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Article

Antimicrobial, Preservative, and Hazard Assessments from Eight Chemical Classes

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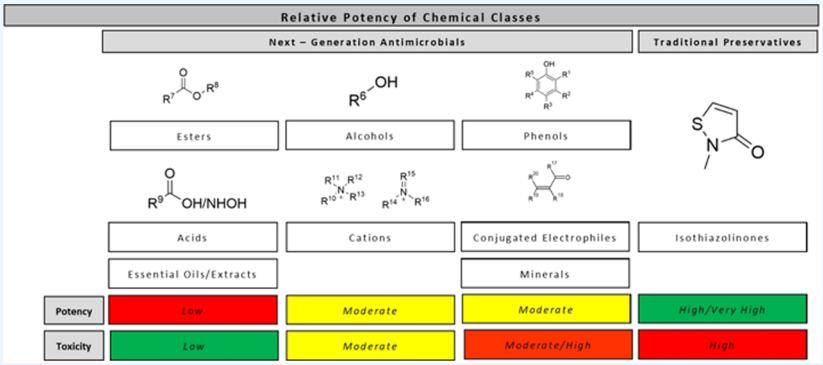

ABSTRACT: Preservatives, such as isothiazolinones and formaldehyde-releasing compounds, provide safety and stability in consumer products by preventing microbial contamination. Yet these ingredients present human and environmental hazards, including allergic contact dermatitis and aquatic toxicity. The development of safer alternatives has been stymied by trade-offs between safety and efficacy. To enable the identification of safer preservatives, substances from eight functional classes were assessed for antimicrobial efficacy and human and environmental hazards. First, 130 substances were evaluated for microbial inhibitory activity against two relevant model microorganisms, *Aspergillus brasiliensis* (filamentous fungi) and *Pseudomonas aeruginosa* (Gram-negative bacteria). High-performing compounds within each class were assessed for hazards across a broad suite of human and environmental health end points. Four promising compounds were selected for further testing based on microbial inhibition, hazard profiles, and commercial availability. These ingredients were tested for biocidal activity in model home care formulations using methods adapted from industrial preservative challenge guidelines (USP-51). Two substances were identified, caprylhydroxamic acid and caprylyl glycol, that provided adequate preservation and improved toxicity profiles compared to isothiazolinone and formaldehyde-releasing preservatives. This study highlights trade-offs between antimicrobial activity and hazards across a broad spectrum of chemical classes relevant to safer preservative development.

INTRODUCTION

Preservatives are used in consumer products, such as paints, coatings, and home and personal care products, to reduce microbial contamination and product spoilage.[1] Traditional preservatives in consumer products include isothiazolinones, formaldehyde-releasing compounds, and parabens.[1,2] These ingredients have come under scrutiny for their hazards to humans and the environment, in particular allergic contact dermatitis.[3,4] Broad-spectrum antimicrobials, including isothiazolinones and quaternary ammonium compounds, can induce nontarget toxicity in aquatic organisms.[5,6]

Less-hazardous ingredients are being sought out by consumers and industry,[7] but identifying safer alternatives has been challenging. Buckley et al. investigated phenolic ester/amide compounds and identified octyl gallate as an effective and lower-hazard preservative compared to existing preservatives.[8] Plant extracts, including essential oils,[4] have also been investigated as alternatives.[9] Biocides traditionally used as disinfectants may also be used in preservative formulas, such as alcohols and quaternary ammonium compounds. Data describing the relative hazards of potential preservatives alongside their performance are needed.

In this work, we evaluated compounds spanning different chemical classes that function as preservatives in personal care products. Eight chemical classes with reported antimicrobial properties were evaluated: phenols, essential oils and extracts,

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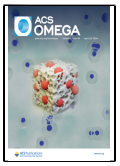

alcohols, organic cations, organic acids, esters, conjugated electrophiles, and minerals.

Phenols, alcohols, and cations have historically been used as disinfectants. Phenols, and their oxidation products, quinones, are common in natural products and exhibit activity against bacteria, viruses, and fungi.[10] These substances function as antimicrobials by targeting cell membranes, proteins, and redox processes.[11,12] Alcohols can also cause microbial membrane damage and rapid denaturation of proteins, and in low concentrations, moderately hydrophobic alcohols (e.g., benzyl alcohol) have been used as preservatives or preservative boosters.[12] Cationic quaternary ammonium compounds induce cell death when their positive charge and hydrophobic nature disrupt microorganism cell membranes.[13,14] This interaction leads to leakage of physiologically important ions (e.g., K^+) and cell death.[15] Polymeric ammonium compounds are nonvolatile and have longer-lasting antimicrobial effects,[13] and can be naturally occurring, like polylysine and chitosan, or synthetic, like polyethylenimine. Chitosan, a polycation derived from chitin, has been widely researched and used in food, agricultural, chemical, and medical industries due to its antimicrobial, antitumor, and hypocholesterolemic properties.[14]

Organic acids (e.g., sorbic, benzoic, propionic) are widely used to preserve personal care and food products, as they are generally inexpensive and effective antimicrobials so long as the acid pK_a is close to, or below, bulk solution pH, as their conjugate base possesses little to no antimicrobial activity. Esters have also recently been reported as food-safe antimicrobials, albeit with reduced potency and scope of protection.[16] Essential oils and extracts contain natural antimicrobial compounds, including terpenes, phenylpropanoids, aliphatic aldehydes, esters, ketones, and aliphatic acids. Present in many botanicals with a long history of use as flavors, fragrances, and preservatives, these antimicrobial subcomponents are only present in low percentages.[17]

Conjugated electrophiles and minerals were included as classes that are currently widely used but have known issues with human and environmental hazards. For instance, methylisothiazolinone (MIT) is a widely used antifungal in the conjugated electrophile class, although its mechanism of action relies on deactivation of biomolecules via its electrophilic sulfur atom.[18] Although MIT is potent against fungal spores, it is also a skin sensitizer and was named Contact Allergen of the Year in 2013 by the American Contact Dermatitis Society.[19] Compounds of cobalt, iron, manganese, nickel, copper, and zinc have been used as preservatives and biocides.[20]

We present order-of-magnitude antimicrobial inhibitory properties at neutral pH for 130 compounds selected from these eight chemical classes. Testing was performed using two microorganisms whose growth is notoriously difficult to inhibit: *Aspergillus brasiliensis* (filamentous fungi) (ATCC 16404) and *Pseudomonas aeruginosa* (Gram-negative bacteria) (ATCC 9027) which secretes a thick polysaccharide capsule. Characteristics relevant to formulation, such as water solubility, color, and odor, were noted such that the feasibility of using identified alternatives could be considered (Supporting Information (SI) Tables S1−S8). Hazard assessments of top-performing substances were completed, and those with the best balance of efficacy and safety underwent preservative challenge testing in home cleaning formulations. We thus provide an assessment of (1) the relative antimicrobial potencies of eight chemical classes against two key organisms, (2) a hazard assessment of selected substances, and (3) preservative challenge tests in personal care product formulations. We anticipate this information to be useful in the development of low hazard, broad-spectrum preservatives for consumer products.

MATERIALS AND METHODS

All chemicals used in this study were of technical grade or higher and were sourced from the following commercial suppliers unless otherwise specified (Sigma-Aldrich, TCI, Fisher). Furoate esters were provided by XF Technologies, Inc. Lauryl alcohol ethoxylate (Genapol LA 070, C12/14 fatty alcohol polyglycol ether) was obtained from Clariant, sodium lauryl sulfate was from Stepan, and alkyl polyglucoside (Glucopon 420 UP) was obtained from BASF. All chemicals were used as received, without any further purification. This approach ensures that our results are representative of practical applications where similar grades of chemicals would be used.

Order-of-Magnitude Antimicrobial Screening. Order-of-magnitude minimum inhibitory concentration (MIC) testing was adapted from previously published methods. These publications contain photographs depicting growth and inhibition of plates and liquid cultures, using the methods described in this work.[8,21−23] Test substances, obtained from chemical suppliers, were treated with 1 mL of Mueller-Hinton broth in a polypropylene tube. pH was adjusted to 7.4 ± 0.5 with HCl or NaOH (0.1 M) before cell inoculation. Test solutions were then inoculated with mold spores or bacterial cells. The initial cell count in the culture tube was ∼10^6 CFU (colony-forming units)/mL before incubation. *Pseudomonas*-containing samples were incubated at 37 °C with shaking (200 rpm) and assessed for growth overnight. *A. brasiliensis* was incubated for 4 days at 25 °C before growth was assessed. In both cases, the appearance of growth was indicated by an increase in turbidity, confirmed by visual inspection and ultraviolet−visible (UV−vis) spectrophotometry (absorbance at 600 nm) and the appearance of mycelium in the case of *Aspergillus*. Complete MIC results are provided in SI Tables S1−S8.

Hazard Analysis. Hazard analysis was completed systematically using authoritative lists, toxicology literature, and online databases including Pharos (Healthy Building Network), the Hazardous Substances Data Bank (HSDB, National Library of Medicine), and information from European Chemicals Agency (ECHA) registration dossiers. End points were grouped similarly to those in GreenScreen as previously described:[8]

- Group I: Human Health Group I
  Carcinogenicity, Mutagenicity & Genotoxicity, Reproductive Toxicity, Developmental Toxicity (including Developmental Neurotoxicity), and Endocrine System Activity.
- Group II and II*: Human Health Group II
  Acute Mammalian Toxicity, Systemic Toxicity & Organ Effects, Neurotoxicity, Skin and Respiratory Sensitization, Skin Irritation, and Eye Irritation.
- Ecotoxicity:
  Acute Aquatic Toxicity, Chronic Aquatic Toxicity, Persistence, and Bioaccumulation.

Hazard ratings were assigned based on the GreenScreen methodology, wherein the assignment of hazard levels

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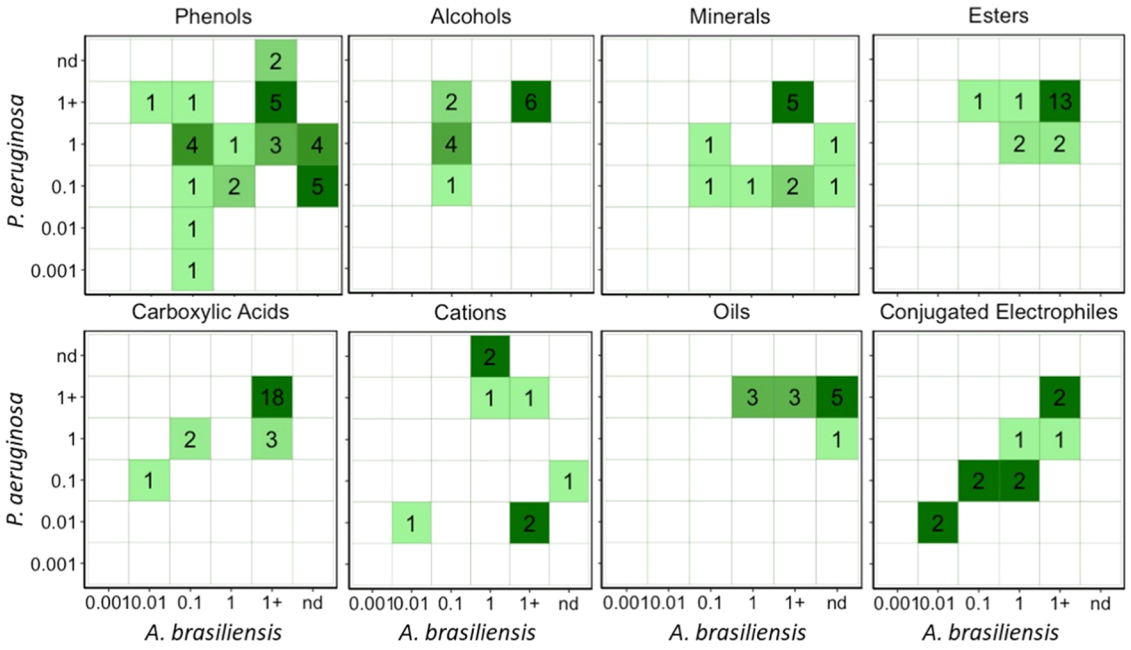

Figure 1. Number of compounds per class that prevented growth at each tested wt %. "nd" indicates that the MIC was not determined.

depended on the weight of evidence. For instance, the assignment of GHS categories by regulatory agencies was treated with high confidence when assessments aligned across multiple data sources (Pharos, ECHA, etc.). Conflicting evidence between data sources or multiple studies reported within a data source resulted in a lower confidence ranking. For chemicals with a GreenScreen assessment, ratings were presented as high confidence, unless conflicting and reliable evidence was found in another data source (e.g., a REACH registration dossier).

Due to a lack of empirical data, endocrine disruption was assessed using predictive tools through the EPA CompTox dashboard. Specifically, models for the activity with respect to androgen and estrogen receptors and assays conducted as part of the Endocrine Disruptor Screening Program for the 21st Century were included as evidence for (active) or against (inactive) the presence of an endocrine-disrupting potential. These ratings were assigned a low confidence level.

Preservative Challenge Test. The protocol for the preservative challenge testing followed the US Pharmacopeia Chapter 51 (USP 51). Where indicated, we evaluated the performance of only two of the most challenging microorganisms tested in the USP 51 protocol: *A. brasiliensis* (ATCC No. 16404) and *P. aeruginosa* (ATCC No. 9027).[24,25]

To assess the potency of a preservative in a personal care product formula, microorganisms were added to a sample of the product formula, to achieve concentration between 1.0×10^5 and 1.0×10^6 CFU/mL of product. The inoculated personal care product formulas were incubated and plated at days 3, 7, and 14. During plating, the number of CFUs were counted to determine the approximate number of viable microbial cells still present in the solution, with a detection limit of 1.0×10^2 CFU/mL. Antimicrobial effectiveness is determined by logarithmic reductions in growth over time.

Statistical Analysis. Statistical analyses were carried out by using Microsoft Excel. The microbial plating determinations, reported as counting colony-forming units (CFU), are reported with an error margin of ±50% CFU/mL, which is represented as the error bars in Figure 3. This considerable error margin is attributed to the inherent variability in microbial growth when measuring multiple order-of-magnitude changes in microbial populations.

Order-of-magnitude minimum inhibitory concentrations (MICs) were determined using dilutions of the following weight percentages in broth: 1, 0.1, 0.01, and 0.001% and consequently have an uncertainty of ± one order-of-magnitude. For example, an MIC of 0.01% has an uncertainty range of 0.1−0.001%. Despite this level of uncertainty, these MIC determinations provided valuable insights into the relative effectiveness of the substances tested over concentrations ranging 4 orders of magnitude. This approach enabled the rapid identification of compounds with promising antimicrobial properties.

RESULTS AND DISCUSSION

Antimicrobial Activity. Thirty-one compounds in the phenol class were screened for antimicrobial efficacy. At nominal levels of 1.0 wt % or lower, 12 compounds were effective against *A. brasiliensis*, 22 were effective against *P. aeruginosa*, and 10 were effective against both organisms (Figure 1). Three isomers (i.e., thymol, carvacrol, and 4-isopropyl-3-methylphenol), salicylic acid and its zinc salt were effective against *A. brasiliensis* at <1.0 wt %, but required higher concentrations to inhibit *P. aeruginosa*. Di- and trihydroxyphenols (2,5-dihydroxybenzaldehyde, 2,3,4-trihydroxybenzaldehyde, orcinol, propyl gallate, and gallic acid) exhibited the opposite tendency (SI Table S1). 1,2- and 1,4-disubstituted phenolics readily oxidize to their corresponding quinone under atmospheric oxygen and neutral pH. We thus evaluated hydroquinone p-benzoquinone, which afforded similar MIC's to the polyphenols. Compounds that lack the substitution to form quinones (3-hydroxy-4-methoxybenzaldehyde and 2,4-dihydroxybenzaldehyde) gave lower activity. Collectively, these results suggested that the high antibacterial activities of 1,2- and 1,4-disubstituted polyphenolics may be due to the formation of their corresponding quinone oxidation products.

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Thymol and propyl gallate were selected for further consideration because they were effective against both organisms at 1.0 wt % or lower and both compounds are food grade, indicating that they could be readily used in commercial products.

Twelve essential oils and extracts were tested for antimicrobial activity. The presence of active components representing different functional classes (i.e., aldehydes, phenols, alcohols) make essential oils and extracts effective against a wide array of microbes.[26] Mechanisms of action typically include antioxidant activity, membrane disruption, or protein deactivation. Of the 12 essential oils and extracts tested, only white willow extract was effective against *P. aeruginosa* at 1.0 wt %, which can be attributed to the presence of salicylic acid. Cedar, tea tree, and rosemary oils were effective against *A. brasiliensis* at 1.0%, which is consistent with previous reports.[27] The low activity of these substances can be attributed to their complex composition, which may contain only a small percentage of the active components, which vary depending on manufacturer, plant harvest conditions, and even cultivar used.

Thirteen alcohols were evaluated based on the use of alcohols as topical disinfectants, such as in hand sanitizers and mouthwash. Short-chain alcohols, such as isopropyl alcohol and ethanol, are bactericidal against microorganisms at high concentrations, typically >40 wt %, and are inhibitory around 10 wt %.[28] Midchain-length alcohols, including 2-phenoxyethanol, and diols (e.g., propylene glycol, caprylyl glycol), are being increasingly used as preservatives or preservative boosters in home and personal care products.[29] Of the 13 alcohols that were evaluated for microbial inhibition, seven inhibited the growth of *A. brasiliensis* at 0.1 wt % and five inhibited growth of *P. aeruginosa* at 1.0 wt % or lower. 2-phenoxyethanol, phenyl propanol, caprylyl glycol, 2-methoxybenzyl alcohol, and 4-ethylbenzyl alcohol all inhibited growth of both test organisms and were moderately hydrophobic ($\log P$ for these compounds ranges from 0.8−2.0). These substances are similar in their amphiphilic character, which includes nonpolar and polar motifs on opposing ends of the molecule. 3,4-dimethoxybenzyl alcohol ($\log P$ = 0.8), which contains opposing polar groups that diminish its amphiphilic character, was ineffective. Relatively hydrophobic and amphiphilic sesquiterpene alcohols (farnesol and nerolidol $\log P$ = 5.3; α-bisabolol, $\log P$ = 5.1) showed no activity, which may be due to their low water solubility, while monoterpenes geraniol ($\log P$ = 3.3) and (−)-nopol ($\log P$ = 3.1) were effective against *Aspergillus* but not *Pseudomonas*. Five and six-carbon glycols were ineffective ($\log P \leq 0.3$) in contrast to caprylyl glycol ($\log P$ = 0.8), which was among the most effective. In general, broad-spectrum antimicrobial activity was obtained with amphiphilic alcohols bearing moderate $\log P$ values (0.8−2.0). Caprylyl glycol and 2-phenoxyethanol were selected for further consideration in this study because they were among the most effective alcohols and data were available to conduct a hazard analysis.

Within the amine/amine class, eight potential preservatives were tested with a focus on polymeric substances. Cationic ammonium biopolymers are gaining increasing attention as low-hazard antimicrobials because they often provide multiple functionalities. For instance, chitosan, a film-forming polymer, has been shown to be potent against bacteria, with MICs of 0.005−0.02 wt % (50−200 ppm), though it can be less effective against fungi, with MICs greater than 0.2 wt % (2000 ppm).[30] For the chemicals in this class that we tested, amines and ammonium compounds tended to be more potent against *P. aeruginosa* than against *A. brasiliensis*. Polylysine was selected for further consideration because of its high relative effectiveness against mold and bacteria, whereas chitosan was considered further because it is widely available, waste-derived, and inexpensive.

Twenty-four organic acids were tested. Carboxylic acids have previously been found to function reliably when the solution pH is comparable to or lower than the acid pK_a (typically ∼5).[17] Carboxylic acids function as antimicrobials by damaging cell membranes and decreasing the microbial internal pH.[31] Our experimental results were consistent with this mode of action. Of the 24 organic acids that were tested in this experiment, three were effective against *A. brasiliensis* and 6 were effective against *P. aeruginosa* when present at 1.0 wt % or lower. Caprylhydroxamic acid (CHA) exhibited the highest efficacy, which can likely be attributed to the higher pK_a of this acid, which is ∼3 units higher than those of the other tested acids. This property results in the protonated hydroxamic acid predominating at neutral pH.

Nineteen esters were assessed for their antimicrobial efficacy. Like the acids that were tested, most of the ester compounds exhibited low antimicrobial potencies. Esters function as antimicrobials by increasing the permeability of the cell membrane, which leads to leaking of intracellular contents.[16] Microbial inhibition was observed in several cases, ethyl-5-methyl-2-furoate and methyl-5-methyl-2-furoate both were observed to have a MIC of 1.0 wt %. In our experiments, it was observed that for both *A. brasiliensis* and *P. aeruginosa*, four of the 19 compounds tested were effective at levels of 1.0 wt % or below. Sorbitan caprylate, which inhibited growth of *A. brasiliensis* at 0.1 wt %, was selected for inclusion in the hazard assessment.

Eleven conjugated electrophiles, including the commonly used preservative methylisothiazolinone (MIT), were evaluated for antimicrobial efficacy. These compounds were effective, as expected based on their current use, but have known hazard properties of concern. MIT is a very potent antifungal because it contains an active thiol that can oxidatively bind with free thiols to form disulfides, which ultimately interacts with enzymatic cysteines in vitro. This process results in the formation of free radicals, the destruction of protein thiols, and finally cell death.[18] Like MIT, many of the other conjugated electrophiles studied in this class are potent antifungals but are also skin sensitizers. For example, dimethyl fumarate is used as a mold inhibitor in shoes and leather furniture, yet has been shown to cause contact dermatitis in humans.[32,33] In our experiments, eight of the 11 conjugated electrophiles tested were effective against *A. brasiliensis* and nine were effective against *P. aeruginosa* when present at concentrations of 1.0 wt % or less. Maleimide and *N*-methylmaleimide were the best performers in the class, with an MIC value of 0.01 wt % for both *A. brasilensis* and *P. aeruginosa*.

The final class of preservatives that we evaluated comprised 12 mineral and metal preservatives. In this experiment, five of the 12 minerals that we tested were effective against *A. brasiliensis* and seven were effective against *P. aeruginosa* at 1.0 wt % or lower. Five compounds inhibited growth of *P. aeruginosa* with an MIC of 0.1 wt %. The minerals tested in this experiment served better as antibacterials than as antifungals.

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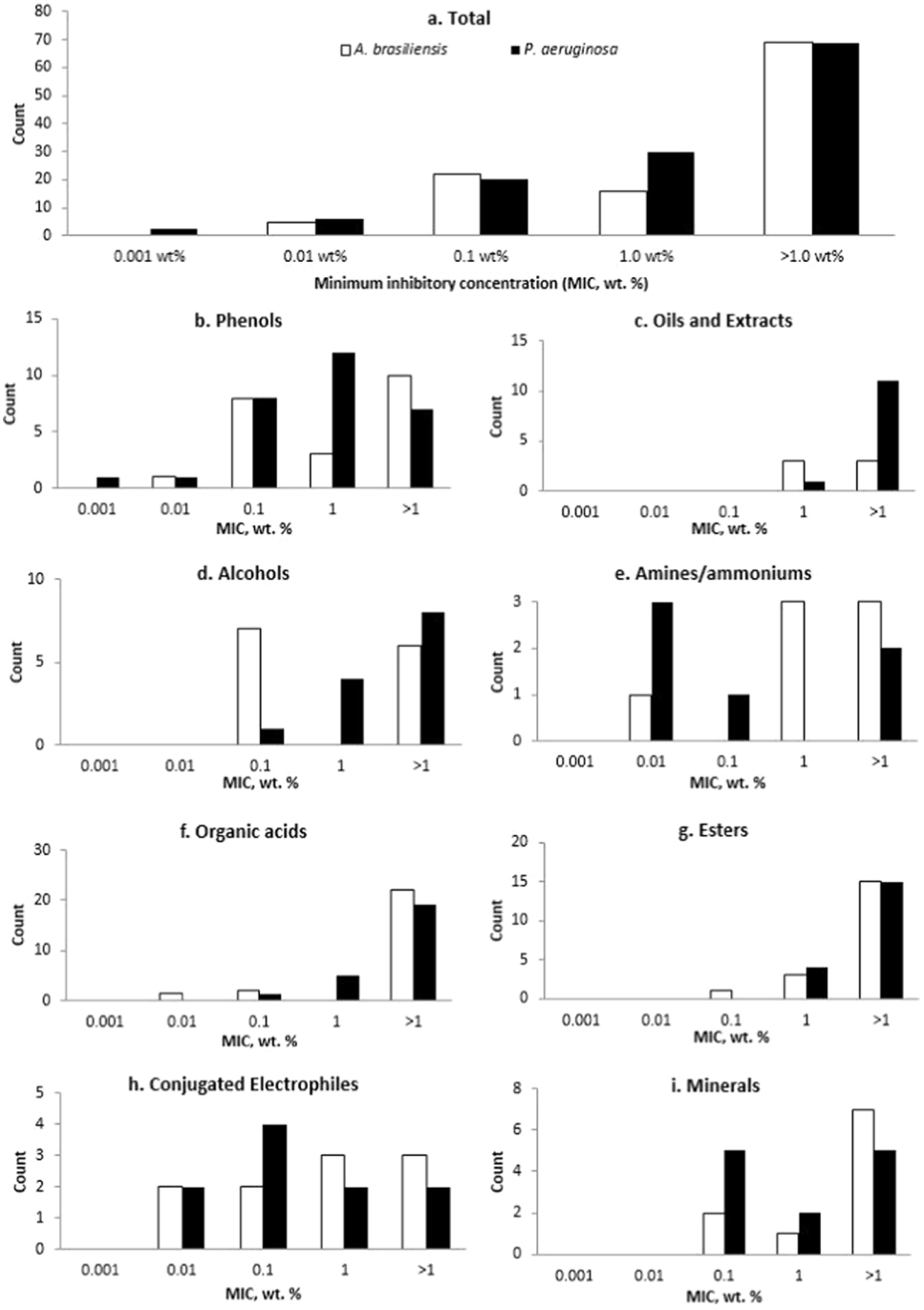

Figure 2. Potencies of (a) total chemicals tested (n = 130) against *A. brasiliensis* and *P. aeruginosa* compared to individual chemical classes (b–i). See SI for the full data set, including structures.

All eight chemical classes contained at least one compound effective against each organism, but most required higher concentrations than the conventional preservative MIT (Figure 2). A subset of phenols and cations inhibited growth at <0.1 wt %, while the few esters that inhibited growth were effective only at 0.1–1.0 wt %. Representative compounds from each class were selected for further consideration in the hazard analysis in order to understand potential trade-offs between safety and efficacy.

Hazard Analysis. The hazard analysis focused on compounds from each class that were effective at <1.0 wt %, with compounds that are food grade or already in commercial use being prioritized. Compounds were also generally selected to ensure that hazard data were available for human group I, irritation and sensitization, and environmental end points. Table 1 provides a summary of the hazard information, whereas full details of the hazard assessment and information sources for each end point are provided in the SI.

Two currently used preservatives, MIT and DMDM hydantoin, were assessed as illustrative examples of thiazolinones and formaldehyde releasers, respectively. Because of their widespread use and registration, these compounds have been well-studied across most hazard end points (e.g., data were available via Pharos and ECHA registration dossiers). MIT exhibited low toxicity to Human Group I end points (carcinogenicity, mutagenicity, reproductive and developmen-

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Table 1. Summary of Hazard Assessment Information for Selected Next-Generation Preservatives, as Compared with Traditional Industrial Preservatives

Class	Chemical	Human Group I					Human Group II					Human Group II*				Environmental			
		C	M	R	D	E	AT	ST1	N1	IrS	IrE	ST	N	SnS	SnR	AA	CA	P	B
Phenols	Thymol		L			L	M			vH	vH			L	M	H	H	L	L
	Propyl Gallate	L	L	L	L	H	M			M	vH			H		vH	vH	H	L
Alcohols	2-Phenoxyethanol	L	L	H	M	L	M		M	L	H	L	L	L		L	L	L	L
	Caprylyl Glycol		L		H	L	L			L	H			L		L		L	L
Minerals	Borax	L	L	H	H	H	L	vH		H	H	H		L		M	M		
	Copper Chloride	L	L	L	L	H	M			H	vH			L		vH	vH		M
Esters	Sorbitan Caprylate	L	L	L	L		L	L		L	L	L	L	L		M	M	L	L
Organic Acids	Potassium Sorbate	L	L	L	L	L	L			L	H			L		L	L	L	L
	Caprylhydroxamic Acid		L		L	L	L			L	H		M	L		H	H	L	L
	Sodium Benzoate	L	L	L	M	L	L			L	H			L		L	L	L	L
Cations	Polylysine																		
	Chitosan							M		H	H					vH			
Oils & Extracts	Tea Tree Oil		L	L			M			H	H			L		H	vH	L	
Conjugated Electrophiles	Maleimide					L	vH			vH	vH			H					L
Current-Use Preservatives	MIT	L	L	L	L	L	vH	M	M	vH	vH		M	H	L	vH	vH	L	L
	DMDM hydantoin	H	M	L	L	L	M	M		M	L	L		M	M		M	L	L

Hazard and Confidence Levels

Very High, Highest Confidence	High, Highest Confidence	Moderate, Highest Confidence	Low, Highest Confidence
Very High, Moderate Confidence	High, Moderate Confidence	Moderate, Moderate Confidence	Low, Moderate Confidence
Very High, Low Confidence	High, Low Confidence	Moderate, Low Confidence	Low, Low Confidence

tal toxicity) and was predicted by CompTox to be inactive with respect to androgen and estrogen receptors, resulting in an evaluation of low endocrine-disrupting potential. In contrast, DMDM hydantoin presents a carcinogenicity and mutagenicity hazard because it releases formaldehyde, which is classified as a human carcinogen.[34] Both preservatives present hazards to irritation and sensitization end points: GHS statements from the EU indicate very high skin and eye irritation and high skin sensitization hazards for MIT, while a GreenScreen evaluation of DMDM hydantoin concluded the compound is moderately hazardous for skin irritation and sensitization. Further, MIT presents a very high hazard, and DMDM presents a high hazard to aquatic life according to authoritative lists. The hazards to irritation and sensitization end points, the aquatic environment, and carcinogenicity/mutagenicity (for DMDM hydantoin) presented by these current-use preservatives provide strong motivation for identifying safer alternative preservatives.

Two phenols (i.e., thymol and propyl gallate) were selected for hazard analysis based on their effectiveness against target organisms at <1.0 wt % and their classification as food-grade ingredients. Both phenols exhibited high and very high hazard levels against irritation and aquatic toxicity end points based on authoritative lists. Propyl gallate is also considered very persistent by ECHA and is a potential endocrine disruptor based on CompTox predictions and in vitro tests.[35] Therefore, while these phenolic compounds could be effective preservatives, they present hazards similar to those of current-use compounds.

Caprylyl glycol and 2-phenoxyethanol from the alcohol class were selected for evaluation based on their relative effectiveness (MIC 0.1 wt % against *A. brasiliensis*, 1.0 wt % against *P. aeruginosa*) and current widespread use in home and personal care products. These two compounds exhibited similar hazard profiles, with some hazards in the Human Group 1 and Group 2 end points, but low environmental hazard levels. Reproductive and developmental effects were observed in mice exposed to phenoxyethanol, but such effects were not observed in rats or rabbits.[36,37] These results were interpreted by ECHA to indicate that 2-phenoxyethanol is likely not a developmental toxicant and classification with respect to this end point was inconclusive. Developmental toxicity tests of caprylyl glycol indicated body weight effects only at high doses (300 and 1000 mg/kg of body weight/day) where the active ingredient was likely acting through disruption of the gut microbiota. Beyond the mixed evidence for reproductive and developmental toxicity, the two alcohols were relatively benign: they exhibited eye irritation but not

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skin irritation or sensitization and present low hazard to the aquatic environment. These findings align with the current certification of caprylyl glycol by the EPA Design for Environment Green Circle.

From the essential oils class, tea tree oil was selected for hazard analysis because it was one of three substances with preservative capabilities against *A. brasiliensis* and because of its popularity in consumer products. None of the essential oils were effective against *P. aeruginosa*. Tea tree oil does not have known hazard properties for Group 1 end points but is highly toxic to aquatic organisms based on its ECHA registration. Further, tea tree oil is irritating to the skin but is not a skin sensitizer. Thus, despite its aquatic toxicity, the hazard profile of tea tree oil is still an improvement over MIT and DMDM hydantoin, which are sensitizing to the skin in addition to exhibiting aquatic toxicity, irritation, and other hazards.

Among the organic acids, three compounds were selected for hazard assessment: potassium sorbate, caprylhydroxamic acid, and sodium benzoate. Caprylhydroxamic acid was of particular interest because of its high pK_a that allows it to be effective as a preservative at circumneutral pH. Potassium sorbate and sodium benzoate are also attractive because they are certified by the EPA Design for Environment Green Circle program as safer alternatives. All three organic acids exhibited generally low human health hazards except for eye irritation. However, unlike potassium sorbate and sodium benzoate, caprylhydroxamic acid is also hazardous to aquatic organisms. These compounds thus represent a trade-off between preservative effectiveness and safety, with caprylhydroxamic acid presenting the greatest efficacy and greatest hazard. Still, the hazard profile of caprylhydroxamic acid may be considered favorable compared to current use isothiazolinones such as MIT, and this compound was selected for further testing in formulation to determine whether it could be a viable alternative.

Sorbitan caprylate was selected from the ester class based on its effectiveness against *A. brasiliensis* and exhibited a favorable hazard profile overall. Based on authoritative lists, sorbitan caprylate is not hazardous to Group 1 or Group 2 end points and poses a moderate hazard to aquatic toxicity. This compound had the safest hazard profile among the compounds evaluated across all eight classes in this study.

From the cation class, only polylysine and chitosan exhibited antimicrobial activity against either organism at 1.0 wt % or below. These two cationic polymers are also naturally derived and have been evaluated as food preservatives. Limited hazard data were available for polylysine or chitosan, and reports of chitosan hazard levels conflicted, leading to a low confidence in hazard assignments. For instance, while chitosan is recognized as a safer alternative by the EPA Design for Environment Green Circle program, it is also classified as an irritant by ECHA and exhibits acute aquatic toxicity, for example, to rainbow trout at low levels (LC_{50} = 0.38 mg/L) according to data on the EPA CompTox dashboard, which may be due to the presence of acids required to dissolve the biopolymer. Another uncertainty inherent in chitosan is that it is an *N*-deacetylated derivative of chitin. The degree of deacetylation of this copolymer and its chain length can alter its properties. More data on these compounds would be required to ascertain whether they are safer alternatives to current-use preservatives.

Maleimide was selected as a representative of the conjugated electrophile class because it inhibited growth of both *P. aeruginosa* and *A. brasiliensis* at 0.01 wt %. However, like methylisothiazolinone, maleimide presents high and very high hazards via skin sensitization and irritation end points, respectively, as indicated by GHS classifications made by the EU, New Zealand, and Korea. Although aquatic toxicity data were not available via the EPA's CompTox Dashboard, this compound has been flagged for potential concern by the Danish Advisory List, which uses QSAR models to predict toxicity. Based on the available data, maleimide is likely to be an effective preservative but is not a safer alternative compared to isothiazolinones.

Finally, borax and copper chloride (mineral preservatives) were evaluated due to their effectiveness in preservative screening tests and their low cost and widespread use. Both minerals exhibited hazards to irritation and environmental end points based on ECHA registration documents. Copper chloride poses a very high aquatic toxicity hazard (ECHA), whereas borax presents a moderate hazard based on GHS statements and LD_{50} values (e.g., LD_{50} was 27 mg/L for rainbow trout according to CompTox). Both minerals were listed on The Endocrine Disruptor Exchange (TEDX) as potential endocrine-disrupting compounds. Borax is also a suspected human reproductive and developmental toxicant based on the observation of developmental effects in three species (rats, mice, and rabbits), as described in its ECHA registration dossier. Overall, minerals such as copper chloride and borax pose both human health and environmental hazards and are not definitively safer than existing solutions.

Overall, several compounds were identified with more favorable hazard profiles than the current-use preservatives. Sorbitan caprylate (an ester) had the safest overall hazard profile. The organic acids also had generally low hazard. From this class, potassium sorbate and sodium benzoate had lower hazards than caprylhydroxamic acid but were not effective as preservatives in inhibition tests. Caprylyl glycol appears generally safe, but more conclusive evidence regarding potential developmental toxicity is needed. Based on these results, sorbitan caprylate, caprylhydroxamic acid, and caprylyl glycol were identified as promising safer alternatives to current preservatives.

Preservative Challenge Testing. Although minimum inhibitory concentration (MIC) values are important to understand the potency of a preservative, testing preservatives in formulations often provides more information about the preservative and how well it will perform during consumer use. Formula compositions play a major role in the potency of preservatives; some preservatives are not compatible with certain surfactant classes, and some preservative-surfactant combinations do not allow for a substantial logarithmic reduction in microbial cell counts.

To evaluate the preservative performance of lead compounds in a model formula, we developed a general surfactant composition from four classes of surfactants: 1.0 wt % alkyl polyglycoside, 1.0 wt % of lauric alcohol ethoxylate (nonionic surfactant), 1.0 wt % sodium lauryl sulfate (anionic surfactant), and 1.0 wt % cocamidopropyl betaine (amphoteric surfactant), for a total of 4.0 wt % unpreserved solids. Typically, inhibiting microbial growth at neutral pH poses a challenge, so the formula was adjusted to pH 7.0 after addition of preservative to ensure pH was not influencing growth. The test preservatives from chosen classes were added at 1.0 wt % to the unpreserved base formula. Four compounds were selected for preservative testing based on their low hazard and potency: caprylhydroxamic acid, caprylyl glycol, sorbitan caprylate, and propyl gallate. Samples were inoculated with 1.0E + 06 CFU/mL of *A.*

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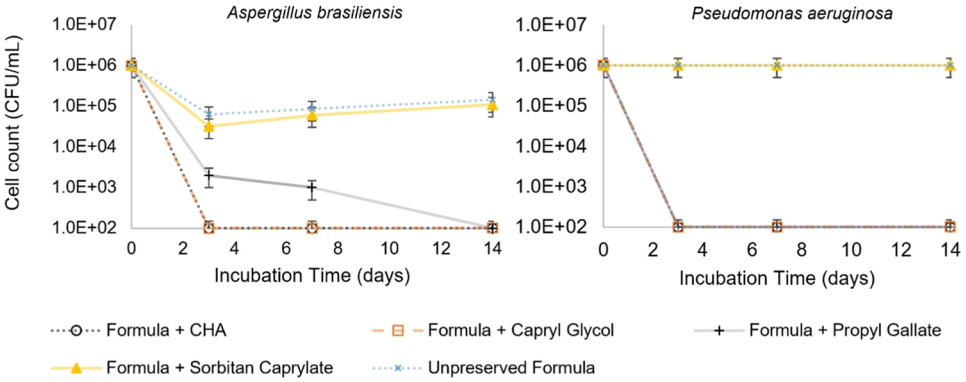

Figure 3. Plate counts of *A. brasiliensis* and *P. aeruginosa*, in two-week preservative tests in 4.0 wt % surfactant formula. Data points at a detection limit of 1.0×10^2 CFU/mL represent zero detectable colonies.

brasilensis and *P. aeruginosa* in accordance with the USP 51 protocol and plated over the course of 2 weeks.

Three of the four preservatives inhibited growth for up to 2 weeks. Caprylhydroxamic acid, caprylyl glycol, and propyl gallate were potent against *P. aeruginosa*, exhibiting a 4-log reduction after 3 days and maintaining the 4-log reduction through day 14 (Figure 3). Sorbitan caprylate did not exhibit an antibacterial effect against *P. aeruginosa* and *A. brasiliensis* at 1.0 wt %. Caprylyl glycol and caprylhydroxamic acid both affected a 4-log reduction against *A. brasilensis* after 3 days of incubation and maintained that reduction through day 14. Propyl gallate showed a 4-log reduction on day 14. These results indicate that CHA, caprylyl glycol, and propyl gallate could potentially be compatible preservatives for use in consumer products.

CONCLUSIONS

Of the eight chemical classes we evaluated, conjugated electrophiles and phenols were generally most effective at inhibiting the growth of *A. brasiliensis* and *P. aeruginosa*, while caprylhydroxamic acid and caprylyl glycol showed the best combinations of performance and hazard. Due to its higher pK_a, caprylhydroxamic acid exhibited antimicrobial activity at neutral pH, while conventional carboxylic acids (e.g., benzoic, sorbic) were much less effective. At 1.0 wt %, caprylhydroxamic acid, caprylyl glycol, and propyl gallate all effectively eliminated *P. aeruginosa* and *A. brasiliensis* to nondetectable levels (~99.99% reduction) in a prototypical surfactant formulation, suggesting their compatibility and effectiveness in home and personal care formulas. Additionally, caprylhydroxamic acid (0.5 wt %) eliminated *A. brasiliensis* in a commercial hand wash formula within 2 weeks, demonstrating its compatibility with a low (<10 wt %) solid, anionic surfactant-based commercial composition. A hazard analysis showed caprylhydroxamic acid and caprylyl glycol to exhibit safer toxicity profiles than current preservatives, such as methylisothiazolinone and formaldehyde releasers. There is thus an opportunity to substitute these substances in place of traditional, relatively hazardous preservatives.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c08672.

Antimicrobial testing methods; order-of-magnitude minimum inhibitory concentration results for eight chemical classes including structures, color, and odor: phenols, alcohols, minerals, esters, acids, oils and extracts, amines/ammoniums, and conjugated electrophiles (PDF)

Hazard analysis based on toxicological data from Pharos, PubChem, CompTox, ECHA, and Master spreadsheet (XLSX)

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