Glutaraldehyde

Health-based recommended occupational exposure limit

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

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Mijnheer de staatssecretaris,

Bij brief van 3 december 1993, nr DGV/BMO-U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid de Gezondheidsraad om gezondheidskundige advieswaarden af te leiden ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen.

In dat kader bied ik u hierbij een advies aan over glutaaraldehyde. Dit advies is opgesteld door de Commissie WGD van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

In verband met de complexiteit van het onderwerp en de beperkte stafcapaciteit heeft de publicatie van het advies langer geduurd dan gebruikelijk.

Ik heb dit advies vandaag ter kennisname toegezonden aan de Minister van Volksgezondheid, Welzijn en Sport en de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieu.

Hoogachtend,

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Dutch Expert Committee on Occupational Standards,
a committee of the Health Council of the Netherlands,
in cooperation with the Nordic Expert Group for criteria
Documentation of Health Risks from Chemicals

to:

the Minister and State Secreatry of Social Affairs and Employment

No. 2005/05OSH, The Hague, May 12, 2005

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues..." (Section 21, Health Act).

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Inhoud

Samenvatting en advieswaarde

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie WGD van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen waaraan mensen via de lucht op hun werkplek kunnen worden blootgesteld. Deze aanbevelingen vormen de eerste stap in een drietrapsprocedure die moet leiden tot wettelijke grenswaarden, aangeduid als maximaal aanvaarde concentraties (MAC-waarden).

In het voorliggende rapport bespreekt de commissie de gevolgen van blootstelling aan glutaaraldehyde en presenteert zij, indien mogelijk, een gezondheidskundige advieswaarde voor die stof. De gezamenlijke evaluatie over de gezondheidskundige implicaties van blootstelling aan glutaaraldehyde, dat in 1997 door de *Nordic Expert Committee* (NEG) is gepubliceerd, is opgenomen in deel 2 van dit advies. Deel 1 bestaat uit een kort overzicht van de relevante onderzoeken, eventueel aangevuld met nieuwe literatuur, die de Commissie WGD gebruikt voor het kunnen afleiden van een gezondheidskundige advieswaarde. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties die vóór februari 2004 zijn verschenen.

Fysische en chemische eigenschappen

Glutaaraldehyde (1,5-pentadial; CAS nr. 111-30-8) wordt gebruikt in de industrie als biocide en als *cross-linking* agens en in de medische en laboratoriumsector voor sterili-

satie van instrumenten. Daarnaast wordt de stof toegepast bij het balsemen en bij het fixeren van organische weefsels. Op de werkplek aanwezigen kunnen aan zure en aan basische, zogeheten geactiveerde, oplossingen van glutaaraldehyde worden blootgesteld en aan dampen van glutaaraldehyde.

Glutaaraldehyde is een kleurloze, olieachtige vloeistof met een prikkelende geur. De geurdrempel ligt op 0, 001 mg/m^3. Glutaaraldehyde verdampt niet makkelijk uit een waterige oplossing tot 50 procent (v/v). Het vriespunt is -14°C en het kookpunt 188°C. De vloeistof is oplosbaar in water en in verschillende organische oplosmiddelen. Glutaaraldehyde is een reactieve verbinding die gemakkelijk interacties aangaat met eiwitten (*cross-linking*).

Grenswaarden

Momenteel geldt in Nederland voor glutaaraldehyde een bestuurlijke grenswaarde van 0,25 mg/m^3 als plafondwaarde (*ceiling*-waarde).

In het Verenigd Koninkrijk gelden grenswaarden van 0,2 mg/m^3 (0,05 ppm), voor tijdgewogen gemiddelden van zowel acht uur als vijftien minuten. In Denemarken en Zweden geldt een *ceiling*waarde van 0,8 mg/m^3 (0,2 ppm). Duitsland heeft een grenswaarde van 0,2 mg/m^3 (0,05 ppm) als een tijdgewogen gemiddelde van acht uur en een *momentary* waarde van 0,8 mg/m^3 (0,2 ppm), welke geen enkel moment overschreden mag worden. De ACGIH beveelt een *ceiling*waarde aan van 0,2 mg/m^3.

Zowel Duitsland, Zweden, het Verenigd Koninkrijk als de Verenigde Staten (ACGIH) hebben glutaaraldehyde aangemerkt als een stof die overgevoeligheidsreacties kan veroorzaken bij huidcontact. Recent heeft Duitsland glutaaraldehyde ook aangemerkt als een stof die overgevoeligheidsreacties kan veroorzaken na inhalatie.

Monitoring

De Occupational Safety and Health Administration (OSHA), het National Institute of Occupational Safety and Health (NIOSH) - beide uit de Verenigde Staten - en de Health and Safety Executive (HSE) uit het Verenigd Koninkrijk hebben methoden beschreven voor gaschromatografische of hoge druk vloeistofchromatografische analyse van glutaaraldehyde uit luchtmonsters. Ook een directe meetmethode is beschreven, maar deze is onvoldoende specifiek. De commissie heeft geen methode voor biologische monitoring gevonden.

Kinetiek

Gegevens over de opname, metabolisme, distributie en uitscheiding van glutaaraldehyde zijn afkomstig van proefdieronderzoek en *in vitro* experimenten. Van radioactief gemerkte glutaaraldehyde oplossingen die werden opgebracht op de huid, werd bij ratten circa 6 procent en bij konijnen circa 40 procent door de huid geabsorbeerd. De opname door de huid is ook *in vitro* bestudeerd met huid die afkomstig was van mensen en van verschillende diersoorten. Minder dan 0,7 procent van glutaaraldehydeoplossingen passeerde de konijnen-, ratten-, cavia- en mensenhuid. Deze geringe huidopname wordt mogelijk veroorzaakt door binding van het reactieve glutaaraldehyde aan de huid.

Onderzoek heeft uitgewezen dat eenmaal opgenomen glutaaraldehyde, door middel van een reeks enzymatische oxidatiestappen in de lever en nieren, wordt omgezet in de eindproducten acetoacetaat of acetaat, en kooldioxide. Bij ratten en konijnen is vastgesteld dat de snelheid van deze omzetting hoog is. Zo werd 80 procent van het intraveneus toegediend radioactieve glutaaraldehyde binnen vier uur in de vorm van kooldioxide uitgeademd. De urine van ratten en konijnen bevatte respectievelijk 8 tot 12 procent en 15 tot 28 procent van de toegediende radioactiviteit. Verder werd vastgesteld dat met name konijnen bij de hoge dosering relatief minder glutaaraldehyde in de vorm van kooldioxide uitademden dan ratten.

Effecten op mensen

Onderzoeken bij mensen na kortdurende of piekblootstelling wijzen uit dat glutaaraldehyde irriterend is. In zowel Zweeds als Brits onderzoek onder ziekenhuis-personeel werkzaam in de endoscopie en koudesterilisatie, is een relatie gevonden tussen irritatie van oog, huid, neus en keel en geometrisch gemiddelde blootstellingsniveaus van 0,05-0,06 mg/m^3 (spreiding <0,001-1,08 mg/m^3). Deze symptomen worden mogelijk veroorzaakt door de sensorisch irriterende werking van glutaaraldehyde. Uit deze onderzoeken komen verder aanwijzingen dat sensorische irritatie vooral het gevolg is van blootstelling aan hoge concentraties, en met name aan pieken. Met de gebruikte meetmethode konden echter uitsluitend (geometrisch) gemiddelde waarden gemeten konden worden over 15 min. Aan hoeveel kortdurende pieken (spreiding 5 sec –12 min) de werknemers zijn blootgesteld en hoe hoog die pieken waren is onbekend. In een onderzoek met niet-rokende vrouwelijke vrijwilligers konden gemeten blootstellingsconcentraties wel gerelateerd worden aan sensorische irritatie. In dit onderzoek is een steile dosis-responsrelatie gevonden: geen irritatie van neus, oog en keel bij een blootstelling van 15 minuten aan 0,4 mg/m^3 en wel sensorische irritatie bij de meeste vrijwilligers bij een blootstelling van 2-25 seconden aan 3 mg/m^3. In het eerdergenoemde Zweedse en in

een Australisch onderzoek werd een andere dosis-responsrelatie vastgesteld: hoe vaker met glutaaraldehyde werd gewerkt, des te meer klachten.

Bij huidcontact kan glutaaraldehyde overgevoeligheidsreacties veroorzaken. Ook kan glutaaraldehyde aanleiding geven tot astmatische symptomen, zoals piepen, hoesten, beklemming op de borst, ademhalingsproblemen en bronchiale hyperreactiviteit. De astmatische luchtwegklachten kunnen erop duiden dat glutaaraldehyde ook overgevoeligheidsreacties bij inademing kan veroorzaken. Aan de hand van klachten en symptomen die voor mensen en proefdieren zijn beschreven is echter niet met zekerheid vast te stellen of dat daadwerkelijk het geval is. Het is namelijk goed mogelijk dat mensen met een toegenomen gevoeligheid van de luchtwegen voor aspecifieke prikkels (bronchiale hyperreactiviteit) door de irriterende werking van glutaaraldehyde een astmatische aanval krijgen. Echter, uit de positieve resultaten van specifieke immunologische testen uitgevoerd bij zowel mensen als proefdieren, en uit het feit dat glutaaraldehyde overgevoeligheid bij huidcontact veroorzaakt, concludeert de commissie dat ervan uitgegaan kan worden dat glutaaraldehyde een stof is die overgevoeligheidsreacties kan veroorzaken bij inademing. Wel heeft glutaaraldehyde waarschijnlijk een zwak sensibiliserende werking, omdat in de praktijk, in verhouding tot het grote aantal mensen dat beroepshalve blootstaat aan de stof, bij slechts weinigen astmatische klachten worden gerapporteerd. Het immunologisch werkingsmechanisme dat aan deze gevoeligheid ten grondslag ligt is onduidelijk.

Er werd geen toename gevonden van spontane abortus of foetale misvormingen bij Finse ziekenhuisverpleegsters die glutaaraldehyde als steriliserend agens hadden gebruikt.

Effecten op dieren

Bij proefdieren leidde acute blootstelling aan glutaaraldehyde tot overeenkomstige effecten als bij mensen: sensorische irritatie aan de ogen en bovenste luchtwegen en overgevoeligheidsreacties en irritatie bij huidcontact. Dit gold voor cavia's, konijnen en muizen. Experimenteel onderzoek in ratten en muizen geeft een steile dosis-responsrelatie te zien, waarin sensorische irritatie wordt gevolgd door ademhalingsproblemen en sterfte na enkele dagen blootstelling aan 4-10 mg/m^3 (muis). Resultaten van onderzoek met muizen wijzen op het optreden van overgevoeligheidsreacties bij inademing. Bij cavia's zijn hiervoor geen aanwijzingen gevonden.

Bij kort- en langdurend inhalatie onderzoek met ratten en muizen zijn voornamelijk effecten op de neus gevonden. Na langdurige blootstelling wordt bij 0,5 mg/m^3 een statistisch significante toename in de incidentie van squameuze metaplasie (afgeplat epitheel) van het neusepitheel in de vrouwtjesmuis gezien. Bij 0,25 mg/m^3, de laagst geteste concentratie, wordt reeds een lichte, maar niet statistisch significante, toename

waargenomen. De ernst hiervan is echter zeer gering. Tevens wordt vanaf 0,25 mg/m^3 een, niet dosis-gerelateerde, significante toename in hyalinedegeneratie (eiwitaccumulatie) van het ademhalingsepitheel in vrouwtjesmuizen gezien. Het is echter onbekend is of hyalinedegeneratie biologisch relevant is voor de mens, terwijl dit verschijnsel bij muizen ook spontaan bij veroudering optreedt. De commissie concludeert dat squameuze metaplasie van het ademhalingsepitheel van de neus het kritisch effect is voor langdurende blootstelling. Verder zijn in dit langdurend inhalatieonderzoek geen tumoren gevonden.

Mutageniteits- en genotoxiciteitstests met bacteriën en zoogdiercellen toonden aan dat glutaaraldehyde mutagene en clastogene eigenschappen heeft en schade aan het DNA kan veroorzaken. Proefdieronderzoek naar mutageniteit en genotoxiciteit leverden in het algemeen negatieve resultaten op.

In het tot nu toe uitgevoerde proefdieronderzoek met ratten, muizen en konijnen zijn bij de daarin toegediende dosering glutaaraldehyde geen effecten op de vruchtbaarheid en de ontwikkeling waargenomen.

Evaluatie

Uit de humane en dierexperimentele gegevens concludeert de commissie dat glutaaraldehyde irriterend is voor de ogen, huid en bovenste luchtwegen en dat het overgevoeligheidsreacties veroorzaakt bij huidcontact en soms ook bij inademing. Zij is van mening dat huidblootstelling voorkomen moet worden en beschermende maatregelen nodig zijn. Verder concludeert ze dat mensen geen verhoogd risico lopen op het krijgen van kanker of reproductiestoornissen wanneer ze beroepsmatig aan deze stof worden blootgesteld.

De commissie meent dat irritatie van ogen, neus en keel (sensorische irritatie) het kritische effect is voor kortdurende blootstelling. Het niveau waarop geen nadelig effect is waargenomen (NOAEL) in onderzoek met niet-rokende vrouwen ligt bij 0,4 mg/m^3. Ze meent dat deze waarde kan dienen als een gezondheidskundige advieswaarde (HBR-OEL). Vanwege de steile dosisresponsrelatie in onderzoek met vrouwelijke vrijwilligers en met proefdieren in combinatie met de ernst van het bij proefdieren waargenomen effect (ernstige ademhalingsproblemen en sterfte) is deze waarde te beschouwen als een plafondwaarde (*ceiling*waarde). Deze *ceiling* is nodig om werknemers te beschermen tegen de piekblootstellingen die karakteristiek zijn voor veel werkzaamheden met glutaaraldehyde. De commissie acht een onzekerheidsfactor voor variatie tussen de individuen niet nodig, omdat niet-rokende vrouwen een gevoelige populatie zijn en omdat extrapolatie van een 15 minuten gemiddelde naar een *ceiling* waarde een extra veiligheidsmarge oplevert.

Uit onderzoek bij mensen komen verder aanwijzingen dat zij meer klachten hebben naarmate ze vaker aan glutaaraldehyde zijn blootgesteld. De commissie beveelt daarom

ook een gezondheidskundige advieswaarde, gemiddeld over een achturige werkdag, aan. Het bedoelde onderzoek geeft echter onvoldoende informatie om zo'n waarde te kunnen afleiden. Er is echter een goed uitgevoerd chronisch onderzoek in proefdieren, dat de commissie voor de afleiding bruikbaar acht. In dit onderzoek is een geen waargenomen nadelig effect niveau (NOAEL) gevonden van 0,25 mg/m^3; het effect was squameuze metaplasie van het neusepitheel bij vrouwtjesmuizen. Omdat dit een lokaal effect aan de oppervlakte van de neus is, acht de commissie een onzekerheidsfactor voor variatie tussen de verschillende soorten niet nodig. De commissie hanteert een factor 3 voor mogelijke verschillen tussen de individuen. Toepassing van deze onzekerheidsfactor levert een gezondheidskundige advieswaarde op van 0,08 (0,25/3) mg/m^3 (TGG 8 uur).

Glutaaraldehyde is een huidsensibiliserende en irriterende stof. De stof kan astma veroorzaken, maar ook reeds bestaand astma verergeren. Reeds gesensibiliseerde werknemers en mensen met astma lopen daardoor een verhoogd risico op de ontwikkeling van klachten bij blootstelling. Ook individuen die overgevoelig zijn voor glyoxal lopen bij blootstelling aan glutaaraldehyde een verhoogd risico op klachten vanwege de kruisreactiviteit van de aldehyden.

Advies

De Commissie WGD stelt een gezondheidskundige advieswaarde voor blootstelling aan glutaaraldehyde in lucht op de werkplek voor van 0,4 mg/m^3 in de vorm van een plafondwaarde (*ceiling*). Daarnaast stelt ze een gezondheidskundige advieswaarde voor van 0,08 mg/m^3 gemiddeld over een achturige werkdag.

Executive summary

Scope

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands sets Health-Based Recommended Occupational Exposure Limits (HBR-OELs) for chemical substances in air at the workplace. These recommendations are made by the Council's Dutch Expert Committee on Occupational Standards (DECOS). They constitute the first step in a three-step procedure that leads to legally binding occupational exposure limits.

In this report the committee discusses the consequences of occupational exposure to glutaraldehyde and recommends a health-based occupational exposure limit. In 1997, the Nordic Expert Group (NEG) published an evaluation of the harmful health effects of glutaraldehyde. This evaluation is included in part 2 of this document. In part 1, a brief summary of relevant effects is given and additional data are described. DECOS assessment of the HBROEL is based on all presented data in part 1 and 2. The committee's conclusions are based on scientific publications prior to February 2004.

Physical and chemical properties

Glutaraldehyde (1,5-pentanedial; CAS no. 111-30-8) is used industrially as biocide, as cross-linking agent, and as (cold) sterilising agent for medical and laboratory supplies. Other applications include embalming agent, histological fixative, and tanning interme-

diate. In the workplace, humans may be exposed to acid or alkaline, so called activated, glutaraldehyde solutions and to vapours derived from these solutions.

Glutaraldehyde is a colourless, oily liquid, with a pungent, aldehyde odour. Its freezing and boiling points are -14°C and 188°C, respectively. Its odour threshold is 0.001 mg/m^3 (0.27 ppb). Glutaraldehyde is soluble in water and various organic solvents. Aqueous solutions up to 50% are not very volatile. Glutaraldehyde is a reactive compound that readily reacts and cross-links proteins.

Current limit values

In the Netherlands, a ceiling value of 0.25 mg/m^3, is presently being used as administrative force for glutaraldehyde. In the UK, maximum exposure limits of 0.2 mg/m^3 (0.05 ppm; both TWA 8 hours and STEL 15 min) are set, and in Denmark and Sweden, a ceiling value of 0.8 mg/m^3 (0.2 ppm). Germany has an eight-hour TWA limit of 0.2 mg/m^3 (0.05 ppm) and a momentary value (value that should not be exceeded at any time) of 0.8 mg/m^3 (0.2 ppm). ACGIH recommends a ceiling value of 0.2 mg/m^3 (0.05 ppm). Germany, Sweden, the UK, and the USA (ACGIH) give a notation indicating that the compound may cause skin sensitisation. Recently, Germany also gave a notation for respiratory sensitisation.

Monitoring

The US Occupational Safety and Health Administration (OSHA), the National Institute of Occupational Safety and Health (NIOSH) and the UK Health and Safety Executive (HSE) have described methods for analysing glutaraldehyde in air, using gas chromatographic or high-pressure liquid chromatographic analysis. Also a direct-reading Glutaraldemeter has been described. This method, however, lacks specificity. The committees did not find a method for biological monitoring of glutaraldehyde.

Kinetics

Data on the kinetics of glutaraldehyde are limited to *in vivo* animal experiments, using intravenous injection or dermal administration, and *in vitro* experiments. Following *in vivo* dermal application of radiolabelled glutaraldehyde solutions on rat and rabbit skin, about 6% (rats) and 40% (rabbits) were calculated to be absorbed. *In vitro*, skin absorption was less than 0.7% for both animal and human skin. The poor skin absorption may be caused by binding of the reactive glutaraldehyde at the site of contact. Results from *in vivo* (intravenous injections) and *in vitro* studies using radiolabelled glutaraldehyde suggest that glutaraldehyde is initially oxidised in the liver and the kidney to glutaric semi-

aldehyde and subsequently to glutaric aldehyde, followed by glutaryl-CoA formation. Via additional oxidations and decarboxylations, glutaryl-CoA is metabolised to ß-hydroxybutyryl-CoA, which is then converted to acetoacetate or to acetate and CO_2. Metabolism occurred rapidly in rats and rabbits.

Following intravenous injection of radiolabelled glutaraldehyde, approximately 80% of the radiolabel was exhaled as CO_2 within four hours. Urinary radiolabel excretion rates were 8-12% and 15-28% in rats and rabbits, respectively. Especially in the rabbit, a higher dose resulted in lower excretion of CO_2 (expressed as % of total dose) compared to rat.

Effects

Human data

In studies in human after short-term or peak exposure, irritant effects have been observed.

In both a Swedish and British study among health-care workers in endoscopy and cold sterilisation, work-related irritational symptoms of the eye, skin, nose, throat and lower respiratory tract could be related to exposure levels with geometric means of 0.05-0.06 mg/m^3 (ranges <0.001-1.08 mg/m^3). Symptoms of the eye, nose and throat are most commonly reported, and probably caused by sensory irritant effects of the glutaraldehyde vapour. These studies further indicated that the sensory irritant effects are due to the higher exposure levels (especially peaks) of the broad exposure range of <0.001-1.08 mg/m^3. With the monitoring method used in these studies, only (geometric) means over 15 min could be measured. To how many peak exposure events the health-care workers were exposed and to what precise peak concentration was unknown. In a study among non-smoking female volunteers, measured exposure levels could be related to sensory irritational effects. In this study, a steep dose-response relationship for irritation was found: no irritation at an exposure for 15 minutes to 0.4 mg/m^3 and irritation in most volunteers after exposure for 2-25 seconds to 3 mg/m^3. Furthermore, in the mentioned Swedish and in an Australian study, the frequency of exposure to glutaraldehyde is related to the number of sensory irritant symptoms.

Case reports on occupational allergic contact dermatitis and positive patch test results from health-care workers showed a skin sensitising potential of glutaraldehyde. Glutaraldehyde can also cause asthmatic symptoms, such as wheezing, coughing, chest tightness, breathing difficulties and non-specific hyperresponsiveness. These asthmatic symptoms might indicate glutaraldehyde as a respiratory tract sensitizer. Yet, human and animal data on respiratory tract sensitisation are not conclusive. The irritating properties of glutaraldehyde may have resulted in work-aggravated asthma in susceptible

individuals. This is pre-existing or concurrent asthma that is aggravated by irritants in the workplace. Nevertheless, based on immunological tests in humans and the few animal studies carried out, and due to the fact that glutaraldehyde may cause allergic skin sensitisation, the committee concludes that glutaraldehyde should be considered as a respiratory sensitizer. However, most probably, it should be considered as a respiratory allergen of low potency in view of the large numbers of occupationally exposed persons in relation to the limited number of reported patients. The immunological mechanism of action is however poorly understood.

No increased risk of spontaneous abortions and foetal malformations was found in Finnish hospital nurses and staff, using glutaraldehyde as a sterilising agent.

Animal data

Data from experiments in animals (rats, guinea pigs, mice) support the findings in humans, in that acute exposure to glutaraldehyde produced sensory irritant effects to the eyes and upper respiratory tract and sensitisation and irritation to the skin. Animal studies also showed a steep dose-response relationship, where sensory irritation was followed by severe respiratory distress and death after exposure to 4-10 mg/m^3 for some days. Studies in mice suggested a possible respiratory sensitising potential. In guinea pigs, however, no such relation was found.

Short-term inhalation studies in rats and mice demonstrated exposure-related lesions in the upper respiratory tract. These lesions included necrosis, inflammation and squamous metaplasia of the epithelium of the nose. Histopathological or clinical pathological assessment did not show evidence for any systemic toxicity.

In a 2 year NTP inhalation study in rats and mice, considerable non-neoplastic lesions in the noses of both species (resembling those in the short-term studies) were demonstrated. At 0.5 mg/m^3 a statistically significant increased incidence in squamous metaplasia in the respiratory epithelium of the nose in female mice was observed. At 0.25 mg/m^3, the lowest dose level tested, also a slight, but non-significant increase was observed. The severity of the effect was however minimal. Furthermore, at 0.25 mg/m^3 a statistically significant, increased incidence of hyaline degeneration of the respiratory epithelium of the nose in female mice was observed. This finding was however not dose-related. The biological relevance of hyaline degeneration (‘hyaline droplets’ in cytoplasm) for humans is, however, unknown, whereas hyaline degeneration also occurs spontaneously during ageing in mice. Based on this information, the committee considered the dose-related, toxicological relevant, squamous metaplasia of the respiratory epithelium of the nose in female mice as the critical effect for long-term exposure. Furthermore, in this 2-year study, no evidence of a carcinogenic potential was found.

Additional *in vitro,* studies showed mutagenic, clastogenic, and DNA-damaging properties. However, *in vivo* data (micronucleus, chromosome aberration, UDS, dominant lethal, and *Drosophila*) generally have shown no activity.

Adequate studies in rats, mice and rabbits did not provide evidence that glutaraldehyde will affect fertility or development.

Hazard assessment

From human and experimental animal data, the committee concludes that glutaraldehyde is an eye, skin, and respiratory tract irritant and a skin sensitizer. It is of the opinion that dermal exposure should be prevented and application of adequate skin protection is warranted. The committee also considers glutaraldehyde as a respiratory tract sensitizer, although probably with a low potency. Furthermore, it concludes that workers are not at risk for developing cancer or effects on reproduction due to occupational exposure to glutaraldehyde.

The committee intends sensory irritation, especially of the eyes, nose and throat, to be the critical effect of short-term exposure to glutaraldehyde and 0.4 mg/m^3 as the NOAEL. It intends this value to serve as a health based exposure level (HBR-OEL). Because of the steep dose-response curves in the female volunteer and short-term animal studies in combination with the severity of the effect (severe respiratory distress and death) in animals, this value is considered a ceiling value. This ceiling level is needed to protect workers against the peak exposures, which are characteristic in many working practices with glutaraldehyde. The committee does not advise an uncertainty factor for inter-individual variation, because non-smoking females have shown to be a sensitive population whereas an extra safety margin is introduced by the use of a 15-minute NOAEL as a ceiling value. Because human data indicate that the frequency of exposure is of relevance in the occurrence of sensory irritational effects, the committee warrants an HBR-OEL (8 h TWA) to be set. The above-mentioned study does not give sufficient information for deriving such value. However, the two-year NTP inhalation study was considered adequate for derivation of an HBR-OEL (8 h TWA). From this study, the NOAEL of 0.25 mg/m^3 for squamous metaplasia of the respiratory epithelium of the nose in female mice, was used as a starting point. Because squamous metaplasia occurs at the nasal epithelia, the committee does not compensate for differences between species. For intraspecies variation, an uncertainty factor of 3 is taken. Application of this factor results in an HBR-OEL of 0.08 (0.25/3) mg/m^3 (8-h TWA).

Glutaraldehyde is a skin sensitizer and an irritant. It can cause asthma but also aggravate already existing asthma. Workers sensitised at an earlier time point, and workers with asthma may be at extra risk for developing symptoms from airborne glutaraldehyde

exposure. Individuals sensitised to glyoxal may have a greater risk for reacting to glutaraldehyde, because of a possibility for cross-reactivity between these aldehydes.

Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards recommends a health-based occupational exposure limit (HBR-OEL) for glutaraldehyde in the air of 0.4 mg/m^3 as a ceiling value and an HBR-OEL of 0.08 mg/m^3 as an eight-hour time-weighted average concentration (8-h TWA).

Part 1 Health Council: Glutaraldehyde

DECOS basis for hazard assessment

Chapter 1

Scope

1.1 Background

In the Netherlands, occupational exposure limits for chemical substances are set using a three-step procedure. In the first step, a scientific evaluation of the data on the toxicity of the substance is made by the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, at the request of the Minister of Social Affairs and Employment (Annex A). The purpose of the committee's evaluation is to set a health-based recommended occupational exposure limit for the atmospheric concentration of the substance, provided the database allows the derivation of such a value.

In the next phase of the three-step procedure, the Social and Economic Council advises the Minister on feasibility of using the health-based limit as a regulatory Occupational Exposure Limit (OEL) or recommends a different OEL. In the final step of the procedure, the Minister of Social Affairs and Employment sets the legally binding OEL.

1.2 Committee and procedure

This document is a co-production of DECOS and the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG). It is a result of an agreement between both groups to prepare jointly criteria documents that can be used by the regulatory authorities in the Netherlands and in the Nordic countries. The members of DECOS and NEG are listed in annex B.

The joint draft document on the harmful effects of glutaraldehyde has been prepared by B Beije, PhD, and P Lundberg, PhD, both from the Department of Toxicology and Chemistry of the National Institute for Working Life, Solna, Sweden. In addition, the draft was reviewed first by DECOS and then by NEG, before the final version was published by the Swedish National Institute for Working Life (Arbete och Hälsa 1997:20) in 1997. The final document is included in part 2 of this report. Part 1 contains a brief summary of the relevant effects from the final document, supplemented with additional data. DECOS, hereafter called the committee, used data from both parts in assessing a health-based recommended occupational exposure limit. In addition, the draft of part 1 was prepared by by JTJ Stouten, MSc, and JHE Arts, PhD, from the Department of Toxicological Risk Assessment of the TNO Nutrition and Food Research Institute, Zeist, The Netherlands.

In 2004, the President of the Health Council released a draft of the report for public review. The individuals and organizations that commented on the draft are listed in annex C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The committee's recommendations on the health-based occupational exposure limit of glutaraldehyde have been based on scientific data, which are publicly available. Data were obtained from the on-line databases Toxline, Medline and Chemical Abstracts, using glutaraldehyde and CAS no. 111-30-8 as key words. The final search was performed in February 2004.

Finally, a list of abbreviations and symbols can be found at the end of this report in annex E.

Chapter 2

Identification, properties and monitoring

2.1 Identification, physical and chemical properties

NEG data

Pure glutaraldehyde (CAS 111-30-8; EINECS 203-856-5) is a colourless, oily liquid, with a pungent, aldehyde odour. Glutaraldehyde is commercial available as aqueous solutions ranging from 1 to 50%. Aqueous solutions up to 50% are not very volatile. Its freezing and boiling points are -14°C and 188°C, respectively. Glutaraldehyde is soluble in water and various organic solvents.

Conversion factors (at 20^0C) 1 mg/m^3 = 0.25 ppm; 1 ppm = 4 mg/m^3

Additional data

Glutaraldehyde is an aliphatic dialdehyde that undergoes most of the typical aldehyde reactions to form acetals, cyanohydrins, oximes, hydrazones and bisulphite complexes. Aqueous solutions of glutaraldehyde are slightly acid pH (3-4) and susceptible to aerial oxidation to give the corresponding carboxylic acid.

Glutaraldehyde reacts with proteins by a cross-linking reaction which is mainly between the free aldehyde group (or active carbonylgroup) and the NH_2 group, and which depends upon time, pH and temperature (Gor80). This reaction is catalysed by acid.

Glutaraldehyde polymerises in water to a glassy form, which regenerates the dialdehyde on vacuum distillation. In solution, glutaraldehyde partially polymerises to oligo-

mers to give a mixture of variable composition. The degree of polymerisation increases with pH and temperature. At a pH of 7.5-8 polymerisation is minimal and the bioactivity optimal*. Above pH 9, polymerisation proceeds comparatively rapidly and solutions eventually lose their biocidal activity.

Recent data have qualitatively established an odour threshold level of 0.001 mg/m^3 (0.27 ppb) (Cai03).

Further details on the identification, physical and chemical properties are described in part 2 of this report.

2.2 EU classification and labelling

Additional data

Symbols	T	Toxic
	C	Corrosive
	N	Environmentally dangerous
Risk phrases[a]	R23/25	Toxic by inhalation and if swallowed
	R34	Causes burns
	R42/43	May cause sensitisation by inhalation and skin contact
	R50	Very toxic to aquatic organisms
Safety phrases	S1/2	Keep locked up and out of reach of children
	S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
	S36/37/39	Wear suitable protective clothing, gloves and eye/face protection
	S45	In case of accident or if you feel unwell, seek medical advice; immediately (show the label where possible)
	S61	Avoid release to the environment. Refer to special instructions/material safety data sheet

[a] Additional R phrases are given for specific concentration limits: R36/37/38 Irritating to eyes (0.5%≤C< 2%), respiratory tract (0.5%≤C<10%) and skin (0.5%≤C<10%); R20/22 Harmful by inhalation (2%≤C<25%) and if swallowed (2%≤C<50%)

2.3 Validated analytical methods

NEG data

The US Occupational Safety and Health Administration (OSHA) and the National Institute of Occupational Safety and Health (NIOSH) have described methods for analysing glutaraldehyde in air, using sampling on coated XAD-2 sorbent tubes, coated silica gel, or coated glass fibre membrane filters and gas chromatographic or high pressure liquid chromatographic analysis. A detection limit of 12 µg/m^3 for a 2 L sample have been

* This *activated* glutaraldehyde is an effective cold steriliser with potent antimicrobial activities.

reported for the OSHA method and 0.04-1.2 mg/m^3 for the NIOSH (OSHA 1990, Cuthbert and Groves 1995; NIOSH, 1994).

Additional data

The Health and Safety Executive (HSE) has published a method for measuring the amount of glutaraldehyde in air, which is based on the OSHA method with some alterations concerning the high performance liquid chromatographic eluent and the calibration procedure (HSE99).

Only one direct-reading instrument is described for the monitoring of glutaraldehyde in air, the Glutaraldemeter (Ano91). The detection range is 0.1-20 mg/m^3 (0.03-5 ppm v/v). The commercially available instrument is simple and convenient to use, but readings are subject to interference from compounds such as alcohols and other aldehydes.

Wellons *et al*., (Wel98) evaluated four personal workplace air monitoring methods for glutaraldehyde: a silica gel tube, a direct reading Glutaraldemeter, a DNPH- impregnated passive diffusion badge and a DNPH-impregnate filter cassette. The badge, silica gel tube and filter cassette methods were found to be accurate under controlled laboratory conditions. At hospital studies, statistically significant differences were found between the badge, silica gel tube and filter cassette methods but the differences were small enough to be acceptable for workplace air monitoring. The handheld meter did not respond to the glutaraldehyde test atmospheres in laboratory, whereas in the hospital its performance could not be demonstrated due to glutaraldehyde concentrations below or slightly above the manufacturer's stated detection limit of 0.1 mg/m^3.

The committees did not find a method for biological monitoring of glutaraldehyde.

Chapter 3

Sources

NEG data

Glutaraldehyde is commonly available as a clear, colourless stable aqueous solution. Usually available as 1, 2, 25 or 50% solutions of glutaraldehyde liquid in water, but other formulations are also present. Alkaline (or activated) solutions of glutaraldehyde (pH 7.5-8.5) are highly effective microbiocidal agents and are widely used in the cold sterilisation of medical, surgical and dental equipment (Norbäck, 1988). Other applications include embalming agent, histological fixative, cross-linking agent and tanning intermediate.

Chapter 4

Exposure

4.1 General exposure

NEG data

Glutaraldehyde is used as a preservative in cosmetics, for example, hair conditioners (CIR, 1996). Furthermore, glutaraldehyde has been used as a therapeutic agent for topical treatment of warts in children, nail infections and onychomycosis, in some dental treatments and for friction blister prevention in soldiers, athletes and ballet dancers.

4.2 Occupational exposure

NEG data

Personal sampling measurements of glutaraldehyde in a British and Danish hospital revealed exposure levels up to 0.17 mg/m^3 and 0.5 mg/m^3 respectively (Leinster *et al*., 1993; Rietz *et al*., 1985). Personal sampling during glutaraldehyde decantation in endoscopy rooms revealed values up to 0.16 mg/m^3 and 0.68 mg/m^3 (Campbell *et al*., 1994; Gannon *et al*., 1995). In a Swedish hospital, short-term (15 min) personal sampling in the breathing zone of the exposed workers during glutaraldehyde handling, revealed a geometric mean of airborne glutaraldehyde exposure of 0.05 mg/m^3 (range: manual sterilisation: <0.01-0.57 mg/m^3; automatic sterilisation: 0.01-0.18 mg/m^3) (Norbäck 1988).

Additional data

In a glutaraldehyde production plant, exposure levels of 0.24 mg/m^3 (0.04-1.36 mg/m^3 15 min personal short-term) and 0.20 mg/m^3 (0.04-0.68 mg/m^3, 8 hour personal) were measured (Tet95). in British hospitals, endoscopy nurses were exposed to 0.06 mg/m^3 (peak GM; range: <0.001-1.08 mg/m^3), and 0.01 mg/m^3 (background GM; range: 0.002-0.1 mg/m^3), respectively (Vya00). In South Australian hospitals, personal short-term sampling revealed values of 0.11 mg/m^3 (GM, operating theatres) and 0.18 mg/m^3 (GM, endoscopy areas) (Pis94, Pis97). In a recent Australian controlled study, peak measurements were performed using a direct Glutaraldemeter and values up to 0.6 mg/m^3 (range 0.04-0.6 mg/m^3) were found (Wat03).

Chapter 5

Kinetics

NEG data

Data available on the kinetics of glutaraldehyde are limited to *in vivo* animal experiments, using intravenous injection or dermal administration, and *in vitro* experiments.

Following *in vivo* application of 0.75 and 7.5% solutions of radiolabelled glutaraldehyde to the occluded skin for 24 hours, 4.1-8.7% (rats) and 33-53% (rabbits) were calculated to be absorbed. Upon dermal application, blood concentrations of radiolabelled glutaraldehyde in animals were 100 to 1000 times less than in those receiving intravenous injections of similar total doses (McKelvey *et al*., 1992). In a flow-through skin penetration chamber using rabbit, rat, or guinea pig skin, less than 0.5% of a 0.7% and less than 0.75% of a 7.5% solution of glutaraldehyde were absorbed through the skin during a 6-hour exposure period. For (undefined) human skin, absorption was approximately 0.2% for both concentrations (Frantz *et al*., 1993). The poor skin absorption may be caused by binding of the reactive glutaraldehyde at the site of contact.

Results from *in vivo* (intravenous injections) and *in vitro* studies using radiolabelled glutaraldehyde suggest that glutaraldehyde is initially oxidised in the liver and the kidney to glutaric semialdehyde and subsequently to glutaric aldehyde, followed by glutaryl-CoA formation. By additional oxidations and decarboxylations, glutaryl-CoA is metabolised to ß-hydroxybutyryl-CoA, which is then converted to acetoacetate or to acetate and CO_2. Metabolism occurred rapidly in rats and rabbits (Karp *et al.,* 1987, Myers *et al*., 1986; NTP 1993; Packer and Greville 1969).

Following intravenous injection of 0.075 or 0.75% radiolabelled glutaraldehyde, approximately 80% of the radiolabel was exhaled as CO_2 within four hours. Urinary radiolabel excretion rates were 8-12% and 15-28% in rats and rabbits, respectively. Especially in the rabbit, a higher dose resulted in lower excretion of CO_2 (expressed as % of total dose) (NTP 1993; Ballantyne *et al*., 1985).

Chapter 6

Effects

In the workplace, humans may be exposed to acid or alkaline, so called activated, glutaraldehyde solutions and to vapours derived from these solutions.

6.1 Observations in man

6.1.1 Irritation

Skin

NEG data

Glutaraldehyde solutions may cause mild to severe irritation in the human skin, depending on the concentration of the solution and the duration of exposure/contact. Repeated exposure of a 10% solution of glutaraldehyde applied to the ankle and heel of twelve subjects (5 days/week for 4 weeks followed by 3 days/week for 4 weeks) revealed mild irritation in five out of twelve subjects only during the second week of application (Reifenrath *et al*., 1985). Of 167 nurses working in endoscopy units, 65% complained of skin irritation. Where measurements were performed, air concentration of glutaraldehyde was less than 0.8 mg/m^3 (0.2 ppm) (Calder *et al*., 1992).

Additional data

In Swedish hospital workers (see page 38, Respiratory tract, for further details), the incidence of skin symptoms such as eczema and rashes on the hands was significantly

greater ($p<0.01$) in individuals exposed to 2% glutaraldehyde solution than in a non-exposed group. According to the authors, the skin symptoms were probably due to the primary irritant effect of the activated glutaraldehyde solution (Nor88).

Pisaniello *et al.* (Pis94, Pis97) carried out a cross-sectional study among 135 endoscopy nurses in 26 South Australian hospitals. Nurses were interviewed with a health/work-practice questionnaire. Furthermore, work-site inspections were undertaken, and personal exposure measurements of glutaraldehyde were carried out when the nurse was actually working with glutaraldehyde solutions (1-2% activated glutaraldehyde). A control group of 132 unexposed nurses in the same hospitals was also interviewed. The geometric means (GM) of the personal exposure in operating theatres during glutaraldehyde handling were 0.11 mg/m^3 (all personal; n=40), 0.06 mg/m^3 (with local exhaust ventilation (LEV); n=9) and 0.14 mg/m^3 (no LEV; n=28). In dedicated endoscopy areas, it was 0.18 mg/m^3 (all personal; n=28), 0.09 mg/m^3 (with LEV; n=12) and 0.37 mg/m^3 (no LEV; n=14). Of the 72 exposure measurements, four were above 0.8 mg/m^3, and ten between 0.4 and 0.8 mg/m^3. Nurses exposed to glutaraldehyde significantly reported more headache, lethargy, and skin symptoms compared with controls. These skin symptoms included dry, cracked skin, skin rash, discolouration and hard skin. Within the subset of exposed nurses for which personal monitoring data were available, there was no evidence that higher inhalation exposures increased the likelihood of symptoms. The authors stated that the lack of dose-response relationships for skin symptoms indicated that skin problems are less likely to be related to airborne exposure than to procedural insufficiencies as splashing or glove misuse. According to the authors, it could not be excluded that survivor bias may have played a role, indicating that those nurses, who have experienced health problems with glutaraldehyde had stopped working.

Waters *et al.* (Wat03) performed a cross-sectional study among 76 nurses from five Australian health care facilities. A number of 38 nurses were recruited from endoscopy and operating theatres where glutaraldehyde was used and 38 control nurses were recruited from area's in which glutaraldehyde was not used. Furthermore, the control nurses had not worked with glutaraldehyde in the previous 12 months. A questionnaire was administered to all nurses. Skin symptoms were 3.6 times more likely to be reported by exposed workers. The skin symptoms included itchy rashes on hands and forearms. This finding was confounded by a significant difference in glove-wearing behaviour between groups, as gloves have recognised irritant and allergic potential. According to the authors latex exposure appears unlikely to explain all skin symptoms, however, skin symptoms were not further investigated. Personal peak exposure measurements of glutaraldehyde were carried out during disinfection phases and different exposure control measures. Peak exposure levels above 0.4 mg/m^3 (range 0.04 mg/m^3 - 0.6 mg/m^3) were found for all methods of exposure control situations, except where a washing machine was used, where a peak reading of 0.32 mg/m^3 was obtained. Authors suggest that the

higher readings could generally be explained by procedural deficiencies including poor practices related to glutaraldehyde use. Exposure levels were determined by an alternative method, the direct reading Glutaraldemeter, which enables peak exposure measurement. Nevertheless, the authors noted that the method used lacks specificity. The committee noted a number of limitations in this study. These included the lack of the precise duration of the (peak) exposure (ranges from 5-735 sec) in relation to the effects observed and the uncertainty of the results of the exposure measurement apparatus.

Eye

NEG data

Of 167 nurses working in endoscopy units, 65% complained of eye irritation. Where measurements were performed, air concentration of glutaraldehyde was less than 0.8 mg/m^3 (0.2 ppm) (Calder *et al*., 1992). In studies at hospitals by NIOSH, a relationship between occupational exposure to glutaraldehyde and irritation of eyes has been demonstrated. Irritation was observed at concentrations of 0.8 mg/m^3 (0.2 ppm) and higher, whereas no symptoms of irritation were observed after reconstruction of the occupational setting and lowering the concentration to 0.4 mg/m^3 (0.1 ppm) or less (NIOSH 1991).

Additional data

Eye irritation was significantly increased in Australian hospital nurses exposed to glutaraldehyde (operating theatres GM 0.11 mg/m^3; endoscopy area's 0.18 mg/m^3) when compared to controls. A dose-response relationship was lacking. However, when exposure was expressed as the number of hours per week of glutaraldehyde usage, the prevalence of 'any eye symptoms' was generally higher in the group exposed for more than two hours per week (Pis94, Pis97; see page 35, Irritation, Skin, for further details).

In a British cross-sectional study, 13.5% of the endoscopy nurses exposed to glutaraldehyde (GM 0.06 mg/m^3, range <0.001-1.08 mg/m^3) reported work-related eye symptoms (Vya00; see page 46 Surveys, for further details).

No work-related eye irritation was found when endoscopy nurses were exposed to glutaraldehyde concentrations up to 0.6 mg/m^3 (Wat03). Furthermore, no increase in eye irritation was found among workers (see Occupational asthma, surveys, below, for further details) exposed to 0.24 mg/m^3 glutaraldehyde in a glutaraldehyde production plant (Tet95).

In a recent study (see page 40, Respiratory tract, for more details) female volunteers were exposed to several concentrations of glutaraldehyde by a vapour delivery device (VDD) for 25 seconds. About 18% of the volunteers detected glutaraldehyde vapour by the eye at about 1 mg/m^3 and 84% at 3 mg/m^3. In a second experiment, female volun-

teers were exposed in an exposure chamber for 15 minutes and ocular detection of glutaraldehyde was recorded. The authors concluded, that irrespective of the role of odour in the 15 min exposures, the threshold for ocular detection is above 0.4 mg/m^3 (Cai03).

Respiratory tract

NEG data

Inhalation of glutaraldehyde at vapour levels below 0.8 mg/m^3 has been reported to cause nose and throat irritation, nausea and headache (Burge *et al*., 1989). Chest discomfort, tightness and breathing difficulty may also occur. In studies at hospitals by NIOSH, a relationship between occupational exposure to glutaraldehyde and irritation of upper respiratory pathways has been demonstrated. Irritation was observed at concentrations of 0.8 mg/m^3 (0.2 ppm) and higher, whereas no symptoms of irritation were observed after reconstruction of the occupational setting and lowering the concentration to 0.4 mg/m^3 (0.1 ppm) or less (NIOSH 1991).

Additional data

Glutaraldehyde vapour may cause peripheral sensory irritant effects in humans. The molecule can interact with the sensory nerve receptors in skin and exposed mucosal surfaces, resulting in sensation at the site of contact together with certain reflexes typical of a peripheral sensory irritant material (eye discomfort, excess lacrimation, discomfort of the nose and possibly chest, rhinorrhea and cough or wheezing; Bal99). The peripheral sensory irritant effects of glutaraldehyde have been studied using an animal model (see section 6.2) and by exposure of human subjects. Ballantyne *et al*., (Bal01b) reported on two unpublished human studies. One study reported by Whitmore (1976) showed a sensory irritant threshold for glutaraldehyde vapour at 1 mg/m^3 and the other study reported by Collwell (1976) indicated a sensory irritant threshold of 1.2 mg/m^3.

The prevalence of airway symptoms, headache, nausea, and fatigue were studied by questionnaire among 107 Swedish hospital workers with (n=39) and without (n=68) exposure to glutaraldehyde, during cold sterilisation work. Among these hospital workers with and without exposure, comparable numbers were atopic (26% and 24%, respectively). Short-term (15 minutes) personal sampling (air sampling rate 1 L/min) in the breathing zone of the exposed workers during glutaraldehyde handling, revealed a geometric mean of airborne glutaraldehyde exposure of 0.05 mg/m^3 (range: manual sterilisation: <0.01-0.57 mg/m^3; automatic sterilisation: 0.01-0.18 mg/m^3). Long-term (3-4 hours) sampling of the exposed group and background level in the work areas were below the actual detection limit of glutaraldehyde (<0.04 mg/m^3; air sampling rate 0.25 L/min). In the exposed group, the prevalence of certain airway symptoms from the nose and throat were higher than in the unexposed group (p<0.05). These symptoms included

nasal catarrh, nasal obstruction, dryness of the throat and irritative cough. General symptoms, such as headache and nausea, were also more common in the exposed group (p<0.01). A dose-response effect was found between the frequency of exposure to glutaraldehyde and the number of symptoms (p<0.01). Neither the severity of the symptoms nor the correlation of the symptoms with exposure levels was addressed (Nor88). The committee is of the opinion that the observed nose and throat symptoms are of a sensory irritant nature.

A preliminary study of occupational exposure to glutaraldehyde was carried out at seven different workplaces in South Australia, of which the data are shown in Table 6.1 (Tka93). The committee noted that in this study airway effects, observed at these levels, were caused by irritation and do not provide evidence that glutaraldehyde can induce occupational asthma.

Table 6.1 Occupational exposure to glutaraldehyde (GA) (Tka93).

profession	personal inhalation dose (mg/m^3)	symptoms
endoscopy nurse (1)	0.004 - 0.19	no
endoscopy nurse (2)	0.04 - 0.42	headache, tingling of face (fine mist of 1% GA)
dental assistant	0.03 - 0.09	dermatitis of hands, due to not wearing gloves
embalmer	below detection limit	no
radiographer	≈ 0.004	no
radiography assistant	≈ 0.004	no
egg collector	0.03	irritation of face and respiratory problems (face wiped of with hands; spray of 0.1-0.3% GA)

In a study of Australian hospital nurses, the incidence of throat symptoms were found more often in glutaraldehyde-exposed workers compared to non-exposed. Within the subset of exposed nurses for which personal monitoring data were available, there was no evidence that higher inhalation exposures increased the likelihood of symptoms. Between both groups, no significant differences were found for the occurrence of nasal and pulmonary symptoms (wheezing and persistent cough). Personal sampling revealed airborne exposure levels of 0.11 mg/m^3 (GM, operating theatres) and 0.18 mg/m^3 (GM, endoscopy areas) (Pis94, Pis97).

British endoscopy nurses exposed to glutaraldehyde reported work-related symptoms of the nose and lower respiratory tract. Lower respiratory tract symptoms included chronic bronchitis, persistent cough, wheeze, shortness of breath and chest tightness. A significant relation between exposed workers and work-related nasal symptoms was found, after adjustment for types of ventilation. According to the authors, these work-related nasal symptoms that were dose-dependent on peak glutaraldehyde concentrations, suggest a direct irritant effect. The mean peak and background airborne glutar-

aldehyde concentrations were 0.06 mg/m^3 (GM; range: <0.001-1.08 mg/m^3), and 0.01 mg/m^3 (GM; range: 0.002-0.1 mg/m^3), respectively (detection limit: 0.001 mg/m^3). (Vya00).

In a cross-sectional study among 76 nurses from five Australian health care facilities (see page 35 Irritation, Skin, for study details) no nose irritation was found when nurses were exposed up to glutaraldehyde levels of 0.6 mg/m^3. Also the other respiratory tract symptoms investigated (nasal burning, throat irritation, cough, wheeze and chest tightness) were not significantly associated with glutaraldehyde exposure. There were significant cross-shift reductions in FVC and FEV_1 in the exposed group. No evidence of a dose-response relationship for symptoms or lung function was found (Wat03). The committee noted some limitations in this study, as the notable high prevalence of respiratory symptoms measured in the unexposed group and the absence of a non-exposed control group at lung function measurements.

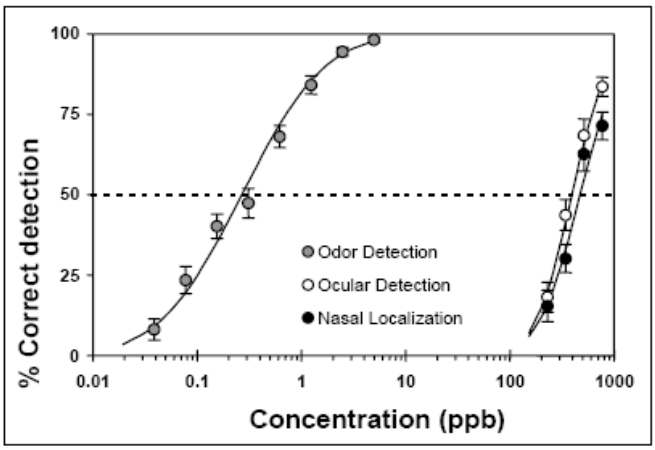
In a recent (unpublished) study, healthy adult female volunteers (n=53) were exposed to several concentrations of glutaraldehyde (odour detection 0.16-20 µg/m^3; nasal and ocular detection 0.92-3.1 mg/m^3) by a vapour delivery device (VDD) for 2-25 seconds (Cai03). The authors called detection by eye and nose "chemesthetic detection". The committee considered this chemesthetic detection to be (sensory) pungency that may result in sensory irritation. (Non-smoking) females were chosen because of their higher sensitivity for chemesthetic detection than males. Glutaraldehyde vapour samples (15 min duration) taken in the VDD outlet were measured using the OSHA64 method. Levels for odour detection, nasal and ocular detection were measured. Figure 6.1 shows the steep dose-response relationships for odour, ocular and nasal detection.

Odour was detected at low vapour concentrations from 0.2 µg/m^3 (0.04 ppb) on. All subjects detected it at 20 µg/m^3 (5 ppb). Eye or nasal pungency, started at concentrations of 0.9 mg/m^3 (229 ppb; 15-18% of volunteers) and was detected by 71-84% of the volunteers at about 3 mg/m^3 (772 ppb).

In a second experiment, female volunteers (n=50) were exposed in an exposure chamber for 15 minutes to concentrations ranging from 0.14-0.4 mg/m^3.

Glutaraldehyde vapour samples (15 min duration) taken in the breathing zone of the subjects were measured using the OSHA64 method. The volunteers recorded during 1 minute intervals the chemesthetic detection of glutaraldehyde by nose, eye or throat as 'yes' or 'no' with a level of confidence, from not certain (rating 1), to moderately certain (rating of 2), to very certain (rating of 3). Hence, if she detected nothing at all in the nose, she gave a rating of 'no' and '3' (no/3). At 0.4 mg/m^3, a slight difference in chemesthetic detection by of nose, eye and throat was reported when compared to the control; from rating 'no/3' to 'no/1' (see figure 6.2). A dose-response relationship was not observed, which might according to the authors be due to interference of odour and chemesthetic detection. The authors concluded, that irrespective of the role of odour in

the 15 min exposures, the threshold for chemesthetic detection of eye, nose and throat is above 0.4 mg/m^3 (Cai03). The authors stated that due to the absence of a rating 'yes' at 0.4 mg/m^3, this level is considered a no effect level for a 15-minute exposure.



Threshold 50%	mg/m^3	ppb
Odour detection	0.001	0.27
Ocular detection	1.58	394
Nasal detection	1.89	472

Figure 6.1 Psychometric functions (average ± sem) for subjects achieving 50% correct detection of glutaraldehyde odour or glutaraldehyde by nose or eye, with the average concentrations for 50% detection. Exposures lasted 2 seconds for odour and nasal detection and 25 seconds for ocular detection (Cai03).

6.1.2 *Sensitisation*

Experimental

NEG data

In studies of 109 and 102 volunteers respectively, skin sensitisation was studied using patch testing. Glutaraldehyde concentrations in aqueous or petrolatum solution were up to 0.5% w/w for induction and 0.5% w/w for challenge. Only one single positive reaction was recorded. Repeating the patch testing with 30 volunteers and higher induction concentrations (5% w/w in petrolatum) resulted in 23% positive skin reactions (Ballantyne and Berman 1984; Marzulli and Maibach 1974).

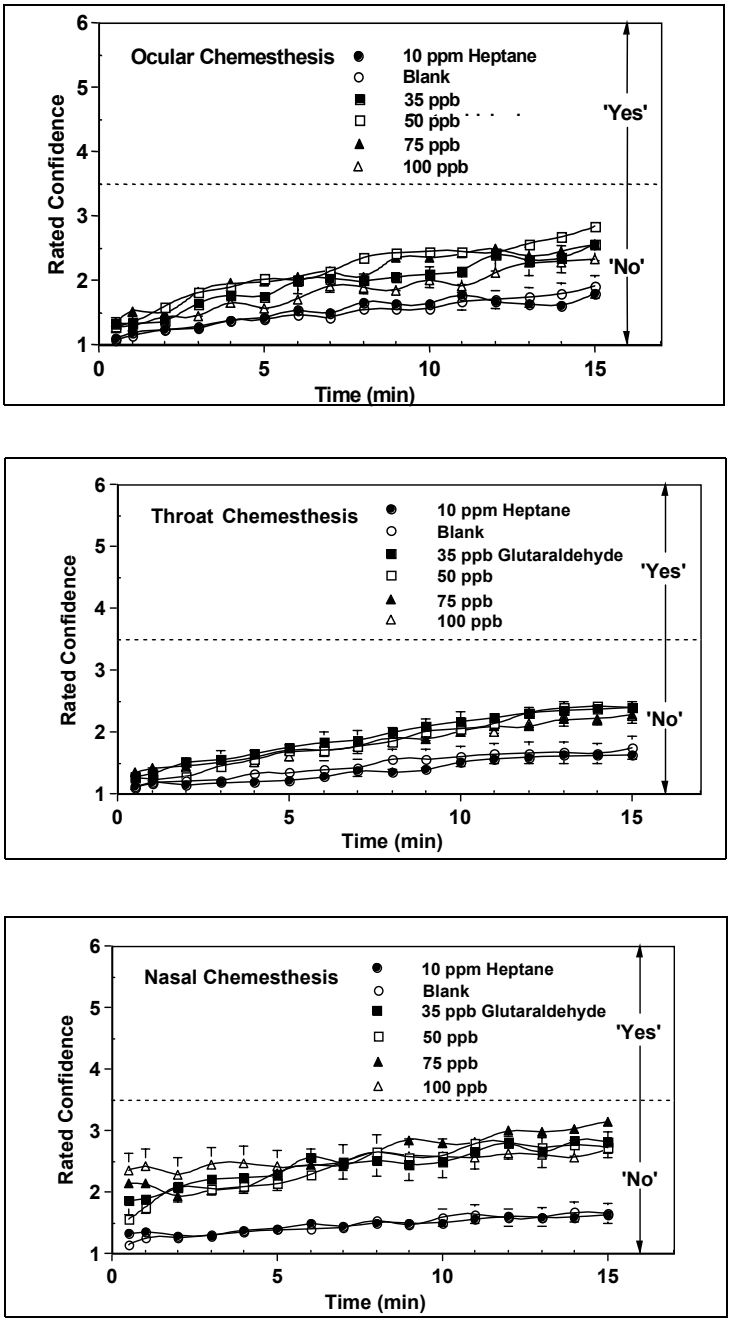


Figure 6.2 Average rated confidence on the transformed scale of 1 to 6 for the various 15 minute exposures (see text). Positive and negative standard errors of the mean are shown for the blank exposures. Negative standard errors are shown for 0.14 mg/m^3 (35 ppb), the lowest level of exposure, and positive errors are shown for 0.40 mg/m^3 (100 ppb), the highest level, for reference (Cai03).

Occupational

NEG data

Several cases of dermatitis due to repeated or prolonged contact to glutaraldehyde or glutaraldehyde containing disinfecting agents have been reported. The symptoms are marked dryness, redness, eczema, infiltration, fissures and skin sensitisation (Bardazzi *et al*. 1986; Burge 1989; Cusano and Luciano 1993; DiPrima *et al*. 1988; Fisher 1990; Fowler 1989; Goncalo *et al*. 1984; Hansen 1983; Potter and Wederbrand 1995; Tam and Freeman 1989; Wahlberg 1985).

Additional data

In Germany, patch test results and important patient history items of about 32,000 patients recorded between 1992 and 1995 in 24 allergy departments, participating in the Information Network of Departments of Dermatology (IVDK), were evaluated. For glutaraldehyde, 1194 health-care workers were tested using an epicutaneous test with 1% glutaraldehyde in vaseline. A significantly increased sensitisation rate common to the health-care sector as a whole of 10% was found compared to 2.6% in about 4000 non health-care workers (Sch98a, b).

In an USA study, patch testing (epicutaneous test with 1% glutaraldehyde in vaseline) was performed on 468 subjects (51 health-care workers, 417 non health-care workers) to assess their allergenicity to glutaraldehyde. Reactions > 1 (on a scale of 1-7 on reaction morphology) to 1% concentration of the allergen were considered allergenic to glutaraldehyde. From the population studied, 17.6% (9/51) health-care workers reacted positive compared to 1.9% (8/417) non health-care workers (Sha00).

In another USA study, patch test evaluations were performed among 101 healthy dental professionals of which 80% had had known exposure to cold sterilisation procedures with glutaraldehyde or formaldehyde. Eleven (10.9%) dental professionals had clear reactions to glutaraldehyde, four (4.0%) were questionably allergic to glutaraldehyde, and two (2%) were definitively allergic to formaldehyde. In a control group of 51 non-dental professionals, one (2%) subject had a reaction to glutaraldehyde, and one other (2%) had a reaction to formaldehyde. The authors found no evidence of cross-reactivity between glutaraldehyde and formaldehyde (Rav03).

In a British cross-sectional study (see page 46 Surveys, for further details), 44% of the endoscopy nurses exposed to glutaraldehyde (GM 0.06 mg/m^3, range <0.001-1.08 mg/m^3) reported work-related contact dermatitis (Vya00).

Also in other publications, occupational allergic contact dermatitis to glutaraldehyde has been reported in a single nurse, an orthodontic assistant, two hairdressers and in one out of fourteen health-care workers respectively (Kan00, Ham03, Kie01, Sti95).

No increase in skin sensitisation was found among workers (see page 46, Surveysj- for further details) exposed to 0.24 mg/m^3 glutaraldehyde in a glutaraldehyde production plant (Tet95). Furthermore, in Swedish hospital workers with glutaraldehyde-related skin symptoms, no skin sensitisation was found using patch testing (Nor88).

6.1.3 *Occupational asthma*

Published reports of occupational asthma associated with the use of glutaraldehyde include case studies and surveys of health-care personnel.

Case studies

NEG data

In many studies (Benson, 1984; Chang *et al*. 1993; Cornacoff *et al*. 1986; Cullinan *et al*. 1992; Gannon *et al*. 1995; Mwaniki and Guthua, 1992; Stenton *et al*. 1994), exposure to glutaraldehyde induced asthmatic symptoms such as wheezing, cough, chest tightness, breathing difficulties, and/or breathlessness, and hyperresponsiveness. Workplace challenge or laboratory provocation tests with glutaraldehyde revealed changes in $FEV_{1,}$ peak expiratory flow, airway responsiveness, and/or nasal airway resistance, in several but not all patients. Although most of these symptoms may point at (specific) hypersensitivity reactions, respiratory irritation cannot be excluded as was stated for instance by Gannon *et al*., 1995 and Chang *et al*. 1993. Conclusive or sufficient evidence of respiratory sensitisation, however, is lacking.

Additional data

A 25-year old female respiratory therapy technician developed asthmatic reactions, after being employed for 3.5 years and using 2% activated aqueous glutaraldehyde solution. She denied to have any other allergy. On lung function testing, she demonstrated a delayed obstructive response after exposure to glutaraldehyde under simulated working conditions. An immunologic mechanism was not demonstrated to be responsible for the reactions. Serum IgG and IgE levels, as well as a scratch test performed with a 2% glutaraldehyde solution were normal, although the methodology applied was not specified. However, the absolute eosinophil count was elevated. After changing position, the frequency and severity of the attacks decreased markedly, although they did not disappear completely. Upon re-exposure, she developed severe life-threatening status asthmaticus. At that time, the eosinophil count and immunoglobulins were normal (Nic86).

Quirce *et al*. (Qui99), described a case of a 61-year old nurse, who experienced sporadic and mild episodes of chest tightness and shortness of breath related to exposure to formalin, ten years after having worked in a renal dialysis unit. Four years after formalin

had been replaced by glutaraldehyde, she developed symptoms of irritation of eyes and upper respiratory tract, dyspnoea on exertion, dry cough and episodic attacks of wheezing, which she associated with glutaraldehyde exposure. Her symptoms were progressively severe, and she had an acute asthma attack requiring hospital admission. After three months recovery, she underwent the specific bronchial challenge test with activated 2% aqueous glutaraldehyde solution for 10 minutes, without reaction. Repeating this challenge one week later, elicited an early asthmatic response that did not occur in two unexposed asthmatic patients. The specific challenge test provoked the appearance of non-specific bronchial hyperresponsiveness (NSBH), which preceded the development of an asthmatic reaction to glutaraldehyde. This increase in NSBH may help to distinguish an asthmatic reaction due to specific mechanisms from bronchoconstriction triggered by an irritant effect. According to the authors, this study indicates the potential of glutaraldehyde to induce asthma.

DiStefano *et al*. (DiS99), studied 24 health-care workers with respiratory symptoms suggestive of occupational asthma due to glutaraldehyde exposure. In eight workers, who underwent a specific bronchial provocation test (SBPT), the diagnosis of occupational asthma was confirmed by a positive reaction (late and dual reaction in five and in three subjects, respectively). The mean level of glutaraldehyde measured during the provocation tests was 0.075 mg/m^3 (range 0.065-0.084 mg/m^3). A control group of unexposed asthmatic subjects was not challenged. However, a non-specific bronchial constriction due an irritant effect seems to be unlikely. The authors considered this because the challenge concentration was low and all reactions observed in the exposed group were late. Sixteen workers performed serial peak expiratory flow rate (PEFR*) measurements every two hours from waking to bedtime over the last two work and rest periods with at least five readings per day using a peak flow meter. In 13 out of 16 remaining workers, PEFR measurements showed a pattern suggestive of work-related asthmatic symptoms. In three workers, there was no physiological confirmation of occupational asthma. Measurements of specific IgE antibodies to glutaraldehyde-modified proteins were positive in seven patients (29.2%), according to a cut-off value of 0.88% RAST binding. Air samples were collected in the workplace during activities likely to produce peak levels of glutaraldehyde. The levels of glutaraldehyde measured were: personal short-term samples of 20 min: mean 0.208 mg/m^3 (range 0.06-0.84 mg/m^3) and personal long-term samples of 34-120 min: mean 0.071 mg/m^3 (range 0.003-0.28 mg/m^3). The authors conclude that in some cases exposures could have reached an irritant level.

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* The PEFR is the maximum rate of airflow that can be achieved during a sudden forced expiration from a position of full inspiration. The measurement of peak expiratory flow rate (PEFR) three to four times per day allows the diagnosis and assessment of the severity of asthma. Untreated asthma is characterised by: 1) greater than 10% diurnal variability in PEFR and 2) lowest values in the morning.

Surveys

NEG data

From 65% of 167 nurses working in endoscopy units, there have been complaints of headache and cough or shortness of breath. Where measurements were performed air concentration of glutaraldehyde was less than 0.8 mg/m^3 (0.2 ppm) (Calder *et al*., 1992).

Additional data

In Great Britain, as a result of cases reported in the Surveillance of Work-related and Occupational Respiratory Disease (SWORD) project, glutaraldehyde is thought to have asthma-inducing effects. The number of occupational asthma cases, claimed to be induced by glutaraldehyde, were 2/554 in 1989 (Mer91), 20/1085 in 1989-1990 (Mer93), and 30/1528 in the period 1989-1991 (Mer94). Sallie *et al*. (Sal94), reported 13 cases of glutaraldehyde-induced asthma out of an estimated total of 1,047 occupational asthma cases in 1993. In the period 1992-1994 and 1995-1997, the number of claimed cases of glutaraldehyde-induced asthma increased to around 4% (128/2,857 and 133/3,002 respectively) (McD00). In 1998, the number of claimed cases increased to around 5% (Mey99). Although diagnostic criteria and exposure concentrations were not reported, the authors indicated the possibility of asthmatic diseases by glutaraldehyde.

DiStephano *et al* (DiS04) reported the results of a British voluntary surveillance scheme for occupational asthma in the West Midlands between 1990-1997. An increase from 1.3 to 5.6% in occupational asthma cases due glutaraldehyde exposure was reported. Diagnosis of occupational asthma included serial peak expiratory flow measurements at and away from work and specific bronchial challenge tests.

Teta *et al.* (Tet95) reported on the incidence of sensitisation and allergic blepharoconjunctivitis among 210 workers assigned to glutaraldehyde production or drumming from 1959 to 1992 (mean exposure in production was 3.8 year and in drumming 6.4 year). There was no indication of glutaraldehyde induced, respiratory sensitisation or allergic blepharoconjunctivitis in workers exposed to mean glutaraldehyde levels of 0.24 mg/m^3 (range: 0.04-1.36 mg/m^3, personal short-term, 15 min measurements (n=88) from 1989 to 1992) or 0.20 mg/m^3 (range 0.04-0.68 mg/m^3, 8 hour personal measurements from 1977 to 1988). The exposure data were only available from 1977 and so were not relevant to the great majority of the subjects in the study. HSE (HSE 1997) evaluated this study and found more deficiencies in the study design. Workers who were assigned to the glutaraldehyde factory after 1978 and left before data collection began were for practical reasons excluded from consideration. Thus any workers who left the factory due to work-related ill-health during the period of which exposure information is available would not have been identified. Furthermore, 5 workers had documented cases of sensitisation related to chemicals other than glutaraldehyde present in the factory.

Due to these limitations of the study design and findings described above, no useful conclusion regarding the identification of a no-effect-level for glutaraldehyde, or even regarding the ability of glutaraldehyde to induce occupational asthma can be drawn.

Pisaniello *et al.* (Pis94, Pis97) carried out a cross-sectional study among 135 endoscopy nurses and 132 control nurses in 26 South Australian hospitals (See Irritation, Skin, for further more details). Between both groups, no significant differences were found for the occurrence of nasal and pulmonary symptoms (wheezing and persistent cough). According to the authors, it could not be excluded that survivor bias may have played a role, indicating that those nurses, who have experienced health problems with glutaraldehyde had stopped working.

In the United Kingdom, a cross-sectional study was carried out, in which 318 endoscopy nurses and 18 former endoscopy nurses with work-related symptoms from 59 endoscopy units were surveyed for respiratory function and immunology in relation to occupational glutaraldehyde exposure. No control group was surveyed. The minimum period of employment was two months and the maximum 19 years (GM: 2.24 years). Examinations were performed by symptom questionnaires, session spirometry, peak flow diaries, skin pricks tests to common allergens and latex, and measurements of total and specific immunoglobulin E to glutaraldehyde and latex. Airborne glutaraldehyde concentrations were measured by personal air sampling of one nurse per unit and presented as "peak" (during glutaraldehyde changeover) and "background" (endoscopy room, excluding glutaraldehyde changeover) concentrations. Twelve out of the eighteen former employees had left their job, because of lower respiratory tract symptoms with a latency of three months or greater. Ten still complained of lower respiratory tract symptoms, despite the fact that nine were no longer in direct contact with glutaraldehyde. The mean peak and background airborne activated glutaraldehyde concentrations were 0.06 mg/m^3 (GM; range: <0.001-1.08 mg/m^3), and 0.01 mg/m^3 (GM; range: 0.002-0.1 mg/m^3), respectively (detection limit: 0.001 mg/m^3). Of the current nurses exposed to glutaraldehyde, 8.5% showed work-related symptoms of the lower respiratory tract symptoms. These included chronic bronchitis, persistent cough, wheeze, shortness of breath and chest tightness. There was no dose-response relation between exposure measures and lower respiratory tract symptoms with the exception of chronic bronchitis, which was in fact the least prevalent symptom. There was no significant difference in lung function (percentage predicted forced expiration volume in 1 second ($ppFEV_1$)) between symptomatic and asymptomatic current workers, whereas there was a significant difference ($p<0.01$) in $ppFEV_1$ between current workers and ex-workers. Occupational peak flow diaries, completed by current workers with work-related symptoms of the lower respiratory tract, showed no evidence of bronchial asthma (<15% variation). There was only one current worker with positive IgE specific to glutaraldehyde. She had work-related symptoms of the eyes and nose, but not of the lower respiratory tract

(Vya00). Because the nurses exhibited irritant symptoms, but neither 'clinical nor investigational indications of asthma', the committee is of the opinion that the findings of this study do not provide evidence that glutaraldehyde is a respiratory sensitizer.

In a cross-sectional study (see Irritation, Skin, for further more details) nurses from five Australian health care facilities were exposed up to glutaraldehyde levels of 0.6 mg/m^3 (Wat03).The investigated respiratory tract symptoms (nasal burning, throat irritation, cough, wheeze and chest tightness) were not significantly associated with glutaraldehyde exposure. There were significant cross-shift reductions in FVC and FEV1 in the exposed group. No evidence of a dose-response relationship for symptoms of lung function was found (Wat03). The committee noted some limitations in the study as the notable high prevalence of respiratory symptoms measured in the unexposed group and the absence of a non-exposed control group at lung function measurements.

Immunological tests

Total and specific IgE antibodies were measured in a group of 20 workers exposed to glutaraldehyde and compared with sera of a group of 21 non-exposed subjects (Cur96). In ten out of twenty of the exposed persons, blood samples were taken at least six months after the last exposure. Although the RAST % binding was not high (net RAST binding > 0.88% was taken to indicate the presence of specific IgE), a significant difference (p=0.026) between exposed and non-exposed subjects with total serum IgE less than 150 kU/L could be detected. Unexposed control sera with total IgE > 150 kU/L produced false-positive results. Out of the thirteen subjects clinically diagnosed to have occupational asthma, two had IgE levels > 150 kU/L, and ten had values < 150 kU/L (one was not tested); specific IgE (positive RAST test) was detected in only two of the latter subjects. A RAST inhibition test carried out in one individual with clinically diagnosed asthma was positive (specific antibody binding by glutaraldehyde modified proteins), but negative when using sera of non-exposed controls with false positive RAST results. The committee agrees with the conclusion of the authors that glutaraldehyde seems to belong to a growing group of low-molecular-weight chemicals for which there is a poor correlation between specific IgE antibody and clinical symptoms of occupational asthma.

Twenty-four health-care workers with respiratory symptoms suggestive of occupational asthma due to glutaraldehyde exposure, have been studied to determine the presence of IgE specific to glutaraldehyde (see Occupational asthma case studies, above, for further details). Specific IgE antibodies to glutaraldehyde-modified proteins were positive in seven patients (29.2%), according to a cut-off value of 0.88% RAST binding (DiS99).

A single-blind, placebo-controlled study was performed on 11 health-care workers occupationally exposed to glutaraldehyde for 6 $\pm$ 3 years with clinically diagnosed occupational asthma and rhinitis due to glutaraldehyde. The control groups comprised of 10 atopic patients with perennial asthma and rhinitis and 10 healthy ones. None of the controls had been occupationally exposed to glutaraldehyde in the past. A 'nasal pool' technique was used to evaluate the examined parameters in nasal washings before and 30 min, 4 and 24 hours after the inhalatory provocation with glutaraldehyde and placebo (0.9% saline). The mean concentration of glutaraldehyde in the air during the challenge tests was 0.32 $\pm$ 0.08 mg/m^3. A significant increase in eosinophil number and percentage, and albumin, eosinophil cationic protein and mast cell tryptase concentrations in nasal lavage fluid from patients with occupational asthma and rhinitis was measured when compared to controls. According to the authors these results suggest an immunological mechanism of glutaraldehyde–induced asthma (Pal01).

From these data, no clear working mechanism can be derived. According to Chan Yeung *et al.* (Cha95), the immunological mechanism of low molecular weight (LWM) substances, such as glutaraldehyde, to cause respiratory sensitisation is less defined. Some LMW substances may acts as haptens and induce specific IgE antibodies by combining with a body protein, whereas (many) other LMW substances may not (or partly) induce specific IgE antibodies (as might be the case with glutaraldehyde). The mechanism may also involve IgG antibodies as well as cell-mediated hypersensitivity.

6.1.4 Carcinogenicity

NEG data

No increased mortality rate and no increased incidence of malignant tumours were found among 186 workers exposed to 0.24 mg/m^3 glutaraldehyde in a glutaraldehyde production plant (Teta *et al*., 1995). Because of the small numbers, the relatively young age of the workers and the relatively short follow-up period (mean 21 year; range 10-34 year), in this study, no useful conclusion on carcinogenicity can be drawn.

6.1.5 Reproductive toxicity

NEG data

No significant increased risk of spontaneous abortions and foetal malformations was found in Finnish hospital nurses and staff, using glutaraldehyde as a sterilising agent (Hemminki *et al*., 1982 and 1985).

Additional data

Russel *et al*. (Rus00), studied the relationship between various occupational exposures and self-reported infertility in nurses from a private gastroendoscopy (GE) clinic (n=14, mean age 35 year) and a hospital (n=183, mean age 35 year) in Brisbane, Australia. The occupational exposures included glutaraldehyde, x-radiation, cytotoxic agents and ultrasound. In the private GE clinic, glutaraldehyde appeared to be the most commonly indicated agent that nurses were exposed to. Thirteen out of 14 nurses were exposed to presumably high levels (due to manual disinfections using open soaking bowls) of glutaraldehyde during a mean period of 5 years. In the hospital, the most common exposure was x-radiation (31% exposed), whereas 20% reported to be commonly exposed to glutaraldehyde (exposure levels unknown). The rate of fertility problems, in this study seems to be unusually high between both GE clinic (70%) and hospital-based nurses (28%) compared to the general population (15%). This may be partly explained by the authors' inclusive definition of infertility based on self-reported problems conceiving. Furthermore, the study findings were hampered due to the poor response rate (33%) and the lack of correction for mixture exposure data. In addition, because the clinic nurses were a very small self-selected group the findings in this group are open to selection bias. Moreover, no other reports have linked glutaraldehyde with infertility and hence these findings require confirmation.

6.1.6 Neurological effects

Additional data

A female anaesthesiologist was only exposed to 2% glutaraldehyde solution in an operating theatre. Adverse neurobehavioral effects, including headache, loss of attention, dizziness, anxiety, drowsiness on the job and alteration of homeostatic reflexes were observed during clinical evaluation of neurobehavioral functions. Ten days after removal from exposure complete recovery occurred (Pro02).

6.2 Animal experiments

6.2.1 Irritation

Skin

NEG data

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 (Ballantyne *et al*.,

1985; Smyth *et al*., 1962). An alkaline 2% glutaraldehyde solution caused moderate skin irritation when applied to the rabbit skin for 24 hour and mild erythema and rash when applied for 6 weeks (Miner *et al*., 1977; Stonehill *et al*., 1963). A severe erythematous reaction with oedema followed by necrosis was observed when an alkaline 24% glutaraldehyde solution was applied to the rabbit skin (Stonehill *et al*., 1963).

Additional data

Primary skin and eye irritation tests in rabbits were performed according to the standard OECD guidelines (OEC84). Application of 0.5 mL glutaraldehyde to the clipped skin of the New Zealand white rabbits (n=6 per group) under occlusive dressing for about 4 hours, induced severe skin irritation and necrosis at 45 and 50% aqueous glutaraldehyde solution. Inflammation was moderate at 25%, slight to moderate at 5 and 10%, minor at 2% and threshold at 1% aqueous glutaraldehyde solution (Bal01a). Because glutaraldehyde solution is alkalinised before use, to optimise biocidal activity, alkalinised glutaraldehyde was also tested in New Zealand white rabbits (n=6). Alkalinisation of 2.2% aqueous glutaraldehyde solution had no significant effect on the skin irritation potential (Bal97).

Eye

NEG data

A 2% acid glutaraldehyde solution produced severe and extensive conjunctival injury of the rabbit eye (Martin, 1978). An alkaline 2% glutaraldehyde solution revealed severe eye irritation in rabbit (Stonehill *et al*., 1963; Miner, 1977). Glutaraldehyde vapour is irritating to the eye at an air concentration of 0.8 mg/m^3 (0.2 ppm). At higher concentrations serious, irreversible injury may occur (Beauchamp *et al*., 1992; Benson 1984, Jachuck *et al*., 1989). As an alternative to the Draize rabbit eye test, glutaraldehyde was found cytotoxic to human corneal endothelial cell cultures (Douglas and Spilman 1983).

Additional data

Instillation of 45% aqueous glutaraldehyde solution (0.001-0.1 mL), in the inferior conjunctival sac or on the surface of the cornea in the eye of the New Zealand white rabbit (n=6), produced severe conjunctival and corneal injury, which persisted for up to 3 weeks. At 2% glutaraldehyde corneal injury was mild and at 5% marked. The lowest concentration producing corneal injury was 1% and the no-effects-concentration was 0.5%. The threshold for conjunctival effects was 0.2% and the no-effects concentration 0.1%. At 1% aqueous glutaraldehyde solution, conjunctival hyperaemia and chemosis were moderate to market, and became more severe with higher glutaraldehyde concentrations (Bal01a). Alkalinisation of 2.2% aqueous glutaraldehyde solution showed con-

junctival inflammation in rabbits (n=6), that was more marked and persistent than 2.2% acid aqueous glutaraldehyde solution, that was more slight and transient (Bal97).

Respiratory tract

NEG data

Measurement of the depression of respiratory rate in ND4 Swiss Webster mice was used as a basis to study the quantitative aspects of the respiratory/sensory irritant effects of glutaraldehyde. Mice were exposed to seven different glutaraldehyde vapour concentrations in ranges of 6.4-146.8 mg/m^3. Based on the exposure concentration-effect relationships the vapour concentration producing a 50% decrease in respiratory rate (RD_{50}) was calculated to be 55.6 mg/m^3 (13.86 ppm) (Werley *et al*., 1995).

In another study in mice exposed to 1.2, 4 and 10 mg/m^3, the breathing frequency, used as an index of respiratory/sensory irritation (Alarie 1973), was measured and a 50% decrease in respiratory rate (15 min oronasal exposure in 2.8-18 mg/m^3 range) RD_{50} of 10.4 mg/m^3 was found (Zissu *et al*., 1994; further described in paragraph 6.2.4). In a distribution study, rats were intra-nasal instilled with 10, 20 or 40 mM glutaraldehyde, followed by an intraperitoneal injection of 5-bromo-2'-desoxyuridine (which is incorporated by cells in the S-phase) after 72 hours. Increased cell proliferation and dose-related, acute inflammatory changes as well as extensive regions of respiratory epithelial hyperplasia and squamous metaplasia were observed after intranasal exposure of rats to 20 and 40 mM glutaraldehyde and incorporation followed by injection (St Clair *et al*., 1990).

6.2.2 *Sensitisation*

Skin

NEG data

The skin sensitising potential of glutaraldehyde was demonstrated in studies in mice and guinea pigs. In these studies, contact hypersensitivity to glutaraldehyde followed a dose-dependent response (Stern *et al*., 1989). Mouse ear swelling tests with glutaraldehyde showed significant increases in the thickness of the ears indicating that the animals were sensitised to glutaraldehyde (Cor *et al*., 1988; Descotes 1988; Gad *et al*., 1986). In a modified Magnusson Kligman test, 72% of the guinea pigs were sensitised against glutaraldehyde. Cross-sensitisation between glyoxal, formaldehyde and glutaraldehyde was also observed (Foussereau *et al*., 1992).

Additional data

Contact hypersensitivity studies in guinea pigs (Hartley strain, females n= 6/group including positive and negative controls) and mice (B6C3F1, female n= 6/group including positive and negative controls) showed a statistically significant dose-related hypersensitivity response at 0.3% in mice and at 3% in both species (Ste87).

The influence of alkalinisation of aqueous solutions of glutaraldehyde on skin sensitising potential was investigated in a guinea pig maximisation test comparing 2.2% aqueous acid glutaraldehyde solution with an 2.2% alkalinised solution of pH 7.8. Acid glutaraldehyde had a greater skin sensitising potential (68% at challenge, and 32% at re-challenge) than alkalinised glutaraldehyde (30% at challenge and 5% at re-challenge) (Bal97, Mye94).

In the local lymph node proliferation assay (LLNA), a method to test the skin sensitising potential (Kim92, Kim98), glutaraldehyde was applied to the ear of mice and subsequently ^{3}H-methylthymidine uptake in the regional auricular lymph nodes was measured. In this assay, glutaraldehyde induced a significant LLNA reaction in a concentration of at least 0.25%, indicating a skin sensitising potential (Dea99, Hil98).

Respiratory tract

NEG data

In the mouse IgE test, regarded a test for respiratory sensitisation potential, glutaraldehyde induced a slight increase in total serum IgE levels which was only significant for the second highest (9.38 mg dermal application) amount tested (Potter and Wederbrand 1995). A study in guinea pigs using inhalation sensitisation and challenge did not produce any evidence of respiratory sensitisation at glutaraldehyde vapour concentrations of 55.6 mg/m^3 (Werley 1995). A weak immunologic response, measured as elicited antibodies, was observed in rabbits injected intramuscularly rabbit serum albumin with 2% glutaraldehyde in Freud's complete adjuvant (CIR 1996).

Additional data

In studies of Dearman *et al*. (Dea97, Dea99), cytokine production profiles of lymph node cells, isolated after topical application of glutaraldehyde or formaldehyde, have been compared with those observed following concurrent exposure to dinitrochlorobenzene (DNCB) and trimellitic anhydride (TMA). The contact allergen DNCB and respiratory allergen TMA are considered model compounds, that induce cytokine secretion patterns consistent with the selective activation of T helper 1 (Th1)- and Th2-type cells, respectively (Dea96). Groups of female BALB/c mice (n=5 for chemical, n=10 for vehicle) were topically treated twice (on day 1 and day 5) with 50 µL of 50% formaldehyde, 15% glutaraldehyde or vehicle in acetone on the shaved flank. Control animals were

treated concurrently with the reference contact allergen 1% DNBC or with the respiratory sensitizer 10% TMA. Ten days after the induction phase treatment, mice were challenged with 25 µL of chemical or vehicle on the dorsum of both ears for 3 consecutive days. Thirteen days after the initiation of exposure, draining lymph node cells (10^7/ml) were cultured for 12-120 h (in the presence or absence of the mitogen concanavalin A). Cytokines in the culture supernatants were analysed by cytokine specific enzyme-linked immunosorbent assay (ELISA). High levels of the Th1-cytokine IFN-gamma, but little of the Th2-type products interleukins 4 and 10 (IL4 and IL10) were provoked by DNCB and formaldehyde. TMA and glutaraldehyde induced the converse pattern of cytokine expression. According to the authors, the induction of selective Th2-type cytokine secretion profiles by glutaraldehyde indicates that glutaraldehyde, in contrast to formaldehyde, may have the potential to cause sensitisation of the respiratory tract.

The study of Ulrich *et al.* (Ulr01), however, does not support the selective Th2-type cytokine secretion discussed above. The authors investigated the cytokine secretion pattern by sensitisation of BALB/c mice and elicitation of contact allergy in sensitised animals. Six female BALB/c mice per group were treated on 3 consecutive days with 50 µL 1% glutaraldehyde, 15% formaldehyde, 0.5% DNCB, 0.5% TMA or other chemicals on the shaved back. Twelve days after the induction phase treatment, mice were challenged with 25 µL 0.5% glutaraldehyde, 15% formaldehyde, 0.5% DNCB or 0.5% TMA on the dorsum of both ears for another 3 days (challenge phase treatment). Mice were sacrificed 24 hour after the last exposure, draining lymph node cells were cultured (10^6 /mL) for 24 hour in the presence of the mitogen anti-CD3 antibodies, and cytokines in the culture supernatants were determined with ELISA. The results indicate that co-expression of Th1 and Th2 cytokines during contact allergy is an important feature of murine contact allergy in BALB/c mice and that glutaraldehyde and other chemicals differ in the degree of induction or expression of these cytokines, but do not induce them in a mutually exclusive manner. The authors further noted some differences in their experimental design compared to Dearman *et al*. (Dea97, Dea99), as the density of incubation of lymph node cells and the different mitogen used.

6.2.3 Toxicity due to acute exposure

Lethal concentrations

Additional data

Several studies have been conducted to assess lethal concentrations of glutaraldehyde. The results (including NEG data) are summarized in table 6.2.

Studies comparing the acute oral toxicity of acid and alkaline buffered solutions of a 2.2% solution of glutaraldehyde have shown that alkalinisation of glutaraldehyde does not influence acute oral toxicity (Bal97).

Acute exposure of rats to glutaraldehyde vapour (concentration range 20-200 mg/m^3) generated at ambient temperature (17-25^{0}C) produced only transient peripheral sensory irritant effects to the eyes and respiratory tract. Similar signs developed in human subjects exposed to glutaraldehyde vapour. In contrast, when the vapour was generated at elevated temperature (60-65^{0}C) severe effects, including mortality (4h LC_{50} range 94-177 mg/m^3) occurred. The peripheral sensory irritant signs and difficulties with breathing persisted for many days post exposure. Histopathology in rats that died included exposure concentration-related acute inflammation and necrosis in the nasal mucosa, larynx trachea and bronchi. These findings clearly indicate that toxicity, and hence a potential hazard, is greater for vapour atmospheres generated at elevated temperature.

Table 6.2 LD_{50} or LC_{50} values reported after acute exposure to glutaraldehyde (Bal01a; Ballantyne *et al*., 1985; Lewis *et al*., 1992; Miner *et al*., 1977; Smyth *et al*., 1962, Stonehill *et al*., 1963; Uemitsu *et al*., 1976.)

Species	Administration route	Reported LD_{50} (LC_{50}) values
rat	inhalation	94-177 mg/m^3 (23.5-44.3 ppm); when vapour generated at 60-65^{0}C no mortalities, only transient peripheral sensory irritant effects when vapour generated at 17-25^{0}C
rat	oral	137-165 mg/kg bw; > 5% aqueous acid solution 67-123 mg/kg bw; 1% aqueous acid 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
mouse	oral	100-110 mg/kg bw; 1% aqueous 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
	percutaneous	898-1,363 mg/kg bw; 45-50% aqueous acid solution 2,314-4,256 mg/kg bw; 25% aqueous acid solution
guinea pig	oral	50 mg/kg bw

Non-lethal concentrations

NEG data

The lungs, liver and kidneys of mice exposed for 24 hour to 33 and 133 mg/m^3 glutaraldehyde were histological examined. At the highest dose level, six out of ten mice showed toxic hepatitis. No remarkable gross changes were observed in lungs or kidneys (Varpela *et al*., 1971). Miner *et al*. (1977) investigated an intra-arterial injection of glutaraldehyde in rats and observed a reversible inhibition of the EEG. Rats that were treated with ^{14}C glutaraldehyde did not show significant changes in liver enzymes (SGPT, SGOT, LDH), however some parameters of the kidney (phenosulfonphtalein-clearance, p–aminohippurate uptake) were affected. Histological examination of the liver did not show abnormalities, kidneys were, however, not examined (Ranly *et al*., 1989). In rats and mice exposed for 4 hour to the evaporation of an alkaline 2% solution of aqueous glutaraldehyde, restlessness and initial body weight loss were found compared to controls (Stonehill *et al*. 1963).

6.2.4 Toxicity due to short-term exposure

Inhalation studies

NEG data

In two-week inhalation experiments, all rats and mice (n=5/sex/group) exposed to 20 and 64 mg/m^3 (5 and 16 ppm) and 6.4, 20, and 64 mg/m^3 (1.6, 5, and 16 ppm), respectively, died before the end of the experiment. The mortality was attributed to respiratory distress. Post-mortem examinations showed lesions of the respiratory tract (minimal to mild squamous metaplasia, hyperplasia, and inflammation or necrosis of the larynx, nasal passages or both) incidences and location depending on the concentration level. The no-observed-adverse-effect-level (NOAEL) was set at 0.64 mg/m^3 (NTP 1993).

In another two-week inhalation study, mice were exposed to 1.2, 4 and 10 mg/m^3 (0.3, 1 and 2.6 ppm). At 10 mg/m^3, 4/10 mice exposed died on the third day of exposure, while the others showed signs of severe toxicity and were killed after 5 days. The breathing frequency, used as an index of sensory irritation (Alarie 1973), was measured and a 50% decrease in respiratory rate (15 min oronasal exposure in 2.8-18 mg/m^3 range) RD_{50} of 10.4 mg/m^3 was found. At 4 mg/m^3, mice showed body weight decrease (20%), marketed excitation by nervously running around, abdominal swelling, rougher hair and looking unhealthier. No signs of systemic toxicity were observed in mice exposed to 1.2 mg/m^3. Histopathological lesions in all exposed mice affected exclusively the respiratory epithelium. Inhalation of 4 mg/m^3 for 14 days caused a marked increase in squamous metaplasia, exudates of keratin strates and inflammatory cells, and

necrosis of the respiratory epithelium in the nasal cavities, which reduced somewhat after two weeks recovery. No concentration related lesions were observed in the lungs of the exposed mice (Zissu *et al*., 1994).

In a thirteen-week inhalation study in rats and mice, with exposure levels between 0.25 and 4 mg/m^3 (0.06 and 1 ppm), mortality was observed in mice exposed to 2 and 4 mg/m^3 (incidences, 2/20 died in week 7-8 and 20/20 died in weeks 1-14 including 8 in week 1, resp). Furthermore, there were decreases in body weight in male rats (4 mg/m^3), female rats (2 and 4 mg/m^3), male mice (0.5, 1 and 2 mg/m^3), and female mice (1 and 2 mg/m^3). Post-mortem examinations did not reveal evidence of systemic toxicity in either of the species, and lesions were limited to the respiratory tract, especially to the anterior nasal passages. The NOAEL was 0.5 mg/m^3 (125 ppb) for rats. For mice, no NOAEL could be established since inflammation in the anterior nasal passages was seen at 0.25 mg/m^3 (62.5 ppb), the lowest exposure level used (NTP 1993, Gross *et al*., 1994).

Additional data

Several short-term (9-12 days) repeated vapour exposure studies conducted in rats and mice, as preliminary to subchronic studies, were shortly mentioned in the review of Ballantyne and Jordan (Bal01b). At vapour concentrations of 8 mg/m^3 and above, mortality was exposure related. The lowest concentration of glutaraldehyde vapour associated with mortality was 2.6 mg/m^3 (one of 20 rats) and all studies showed a steep slope on the vapour concentration-mortality data. Based on these data, the authors suggest a greater degree of lethal toxicity from glutaraldehyde vapour generated at elevated temperature (ca 50^{0}C) than for vapour generated at ambient temperature. Histopathological lesions in the respiratory epithelium were found and there was no evidence for systemic toxicity by histopathology of clinical pathology assessments. Threshold concentrations for effects on nasal mucosa were 1.2 mg/m^3 for rat and 2 mg/m^3 for mice. The no effect concentration was 0.64 mg/m^3 (0.16 ppm) in both rats and mice.

Greenspan *et al.* (Gre85) conducted a subchronic inhalation study in Fischer 344 rats (both sexes; animal numbers not specified) at glutaraldehyde vapour concentrations of 0.08, 0.2 and 0.8 mg/m^3. Exposures were for 6 hours/day, 5 days/week for 14 weeks. Perinasal wetness and statistically significant decreases in body weight were observed at 0.2 and 0.8 mg/m^3, but there was no evidence of respiratory tract inflammation or systemic toxicity.

Halatek *et al*. (Hal03) evaluated effects of glutaraldehyde inhalatory exposure (0.1 mg/m^3 or 0.4 mg/m^3, 6 hours/day, 5 days/week for 28 days) in the lungs of rats (numbers not reported) exposed corresponding to the occupational shift cycle, at time point 24 h, 48 h, and 7 days postexposure (PE). At these time points rats were sacrificed, trachea were cannulated and bronchoalveolar lavage (BAL) was performed. Furthermore, lungs were prepared for electromicroscopical examination. At 24 h PE in 0.4 mg/m^3 exposed

rats, numerous vacuoles and dilated spaces in epithelial cells in bronchioles showing a destructive effect of glutaraldehyde on the cellular membrane were observed. After 48 h PE at the 0.4 mg/m^3 exposure, lipid vacuoles were observed, in the Clara cells of the bronchial epithelium, and in endothelial cells of the alveolar capillaries. According to the authors the lipid vacuoles are probably attributable to disturbed lipid metabolism. Many foci of collagen fibres were observed already after 7 days postexposure. The inflammatory response and repair was monitored using two biomarkers: Clara-cell protein (CC16) and hyaluronic acid. The study showed that the inflammatory repair response contributed to progenitor Clara cells and that hyaluronic acid plays a role in the development of fibrotic changes in the lung of rats. Glutaraldehyde exposure had no effect on total protein content in BAL. This suggest that there was no significant change in epithelia permeability for protein. No significant effects were observed at 0.1 mg/m^3.

Oral studies

NEG data

No adverse effects were found in the nervous system of rats (n=3/group) giving drinking water containing 0.05, 0.1 or 0.25% glutaraldehyde for 11 weeks (Spencer *et al*., 1978).

Dermal studies

NEG data

Werley *et al*. (1996) performed a 26-day epicutaneous application study with glutaraldehyde in F344 rats. The reported effects (local skin irritation, minimal erythema and oedema) were minor and are common findings in rodents receiving cutaneous applications of irritant materials (Her95). Also no evidence of systemic toxicity was observed when 0.5 ml of a 2% alkaline glutaraldehyde solution was daily applied for 6 weeks on the clipped skin of albino rabbits (Stonehill *et al*., 1963).

6.2.5 *Toxicity due to long-term exposure and carcinogenicity*

Inhalation studies

Additional data

The National Toxicology Program (NTP) has conducted a well-performed toxicology and carcinogenesis inhalation study (Bir00, NTP99). Male and female F344/N rats and B6C3F$_1$ mice (n=50/sex/group/species) were whole-body exposed to (activated GA) vapour* concentrations of 0, 1, 2, or 3 mg/m^3 (0, 0.25, 0.5, or 0.75 ppm) and 0, 0.25, 0.5, or 1 mg/m^3 (0.0625, 0.125, or 0.250 ppm), respectively, for 6 hours/day, 5 days/week,

for 104 weeks. The 13-week inhalation study preceding this 2-year study is described in part 2 of this report (Gross *et al*., 1994, NTP, 1993). Animals were observed twice daily. Until the end of the studies, body weight and clinical observations were recorded every 4 weeks through week 89, and every 2 weeks from week 92 (rats) or 93 (mice). A complete necroscopy of all organs and tissues, and microscopic examination on all major tissues were performed on all rats and mice.

Mean body weights of all exposed male rats, female rats exposed to 2 and 3 mg/m^3 and female mice exposed to 1 mg/m^3 were generally less than those of the controls throughout the study. Only in the female rat groups exposed to 2 and 3 mg/m^3, survival was significantly decreased compared with controls. No clinical findings were attributed to glutaraldehyde exposure, except in male (8/50) and female (5/50) rats exposed to 3 mg/m^3, which were having breathing problems. These breathing problems were likely related to nasal lesions, and animals were removed from the study in a moribund condition between weeks 13 and 21. No inhalation exposure-related neoplastic lesions were observed in either rats or mice. However, exposure to glutaraldehyde resulted in considerable non-neoplastic lesions in the noses of rats and mice, as shown in Table 6.3 and 6.4. Data from Table 6.3 show that at the lowest dose given to both male and female rats (1 mg/m^3), significantly more animals developed hyperplasia and inflammation in the squamous epithelium compared with controls. At dose levels of 2 and 3 mg/m^3, also a significantly increased number of rats developed respiratory and olfactory epithelium effects compared with control groups. Concerning mice, an increased number of male mice, exposed to 1 mg/m^3, developed squamous metaplasia in the respiratory epithelium compared with the control group (see Table 6.4). This was the only statistical significant effect found in male mice. In female mice, however, more effects were observed. A, non dose-related, statistically significant increased incidence of hyaline degeneration of the respiratory epithelium of the nose in female mice was observed in all dose groups (0.25, 0.5 and 1.0 mg/m^3). Furthermore, statistically significant increased incidences of squamous metaplasia in the respiratory epithelium (0.5 and 1.0 mg/m^3) and inflammation (1 mg/m^3) were observed in female mice groups. Increased incidences of squamous metaplasia already started at 0.25 mg/m^3. The severity is minimal and hardly different from the control group. Although most effects showed a dose-response relationship, the authors did not report on the statistical analyses.

* The vapour was generated by heating glutaraldehyde resulting in activated glutaraldehyde.

Table 6.3 Incidences and severity of non-neoplastic nasal lesions of the nose in F344/N rats, exposed to glutaraldehyde for two years (Bir00, NTP99).

	males				females			
concentration mg/m^3	0	1	2	3	0	1	2	3
numbers examined	50	50	50	50	50	50	50	49
squamous epithelium								
hyperplasia	3 (2.0[a])	11*(1.6)	39**(2.2)	48**(2.9)	3 (1.3)	15**(1.7)	29** (2.0)	45** (2.7)
inflammation	6 (2.0)	17*(1.5)	41**(2.7)	49**(3.6)	6 (2.5)	26**(1.5)	42** (2.1)	48** (3.2)
respiratory epithelium								
hyperplasia	6 (2.0)	5 (2.0)	17**(1.9)	35**(1.9)	1 (3.0)	6 (1.7)	15** (1.9)	29** (1.9)
inflammation	17 (2.1)	10*(1.5)	25 (2.4)	43**(3.2)	5 (2.2)	9 (1.7)	26** (2.1)	42** (2.5)
squamous metaplasia	1 (2.0)	2 (1.5)	11**(2.0)	24**(2.2)	1 (2.0)	1 (3.0)	11** (1.6)	16** (2.3)
goblet cell hyperplasia	1 (1.0)	0	6 (1.8)	6* (1.2)	1 (2.0)	3 (1.3)	5 (1.4)	8** (1.6)
olfactory epithelium								
hyaline degeneration	4 (1.0)	8 (1.3)	9 (1.1)	14**(1.1)	4 (1.0)	5 (1.0)	12* (1.1)	15** (1.1)

Significantly different (*, $p \leq 0.05$;**, $p \leq 0.01$) from control group.
Microscopical examination of rats removed from study in moribund condition, were also included
[a] Average severity grade of lesions in affected animals: 1 = minimal, 2= mild, 3= moderate, 4= marked

Table 6.4 Incidences and severity of non-neoplastic nasal lesions of the nose in B6C3F$_1$ mice, exposed to glutaraldehyde for two years (Bir00, NTP99).

	males				females			
concentration mg/m^3	0	0.25	0.50	1.0	0	0.25	0.50	1.0
numbers examined	48	50	50	50	50	49	50	50
respiratory epithelium								
squamous metaplasia	2 (1.0)[a]	5 (1.0)	6 (1.2)	9* (1.1)	7 (1.1)	11 (1.0)	16* (1.3)	21**(1.5)
hyaline degeneration	-[b]	-	-	-	16 (1.4)	35**(1.4)	32**(1.3)	30* (1.1)
turbinate necrosis	0	0	2 (2.0)	0	0	3 (2.0)	1 (1.0)	4 (1.5)
inflammation	6(1.1)	4(1.0)	3(1.3)	5(1.0)	6 (1.2)	7 (1.3)	13 (1.4)	14* (1.4)

significantly different (*, $p \leq 0.05$; **, $p < 0.01$) from control group.
[a] Average severity grade of lesions in affected animals: 1 = minimal, 2= mild, 3= moderate, 4= marked
[b] No data present

In a separate study, B6C3F$_1$ mice were whole-body exposed to 0 and 0.4 mg/m^3 (0.1 ppm), 6 hours/day, 5 days/week, for 52 (n=50/sex/group) or 78 (n=30/sex/group) weeks. Treatment did not affect mortality rates. In the animals exposed for 78 weeks, statistically significant decreases were observed in the body weights of the female animals,

while increases were observed in the males (no data were presented on the 52-week group). In neither group, effects indicative of nasal (discharge, swelling, purities) or respiratory (dyspnoea) irritation were observed at the weekly observations. There were no treatment-related increases in incidences of any tumour. As in the former study, only histological changes in the nasal passages were found. These lesions were limited to the vestibule and included hyperplasia of the squamous epithelium lining of the dorsal wall and the lateral aspect of atrioturbinate together, with necrosis and exfoliation of epithelial cells and granulocytes in the lumen. These vestibular alterations were observed in female animals only, and increased in incidence (52 weeks: 15/48 versus 2/49 controls; 78 weeks: 14/28 versus 6/28 controls) and severity with increased exposure duration. Increase in foam cells or alveolar macrophages as well as focal or diffuse interstitial fibrosis were observed in the lungs of 95% of control and exposed rats (Zis98).

Oral studies

Additional data

Drinking water studies have been conducted in F344 rats, CD-1 mice and Beagle dogs (animal numbers not specified), with dosing up to 3 months. Actual daily dosages used were around 80-120 mg/kg bw/day for rats and mice and around 20-30 mg/kg bw/day for dogs. The major findings in all species were decreased food and water consumption (probably related to an aversion to the taste and/or irritation of glutaraldehyde), decreased body weight and decreased urine volume with increased specific gravity. There was no clinical, haematological, biochemical or morphological evidence for target organ or tissue systemic toxicity in any species. According to the authors, all the findings were compatible with decreased urine output secondary to decreased water consumption from an aversion to glutaraldehyde in the drinking water (Her96).

Van Miller *et al.* (Mil02), published a two-year carcinogenicity drinking water study, which was only briefly mentioned in the NEG data (CIR 1996, part 2 of this report). In this study, F344 rats (n=100/sex/group) were given mean (actual) amounts of approximately 4, 17, or 64 (males) and 6, 25, or 86 (females) mg/kg bw/day, 7 days/week, for 104 weeks. Treatment did not affect survival rates. In the mid- and high-dose groups, decreases in body weight, body weight gain, and food consumption were observed throughout the study, while there was a decrease in water consumption in all groups. Haematology and clinical chemistry parameters were not affected. Gross and histological evidence for gastric irritation (thickening of the stomach wall; ulceration of the mucosa) was observed in many of the animals of the mid- and high-dose groups euthanised at 52 weeks (30%), 78 weeks (10-20%), 104 weeks (10%) and in animals that had died during the study (40%). Upon microscopic examination at week 104, an increased incidence of mucosal hyperplasia was seen in the animals of the high-dose

group (males: low, 1/52; mid, 1/51; high, 7/51; controls, 1/56; females: low, 0/47; mid, 1/52; high, 7/56; controls, 1/62). As to neoplastic lesions, a high incidence of large granular lymphocyte leukaemia (LGLL) in liver and spleen was found in all dose groups, including the controls (males: low, 51%; mid, 40%; high, 46%; controls, 43%; females: low, 41%; mid, 41%; high, 54%; controls, 23%). However, the toxicological significance of this finding was questioned by the authors in view of the known high spontaneous incidence rate of this tumour in this rat strain, the rather low incidence in the female control group, and a lack of a clear dose-response relationship.

The lack of a dose-response relationship was supported by a pathological working group who reinvestigated the histological tissues from spleen, liver and lungs of females rats (Hardisty *et al* 2003, unpublished study report evaluated by DFG 2004).

To investigate the spontaneous incidence rate of the LGLL tumours in F344 rats, Kasper *et al* (Kas03, unpublished study report evaluated by DFG 2004) performed a two-year carcinogenicity drinking water study using the same dose levels and experimental design in Wistar rats. No treatment related increases in incidences of any tumour were observed. Moreover, no large granular lymphocyte leukaemia (LGLL) were found. Based on this study, DGF concluded that the high incidence of LGLL observed in the study of van Miller *et al* (Mil02) was much more likely due to a high spontaneous incidence rate of this tumour in F344 rats.

6.2.6 *Genotoxicity*

In vitro data

NEG data

Glutaraldehyde is 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 (Galloway *et al*., 1987; Haworth *et al*., 1983; Levin *et al*., 1982; Marnett *et al*., 1985; McGregor *et al*., 1988; NTP, 1993; St Clair *et al*., 1991).

Additional data

Vock *et al*. (Voc99) investigated the time-dependent dose-response relationships for the induction of DNA double-strand breaks and for viability of a number of aldehydes and di-epoxides in cultured human lung epithelial cells (A549 commercial celline) in order to discriminate between genotoxic and cytotoxic mechanisms of DNA fragmentation. Glutaraldehyde (100 µM) was added to exponentially growing cell monolayers and cells were harvested 8, 24 and 72 hr after treatment initiation. Glutaraldehyde was found to induce double-strand breaks. However, this occurred only after cell viability was

reduced to less than 60% of control values, and the authors concluded that these breaks were not caused by genotoxic mechanisms, but were due to extragenomic damage and loss of viability.

As a bifunctional aldehyde, glutaraldehyde exhibits DNA reactive genotoxic activity that may involve, at least in part, DNA protein cross-linking in cell cultures (StC91). However, in human leukaemia cells (HL60), glutaraldehyde (100 µM) does not show DNA protein cross-linking (Sch00). In the comet-assay with a primary culture of human leucocytes, reduction of DNA-migration together with a decrease in Spot diameter was recorded > 250 µM glutaraldehyde. According to the authors, this might be caused by the known DNA damaging and cross-linking properties of glutaraldehyde (Fre00).

Mutagenic activity occurred in *Salmonella typhimurium* strain TA100, TA102, TA1535/PSK1002, BA9, BA13 and *Escherichia coli* strain WP2uvrA, independent of metabolic activity (Hem80, Hud88, Jun92, Kos82, Rui85, Sak88, Ver02, Wil90). Mammalian cellines have given variable results. With Chinese hamster ovary (CHO) cells, the results depend on the gene locus, with no activity being demonstrated at the HGPRT locus and weak activity at the thymidine kinase (TK) locus (Ver02). Increases in sister chromatid exchanges generally do not occur, and chromosomal aberration tests *in vitro* vary from no activity to weak or equivocal results (Gal85).

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 *in vitro* did not require S9 activation.

In vivo data

NEG data

Glutaraldehyde is not considered genotoxic in vivo, based on the absence of dose-related increases in micronuclei or chromosomal aberrations in mice and rats respectively (Vergnes *et al*., 1993). Furthermore, glutaraldehyde did not show any genotoxic activity in Drosophila tests (NTP 1993, Yoon *et al*., 1985; Zimmering *et al*., 1989).

Additional data

Glutaraldehyde has been tested for its potential to induce chromosomal aberrations in bone marrow, following intraperitoneal injection into male $B6C3F_1$ mice (n=10/group). Fifty first-division metaphase cells were scored from each of eight animals per treatment. Responses were evaluated as the percentage of aberrant cells, excluding gaps. A dose range-finding study was included, but data on general or bone marrow toxicity

were not given. In the 1st trial, with single doses of 0, 15, 30, or 60 mg/kg bw and a harvest time of 17 hours, there was no significant increase in the number of aberrant cells. The 2nd trial, with doses of 0, 15, 30, 50, or 60 mg/kg bw and a harvest time of 36 hours, showed dose-related increases in the percentage of aberrant cells, which were statistically significant at the two highest doses when pair-wised compared to controls. Testing by a one-tailed trend test showed significance as well. In the 3rd trial with the same dose range and a harvest time of 36 hours, an increase in chromosomal aberrations was found at the highest dose only (p=0.004) (NTP99).

The potential to induce micronuclei has been investigated as well. In a subset of the $B6C3F_1$ mice (n=5), treated in the above-mentioned second trial, small increases in the frequency of micronuclei were found in the bone marrow polychromatic erythrocytes. However, these increase were not dose-related and did not reach statistical significance by pair-wise comparison of treated group to solvent control group or by a one-tailed trend test. In two additional trials, in which male $B6C3F_1$ mice (n=5/group) were given three intraperitoneal injections of 5, 10, or 20 mg/kg bw each at 24-hour intervals, no statistically significant increases in the frequency of micronuclei was observed in either of the trials. Animals were killed 24 hours after the final injection, and 2,000 polychromatic erythrocytes per animal were scored. Data on general or bone marrow toxicity were not presented. Finally, there were no significant increases in the frequency of micronuclei in normochromatic peripheral blood erythrocytes of female mice following a thirteen-week inhalation exposure up to 2 mg/m^3 (0.5 ppm) (NTP99). The same laboratory reporting the positive results following intraperitoneal injection of glutaraldehyde in mice reported it negative for micronuclei induction (MN) in the same tissue, using the same doses and equivalent sampling times. Furthermore, other acute and subacute NTP studies that evaluated MN at the same laboratory using the same doses and similar dosing regimes and sampling times failed to reproduce the positive finding (NTP99). The weight of evidence supports the spurious nature of the single finding of chromosomal aberrations after intraperitoneal injection of glutaraldehyde.

Other data on the induction of micronuclei (mice) or chromosomal aberrations (rats) were based on further studies of Vergnes and Ballantyne (Ver02). In a standard OECD mouse peripheral blood micronucleus test, groups of Swiss Webster mice (n=5/sex/group for mid and low dose and n=8/sex/group for high dose) were given aqueous glutaraldehyde solution by gavage dose of glutaraldehyde at 40, 80 and 125 mg/kg bw (corresponding to 25, 50 and 85% of the LD50). At 30, 48 and 72 hour after dosing peripheral blood samples were collected and analysed. No increases in micronucleated polychromatophils were observed. In a standard bone marrow cytogenetic study, CD rats (n=15/sex/group) received a single dose of glutaraldehyde: 12.5, 30 or 60 mg/kg bw for males and 7.5, 20 or 40 mg/kg bw for females. Sampling at 12, 24 and 48 h after dosing

revealed no increase in chromosomal aberrations, indicating the absence of an *in vivo* clastogenic potential. HSE (HSE97) reviewed this study and concluded that, based on changes in mitotic indices observed at some time points in the chromosomal aberration rat study, at least some glutaraldehyde or a metabolite had reached the bone marrow.

No differences in Unscheduled DNA Synthesis (UDS), assessed by radiography in hepatocytes, were found between controls and male F344 rats, given single oral (gavage) doses of 30, 150, or 600 mg/kg bw, at 2 and 12 hour after treatment (Mir89).

In a dominant lethal assay the genotoxicity of glutaraldehyde in germ cells was tested. The authors indicate that single oral doses of glutaraldehyde (30 or 60 mg/kg bw) administered to male mice did not cause a dominant lethal effect. The positive control substance produced appropriate responses, but the significance of the negative results for glutaraldehyde is uncertain, given that it is not known whether any glutaraldehyde reached the target tissue (the testis) in this assay (Tam78).

Overall, the clearly negative results of recent, good quality bone marrow cytogenetics and peripheral micronucleus tests together with those of the liver UDS and dominant lethal assay, provide assurance that the genotoxic effects shown by glutaraldehyde *in vitro* are unlikely to be expressed *in vivo*. According to Ballantyne and Jordan (Bal01b), the absence of genotoxic effects *in vivo* may be partly related to the rapid metabolism and protein characteristics of glutaraldehyde.

6.2.7 *Reproductive toxicity*

NEG data

There were no effects on fertility or embryonic/foetal viability in a dominant lethal assay in mice (Tamada *et al.,* 1978). Rats given subcutaneous doses up to 125 mg/kg bw/day for 35 days showed gonadal effects in both sexes at the two highest dose levels (Uemitsu *et al*., 1976). In teratogenicity studies in mice and rats given oral doses up to 100 mg/kg bw/day no developmental effects other than those accompanied by maternal toxicity were found (Ema *et al*., 1992; Marks *et al*., 1980).

Additional data

In the thirteen-week NTP inhalation study (for details see section 6.2.5) with exposure concentrations of up to 2 or 4 mg/m^3, no effects were observed on male rats and mice testis, epididymis, and caudal epididymis weights, spermatid counts, spermatid head counts, or spermatozoal motility. In female rats, there was no effect on the length of the oestrus cycle or on the length of the several stages as a proportion of the whole cycle. Female mice exposed to 1 or 2 mg/m^3, showed longer oestrus and dioestrus cycles and

shorter metoestrus and proestrus cycles when compared to controls. No such effects were observed in animals exposed to 0.25 mg/m^3 (NTP93, Gro94).

In the two-year inhalation study (for details see section 6.2.5), no histological changes were reported in the reproductive organs of male and female rats and mice exposed to concentrations up to 3 or 1 mg/m^3, respectively (NTP99, Bir00).

The two-generation reproduction drinking water study using rats, very briefly discussed in the NEG data (CIR 1996; part 2 of this report), has been published in the open literature by Neeper-Bradley and Ballantyne (Nee00). Adult male and female CD rats (n=28/sex/group) were given glutaraldehyde in drinking water at concentrations of 50, 250 or 1000 ppm for a 10-week breeding period and through mating, gestation and lactation. The actual daily doses were approximately 4, 17.5, or 69 mg/kg bw (males, F_0) and of approximately 7, 28, or 98 mg/kg bw (females, F_0). The F_1-offspring, selected one week after weaning of the F_1 pups to be parents (n=28/sex/group) for the F_2 generation, received the same concentrations in drinking water (actual daily doses of approximately 4.5, 23, or 71 mg/kg bw for males and approximately 7, 30, or 100 mg/kg bw for females) from the prebreed, breeding, gestational, and lactation periods to weaning. Parental observations included regular clinical examinations, body weight measurements, and food and water consumption recordings. Parental animals were necropsied; reproductive tissues and any gross lesions were examined on histological changes. Pups were treated in a similar way. Reproductive endpoints examined concerned mating, fecundity, fertility, gestation, live birth, and pup survival. Treatment did not result in mortality or clinical signs of toxic and/or pharmacological effects, or in histological changes. No effects were observed on parental fertility and mating performance, on gestation length, on litter size and viability, or on pup survival. The NOAELs of 50 ppm (4-7 mg/kg bw/day) and 250 ppm (23-30 mg/kg bw/day) for adult and offspring toxicity, respectively, were based on reduced water consumption and occasionally slightly decreased body weights at the next higher dose levels.

Citing unpublished industrial reports, Ballantyne and Jordan (Bal01b) presented additional data on developmental toxicity in rats and rabbits.

No effects indicative of maternal or developmental toxicity were found in pregnant Wistar rats (n=25/group) following administration of glutaraldehyde in the drinking water at approximately 0, 5, 26, or 68 mg/kg bw/day, on gestational days (GD) 6-16. Effects on dams and foetuses were assessed at GD 20. The only effect observed was a decrease in water consumption at the two higher dose levels. (Bal01b).

Pregnant Himalayan rabbits (n=15/group) were orally (gavage) given daily doses of 0, 5, 15, or 45 mg/kg bw, on GD 7-19. Evaluating the effects on dams and foetuses on GD 29, no adverse effects were seen at the lower two doses. At 45 mg/kg bw, there were mortality (5/15 animals) and severe body weight loss and diarrhoea. Post implantation loss was greatly increased, due to massive increase in resorption rate, with 9/10 survi-

ving dams not producing viable foetuses. The remaining animal had four live foetuses with significantly reduced body weights, but without malformations (Bal01b).

Citing an English translation of a Russian study, HSE reported a small increase in incidences in foetal abnormalities following exposure of rats (n=20, strain not given) to oral (gavage) doses of 0.03 or 14 mg/kg bw/day, on GD 1-19 (evaluations at GD 20). At 14 mg/kg bw/day, one out of 181 foetuses had encephaly and another microencephaly. In addition, there was a statistically significant increase in haemorrhages, but it was not clear whether they were internal or external. At the low dose, microencephaly and brain rupture were seen in two and one out of 176 foetuses, respectively. No foetal abnormalities were observed in the animals of the control group. There were no data on (any) maternal toxicity (HSE97).

6.3 Other relevant studies

NEG data

The cytotoxicity of glutaraldehyde was studied in various in vitro cell systems. Cytotoxicity was observed at media concentrations of glutaraldehyde greater than 1.2 µg/mL (human fibroblasts; Jeng *et al*., 1987), 3 ppm (3T3 fibroblast; Speer *et al*., 1980) 0.1 µg/mL (bovine endothelial cells; Eybl *et al*., 1989) or 0.01 µl/mL (pig skin fibroblast; Cooke *et al*., 1983).

6.4 Summary

In the workplace, humans may be exposed to acid or alkaline, so called activated, glutaraldehyde solutions and to vapours derived from these solutions.

General toxicity: human data

Several case and epidemiological studies on glutaraldehyde toxicity have been performed. Almost all studies reported effects after short-term or peak exposure. These effects include irritation (of the skin, the eyes, the nose and the throat), sensitisation (of the skin and respiratory tract) and asthmatic symptoms, such as wheezing, coughing, chest tightness, breathing difficulties, and non-specific bronchial hyperresponsiveness. Below, details on the irritational effects and on sensitisation are given.

On irritation, four adequate epidemiological studies on occupational exposure and a volunteer study were carried out (Cai03, Nor88, Pis94/Pis97, Vya00, Wat03). In the epidemiological studies of Norbäck (Nor88) and Vyas *et al*. (Vya00), health-care workers in endoscopy and cold sterilisation were followed. In both studies, work-related irritational

symptoms of the eye, skin, nose, throat and lower respiratory tract could be related to exposure levels with geometric means of 0.05-0.06 mg/m^3 (ranges <0.001-1.08 mg/m^3). Symptoms of the eye, nose and throat are most commonly reported, and probably caused by sensory irritative effects of the glutaraldehyde vapour. Vyas *et al.* (Vya00) further recorded a significant relationship between peak glutaraldehyde concentrations and nasal irritation, whereas Norbäck (Nor88) found a significant relationship between the exposure frequency (days of exposure) and the number of symptoms.

In addition, Pisaniello *et al*. (Pis94/Pis97) found a significant relationship between exposure frequency (hours exposure/week) and the number of symptoms (i.e. 'any eye' irritation) after exposure of health-care workers to 0.11-0.18 mg/m^3 (GM). Furthermore, irritation of the eyes, throat and skin were significantly increased in exposed workers.

In a cross-sectional study of Waters *et al.* (Wat03), no significant increase in work-related symptoms of the eyes, nose and throat was recorded after health-care workers were exposed to high glutaraldehyde levels of 0.6 mg/m^3 for short peak intervals. Both the exposed and unexposed groups reported a high prevalence of irritational symptoms. In this study an alternative method (direct reading Glutaraldemeter) for exposure measurements was used, which enables peak exposure measurements. The authors further described the work practices in endoscopy or cold sterilisation units, which are such that in 15 minutes *several* (peak) exposure events (ranges between 5-735 sec) are likely to occur.

In a clinical study of Cain *et al*. (Cai03), non-smoking female volunteers were exposed to several, constant (no peaks), glutaraldehyde concentrations. In the first experiment of this study, females were exposed for 2-25 sec by a vapour delivery device at several, constant glutaraldehyde concentrations. Odour was detected at low exposure concentrations of 0.2-20 µg/m^3, whereas nose and eye irritation were detected at much higher exposure levels (0.9-3 mg/m^3). Since all volunteers detected odour at about 20 µg/m^3, odour-interference did not affect further experiments at higher dose levels and longer exposure duration. A steep dose-response relationship for irritation was found: 15-18% of the volunteers reported eye/nose irritation at 0.9 mg/m^3, whereas most volunteers (71-84%) reported eye/nose irritation at 3 mg/m^3. In a second experiment, the effect of duration of exposure to glutaraldehyde was tested among female volunteers. After 15 min exposure to several glutaraldehyde concentrations, no irritation of eye, nose or throat was observed at 0.4 mg/m^3, the highest concentration tested, when compared to the control group.

Indications for both skin and respiratory tract sensitisation have been reported. Concerning sensitisation of the skin, several case reports on occupational allergic contact dermatitis were published. Furthermore, positive patch test results from health-care workers using glutaraldehyde were reported. Concerning sensitisation of the respiratory tract that may result in occupational asthma, data are not conclusive. Specific immuno-

logical tests carried out demonstrated specific IgE levels in about 25% of the patients with occupational asthma (Cur96, DiS99). The authors of these studies stated that glutaraldehyde might behave like many other low-molecular-weight chemicals, in that there is a poor correlation between specific IgE antibody and clinical symptoms of occupational asthma. Another study reported increased concentrations of immunological cells (eosinophil number and percentage, albumin, eosinophil cationic protein and mast cell tryptinase) in nasal lavage fluids of workers with occupational asthma (Pal01). Clinical evidence that glutaraldehyde may be a respiratory sensitizer comes from several case studies and one epidemiological study in which workplace challenge or laboratory provocation tests were performed. Challenges revealed changes in FEV_1, peak expiratory flow, non-specific bronchial responsiveness, nasal airway resistance, as well as late and dual asthmatic reactions in several but not all patients (Ben84, Chan-Yeung *et al*., 1993, *Cornacoff et al*., 1986, Cullinan *et al*., 1992, DiS99, Gan95, Nic86, Qui99, Stenton *et al*., 94). Many of the positive responses occurred in patients having an asthmatic history. Glutaraldehyde measurements were performed in a limited number of studies: workplace exposure levels reported were about 0.21 mg/m^3 (range 0.06-0.82 mg/m^3), and challenge levels ranging between 0.065-0.40 mg/m^3. A dose-response relationship was not established. Cross-sensitisation between glyoxal and glutaraldehyde has been observed in a human study.

There are no adequate long-term human studies available.

General toxicity: animal studies

Data from experiments in animals support the findings in humans, in that acute exposure to glutaraldehyde vapour produced sensory irritant effects to the eyes and upper respiratory tract. Furthermore, studies in rabbits, guinea pigs and mice with glutaraldehyde solution confirmed the eye and skin irritating and skin sensitising potential. Animal studies also showed a steep dose-response relationship where sensory irritation was followed by severe respiratory distress and death after exposure to 4-10 mg/m^3 for some days. Cross-sensitisation between glyoxal, formaldehyde and glutaraldehyde was observed in guinea pigs. Animal (and human) studies, in which evidence on respiratory sensitisation could have been obtained, were not conclusive. These included slightly increased IgE levels in mice, selective Th2-type cytokine secretion patterns in some but not all mice studies as well as the absence of changes in respiratory rate in a guinea pig sensitisation study.

Concerning short-term exposure, several inhalation studies in rats and mice have been performed. All studies demonstrated exposure-related lesions in the upper respiratory

tract. The threshold levels obtained for upper respiratory tract lesions in the 13 week studies were 1 mg/m^3 in rats and 0.25 mg/m^3 in mice. These lesions included necrosis, inflammation and squamous metaplasia of the epithelium of the nose. Histopathological or clinical pathological assessment did not show evidence for any systemic toxicity. Concerning long-term exposure, a well-performed NTP animal study was carried out. In this study, rats and mice were whole-body exposed to 0, 1, 2 or 3 mg/m^3 (rats) and 0, 0.25, 0.5 or 1 mg/m^3 (mice) glutaraldehyde vapour for 104 weeks (6h/day, 5d/week). Exposure resulted in considerable non-neoplastic lesions in the noses of both species resembling those in the short-term studies. For rats, these included nasal lesions on the squamous epithelium (hyperplasia and inflammation), respiratory epithelium (hyperplasia, inflammation, squamous metaplasia and goblet cell hyperplasia) and olfactory epithelium (hyaline degeneration). Non-neoplastic nasal lesions in mice included the respiratory epithelium (squamous metaplasia and hyaline degeneration), turbinate necrosis and inflammation. At 0.25 mg/m^3, the lowest dose level tested, a statistically significant increased incidence of hyaline degeneration of the respiratory epithelium of the nose in female mice was observed. This finding was, however, not dose-related. Furthermore, an increase in the incidence of squamous metaplasia in the respiratory epithelium of the nose in female mice was observed, which became statistically significant at 0.50 mg/m^3 (Bir00). The severity of squamous metaplasia, at 0.25 mg/m^3, however, was minimal. Another long-term inhalation study in mice (78 weeks, 6h/day, 5d/week) showed comparable effects at 0.4 mg/m^3, the only dose level tested.

Carcinogenicity and genotoxicity

No increased incidence of malignant tumours or in mortality was found in 186 workers exposed to 0.24 mg/m^3 (0.8 ppm) glutaraldehyde for 4-6 years in a glutaraldehyde-producing plant. Due to the small number of workers, on which the mortality analysis was conducted, their young age (mean 21 years) and the relatively short follow-up period (10-34 years), the committee considered these results of limited value. In addition, no evidence of a carcinogenic potential was found in the 2-year whole-body inhalation exposure study in rats and mice described above. The high incidence of large granular lymphocyte leukaemia observed in female F344 rats in a 2-year oral carcinogenicity study, was not considered toxicologically significant in view of the known high spontaneous incidence rate of this tumour in this specific rat strain and the lack of a dose-response relationship.

Additional *in vitro* genotoxicity studies (bacterial mutagenicity, forward gene mutation, sister chromatide exchange, chromosome aberration and DNA repair) have shown variable results ranging from no effects through positive effects. *In vivo* data (micronucleus, chromosome aberration, UDS, dominant lethal, and *Drosophila*) generally have

shown no activity. Inhalation of glutaraldehyde will result in local reactions in the nasal epithelium, and not into significant systemic exposure.

Reproductive toxicity

No increased risk of spontaneous abortions and foetal malformations was found in Finnish hospital nurses and staff, using glutaraldehyde as a sterilising agent.

Concerning animals, female mice exposed to 1-2 mg/m^3, showed longer oestrus and dioestrus cycles and shorter metoestrus and proestrus cycles when compared to controls. No such effects were observed in animals exposed to 0.25 mg/m^3. Developmental studies in mice, rats and rabbits showed that glutaraldehyde was not teratogenic. In a two-generation reproduction drinking water study, rats did not show adverse reproductive effects. Finally, in a two-year whole-body inhalation exposure study in rats and mice, no histological changes were reported in the reproductive organs.

Chapter 7

Existing guidelines, standards and evaluations

7.1 Working population

The existing occupational exposure limits of glutaraldehyde are presented in the tabel below.

In the Netherlands, a ceiling value of 0.25 mg/m^3, is presently being used as administrative force for glutaraldehyde (SZW04). The current ACGIH threshold limit value (TLV) for glutaraldehyde is 0.20 mg/m^3 (0.05 ppm), as a ceiling value, with an A4 designation indicating that glutaraldehyde is not classifiable as a human carcinogen. HSE (HSE03) established maximum exposure limits (MELs) of 0.20 mg/m^3 (0.05 ppm; TWA 8-hour) and 0.20 mg/m^3 (0.05 ppm; STEL). In Germany, a momentary value of 0.8 mg/m^3 (0.2 ppm) was set as a peak limitation, a value that should not be exceeded at any time, and a TWA value of 0.2 mg/m^3 (0.05 ppm; DFG03). In all these cases, glutaraldehyde was "labelled" as a sensitizer (ACG03, DFG03, HSE03).

tabel 7.1 Existing occupational exposure limits.

Country -organisation	OEL mg/m^3	OEL ppm	average time	type of OEL	note[a]	year of adoption[b]	ref[c]
The Netherlands							
- Ministery	0.25	1	Ceiling	-	administrative	-	SZW04
Germany							
- DFG	0.2	0.05	8 h	MAK	3B; C; Sah	2002	DFG03
	0.8	0.2	[d]	Peak limitation[e]			
-AGS	0.4	0.1	-	Peak limitation	C		AGS03
Norway	0.8	0.2	Ceiling	-	nonactivated	-	Dir94
	0.25	-			activated		
Sweden	0.8	0.2	Ceiling	-	Sens	1990	SNB00
Denmark	0.8	0.2	Ceiling	-	-	-	Arb02
Finland	0.42	0.1	15 min	-	-	-	Sos00
United Kingdom							
- HSE	0.2	0.05	8 h	MEL	Sens	1997	HSE03
	0.2	0.05	15 min	MEL			
USA							
- ACGIH	0.2	0.05	Ceiling		Sens, A4	-	ACG03
- NIOSH	0.8	0.2	Ceiling	-	-	-	ACG03

[a] Sens = substance can cause sensitisation by dermal contact and/or inhalation exposure, based on weight of scientific evidence
Sah= substance can cause sensitisation by dermal contact and inhalation exposure
A4 not classifiable as human carcinogen due to lack of data
3B Substance for which *in vitro* tests or animal testing have yielded evidence for carcinogenic effects that is not sufficient for classification of the substance in one of the other categories
C No reason for a risk or damage to developing embryo or foetus when MAK and BAT values are observed

[b] Year that this limit was officially adopted

[c] Reference to the most recent official publication of occupational exposure limits

[d] A momentary value (concentration which should not be exceeded at any time) of 0.83 mg/m^3 was established

[e] Max 4/shifts with 1-h interval

Chapter 8

Hazard assessment

8.1 Assessment of health risks

Introduction

In the workplace, humans may be exposed to acid or alkaline, so called activated, glutaraldehyde solutions and to vapours derived from these solutions. Based on the physico-chemical properties of glutaraldehyde*, the committee concludes that all forms of glutaraldehyde that enter the body will be activated. Hence, the health-based-occupational exposure limit(s) derived from studies with exposure to inactive glutaraldehyde (vapour) will also protect against effects of exposure to activated (alkaline) glutaraldehyde (vapour).

Effects after short-term exposure

In table 8.1, details of the most relevant human studies on irritational symptoms are given.

In the epidemiological studies of Norbäck (Nor88) and Vyas *et al*. (Vya00), healthcare workers in endoscopy and cold sterilisation were followed. In both studies, work-

* The pH of the body is comparable to the pH of the activated glutaraldehyde (pH 7.5-8.5). Therefore, the committee assumes that inactive (acid) glutaraldehyde that enters the body will be primarily present in its activated form at the pH of the body.

related irritation of the eye, skin, nose, throat and lower respiratory tract could be related to exposure levels with geometric means of 0.05-0.06 mg/m^3 (ranges <0.001-1.08 mg/m^3). Symptoms of the eye, nose and throat are most commonly reported, and probably caused by sensory irritative effects of the glutaraldehyde vapour. Vyas *et al.* (Vya00) further recorded a significant relationship between peak glutaraldehyde concentrations and nasal irritation, whereas Norbäck (Nor88) found a significant relationship between the exposure frequency (days of exposure) and the number of symptoms.

In addition, Pisaniello *et al*. (Pis94/Pis97) found a significant relationship for exposure frequency (hours exposure/week) and the number of symptoms (i.e. 'any eye' irritation) after exposure of health-care workers to 0.11-0.18 mg/m^3 (GM). Furthermore, irritation of the eyes, throat and skin were significantly increased in exposed workers.

Table 8.1 Irritational symptoms reported in humans, who have been occupationally exposed to glutaraldehyde (GA).

Ref	Exposed subjects	Sampling method/ duration	Airborne concentrations mg/m^3	Reported symptoms (type of symptom)
Nor88	Cold sterilization hospital workers: n=39, exposed; n=68, unexposed	Personal (15 min) sampling during short-term (15 min) exposure (~OSHA64)	GM[a]: 0.05 range <0.01-0.57 background:<0.04	Nose (28%: catarrh and obstruction) throat (26%:smarting) and skin (40%: eczema, rashes) symptoms, headache (36%) and nausea (13%) were significantly increased in exposed workers. Significant relation between exposure frequency (days of exposure) and number of symptoms (p<0.01).
Vya00	Endoscopy nurses: n=318, exposed; n=18, ex-workers No control	Personal short-term sampling during GA changeover[b] (OSHA64)	GM: 0.06 range:<0.001-1.08 background: 0.01	Thirty percent of exposed workers showed work related symptoms of the eyes (13.5%:irritation), nose (19.8%:irritation) and lower respiratory tract (8.5%: persistent cough, chronic bronchitis, wheeze, shortness of breath, chest tightness). Significant relation between peak GA concentrations and nasal irritation.
Pis94/ Pis97	Endoscopy, operating theatre nurses: n=135, exposed; n=132, unexposed	Personal short-term sampling during 1-15 min actual GA working (OSHA64)	Endoscopy area's: GM:0.18 (LEV[c]: 0.09; no LEV: 0.37) Operating theatres GM: 0.11 mg/m^3 (LEV: 0.06; no LEV: 0.14)	Eye (52%: itchy and burning), throat (24%: burning itchy and sore throat) and skin (68%: dry, cracked and hard skin, discolouration) symptoms, headache (60%) and lethargy (57%) were significantly (p<0.05) increased in exposed workers. Significant relation between exposure frequency (exp hours/wk) and symptoms prevalences (f.i. 'any eye' symptoms)

Wat03	Endoscopy, operating theatre nurses: n=38, exposed; n=38, unexposed	Personal sampling during actual exposure peaks (direct Glutaraldemeter)	Peaks up to 0.6 (range 0.04 - 0.6)	No significant increase in work related symptoms of the eyes (irritation), nose (irritation, burning), throat (irritation, cough), or headache in exposed. Unexposed also showed high prevalences in these symptoms. Skin symptoms (local effects on hand and forearm) significantly increased (3.6 x) in exposed, were partly confounded by difference in glove–wearing behaviour.
Cai03	Female volunteers:			
	n=53 exposed by vapour delivery system	Exposure 2 sec nose; 25 sec eye Device sampling during 15-30 min	3	71% correctly detected GA by nose 84% correctly detected GA by eye
		Exposure 15 min	0.9	15% correctly detected GA by nose 18% correctly detected GA by eye
	n=50 exposed in exposure chamber	Personal sampling, (OSHA64)	<0.4	No correct detection of GA by nose, eye or throat.

[a] GM geometric mean

[b] Sampling and exposure duration not specified but considered ≥ 15 minutes based on Gan95, Wat03

[c] LEV local exhaust ventilation

In a cross-sectional study of Waters *et al.* (Wat03), no increase in work-related symptoms of the eyes, nose and throat was recorded after health-care workers were exposed to high glutaraldehyde levels of 0.6 mg/m^3. Both the exposed and unexposed groups reported a high prevalence of irritational symptoms. In this study an alternative method (direct reading Glutaraldemeter) for exposure measurements was used, which enables peak exposure measurements. The authors further described the work practices in endoscopy or cold sterilisation units, which are such that in 15 minutes *several* (peak) exposure events (ranging between 5 sec – 12 min) are likely to occur. The committee however noted some limitations of the study. These included the high prevalence of irritational symptoms recorded in the unexposed control group, the lack of the precise duration of the (peak) exposure in relation to the effects observed and the uncertainty of the results of the exposure measurement apparatus.

In a clinical study of Cain *et al*. (Cai03), non-smoking female volunteers were exposed to several, constant (no peaks), glutaraldehyde concentrations. After isolated exposure of the eyes (25 sec) or nose (2 sec) by a vapour delivery device, a steep dose-response relationship for irritation was found: 15-18% of the females reported sensory irritation at 0.9 mg/m^3, whereas most females (71-84%) reported sensory irritation at 3 mg/m^3. In a further experiment in an exposure room (simultaneous exposure of nose,

eyes and throat) lasting 15 minutes, no sensory irritation was observed up to 0.4 mg/m^3, the highest concentration tested, when compared to the control group.

From the human data, the committee considers sensory irritation as the critical effect for short-term exposure. From the studies of Norbäck (Nor88) and Vyas *et al* (Vya00), the committee presumes that the sensory irritant effects are caused by the higher exposure levels, (especially peaks) within the broad exposure range of <0.001-1.08 mg/m^3. Furthermore, the committee assumes that the health-care workers in both studies were exposed to peaks (the duration of a single peak ranges between 5 sec – 12 min according to Wat03), but that the (OSHA64) detection method used only measures the geometric mean over (minimal) 15 minutes sampling time. Because in 15 minutes several peak events may occur, this detection method is not suitable to determine the precise values of each peak exposure event. Based on the findings of Cain *et al*. (Cai03) with precisely measured exposure effect levels, the committee assumes that the sensory irritant effects observed in the studies of Norbäck (Nor88) and Vyas *et al*. (Vya00) are caused by exposure peaks that are higher than 0.4 mg/m^3. This level was indirectly supported by a cross-sectional study of Waters *et al* (Wat03), in which no sensory irritational effects were observed up to 0.6 mg/m^3 .

From the studies of Vyas *et al* (Vya00) and Pisianello *et al.* (Pis94, Pis97), the committee further concludes that the frequency of exposure to glutaraldehyde is related to the number of sensory irritant symptoms.

Animal studies support the findings in humans in that acute exposure to glutaraldehyde vapour causes sensory irritant effects on the eyes and respiratory tract. Furthermore, animal studies also showed a steep dose-response relationship where sensory irritation was followed by severe respiratory distress and death after exposure to 4-10 mg/m^3 for some days.

Indications for both skin and respiratory tract sensitisation have been reported. The committee considers glutaraldehyde to be a human skin sensitizer based on case reports on occupational allergic contact dermatitis and positive patch test results. Positive hypersensitivity studies in animals supported these findings. It further assumes that the cross-sensitisation between glyoxal and glutaraldehyde observed in animals could be relevant in humans.

Human and animal data on respiratory tract sensitisation, which could result in occupational asthma, are not conclusive. Evidence that glutaraldehyde is a respiratory tract sensitizer is derived from some positive immunological test results carried out in

humans and a few animal species. However, the immunological mechanism of action is not well understood. Clinical evidence comes from several case studies and one epidemiological study where challenge tests revealed changes in FEV1, peak expiratory flow, non-specific bronchial responsiveness, nasal airway resistance, as well as and late and dual asthmatic reactions. Although most of these clinical asthma-like symptoms may point at (specific) respiratory sensitisation, the committee cannot rule out that the irritating properties of glutaraldehyde may have resulted in work-aggravated asthma in susceptible individuals (Cha95). This is pre-existing or concurrent asthma that is aggravated by irritants in the workplace. However, based on the positive immunological tests in humans and the few animal studies, and due to the fact that glutaraldehyde is suggested to be a skin sensitizer, the committee considers glutaraldehyde as a respiratory sensitizer. Because only a few cases with possible respiratory sensitisation symptoms have been reported in relation to the large numbers of persons exposed to glutaraldehyde over a period of many years, the committee considers the risk of glutaraldehyde to cause respiratory sensitisation extremely low. Further, the committee noted the poorly understood immunological mechanism of action.

Effects after longer-term exposure

There are no adequate long-term human studies on glutaraldehyde exposure available.

In several (9 days to 13 weeks) inhalation studies in rats and mice, exposure-related lesions in the upper respiratory tract at levels of 1 mg/m^3 rats and 0.25 mg/m^3 in mice have been demonstrated. These lesions included necrosis, inflammation and squamous metaplasia of the epithelium of the nose. Comparable findings were demonstrated in a well-performed 2-year (104 weeks, 6h/day, 5d/week) whole-body exposed, NTP inhalation study in rats and mice. Exposure resulted in considerable non-neoplastic lesions in the noses of both species. At 0.5 mg/m^3 a statistically significant increased incidence in squamous metaplasia in the respiratory epithelium of the nose in female mice was observed. At 0.25 mg/m^3, the lowest dose level tested, also a slight, but non significant increased incidence was observed. The severity of the effect was minimal. Furthermore, at 0.25 mg/m^3 a statistically significant, increased incidence of hyaline degeneration of the respiratory epithelium of the nose in female mice was observed. This finding was however not dose-related. The biological relevance of hyaline degeneration (‘hyaline droplets’ in cytoplasm) for humans is, however, unknown, and hyaline degeneration also occurs spontaneously during ageing in mice. Based on this information, the committee considered the dose-related, toxicological relevant, squamous metaplasia of the respiratory epithelium of the nose in female mice as the critical effect for long-term exposure.

No evidence of a carcinogenic potential was found in a 2-year whole-body inhalation exposure study in rats and mice (described above), nor in a 2-year drinking water study in rats. Additional *in vitro* studies showed mutagenic, clastogenic, and DNA-damaging properties. However, since no genotoxic or carcinogenic activity was demonstrated in well-performed *in vivo* studies, the committee concludes that workers are not at risk for developing cancer due to occupational exposure to glutaraldehyde.

Adequate animal data did not provide evidence that glutaraldehyde will affect reproduction.

Conclusion

From the human (and animal) data, the committee considers sensory irritation, especially of the eyes and upper respiratory tract (nose and throat), the critical effect for short-term exposure to glutaraldehyde vapour. Further, the committee concludes that both steep dose-response curves in the female volunteer and short-term animal studies in combination with the severity of the effect (severe respiratory damage and death) in animals, warrant a ceiling value to be set. This ceiling level is needed to protect workers against the peak exposures, which are characteristic in many working practices with glutaraldehyde.

Because human data indicate that the frequency of exposure to glutaraldehyde is of importance in the occurrence of sensory irritant effects, the committee advises that also a health-based recommended occupational exposure limit for long-term exposure (HBR-OEL, 8-h TWA) is warranted.

8.2 Recommendation of a ceiling value and an HBR-OEL

The committee concludes from the steep dose-response curves in both the volunteer and short-term animal studies, in combination with the severity of the effect (severe respiratory damage and death) in animals, that a ceiling value is warranted. In deriving this ceiling value, the committee uses the human studies in which effects after short-term or peak exposure have been reported and personal exposure levels have been measured. The committee considers the female volunteer study with 15 minutes exposure time of Cain *et al*. (Cai03) the most relevant for establishing this ceiling value, with 0.4 mg/m^3 being the no-observed-adverse-effect-level (NOAEL) for sensory irritation. Because an additional safety margin is introduced by the use of a 15 minute NOAEL as a ceiling value and non-smoking females are a sensitive population*, the committee does not advise an uncertainty factor for inter-individual variation. Moreover, the lethal effects in

mice (which were taken into consideration by deciding to set a ceiling) occur at a still somewhat higher exposure, providing additional safety. Hence, the committee recommends a ceiling value of 0.4 mg/m^3. This level should never be exceeded.

In deriving an HBR-OEL, the committee uses the well-performed long-term inhalation study in mice from the National Toxicology Program (NTP), which it considers representative for chronic exposure of human. From this study, a NOAEL of 0.25 mg/m^3, the lowest dose level tested, can be derived. At this level, the incidence in squamous metaplasia in the respiratory epithelium was not statistically higher, and the severity was minimal.

For the assessment of the HBR-OEL, several aspects have to be considered, because the toxicity data should be extrapolated to workers. Because mice are obligatory nose breathers, the committee is of the opinion that mice are more sensitive for nasal effects than humans. Since squamous metaplasia occurs at the nasal surface, the committee does not compensate for differences between species. For intraspecies variation, an uncertainty factor of 3 is taken. Application of these factors results in an HBR-OEL of 0.08 (0.25/3) mg/m^3 (8-h TWA).

Because glutaraldehyde is a skin irritant and sensitizer, the committee is of the opinion that dermal exposure to glutaraldehyde should be prevented and application of adequate skin protection is warranted.

8.3 Groups at extra risk

Glutaraldehyde is a skin sensitizer and an irritant. It can cause asthma but also aggravate already existing asthma. Workers sensitised at an earlier time point, and workers with asthma may be at extra risk for developing symptoms from airborne glutaraldehyde exposure. Individuals sensitised to glyoxal may have a greater risk for reacting to glutaraldehyde, because of a possibility for cross-reactivity between these aldehydes.

8.4 Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards recommends a health-based occupational exposure limit (HBR-OEL) for glutaraldehyde in the air of 0.4 mg/m^3 as a ceiling value and an HBR-OEL of 0.08 mg/m^3 as an eight-hour time-weighted average concentration (8-h TWA) for either activated or inactive glutaraldehyde.

* Females have shown a higher chemesthetic sensitivity than males (Gar82)

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A	Request for advice
B	The Committees
C	Comments on the public draft
D	Definitions
E	Abrreviation
F	Part 2 Arbete och Hälsa: Glutaraldehyde

Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of

genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in annex B.

Annex **B**

The committees

Dutch expert committee on occupational standards

- GJ Mulder, *chairman*
  professor of toxicology; Leiden University, Leiden
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- LJNGM Bloemen
  epidemiologist; Environ Netherlands BV, Zeist
- PJ Boogaard
  toxicologist; Shell International BV, The Hague
- PJ Borm
  toxicologist; Centre of Expertise in Life Sciences, Hogeschool Zuyd, Heerlen
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- DJJ Heederik
  professor of risk assessment in occupational health; IRAS, Utrecht University, Utrecht
- TM Pal
  occupational physician; Netherlands Center for Occupational Diseases, Amsterdam
- IMCM Rietjens
  professor of toxicology; Wageningen University, Wageningen.

- H Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague
- T Smid
  occupational hygienist; KLM Health Safety & Environment, Schiphol and professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Dow Chemical, Terneuzen
- RA Woutersen
  toxicologic pathologist; TNO Nutrition and Food Research, Zeist
- P Wulp
  occupational physician; Labour Inspectorate, Groningen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague
- TMM Coenen, *scientific secretary*
  Health Council of the Netherlands, The Hague
- JM Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

Nordic Expert Group

- G Johanson (chairman)
  professor of occupational toxicology; Karolinska Institute and National Institute for Working Life (Sweden)
- V Kristjansson
  organic chemist; Administration of Occupational Safety and Health (Iceland)
- K Savolainen
  toxicologist, professor; Finnish Institute of Occupational Health (Finland)
- V Skaug
  toxicologist, occupational physician; National Institute of Occupational Health (Norway)
- K Sørig Hougaard
  toxicologist; National Institute of Occupational Health (Denmark)
- J Järnberg, s*cientific secretary*
  National Institute for Working Life, Solna (Sweden)

The first part of the document was prepared by JTJ Stouten, MSc and JHE Arts, PhD, from the Department of Toxicological Risk Assessment of the TNO Nutrition and Food Research Institute, Zeist, The Netherlands.
Secretarial assistance: F. Smith.
Lay-out: M Javanmardi.

Annex C

Comments on the public draft

A draft of the present report was released in 2004 for public review. The following organisations and persons have commented on the draft document:

- W Clous, Dow Europe, Switzerland
- RD Zumwalde, National Institute for Occupational Safety and Health, USA

Annex **D**

Definitions

Definitions of the World Health Organisation (1996):

- hypersensitivity: abnormally increased response to a stimulus;
- inducer: an inducer leads to the *de novo* generation of an altered state of reactivity to a specific substance;
- sensitisation: induction of specialised immunological memory in an individual by exposure to an allergen;
- specific hypersensitivity: the induction of an altered state of specific reactivity in an individual by exposure to inducing substances and preparations; specific hypersensitivity may be allergic sensitisation or of uncertain mechanism.

The two categories of asthma in the workplace are, according to Chan-Yeung and Malo (Cha95):

- occupational asthma: characterised by variable airflow limitation, bronchial hyperresponsiveness, or both, due to conditions in a particular work environment, not to stimuli outside the workplace. Two types of occupational asthma can be distinguished according to whether there is a latency period:
  - asthma with latency: develops after a period of exposure that may vary from a few weeks to several years. It includes all instances of immunologic asthma ('immune-mediated asthma'), and can be subdivided into IgE-dependent and IgE-independent reactions.
  - asthma without latency: follows exposure to high concentrations of irritant gases, fumes, or chemicals on one or several occasions ('irritant-induced asthma');

- work-aggravated asthma: pre-existing or concurrent asthma that is aggravated by irritants or physical stimuli in the workplace.

Note

In any case, since EC-labelling criteria, unfortunately, do not discriminate between airway sensitizers, inducing immune-mediated reactions, and other inducers of airway hypersensitivity reactions (physical stimuli, irritants, etc.), since they may all induce the same physiological reactions in individuals, glutaraldehyde fits the criteria to be a respiratory sensitizer (R42).

In the case of sensitizers there is inter-individual variability in induction and in provocation of a response in sensitised subjects, with considerable variation in threshold (WHO, 1996). However, since specific airway hypersensitivity reactions were observed almost exclusively in atopic subjects with pre-existing or concurrent asthma, this may have implications in establishing occupational exposure limits.

Annex **E**

Abbreviations

bp	boiling point
EC_{50}	concentration at which a described effect is found in 50% of the exposed animals or at which the effect is decreased up to 50% of the control value
HBR-OEL	health based recommended occupational exposure limit
h	hour
IC_{50}	concentration at which inhibition of a certain function is found up to 50% of the control value
LC_{50}	lethal concentration for 50% of the exposed animals
LC_{10}	lowest lethal concentration
LD_{50}	lethal dose for 50% of the exposed animals
LD_{10}	lowest lethal dose
LOAEL	lowest observed adverse effect level
MAC	maximaal aanvaarde concentratie (maximal accepted concentration)
MAEL	minimal adverse effect level
MAK	Maximale Arbeitsplatz Konzentration
MOAEL	minimal observed adverse effect level
MTD	maximum tolerated dose
NAEL	no adverse effect level
NEL	no effect level
NOAEL	no observed adverse effect level
OEL	occupational exposure limit
PEL	permissible exposure limit
ppb	parts per billion (v/v)10^{-9}

ppm	parts per million (v/v)10^{-6}
RD_{50}	concentration at which a 50% decrease of respiratory rate is observed
REL	recommended exposure limit
STEL	short term exposure limit
tgg	tijd gewogen gemiddelde
TLV	threshold limit value
TWA	time weighted average
V_{max}	maximal reaction velocity of an enzyme

Organisations

ACGIH	American Conference of Governmental Industrial Hygienists
CEC	Commission of the European Communities
DECOS	Dutch Expert Committee on Occupational Standards
DFG	Deutsche Forschungsgemeinschaft
EPA	Environmental Protection Agency (USA)
FDA	Food and Drug Administration (USA)
HSE	Health and Safety Executive (UK)
IARC	International Agency for Research on Cancer (WHO)
INRS	Institut National de Recherche et de Sécurité (France)
NIOSH	National Institute for Occupational Safety and Health (USA)
NTP	National Toxicology Programme (USA)
OECD	Organisation for Economic Cooperation and Development
OSHA	Occupational Safety and Health Administration (USA)
RTECS	Registry of Toxic Effects of Chemical Substances
SER	Social and Economic Council (Sociaal-Economische Raad NL)
WATCH	Working Group on the Assessment of Toxic Chemicals (UK)
WHO	World Health Organisation

Toxicological terms

bid	*bis in diem* (twice a day)
bw	body weight
CARA	chronic non-specific respiratory diseases
CHD	coronary heart disease
CNS	central nervous system
ECG	electrocardiogram
EEG	electro encephalogram
FCA	Freunds Complete Adjuvans
FEV	forced expiratory volume
FSH	follicle stimulating hormone
GD	gestation day(s)
GPMT	Guinea pig maimisation test

GSH	glutathione
HLiA	hamster liver activated
IHD	ischaemic heart disease
im	intramuscular
ip	intraperitoneal
ipl	intrapleural
it	intratracheal
iv	intravenous
LH	lutheinising hormone
MAC	minimal alveolar concentration
MFO	mixed function oxidase
NA	not activated
PNS	peripheral nervous system
po	*per os* (= oral)
RBC	red blood cells
RLiA	rat liver activated
SCE	sister chromatid exchange
sc	subcutaneous
UDS	unscheduled DNA-synthesis

Statistical terms

GM	geometric mean
OR	Odds Ratio
RR	Relative Risk
SD	standard deviation
SEM	standard error of mean
SMR	standard mortality ratio

Analytical methods

AAS	atomic absorption spectroscopy
BEEL	biological equivalent exposure limit
BEI	biological exposure index
BEM	biological effect monitoring
BM	biological monitoring
ECD	electron capture detector
EM	environmental monitoring
FID	flame ionisation detector
GC	gas chromatography
GLC	gas liquid chromatography
GSC	gas solid chromatography
HPLC	high performance liquid chromatography

IR	infrared
MS	mass spectrometry
NMR	nuclear magnetic resonance
PAS	personal air sampling
TLC	thin layer chromatography
UV	ultraviolet

Statistical terms

GM	geometric mean
OR	Odds Ratio
RR	Relative Risk
SD	standard deviation
SEM	standard error of mean
SMR	standard mortality ratio

Additional abbreviations in the present report

$IgEV_1$	immunoglobulin
LEV	local exhaust ventilation
$ppFEV_1$	percentage predicted FEV_1
RAST	radio allergosorbent test

Part 2 Arbete och Hälsa: Glutaraldehyde

DECOS and NEG basis for an occupational standard

1997:20

Brita Beije

Per Lundberg

1997:20

DECOS and NEG Basis for an Occupational Standard

Glutaraldehyde

Brita Beije
Per Lundberg

Nordic Council of Ministers

ARBETE OCH HÄLSA VETENSKAPLIG SKRIFTSERIE
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National Institute for Working Life

The National Institute for Working Life is Sweden's center for research and development on labour market, working life and work environment. Diffusion of information, training and teaching, local development and international collaboration are other important issues for the Institute.

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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.

V.J. Feron
Chairman
DECOS

Per Lundberg
Chairman
NEG

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 °C
Boiling point	188 °C
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 $\lambda = 365$ 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 °C 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 glutaraldehyde. 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 0.2	0.4 0.8	S 5 min short term	1996	7
Iceland	0.2	0.8	Ceiling	1989	3
Netherlands	-	0.25	Ceiling	1996	4
Norway	0.2 -	0.8 0.25	Ceiling Activated glutaraldehyde	1995	5
Sweden	0.2	0.8	Ceiling; S	1996	6
USA (ACGIH)	0.2 0.05	0.82 0.2	Ceiling intended change	1996	8
(NIOSH)	0.2	0.8	Takvärde	1994	9
(OSHA)	-	-		1994	9

S = risk for sensitisation

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c. Book
1. Griffin MJ. *Handbook of human vibration.* London: Academic, 1990.
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Figures and tables
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