

Reviewing the evidence of antimicrobial activity of glycols

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Abstract

In the 1940s and 1950s, researchers seeking safe and novel ways to eliminate airborne pathogens from enclosed spaces, investigated glycol vapours as a method of disinfection. More recently, the COVID-19 pandemic highlighted the need for a non-toxic aerial disinfectant that can be used in the presence of people. This scoping review is intended to analyse the early and more recent literature on glycol disinfection, scrutinizing the methodologies used, and to determine if the use of glycols as modern-day disinfectants is justified.

PRISMA-ScR guidelines were used to assess the 749 articles retrieved from the Web of Science platform, with 46 articles retained after the search strategy was applied. Early studies generally demonstrated good disinfection capabilities against airborne bacteria and viruses, particularly with propylene glycol (PG) vapour. Vapour pressure, relative humidity, and glycol concentration were found to be important factors affecting the efficacy of glycol vapours. Contact times depended mainly on the glycol application method (i.e. aerosolization or liquid formulation), although information on how glycol efficacy is impacted by contact time is limited. Triethylene glycol (TEG) is deemed to have low toxicity, carcinogenicity, and mutagenicity and is registered for use in air sanitization and deodorization by the US Environmental Protection Agency. Glycols are also used in liquid formulations for their antimicrobial activity against a wide range of microorganisms, although when used as a non-active excipient in products, their contribution to antimicrobial efficacy is rarely assessed. The appropriate use of liquid glycol-containing formulations was found to positively impact the antimicrobial capabilities of disinfectants when used at temperatures <0, food preservatives, and dental medicaments. Providing modern delivery technology can accurately control environmental conditions, the use of aerosolized glycol formulations should lead to successful disinfection, aiding infection prevention, and control regimens.

Keywords: glycols; disinfection; antimicrobial; sanitization

Introduction

Antimicrobials can be defined as any substance that inhibits the growth of microorganisms or kills them. The term microbicide or biocide is reserved for compounds that kill microbes, with bactericidal, virucidal, and fungicidal compounds active against bacteria, viruses, and fungi, respectively. The term disinfectant is typically reserved for agents that kill microorganisms on non-living surfaces (McDonnell and Russell 1999). There are many types of disinfectant chemicals that can be divided according to their microbicidal efficacy against specific pathogens, and a wide range of applications, from high- to low-level disinfection (Maillard and Pascoe 2023). Most biocides are delivered as a formulated liquid or solid for surface or water disinfection, but only a few have been used for aerial disinfection. These include formaldehyde, chlorine dioxide, hydrogen peroxide, ozone, peracetic acid, and lactic acid, which tend to be highly toxic or irritant at their in-use concentrations (McDonnell 2007, Buklaha et al. 2022), but also hexylresorcinol and propylene glycol, which in the 1970s were considered to be effective as fumigants, although not against a broad spectrum of microorganisms, but importantly non-irritant and non-toxic (Hugo and Russell 1999). Following the COVID-19 pandemic, there is a renewed interest for developing non-toxic aerial disinfectants that can be used in the presence of humans or animals (Buklaha et al. 2022).

Structurally, glycols are alcohols with two hydroxyl groups located on adjacent carbon atoms. Propylene glycol (PG) is a commonly used excipient in a variety of products, including

applications in the cosmetics and pharmaceutical industries. In a formulation, PG is used not only for its antimicrobial activity but also as humectant, plasticizer, solvent, and stabilizer for vitamins (Rowe et al. 2012). As an aerial disinfectant, the renewed interest in glycols derives from their non-toxic properties compared to oxidizing and alkylating agents, such as ozone, hydrogen peroxide, or formaldehyde, but also their documented antimicrobial efficacy. Their microbicidal efficacy when used in aerosol form to decontaminate air was first evaluated in the 1940s and 1950s, but overall, their efficacy as an aerosol has not been widely investigated in comparison to oxidizing and alkylating agents used for aerial disinfection. In addition, whilst early studies investigated the factors affecting the microbicidal efficacy of aerosolized glycols, including relative humidity, hygroscopicity, and vapor pressure, the mode of action of such compounds remains largely unknown.

This review aims to establish if glycols should be repurposed as antimicrobials suitable for disinfection purposes in the 21st century.

As such, the scientific literature relating to the disinfectant and antimicrobial properties of glycols was evaluated with an aim to answer the following research questions:

- Is there evidence for the use of aerosolized glycols for environmental disinfection purposes?
- If evidence exists, what factors contribute to successful disinfection with glycols?
- What evidence exists for glycols as antimicrobial agents in liquid form?

Received 12 January 2024; revised 12 February 2024; accepted 3 April 2024

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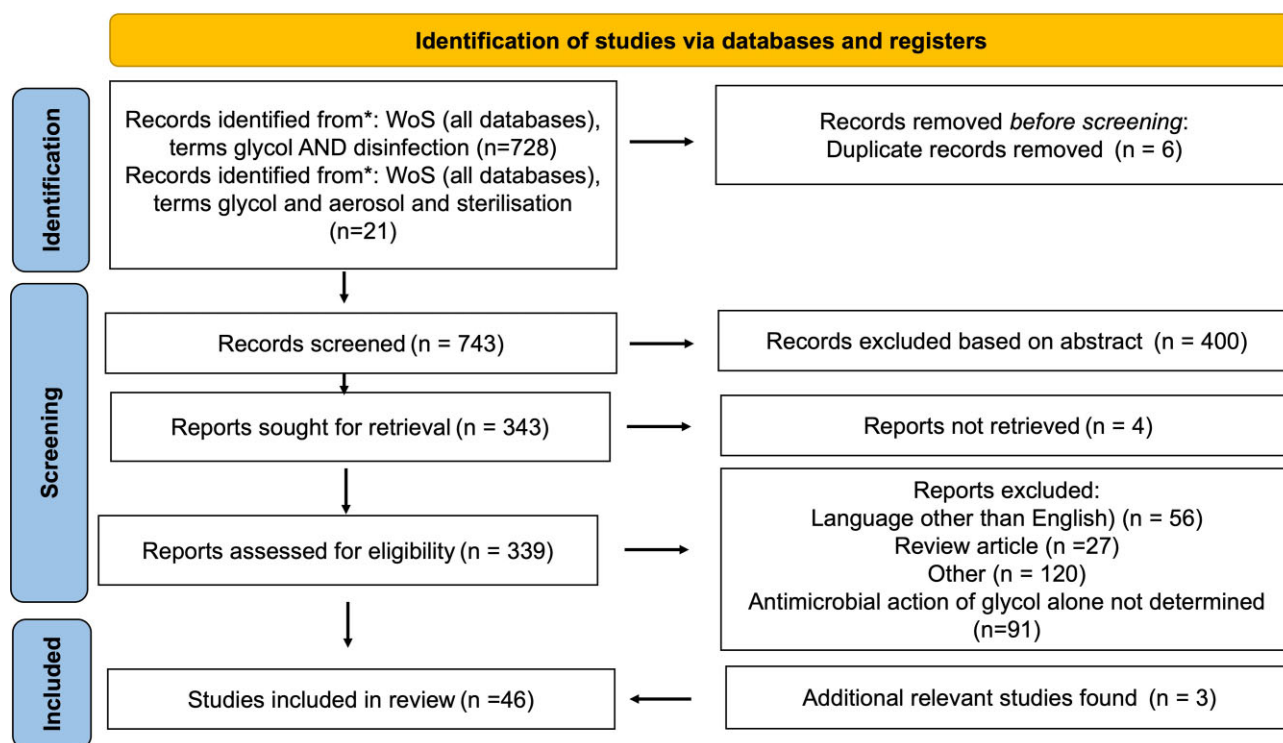


Figure 1. PRISMA diagram representing the article selection based on search terms and inclusion and exclusion criterias.

Materials and methods

Screening and eligibility criteria

This scoping review was designed to retrieve articles relating to the antimicrobial properties of glycols and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement extension for scoping reviews (PRISMA-ScR) (Tricco *et al.* 2018).

The search engine ‘Web of Science’ was selected for literature searches, with titles, abstracts, summaries, and keywords used to conduct the searches. The searches were performed between 9 December 2022 and 24 March 2023. All peer-reviewed articles from all years were eligible for selection. Articles written in a language other than English were excluded unless an English translation was available.

Search terms

The search strategy for the literature review is illustrated in Fig. 1. An initial search was performed using the terms ‘glycol’ and ‘antimicrobial’ or ‘antibacterial’, but the search was later refined to produce more relevant documents using the search terms ‘glycol’ and ‘disinfection’. A second search was performed using the terms ‘glycol’ and ‘aerosols’, and ‘sterilization’ to retrieve documents that were missed out in the initial search. Additional relevant articles that did not appear in the initial searches but were found via the reference sections of some of the retrieved articles were also included. For the searches, all databases and all years were selected. The records retrieved from the search were further analysed, and the inclusion/exclusion criteria were applied. Exclusion criteria were as follows: non-English language, meeting abstract, book, patent, editorial material, review article, and abstract. Documents were also excluded if the antimicrobial action of glycol alone was not evaluated.

The use of polyethylene glycol polymers as anti-fouling coatings has long been studied. The applications of such anti-fouling coatings are vast, including numerous studies linked to biofilm prevention in medical devices. Due to the expanse of literature covering this topic (>1300 articles in a separate search), this area will not be discussed during this review, and articles relating to this area were excluded.

Data review and recording

The search strategy was agreed upon by three researchers, with a single researcher conducting the literature searches, data extraction, and recording. All researchers discussed findings and agreed upon the final articles retained. Mendeley reference manager was used to manage all references and Microsoft Excel was used for article data extraction.

Results and discussion

The 1940s were a critical decade for the investigation of glycols as aerosolized disinfectants (Fig. 2). The 1980’s brought about an interest in glycols for the use in cooling systems, food preservatives, and as antifreeze agents in disinfectants, whilst a renewed interest emerged from late in the 2nd decade of the 21st century in glycols for the sterilization of air and surfaces (Fig. 2). But overall, pertinent peer-reviewed literature on the microbicidal efficacy of glycol remains limited.

Categories of articles retrieved

From the articles retrieved during the literature searches, it was apparent that the antimicrobial properties of glycol had been investigated for use in multiple applications across a variety of sectors (Fig. 3). Disinfection of healthcare, educational, and farming environments were reported by the use

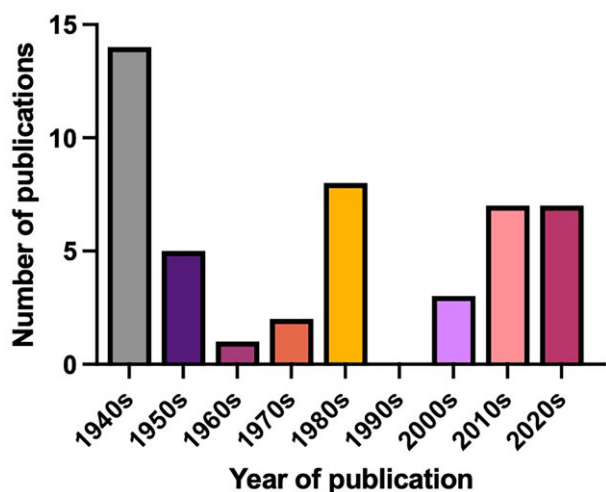


Figure 2. Number of publications retrieved during the literature search for each decade from 1940 to the present day.

of aerosolized glycol formulations. Research into the addition of glycol to aerosolized disinfectants for decontamination of cold-chain environments had also been an area of investigation. In addition, multiple studies report on the utilization of glycols for dental applications, particularly in the areas of endodontic disinfection.

The largest application (47%–22/46) of glycols was for disinfection of air. A total of 15% (7/46) of the studies were dedicated to surface disinfection and 7% (3/46) articles were

related to the disinfection of both air and surfaces (Fig. 4). The use of glycols to reduce the microbial burden of environments with temperatures $<0^{\circ}\text{C}$ (i.e. sub-zero environments) and their use in cooling systems was a focus in 15% (7/46) of the retained articles. Another notable application of glycols as antimicrobials was in the field of dentin tubule disinfection and other applications in dentistry. The use of glycols in the food industry, general studies on the bactericidal actions of glycols with no specific application, and other categories, including disinfection of laboratory personnel accounted for the remaining papers (Fig. 4).

Glycols as aerosol disinfectants

To prevent transmission of infection and disease in enclosed spaces in the presence of humans or animals, researchers in the early 1940s began to focus their efforts on the use of glycols as aerosol disinfectants, largely based on reports of low toxicity and favourable hygroscopic properties. Of the 24 articles reviewed in this study relating to aerosolized glycols, 15 included PG and 16 included triethylene glycol (TEG). Alternative glycols, including ethylene glycol, dipropylene glycol (DPG), trimethylene glycol, and 2,3-butylene glycol, were also investigated in some studies. A summary of studies that investigated vaporized glycols for disinfection purposes is listed in Table 1.

Triethylene glycol was first registered with the US Environmental Protection Agency (EPA) for use as an aerial disinfectant in 1947, and over 105 pesticide chemical companies later registered TEG as an active ingredient in their products.



Figure 3. Industries investigating glycol as an antimicrobial or disinfectant.

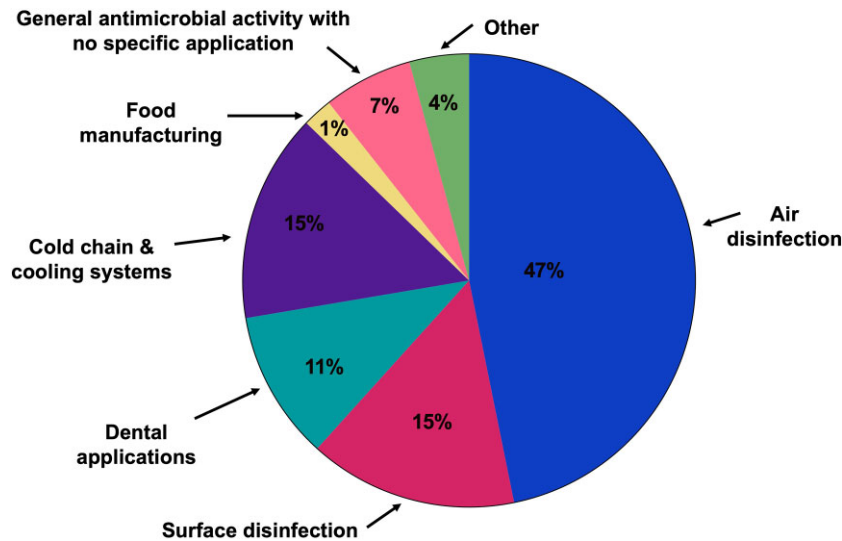


Figure 4. Categories of articles reviewed following application of the inclusion/exclusion criteria on retrieved publications.

However, most registrations were later cancelled until a review by the EPA in 2003. The EPA concluded that TEG had low toxicity, carcinogenicity, and mutagenicity potential, and TEG was therefore re-registered for use in air sanitization and deodorization, and in combination with other ingredients as fungicides, virucides, and miticides for disinfection of hard non-porous surfaces when used as a pressurized liquid (US Environmental Protection Agency 2003). The low toxicity of glycols was established in the 1930s (Hanzlik et al. 1939).

Robertson and colleagues (1941a) were early pioneers in the use of propylene glycol, trimethylene glycol, and ethylene glycol (EG) to disinfect enclosed spaces. They employed a glass-walled, 60-L capacity chamber sprayed with an aerosolized bacterial suspension ($200\,000\text{ cfu m}^{-3}$) to create a uniform distribution with the aid of a rotating fan, followed by introduction of an aerosolized glycol. Efficacy against pathogenic and non-pathogenic bacterial strains, including *Staphylococcus albus* (now *S. epidermidis*), *Pneumococcus* types I and III, haemolytic *streptococci*, haemolytic *staphylococci*, *Streptococcus viridans*, *Bacillus coli*, *Micrococcus catarrhalis*, and *Bacillus subtilis* (vegetative form) were tested. All species were killed rapidly, with only a few colonies detected immediately after glycol disinfection and no viable bacteria recovered 15 and 30 min after application (Robertson et al. 1941a). Puck et al. (1942) came to the same conclusions using a similar setup. The impact of different parameters on the antimicrobial activity of PG was investigated in the 1940s. This included temperature, humidity, glycol concentration in the air, bacterial concentration suspended in the air, and volume of bacterial suspension sprayed into the test chamber (Puck et al. 1943). PG loses its bactericidal efficacy as its concentration decreased below the saturation point, from 0.66 mg L^{-1} (saturated) to $0.25\text{--}0.27\text{ mg L}^{-1}$ (unsaturated) and concentration of 0.16 mg L^{-1} showing no activity (Puck et al. 1943). PG was also more efficient against bacteria in small droplets, low temperatures (15°C compared to 37°C), and at higher relative humidity (42% RH compared to 15% RH) (Puck et al. 1943). Subsequent experiments using *Pneumococcus* type I as the model organism, showed that at very low RH levels, PG vapour can exert rapid bactericidal action if the PG is at a sufficiently high concentration (Puck

et al. 1943). Chambers pre-filled with aerosolized glycol also demonstrated rapid disinfection when bacterial suspensions were sprayed into the enclosed space after the deployment of the glycol vapour (Puck et al. 1943).

In-situ studies

One of the first studies on the efficacy of PG for aerial disinfection *in situ* comes from experiments that sought to eradicate airborne pathogens from a hospital ward with PG vapour. The study used mice morbidity and mortality as a marker of infection (Henle et al. 1942; Table 1) and was set in a sealed ward ($47 \times 27 \times 11$ feet; total volume $\sim 4000\text{ ft}^3$) divided into separate cubicles. Airborne streptococci ($>3000\text{ cells/cubic foot}$) atomized in one cubicle (representing the first infected patient) resulted in most mice dying from streptococcal pneumonia and septicaemia in adjacent and non-adjacent cubicles. Conversely, wards disinfected with vaporized PG (placed in the centre) completely prevented streptococcal-induced death. In the same study, PG vapour protected mice from influenza type A infection, with only 3 out of 70 animals succumbing after exposure. In comparison, all animals died in the untreated group (Henle et al. 1942).

Harris and Stokes (1945) conducted a study over a 3-year period on the protective effects of PG and TEG vapour released into hospital wards. PG was used for the first two winters and TEG vapour for the last. The study demonstrated that vaporized PG released into wards protected patients from the common cold, tracheobronchitis, otitis media, and pharyngitis, with only 13 reported infections in comparison to 132 infections in patients located in control wards (Table 2). The reduction in circulating bacteria was confirmed by settling plate counts, with a 6-fold reduction in bacterial counts in the PG-treated wards vs. untreated wards. Plate counts were also lower in TEG-treated wards, but only by a magnitude of 4-fold due to problems with maintaining the TEG concentration in air (Harris and Stokes 1945).

A similar monitoring study using TEG vapour was performed in a rural school in Upstate New York, with monitoring occurring over a 10-year period (Gilcreas and Read 1955). Although a 60% reduction in total bacteria and a 55%

Table 1. Summary of retrieved articles that studied the disinfection efficacy of vaporized glycols.

Glycol	Microorganism	Glycol concentration	Relative humidity (RH)	Temperature	Chamber or room dimension/Volume	Study results	Reference
Application: air disinfection PG, EG, and 2,3 butylene glycol	<i>S. albus</i> , <i>Pneumococcus</i> types I and III, haemolytic <i>streptococci</i> and haemolytic <i>staphylococci</i> , <i>S. viridans</i> , <i>B. coli</i> , <i>M. catarrhalis</i> , and <i>B. subtilis</i> (vegetative form)	1 g per 2 000 000 cm ³ of air	Unknown	Unknown	60 l	<ul style="list-style-type: none"> <i>S. albus</i>. PG group: 0 colonies, (<i>t</i> = 0, 15, and 30 min). Control group, <i>t</i> = 0, 15, and 30 min (764, 532, and 336 colonies, respectively). Other bacteria similarly affected by PG. TEG, trimethylene glycol, 2,3-butylene glycol, and EG vapour gave similar results. 	Robertson et al. (1941a)
PG	Influenza	1 g per 2 000 000–3 000 000 cm ³ of air	Unknown	Unknown	60 l	<ul style="list-style-type: none"> PG exposed mice remained healthy. Non-exposed mice died within 6–10 days. 	Robertson et al. (1941b)
PG	Haemolytic <i>streptococci</i> and Influenza A	1 g per 2 000 000–5 000 000 cm ³ of air	Unknown	Unknown	47 × 27 × 11 ft (14 000 ft ³)	<ul style="list-style-type: none"> Treated air: 0–2 organisms recovered from 0.03 m³ (90–120 min after onset); untreated: too many cells to count. Settling plate counts in 5 min; treated: 0–1 organisms; untreated: >2000 organisms. PG treated air: all mice survived 10 days (three mice showed influenza lesions); untreated: almost all dead (3–7 days). 	Hentle et al. (1942)
PG	<i>S. albus</i> <i>Pneumococci</i> <i>Streptococci</i> and <i>Staphylococci</i>	0.16–0.66 mg L ⁻¹ air	44–52%	27–30°C	10 × 10 × 8 ft	<ul style="list-style-type: none"> PG concentration > saturation, 99.3% reduction in 15 s, and 100% within 5 min. Bactericidal action decreased as PG concentration decreased. <26.7°C and RH 45–70 were most favourable conditions. Pneumococci killed by PG as low as 1 g per 20 million cm³ of air; 1 g per 1–5 million and 1 g per 1–10 million for killing of <i>Streptococci</i> and <i>Staphylococci</i>. 	Puck et al. (1943)

Table 1. Continued

Glycol	Microorganism	Glycol concentration	Relative humidity (RH)	Temperature	Chamber or room dimension/Volume	Study results	Reference
PG	<i>C. xerosis</i>	Hot plate aerosolization: 100 mg·m ⁻³ of air and paints: 1000 mg per 0.55 m ³ of air	35–70%	20–28°C	3.03 m ³	<ul style="list-style-type: none"> PG aerosolized by hot plate was as good as mechanically produced atoms. Phenol paint gave a quicker kill than glycol paint, but glycol was superior to phenol when a smaller quantity was evaporated (>95% kill during 5–8 min). 	Baker and Twort (1944)
PG and TEG	Total airborne bacteria	PG: 0.048–0.094 mg L ⁻¹ air and TEG: 0.0018–0.0033 mg L ⁻¹ air	30–40%	RT	Unknown	<ul style="list-style-type: none"> Lower rate of infection in wards with glycol vapor (13 vs. 132 infections). Reduction of bacteria on settling plates was greater with PG treated vs. TEG treated wards. Difficulty maintaining TEG at the required bactericidal concentration in the air. 	Harris and Stokes (1945)
TEG	<i>Penicillium notatum</i> spores	0.01–0.056 mg L ⁻¹ air	17–98%	Unknown	2 ft ³	<ul style="list-style-type: none"> Optimum activity occurred at 0.035 mg l⁻¹ air, 55–60% RH. 	Mellody and Biggs (1946)
TEG	Meningopneumonitis virus and Psittacosis virus	> Saturation in air	35–60%	Unknown	Unknown	<ul style="list-style-type: none"> Average 62% (0–93%) reduction in virus. 73% (55–98%) reduction in infection response in mice. 	Rosebury et al. (1947)
PG	<i>S. albus</i>	0.3 mg L ⁻¹ of air	Unknown	82°F (27.7°C)	2 ft ³	<ul style="list-style-type: none"> 73% reduction after 15 s, 95% reduction after 5 min; 100% reduction in 15 min. 	Puck (1947a)

Table 1. Continued

Glycol	Microorganism	Glycol concentration	Relative humidity (RH)	Temperature	Chamber or room dimension/Volume	Study results	Reference
PG, DPG, and TEG	<i>S. albus</i>	0–0.21 mg L ⁻¹ of air	28%	72°F (22.2°C)	640 ft ³	<ul style="list-style-type: none"> • TEG has lowest minimum vapor pressure required for 95% reduction in air and lowest theoretical concentration in the air. 	Puck (1947b)
TEG	Total airborne bacteria	>50% saturation in air	Unknown	Unknown	8400, 11 880 and 11 352 ft ³	<ul style="list-style-type: none"> • 60% reduction in total airborne bacteria. • 55% reduction in settling bacteria. • 11% reduction in <i>Streptococci</i>. • No change in incidence of disease. 	Gilcreas and Roberts (1950)
TEG	<i>S. marcescens</i>	>Saturation point	25 and 80%	20°C	12 inch diameter, 24 inches long, and 35 l volume	<ul style="list-style-type: none"> • Compounds with VP lower than that of water—RH has a profound effect on their ability as aerial disinfectants. • Hygroscopic compounds (TEG)—effectiveness decreased with increasing RH. • Non-hygroscopic compounds—effectiveness increased with increasing RH. 	Kethley et al. (1956)
PG and TEG	<i>S. marcescens</i> , <i>E. coli</i> , and bacteriophage BT-3	0.4% concentrate (heat vaporization —40 µg L ⁻¹ in air and nebulizer —3.4 µg L ⁻¹ in air)	40–90%	25°C	33.3 m ³	<ul style="list-style-type: none"> • <i>S. marcescens</i>: glycol spray enhanced survival to 107% of the water control. Glycols were most effective when RH 40–60%. At 90% RH, performance was poor. 	Braymen and Songer (1970)
(PG and TEG	<i>Streptococcus mitis</i> , <i>Streptococcus epidermidis</i> , and <i>Bacillus subtilis</i> spores	8.8% PG and TEG (air-borne concentration 10 µg L ⁻¹ air)	30–40%	75°F (23.9°C)	700 ft ³	<ul style="list-style-type: none"> • Bacterial reduction of glycol treated airborne bacteria was the same as water aerosol treated samples. 	Pelleu et al. (1974)

Table 1. Continued

Glycol	Microorganism	Glycol concentration	Relative humidity (RH)	Temperature	Chamber or room dimension/Volume	Study results	Reference
Iodo-TEG	<i>E. coli</i>	2 mg m ⁻³	Unknown	Unknown	Chamber: 1.6 m ³ and barn: 1738 m ³	<ul style="list-style-type: none"> 91% bacterial reduction with electro-aerosols vs 68% reduction for normal aerosol. 	Yarnykh and Kel'bikhanov (1982)
TEG	HCoV-229E (SARS-CoV-2 human surrogate)	161.87–56.78 pg delivered 3% TEG also added to cocktail of antimicrobials	Unknown	Unknown	N/A	<ul style="list-style-type: none"> TEG alone -0.83 log₁₀ reduction in mina and 1.07 log₁₀ reduction in 5 min. TEG increased efficacy of EWNS nano-sanitizers when used with other antimicrobials (3.8 log₁₀ reduction in 5 min). 	Vaze et al. (2022)
PG	SARS-CoV-2, Influenza A, and Epstein Barr virus	10–60% 0–11 mg L ⁻¹ air	Unknown	20°C, 32°C, and 37°C	Unknown	<ul style="list-style-type: none"> 60% PG–5 log₁₀ reduction of IAV in 5 min at 32–37°C, undetectable at 30 min. Inhalation of IAV and 20% PG-greater survival of mice. Airborne SARS-CoV-2 and IAV inactivated in dose-dependent manner. 	Styles et al. (2023)
TEG	MS-2 bacteriophage	0.025–0.287 mg m ⁻³	30–40%	23°–26°C	Lab 1: 3 m (H) × 3 m (W) × 2.4 m (D) made from polycarbonate plastic with a thickness of 0.038 m Lab 2: 2.7 m (H) × 2.7 m (W) × 2.1 m (D) stainless steel	<ul style="list-style-type: none"> 2–3 log₁₀ reduction in 30–60 min. 	Desai et al. (2023)

Table 1. Continued

Glycol	Microorganism	Glycol concentration	Relative humidity (RH)	Temperature	Chamber or room dimension/Volume	Study results	Reference
PG and TEG	Application: air and surface disinfection (Poultry incubator) <i>Salmonella gallinarum</i> and <i>S. pullorum</i>	Unknown	Unknown	Unknown	Unknown	<ul style="list-style-type: none"> In incubators 22/32 no detectable <i>S. gallinarum</i>. 4/8 chicks exposed to PG had reduced organisms on their fluff. Overall PG did reduce <i>S. gallinarum</i> but not as effectively as formalin. <p>TEG vapour condensed.</p>	Moore (1947)
PG and TEG	Application: surface disinfection <i>S. pullorum</i>	Unknown	Unknown	RT	2.3–5.25 ft ³	<ul style="list-style-type: none"> In sealed jars, PG and TEG vapour sterilized contaminated eggshells, string, and filter pads. Poor performance in bacteriological incubators. 	Gwatkin (1947)
TEG (in Ibcol mixture)	<i>E. coli</i> <i>Salmonella pullorum</i> , moulds (<i>Mucor</i> , <i>Penicillium</i> , <i>aspergillus</i> , and <i>Rhizopus</i>)	chloro-xylene/TEG/water 1:2:2 ratio chloro-xylene:TEG 3:2 ratio	Unknown	Unknown	1900–5000 ft ³ (incubators) 500–2450 ft ³ (rearing rooms)	<ul style="list-style-type: none"> 1:2:2 chloro-xylene/TEG/water: 40–43% reduction in <i>E. coli</i> at 30 min, 53–66% at 24 h. 3:2 chloro-xylene/TEG: 14–75% reduction in <i>E. coli</i> kill at 30 min, 100% kill at 24 h. 3:2 chloro-xylene/TEG: 11–67% reduction in <i>S. pullorum</i> at 30 min, 0–64% over 24 h. 	McKenzie et al. (1959)
TEG	H1N1 influenza virus	2 ppm	58–65%	25°C	130 l	1.31 log ₁₀ reductions per hour.	Rudnick et al. (2009)
TEG	MRSA, <i>K. pneumoniae</i> , <i>E. coli</i> , and <i>A. baumannii</i>	25%	Unknown	Unknown	4 in × 4 in × 7 in	<ul style="list-style-type: none"> Atomized 25% TEG completely decontaminated coupon surface materials contaminated with CRE <i>K. pneumoniae</i>. MRSA persisted on the majority of coupon surfaces after 25% TEG atomization, but a 42% bacterial reduction occurred for the MRSA contaminated BS coupon. 	Kumaraswamy et al. (2018)

Table 1. Continued

Glycol	Microorganism	Glycol concentration	Relative humidity (RH)	Temperature	Chamber or room dimension/Volume	Study results	Reference
PG	Application: cold chain disinfection Porcine reproductive and respiratory syndrome virus	10%	Unknown	-20°C	0.28 × 0.5 × 0.3 m	<ul style="list-style-type: none"> 10% PG alone—PRRSV detected in 18/20 trailers. Tailers treated with PG/disinfectant combination—no virus detected. 	Dee et al. (2005)
PG	Avian influenza virus	30%	Unknown	-20°C and 20°C	N/A	<ul style="list-style-type: none"> No effect of PG alone at -20°C. 4-6 log₁₀ reduction when added to disinfectant solution. 	Guan et al. (2015)
PG TEG	Porcine epidemic diarrhoea virus, swine influenza virus, <i>E. coli</i> , <i>S. aureus</i> , and <i>B. subtilis</i> spores	50.9% PG/51.1% DDAB 30% TEG; 16-23% PAA	Unknown	-20°C	Unknown	<ul style="list-style-type: none"> DDAB-PG and PAA-TEG: PEDV and SIV inactivated within 15-60 min. DDAB-PG: <i>S. aureus</i>: 5 log₁₀ reduction; <i>E. coli</i>: >4 log₁₀ reduction in 15-60 min; <i>B. subtilis</i> spores: 1-2 log₁₀ reduction. PAA-TEG: <i>B. subtilis</i> spores: 3 log₁₀ reduction; <i>S. aureus</i> and <i>E. coli</i>: 1.5-2 log₁₀ reduction. 	Hu et al. (2022)

DPG: dipropylene glycol; EG: ethylene glycol; EWNS: engineered water nanostructures; MRSA: methicillin-resistant *Staphylococcus aureus*; PAA: peracetic acid; PEDV: porcine epidemic diarrhoea virus; PG: propylene glycol; PRRSV: porcine reproductive and respiratory syndrome virus; RH: relative humidity; RI: room temperature; SIV: swine influenza virus; TEG: Triethylene glycol; and VP: vapour pressure.

Table 2. Summary of retrieved articles that studied the antimicrobial efficacy of liquid glycol preparations.

Glycol	Application	Microorganism	Glycol concentration	Temperature	Study results	Reference
PG and TEG	No specific application	<i>T. bacilli</i>	60–100%	70°F (2°C)	<ul style="list-style-type: none"> 80% PG (5 min) and TEG (15 min) caused completed cell death. Lesions in guinea pigs injected with treated bacterial suspensions had fewer lesions than the untreated animals. 	Potter (1944)
PEG 300	No specific application	<i>B. stearothermophilus</i> and <i>B. subtilis</i>	100%	Room temp.	<ul style="list-style-type: none"> <i>B. subtilis</i> spores not recovered after 1 week submersion in PEG-300. Spores stored in sesame oil were recovered after 3 weeks. 	Robison and Weinswig (1969)
PEG 200 and PEG 400	No specific application	<i>S. aureus</i>	Unknown	30°C	<ul style="list-style-type: none"> PEG-200 and PEG-400 prevented growth of two <i>S. aureus</i> strains ($a_w \leq 0.93$). Another strain grew slowly in PEG-400 at a_w 0.93 and no growth recorded at a_w 0.93. Lowering water activity not completely responsible for antimicrobial actions. 	Vaamonde et al. (1984)
Glycol derivatives	No specific application	<i>S. aureus</i>	Unknown	Room temp.	<ul style="list-style-type: none"> 3 log₁₀ reduction with glycol derivatives. Glycol treatment did not prevent transfer of bacteria across surfaces. 	Exner et al. (1970)
DG	No specific application	<i>M. abscessus</i>	Unknown	37°C	<ul style="list-style-type: none"> Ineffective against <i>M. abscessus</i>. 	Caskey et al. (2018)
EG	Antifreeze in cooling system	<i>Pseudomonas</i> sp.	5–10%	25°C	<ul style="list-style-type: none"> EG > 10% inhibited growth. <i>Pseudomonas</i> flourished at EG concentrations between 5 and 10%. 	Du Moulin et al. (1981)
EG	Antifreeze in cooling system	<i>S. typhimurium</i>	30:70% EG/water	–1–1°C	<ul style="list-style-type: none"> <i>S. typhimurium</i> injured or inhibited but not killed by EG at low temperatures. 	Zottola and Smith (1986)

Table 2. Continued

Glycol	Application	Microorganism	Glycol concentration	Temperature	Study results	Reference
PG	Antifreeze in cooling system	<i>S. typhimurium</i>	0–50%	–1–37°C	<ul style="list-style-type: none"> At 37°C, >5% PG inhibited growth. At –1°C, >20% PG led to complete kill after 4 h when a_w 0.96. 	Airoldi and Zottola (1989)
PG	Antifreeze in cooling system	Psychrotrophs, mesophiles, coliforms, and <i>S. almonellae</i>	4.5–34.8%	10–37°C	<ul style="list-style-type: none"> <0.21 and >240 colonies/100 ml of coolant. PG > 30% should be used in HTST pasteurizers. 	Stranz et al. (1989)
PG	Food preservative	<i>C. botulinum</i>	5–50%	30°C	<ul style="list-style-type: none"> 14.3% PG inhibited <i>C. botulinum</i>. 9.1% PG inhibitory when combined with 24.2 ppm sodium nitrate. 	Hall and Maurer (1986)
PG, PEG-400, and PEG-1000	Dental disinfection	<i>S. mutans</i> , <i>E. faecalis</i> , and <i>E. coli</i>	2.5–100%	37°C	<ul style="list-style-type: none"> PG: MBC <i>E. coli</i> 100%, <i>S. mutans</i> 50%, and <i>E. faecalis</i> 25%. PEG-400: MBC <i>E. coli</i> 100%, <i>S. mutans</i> 100%, and <i>E. faecalis</i> 100%. PEG-100: MBC <i>E. coli</i> 100%, <i>S. mutans</i>, and <i>E. coli</i> 25%. 	Nalawade et al. (2015)
PG	Dental disinfection	<i>F. nucleatum</i>	$1.5 \pm 0.4 \mu\text{g ml}^{-1}$	37°C	<ul style="list-style-type: none"> No zone of inhibition produced by PG. 	Zaruba et al. (2015)
PG	Dental disinfection	<i>E. faecalis</i>	10%	37°C	<ul style="list-style-type: none"> Minimal biocidal activity (1–2.5% cell death at day 1, 8–10.3% death after 7 days). PG enhanced penetration of dentin tubules by TAP and increased TAP release. 	Nagarathinam et al. (2019)
PG	Dental disinfection	<i>E. faecalis</i>	PG + $1 \mu\text{l mg}^{-1}$ calcium hydroxide	37°C	<ul style="list-style-type: none"> PG increased release of calcium hydroxide. 	Vaghela et al. (2011)
PG-monooleate	Plaque anti-adherent	<i>S. mutans</i> and <i>S. sanguis</i>	0.98– $1.0 \times 10^{-3}\%$ w/v	35°C	<ul style="list-style-type: none"> No MIC obtained at the concentrations tested. 	Lim et al. (1982)

a_w : water activity; DG: diethylene glycol; EG: ethylene glycol; PG: propylene glycol; PEG: polyethylene glycol; and MIC: minimum inhibitory concentration.

reduction in the settling plate count were noted in TEG-treated classrooms, streptococci bacteria were only reduced by 11% compared to untreated classrooms. Over the 10-year period, there was no reduction in the incidence of disease, likely due to secondary sources of infection, such as close contact on school buses. Disinfection of buses with TEG vapour was attempted during this study, but the required concentration of aerosolized TEG could not be controlled (Gilcreas and Read 1955).

Electrical dispersal of iodo-triethylene glycol was investigated as a method to disinfect the air in a barn used to raise calves, resulting in a 91% reduction in total bacterial cell count compared to a 68% reduction when the air was decontaminated with glycols generated using conventional methods (Yarnykh and Kel'bikhanov 1982).

In situ studies investigating the efficacy aerosolized glycols did not always yield positive control of bacterial contaminants. The use of two commercially available glycol sprays, Ozium (containing 8.8% PG and TEG) and air-fresh (containing PG and TEG in unspecified amounts), failed to disinfect closed rooms containing airborne *Streptococcus mitis*, *S. epidermidis*, and *B. subtilis* spores, and produced disappointing results (Pelleu et al. 1974). Although the glycol aerosol dosages applied were above those stated by the manufacturers, the researchers concluded that the concentrations of glycol in the air did not reach sufficient concentrations required for biocidal activity. When compared to other studies using vaporized PG and TEG as disinfectants (Puck et al. 1943, Harris and Stokes 1945), the concentration of airborne glycol was between 5- and 30-fold lower and likely attributable to the failure of PG and TEG to disinfect the contaminated rooms.

Disinfection of poultry incubators

In 1947, the use of vaporized glycols to disinfect poultry incubators to prevent *Salmonella pullorum* infection was considered as a potential replacement for using toxic formaldehyde. Gwatkin (1947) introduced vapour from boiled PG to TEG into desiccator jars and successfully sterilized eggshells, string, and filter pads contaminated with *S. pullorum* (Table 1). However, the same experiments performed in bacteriological incubators failed to sterilize the test pieces. Liquid glycol was also investigated, but little effect was observed against *S. pullorum*. Overall, Gwatkin (1947) concluded that PG and TEG had little value as incubator disinfectants to replace the more efficacious but toxic formaldehyde. However, the experiments conducted in this early study failed to measure the concentration of glycol produced into the air, and to adequately control parameters that impact glycol efficacy, such as temperature and relative humidity. In the same year, Moore published data on the use of PG and TEG vapour as incubator fumigants for the hatchery industry, as a potential replacement for the previously used formalin (Moore 1947). Initial experiments reported no toxic effects on chicks 3 weeks post-exposure to PG or TEG for 24–48 h, although 1 chick out of a total of 89 died of an unknown cause in the PG group. Exposure of *Salmonella gallinarum* to PG vapour in a laboratory incubator and six commercial incubators of varying makes, models, and capacities generally yielded positive results, particularly with long exposure times (i.e. hours instead of minutes), although there was some variation in the effectiveness of PG vapour across the different incubators. *Staphylococ-*

cus aureus growth was noted in some incubators where PG vapour had been released, suggesting that it may be more resistant to disinfection by glycol vapours than *S. gallinarum* compared to formalin, which was the standard disinfectant used at the time. In addition, the author noted that TEG vapour condensed in chick incubators before a bactericidal concentration could be reached; however, PG was deemed to be suitable and an appropriate alternative to using the toxic formalin (Moore 1947).

Further investigations of TEG as a surface disinfectant in poultry premises combined mixtures of chloro-xyleneol, TEG, and water in ratios of 1:2:2 where small particle aerosols were generated, and in a ratio of 3:2 chloro-xyleneol and TEG where large particles were produced. The chloro-xyleneol/TEG/water mixture only killed 53–66% (i.e. $<1 \log_{10}$ reduction) of *Escherichia coli* following 24 h of exposure, whereas 100% of *E. coli* cells were killed following 24 h of treatment with the chloro-xyleneol/TEG mixture. *Salmonella pullorum* was less susceptible with only 0–64% reduction in viable bacteria over 24 h (Table 1). The researchers also noted that the position of infected surfaces in relation to the aerosol generator affected the performance of the disinfectant. It was recommended that moving the position of the generator could improve efficacy (McKenzie et al. 1959).

In-vitro and *in-vivo* studies

The majority of studies measuring the disinfection efficacy of aerosolized glycols used environmental chambers, where temperature and relative humidity were controlled. Although methods to vaporize glycols varied across studies, most studies used a method to heat liquid glycol to produce a vapour that could be dispersed around the chamber. The disinfection protocols studied a variety of microorganisms, including bacteria, viruses, and fungi.

Staphylococcus albus, later renamed *S. epidermidis*, was the bacteria chosen in early studies by various researchers. As previously mentioned, Robertson et al. (1941a) showed PG vapour to rapidly disinfect chambers containing different vegetative bacterial species (Table 1). TEG and EG vapours were equally efficacious as disinfectants against such bacteria (Robertson et al. 1941a). Puck and colleagues (1943) investigated the effect of PG concentration in the air on the rate of disinfection of chambers contaminated with *streptococci*, *staphylococci*, and *pneumococci*, and showed that PG concentration in the air above saturation caused rapid disinfection (Puck et al. 1943). Baker and Twort (1944) employed a variety of methods to disperse glycol vapours into the air of the chambers: methylated spirit solutions of EG, PG, and diethylene glycol (DG) were painted onto the base of the chambers so that the concentration of glycol in the air reached $>1000 \text{ mg m}^{-3}$. Mechanical and hot-plate atomization were also investigated as methods to create glycol vapours within the experimental chamber. Evaporation of glycol at room temperature and painting of the glycols required a longer contact time to produce an equivalent kill rate in *Corynebacterium xerosis* emulsified in saliva than that of mechanically produced or heated glycol. After 45 min, no viable bacteria were recovered from EG or PG painted chambers, but 4.6% of viable cells were recovered from DG painted chambers (Baker and Twort 1944) (Table 1).

The disinfection efficacies of aerosolized formulations of dipropylene glycol (DPG) and ethanol were probed us-

ing a novel protocol to improve microbial recovery post-treatment. *Escherichia coli* and *S. aureus* were embedded in an alginate matrix to overcome some of the challenges faced when recovering airborne bacteria. During the investigation, the aerosolized 4% DPG/35% ethanol formulation reduced bacterial load by $>3 \log_{10}$ and proved to be more effective than DPG or ethanol alone (Rubiano et al. 2020).

In order to improve glycol vapour efficacy and air disinfection, electrical dispersal of iodo-triethylene glycol at air concentrations of 2 mg m^{-3} was investigated by Yarnykh and Kel'bikhanov (1982). Both the *E. coli* suspension and glycol suspension underwent separate unipolar electrification and the reduction in bacterial viability was greater than using glycol aerosolized by conventional methods. The increase in bactericidal activity was attributed to an increase in electric charges of opposite polarity between the bacterial target on the glycol droplets, resulting in increased contact and adherence to the bacterial surface.

Viruses have also been shown to be inactivated by vaporized glycols. Both *in vivo* and *in vitro* studies have been conducted, including an early study by Robertson and colleagues (1941b) demonstrating the protective effects of PG vapour to airborne influenza virus in mice. Glycol vapour at 1:2 000 000 in air allowed mice to remain healthy 8 days post-exposure, and normal healthy lungs were observed upon autopsy. In comparison, a 100% death rate occurred in mice exposed to the virus without the protective PG vapour (Robertson et al. 1941b).

TEG vapour, added to a chamber in excess of saturation, was later investigated as a mechanism to reduce airborne meningopneumonitis and psittacosis virus levels (Rosebury et al. 1947). Mice exposed to atomized viruses in a sealed chamber were sacrificed 4–11 days after exposure and infection was confirmed by focal lesions on lung surfaces, with a 55–98% reduction in infection reported. Airborne virus measurements resulted in an average 62% reduction following TEG exposure (Rosebury et al. 1947).

The bactericidal action of TEG vapour against fungi was investigated by Mellody and Bigg (1946). *Penicillium notatum* spores were killed by TEG vapour concentrations between 0.01 and 0.054 mg L^{-1} , with optimum performance occurring at 0.035 mg L^{-1} and 55–60% RH. It was observed that RH played a more critical role for air disinfection of fungi with TEG vapour compared to bacteria and viruses. In addition, it was established that 85% TEG in water was the optimal antifungal concentration against fungal spores in suspension (Mellody and Bigg 1946).

Surface disinfection

In addition to the use of aerosolized glycols for the disinfection of air, they have also been studied as surface disinfectants. In one study, bacteria deposited onto coupons constructed from polycarbonate, polyethylene terephthalate, stainless steel, borosilicate glass, and natural rubber were subjected to atomized 25% TEG for 30 min, with cycles of 2 min on/off periods (Kumaraswamy et al. 2018). The coupons were retrieved 30 min after the last atomization cycle and the bacteria were enumerated. No carbapenem-resistant *Klebsiella pneumoniae* or methicillin-resistant *Staphylococcus aureus* (MRSA) were recovered from any of the surfaces, with the exception of MRSA on borosilicate glass, for which only a 40% reduction in viability was observed. However, the absence of a

neutralizing step to quench the activity of TEG means the bactericidal efficacy of TEG was likely overstated (Kumaraswamy et al. 2018).

The ability of aerosolized TEG to disinfect stainless steel surfaces contaminated with dried H1N1 influenza virus was conducted by Rudnick et al. (2009). Low vapour concentrations of 2 ppm in air were shown to reduce the viral load by $\sim 1.31 \log_{10}$ per hour under ambient conditions of 25–29°C, a 16-fold increase in the natural inactivation rate. Yet again, a neutralizing step was not performed after the virus exposure to TEG. It was concluded that such low vapour concentrations would expect to produce minimal damage to avionics of an airplane, and therefore, low-concentration TEG would be a suitable method to disinfect aeroplane surfaces of viruses such as influenza (Rudnick et al. 2009).

Factors affecting efficacy of glycol aerosols as disinfectants

In 1947, Theodore Puck published two articles to address the parameters that impact glycol vapours biocidal efficacy. He stated: ‘the effectiveness of any substance as an aerial germicide depends directly on the degree of condensation of its vapour on the air-suspended particles, and on the rate at which the resulting concentration of germicide can bring about death of the microorganisms’ (Puck 1947a–b). A summary of factors that impact the biocidal action of aerosolized glycols is depicted in Fig. 5.

Effect of vapour pressure

Vapour pressure is defined as the measurement of a liquid’s ability to change into the gaseous or vapour phase. Generally, for a liquid in a closed container, vapour pressure increases as temperature increases. Puck (1947b) demonstrated the different efficacies of three glycol vapours, namely TEG, DG, and PG, on air disinfection of chambers containing airborne *S. albus*. At 72°F (22°C) and RH 28%, the vapour pressures of TEG, DG, and PG were 0.001, 0.02, and 0.1 mm Hg, respectively. The theoretical minimum concentrations of the glycols in air required for a 95% reduction of airborne bacteria were calculated to be 0.006, 0.027, and 0.19 mg L^{-1} , respectively. These results clearly demonstrated the minimum concentration of airborne glycol in the air for a 95% kill rate increases as vapour pressure increases. Puck (1947b) discussed that compounds with low vapour pressure require smaller quantities to produce the desired kill rate, and therefore, the condensation of such vapours on cold surfaces would be small. However, if vapour pressure is too low, a reduction in the rate of kill may result, as the velocity of condensation of vaporized glycol on air-suspended bacteria droplets is dependent upon the collision rate between the vapour and the droplet. This can explain why TEG, with a lower effective kill concentration than PG, has a slower rate of action than PG when at the same % saturation in air (Puck 1947b).

Concentration of glycol vapour in the air

Further experiments by Puck (1947a) investigated the concentration of germicide in the air and its effects on the viability of aerial bacteria. He concluded that chambers sprayed with increasing concentrations of PG, DG, and TEG vapour, showed a direct correlation with the rate of kill. This was attributed to an increased concentration of germicide in the condensate; hence, a higher bactericidal action (Puck 1947a).

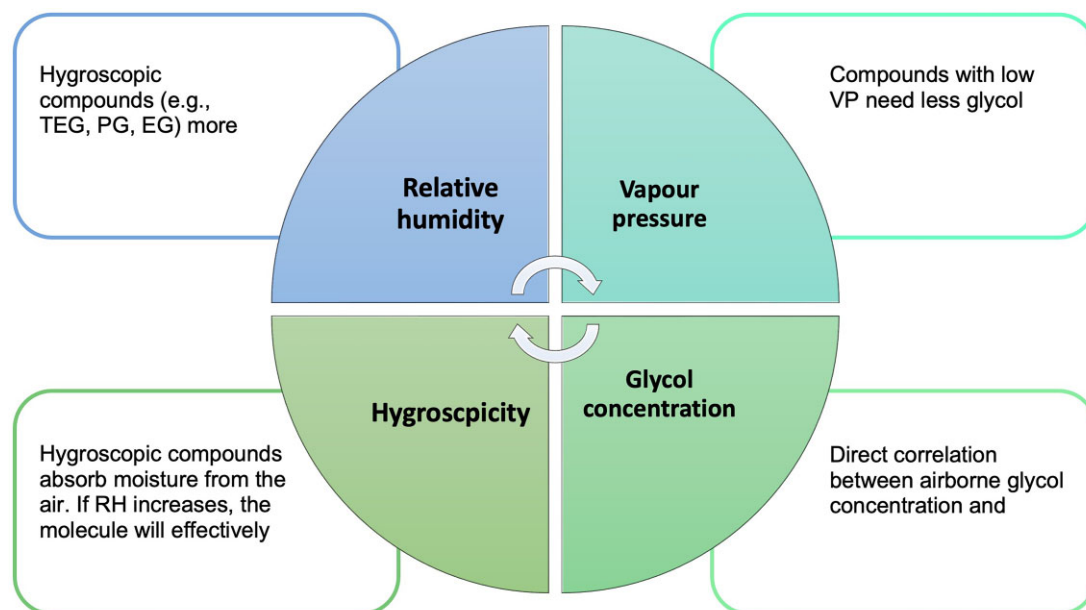


Figure 5. Factors affecting disinfection efficacy of aerosolized glycols.

Relative humidity and hygroscopicity

To understand the effect of relative humidity (RH) on compounds used for aerial disinfection, Kethley et al. (1956) investigated the efficacy of a range of hygroscopic compounds (i.e. those absorb moisture from the air) and non-hygroscopic ones. TEG, PG, and EG, all hygroscopic compounds, showed greater kill rates of aerosolized *Serratia marcescens* at 25% RH than at 80% RH. Aerosolized TEG was more effective at 25% RH than PG, which itself demonstrated greater efficacy at the same RH than EG. All non-hygroscopic compounds tested performed better at 80% RH than at 25% RH. The investigators concluded that RH is a key factor in performance of an aerial disinfectant. For non-hygroscopic compounds, the concentration of the compound in the airborne particle increases as RH increases; thus, the rate of kill also escalates. However, for hygroscopic compounds, an increase in RH results in a greater amount of water in the airborne particle, actually leading to a decreased concentration of the compound, and an overall decline in biocidal activity (Kethley et al. 1956). Although these observations from Kethley and colleagues (1956) between glycols and RH seem straightforward and based on specific experimental setup, Puck et al. (1943) indicated that the relationship between RH and bactericidal efficacy of glycols is not linear and depends on other parameters, as mentioned in an earlier section.

High RH levels were also thought to be responsible for the failure of glycols to improve the efficacy of commercially available disinfectant-detergent solutions (Gwatkin 1947, Braymen and Songer 1970). Surfaces contaminated with microorganisms and subsequently disinfected using high-pressure devices were thought to generate microbial aerosols that were resistant to most disinfectant-detergent solutions. The addition of glycols to commercially available disinfectants was investigated for their ability to disinfect rooms containing aerosolized *S. marcescens* and *E. coli* bacteriophage T3 (Braymen and Songer 1970). PG and TEG added to water at a concentration of 0.4% actually increased the survival of *S. marcescens*. PG added to disinfectant solutions in con-

centrations of 0.1–0.4% also increased survival to 84–104% compared to the number of bacteria recovered from treatment with water only. Similarly poor results were obtained against T3 phages. It was concluded that the high RH that rose from 55–60% to >90% after spraying was the cause of disinfection failure. An RH between 40 and 60% was thought to be the optimal range for glycol disinfection (Braymen and Songer 1970).

Inactivation of SARS-CoV-2 and other viruses

In January 2021, with the COVID-19 pandemic still of global concern, the EPA issued an emergency exemption for the use of Gringard Pure™, a blend of TEG and other inert ingredients, to aid in the control of the virus. Seven states in the USA were granted approval for its use in indoor spaces where social distancing and other guidelines were difficult to control. An article was later published on the efficacy of Gringard Pure™ (GP) to inactivate MS2 bacteriophage used as a surrogate of SARS-CoV-2. Airborne MS2 was reduced by 99–99.9% (2–3 log₁₀) following either its introduction into air containing GP, or vaporized GP introduced after the aerosolized bacteriophage. The concentrations of GP were between 0.02 and 0.5 mg m⁻³ of GP (equivalent concentration of TEG: 0.025–0.287 mg m⁻³) (Desai et al. 2023).

Inactivation of SARS-CoV-2 on surfaces was also a key route to prevent transmission of the virus. A novel technology, utilizing antimicrobial agents encapsulated in nanoscale structures, termed engineered water nanostructures (EWNS), investigated TEG as a potential compound to inactivate the human HCoV-229E coronavirus that had been dried onto stainless steel surfaces. As a single agent, the aerosolized EWNS containing 3% TEG resulted in a 1 log₁₀ reduction in HCoV-229E in 5 min. The combination of 3% TEG added to a cocktail of other antimicrobial ingredients (10% H₂O₂, 1% citric acid, 0.1% lysozyme, and 0.0025% nisin), which resulted in a droplet size of 42.71 ± 3.36 nm, increased the virucidal activity of the EWNS to 3.8 log₁₀ reduction (limit of detection) after 1 min of exposure. The dose of TEG delivered to the

tivated coronavirus porcine epidemic diarrhoea virus (PEDV) and a swine influenza virus (SIV) within 60 min at -20°C , and showed some bactericidal *E. coli* and *S. aureus* and sporicidal activity against *B. subtilis* spores (Hu et al. 2022). Unfortunately, the PG or TEG were not tested on their own.

Whilst the combination of some glycols with disinfectants for the disinfection of sub-zero environments seems beneficial, there have been concerns that glycols can support bacterial survival and growth at low temperatures. In 1979, an outbreak of *Pseudomonas* spp. in a Boston hospital was linked to the closed chilled water system and thought to be due to overdilution of the ethylene glycol antifreeze during a routine system maintenance. As a result, researchers investigating the effect of EG concentration on *Pseudomonas* sp. growth established that the microorganisms flourished at EG concentrations between 5 and 10%, whilst a concentration $>10\%$ inhibited growth. It was concluded that EG was likely to have been diluted from the 50% level that was initially added to the water system during a routine maintenance, leading bacterial growth (Du Moulin et al. 1981). In another study, investigating outbreaks of *Salmonella typhimurium* in pasteurized milk, Zottola and Smith (1986) tried to establish if the cooling medium (30:70% EG/water mixture) used in the cooling system could be a potential source of contamination in dairy plants. Whilst the results were difficult to analyse due to issues with recovering the bacteria, it was concluded that *S. typhimurium* was injured or inhibited by the EG but not killed. It was concluded that the persistence of the bacteria in glycol/water mixtures at sub-zero temperatures may have caused contamination of cooling systems in dairy processing plants and contributed to *Salmonella* outbreaks in pasteurized milk (Zottola and Smith 1986).

Further investigations showed that at 37°C , $>5\%$ PG was sufficient to inhibit growth of *S. typhimurium* and that PG concentrations $>20\%$ and water activity (A_w) <0.96 led to no recoverable bacteria after 4 h of contact time. At -1°C , the bacteria population decreased over time, with the fastest rate of decline observed in the groups subjected to the highest PG concentrations (Airoldi and Zottola 1989). Additional studies concluded that water/glycol mixtures in cooling systems in high temperature short time (HTST) pasteurizers should use PG at concentrations above $>30\%$ to reduce the chances of microbial contamination (Stranz et al. 1989).

Food industry

Inhibition of *Clostridium botulinum* in cured meats relies on the addition of nitrates for their antimicrobial activities. In 1984, Hall and Maurer sought to identify alternative antimicrobials that would be suitable to be added to turkey frankfurter slurries, and a variety of spice extracts, lauricidin and PG, were investigated (Hall and Maurer 1986). The study was based on adding *C. botulinum* spores to turkey frankfurter slurries with and without antimicrobials, and after incubation for 4 days at 30°C , slurries were centrifuged and the supernatants injected into mice. It is unclear why germination and culturability of the spores were not studied *in vitro* before moving to an infectious model. The study showed that the addition of 14.3% PG alone into the slurry enabled mice to survive. Furthermore, 6.2% PG in combination with 31.2 ppm sodium nitrate, or 9.1% PG in combination with 24 ppm sodium nitrate also enabled mice to survive. Hall and Maurer (1986) suggested that 14.3% PG lowered a_w sufficiently

(to 0.88/0.89 a_w) to prevent *C. botulinum* spore germination (spore germination inhibited at a_w 0.93) or the production of toxin (toxin production inhibited at a_w 0.95) (Troller 1973). However, lowering a_w does not necessarily explain the beneficial effect of combining lower PG concentration with sodium nitrate. Using other means to reduce A_w to prevent *C. botulinum* germination or toxin production was not exploited further by the authors. In addition, as mentioned earlier, Vaamonde et al. (1984) concluded that a reduction of A_w by another glycol, could not solely explain the efficacy of PEG200 and PEG400 against *S. aureus*.

Uses in dentistry

In the field of endodontic dentistry, successful procedures require thorough disinfection of the root canals and surrounding regions, including deep within the dentine tubules where bacteria can reside. Intracanal medicaments containing calcium hydroxide or antibiotics are usually employed as antibacterial agents for such scenarios, but their carrier vehicles can also affect their disinfection capabilities and have been the subject of numerous studies (Vaghela et al. 2011, Nagarathinam et al. 2019). The use of glycols has been mainly evaluated as a vehicle to deliver other antimicrobials, but their intrinsic antimicrobial efficacy has been investigated in the process, although using simple *in vitro* experimental protocols, which would not reflect their use in practice.

PG, PEG400, PEG1000, and PG with PEG400 showed some bactericidal efficacy against *Streptococcus mutans*, *S. aureus*, *Enterococcus faecalis*, and *E. coli* in a microdilution broth experiments to determine their minimal bactericidal concentration (MBC). However, the MBC corresponded mostly to the concentrated solution (100%), whilst PG at 25 and 50% exerted bactericidal activity against *S. mutans*, *E. faecalis*, and *E. coli*, respectively, and PEG1000 was bactericidal against *S. mutans* and *E. coli* at a 25% concentration. The combination of PG with PEG400 did not enhance any activity, and reduced the efficacy of PG (Nalawade et al. 2015). Lim and colleagues (1982) showed that propylene glycol monooleate, a non-ionic surfactant used in the dental field as a plaque anti-adherent, had no inhibitory activity against *S. mutans* and *Streptococcus sanguis* at concentrations between 0.98 and $0.1 \times 10^{-3}\%$ w/v. Using a crude agar-based assay, Zaruba et al. (2015) showed no activity of PG against *Fusobacterium nucleatum* although the ability of PG to diffuse in the agar was not established. In another study, it was also shown that PG (10%) did not affect *E. faecalis* biofilms (Nagarathinam et al. 2019). Whilst PG does not seem to show any appropriate bactericidal efficacy on its own against a range of bacterial pathogens pertinent to dental applications, PG has been shown to improve the delivery of antibiotics (Nagarathinam et al. 2019) and calcium hydroxide (Vaghela et al. 2011).

The incorporation of 10% PG into triple antibiotic pastes (TAP) for dentin tubule disinfection improved the ability of TAP to penetrate the dentin and contributed to the sustained release of the antibiotics (Nagarathinam et al. 2019).

Study limitations

We only used one database to conduct the literature searches, although 'Web of Science' encompasses multiple databases that provide reference and citation data from academic journals and other documents, such as conference proceedings,

which were excluded from our search. In addition, the database searches and data extraction were only conducted by a single reviewer, which may have added some bias to the articles retained.

Conclusions

From the studies retained in this review, the effectiveness of glycols as antibacterial agents or disinfectants depends on their mode of application and environmental factors. One of the main advantages of glycols, such as PG and PEG, is their lack of toxicity when vaporized, a property that has led to their approved use by the US EPA. The use of microbicidal fumigants in the presence of humans or animals is attractive to the industry and end users, hence the renewed interest in these compounds. Generally, glycols in a vaporized form show a better microbicidal efficacy compared to a liquid form. There appear to be no studies investigating the difference between the vaporized and liquid forms in their interactions with a microbial target. The microbicidal action of glycols has not been well studied, but it is thought to be associated with an alteration of membrane function in bacteria and fungi (Levinson *et al.* 2006). However, TEG might also have an interaction with proteins as it has activity against non-enveloped viruses (Desai *et al.* 2023).

Studies reporting aerial disinfection by vaporized glycols, particularly PG and TEG, showed antimicrobial efficacy when the studies were conducted *in vitro*. However, generally, studies exploring the microbicidal efficacy of vaporized glycols, lack a neutralizing step, which might have resulted in a better reported efficacy. Vaporized PG, EG, and TEG were all shown to be highly efficacious against numerous bacterial species (Table 1). In addition, early studies reporting on protective effects of PG and TEG vapour against viral pathogens all showed reductions in viral disease amongst animals in the treatment groups (Table 1). However, there are many factors that impact the efficacy of glycols delivered as vapour (Puck *et al.* 1943), which means the application of vaporized glycols to *in situ* applications did not always result in a positive outcome. Glycol concentration has been shown to be directly proportional to bactericidal activity. In addition, glycols with a low vapour pressure require less compound to exert the same bactericidal activity as those with higher vapour pressures, thus explaining why less PG vapour is required for complete biocidal activity than TEG vapour. The RH is a crucial factor in aerial disinfection, and a fine balance exists between too high and too low RH. Generally, glycol vapours are hygroscopic and exert optimal activity between 40 and 60% RH. The delivery of effective concentrations in studies *in situ* was not always possible or difficult to maintain, delivery parameters not always controlled, and glycol concentrations were not always quantified. This led to variability in results and questions about the suitability of using PG and TEG vapours in the poultry industry, for example.

Overall, glycols are less microbicidal in liquid form on their own, but glycols have been useful when combined with other antimicrobials. In particular, their combined use with disinfectants in sub-zero environments have been positive to control bacterial and viral pathogens, although glycol concentration needs to be maintained >30% to avoid bacterial proliferation (du Moulin *et al.* 1981, Airoidi and Zottola 1989, Stranz *et al.* 1989). *In vitro* findings on the use of glycol as a delivery system for other antimicrobials in the dental field have shown

a better and sustainable delivery of the antimicrobials (Nagarathinam *et al.* 2019).

Overall, the use of aerosolized glycols for disinfection of air and surfaces has merit, but the control of the factors impacting efficacy and pertaining to a high concentration to be effectively delivered is paramount. Such an application is attractive since a glycol-based vaporized formulation could be used in the presence of people and animals. The use of glycols in their liquid form in addition to other antimicrobials, has provided positive results in sub-zero environments. Other applications using glycols have a delivery system remains at a research stage.

The main gap in knowledge identified in this review is the lack of understanding of the mechanism of microbicidal activity of glycols, particularly in a vapour phase or aerosolized form that would inform the bactericidal and virucidal efficacy observed in early publications.

Conflict of interest: This study was funded by Reckitt Benckiser LLC (USA). K. Ijaz and J. McKinney are employees of Reckitt Benckiser LLC (USA).

Author contributions

Katrina Duggan (Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing), M. Khalid Ijaz (Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft), Julie McKinney (Funding acquisition, Project administration, Resources, Supervision), and Jean-Yves Maillard (Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing)

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