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DECOS and NEG Basis for an Occupational Standard

Glutaraldehyde

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Nordic Council of Ministers

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Preface

An agreement har been signed by the Dutch Expert Committee for Occupational Standards (DECOS) of the Dutch Health Council and the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG). The purpose of the agreement is to write joint scientific criteria documents which could be used by the national regulatory authorities both in the Netherlands and in the Nordic Countries.

The evaluation of health effcts of Glutaraldehyde is a product of this agreement. The draft document was written by Drs Brita Beije and Per Lundberg at the Department of Toxicology and Chemistry, National Institute for Working Life, Solna, Sweden. The document has been reviewed by the Dutch Expert Committee as well as by the Nordic Expert Group.

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Contents

1. Introduction

The first report of the synthesis of glutaraldehyde appeared in 1908, but it was not until the early 1960s that the commercial use of glutaraldehyde, as a tanning agent, was recognised. This was followed by many other uses, such as a fixative in electron microscopy, a cross-linking agent for proteins and enzymes, as a disinfectant for instruments in the health care system. When the use of formaldehyde was questioned in the early 1970s due to potential health risks, the use of glutaraldehyde was further increased.

2. Chemical identification

Common name:	glutaraldehyde
CAS number:	111-30-8
Synonyms:	glutaral, glutardialdehyde, glutaric dialdehyde, 1,5-pentanedial, 1,5-pentanedione, 1,3-diformylpropane, sonacide
Molecular formula:	$C_5H_8O_2$
Structural formula:	$CHO-(CH_2)_3-CHO$
Molecular weight:	100.13

3. Physical and chemical properties

Freezing point	-14 ^oC
Boiling point	188 ^oC
Density (specific gravity): (water =1)	0.72
Vapour density:	3.4 (air=1)
Vapour pressure: (at 20°C)	0.00016 kPa (2 % solution) 0.002 kPa (50 % solution)

Saturation vapour conc:	6.6 mg/m^3 (1.6 ppm) (20 % solution) 82 mg/m^3 (20 ppm) (50 % solution)
Partition coefficient n-octanol/water	log $P_{o/w}$ = 0.01
pH value:	3-4 (in solutions) 7.5-8 (activated solutions)
Conversion factors: (at 20°C)	1 mg/m^3 = 0.25 ppm 1 ppm = 4.0 mg/m^3

Glutaraldehyde is a colourless, oily liquid, with a pungent, aldehyde odour. The odour threshold value is 0.04 ppm (1, 7). Glutaraldehyde is soluble in water and ethanol in all proportions. Glutaraldehyde is also soluble in benzene, ether, and similar organic solvents. Glutaraldehyde is corrosive. Glutaraldehyde can react violently with strong oxidisers, heat is produced in the presence of strong alkalies or strong acids, and glutaraldehyde may initiate polymerisation in the presence of amines. Glutaraldehyde does not burn and there is no danger of explosion or auto ignition. The combustion and thermal products are carbon monoxide and carbon.

4. Occurrence, production and use

Glutaraldehyde is commonly available as a clear, colourless aqueous solution. Usually available as 1%, 2%, 25% or 50% solutions of glutaraldehyde liquid in water, but other formulations are also available. Commercial solutions may contain other chemicals which may affect the overall toxicity and characteristics of the solution.

Alkaline solutions of glutaraldehyde (pH 7.5-8.5) is a highly effective micro-biocidal agent and widely used in the cold sterilisation of medical, surgical and dental equipment (70). Glutaraldehyde is used as a slimicide in the paper industry (30).

Glutaraldehyde is widely used as a disinfectant and sterilising agent (usually as a 2% solution) in medical and dental settings, in embalming (25% solution), as an intermediate and fixative for tissue fixing in electron microscopy (20, 50, and 90% solutions) and in X-ray films, in the tanning industry, in the manufacture of adhesives and sealants, as a biocide in water cooling towers, as a cross-linking agent, and in microcapsules containing flavouring agents.

Glutaraldehyde is used as an agent to cross-link collagen strands, thereby strengthening tissues for use in bioprosthetic devices (48, 56).

Glutaraldehyde has been used in systemic chemotherapy to treat drug-loaded erythrocytes in order to produce specific targeting of the red blood cells to the liver in rodents and other animal species (108), as well as in one man (97). Glutaraldehyde treatment was able to reduce the release of the drug, and the efflux

rate from the treated cells was dependent on the glutaraldehyde concentration. The lowest rate was detected at 0.3% glutaraldehyde.

Glutaraldehyde has been used as a therapeutic agent for topical treatment of hyperhidrosis (excessive sweating), for topical treatment of warts in children, for topical treatment of onychomycosis (fungal nail infection), for friction blister prevention in soldiers, athletes and ballet dancers.

5. Occupational exposure

In six hospitals in the Southeast of England, 77 samples were collected at 14 locations. Of these samples, 39 were collected with personal sampling devices. Sampling periods were from 4 to 26 minutes, and the exposure concentrations measured were between 0.003 and 0.17 mg/m^3. The highest exposure during the survey was 0.17 mg/m^3, which was found during the cleaning of suction bottles with Cidex (trade name for a glutaraldehyde solution). The lowest exposure was 0.003 to 0.006 mg/m^3, which was recorded for those working with *x* ray processing chemicals (53).

Measurements of glutaraldehyde have been performed in Danish hospitals, different departments. Both personal sampling and stationary sampling was used. The highest air concentration of glutaraldehyde was found in a Surgical Department, where 0.250 to 0.500 mg/m^3 were found (80).

In short-time measurements during manual cold sterilisation work with a 2 % solution of glutaraldehyde the concentration was low in all samples. The geometric mean of 16 glutaraldehyde measurements was 0.05 mg/m^3. The highest value, 0.57 mg/m^3, was measured during the cold sterilisation of a gastroscope. During automatic cold sterilisation the glutaraldehyde exposure levels were from 0.01 to 0.18 mg/m^3. Personal sampling was used in this study (70).

When glutaraldehyde was decanted into a bowl, an endoscope disinfected and the used solution disposed by pouring into a sluice the air concentration was monitored to be 0.68 mg/m^3 (14). In another study of endoscopy suites a short-term level of 0.16 mg/m^3 was measured (39). In both these studies personal sampling was used.

Cleaning procedures in operating theatres were performed with disinfectants containing both formaldehyde and glutaraldehyde. Personal measurements revealed peak concentrations of glutaraldehyde up to 0.03 ppm. The time-weighted average during an 8-h shift the mean value of glutaraldehyde was 0.01 ppm (10).

In the atmosphere above a commercial sterilizing product at concentrations about 2 % glutaraldehyde it is suggested that air concentrations of up to 2 ppm (8 mg/m^3) glutaraldehyde can be formed (81).

The air levels of glutaraldehyde in X-ray darkrooms has been measured by personal and stationary samplers to be 0.16 mg/m^3 (short term, median level) during decantation in endoscopy suites and <0.009 mg/m^3 in darkrooms (39).

6. Sampling and analysis of substance at work place

There is a fully validated method (73) involving the collection on two glass fibre membrane filters, each coated with 2,4-dinitro phenyl hydrazine and phosphoric acid. Acetonitrile is used for desorption. Analysis is done by high pressure liquid chromatography (HPLC) using a UV detector. The detection limit is 18 $\mu g/m^3$ (4.4 ppb) for a sampling volume of 15 L at a sampling rate of 1 L/min. Later (23), a detection limit of about 3 ppb for a 2 L sample has been reported for the OSHA-method.

In another method XAD-2 sorbent tubes coated with 2,4-dinitrophenylhydrazine (DNPH) are used for collection and the hydrazones formed are desorbed with toluene or diethylether. Analysis is performed with gas chromatography using a flame ionisation detector. In a later method from NIOSH, the sampling is performed on a silica gel coated with 2,4-dinitrophenylhydrazine-HCl. After extraction with acetonitrile the glutaraldehyde dinitrophenylhydrazone is detected by HPLC-UV at 365 nm. The working range for a 20 L sample is 0.01 to 0.3 ppm (0.04 to 1.2 mg/m^3) (3, 69).

Quantitative determination of glutaraldehyde, formaldehyde and acrolein in air samples has been described. Known volumes of air are drawn through sampling tubes, containing Amberlite XAD-2, coated with DNPH as adsorption material. The hydrazones formed are desorbed using acetonitrile as eluent. The separation of the three compounds is performed on a RP (Reversed Phase) C-18 column. For detection at λ = 365 nm an UV spectrophotometer is used. The detection limits, based on a 3 l air sample and an injection volume of 15 µl, were estimated to 0.02 mg/m^3 (glutaraldehyde), 0.04 mg/m^3 (formaldehyde) and 0.015 mg/m^3 (acrolein) (80).

In a Norwegian method (96) samples were collected on a Sep-Pak DNPH-Silica cartridges followed by elution with acetonitrile and analysis by HPLC. The recovery was 87±5 %.

7. Toxicokinetics

7.1. Uptake and distribution

The uptake of glutaraldehyde has been investigated in a variety of biological systems.

In an in vitro study in skin samples of F344 rats, CD-1 mice, rabbits, guinea pigs and humans less than 1 % of the applied glutaraldehyde penetrated the skin (92). This abstract only states two dose levels but not the concentrations.

A flow-through skin penetration chamber has been used to determine the in vitro skin penetration over a 6 h exposure period of 0.75 and 7.5 % [1,5-^{14}C]-glutaraldehyde on excised skin from Fischer 344 rats, CD-1 mice, Hartley guinea pigs, New Zealand white rabbits, and human beings. Total recovery from all species ranged from 75-92 % for both concentrations. Overall, <0.5 % of 0.75 %

glutaraldehyde and <0.7 % of the 7.5 % solution was absorbed through the skin. For human beings, approximately 0.2 % of the applied radioactivity penetrated the skin for both doses tested, largely due to binding to the skin (36).

Percutaneous penetration of glutaraldehyde has been studied using isolated stratum corneum and epidermis prepared from whole human skin obtained at autopsy (79). Isolated stratum corneum from chest and abdomen as well as epidermis from abdomen were treated in vitro with 450 µl of a 10 % aqueous glutaraldehyde solution. The percutaneous penetration was 12 % of applied dose for stratum corneum from chest and 13.8 and 3.3 % of applied dose for stratum corneum from abdomen of two different individuals. The percutaneous penetration of glutaraldehyde through abdominal epidermis from three different individuals was 3.3, 4.4, and 2.8 % of applied dose.

7.2. Biotransformation

Extensive metabolism of glutaraldehyde to CO_2 has been described in in vivo and in vitro studies using ^{14}C-glutaraldehyde as a tracer (51, 66, 71, 74). Although direct identification of the metabolites has not been accomplished, the probable metabolic pathway involves a series of oxidation, decarboxylation and hydroxylation reactions (7, 71). The initial step is probably oxidation of glutaraldehyde to glutaric semialdehyde, followed by oxidation to glutaric acid, which can undergo further metabolism by synthesis of a Coenzyme A thioester. The glutaryl CoA produced is then oxidised by glutaryl CoA dehydrogenase to give glutaconyl CoA, which is then decarboxylated to crotonyl CoA (9, 71). The crotonyl CoA is then converted by enoyl CoA hydratase to β-hydroxybutyryl CoA, which can be subsequently used for synthesis of acetoacetate or be degraded to acetate and then to CO_2.

Evidence that glutaraldehyde undergoes oxidation derives from *in vitro* studies in rat liver mitochondria in which an increase in oxygen consumption was measured. The oxidation of glutaraldehyde involves the electron transport system and results in reduction of NAD^+ and consumption of two atoms of oxygen per molecule of glutaraldehyde. Glutaraldehyde was oxidised extensively to CO_2 in rat tissue slices, with the greatest activity occurring in the kidney and then the liver. The activity was localised in the mitochondrial fraction of the kidney (51, 71, 74).

Material balance studies and pharmacokinetic studies were conducted with groups of Fischer 344 rats and New Zealand white rabbits using both intravenous (i.v.) and epicutaneous dosing. The animals received an i.v. dose of either 0.075 or 0.75 % glutaraldehyde in the tail vein or ear vein, respectively. Concentrations of 0.75 and 7.5 % glutaraldehyde were applied to the skin under a 24 h occlusive period. After i.v. administration up to 80 % of the dose was recovered as $^{14}CO_2$. The calculated dermally absorbed doses ranged from 4.1-8.7 % in the rat and 33-53 % in the rabbit. The mean concentration of radiochemical in animals receiving epicutaneous [^{14}C]glutaraldehyde were 100-1000 times less than those following i.v. injection of corresponding concentrations of glutaraldehyde (62).

The systemic distribution of glutaraldehyde has been studied in male Sprague-Dawley rats (78) being exposed to ^{14}C-glutaraldehyde, which was either deposited

in the maxillary left first molar, or infused into the jugular vein. A 0.4 µl aliquot of 4 % glutaraldehyde was administered in the pulp chamber and the intravenously administered dose was 10, 15, 20, 25, 30 , or 50 % of the amount deposited in the pulp chamber. The systemic distribution was estimated to 25 % of the applied dose in the maxillary molar, which amounts to a body load of 40 nanomoles of glutaraldehyde. After 45 min the tissue/fluid ratio (g tissue/ ml serum) of isotopic activity was 3 for the liver and 4.5 for the kidney. Similar ratios were also found for the metabolic clearance studies. Glutaraldehyde was rapidly metabolised to carbon dioxide (77, 78).

7.3. Tissue clearance and elimination

Following intravenous administration of 0.075 % and 0.75 % solutions of ^{14}C-glutaraldehyde to F344 rats and New Zealand white rabbits (0.2 ml for rats and 2.5 ml for rabbits), the majority of the radiolabel was excreted as CO_2, with approximately 80 % being exhaled in the first 4 hours. Urinary excretion of radiolabel ranged from 8 % to 12 % in the rat and 15 % to 28 % in the rabbit. Excretion of CO_2 as a percentage of total dose was less at the higher dose, particularly in the rabbit (5, 71).

In the studies by Ranley et al (77, 78) described above the clearance of ^{14}C-glutaraldehyde from liver, kidney, serum, and muscle was followed for 7 days after the exposure. After 1 hr, 81 % of the initial tissue load remained in the liver, 42 % in the kidney, 24 % in serum, and 67 % in muscle. After 7 days, the radioactivity remaining in the liver was 8 %, in the kidney 7 %, in serum 1 %, and in muscle 0.7 %. The ^{14}C from glutaraldehyde was exhaled as carbon dioxide or excreted in the urine (metabolite not given). At the end of 24 hr, 42 % of the administered radioactivity was eliminated and 90 % was cleared from body tissues in 3 days. After 6 days both routes were still being used for elimination (77, 78).

8. Methods of biological monitoring

Today, there is no suitable method described for biological monitoring of glutaraldehyde.

9. Mechanisms of toxicity

Glutaraldehyde can react and cross-link proteins. It can react with the α-amino groups of amino acids, the N-terminal amino groups of peptides and the sulfhydryl group of cysteine. The predominant site of reaction in proteins is the ε-amino group of lysine, although reactions may also occur with tyrosine, histidine and sulfhydryl residues (7, 42, 71, 75).

Products are formed on reaction of glutaraldehyde with deoxyadenosine, deoxyguanosine and deoxycytidine but not with deoxythymidine. The adducts

formed with deoxyadenosine are unstable but those formed on reaction with deoxyguanosine are relatively stable (7. 47. 71).

In the study by St. Clair and coworkers (87) it was shown that glutaraldehyde instilled in the nose of rats, induced lesions (inflammation, epithelial degeneration, respiratory epithelial hypertrophy and squamous metaplasia) that resembled, both in nature and in severity, the changes observed after acute inhalation exposure of rats to carcinogenic concentrations of formaldehyde gas (15, 64). Glutaraldehyde induces regenerative cell proliferation (87) and is about an order of magnitude more toxic to the nasal epithelium than formaldehyde (107).

10. Effects in animals and in vitro studies

10.1. Irritation and sensitisation

Irritation

Glutaraldehyde solutions may cause mild to severe irritation to the skin, depending on the concentration of the solution and the duration of exposure/contact. Dermal exposure to 25% glutaraldehyde solution or more caused necrosis in rabbits (5, 83). Glutaraldehyde vapour is irritating to the eye at an air concentration of 0.2 ppm (0.8 mg/m^3). At higher concentrations serious, irreversible injury may occur (7, 8, 49).

An alkaline 2 % glutaraldehyde solution was applied to the intact and abraded skin of rabbits for 24 h and irritation was scored at 24 and 48 h. Glutaraldehyde was a moderate skin irritant; the primary irritation index was 2.125 (maximum possible score 8.0) (63).

A 0.5 ml dose of a 2 % aqueous alkaline solution of glutaraldehyde was applied for 6 weeks to the clipped dorsal skin of 20 albino rabbits. The solution was spread with a brush and allowed to dry. The skin was examined daily. After the first application the skin and hair were stained faint yellow. The stain became more intense and turned golden brown during the 6 weeks, and it persisted for up to 35 days after the last application. Erythema was mild and a mild rash was observed following the first few applications. A severe erythematous reaction with edema followed by necrosis and scarring was observed when 24 % glutaraldehyde was applied to the skin of rabbits (90).

A single drop of a 2 % acid glutaraldehyde solution was placed in one conjunctival sac of each of two Dutch belted rabbits and the eyes were observed periodically for 72 h whereafter the animals were killed. Edema and swelling of the conjunctiva were observed 6 h after glutaraldehyde administration. Swelling and exudate was moderate at 18 h and the cornea was cloudy at 24 h. At 72 h the cornea remained cloudy and the conjunctiva was red and inflamed. The 2 % acid glutaraldehyde solution, thus, produced severe and extensive conjunctival injury (59).

In another study a 0.1 ml volume of an alkaline 2 % glutaraldehyde solution was placed in one conjuctival sac of the eye of each of 12 rabbits. After 30 seconds the

eyes of 3 rabbits were rinsed. Severe corneal opacity and irritation of the iris and conjunctiva were observed in unrinsed eyes after 7 days. Irritation of the conjunctiva, which was similar in rinsed eyes, lasted 7 days. The authors concluded that the alkaline 2 % glutaraldehyde solution was a severe ocular irritant (63).

When 0.1 ml volume of an alkaline 2 % glutaraldehyde solution was instilled into the conjunctival sac of one eye of five albino rabbits, inflammation lacrimation and edema were observed. A severe eye irritation was caused by this glutaraldehyde solution in rabbits (90).

In an alternative to the Draize rabbit eye test, glutaraldehyde was cytotoxic to human corneal endothelial cell cultures (28).

In a respiratory irritation study, groups of four ND4 Swiss Webster mice were exposed to seven different glutaraldehyde vapour concentrations in the range of 1.6 to 36.7 ppm (6.4 to 146.8 mg/m^3) while the respiratory rate was measured. Concentration related decreases in the respiratory rate were measured with a maximum at 3 to 20 minutes. The 50 % decrease in respiratory rate, RD_{50}, was calculated to be 13.9 ppm (55.6 mg/m^3) (103).

Sensitization

Female albino Hartley strain guinea pigs were sensitised with 0.3, 1.0, and 3.0 % glutaraldehyde and challenged with 10 % glutaraldehyde (89). The guinea pigs received 100 µl by direct dermal application for 14 consecutive days, followed by a rest period for 7 or 14 days. The primary irritancy response, determined by visual scoring or radioisotopic assay, indicated a minimal irritation concentration of 10 % glutaraldehyde, and a maximal non-irritating concentration of 3 % glutaraldehyde. The irritancy index was 3 for 0 % glutaraldehyde, almost 5 for 1.0 % glutaraldehyde ($p<0.05$ vs 0 %), and about 7 for 10 % glutaraldehyde ($p<0.01$ vs 0 %). Furthermore, contact hypersensitivity to glutaraldehyde followed a dose-dependent response. The hypersensitivity index was <0.5 for 0 % glutaraldehyde, about 1 for 1.0 % glutaraldehyde ($p<0.05$ vs 0 %), and about 2.6 for 3.0 % glutaraldehyde ($p<0.01$ vs 0 %). (The irritancy and hypersensitivity indices are calculated for each animal. The mean of the left (treated) to right (untreated) ratios of the biopsis of the vehicle group animals were calculated and this non-specific contribution was subtracted from the left to right ratio of every other animal. The resulting value was the index for the animal.)

In the same study (89) female B6C3F1 mice were sensitised with 0.3, 1.0, and 3.0 % glutaraldehyde and challenged with 10 % glutaraldehyde. The vehicle used was one part olive oil and four parts acetone. The mice received 20 µl by direct dermal application for 5 or 14 consecutive days, followed by a rest period for 4 or 7 days. The primary irritancy response, determined by visual scoring or radioisotopic assay, indicated a minimal irritation concentration of 10 % glutaraldehyde, and a maximal non-irritating concentration of 3 % glutaraldehyde. The irritancy index was almost 2 for 0 % glutaraldehyde, 3 for 1.0 % glutaraldehyde, and about 5.5 for 10 % glutaraldehyde ($p<0.01$ vs 0 %). Furthermore, contact hypersensitivity to glutaraldehyde followed a dose-dependent response. The hypersensitivity index was about 0.1 for 0 % glutaraldehyde, about 0.6 for 0.3 %

glutaraldehyde ($p<0.01$ vs 0 %), and about 2.2 for 1.0 and 3.0 % glutaraldehyde ($p<0.01$ vs 0 %).

Glutaraldehyde has been used to validate the mouse ear swelling test. To the shaved and tape-stripped abdomens of 10 mice 100 µl of 1 % glutaraldehyde was applied and allowed to dry. This procedure was repeated for 3 consecutive additional days. After a 7-day nontreatment period 20 µl of 10 % glutaraldehyde was applied to the left pinna of each animal. The right pinna was treated with 70 % ethanol, the vehicle. The thickness of both pinna was measured 24 and 48 h later. Of the animals, 67 % were sensitized to glutaraldehyde and the degree of pinna swelling was 125 % (37).

It has been suggested (4) that the threshold concentration for glutaraldehyde in aqueous solution to induce dermal sensitization is in the range of 0.1 to 1.0 %.

Production of IgE antibodies has been studied in mice (BALB/c). Solutions of glutaraldehyde (water:acetone; 50:50) were applied twice on the shaved flank of the mice (day 1 and day 7). Serum was collected 14 days after the initial administration and total serum IgE antibody content was evaluated by an enzyme-linked immunosorbent assay. Glutaraldehyde-treated mice had slightly higher concentration of serum IgE antibodies than controls. A total of 9.38 mg glutaraldehyde produced a small but significant elevation in IgE (76).

In a study the radioisotopic incorporation method was compared with the mouse ear swelling test for its ability to detect weak sensitizers. (In the radioisotopic incorporation method the infiltration of radiolabeled cells in the ear is evaluated.) Filter discs treated with 10 % glutaraldehyde (25 µl) were attached to the shaved and tape-stripped abdomens of 24 Balb/c mice. Glutaraldehyde was applied for 3 additional consecutive days. One day prior to challenge, the discs were removed and the mice were injected i.p. with 1 mg FUdR (5-fluorodeoxyuridine)/kg bw followed by 1 µCi [^{125}I]-iododeoxyuridine 1 h later. On the following day 25 µl of 2 % glutaraldehyde was applied to the left pinna of each animal. The mice were killed 24-48 h later and the thickness of the pinna was measured. Reactivity to glutaraldehyde was not detected by the radioisotopic assay, but in the ear swelling test the mice were slightly responsive (20).

In another study the mouse ear sensitization assay was used to determine the sensitization potential of glutaraldehyde. The right pinna of 18 female Balb/c mice were topically treated with 1 % glutaraldehyde on days 0 and 2. A scapular subcutaneous injection of Freund's complete adjuvant was also administered on day 2. On day 9 the thickness of the pinnae was measured followed by topical application of 10 % glutaraldehyde. When ear thickness was measured 24 h later there was a significant increase in thickness (25).

With a modified Magnusson-Kligman test on guinea pigs the sensitising capacity of glutaraldehyde was tested. A 10 % solution of glutaraldehyde was used on 30 animals. Glutaraldehyde was found to be a potent allergen as 72 % of the animals were sensitised. Cross-sensitization was shown between glyoxal, formaldehyde and glutaraldehyde (34).

The respiratory sensitizing potential of glutaraldehyde vapour was studied in male Hartley guinea pigs. The animals were exposed for one hour per day for five

consecutive days to an inducing vapour concentration of 13.9 ppm (55.6 mg/m^3). Challenge exposures to 4.4 ppm (17.6 mg/m^3) at 14, 21 and 35 days after the final induction exposure did not produce any evidence of respiratory sensitisation (103).

10.2. Effects of single exposure

LD_{50} values reported are presented in Table 1.

No evidence of systemic effects was observed in rats and rabbits that received a single dermal application of glutaraldehyde as a 2 % aqueous solution (dose unspecified) (90).

Increasing volumes of a stabilized 2 % glutaraldehyde solution were applied under an occlusive wrap to the shaved skin of rabbits for 48 h. There were no deaths when 50 ml glutaraldehyde solution/kg bw, the largest practical achievable dose, was applied (63).

Intra-arterial injection of glutaraldehyde to mature, non-inbred male rats (0.1 or 0.2 ml/100 g body weight (bw) of a 0.02% solution) gave rise to a temporary reduction in the amplitude of the EEG, which was more marked after 0.2 ml glutaraldehyde/100 g bw. The EEG was back to normal after17-22 min. Formaldehyde had a synergistic effect. The authors suggested, as one possibility, a mechanism of inhibition of the EEG linked with competitive blocking of membrane receptors by products of the aldehyde-mediator interaction. The inhibition of EEG was fully reversible (52).

Table 1. LD_{50} values reported after exposure to glutaraldehyde. Data from refs 5, 55, 63, 83, 90, 98.

Species	Adm. route	Reported LD_{50} (LC_{50}) value(s)
rat	oral	123 mg/kg bw; 1 % water solution
		252 mg/kg bw; 2 % saline solution
		≈ 2000 mg/kg bw; 2 % alkaline solution
		134-600 mg/kg bw; 25 % solution
	dermal	2500 mg/kg bw
	i.v.	17.9 mg/kg bw
	inhalation	24-40 ppm (96-160 mg/m^3)*
mouse	oral	100-110 mg/kg bw; 1 % water solution
		352 mg/kg bw; 2 % saline solution
	i.p.	13.9 mg/kg bw
	i.v.	15.4 mg/kg bw
	s.c.	1430 mg/kg bw
rabbit	dermal	600-2560 mg/kg bw
guinea pig	oral	50 mg/kg bw

* [In unpublished reports from the industry the 4 h LC_{50} for rats is reported to be 280-800 mg/m^3. The animals were exposed head-nose-only to an aerosol of glutaraldehyde.]

In the study by Ranly et al (78) (described above in chapter 7.2) no significant glutaraldehyde effects were observed on serum glutamic-oxalacetic transaminases (SGOT), serum glutamic-pyruvic transaminases (SGPT) or serum creatinine, and neither on urinary protein and urinary lactate dehydrogenase (LDH). When livers were examined histologically no evidence of abnormality was found. However, the uptake of p-aminohippurate was significantly higher in the exposed group and the clearance of phenosulfonphtalein (PSP) was significantly lower in the group receiving 2 µmoles of glutaraldehyde, but not in those receiving 0.4 µmoles of glutaraldehyde. The clearance of PSP from the blood was used as an endogenous test of kidney function, but no kidney histopathology was performed.

Male Fisher 344 rats have been exposed to 10, 20 or 40 mM glutaraldehyde by intra-nasal instillation (87). For the histopathology and cell proliferation studies, rats received, 72 hours after the glutaraldehyde instillation, an intraperitoneal injection of 5-bromo-2´-deoxyuridine, which is incorporated by cells in S-phase. Two hours later the rats were killed and the nasal cavity was prepared for examination by light microscopy. The lesions scored were; squamous metaplasia, rhinitis, epithelial erosions, epithelial hyperplasia, and goblet cell hypertrophy. The distribution of glutaraldehyde induced nasal epithelial lesions corresponded with the localisation of dyes in the deposition study. At 0 and 10 mM glutaraldehyde, no lesions were observed. Acute inflammatory changes (neutrophilic infiltrates and epithelial erosion) as well as extensive regions of respiratory epithelial hyperplasia and squamous metaplasia were observed after exposure to 20 or 40 mM glutaraldehyde. The effects were dose-related. Increased cell proliferation was also observed after 20 and 40 mM glutaraldehyde.

An alkaline 2 % aqueous glutaraldehyde solution was allowed to evaporate freely at room temperature in a closed system and rats and mice were exposed for 4 h. No gross effects were observed. Twelve rats exposed to vapours of 1.5 ml glutaraldehyde solution per litre of air were slightly more restless than controls. An initial weight loss was observed in 5 of the 12 rats. None of the 5 mice exposed to glutaraldehyde died. Two mice had an initial weight loss. Higher concentrations of glutaraldehyde produced more signs of respiratory tract irritation (90).

Groups of 10 male NMRI mice were exposed for 24 h to 33 and 133 µg/L (mg/m^3). There were 20 control mice. The lungs, liver and kidneys were evaluated histopathologically. No remarkable gross changes were observed in the lungs or kidneys but 6 mice exposed to the high dose had toxic hepatitis, that may have been reversible (99).

10.3. Effects of short-term exposure

10.3.1. In vitro studies

The cytotoxicity of glutaraldehyde in primary human pulp fibroblast cultures has been studied by Jeng and coworkers (50). The evaluation of cytotoxicity was based on the staining of the cells, and cell morphology. The fibroblasts, which were derived from third molar pulps, were treated with a 2.5 % ^{14}C-(1,5) glutaraldehyde solution (250 µCi/ml). The maximum non-toxic concentration in solution was 0.65

µl/ml, and in agar overlay technique 1.20 µl/ml. In another study fibroblasts (3T3 cells) were incubated for 24 hr with glutaraldehyde. A concentration of 3.0 ppm (in the medium) inhibited almost completely the growth of the fibroblasts, as measured by incorporation of ^{3}H-thymidine (84).

Cells from a human embryonic lung (WI-38) fibroblast culture were exposed to serial dilutions of a 2.5 % glutaraldehyde solution (91). Cytotoxicity was measured as the inhibition of mitochondrial dehydrogenase activity. Maximum non-toxic concentration was 0.98 mM (3.91 µl/ml, 4 hr), 1.03 mM (4.11 µl/ml, 8 hr), and 0.85 mM (3.40 µl/ml, 24 hr).

Glutaraldehyde is commonly used to produce intramolecular and intermolecular cross-links in collagen-based biomaterials. The cytotoxicity of glutaraldehyde-treated pig dermal collagen (19) has been studied by measuring ^{3}H-thymidine incorporation in adult human skin fibroblasts, when grown for 1 or 3 days in the presence of the collagen. The glutaraldehyde concentrations were 0.001-0.05 %. After 1 day exposure to the lowest glutaraldehyde-concentration, the ^{3}H-thymidine incorporation was reduced to approximately 60 % at the lowest concentration, to about 30 % at 0.01 % glutaraldehyde and to about 29 % at the highest glutaraldehyde concentration. After 3 days of exposure, ^{3}H-thymidine incorporation was reduced to approximately 50 % at the lowest concentration, to about 20 % at 0.01 % glutaraldehyde and to about 7 % at the highest glutaraldehyde concentration. In some other studies of the cytotoxicity of glutaraldehyde, effects were observed at media concentrations greater than 10-20 ppm (104) and at 3 ppm (84).

The in vitro cytotoxicity of glutaraldehyde (31) on bovine aortic endothelial cells has been evaluated by proliferation capacity, cellular ATP content, PGI_2 release and cyclic AMP synthesis. Continuous incubation of the cells with 0.1-1.0 µg glutaraldehyde/ml caused a statistically significant decrease in cell proliferation. More than 0.5 µg/ml glutaraldehyde led to a statistically significant increase in ATP content. A concentration dependent increase in PGI_2 release and cyclic AMP content was also observed, and at glutaraldehyde concentration over 0.1 µg glutaraldehyde/ml induced disproportionate amounts of PGI_2 and cyclic AMP, indicating a disturbance of cell functions.

10.3.2. Animal studies

A 0.5 ml dose of a 2 % alkaline glutaraldehyde solution was applied daily for 6 weeks to the clipped skin of albino rabbits. No evidence of systemic toxicity was observed (90).

Applications of 2.5, 5.0 and 7.5 % aqueous solutions of glutaraldehyde were ggiven under occlusion to Fischer 344 rats at doses equivalent to 50, 100 and 150 mg/kg bw during 6 h/day for 20 application over a period of 26 days. No treatment-related mortalities or clinical signs of systemic toxicity were found. Local skin irritation, mainly erythema and edema was minimal and present only intermittently during the treatment period (102).

In 2-week inhalation studies, groups of five rats (F344) and five mice (B6C3F1) of each sex were exposed to vapours of glutaraldehyde by inhalation at concentrations of 0, 0.16, 0.5, 1.6, 5 and 16 ppm (0, 0.64, 2.0, 6.4, 20 and 64 mg/m^{3}) for

6 hours per day, 5 days per week. All rats and mice exposed to 5 or 16 ppm glutaraldehyde died before the end of the studies, as did all mice exposed to 1.6 ppm. Rats exposed to 1.6 ppm did not gain weight. Deaths were attributed to respiratory distress. Lesions noted in the nasal passage and larynx of rats and mice included necrosis, inflammation and squamous metaplasia. At higher exposure concentrations similar lesions were present in the trachea of rats and mice and in the lung and on the tongue of rats. At 0.5 ppm nasal hyperplasia was seen in 3 male rats and squamous metaplasia in 2 males and one female. One female rat had necrosis/inflammation in larynx. There were higher incidences of these effects at higher concentrations. At 1.6 ppm all rats had necrosis in the nasal passages and squamous metaplasia was seen in 2 male and all female rats. At 5.0 ppm similar effects were seen in trachea. The NOEL in this study appears to be 0.16 ppm (71).

Male Swiss OF1 mice (107) were exposed for 6 h/day to; a) 0.3±0.1 ppm, 0.9±0.2 ppm or 2.6±0.2 ppm glutaraldehyde for 4 consecutive days; b) 0.3±0.1 ppm or 1.0±0.2 ppm glutaraldehyde for 5 consecutive days in the first week and for 4 consecutive days in the second week; c) 0.3±0.1 ppm or 0.9±0.2 ppm glutaraldehyde for 5 consecutive days in the first 2 weeks and for 4 consecutive days in the third week. Some mice (4/10) exposed to 2.6 ppm glutaraldehyde were dying on the third day. As the surviving mice showed signs of severe toxicity (weight decrease, mouth breathing etc), they were killed after 5 days. The breathing frequency was used as an index of sensory irritation (Alarie 1973). In the concentration range 0.7-4.5 ppm glutaraldehyde (15 min oronasal exposure), a concentration dependent expiratory bradypnea was observed. RD_{50} (50 % decrease in respiratory rate) was 2.6 ppm. After 14 days of exposure to 1.0 ppm glutaraldehyde, the decrease in body weight was about 20 %. Exposed mice showed marked excitation by nervously running around, abdominal swelling, rougher hair, and looking unhealthier. No signs of systemic toxicity were observed in mice exposed to 0.3 ppm glutaraldehyde. Histopathological lesions were observed in all mice exposed to 0.3, 1.0, 2.6 ppm. The lesions affected exclusively the respiratory epithelium covering the septum, the naso- and maxilloturbinates and also to a lesser extent the lateral wall, but not the olfactive one. The severity of lesions increased with glutaraldehyde concentration from 0.3 ppm to 1.0 ppm and remained constant from 1.0 to 2.6 ppm in the surviving mice, but it did not depend on exposure time. Inhalation of 1.0 ppm glutaraldehyde for 14 days caused a marked increase in squamous metaplasia, exudate of keratin strates and inflammatory cells, and necrosis of the respiratory epithelium in the nasal cavities. After 1 and 2 weeks recovery, the effects remained. However, after two weeks the severity of squamous metaplasia was somewhat reduced, necrosis was even further reduced and the increase in keratin exudate was completely reverted. No concentration-related lesions were observed in the lungs of the exposed mice (107).

Groups of 3 rats were given drinking water containing 0.05, 0.1 or 0.25 % glutaraldehyde for 11 weeks. The rats were then killed and the nervous system tissue was examined microscopically. No signs of adverse effects were found (85).

Feigal and Messer (32) have shown that glutaraldehyde used as a pulpotomi agent penetrates into surrounding tissue in small but measurable amounts.

10.4. Effects of long-term exposure and carcinogenicity

In 13-week studies, groups of 10 rats (F344) and 10 mice (B6C3F1) of each sex were exposed to vapours of glutaraldehyde by inhalation at concentrations of 0, 62.5, 125, 250, 500 and 1000 ppb for 6 hours per day, 5 days per week. There were no exposure-related deaths in rats but all mice exposed to 1000 ppb and two female mice exposed to 500 ppb died before the end of the study. Body weight gains were reduced in male rats exposed to 1000 ppb, in female rats exposed to 500 or 1000 ppb, in male mice exposed to 125, 250 or 500 ppb and in female mice exposed to 250 or 500 ppb. There was no evidence of systemic toxicity in rats or mice by histopathologic or clinical pathology assessments. Exposure-related lesions in the respiratory tract were, however, observed and resembled those in the 2-week studies. A NOAEL for respiratory lesions was decided to be 125 ppb in rats. No NOAEL was reached in mice as inflammation was found in the anterior nasal passage at concentrations as low as 62.5 ppb. The inflammation was characterized by focal accumulation of neutrophils in the nares, particularly in females. The neutrophilic infiltrate became progrssively more severe and was associated with increased epithelial cell replication in the anterior nasal passages (71).

From the histopathology of the respiratory tract of the animals in the NTP-study there is a separate report (41). Treatment-induced lesions, including epithelial erosions, inflammation, and squamous metaplasia, were confined to the anterior third of the nose and were present in both sexes and in both rats and mice. No histopathological evidence of glutaraldehyde-induced responses was observed in the trachea, central airways, or lungs, while the larynx showed minimal changes. Neutrophilic infiltration of the squamous epithelium of the nasal vestibule, present in both rats and mice, became progressively more severe with increasing exposure time. Lesions induced by glutaraldehyde were more anterior in the nose than those reported for formaldehyde.

A carcinogenesis study (inhalation) performed for the NTP has not yet been finalised (72).

[A two-year carcinogenicity study has been performed by industry. The results are not published but they are referred to by the US Cosmetic Ingredient Review Expert Panel (17). Fischer rats were given 50, 250, andd 1000 ppm glutaraldehyde in drinking water. Large granular lymphocytic leukemia (LGLL) was found in dosed females at necropsy. The incidence of LGLL is high in untreated controls. The conclusion was: The nature of the response and the factors associated with it, suggest that this was not a direct chemical carcinogenic effect but resulted from a modifying influence on determinants normally controlling the expression of this spontaneously occurring neoplasm.]

10.5. Mutagenicity and genotoxicity

Glutaraldehyde was tested for inductions of mutations in Salmonella typhimurium in three laboratories. In one laboratory positive results were obtained with strain TA100 with and without liver S9 from Aroclor 1254-induced male Sprague Dawley

rats or Syrian hamsters. In the second laboratory no increase in mutations was observed in TA100 in the absence of S9 or with 10 % Aroclor-induced hamster S9. A small increase in mutations was noted in TA100 in the presence of 10 % Aroclor-induced rat S9. In both laboratories negative results were obtained with the strains TA1535, TA1537 and TA98 with and without S9. The third laboratory reported positive results in strains TA100, TA102 and TA104 with and without Aroclor-induced hamster or rat liver S9 (44, 71).

In a liquid preincubation procedure with the base substitution strain Salmonella typhimurium TA104, it was shown that glutaraldehyde at its maximum non-toxic dose (>0.5 μmoles) induced 4 150 revertants/μmol glutaraldehyde. The average spontaneous reversion value of 304 had been subtracted. In the liquid preincubation procedure the test substance and the bacteria were incubated at 37 ºC for 20 minutes. Following incubation histidine and biotine were added, the mixture plated and revertants recorded after 48 hours. The strain TA104 carries a nonsense mutation (-TAA-) at the site of reversion that is present in a single copy on the chromosome (58). In Salmonella typhimurium TA102, which detects oxidative mutagens, glutaraldehyde (25 μg/plate) induced His^+ revertants (389 revertantss/plate, the spontaneously induced 240 revertants/plate subtracted), which was equal to the number of revertants induced by formaldehyde. The strain TA104 contains A-T base pairs at the site of mutation (54).

The cytotoxic and genotoxic effects of glutaraldehyde have been studied in vitro in the human TK6 lymphoblast cell line and in primary cultures of rat hepatocytes. TK6 lymphoblasts were exposed to glutaraldehyde for 2 hours in serum-free GSH-free media. Cytotoxic effects were observed at concentrations as low as 10 μM with only 10 % cell survival at 20 μM. Glutaraldehyde-induced DNA-protein cross-linking increased linearly over the concentration range from 0 to 25 μM. Glutaraldehyde induced a marginal increase in unscheduled DNA synthesis in the in vitro hepatocyte DNA repair assay, but only at the two highest concentrations of 50 and 100 μM, indicating the induction of some excision-repair activity (71, 86).

Glutaraldehyde induced mutations at the $TK^{+/-}$ locus of mouse L5178Y cells at a concentration of 8 μg/mL in the absence of S9 activation. Glutaraldehyde induced sister chromatid exchanges (SCEs) in Chinese hamster ovary cells with and without S9 activation (38, 61, 71).

Glutaraldehyde was tested for its ability to induce sex-linked recessive lethal mutations in germ cells of male Drosophila melanogaster treated as newly emerged adult flies by feeding or injection or treated as larvae by feeding. All three tests were negative. (71, 105, 106).

The genotoxic potential of 50 % aqueous glutaraldehyde was assessed in vivo using a micronucleus test in mice and a bone marrow chromosomal aberrations test in rats. Glutaraldehyde was given to male and female Swiss-Webster mice (numbers not given) as a single dose by peroral intubation at 80, 160 and 250 mg/kg bw. Glutaraldehyde did not produce dose-related increases in the frequency of micronucleated polychromatophilic erythrocytes sampled 30, 48 or 72 hour after treatment. The single doses of glutaraldehyde given by peroral intubation to Sprague Dawley rats were 25, 60 or 120 mg/kg bw for males and 15, 40 and

80 mg/kg bw for females. There were no dose-related increases in the frequency of chromosomal aberrations in rats assessed at 12, 24 or 48 hours after treatment (100).

In summary, glutaraldehyde was shown to be genotoxic in vitro inducing mutations in bacterial cells and producing mutations, sister chromatid exchanges and chromosomal aberrations in mammalian cells. Its mutagenic activity was independent of S9 activation.

10.6. Reproductive and developmental toxicity

The effects of glutaraldehyde in male reproductive function using the dominant lethal assay have been studied in male mice, administered a single dose (per os) of 30 or 60 mg glutaraldehyde per kg bw and mated for the next 6 weeks with virgin females. There were no evidence of reduced fertility and no significant effects on embryonic/foetal viability (94).

In a subacute toxicity study (98), groups of Sprague-Dawley rats were given daily doses s.c. of 1, 5, 25 or 125 mg glutaraldehyde/kg bw for 35 days. A control group received saline. Changes on testes/sperm duct/epididymis, and prostate/ seminal vesicles/Cowpers gland/urethra were seen in male rats in the two highest dose groups. In female rats, changes in uterus/cervix/vagina were seen at these dose levels.

In a study CD1-mice were given by gastric intubation 16, 20, 24, 40, 50 or 100 mg glutaraldehyde/kg bw of a product containing 2 % glutaraldehyde on days 6 through 15 of gestation. Nonionic ethoxylates of isomeric linear alcohols [$(CH_3)_2(CH_2)_nO(CH_2CH_2O)_{12}H$ with $11 \leq n \leq 15$] and possibly orthophosphoric acid were also present. The mice were killed at day 18 of gestation. At daily doses of 40 mg/kg bw six of 35 animals died as did 12 of 48 mice given 50 mg/kg bw and 19 of 35 given 100 mg/kg bw. There was a significant ($p<0.05$) reduction in average weight gain during pregnancy. The unborn offspring of dams treated at the highest dose level were also adversely affected. At the lowest exposure group (16 mg/kg bw) there was a significant decrease in the average fetal weight. The group given 100 mg/kg bw produced a significant increase in the average percent malformed fetuses. Mainly due to the toxic effects on mothers, including deaths, the authors concluded that the glutaraldehyde-containing product was not teratogenic toward the CD1-mouse (57).

In another study (29) pregnant rats were given glutaraldehyde by gastric intubation at a dose of 0, 25, 50 or 100 mg/kg bw on days 6 through 15 of pregnancy. Maternal toxicity occurred in the 100 mg/kg group as evidenced by a significant increase in maternal death and a significant decrease in maternal body weight gain and food consumption. A significantly lowered fetal weight was also found in the 100 mg/kg group. No significant change induced by glutaraldehyde was detected in the incidence of postimplantation loss. Morphologic examinations of fetuses revealed no evidence of teratogenicity of glutaraldehyde. The authors concluded that glutaraldehyde has no teratogenic effects on rat offspring even at a dose which induced severe maternal toxicity.

[Oral doses of 25 and 50 mg/kg of glutaraldehyde given to rats on days 6 to 15 of gestation were maternally toxic but not fetotoxic as reported in an unpublished industrial report.]

[In another unpublished study, reported by the Cosmetic Ingredient Review Expert Panel (17) groups of CD rats were given glutaraldehyde in drinking water; 0, 50, 250 and 1000 ppm for 10 weeks. The rats were then paired within each dose group. Treated water was administered throughout the mating, gestation and lactation periods. Groups of F_1 rats were administered glutaraldehyde at the same concentrations as their parents. They were allowed to mate and produce a F_2 generation. The investigators concluded that the NOAEL for adult and offspring toxicity was 50 ppm and 250 ppm glutaraldehyde in drinking water, respectively. The NOAEL for reproductive effects was > 1000 ppm in drinking water]

10.7. Immunotoxicity

No studies on immunotoxic effects of glutaraldehyde have been found in the literature. It should be noted, however, that total serum IgE antibody content was elevated in mice given dermal application of 50 % glutaraldehyde in acetone (76).

New Zealand rabbits (n=2) were injected intramuscularly with 10 mg of rabbit serum albumin (RSA) treated with 2 % glutaraldehyde in Freund's complete adjuvant. At weeks 2, 3 and 4 the rabbits were given subcutaneous injections of 10 mg of antigen without adjuvant. Blood samples were taken at weeks 5, 6 and 7. The sera from each rabbit were pooled and analyzed for elicited antibodies using enzyme-linked immunosorbent assay (ELISA) and horseradish peroxidase assay. A weak immunologic response was observed. The average IgG concentration in response to glutaraldehyde treated RSA was 0.02 mg/ml serum (17).

11. Observations in man

11.1. Effects by contact and systemic distribution

Glutaraldehyde solutions may cause mild to severe irritation in the skin, depending on the concentration of the solution and the duration of exposure/contact. Inhalation of glutaraldehyde at vapour levels below 0.8 mg/m^3 (0.2 ppm) has been reported to cause nose and throat irritation, nausea and headaches (11). Chest discomfort and tightness and breathing difficulty may also occur.

When glutaraldehyde treated drug-loaded erythrocytes were used in systemic chemotherapy of a near-terminal male patient (97), no side-effects were observed due to glutaraldehyde.

A very specific effect of glutaraldehyde has been described, where endoscopes, sterilised by glutaraldehyde, have produced corrosive mucosal lesions in form of necrotic hemorrhagic colitis. The effect in this case is on the patient not on the occupationally working personnel and caused by direct contact with glutaraldehyde (12, 27).

11.2. Effects of repeated exposure on organ systems

From 65 % of 167 nurses working in endoscopy units there have been complaints of eye irritation, skin irritation, headache and cough or shortness of breath. Where measurements were performed the air concentration of glutaraldehyde was less than 0.2 ppm (13).

In an irritancy test (79), a 10 % solution of glutaraldehyde was applied to the anterior, lateral, and posterior ankle and posterior heel of twelve subjects (3 black and 9 white). The application was done 5 days/week for 4 weeks, and thereafter 3 days/week for further 4 weeks. No irritation (erythema, pruritus, or isolated vesicles and papules) was observed during the first week. However, in 11 subjects the skin was discoloured after 5 applications. During the second week, all subjects were significantly discoloured, and 5 out of 12 had minimal irritation on the anterior ankle. One of the five subjects became sensitised to glutaraldehyde. During the remaining 6 weeks of the study, the application was only done to areas of thick stratum corneum (medial, posterior, and lateral heel and posterior ankle). The irritation of the anterior ankle subsided during the third and fourth weeks of the study. During the last four weeks of application, there was no evidence of irritancy, even among those who had previously experienced some irritation. The skin colour returned to normal within two weeks of the final glutaraldehyde application (= 10 weeks after the first application).

There are several cases of dermatitis due to repeated or prolonged contact with glutaraldehyde or glutaraldehyde-containing disinfectant agents. The symptoms are marked dryness, redness, eczema, infiltrations, fissures and skin sensitisation (6, 11, 24, 26, 33, 35, 40, 43, 67, 93, 101).

In a study tests on 109 volunteers were conducted using 0.1 %, 0.2 % and 0.5 % (w/w) aqueous solutions of glutaraldehyde with the same concentration being used for induction and challenge. For induction the glutaraldehyde doses were applied to the skin of the backs under occlusion for 48 to 72 hours. A total of ten induction applications were made over a 3 week period. Two weeks after removal of the final induction patch, a challenge patch was applied under occlusion for 48 hours to a site not used for induction. The reaction was recorded 24 hours after removal of the challenge patch. The two lowest doses produced no evidence for a sensitization reaction, but at 0.5 % there was a definite reaction to the challenge patch in one of the 109 subjects. While 0.1 % and 0.2 % glutaraldehyde were not significantly irritating to the skin, 0.5 % produced mild to moderate local erythema in 16 of the 109 subjects (4).

Glutaraldehyde was tested for sensitization in 102 male subjects. Ten occlusive induction patches containing 0.1 % glutaraldehyde in petrolatum were applied to the upper lateral portion of the arm for 48 to 72 h over 3 weeks. A nontreatment period of 2 weeks was followed by an occlusive challenge patch containing 0.5 % glutaraldehyde in petrolatum. Skin reactions were graded on a scale of 1 to 4 and a grade 2 or greater was considered positive. No sensitization was observed among the 102 men. The experiment was repeated with 30 men and with 5.0 % glutaraldehyde

induction patches and a 0.5 % glutaraldehyde challenge patch. Seven (23.3 %) of the men were sensitized (60).

In studies at hospitals by NIOSH a relationship between exposure to glutaraldehyde and irritation of eyes and upper respiratory pathways has been demonstrated. The occupational concentration of glutaraldehyde was 0.2 ppm or higher. After reconstruction of the occupational setting the concentration was lowered to 0.1 ppm or less and there were no symptoms of irritation (68).

There is limited evidence from case reports that glutaraldehyde would cause respiratory sensitisation (8, 11, 22, 49). However, two of four nurses, complaining of respiratory symptoms, reacted positive in a provocation test when exposed to glutaraldehyde vapour, as measured by changes in $FEV_{1.0}$ and nasal airway resistance (an index of nasal obstruction) (21). The respiratory symptoms included sneezing, wheezing, chest tightness and breathing difficulties.

In an outpatient clinic in Nairobi glutaraldehyde for instrument decontamination was left in an open vessel in a room of 11.4 m^3, ventilated through 1.44 m^2 windows, which remained open for about 10 h daily during a 5-day week. Five persons (doctors and nurses) were working in that room. The symptoms reported were itching and watery eyes, sneezing, headaches, nausea, coughing, breathlessness, acute rhinitis, bronchitis and nasal irritation. The authors (65) believe that the adverse reactions to glutaraldehyde probably corresponded to development of hypersensitivity to glutaraldehyde. Provocation tests were, however, not performed.

There are also cases of occupational asthma due to exposure to glutaraldehyde used as a sterilising agent. The diagnosis of occupational asthma was documented by tests for preshift and postshift spirometry, serial measurements of peak expiratory flow rate and nonspecific bronchial hyperresponsiveness, and workplace challenge (16, 39).

There is another case report on one endoscopy nurse who developed symptoms suggestive of occupational asthma after seven years of exposure to glutaraldehyde. The exposure had increased during the last 18 months. During that time, she developed symptoms of breathlessness, wheeze, chest tightness and cough. Chest tightness and wheezing developed immediately on exposure to glutaraldehyde and wore off after one or two hours. The asthmatic symptoms were accompanied by hoarseness, sore eyes, sore throats and sneezing (88).

Occupational exposure to glutaraldehyde has been reported to cause palpitations or tachycardia. The symptoms ceased when the exposure to glutaraldehyde ceased. No exposure data were given (18).

11.3. Genotoxic effects

No studies on genotoxic effects of glutaraldehyde to man have been found in the literature.

11.4. Carcinogenic effects

In a mortality study of 186 workers exposed to glutaraldehyde (0.2 ppm) in a glutaraldehyde-producing plant between 1959 and 1978, there was no increased insidence of malignant tumours. Nor was there an increased mortality rate according to an unpublished industrial study presented in the German MAK-committee (82). This study has later been published (95). The mortality analysis included 186 males assigned to glutaraldehyde production or drumming from 1959 to 1978, who were followed through 1988. Traditional SMR adjusting for age and calendar year were conducted using US mortality rates for white males through 1989 for calculation of expected deaths. To control for healthy worker effect and unmeasured confounders, internal comparisons using the men from the Kanawha Valley cohort never assigned to the glutaraldehyde unit as a referent group were also conducted. There were 14 deaths among the 186 study subjects. There were 4 cancer deaths versus 6.1 expected. The four cancers included one each due to stomach, lung and brain and a death due to lymphosarcoma.

11.5. Reproductive and developmental effects

The assessment of spontaneous abortions and foetal malformations have been studied in Finnish hospital nurses and staff who had been exposed to glutaraldehyde used as a sterilising agent. No increase in risk of either endpoint was found (45, 46, 71).

There is no information available on human reproductive toxicity.

12. Dose-effect and dose-response relationships

From animal inhalation studies the relationship between exposure dose and effect is given in Table 2. LD_{50} values (oral) varies between 50 mg/kg bw in guinea pigs and about 100 mg/kg bw in rats and mice. The TD_{LO} in rats is given to 54.6 mg/kg bw.

The dermal LD_{50} in rats and rabbits is approximately 2500 mg/kg bw.

Table 2 Effects of inhalation exposure to glutaraldehyde in rats and mice.

Species	Exposure	Effect	Ref
rat (n.s.)	24-120 ppm; 4 h	LC_{50}	5
mouse Swiss	2.6 ppm; 15 min	RD_{50}	107
rat F344	1.6 ppm; 6h/d;5d/w;2w	no weight gain	71
mouse BCF	1.6 ppm; 6h/d;5d/w;2w	10/10 animals died	71
mouse BCF	1.0 ppm; 6h/d;5d/w;13w	20/20 animals died	71
rat, m F344	1.0 ppm; 6h/d;5d/w;13w	reduced body weight gain	71
mouse Swiss	1.0 ppm; 14 days	squamous metaplasia in the nose; necrosis of respiratory epithelium	107
rat F344	0.5 ppm; 6h/d;5d/w;13w	squamous metaplasia in the nose	71
rat, f F344	0.5 ppm; 6h/d;5d/w;13w	reduced body weight gain	71
mouse Swiss	0.3 ppm; 4 days	lesions in respiratory epithelium	107
mouse, f BCF	0.25 ppm; 6h/d;5d/w;13w	reduced body weight gain	71
rat F344	0.25 ppm; 6h/d;5d/w;13w	nasal inflammation	71
mouse, m BCF	0.125 ppm; 6h/d;5d/w;13w	reduced body weight gain	71
rat F344	0.125 ppm; 6h/d;5d/w;13w	NOAEL for respiratory lesions	71
mouse BCF	0.0625 ppm; 6h/d;5d/w;13w	nasal inflammation	71

n.s. = strain not stated

13. Previous evaluations by (inter)national bodies

The German MAK-committee has evaluated glutaraldehyde in 1993 (82). The MAK-value (0.1 ppm) is based on irritative effects in eyes, nose and respiratory epithelium. Due to the irritative effects there is also a 5 min short-term value of 0.2 ppm. The teratogenic and embryotoxic risk is evaluated as none. Glutaraldehyde is marked as a sensitiser (allergen).

In a revision 1992 of the documentations for the ACGIH TLVs, a ceiling value (0.2 ppm) is recommended for glutaraldehyde vapour based on the irritation threshold of glutaraldehyde (1).

14. Evaluation of human health risks

14.1 Groups at extra risk

Individuals sensitised to formaldehyde or glyoxal seem to have a greater risk for reacting to glutaraldehyde. There seems to be a possibility for cross-reactions between these aldehydes. Glutaraldehyde as such is, however, said to be a strong human sensitiser.

14.2 Assessment of health risks

Data from occupational exposure are scarce. Direct skin contact with glutaraldehyde should be avoided. Also water solutions of glutaraldehyde can irritate and affect the skin. There is also the risk of being sensitized.

Vapours of glutaraldehyde causes eye, nose and throat irritation, nausea and headaches. The LOEL (lowest observed effect level) for the irritative effects is below 0.2 ppm which is comparable to animal data. Glutaraldehyde vapours may also cause asthma. Symptoms include sneezing, wheezing, chest tightness and breathing difficulties.

From animal studies histopathological effects in the nose have been demonstrated in rats and mice. The lesions included epithelial erosions, inflammation, and squamous metaplasia in the anterior third of the nose. Lesions were of a similar kind as caused by formaldehyde, although they were more anterior than those reported for formaldehyde.

Glutaraldehyde is genotoxic in vitro and induces mutations in both bacterial and mammalian cells. Glutaraldehyde also produces sister chromatid exchanges and chromosomal aberrations in mammalian cells in vitro. However, an in vivo micronucleus test in mice and a bone marrow chromosomal aberration test in rats yielded negative results.

The results from an ongoing carcinogenesis study has not yet been reported.

14.3. Scientific basis for an occupational exposure limit

There are very few data which can be used as a scientific basis for an occupational exposure limit for glutaraldehyde. The critical effect, based on these data, is irritation of the skin, the eyes and the mucous membranes. The LOEL for irritative effects is below 0,2 ppm. However, from data on mice, 13 weeks inhalation of 0.0625 ppm (lowest tested) glutaraldehyde caused nasal inflamation Moreover, glutaraldehyde is a skin allergen and may cause respiratory allergic reactions.

15. Research needs

No long-term (more than 13 weeks) inhalation studies have been performed in animals and there is a lack of epidemiological data from exposure to glutaral-dehyde. The local effect in the nasal mucosa should be compared to the effects of e.g. formaldehyde, thereby also investigating the toxicological mechanisms involved.

16. Summary

Beije B, Lundberg P. Glutaraldehyde. DECOS and NEG Basis for an Occupational Standard. *Arbete och Hälsa* 1997;20, pp 1-30.

Glutaraldehyde is used, among other things, as a fixative in electron microscopy, a disinfectant for instruments and in chemical industry. It is a skin and mucous membrane irritant. Glutaraldehyde is a skin allergen and may cause respiratory allergic reactions. In rats and mice histopathological effects in the nose have been demonstrated. Glutaraldehyde is genotoxic in vitro and induces mutations in both bacterial and mammalian cells. It also produces sister chromatid exchanges and chromosomal aberrations in mammalian cells in vitro. Based on relatively few available data the critical effect when occupationally exposed is irritation of the skin, the eyes and the mucous membranes.

Keywords : Human toxicity, irritation, metabolism, mutagenicity, occupational exposure, occupational exposure limit, risk evaluation, sensitization

17. Summary in Swedish

Beije B, Lundberg P. Glutaraldehyde. DECOS and NEG Basis for an Occupational Standard. *Arbete och Hälsa* 1997;20, s 1-30.

Glutaraldehyd används bl a som fixativ i elektronmikroskopi, som desinfektionsmedel för instrument och i kemisk industri. Ämnet irriterar hud och slemhinnor. Glutaraldehyd är en hudallergen och kan ge allergiska reaktioner i andningsvägarna. Hos råtta och mus har histopatologiska effekter i nosen påvisats. Glutaraldehyd är genotoxisk in vitro och inducerar mutationer i såväl bakterier som mammalieceller. Det ger även systerkromatidutbyten och kromosomaberrationer i mammalieceller in vitro. Baserat på relativt få tillgängliga data är den kritiska effekten vid yrkesmässig exponering irritation av hud, ögon och slemhinnor.

Nyckelord : Humantoxicitet, hygieniskt gränsvärde, irritation, metabolism, mutagenicitet, riskvärdering, sensibilisering, yrkeshygienisk exponering

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19. Data bases used in search for literature

In the search for literature the following data bases were used:

- NIOSHTIC
- Cancerline
- Chemical Abstracts
- Medline
- Toxline
- RTECS

The latest search was performed February 17, 1997, at the library of the Swedish National Institute for Working Life. In order not to miss any references the only search-words used were "111-30-8" (the CAS nr) and "glutaraldehyde".

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Appendix 1.

Permitted or recommended maximum levels of glutaraldehyde in air.

Land	ppm	mg/m^3	Kommentarer	År	Ref.
Denmark	0.2	0.8	Ceiling	1994	1
Finland	0.1	0.42	Short term	1996	2
Germany	0.1	0.4	S	1996	7
	0.2	0.8	5 min short term		
Iceland	0.2	0.8	Ceiling	1989	3
Netherlands	-	0.25	Ceiling	1996	4
Norway	0.2	0.8	Ceiling	1995	5
	-	0.25	Activated glutaraldehyde		
Sweden	0.2	0.8	Ceiling; S	1996	6
USA (ACGIH)	0.2	0.82	Ceiling	1996	8
	0.05	0.2	intended change		
(NIOSH)	0.2	0.8	Takvärde	1994	9
(OSHA)	-	-		1994	9

S = risk for sensitisation

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