ia.,Arshava B.M., Kliuchnikov V.N., Jurina F.G., Kaliya T.K. (1990), Jurn. prikl. spectroscopii 52, (2), p 327

Smyth, H.F., Carpenter, C.P., Weil, C.S., Pozzani, U.C., Striegel, J.A. (1962) Range-finding toxicity data: list VI. Am. Ind. Hyg. Assoc. J. 23, 95-107

Smyth, H.F., Seaton, J., Fischer, L. (1941): J. Ind. Hyg. Toxicol. 23, 259-268

Société Française Hoechst (1979): Internal report of IRCHA (Etude.B. 7637)

Société Française Hoechst (1980) Unpublished report by Centre d'Etudes Biologiques

Société Française Hoechst (1982) Détermination de la toxicité aigue chez le rat du produit "HF 0002" (par voie orale) unpublished report No 4028 TAR performed by Centre d'études biologiques

Société Française Hoechst (1986a): Unpublished report by CIT No. 1784 MVA (HOE 87.0418)

Société Française Hoechst (1986b) Unpublished report by CIT No. 2018 MAS (HOE 86.0783)

Société Française Hoechst (1987): Unpublished report by CIT No. 2619 TSR (HOE 87.1678)

Société Française Hoechst (1992) Internal report: SFH - CRA - SCA - HF002 - ChD85/92

Société Française Hoechst (1992): Internal report SFH - CRA - SCA - HF002 - ChD76/92

Société Française Hoechst (1992): Internal report: SFH - CRA- SCA - HF002 - ChD136/92

Société Française Hoechst (1992): Safety Data Sheet of May 1992

Société Française Hoechst (1993): Test to evaluate Acute Toxicity (72 hours) in Freshwater Unicellular Algae. Report No. D009.

Spolter, P.D., Adelman, R.C., Weinhouse, S. (1965) Distinctive properties of native and carboxypeptidase-treated aldolases of rabbit muscle and liver. J. Biol. Chem. 240, pp 1327-1337

Suwa, Y., Nagao, M., Kosugi, A., Sugimura, T. (1982 Sulfite suppresses the mutagenic property of coffee. Mutat. Res., 102, pp 383-391

Syracuse Research Corporation (1994a) "AOP"

Syracuse Research Corporation (1994b) "LogKow"

Takahashi, N., Okamiya, H., Furukawa, F., Toyoda, K., Sato, H., Imaida, K., Hayashi, Y. (1989) Effects of glyoxal and methylglyoxal administration on Gastric carcinogenesis in Wistar rats after initiation with N-methyl-N'-nitro-N-nitrosoguanidine. Carcinogenesis 10, pp 1925-1927

Taylor, R.T. & Wu, R. (1980) Mutagen induced reversion of a Chinese hamster ovary triple auxotroph. Environ. Mutagen. 2, p 236

Taylor, R.T., Wu, R., Hanna, M.L. (1983) Induced reversion of a Chinese hamster ovary triple auxotroph: response to 1,2-dicarbonyl compounds versus the CHO-S/HGPRT locus. Environ. Mutagen. 5, p 504

Thomas, J.A. (1958) L'action inhibitrice du glyoxal sur les macromolécules biologiques. Symp. Soc. Exptl. Biol., 12, pp 242-255

Torosyan, A.T. (1979) Mutagenic alterations in antibody synthesis. Sov. Genet., 15, pp 41-46

Tucker, J.D., Taylor, R.T., Christensen, M.L., Strout, C.L., Hanna, M.L., Carrano, A.V. (1989) Cytogenetic response to 1,2-dicarbonyls and hydrogen peroxide in Chinese hamster ovary AUXB1 cells and human peripheral lymphocytes. Mutat. Res. 224, pp 269-279

Ueno, H., Segawa, T., Hasegawa, T., Nakamuro, K., Maeda, H., Hiramatsu, Y., Okada, S., Sayato, Y. (1991a) Subchronic oral toxicity of glyoxal via drinking water in rats. Fund. Appl. Toxicol. 16, pp. 763-772

Ueno, H., Nakamuro, K., Sayato, Y., Okada, S. (1991b) Characteristics of mutagenesis by glyoxal in Salmonella typhimurium: contribution of singlet oxygen. Mutat. Res. 251, pp 99-107

Ueno, H., Nakamuro, K., Sayato, Y., Okada, S. (1991c) DNA-lesions in rat hepatocytes induced by in vitro and in vivo exposure to glyoxal. Mutat. Res. 260, pp 115-119

Union Carbide Company (1966) Unpublished report No. 29-1 (HOE 87.0733)

Union Carbide Corporation (1955) Unpublished report No 18-48 performed by Mellon Institute of Industrial Research (NTIS/OTS 953-5072)

Union Carbide Corporation (1965) Unpublished report Nr. 26-103 performed by Mellon Institute of Industrial Research.

Union Carbide Corporation (1973) Unpublished report Nr 36-52, Mellon Institute of industrial researches

von der Hude, W., Behm, C., Gürtler, R., Basler, A. (1988) Evaluation of the SOS chromotest. Mutat. Res. 203, pp 81-94

Wangenheim, J. & Bolcsfoldi, G. (1988) Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. Mutagenesis 3, pp 193-205

Windholz (1983): The Merck Index, 10th ed., 647

Wingard, C., Hitchcock, P., Teague, R.S. (1955) A survey of aldehydes with respect to their action on the blood pressure. Arch. Int. Pharmacodyn. 102, pp 65-84

Yamaguchi, T. & Nakagawa, K. (1983) Mutagenicity of and formation of oxygen radicals by trioses and glyoxal derivatives. Agric. Biol. Chem. 47, pp 2461-2465

Zimmermann, F.K. & Mohr, A. (1992) Formaldehyde, glyoxal, urethane, methyl carbamate, 2,3-butanedione, 2,3-hexanedione, ethyl acrylate, dibromoacetonitrile and 2-hydroxy-propionitrile induce chromosome loss in Saccharomyces cerevisiae. Mutat. Res., 270, pp 151-166