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Review

Effects of Glutaraldehyde Exposure on Human Health

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Abstract: Effects of Glutaraldehyde Exposure on Human Health: Tomoko TAKIGAWA, *et al.* Department of Public Health, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences—Glutaraldehyde (GA) is widely used in the industrial, scientific and biomedical fields. Many adverse health effects on humans have been reported in association with biomedical uses of GA, with 2–3.5% aqueous GA solution generally used for cold sterilization and GA exposure ranges of 0.001 to 2.6 ppm for this type of use. GA is metabolized extensively to CO_2, but urinary excretion of it is low. Sensory irritant effects, sensitization of skin and respiratory organs and other symptoms have been reported among endoscopy nurses and medical radiation technologists. The prevalence of chronic bronchitis and nasal symptoms in humans is significantly correlated with peak concentrations of GA exposure. The extent of primary skin irritation depends on the duration and site of contact, and the severity of symptoms is dose-related. Chronic inhalation affects the nose and respiratory tract, and lesions become severe with prolonged duration of exposure. Increases in neither mortality nor tumor incidence have been found in workers with less than 0.2 ppm GA exposure, no evidence of carcinogenic activity has been obtained in experimental animal studies. There has been no clear evidence of genetic toxicity of GA in either *in vitro* or *in vivo* studies, and neither developmental nor reproductive toxicity has been found in humans or animals. To prevent hazards from GA exposure, use of closed-system, fully automated washing machines is recommended, since numerous symptoms have been found in individuals with less than 0.05 ppm GA exposure, the recommended peak exposure limit in many countries. *(J Occup Health 2006; 48: 75–87)*

Key words: Glutaraldehyde, Toxicity, Occupational exposure, Cold sterilization, Endoscopy unit, X-ray department

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Glutaraldehyde (GA) is a colorless liquid with a pungent odor. It has a wide spectrum of medical, scientific and industrial applications. GA is the best disinfectant for cold sterilization of medical equipment[1)] and is also used as a fixative in histochemistry and electron microscopy, a developer and fixer in X-ray film processing, a linking material, a leather tanning agent and as an ingredient in cosmetic, toiletry and chemical specialty products[2)]. It is irritating and corrosive to the skin, eyes and respiratory tract[3, 4)] and is recognized as a cause of health problems in those handling it. Many regulatory organizations including the Japanese Ministry of Health, Labour and Welfare (MHLW) have therefore set limits on exposure to GA to prevent its irritating effects[5–8)].

Recently, not only irritation and sensitization but also darkroom disease (DRD) among radiographers, associated with various symptoms including indefinite complaints, has been reported to be related to GA exposure, though the relationship between DRD and GA exposure has not been clarified[9, 10)]. In addition, onset of multiple chemical sensitivity (MCS) has been reported among nurses using GA[11, 12)], however, there was no description about work environmental conditions.

Aldehydes are one of the major pollutants of indoor air and cause sick building syndrome (SBS) and sick house syndrome, the major symptoms of which are irritation and indefinite complaints[13–15)]. Since the symptoms of DRD are very similar to those of SBS, GA, one of the aldehydes, may contribute to the onset of DRD. Prolonged low exposure to formaldehyde affects regulation of hypothalamic-pituitary-adrenal axis activity in the female mouse, which may be a suitable animal model for SBS and/or MCS[16)]. Thus, not only formaldehyde but also GA may cause MCS. However, investigations of GA exposure have not been thoroughly conducted from the perspective of MCS. Moreover, another aldehyde, ortho-phthalaldehyde (OPA) has begun

to be used as an alternative to GA, without the establishment of effective preventive measurements for GA exposure. This article therefore reviews the toxicological and medical aspects of GA, for the purpose of preventing adverse health effects of GA and its substitutes.

Data Sources

Information was obtained by a search of electronic databases (PubMed and Japana Centra Revuo Medicina) for medical journals, examination of official guidelines and internet resources. The searches were supplemented with manually investigation of the references cited in the original articles. We included studies written in English and Japanese.

Physical and Chemical Properties

The principal physical and chemical properties of GA are summarized below[3, 17].

CAS No:	111-30-8
Structural formula:	$OHC(CH_2)_3CHO$
Molecular weight:	100.1
Boiling/Freezing point:	187–189°C/–14°C
Density:	0.72 (water=1)
Solubility:	Soluble in water, alcohol and ether
Vapor pressure:	2.3 kPa (20°C)
Vapor density:	3.45 (air=1)

A 2% solution of GA, widely used as a microbicidal agent, is stable for long periods before buffering, when stored in closed containers in a cool place. This solution is mildly acidic, less pungent than a buffered solution and not sporicidal. When GA is used, a buffer agent consisting mainly of sodium bicarbonate is added for alkalinization and activation. The resulting buffered solution has strong biocidal activity against bacteria, viruses, fungi and spores, and its antimicrobial activity is maintained for 14 to 28 d[1, 18].

Pharmacokinetics and Metabolism

There have been few reports on the pharmacokinetics and metabolism of GA, with no information available on the disposition of GA after peroral and inhalational exposure in humans or animals.

Rats were infused via the jugular vein with 10 μCi of ^{14}C labeled GA ([^{14}C]1,5-GA)[19]. The level of ^{14}C in red blood cells was two to three times higher than that in plasma at 5 min to 3 d after infusion. There was a 6-fold reduction in both cellular and humoral ^{14}C radiolabel within 3 d. During the first hour and by 6 h post-dosing, approximately 14% and 29%, respectively, of the infused ^{14}C radiolabel was excreted in urine. Even after 3 d, renal excretion of ^{14}C was proceeding at a rate of 0.2% of dose per h. Only 3% of ^{14}C in the urine was recovered as GA by thin-layer chromatography.

After intravenous administration of 0.075–0.75% [^{14}C]GA (0.2 m*l* for rats and 2.5 m*l* for rabbits), the largest portion of the administered dose was recovered as $^{14}CO_2$ (64.4–78.0% in rats and 22.4–70.9% in rabbits during the first 24 h)[20]. Lesser amounts were eliminated in urine, i.e., 7.3–12.0% and 15.5–28.1% of dose in rats and rabbits, respectively. After occluded application of ^{14}C radiolabeled GA to the dorsal skin of rats (0.075–7.5%, 0.2 m*l*) and rabbits (0.75–7.5%, 2.5 m*l*) by 24 h, principal radiochemical recovery was from the skin, at 45.1–60.6% and 31.0–45.0%, respectively. Calculations made in that report estimated the total proportions of [^{14}C]GA absorbed percutaneously were 0.3–2.1% for rats and 2.5–15.6% for rabbits. The terminal half-life values were 9.6–12.0 h in rats and 14.1–29.2 h in rabbits for intravenous administration and 39.4–112.4 h in rats and 17.3–99.0 h in rabbits for percutaneous administration.

Frantz *et al.*[21] reported *in vitro* permeabilities of 0.75% and 7.5% for [^{14}C]1,5-GA in skin from humans and various animals. Recoveries were 0.05–1.55% in all species studied. For humans, recovery was approximately 0.2% at both GA concentrations, and it was lower than those of the other animals tested.

The major route of metabolism of GA is oxidization by the kidney and liver to glutaric-γ-semialdehyde, and then to glutaric acid, which is used to synthesize glutaryl CoA, with further metabolism to glutaconyl CoA, crotonyl CoA, β-hydroxybutyryl CoA, and acetyl CoA, and finally CO_2[2].

Toxicity

Acute toxicity

There are several reports on accidental acute exposure to GA in humans. In a case in which approximately 100 m*l* of GA (Cidex) was spilled on a child's face by mistake during surgery, fever, vomiting, tachypnea and tachycardia were noted for 6 h after the accident, and chemical pneumonia was diagnosed[22]. The child finally recovered without sequelae. It was reported that colitis was induced by retention of 2% GA disinfectant in endoscope channels[23, 24].

The acute toxicity of GA has been investigated in many studies with various animal species[2, 25–27]. Acute peroral LD_{50} in rats treated with GA concentrations from 1 to 50% ranged from 12.3 to 1.19 m*l*/kg, or from 99 to 733 mg GA/kg, respectively. LD_{50} values expressed in m*l*/kg increased with dilution rates, while those expressed in mg GA/kg decreased with increasing dilution rates. Therefore, if water is taken following accidental ingestion of GA in high concentration, the toxicity of GA may be enhanced[25]. Signs of acute oral toxicity in rats and mice given 2% GA solution included suppression of spontaneous behavior, piloerection and abdominal swelling[26]. Whether aging affects sensitivity to GA was examined[28]. Young

Table 1. Human skin irritation and sensitization by experimental exposure to glutaraldehyde

Subject	Test site	GA conc (Administration)	Result	Ref
12 volunteers, all male except one	anterior, lateral and posterior ankle, posterior heel	10% (5 d/wk, 4 wk, without occlusion)	11 subjects: discoloration (during the 1st wk) 5 subjects: minimal irritation on the anterior ankle; one subject became sensitized (during the 2nd wk)	31)
Same volunteers as above (except one)	posterior ankle, medial, lateral and posterior heel	10% (3 d/wk, 4 wk, without occlusion)	No irritation	31)
6 young females	forearm	10% (twice daily for 4 d, without occlusion)	2 subjects: allergic contact dermatitis	32)
8 young adult volunteers	forearm	5% (twice daily for 4 d, without occlusion)	1 subject: allergic contact dermatitis	32)
10 volunteer medical students with hyperhidrosis	sole, palm, forearm	1–10% (once, without occlusion)	All: no irritation with brown discoloration (>2%)	33)
109 male and female volunteers	back	0.1–0.5% (10 applications over 3 wk for induction and one for challenge after 2 wk, with occlusion)	0.1%: no reaction to induction and challenge 0.2%: doubtful findings in 2 for induction and no reaction to challenge 0.5%: doubtful findings in 9, definite erythematous reaction in 7 on induction and doubtful findings in 1, local erythema with edema in 1 on challenge	34)

GA conc: Glutaraldehyde concentration used in test. Ref: Reference number.

(5–6 wk) and old (57–60 wk) rats were administered GA by gavage (young: 200–1,600 mg/kg, old: 50–400 mg/kg). The LD_{50} values were 283 mg/kg for young and 141 mg/kg for old rats.

Acute dermal LD_{50} in rabbits treated with GA concentrations from 25 to 50% ranged from 16.0 to 1.59 m*l*/kg, or from 4,256 to 898 mg GA/kg, respectively[27]. Unlike the case for oral administration, dilution was not related to the LD_{50} expressed in mg GA/kg. The report's authors concluded that the GA concentration applied to the skin was a more important determinant of dermal toxicity than the absolute amount of material applied[25].

Because of the low vapor pressure of GA (0.02 hPa for a 50% aqueous solution at 20°C), the atmospheric concentration of GA vapor generated from aqueous solutions depends on the vapor pressure of water[29]. There are two methods for obtaining a particular GA concentration in an exposure chamber: a static method, in which GA solution is placed in the chamber and vapor is allowed to equilibrate, and a dynamic method, in which the solution is used to generate inspirable aerosols by means of compressed air. In both static and dynamic experiments, mortalities were unusual after 4–8 h exposure, and signs of exposure included blepharospasm, excess lacrimation and salivation, nasal discharge and abdominal and mouth breathing[25, 27]. When male rats were treated with GA solution (10–40 mM) by intra-nasal instillation, GA higher than 20 mM induced extensive lesions such as inflammation at the site of treatment of the nasal mucosa[30]. In the same study, formaldehyde induced similar lesions at doses of 200 mM or higher, and it was concluded that GA was approximately 10 times more toxic to the nasal epithelium than formaldehyde. When rats were exposed to saturated GA vapor generated at 23°C, no deaths occurred. However, 4/12 rats died when they were exposed to GA vapor at 65°C. These findings suggest that toxic substances may be produced at higher temperatures of generation of GA vapor and enhance its toxicity[25].

Irritation and sensitization

1. Skin

GA has been used to treat hyperhidrosis because of its antiperspirant effect, and has been investigated in dermatological studies (Table 1). The findings obtained indicated little irritation by and low sensitivity to GA. Although Juhlin and Hansson[33] observed no allergic reactions to GA, even in patients sensitive to formaldehyde, they noted that evaluation of their findings concerning sensitivity was difficult because the dose used in their experiments was too small (1–10% with occlusion). GA is also used to treat warts[35, 36]. There were no cases of sensitization to buffered 10% GA solution[37], although a 20% solution produced necrosis[38]. Reaction to applied GA depends on the thickness of the skin. Irritation and sensitization were observed on the anterior ankle but not on the posterior ankle or medial, lateral or posterior heel[31]. In a factory with indoor GA

Table 2. Case reports of allergic contact dermatitis due to glutaraldehyde

Sex	Age	Working as/on	GA conc	Affected site	Patch test results	Ref
F	–	theater nurse	–	fingers, hands, arms, chin	P (1% GA), N (2% FA)	40)
F	–	theater nurse	–	fingers, hands	P (1% GA), N (2% FA)	40)
F	24	dental assistant	2% (Cidex)	fingers, hands, arms, legs	P (2% GA)	41)
F	24	dental assistant	2%	fingers, hands	P (1, 0.25% GA), N (0.25% FA)	42)
F	48	dental assistant	2%	fingers, hands	P (1, 0.25% GA), N (0.25% FA)	42)
F	50	dental assistant	2%	fingers, hands	P (1, 0.25% GA), N (0.25% FA)	42)
M	35	–	10% aq	foot	P (1, 0.25% GA), N (0.25% FA)	42)
M	61	–	10% aq*	around the nails	P (1, 0.25% GA), N (0.25% FA)	42)
–	–	radiologist	2% (Cidex)	fingers	P (1% GA)	43)
–	–	x-ray technician	2% (Cidex)	fingers	P (1% GA)	43)
F	27–29	cleaning woman	Cidex	hands, arms	P (1,10%, as is Cidex*, 0.1, 0.5, 1% GA, 2% FA)	44)
F	27–29	cleaning woman	Cidex	hands, arms	P (1,10%, as is Cidex*, 0.1, 0.5, 1% GA, 2% FA)	44)
F	27–29	cleaning woman	Cidex	hands, arms	P (1,10%, as is Cidex*, 0.1, 0.5, 1% GA)	44)
F	50	disinfection	Cidex	hands, arms	P (10%, as is Cidex*, 1% GA)	44)
F	39	nurse-aid	Cidex	hands, arms, face, neck	P (10%, as is Cidex*, 1% GA)	44)
F	25	disinfection	Cidex	hands	P (Cidex)	45)
F	34	nurse	Cidex, Diba	hands, feet	P (Cidex, Diba, GA)	45)
F	22	–	<1% (hair conditioner)	scalp (eczematous change, hair loss)	P (0.1, 0.5, 1% GA), N (0.05% GA)	46)
F	56	disinfection	2% (Cidex)	hands, arms, face, neck	P (1% GA)	47)
F	23	dental assistant	1% (Wavicide)	hands	P (1% GA, FA)	48)
F	20	dental nurse	2% (Aldecyde)	hands, face, arms	P (1% GA)	48)
M	31	dental surgeon	1% (Wavicide)	hands	P (1% GA)	48)
F	53	endoscopy nurse	1% (Wavicide)	hands, face, arms	P (1% GA)	48)
F	18	dental nurse	1% (Wavicide)	hands, face, arms	P (1% GA)	48)
F	23	dental nurse	1% (Wavicide)	hands, wrists	P (1% GA)	48)
F	36	dental nurse	Cidex	hands, arms, face	P (0.5% GA, 2% Cidex)	49)
M	20	–	20% (Verutal)	leg	P (0.3, 1% GA, FA)	50)
F	26	hairdresser	Aldesan	hands, face	P (0.2% GA)	51)
F	46	hairdresser	Lysoformin	hands, face	P (0.2% GA)	51)
F	49	disinfection	Steriscope	fingers, hands, arms	P (0.002% GA)	52)

GA conc: Glutaraldehyde concentrations used in workspaces. Ref: Reference number. M: Male. F: Female. ag: Agueous solution. P: Positive. N: Negative. GA: Glutaraldehyde. FA: Formaldehyde. *Buffered solution.

concentrations generally below 0.2 ppm, no cases of GA-induced skin sensitization were found in an examination of the medical records of 218 workers between 1959 and 1992[39].

A case report of GA-related allergic contact dermatitis appeared as early as 1968[40], and many such reports have subsequently been made (Table 2). Most cases resulted from occupational exposure, e.g., disinfection of medical equipment with GA or use of such disinfected equipment, and some accompanied treatment of dermatological diseases. The cross-reaction between GA and formaldehyde has been studied[40, 53]. Examinees reacted to patch tests using 1% aqueous GA, but were negative in subsequent tests with 2% formalin. On the other hand, some patients with GA-induced contact dermatitis reacted to formaldehyde[44, 48, 50, 54].

In contact studies, the degree of signs observed depended on the concentration of GA solution in experiments on the shaven skin of rabbits[25, 27]. No effect was observed in rabbits treated with 1% GA for 4 h, but symptoms such as necrosis, erythema and edema were observed with 50% GA. When female guinea pigs and female mice were sensitized with 0.3–3.0% GA and challenged with 10% solution, both species demonstrated dose-dependent contact hypersensitivity[55]. The acidity of GA solution is also an important determinant of chemical hazard. In an experiment comparing unbuffered (acidic, approximately pH 4) and buffered (alkaline, approximately pH 8) solution, 2.2% unbuffered and buffered GA yielded skin irritation, but unbuffered solution had greater skin sensitizing potential[56].

Table 3. Case reports of occupational asthma induced by glutaraldehyde

Sex	Age	Occupation	Symptoms	GA conc (%)	Ref
Use: Sterilization of medical equipment					
F	–	endoscopy nurse	irritated eyes	–	63)
F	33	endoscopy nurse	severe nasal symptoms, chest tightness	2	64)
F	30	endoscopy nurse	perennial rhinitis, asthma	2	64)
F	43	endoscopy nurse	asthma, rhinitis	2	64)
F	37	endoscopy nurse	chest tightness	2	64)
F	33	respiratory technologist	worsening of asthma with daily symptoms	3.6	65)
F	46	endoscopy nurse	breathlessness, wheezing, chest tightness, cough, hoarseness, sore eyes and throat, sneezing	–	66)
F	45	endoscopy nurse	asthmatic symptoms	–	9)
F	31	endoscopy nurse	asthmatic symptoms	–	9)
F	29	ENT nurse	asthmatic symptoms	–	9)
F	25	theater nurse	asthmatic symptoms	–	9)
F	42	endoscopy nurse	asthmatic symptoms	–	9)
F	61	nurse (working in a renal dialysis unit)	irritation of the eyes and upper respiratory tract, dyspnea on exertion, dry cough, episodic attacks of wheezing	2	67)
F	32	laboratory technician	episodic chest tightness, wheezing	2.5	68)
Use: Radiographic developer and fixer					
F	25	radiographer	difficulty breathing	11	69)
M	39	radiographer	chronic dyspnea	–	70)
F	53	darkroom technician	–	–	9)
F	40	X-ray secretary	–	–	9)

GA conc: Glutaraldehyde concentrations used in workspaces. Ref: Reference number. M: Male. F: Female.

2. Eye

In reports by the National Institute for Occupational Safety and Health in the United States (US NIOSH)[57–60], eye irritation was noted to occur in medical workers using GA. For instance, in one hospital, 28 of 44 workers (64%) using GA at least once a week complained of eye irritation while using GA solution[58]. Cases of keratopathy[61] and conjunctivitis[62] were caused by use of medical equipment with incomplete washing and removal of 2% GA solution.

In experiments on rabbit eyes, the extent of the eye injury depended on the GA concentration[25, 27]. No effect was observed in the rabbits treated with 0.1 m*l* of 1% GA, but symptoms such as diffuse corneal injury and chemosis were observed with 45% GA. In the eyes of animals of various species, 2.2% buffered GA solution (alkaline) had greater corneal injury potential than unbuffered GA solution (acidic)[56], and 2% buffered solution caused severe corneal opacification, conjunctivitis and iritis[18].

3. Respiratory tract

There was no respiratory sensitization in 218 males assigned to GA production units with general indoor GA levels below 0.2 ppm[39]. Some cases of respiratory irritation were reported in the US NIOSH surveys[58, 59]. Among 44 workers who used GA, 28 (64%) reported nasal irritation, 18 (41%) pharyngeal irritation and 7 (16%) sore throat[58].

Following the first case of obstructive airway reaction to GA in a female in charge of an endoscopy unit[63], many cases of occupational asthma induced by GA vapor inhalation have been reported (Table 3). When provocation testing with alkaline GA was performed on four nurses with asthma or rhinitis after working in endoscopy units, two had positive reactions[64]. A respiratory technologist, whose respiratory function improved when she was away from work, underwent a workplace challenge test, in which she was exposed to the 3.6% GA used to clean bronchoscopes at her workplace, and her forced expiratory volume in 1 second (FEV_1) fell progressively during the day[65].

An initial inhalation challenge test was conducted on an endoscopy nurse who had findings suggestive of occupational asthma, and caused an immediate fall in FEV_1 from 3.6 to 1.5 *l*[66]. Subsequent double-blind inhalation challenge tests with activated GA vapor (0.01–0.32 ppm) caused asthmatic reactions following challenges at 0.032 ppm. However, the nurse had no

clear reactions to the challenge test series repeated three weeks later, making her diagnosis unclear.

Seven workers had positive specific bronchial challenge tests for GA, and three of them were also reported to be positive to formaldehyde[9].

Peripheral sensory irritation by GA is known to induce ophthalmic and nasal discomfort and cough. To quantitate sensory irritant effects, depression of the respiratory rate is measured. When male mice were exposed to GA vapor at 1.6–36.7 ppm in an irritation study, concentration-related decreases in respiratory rate were measured, and the 50% decrease in respiratory rate (RD_{50}) was calculated to be 13.9 ppm[71]. In mice, RD_{50} was found to be 2.6 ppm in a range of concentrations from 0.7–4.5 ppm[72]. In a sensitization study using male guinea pigs, no evidence of respiratory sensitization was obtained with exposure to GA vapor of 13.9 ppm for one hour a day for five consecutive days and subsequent challenge exposures to 4.4 ppm at 14, 21 and 35 d after the initial induction exposure[71].

4. Photosensitization

GA formulations (0.005–0.05%) were applied for 24 h with occlusion to two sites of the dorsal skin of 52 volunteers[29]. One of the application sites was irradiated with UVA (24 J/cm^2), without observable phototoxic reaction.

Photosensitivity was also examined. GA solutions (0.1–0.5%) were applied six times during three weeks to the dorsal skin of 99 volunteers, and the application sites were exposed twice to erythematogenic doses of UV light (290–400 nm)[29]. After 10–13 d, GA was applied to a remote skin site in the same fashion, and the site was irradiated with 6 J/cm^2 UVA (320–400 nm). No photosensitivity was observed.

Chronic toxicity

There is no information available on chronic toxicity of GA to humans.

A repeated exposure study with rats and mice of each sex was carried out by the National Toxicology Program in the US (US NTP)[73]. The animals were exposed to GA vapor with whole-body inhalation at 62.5–1,000 ppb. There was no exposure-related mortality in rats, but all mice exposed to 1,000 ppb and two female mice exposed to 500 ppb died before the end of the study. On histological and clinical pathological examination, there was no evidence of systemic toxicity of GA in rats or mice; however, exposure-related lesions were observed in the respiratory tract. Mice tended to be more sensitive than rats, due to their smaller nasal airways, which were easily blocked by cell debris and keratin. In a similar GA inhalation experiment[74], neutrophilic infiltration was present in the squamous epithelium of the nasal vestibule in both species and became progressively more severe with increasing exposure time. These lesions appeared at a site in the nose more anterior than that reported for formaldehyde.

In a study of rats subjected to subcutaneous applications of GA in NaCl solution over 35 d at daily doses ranging from 1–125 mg/kg, the 25 and 125 mg/kg dose groups exhibited remarkable inflammation and necrosis at the sites of injection. Significant changes included an increase in white blood cells, decreases in hematocrit and hemoglobin levels and lymphocytes on hematological examination, and abnormalities in the spleen, thymus, prostate and kidneys on histological examinations[26].

There were no systemic lesions in rats given a diet containing 0.5–5% GA for about three months or 0.25% GA solution as drinking water for 11 wk[2].

Genotoxicity and mutagenicity

Although there has been no report on genetic toxicity of GA to humans, it has been investigated extensively in animals[75, 76]. Both positive and negative results have been reported in *in vitro* mutagenicity studies[76–86], while almost all *in vivo* tests have yielded negative results[75, 76, 87, 88].

GA exhibited mild to strong mutagenic effects with and without S9 metabolic activation in *S. typhimurium* strain TA102[77–79]. In TA100, negative results were reported both with and without S9[81], while weakly positive results were reported with S9[76]. GA was mutagenic without S9 in TA104, which exhibited higher sensitivity to carbonyl mutagenesis than TA100 did[82]. GA was not mutagenic with or without S9 in TA98, TA1535, TA1537 and TA1538[76, 81].

GA was positive in the DNA repair test by liquid *rec*-assay[83] and by *umu* test without S9 activation[84]. GA was mutagenic in *E. coli* WP2 tester strains[79], but yielded negative results in the SOS chromotest with *E. coli* PQ37[80].

GA did not induce mutation in *in vitro* chromosomal aberration tests, in sister chromatid exchanges (SCE) tests, or forward gene mutation assays in cultured Chinese hamster ovary cells[76, 81]. SCE and a low frequency of chromosomal aberration were induced by high concentrations of GA, 3.6–16 mg/*l*, without metabolic activation[75]. Gene mutation was induced by GA in L5178Y *tk*+/*tk*– mouse lymphoma cells[85] and the human TK6 lymphoblast cell line[86]. Since GA induced a marginal increase in unscheduled DNA synthesis in the *in vitro* hepatocyte DNA repair assay (50, 100 μM), DNA-reactive genotoxic activity of GA was suggested to involve DNA-protein cross-linking[86].

Although genetic toxicity was not found in standard *in vivo* tests, such as the mammalian erythrocyte micronucleus test, rodent dominant lethal test and sex-linked recessive lethal test in *Drosophila melanogaster*, chromosomal aberrations were increased in mouse bone marrow cells after intraperitoneal injection of GA[75].

When GA was injected by the gastric tube method, no increase was found in the number of micronucleated bone marrow polychromatic erythrocytes in mice or of bone marrow cells with chromosomal aberration in rats[76]. GA failed to induce unscheduled DNA synthesis when administered by gastric intubation to rats and mice[87]. In another study, GA was found not to be mutagenic, since injected [^{14}C]GA was taken up by hepatic cells and did not reach the nucleus[88].

Carcinogenicity

Information on carcinogenicity of GA in humans is available from only one report. Among 186 persons employed in the factory mentioned above between 1959 and 1978, where indoor GA concentrations did not exceed 0.2 ppm, there was no increase in mortality or incidence of malignancy[39].

In animal studies, rats and mice were exposed to GA vapor at 250–750 ppb and 62.5–250 ppb, respectively, both for 6 h/d, 5 d/wk, for 104 wk[75, 89]. In rats, incidences of non-neoplastic nasal lesions increased primarily within the anterior portion of the nose. Other significant lesions included dose-related hyperplasia and inflammation of the squamous and respiratory epithelia and squamous metaplasia of the respiratory epithelium. Goblet cell hyperplasia and olfactory epithelial hyaline degeneration occurred with lesser frequency. The number of inflammatory cells and the extent of the infiltration were related to exposure concentration. Similar histopathological changes were noted in mice. Increased incidences of squamous metaplasia of the respiratory epithelium occurred in both sexes at 250 ppb and in females at 125 ppb. Hyaline degeneration of the respiratory epithelium was increased in all exposed groups of females, but not in a dose-related fashion. In these studies, it was concluded that there was no evidence of carcinogenic activity of GA under the conditions examined.

In a study of mice exposed to GA vapor at 100 ppb for 6 h/d, 5 d/wk, for 52 or 78 wk, similar histopathological findings were obtained[90]. Hyperplasia of the squamous epithelium lining the dorsal wall and lateral aspect of the atrioturbinate was observed in females and confined to the nasal vestibule. Additionally, epidermal erosion and ulceration along with squamous and inflammatory exfoliation were noted in the nasal cavity. The severity of these changes depended on the length of GA exposure.

When rats were given GA in drinking water at concentrations of 50, 250 and 1,000 ppm for 52–104 wk, average daily GA consumptions were 4, 17 and 64 mg/kg for males and 6, 25 and 86 mg/kg for females, respectively. Gross and histological findings of gastric irritation were found principally in the rats of the 1,000 ppm group that were euthanized at 104 wk or died during the study[91]. Bone marrow hyperplasia and renal tubular pigmentation were observed and were most likely related to the low-grade hemolytic anemia that accompanies large granular lymphocytic leukemia (LGLL)[92]. The incidence of LGLL in the spleen was elevated at all exposure levels at 104 wk. However, it was difficult to conclude that GA was responsible for the LGLL occurrence, since (i) the incidence of LGLL was increased only in females, and (ii) LGLL occurs naturally in the rat[93].

Reproductive and developmental toxicity

The frequencies of spontaneous abortions were 11.3% among hospital sterilizing staff exposed to ethylene oxide, glutaraldehyde or formaldehyde, and 10.6% for nursing auxiliaries (controls)[94]. When the staff performed sterilizing procedures during pregnancy, the frequency was 16.7% compared with 5.6% for non-exposed pregnancies. The increase in frequency of spontaneous abortion was correlated with exposure to ethylene oxide but not with that to glutaraldehyde or formaldehyde.

In a case-control study of nurses with spontaneous abortion or children with congenital anomalies and nurses with normal births as controls, no significant increase in risk of spontaneous abortion or of malformation in the offspring was observed after exposure to sterilizing gases and soaps[95]. In these studies, information on exposure was obtained from questionnaires or interviews, and measurement of environmental exposure was not performed.

Adult male and female rats (F_0) and their offspring (F_1) were administered GA in drinking water at concentrations of 0, 50, 250 or 1,000 ppm for a 10-wk prebreeding period and throughout mating, gestation and lactation[96]. Average daily consumption of GA ranged from 4.25 to 98.37 mg/kg/d for F_0 parents and from 4.53 to 99.56 mg/kg/d for F_1 parents. Although parental body weights, body weight gains and food consumption were significantly reduced at 1,000 ppm for F_0 and F_1 parents, there were no effects on parental fertility or mating performance or on pup viability or litter size in any generation. There was no evidence of reproductive toxicity, and the NOEL for this study was therefore >1,000 ppm.

Pregnant mice were given Sonacide (2% GA) by gavage on days 6–15 of gestation[97]. Doses of 100 mg/kg/d of GA killed 19 of 35 dams and significantly reduced the mean weight gain of surviving mothers. In addition, this dose significantly increased the number of fetuses with stunted growth, and the incidence of congenital malformations was elevated.

When pregnant rats were given 0, 25, 50 or 100 mg/kg of GA by gastric intubation on days 6–15 of gestation, a significant increase in maternal death, significant decreases in maternal body weight gain and food consumption, and significantly decreased fetal weight were observed in the 100 mg/kg group[98]. Since no congenital malformations were found in any group administered GA, it was concluded that GA had no

teratogenic effects on rat offspring even at a dose which induced severe maternal toxicity.

Epidemiological Studies on Workers in Biomedical Fields

Workers in endoscopy units

A cross-sectional study of 135 nurses (including 105 nurses working in operating rooms) using GA solutions for cold instrument sterilization was conducted in 26 hospitals in South Australia[99]. Three hospitals used 2% GA solution, and the remaining 23 hospitals used 1% GA solution. Geometric mean concentration of personal exposure was 0.032 ppm, while environmental concentration was 0.008 ppm. In cases in which a local air exhaust system was installed, exposure levels in operating rooms and endoscopy units were 0.014 ppm and 0.022 ppm, respectively. Without such an exhaust system, exposure levels in operating rooms and endoscopy units increased to 0.034 ppm and 0.093 ppm, respectively. Nurses exposed to GA had significantly higher prevalence of headache, fatigue and irritation of skin, eye and throat than those without GA exposure. However, the prevalence of these symptoms was, with the exception of skin irritation, inversely correlated with personal GA exposure. The authors suggested that there might be survivor bias or the most GA-tolerant nurses were in the exposed group.

A total of 319 individuals currently working as endoscopy nurses and 18 ex-employees were surveyed by symptom questionnaires and underwent respiratory function testing, skin prick tests, immunological testing and measurement of exposure to GA[100]. Work-related contact dermatitis was reported by 44% of current and ex-workers. The prevalence of sensory irritation in ex-workers was much higher than that in current workers. The mean percentage predicted FEV_1 for ex-workers was significantly lower than that for current workers. The mean peak GA concentration measured over the short period of a biocide changeover was 0.015 ppm (range: <0.00024–0.024 ppm), and the mean background concentration was 0.0024 ppm (range: <0.00049–0.26 ppm). Significant relationships were found between peak GA concentration and work-related chronic bronchitis (relative risk (RR)=1.6) and nasal symptoms (RR=1.2), but not with other symptoms. Peak GA concentrations were significantly higher in units that used both negative-pressure rooms and decontaminating unit ventilation.

We investigated which type of biocide was used in endoscopy units in 112 hospitals[101]. Fifty (45%) hospitals used GA, 29.5% used GA alone and 15.2% used GA with OPA or peracetic acid (PAA).

Koda *et al.*[102] reported that GA was not detected (<0.2 ppm) in a well-ventilated endoscopy unit, but 0.1–0.8 ppm GA was detected in another unit without a general ventilation system. The workers in the poorly ventilated endoscopy unit complained of irritation, headache and skin symptoms.

Decontamination of instruments for endoscopy is performed in two ways, by dipping instruments in a container and with an automatic decontamination machine. The dipping method is expected to generate higher airborne GA concentrations than the automatic machine method. Work environment GA concentrations were measured during decontamination of endoscopy instruments in 19 hospitals in Japan[101]. The mean airborne GA concentrations in rooms with use of an automatic machine and the dipping method were 2.9 ppb (range: 0.03–14.6 ppb, n=13) and 10.3 ppb (range: 2.0–36.1 ppb, n=6), respectively. The mean air GA concentration in the rooms with use of the dipping method was significantly higher than that in those with use of the machine method, but in some hospitals high GA concentrations were found in the units using machinery washing. The workers complained of dry skin, irritation of the throat, eye irritation and itching of hands during the work of the biocide changeover.

Workers in X-ray departments

Since the 1980's, in the United Kingdom (UK), radiographers and darkroom technicians engaged in processing of X-ray films have been reported as developing a variety of symptoms, including asthma, sinus pain, sore eyes, blurred vision, aching and ringing ears, pulmonary and ear infections, weight loss, fatigue, dry skin, inflamed nostrils and nausea, together known as "darkroom disease"[103–106].

Not only GA but many other chemicals, such as acetic acid, hydroquinone, glycol, sodium sulphite and potassium hydroxide are constituents of the developers used in X-ray film processing[69, 70, 107, 108]. Recently, GA concentrations in X-ray developers have risen to compensate for the reduced silver content of film[9, 107]. Development of occupational asthma has been reported in workers in X-ray departments, as shown in Table 3. Although airborne levels of GA in the breathing zone were reported to be less than 0.002 ppm[9], GA concentration above the developer solution tank was 0.13 ppm[108].

It was reported that radiographers (n=588) consistently exhibited increased prevalence of respiratory, musculoskeletal and other somatic symptoms compared with a control group of physiotherapists (PTs) (n=628), and that such symptoms were related to specific aspects of exposure at work to radiographic processing chemicals[105]. Since inadequate ventilation and frequent detection of X-ray processing chemical odor were significantly related to these symptoms, chemical exposure may have caused these symptoms. On the other hand, Nallon *et al.*[104] reported that radiographers were no more symptomatic than a group of hospital staff not

exposed to processing chemicals, since radiographers had significantly higher incidences than PTs for the symptoms of bad taste and sore eyes, but PTs had significantly higher incidences of sore throat and nasal discharge. Moreover, a mail survey[106] of 1,483 medical radiation technologists (MRTs) and 1,545 PTs revealed that MRTs had excessive numbers of symptoms consistent with DRD. There were significant associations between these symptoms and self-reporting of increased psychosocial stressors and increased workplace exposure to expected mucous membrane irritants. Thus, aside from chemical exposure in the workplace, workload and psychosocial stressors may affect the occupational health of radiographers.

Assessment of Exposure

Because the most important toxicity of GA is irritation and sensitization of the eyes, skin and respiratory tract, peak concentration should be determined for exposure management, and many countries including Japan are in fact making use of ceiling concentrations or limits to short-term exposure levels (STEL).

According to guidelines for preventive measurement of GA exposure issued by the MHLW[8], GA should be measured at the place and time when its level is highest, and the obtained value should be compared with the guideline value of 0.05 ppm. A Threshold Limit Value-Ceiling of 0.05 ppm is set for GA by the American Conference of Governmental Industrial Hygienists (ACGIH) in the US[6], 8 h Time Weighted Average (TWA) and 15 min period of 0.05 ppm by the Health and Safety Executive (HSE) in the UK[5], and Maximale Arbeitsplatzkonzentrationen (MAK; maximum concentration at the workplace) of 0.05 ppm and momentary value of 0.2 ppm by the Deutsche Forschungsgemeinschaft (DFG) in Germany[7].

Gannon *et al.*[9] reported five cases of occupational asthma due to GA in endoscopy departments, in which median short-term levels were 0.04 ppm during decantation in endoscopy suites. Not only Pisaniello *et al.*[99] but also Vyas *et al.*[100] reported that nurses exhibited significantly higher prevalences of various symptoms with levels of exposure below 0.05 ppm GA. This level is lower than the recommended ones noted above.

Kumagai *et al.*[109] tested whether urinary GA can be used as an indicator of biological exposure with six workers engaged in cold sterilization of endoscopy instruments. When personal exposure levels were 1–5 ppb for 8-h TWA and 6–22 ppb for STEL, urinary GA concentrations of the six workers ranged from the not detected (ND) level to 4.5 μg/g creatinine at the start of work, and from ND to 4.3 μg/g creatinine at the end of the work. The urinary GA levels of 12 volunteers were equal to the workers' levels of from ND to 4.3 μg/g creatinine. That report's authors therefore concluded that urinary GA cannot be used as an index of biological exposure in cases of low exposure since increase in urinary GA with exposure was not detected at less than 50 ppb exposure.

There has been no examination of glutaric acid as other biomarker. However, glutaric acid cannot be a proper biomarker because it is generated from lysine through the metabolic pathway and glutaric aciduria is a congenital disease in which large quantities of glutaric acid are excreted in urine[110]. No report on other biomarkers of GA metabolites is available.

Preventive Measures

GA is an eye, skin and respiratory tract irritant and skin and respiratory tract sensitizer. Generally, alkalinized 2–3.5% GA aqueous solution is used for cold sterilization of endoscopy instruments. GA concentrations of commercial products range from 3 to 20%, and a 20% GA solution is diluted to 2% at use. Since these levels of GA solution produce moderate to severe irritation of the skin, wearing gloves is essential to prevent hazards to the skin. When the permeability of gloves was tested with 2% or 3.4% GA solutions, nitrile rubber, butyl rubber, a synthetic surgical glove and polyethylene were each impermeable for at least 4 h, but latex gloves exhibited breakthrough at 45 min[111]. With 50% GA, only butyl rubber and nitrile rubber were impermeable for 4 h[111]. When changing sterilization solutions, workers are exposed to high concentrations of GA solution, and should therefore wear butyl rubber or nitrile rubber gloves.

In addition, airborne GA concentrations can be high during the changing of GA solutions or dipping of instruments by hand. Since the vapor pressure of GA is low but its airborne concentration depends on the temperature of aqueous solution[29], the temperature of the solution should be kept low, and a respirator may be necessary. When auto decontamination machines are used, push-pull-type ventilation is recommended by the MHLW[8]. Since very low levels of GA exposure, such as below 0.024 ppm, can generate airway symptoms[100], and there is no information about NOAEL on irritation and sensitization, it is hard to establish threshold levels of GA exposure. In order to keep GA exposure as low as possible, we recommend the use of closed-system, fully automated washing machines.

In order to suppress volatilization of GA in X-ray film processing rooms, room temperature should be kept at 20–24°C with 40–70% humidity and a rate of ventilation of 10–15 times/h[107]. A survey of X-ray film processing departments in four hospitals revealed that GA was detected in neither ambient air nor in the exhaust duct from automatic film processors[108]. Thus, automation of film processing and improvement of developers and fixers can reduce GA exposure. Since the most available factors to determine GA exposure levels are the number of the films to develop per machine, time spent in processing

departments and installation of local exhaust ventilation[112], these factors are used as indicators of reduction of exposure of workers to GA.

Recently, three alternatives to GA, i.e., OPA, succinic dialdehyde (SDA) and PAA, have been introduced for use in endoscope disinfection. Use of them may increase significantly in the future. However, the HSE[113] suggested that OPA and SDA may have the potential to cause occupational asthma, based on knowledge that both aldehydes exhibited irritation effects and had similar molecular structures to GA. The HSE considered PAA likely to cause chronic inflammation of the upper respiratory tract following repeated exposure[113]. In fact, OPA has been reported to cause anaphylaxis[114]. Although little is known concerning the risks to employees of GA alternatives, their use is widespread[115]. Toxicological studies of these alternatives are therefore required.

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