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RESEARCH ARTICLE

A Preliminary Quantitative Risk Assessment for Inhalation Exposure to Glutaraldehyde

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ABSTRACT

Glutaraldehyde (Chemical Abstracts Service [CAS] registry number 111-30-8) has various occupational uses and is associated with adverse health effects including respiratory tract irritation, asthma, and chronic obstructive pulmonary disease. A quantitative risk assessment was conducted to evaluate the likelihood of adverse health effects associated with differing levels of occupational inhalation exposure to glutaraldehyde. Dose–response models were fit to data from a 2-year glutaraldehyde inhalation exposure bioassay conducted by the National Toxicology Program. The benchmark concentration lower bound values of 32 and 44 parts per billion (ppb) were based on bioassay data for female rats and mice that developed squamous epithelium inflammation and respiratory epithelium squamous metaplasia, respectively. These values were used as a point of departure to determine exposure levels relevant to the occupational setting. Extrapolation from rodents to humans assumed a 40-h workweek and an 8-fold uncertainty factor to account for interspecies and interindividual variability. Adjusted benchmark lower bound concentrations of 3 and 4.1 ppb were calculated for inhalation exposure to glutaraldehyde using the endpoints observed in rat and mouse models. Due to the extrapolation parameters used in deriving this result, these findings have applicability for exposure to glutaraldehyde in the occupational setting.

1 | Introduction

Glutaraldehyde (Chemical Abstracts Service registry number 111-30-8; National Institute for Occupational Safety and Health 2019) is a clear, viscous, colorless liquid with a pungent odor (International Labour Organization, World Health Organization 2022). It is used in various occupational contexts including disinfecting medical equipment, as a cross-linking and tanning agent, as a biocide in metalworking fluids and oil and gas pipelines, as an anti-microbial agent in water-treatment systems, as a tissue fixative in histology and pathology laboratories, as a hardening agent in developing x-rays, and as a component of embalming solutions, in paper manufacturing, in the preparation of grafts and other bioprosthetic devices, and in other clinical applications (Aranke et al. 2021; Beauchamp et al. 1992; Di Martino 2021; Gupta, Perwez, and Sardar 2020).

Glutaraldehyde has been reported to have various adverse health effects. It is a known human skin and respiratory irritant (Beauchamp et al. 1992). While evidence does exist in the published literature that irritant and inflammatory changes in the upper respiratory tract can affect the long-term human respiratory health of the lower airways, such as the risk for asthma, the specific pathways underlying such associations are areas of evolving clinical understanding and research (Saranz et al. 2017). Case reports and case series have described occupational asthma associated with glutaraldehyde exposure (Chan-Yeung et al. 1993; Copeland and Nugent 2015;

Corrado, Osman, and Davies 1986; Gannon et al. 1995; Sutton et al. 2007). An analysis of data from a cohort of nurses suggested an association of chronic obstructive pulmonary disease (COPD) with regular chemical disinfectant use, including use of glutaraldehyde (Dumas et al. 2019). Glutaraldehyde is structurally like formaldehyde. Formaldehyde has been designated a human carcinogen by the International Agency for Research on Cancer (Cogliano et al. 2005) based on early in vitro studies suggesting possible tumorigenic effects. Due to the structural similarity of glutaraldehyde to formaldehyde, studies have examined whether glutaraldehyde may be carcinogenic (National Toxicology Program 1999; Van Miller et al. 2002; Zeiger, Gollapudi, and Spencer 2005). The National Toxicology Program (NTP) has concluded that there was no evidence of carcinogenic activity of glutaraldehyde under the conditions of 2-year inhalation toxicology studies in male or female F344/N rats, or B6C3F1 mice (National Toxicology Program 1999).

Despite published cases on glutaraldehyde effects in humans, no human data found in the published literature were suitable for dose–response modeling. Dose–response relationship data on respiratory health effects were found in experimental studies in animals. Chronic, 2-year, NTP studies conducted via whole-body air exposure reported a range of non-carcinogenic adverse effects in rats and mice. Effects in rats included respiratory epithelial remodeling, hyperplasia and metaplasia; olfactory epithelial degeneration; and nasal vestibular/anterior nares epithelial inflammation, while effects in mice spanned respiratory health effects of nasal passage/turbinate respiratory epithelial inflammation, exfoliation, erosion, and metaplasia, as well as laryngeal inflammation and metaplasia (National Toxicology Program 1999). Inflammatory and hyperplastic effects are markers of irritation (Renne et al. 2009). The health effects observed in these studies indicate that inhalation exposure to glutaraldehyde causes damage to the upper respiratory tract. These types of changes are also biologically relevant for adverse upper respiratory tract effects in humans (Harkema, Carey, and Wagner 2006; Renne et al. 2009; Saranz et al. 2017). This paper describes a quantitative risk assessment conducted using data from these NTP (1999) animal studies to extrapolate to the context of occupational inhalation exposure to glutaraldehyde.

2 | Methods

2.1 | Data Sources and Health Effects

Data considered for this analysis were those with measurements of inhaled glutaraldehyde exposure dose and respiratory system responses. Targeted literature searches were conducted for dates through August 8, 2022, of the OVID system, focusing on the PubMed, Embase, and CAB Abstracts databases using the terms glutaraldehyde, exposure, and quantitative risk assessment. The purpose of this search was to identify critical data for this dose–response analysis and any other risk assessments that may have been published in the literature. The literature searches in different databases were intended to identify dose–response data appropriate for quantitative analyses, as opposed to a comprehensive review of occupational glutaraldehyde exposure and outcome information.

Human studies with data suitable for this quantitative risk assessment were not found. However, suitable animal studies with dose–response data for inhaled glutaraldehyde exposure were identified. Study authors were not contacted as published studies and publicly available information were useable for this analysis.

Acute toxicity data for respiratory depression does exist for glutaraldehyde, in the form of the RD_{50}. Inhalation exposure to a peripheral sensory irritant leads to a reflex decrease in breathing rate due to trigeminal nerve stimulation. The RD_{50} is the concentration of irritant that decreases the breathing rate by 50%.

Concentration-related decreases in respiratory rate were detected when Swiss Webster mice were exposed by head-only inhalation exposure to concentrations of GA vapor ranging from 1.6 to 36.7 ppm for 30 min (Werley, Burleigh-Flayer, and Ballantyne 1995). Tolerance did not develop in most animals. The RD_{50} was 13.9 ppm (55.6 mg/m^3). Additionally, an RD_{50} of 2.6 ppm was reported in Swiss mice exposed via head-only inhalation to GA vapor concentrations from 0.7 to 4.5 ppm for 60 min (Zissu, Gagnaire, and Bonnet 1994). Since the RD_{50} values reported in the literature are far higher than the exposure concentrations causing toxicity in chronic exposure studies (detailed below), the RD_{50} data were not considered appropriate for establishing an OEL for lifetime occupational exposures.

The NTP (1993) published results from 13-week studies that used whole-body inhalation exposure in F344/N rats and B6C3F1 mice of both sexes ($N = 10$ per dose group per sex). Rats and mice were exposed by inhalation to 0, 62.5, 125, 250, 500, or 1000 parts per billion (ppb) of glutaraldehyde for 6 h per day, 5 days per week, for 13 weeks. Lesions reported in the respiratory tract of test animals were evaluated for the present analysis. The most severe lesions in rats occurred in the anterior nasal passages, involving respiratory and olfactory epithelium. The nasal cavity lateral wall and nasoturbinate tips were the most frequent sites of hyperplasia and squamous metaplasia. Mice were more sensitive than rats, potentially due to smaller airways predisposed to blockage with cellular debris, bacteria, and keratin. A no-observed-adverse-effect level (NOAEL) of 125 ppb for lesions in rats contrasted with inflammation in the anterior nasal passage occurring in mice at a lowest-observed-adverse-effect-level (LOAEL) of 62.5 ppb (lowest concentration tested).

The NTP (1999) also published results from a 2-year chronic study in F344/N rats and B6C3F1 mice of both sexes exposed to glutaraldehyde vapor by whole-body inhalation. F344/N rats underwent whole-body inhalation exposure to 0, 250, 500, or 750 ppb glutaraldehyde (Table 1). B6C3F1 mice underwent whole-body inhalation exposure to 0, 62.5, 125, or 250 ppb (Table 2). These studies had an $n = 50$ per dose group per sex, except for an $n = 49$ for the dose group of 750 ppb in female rats, $n = 48$ for the dose group of 0 ppb in male mice, and $n = 49$ for the dose group of 62.5 ppb in female mice. Exposures were 6 h per day, 5 days per week, for 104 weeks. In rats, these studies reported lung alveolar/bronchiolar adenomas in one male rat in each of the 250 and 500 ppm groups, two in the 750 ppm group, and one in a female rat in the 500 ppm group. One male rat in the 750 ppm group that had an adenoma also had a carcinoma in the lung. Neoplasm incidences were within historical control ranges, and no trends in carcinogenic respiratory tract responses were detected using

TABLE 1 | Incidence of adverse effects in the nasal region in F344/N rats in 2-year inhalation study data.[a]

Adverse effects[b]	Male/female	Incidence in each dose group (ppb glutaraldehyde) 0	250	500	750	Trend test *p*-value[c]
Squamous epithelium hyperplasia	Male	3	11	39	48	< 0.001
	Female	3	15	29	45	< 0.001
Squamous epithelium inflammation	Male	6	17	41	49	< 0.001
	Female	6	26	42	48	< 0.001
Respiratory epithelium hyperplasia	Male	6	5	17	35	< 0.001
	Female	1	6	15	29	< 0.001
Respiratory epithelium inflammation	Male	17	10	25	43	< 0.001
	Female	5	9	26	42	< 0.001
Respiratory epithelium squamous metaplasia	Male	1	2	11	24	< 0.001
	Female	1	1	11	16	< 0.001
Respiratory epithelium goblet cell hyperplasia	Male	1	0	6	6	0.005
	Female	1	3	5	8	0.005
Olfactory epithelium hyaline degeneration	Male	4	8	9	14	0.006
	Female	4	5	12	15	0.008

Abbreviation: ppb, parts per billion.
[a]Data source: National Toxicology Program (1999)
a. *N* per sex per dose group = 50 animals; exceptions: *N* = 49 for the dose group of 750 ppb in female rats
b. Timing of exposures: 6 h per day, 5 days per week, for 104 weeks
c. Survival rates for dose/number out of 50:
i. Males: 0/12, 250/14, 500/9, 750/6
ii. Females: 0/26, 250/31, 500/15, 750/14
[b]All nasal endpoints reported in the data source are presented.
[c]Cochran Armitage one-sided exact test for trend in incidence across dose groups.

statistical hypothesis tests. Glutaraldehyde inhalation did not significantly alter the number of benign and malignant lung tumors in male or female mice. No neoplasms were reported in the nose of male or female mice in any exposure group.

Several non-neoplastic respiratory tract lesions in both rats and mice exhibited a detectable trend of increasing response with increasing exposure (Tables 1 and 2). In both male and female rats, increased incidences of non-neoplastic nasal lesions were reported, mostly in the anterior section of the nose in rats exposed to 500 and 750 ppb and to a lesser extent in rats exposed to 250 ppb (National Toxicology Program 1999). The lesions included hyperplasia and inflammation of the squamous and respiratory epithelia and squamous metaplasia of the respiratory epithelium. Increased squamous metaplasia of the respiratory epithelium was observed in male and female mice exposed to 250 ppb and female mice exposed to 125 ppb. In female mice exposed to 250 ppb, there was increased incidence of inflammation of the nose. In all exposed groups of female mice, the incidence of hyaline degeneration of the respiratory epithelium was increased.

The 2-year rat and mouse studies (National Toxicology Program 1999) were considered to have the best data for this quantitative risk assessment intended to have relevance for the occupational setting. Since the 2-year exposure is more comparable than a shorter exposure to a full working lifetime, it is preferred to the 13-week data. While lung effects were observed in the rat model in these studies, they were not observed in mouse models. Since nasal effects were reported in two rodent species, extrathoracic effects were the focus of this analysis. Extrathoracic effects are defined as those occurring in the upper respiratory system, including the nasal and oral passages, the pharynx, and the larynx (Cheng 2003). Endpoints reported in the 2-year NTP studies (1999) were examined using the Cochran-Armitage trend test (Armitage 1955; Cochran 1954) using the Benchmark Dose Software (BMDS) version 2.6.0.86 (US Environmental Protection Agency, 2015). This earlier version of BMDS was initially used to conduct trend tests on these data at the start of this work; however, recent versions of BMDS available through to the time of the present analysis had not added this option. Although BMD modeling was conducted using a more recent version of BMDS, the trend test results obtained using version 2.6.0.86 are included here.

2.2 | Risk Assessment Methods

2.2.1 | Dose–Response Modeling and Choice of Point of Departure

The risk assessment followed benchmark dose–response regression modeling procedures (Crump 1984; Gaylor et al. 1998; National Institute for Occupational Safety and Health 2020).

TABLE 2 | Incidence of adverse effects in the nasal region in B6C3F1 mice in 2-year inhalation study data.[a]

Adverse effects[b]	Male/female	Incidence in each dose group (ppb glutaraldehyde) 0[a]	62.5	125	250	Trend test *p*-value[c]
Respiratory epithelium squamous metaplasia	Male	2	5	6	9	0.020
	Female	7	11	16	21	< 0.001
Respiratory epithelium hyaline degeneration	Male	ND				
	Female	16	35	32	30	0.025
Inflammation	Male	ND				
	Female	6	7	13	14	0.015

Abbreviations: ND, no data reported in original study; ppb, parts per billion.
[a]Data source: National Toxicology Program (1999)
a. *N* per sex per dose group = 50 animals; exceptions: *N* = 48 for the male dose group of 0 ppb, *N* = 49 in the female dose group of 62.5 ppb
b. Timing of Exposures: 6 h per day, 5 days per week, for 104 weeks
c. Survival rates for dose/number out of 50:
i. Males: 0/31, 62.5/27, 125/40, 250/38
ii. Females: 0/34, 62.5/37, 125/35, 250/32.
[b]All nasal endpoints reported in the data source are presented.
[c]Cochran Armitage one-sided exact test for trend in incidence across dose groups.

Briefly, parametric regression models were fit to bioassay data to estimate the benchmark concentration (BMC) corresponding to 10% extra risk (i.e., the benchmark response, BMR). Extra risk is calculated as $[P(d) - P(0)]/[1 - P(0)]$, where $P(d)$ is the probability of an adverse response at dose, d, and $P(0)$ is the probability of response at zero dose (i.e., background risk).

Dose–response modeling and estimates of BMCs were obtained using Version 3.2.0.1 of the US Environmental Protection Agency (USEPA) Benchmark Dose Software (BMDS) (US Environmental Protection Agency 2022). The suite of models examined included the dichotomous Hill, gamma, logistic, log-logistic, multistage, probit, log-probit, quantal-linear, and Weibull dose–response functions. Here, these fits assumed a binomial response distribution, and these models were used with default restrictions. Frequentist models with chi-square goodness-of-fit test p values of 0.1 or greater were considered to fit the data adequately. To account for potential model misspecification, BMC and benchmark concentration lower bound (BMCL) values were estimated using the Bayesian model-averaging (MA) method in BMDS, which weights multiple models based on posterior model probabilities. For the MA method, all available frequentist models in the BMDS software were included. For comparison, BMC and BMCL values were also estimated from individual model fits using the BMDS software.

In individual regression model results, functions labelled as viable, recommended models were selected for each endpoint, where viable, recommended models are defined following guidelines in the BMDS User Guide (US Environmental Protection Agency 2022). The endpoints with the smallest 95% lower bound on the BMC, the BMCL, were selected as sensitive endpoints. One endpoint was selected from the series of studies in rats and one from the series of studies in mice. Characteristics of the estimated dose that were considered included aspects of the data and modeling results and the BMCL value of which served as the point of departure (PoD), to account for modeling uncertainty. Additional aspects of data and modeling results that were considered included (1) the range of viable model BMCLs, (2) comparison of the viable model AIC (Akaike information criterion) values, and (3) the ratio between the BMC/BMCL and the nearest non-zero exposure data point. The BMC and BMCL values of these endpoints were used for calculating adjusted values with relevance for the occupational setting.

2.2.2 | Exposure Duration and Dose Adjustment Factor

Calculation of adjusted BMC and BMCL values require the application of a factor to account for exposure duration and a dose adjustment factor (DAF). Toxicologically based BMC and BMCL estimates reflect experimental conditions, including the number of hours per day and number of days per week that animals were exposed. For the NTP studies (National Toxicology Program 1999), an experimental protocol was used with exposures of 6 h per day, 5 days per week, for a total of 30 h per week (National Toxicology Program 1993; National Toxicology Program 1999). To extrapolate to an exposure duration for a 40-h workweek, the PoD value was multiplied by 30/40 or 0.75.

Glutaraldehyde inhalation toxicity for site of contact effects does not appear dependent on systemic metabolism. Instead, observed adverse effects in chronic bioassay study results involve inflammation, remodeling, hyperplasia, and metaplasia of the respiratory and olfactory epithelium from local contact; pathological changes frequently observed as a response to irritant gases (Monticello, Morgan, and Uraih 1990; Renne et al. 2009). Thus, for gases producing extrathoracic effects, the animal to human interspecies extrapolation is based on a 1:1 equivalency of the inhaled concentration in humans and rodents (US Environmental Protection Agency 2012). Thus, a PoD_{adj} is derived to be the animal POD with only a DAF = 0.75.

2.2.3 | Method for Calculation and Application of Uncertainty Factors

The determination of a POD often incorporates the adjustment of BMC and BMCL values and can require the use of uncertainty factors (UF). The UF accounts for interspecies (animal-to-human) uncertainty and interindividual variability. The calculation of the total uncertainty factor (UF_{Total}) in this study followed previously promulgated methods (International Programme on Chemical Safety 2005). The scheme, originally proposed by Renwick (1993), separately considers the contribution of interspecies and interindividual differences in toxicokinetics and toxicodynamics.

The interspecies (animal-to-human) uncertainty factor (UF_{AH}) is subdivided into values of 2.5 for toxicodynamics (UF_{AHTD}) and 4.0 for toxicokinetics (UF_{AHTK}) (Dankovic et al. 2015). The endpoint for this risk assessment, however, was a site of contact effect. An UF_{AHTK} of 1 was used to represent the assumption that toxicokinetic mechanisms for a site of contact effect are similar across species. This resulted in an interspecies $UF_{AH} = UF_{AHTD} * UF_{AHTK} = 2.5 * 1 = 2.5$.

The interindividual UF (UF_{Indiv}) also has subcomponents of toxicokinetics ($UF_{IndivTK}$) and toxicodynamics ($UF_{IndivTD}$), with values of 3.2 for each (Dankovic et al. 2015). Occupational risk assessments, when based on site of contact effects, however, have used lower values such as 1 for the $UF_{IndivTK}$ portion of the UF_{Indiv} (Dankovic et al. 2015). For this analysis, the value of 3.2 was applied for the $UF_{IndivTD}$ and the value of 1 was applied for the $UF_{IndivTK}$ components and the total $UF_{Indiv} = UF_{IndivTD} * UF_{IndivTK} = 3.2 * 1 = 3.2$.

Combining the interspecies and interindividual uncertainty factors, the $UF_{Total} = UF_{AH} * UF_{Indiv} = 2.5 * 3.2 = 8$. The determination of adjusted BMC and BMCL values for an 8-h time-weighted average (TWA8) is based on the PoD derived from model averaging results (National Institute for Occupational Safety and Health 2020). The PoD_{adj} in this study was the PoD multiplied by the exposure duration and DAF. The calculation of the adjusted BMC and BMCL values equaled the PoD_{adj} (for the BMC or BMCL, respectively) divided by the UF_{Total}, which was a calculation of $PoD_{adj}/8$.

3 | Results of Risk Assessment

3.1 | Choice of Critical Endpoints and Dose–Response Modeling

Endpoints from the nasal region in the 2-year study of inhalation exposure to glutaraldehyde in rats and mice (National Toxicology Program 1999), with a significant ($p < 0.05$), positive, one-sided Cochran-Armitage trend test (Cochran 1954; Armitage 1955; US Environmental Protection Agency 2020) for incident counts across dose groups, were chosen for evaluation in dose–response models. Trend tests were significant for all adverse nasal effects reported for male and female rats and mice (Tables 1 and 2).

Table 3 presents viable, recommended models from the results of dose–response modeling analyses using the BMDS 3.2.0.1

TABLE 3 | Results of model average benchmark dose procedures for adverse effects in the nasal region in rats and mice after 2-year inhalation exposure to glutaraldehyde.

Animal	Adverse effect	Sex	Viable, recommended frequentist model from analysis using BMDS version 3.2.0.1; U/R; *p*-value	Glutaraldehyde (ppb)			
				FM BMC	FM BMCL	MA BMC	MA BMCL
Rats	Squamous epithelium hyperplasia	M	Logistic; U; 0.5	157	123	161	111
		F	Probit; U; 0.6	141	115	138	81
	Squamous epithelium inflammation	M	Multi D3; R; 0.6	160	75	126	79
		F	**Multi D1; U; 0.1**	**31**	**25**	**71**	**32**
	Respiratory epithelium hyperplasia	F	Probit; U; 0.9	274	227	264	165
	Respiratory epithelium inflammation	F	Multi D2; R; 0.4	192	139	221	138
	Respiratory epithelium squamous metaplasia	M	Logistic; U; 0.8	382	319	364	279
		F	Multi D2; R; 0.3	384	282	398	294
	Respiratory epithelium goblet cell hyperplasia	F	Multi D1; U; 0.9	545	339	663	440
	Olfactory epithelium hyaline degeneration	M	Probit; U; 0.8	424	317	459	263
		F	Probit; U; 0.7	370	148	390	246
Mice	Respiratory epithelium squamous metaplasia	M	Gamma; U; 0.9	149	16	222	119
		F	**Multi D1, 3; R; 0.95**	**64**	**42**	**87**	**44**
	Olfactory epithelial hyaline degeneration	F	Log-logistic; R; 0.7	108	58	150	74

Note: The results for the most sensitive endpoint, in each species, are bolded.
Abbreviations: BMC, benchmark concentration; BMCL, lower 95% confidence bound on the BMC; D1, degree = 1; D2, degree = 2; D3, degree = 3; FM, frequentist model; MA, model average; Multi, multistage; ppb, parts per billion; *p*-value, chi-square goodness of fit *p*-value for model from BMDS analysis using version 3.2.0.1 (US Environmental Protection Agency 2022); R, restricted; U, unrestricted.

software (US Environmental Protection Agency 2022) for each endpoint evaluated. One endpoint in female rats and one in female mice were found to be the most sensitive endpoints, based on the BMCL values of the viable, recommended models. In female rats, the endpoint was squamous epithelium inflammation (function: multistage [degree = 1; unrestricted]; goodness-of-fit *p* value: 0.1; BMC: 31; BMCL: 25). In female mice, the endpoint was respiratory epithelium squamous metaplasia (function: multistage [degree = 1 and degree = 3; restricted]; goodness-of-fit *p* value: 0.95; BMC: 64; BMCL: 42). We note that the NTP did not report severity grade information for the endpoint of squamous metaplasia; therefore, a dichotomous modelling approach was used for this risk assessment. The BMC and BMCL values from the individual model fits were similar to the model average results for both endpoints (Table 3). Individual models demonstrated a range of BMC and BMCL values (results not presented). These models contribute model-variability that is reflected in MA results (US Environmental Protection Agency 2020). Simulation studies by Wheeler and Bailer (2009) have concluded that the lower bound estimate (BMCL) from a MA approach provides superior statistical coverage compared to selecting an individual frequentist model. Therefore, the MA results were the primary results of this analysis and the basis for conclusions regarding the toxicity of glutaraldehyde.

Figures 1 and 2 depict graphical representations of dose–response curves for viable, recommended individual functions that resulted from assessing each of these endpoints. All curves show a reasonable shape reflecting the monotonically increasing dose–response relationship expected based on trend test results.

The MA results for the endpoint of squamous epithelial inflammation in female rats were a BMC of 71 ppb and a BMCL of 32 ppb (Table 3). The dose–response curve for the endpoint of squamous epithelial inflammation in the female rat study is presented in Figure 1. The greater incident counts in the two highest dose groups result in the upper portion of the dose–response curve approaching very close to a 100% response. The BMC and BMCL values, however, occur at very low values relative to the lowest non-zero concentration group used in the study. The modeling of the data for this endpoint raised a concern that use of these results may lead to an unreliable calculation of adjusted BMC and BMCL values. EPA guidelines for BMDS (US Environmental Protection Agency 2020) suggest that a BMCL < one-third of the lowest non-zero dose used in the study raises a concern about the BMCL and a BMCL < 10% of the lowest non-zero dose raises a very serious concern about the adequacy of the BMC/BMCL values as a basis for extrapolation. In this case, the BMCL was exactly 10% of the dose used in the lowest dose group, which raised a concern due to the lack of any dose–response data in the vicinity of the BMCL.

The MA results for the endpoint of respiratory epithelium squamous metaplasia in female mice were a BMC of 87 ppb and a BMCL of 44 ppb (Table 3). In the individual-model-based analysis, the BMDS guideline-based viable, recommended model had results using restricted functions for this endpoint. As seen by the dose–response curves in Figure 2, both multistage models with degree = 1 and degree = 3 resulted in BMC and BMCL values of 64 and 42 ppb, respectively (Table 3). The MA (44 ppb) and frequentist (42 ppb) results were almost identical for the BMCL. These results were similar to the low dose used in the mouse study (62.5 ppb) and were therefore less likely to produce a biased result compared to the that of the endpoint from the female rat data. Thus, respiratory epithelium squamous metaplasia in female mice would be the most appropriate endpoint when considering subsequent calculations of adjusted BMC and BMCL values.

3.2 | Determination of Occupationally Adjusted BMC and BMCL Values

To calculate an adjusted BMCL value, the PoD used the BMCL of 32 ppb obtained from the MA analysis of the female rat squamous epithelium inflammation and the BMCL of 44 ppb, obtained from the MA analysis of the female mice respiratory epithelium

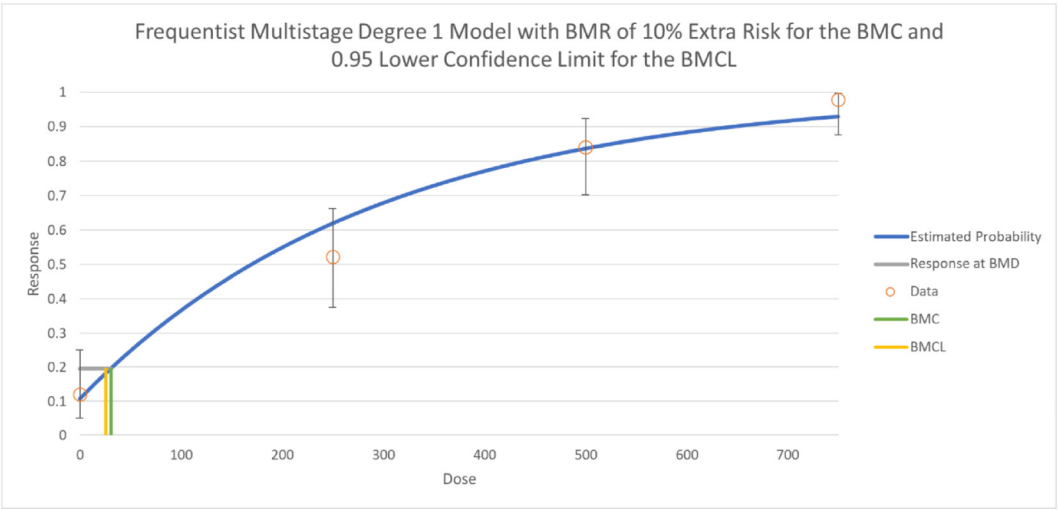
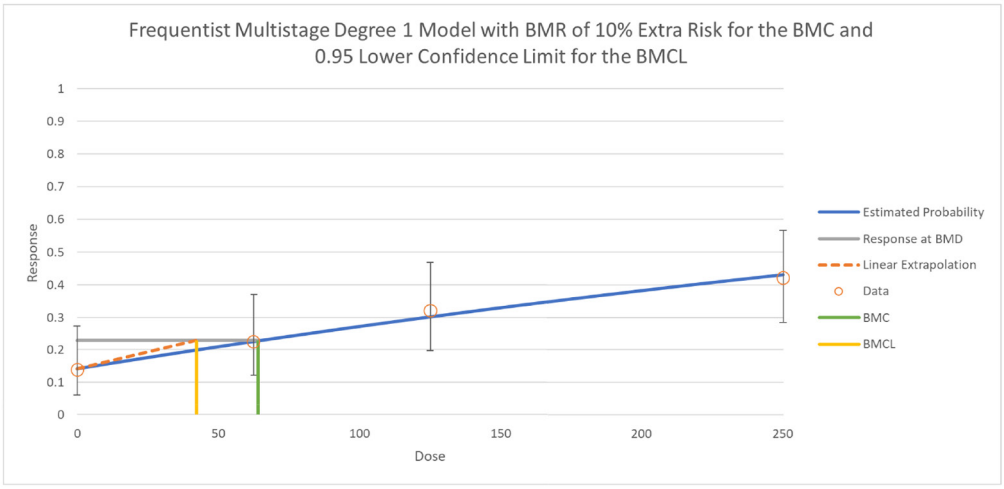


FIGURE 1 | Dose–response curve for endpoint of squamous epithelium inflammation in the nasal region of female rats. The curve presented is from the viable, recommended function for dose–response modeling using 2-year bioassay data of inhalation glutaraldehyde exposure in rats. The model was generated specifying a BMR of 10% extra risk and a 95% lower confidence limit for the BMCL. This graphical representation was generated using BMDS 3.2.0.1 (US Environmental Protection Agency 2022). BMC, benchmark concentration; BMCL, lower 95% confidence bound on the BMC.

A



B

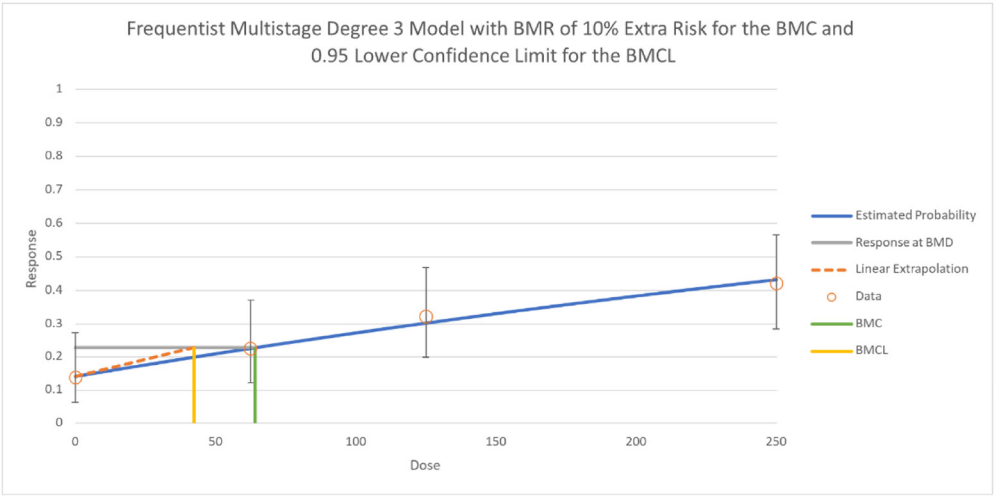


FIGURE 2 | Dose–response curves for the endpoint of respiratory epithelium squamous metaplasia in the nasal region of female mice. The curves presented are from viable, recommended functions for dose–response modeling using 2-year bioassay data of inhalation glutaraldehyde exposure in a female mouse model. The models were generated specifying a BMR of 10% extra risk and the 95% lower confidence limit for the BMCL. Graphs for multi-stage models degree = 1 and degree = 3 are in panels A and B, respectively. Graphical representations were generated using BMDS 3.2.0.1 (US Environmental Protection Agency 2022). BMC, benchmark concentration; BMCL, lower 95% confidence bound on the BMC.

squamous metaplasia from the 2-year NTP chronic bioassay data. The PoD was then adjusted as described in Section 2 for exposure duration (multiplying by a factor of 0.75) and for the DAF of 1 for rodent to human extrapolation. The adjusted PoD values were 32 ppb × 0.75 × 1 = 24 ppb and 44 ppb × 0.75 × 1 = 33 ppb. Applying the composite uncertainty factor of 8 yielded adjusted BMCL values of 24 ppb/8 = 3 ppb and 33 ppb/8 = 4.1 ppb. The BMC-based adjusted values equaled 6.7 ppb (BMC = 71) and 8.2 ppb (BMC = 87) for these same endpoints.

4 | Discussion

This quantitative risk assessment estimated adjusted BMCL values of 3 and 4.1 ppb for two endpoints representing respiratory irritant effects that, due to the approach to adjusting the PoD values, has relevance for occupational inhalation exposure to glutaraldehyde. Calculation of these adjusted BMCL values used the MA-derived BMCL of 32 and 44 ppb for the endpoints of nasal squamous epithelium inflammation in female rats and respiratory epithelium squamous metaplasia in female mice, respectively, as PoD values. The BMC and BMCL values were determined from dose–response modeling of the incidence of these endpoints from 2-year chronic inhalation glutaraldehyde exposure in rat and mouse models. Of note, the data for the endpoint of respiratory epithelium squamous metaplasia in female mice had appropriate distribution of incident counts across dose groups, adequate fit of the dose–response model, and a BMCL that was among the most sensitive of all endpoints examined. Extrapolation of the PoD values to the adjusted BMC and BMCL values assumed a 40-h workweek, mouse to human DAF of 1, and an 8-fold uncertainty factor, to account for interspecies and interindividual variability.

We note that in environmental risk assessments, as opposed to occupational, the UF_{AH} would typically be set at 3.2 rather than 2.5. The assumption of an UF_{AH} of 2.5 is consistent with NIOSH

guidelines for occupational risks assessment (National Institute for Occupational Safety and Health 2020). Assumptions regarding duration of exposure would also be different for environmental exposures.

An older method of determining a PoD for risk assessment, prior to the advent of BMD modeling, was to base the PoD on either a NOAEL, preferably, or failing that a LOAEL with an additional uncertainty factor. For example, toxicity was observed at all non-zero concentrations in the female rat squamous epithelium inflammation data (Table 1). Although NIOSH risk assessment guidelines specify the use of BMD modeling methods (National Institute for Occupational Safety and Health 2020), it is of interest to note that treating the lowest exposure concentration in the rat bioassay as a LOAEL and applying a 10-fold UF would result in a PoD identical to the BMCL from the squamous epithelium inflammation endpoint in female rats, 25 ppb. Applying the adjustments described above for occupational exposure would therefore result in the same adjusted BMCL as described above for the endpoint of squamous epithelium inflammation in female rats, 3 ppb.

The current National Institute for Occupational Safety and Health (NIOSH) REL for glutaraldehyde is 0.2 ppm as a ceiling limit (National Institute for Occupational Safety and Health 2019). The NIOSH REL is based on the 1988 Occupational Safety and Health Administration (OSHA) PEL Project update (National Institute for Occupational Safety and Health, 2018). The 1988 PEL for glutaraldehyde was a ceiling limit of 0.2 ppm, which was vacated as a PEL in 1989, but this value and the data that were used to develop it became the basis for the NIOSH REL (National Institute for Occupational Safety and Health 2018). The NIOSH REL assumes that aldehydes as a group possibly behave similarly to acetaldehyde, which is associated with nasal tumors in rats and laryngeal tumors in hamsters (National Institute for Occupational Safety and Health, 2019). Of note, although squamous metaplasia is believed to play a role in the pathogenesis of formaldehyde-induced squamous cell carcinomas of the rodent nose (Feron et al. 1988), no data presently suggests that glutaraldehyde is associated with nasal tumors.

Currently, glutaraldehyde has no permissible exposure limit (Occupational Safety and Health Administration 2022). OSHA method number 64 has reliable quantitation limits of 4.4 ppb for a target concentration of 200 ppb per sample, 0.44 ppb (for short-term samples with a target concentration of 10 ppb per sample), and 0.027 ppb (for long-term samples with a target concentration of 2 ppb per sample) (Occupational Safety and Health Administration 1998). NIOSH method 3652 for sampling and analyzing glutaraldehyde (National Institute for Occupational Safety and Health 1994) has an estimated limit of detection of 4.9 ppb for a target sample concentration range of 13–392 ppb. The OSHA and NIOSH methods have some differences with respect to sampling and derivatization techniques that could account for the differences in levels of quantitation/detection. The OSHA short- and long-term sampling methods would be adequate for quantification of either a 3- or 4.1-ppb 8-h exposure.

The Agency for Toxic Substances and Disease Registry (ATSDR) has derived Minimal Risk Levels (MRLs) for glutaraldehyde (Wilbur et al. 2017). MRL calculations presented by ATSDR apply to the general population, utilize a no-adverse-effect-level or lowest-adverse-effect-level value as a PoD, use an UF appropriate for protecting the general population, and assume an exposure period of 24 h a day for a 70 year lifetime. The MRL for chronic exposure to glutaraldehyde vapor is 0.00003 ppm, or 0.03 ppb.

Squamous epithelium inflammation and respiratory epithelium squamous metaplasia are markers of respiratory tract irritation (Renne et al. 2009). Inflammation has been described as a characteristic of chronic irritation in the respiratory tract (Kim, Rogers, and Criner 2008). Zissu, Gagnaire, and Bonnet (1994) reported respiratory epithelium squamous metaplasia associated with glutaraldehyde inhalation exposure in mice. These researchers reported similar severity for histopathologic effects 1 and 2 weeks after cessation of a 14-day exposure, with some effect resolution reported 4 weeks post-cessation, although which effects remained were not specified. Similarly, squamous metaplasia induced by even short-term formaldehyde inhalation in rats can persist up to 126 weeks after exposure (Feron et al. 1988). Since squamous metaplasia involves the replacement of one mature cell type by another mature cell type (Giroux and Rustgi 2017), persistence even after a recovery period is not surprising. Indeed, in smokers with COPD, cessation of smoking reduces, but does not eliminate, lung airway squamous metaplasia (Lapperre et al. 2007). A recent review of cyclic mechanisms of injury and abnormal repair suggests that inflammatory and metaplastic changes are important in chronic irritation of the respiratory tract (Raby et al. 2023).

Direct-acting cytotoxic vapors like formaldehyde and glutaraldehyde tend to react with the cells that line the passageways through which they are inhaled (National Research Council 2014). Therefore, rats and mice, which are obligate nasal breathers, develop changes in the epithelium of the nose when inhaling cytotoxic compounds. In contrast, humans, who are generally oropharyngeal breathers, with some people always breathing through their mouth (Hubbs et al. 2019), may suffer damage to the upper respiratory tract. The cytotoxicity of glutaraldehyde and the observation of occupational lung disease in exposed workers suggest that it may reach the upper airways of workers.

Development of other illnesses, for example, occupational asthma and COPD, are a concern for irritants that reach the lung (Dey et al. 2022; Moscato et al. 2016). Histopathologic changes that are features of COPD can include epithelial squamous metaplasia (Dey et al. 2022; Lapperre et al. 2007). Further, asthma and COPD are heterogenous diseases that can occur independently or with overlapping characteristics, but with airway obstruction occurring in both (Dey et al. 2022). A recent systematic review and meta-analysis found, across 14 studies out of a pool of 9440 screened, that exposure to glutaraldehyde increased risk for asthma in nurses (compared to other occupations) with an observed OR = 1.91 [95% CI: 1.35–2.70] (Romero Starke et al. 2021). Conversely, analysis of 277,744 person-years of follow-up (2009–2015) of 370 nurses from the prospective Nurses' Health Study II, which collected occupational information using a Job-Task-Exposure-Matrix and self-report of physician-diagnosed incident asthma, found no significant association between exposure to disinfectants and incident asthma (Dumas et al. 2020). In this study by Dumas et al. (2020), work exposure to disinfectants were assessed individually in a questionnaire to

which participants responded. In other analyses of a different subset of Nurses' Health Study II data, however, exposure to glutaraldehyde was associated with poor asthma control (Dumas et al. 2017). However, another analysis based on this same prospective nurses' study, with another subset of data for 368,145 person-years of follow-up for 582 nurses, suggested that regular use of chemical disinfectants may be associated with developing COPD (Dumas et al. 2019). In this analysis, examination of data for 125,281 person-years for 192 cases found a significant association between exposure to glutaraldehyde and incident COPD (multivariable-adjusted hazard ratio of 1.25, 95% CI: 1.04–1.51; model was adjusted for age, smoking status and pack-years [continuous], race, ethnicity, and body mass index).

Glutaraldehyde is considered a low molecular weight asthmagen (Moscato 2013). Friedman-Jiminez et al. (2015) have discussed that occupational asthma can be irritant-induced (non-allergenic) and sensitizer-induced (allergenic), although conflicting reports exist regarding the status of glutaraldehyde as a respiratory sensitizer (Occupational Safety and Health Administration 2012; Teta et al. 1995; Vandenplas et al. 2013). Thus, the use of squamous epithelium inflammation and squamous epithelial metaplasia, as endpoints relevant to irritant effects, for risk assessment is appropriate. Further investigation is needed on dosimetry of inhaled glutaraldehyde and on chemical specific mechanisms of action for the association of inhaled glutaraldehyde exposure with asthma and COPD.

Strengths of this analysis include the use of chronic bioassay data from an inhalation exposure study for dose–response modeling. Limitations of this analysis include lack of suitable human data for quantitative risk assessment, lack of cross-species information on respiratory penetration/absorption, and lack of knowledge of the inter-relationships of irritant, sensitizing, and obstructive effects of inhalation glutaraldehyde exposure.

A key question is whether glutaraldehyde may have greater toxicity in humans than in rodent models. It is possible that glutaraldehyde penetrates more deeply in the human respiratory tract than in the rodent respiratory tract, since histopathology studies suggest that formaldehyde may penetrate more deeply in the rhesus monkey respiratory tract than in the rat (Monticello, Miller, and Morgan 1991). In contrast, computational fluid dynamics model studies suggest that inhaled formaldehyde may have similar transport patterns in the rat and human nose (Kimbell 2006). Computational fluid dynamics studies of flux rates in rodent versus human anterior nasal airways also supports the use of a DAF = 1 (Corley et al. 2012). In the absence of specific knowledge of patterns of inhaled glutaraldehyde penetration into the human respiratory tract compared to rodents, a dose adjustment factor of 1 is appropriate for exposures exerting their effects at the site of contact and in the extrathoracic region (US Environmental Protection Agency 2012).

5 | Conclusions

No other quantitative risk assessments relevant to occupational exposure to glutaraldehyde were found in the published scientific literature. This risk assessment used data from a 2-year glutaraldehyde inhalation exposure study (National Toxicology Program 1999), specifically for the endpoints of squamous epithelium inflammation in female rats and respiratory epithelium squamous metaplasia in female mice and utilized benchmark dose models and model averaging techniques to calculate adjusted BMCL values. These values were adjusted for occupational exposure duration, with an uncertainty factor for interspecies and interindividual variability applied. Thus, they have relevance for occupational exposure to glutaraldehyde and irritant effects.

Author Contributions

Sudha P. Pandalai: conceptualization, data curation, formal analysis, methodology, project administration, resources, software, supervision, validation, visualization, roles/writing–original draft, writing–review and editing. **David A. Dankovic:** conceptualization, data curation, formal analysis, methodology, project administration, resources, software, supervision, validation, visualization, roles/writing–original draft, writing–review and editing.

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Disclosure

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in the National Institute of Environmental Health Sciences/National Toxicology Program at https://ntp.niehs.nih.gov/, reference numbers NTP Toxicity Report No. 25, NIH Publication No. 93-3348 and NTP Toxicity Report No. 490, NIH Publication No. 99-3980.

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