Emerging Pollutants in Grey Wastewater

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Maintaining good personal hygiene is essential to prevent infectious diseases from occurring and spreading. Everyday practices such as cleaning objects used often, washing face and hair, and brushing teeth, require using appropriate synthetic chemicals (personal health care products, PCP). Emerging micropollutants (EMPs) are compounds that have recently been classified as harmful to the environment and, consequently, the health of human beings. One specific group is micro-pollutants (M.P.s): contaminants found in trace concentrations (microgram to nanogram per litter or kg). The most detected micropollutants in greywater are Triclosan (biocide), Methylparaben, and Propylparaben (preservatives), Galaxolide and Tonalide (fragrances), as well as Oxybenzone and Octocrylene (U.V. filters) and Benzalkonium chloride. Biocides are active chemicals that control the growth of bacteria or kill them. Preservatives are compounds that inhibit the growth of any infectious microorganisms that may be present. Fragrance ingredients are extensively used in PCPs. Ultraviolet (UV) filters are compounds that block or absorb ultraviolet light. Benzalkonium chloride is used primarily as a disinfectant and is a common ingredient in domestic applications like personal hygiene products or fabric softeners.

emerging pollutants | antibiotic resistance | treated greywater | resistance genes

1. Cross-Resistance

Concerns about the effect of antimicrobials on the increasing resistance to antibiotics of bacteria were raised more than 50 years ago [1]. Giuliano and Rybak [2] showed that there is a potential link between triclosan and antibiotic resistance. Lu et al. [3] report bacterial mutants resistant to quinolone and mupirocin that have decreased susceptibility to triclosan. Exposure to benzalkonium chloride and triclosan resulted in increased resistance to erythromycin and ciprofloxacin strains of *Campylobacter jejuni* and *Campylobacter coli* [4]. Triclosan exposure in strains of *E. coli* and *P. aeruginosa* increased ten times the resistance to chloramphenicol and tetracycline [5]. However, there is still a lack of evidence on the role of EMPs and their direct effect on antibiotic resistance.

2. Ecotoxicological Effects of EMPs

Triclosan (TCS) is a common antimicrobial chemical in numerous PCPs (soaps, sanitizers, and toothpaste) [6] and is widely detected in aquatic environments at µg/L [7][8] to mg/L level [9]. It can serve as an external pressure to co-select for triclosan resistance and antibiotic resistance in many bacteria [10][11][12]. Triclosan induces oxidative stress, causing genetic mutations in a few genes, such as *marR, frdD, fabI, acrR,* and *soxR* [13][14][15]. The gene *fabI* is an acyl carrier protein reductase gene, a key enzyme in fatty acid synthesis in bacteria [16]. The interference with fatty acid synthesis results in modifications of the membrane structure, which causes less antibiotic uptake [17].

Subinhibitory concentrations of triclosan decrease the susceptibility of *E. coli* to ciprofloxacin, kanamycin, and gentamicin due to alteration of the membrane structure and biofilm formation [18]. Likewise, *Stenotrophomonas maltophilia* exposed to triclosan resulted in overexpression of the multidrug efflux pump SmeDEF and reportedly reduced the susceptibility to chloramphenicol, tetracycline, and ciprofloxacin [19]. Similarly, *Salmonella enterica* exposed to triclosan with increasing concentrations showed overexpression of the AcrAB efflux pump and reportedly reduced susceptibility to chloramphenicol, tetracycline, and ampicillin [20]. An efflux pump overexpression suggests the co-selective potential for more antimicrobial chemicals. In addition, *E. coli* exposed to triclosan also resulted in overexpression of the multidrug efflux pump and a transcription of genes encoding beta-lactamases. In contrast, the expression of genes related to membrane permeability decreases [15]. Hartmann et al. [21] documented that exposure to antimicrobials methyl-, ethyl-, propyl-, butylparaben, triclocarban, and triclosan increases ARGs in the microbiome.

Parabens are widely used as preservatives in many pharmaceutical, food, and cosmetic products due to their low toxicity [22]. They are often used in small amounts and primarily prevent bacteria growth and prolong shelf life. The mode of action against microorganisms mostly interferes with cellular membrane transfer processes. The effectivity of the paraben is correlated with the size of the chemical; propylparaben is considered more active against most bacteria than methylparaben [23]. Propylparaben specifically induces the permeabilization of bacterial membranes, causing the release of potassium [24][25]. The mechanisms of microbial resistance to parabens need to be better understood. Parabens are less active toward Gram-negative than Gram-positive bacteria [26][27]. Paraben resistance has been linked to the cell wall characteristics and non-specific efflux systems [28]. However, so far, only a few cases of resistance to parabens have been reported, occurring in the specific strains of *P. aeruginosa*, *Burkholderia cepacia*, and *Cladosporium resinae* [22]. Wu et al. [29] reported biodegradation of methyl- and propylparaben under aerobic and anoxic conditions, and benzoic acid was identified as one of the significant degradation products, thus reducing the efficacy of the compound.

Tonalide and galaxolide are the most used synthetic fragrance compounds in various PCPs, such as detergents, perfumes, deodorants, skin creams, and soaps [30]. A tonalide concentration has been detected in wastewater treatment plants (WWTP), ranging between 0.086 and 12.5 µg/L and 0.043 and 16.6 µg/L for galaxolide [31]. They are moderately soluble in water and thus increase the possibility of accumulation in the environment [32]. Tonalide and galaxolide are volatile lipophilic compounds and can, therefore, relatively easily penetrate through the cell wall of microorganisms. They are more active against Gram-positive than Gram-negative bacteria [33]. Tonalide and galaxolide disrupt and damage the structure of the membrane, resulting in a loss of ions, collapse of the proton pump and cytoplasm leakage, enzyme inhibition, and proton exchange disruption [34]. The metabolic pathway of hydroxylation is mainly causing the biotransformation of galaxolide, which is attributed to the existence of the cytochrome P-450 enzymes, which are linked to the inactivation of antibiotics [35].

Oxybenzone and octocrylene are filters that absorb U.V. radiation between 280 and 400 nm. U.V. filters are the main components of sunscreen due to their absorbing properties, but they are also found in other industrial products such as plastics and paints. Lozano et al. [36] were the first to analyze the effect of these compounds on

microbes, reporting that they affected only gram-negative microbes. A correlation has been detected between genome size and the appearance of resistance mechanisms [37].

Quaternary ammonium compounds (QACs) are a group of chemicals found in most household cleaning products because of their different functions. They can act as disinfectants, surfactants, or preservatives [38]. Several genes, such as *qacE*, *qacE11*, *qacF*, *qacG*, and *qacH*, have been reported to confer resistance to QACs in Gram-negative bacteria, with *qacE11* being the most widespread [39]. These genes belong to the small multidrug resistance (SMR) family [40], and their resistance to QACs is efflux-mediated [41]. The use of QACs drives the spread of class I integrons, responsible for a significant part of antimicrobial resistance in Gram-negative bacteria [42][43]. Benzalkonium chlorides (BAC) are the most commonly used QACs [44]. Isolates of *P. aeruginosa* exposed to increasing concentrations of BAC caused mutations in the *pmrB* gene and physiological adaptations that contributed to a higher tolerance to antibiotics [45]. Additionally, Guerin et al. [46] report the susceptibility of *Listeria monocytogene* to various antibiotics such as ciprofloxacin, gentamicin, or kanamycin after exposure to BAC. Efflux pump expression most likely causes antibiotic resistance to BAC, accompanied by the minor role of reduced membrane permeability [47]. Efflux genes such as *qacG*, *acrA*, *qacH*, and *acrB* have been identified in bacteria resistant to BAC [48].

Table 1 summarizes the EMPs their effect on bacteria and the defense mechanism against EMPs. In summary, non-antibiotic chemicals induce antibiotic resistance, and the cell wall is the first encounter between the bacteria and the chemical and is, thus, an essential mechanism of resistance.

Table 1. EMPs and their effect on bacteria and the defense mechanism of the bacteria.

Active Compound	Disinfectant Working Mechanism	Bacterial Adaptation to Disinfectant	References
Triclosan	- Oxidative stress in bacterial cell - Genetic mutation of the enoyl-acyl carrier protein (ACP) reductase genes - Fatty acid synthesis disruption	- Active efflux pumps - Membrane permeability decrease - Biotransformation, horizontal gene transfer - Increased target expression (overexpressed genes mufA1 and mufM)	[15][49][50][51][52]
Methyl- and propylparaben	- Membrane disruption - Cell leakage -	- Change of cell wall characteristics - active efflux pumps	[23][24][25]

Active Compound	Disinfectant Working Mechanism	Bacterial Adaptation to Disinfectant	References
	Induction of potassium efflux		
Tonalide and Galaxolide	- Membrane disruption - Enzyme inhibition - Proton exchange disruption.	- Existence of cytochrome P-450 (biotransformation)	[34][35]
Oxybenzone and octocrylene	- General toxic effects like reduced growth, energy, and DNA metabolism.	- Multidrug transporters - ROS responsive elements - Periplasmic stress response regulons	[36][37][53]
Benzalkonium chlorides	- Spread of intI1 gene - Cytoplasmic membrane damage	- Increasing horizontal gene transfer - Downregulation of membrane porins - Overexpression of efflux pumps	[54][55][56][57]

References

1. Russell, A.D. Antibiotic and Biocide Resistance in Bacteria: Introduction. Available online: https://academic.oup.com/jambio/article/92/s1/1S/6721470 (accessed on 23 January 2023).
2. Giuliano, C.A.; Rybak, M.J. Efficacy of triclosan as an antimicrobial hand soap and its potential impact on antimicrobial resistance: A focused review. Pharmacotherapy 2015, 35, 328–336.
3. Lu, J.; Wang, Y.; Li, J.; Mao, L.; Nguyen, S.H.; Duarte, T.; Coin, L.; Bond, P.; Yuan, Z.; Guo, J. Triclosan at environmentally relevant concentrations promotes horizontal transfer of multidrug resistance genes within and across bacterial genera. Environ. Int. 2018, 121, 1217–1226.
4. Mavri, A.; Smole Možina, S. Development of antimicrobial resistance in Campylobacter jejuni and Campylobacter coli adapted to biocides. Int. J. Food Microbiol. 2013, 160, 304–312.

5. Alderton, I.; Palmer, B.R.; Heinemann, J.A.; Pattis, I.; Weaver, L.; Gutiérrez-Ginés, M.J.; Horswell, J.; Tremblay, L.A. The role of emerging organic contaminants in the development of antimicrobial resistance. Emerg. Contam. 2021, 7, 160–171.
6. Cameron, A.; Barbieri, R.; Read, R.; Church, D.; Adator, E.H.; Zaheer, R.; McAllister, T.A. Functional screening for triclosan resistance in a wastewater metagenome and isolates of Escherichia coli and Enterococcus spp. From a large Canadian healthcare region. PLoS ONE 2019, 14, 1144.
7. Liu, J.L.; Wong, M.H. Pharmaceuticals and personal care products (PPCPs): A review on environmental contamination in China. Environ. Int. 2013, 59, 208–224.
8. Singer, H.; Müller, S.; Tixier, C.; Pillonel, L. Triclosan: Occurrence and fate of a widely used biocide in the aquatic environment: Field measurements in wastewater treatment plants, surface waters, and lake sediments. Environ. Sci. Technol. 2002, 36, 4998–5004.
9. Kumar, K.S.; Priya, S.M.; Peck, A.M.; Sajwan, K.S. Mass loadings of triclosan and triclocarbon from four wastewater treatment plants to three rivers and landfill in Savannah, Georgia, USA. Arch. Environ. Contam. Toxicol. 2010, 58, 275–285.
10. Schweizer, H.P. Triclosan: A widely used biocide and its link to antibiotics. FEMS Microbiol. Lett. 2001, 202, 1–7.
11. Halden, R.U. On the need and speed of regulating triclosan and triclocarban in the United States. Environ. Sci. Technol. 2014, 48, 3603–3611.
12. Birošová, L.; Mikulášová, M. Development of triclosan and antibiotic resistance in Salmonella enterica serovar Typhimurium. J. Med. Microbiol. 2009, 58, 436–441.
13. McMurry, L.M.; Oethinger, M.; Levy, S.B. Triclosan targets lipid synthesis. Nature 1998, 394, 531–532.
14. Fahimipour, A.K.; ben Maamar, S.; McFarland, A.G.; Blaustein, R.A.; Chen, J.; Glawe, A.J.; Kline, J.; Green, J.L.; Halden, R.U.; van den Wymelenberg, K.; et al. Antimicrobial Chemicals Associate with Microbial Function and Antibiotic Resistance Indoors. mSystems 2018, 3, e00200-18.
15. Lu, J.; Jin, M.; Nguyen, S.H.; Mao, L.; Li, J.; Coin, L.J.M.; Yuan, Z.; Guo, J. Non-antibiotic antimicrobial triclosan induces multiple antibiotic resistance through genetic mutation. Environ. Int. 2018, 118, 257–265.
16. Rana, P.; Ghouse, S.M.; Akunuri, R.; Madhavi, Y.; Chopra, S.; Nanduri, S. FabI (enoyl acyl carrier protein reductase)—A potential broad spectrum therapeutic target and its inhibitors. Eur. J. Med. Chem. 2020, 208, 15.
17. Zhu, L.; Bi, H.; Ma, J.; Hu, Z.; Zhang, W.; Cronan, J.E.; Wang, H. The two functional enoyl-acyl carrier protein reductases of Enterococcus faecalis do not mediate triclosan resistance. mBio

2013, 4, e00613-13.

18. Li, M.; He, Y.; Sun, J.; Li, J.; Bai, J.; Zhang, C. Chronic Exposure to an Environmentally Relevant Triclosan Concentration Induces Persistent Triclosan Resistance but Reversible Antibiotic Tolerance in Escherichia coli. Environ. Sci. Technol. 2019, 53, 3277–3286.
19. Sanchez, P.; Moreno, E.; Martinez, J.L. The biocide triclosan selects Stenotrophomonas maltophilia mutants that overproduce the SmeDEF multidrug efflux pump. Antimicrob. Agents Chemother. 2005, 49, 781–782.
20. Karatzas, K.A.G.; Webber, M.A.; Jorgensen, F.; Woodward, M.J.; Piddock, L.J.V.; Humphrey, T.J. Prolonged treatment of Salmonella enterica serovar Typhimurium with commercial disinfectants selects for multiple antibiotic resistance, increased efflux and reduced invasiveness. J. Antimicrob. Chemother. 2007, 60, 947–955.
21. Hartmann, E.M.; Hickey, R.; Hsu, T.; Betancourt Román, C.M.; Chen, J.; Schwager, R.; Kline, J.; Brown, G.Z.; Halden, R.U.; Huttenhower, C.; et al. Antimicrobial Chemicals Are Associated with Elevated Antibiotic Resistance Genes in the Indoor Dust Microbiome. Environ. Sci. Technol. 2016, 50, 9807–9815.
22. Valkova, N.; Lépine, F.; Valeanu, L.; Dupont, M.; Labrie, L.; Bisaillon, J.G.; Beaudet, R.; Shareck, F.; Villemur, R. Hydrolysis of 4-Hydroxybenzoic Acid Esters (Parabens) and Their Aerobic Transformation into Phenol by the Resistant Enterobacter cloacae Strain EM. Appl. Environ. Microbiol. 2001, 67, 2404–2409.
23. Nowak, K.; Jabłońska, E.; Ratajczak-Wrona, W. Controversy around parabens: Alternative strategies for preservative use in cosmetics and personal care products. Environ. Res. 2021, 198, 110488.
24. Bredin, J.; Davin-Régli, A.; Pagès, J.M. Propyl paraben induces potassium efflux in Escherichia coli. J. Antimicrob. Chemother. 2005, 55, 1013–1015.
25. Bolujoko, N.B.; Unuabonah, E.I.; Alfred, M.O.; Ogunlaja, A.; Ogunlaja, O.O.; Omorogie, M.O.; Olukanni, O.D. Toxicity and removal of parabens from water: A critical review. Sci. Total Environ. 2021, 792, 148092.
26. Soni, M.G.; Carabin, I.G.; Burdock, G.A. Safety assessment of esters of p-hydroxybenzoic acid (parabens). Food Chem. Toxicol. 2005, 43, 985–1015.
27. Selvaraj, K.K.; Sivakumar, S.; Sampath, S.; Shanmugam, G.; Sundaresan, U.; Ramaswamy, B.R. Paraben resistance in bacteria from sewage treatment plant effluents in India. Water Sci. Technol. 2013, 68, 2067–2073.
28. Blanco, P.; Hernando-Amado, S.; Reales-Calderon, J.A.; Corona, F.; Lira, F.; Alcalde-Rico, M.; Bernardini, A.; Sanchez, M.B.; Martinez, J.L. Bacterial multidrug efflux pumps: Much more than antibiotic resistance determinants. Microorganisms 2016, 4, 14.

29. Wu, Y.; Sun, Q.; Wang, Y.; Deng, C.; Yu, C.P. Comparative studies of aerobic and anaerobic biodegradation of methylparaben and propylparaben in activated sludge. Ecotoxicol. Environ. Saf. 2017, 138, 25–31.
30. Rimkus, G.G. Polycyclic Musk Fragrances in the Aquatic Environment. 1999. Available online: www.elsevier.com/locate/toxlet (accessed on 5 January 2023).
31. Clara, M.; Gans, O.; Windhofer, G.; Krenn, U.; Hartl, W.; Braun, K.; Scharf, S.; Scheffknecht, C. Occurrence of polycyclic musks in wastewater and receiving water bodies and fate during wastewater treatment. Chemosphere 2011, 82, 1116–1123.
32. Alfiya, Y.; Gross, A.; Sklarz, M.; Friedler, E. Reliability of on-site greywater treatment systems in Mediterranean and arid environments—A case study. Water Sci. Technol. 2013, 67, 1389–1395.
33. Dhifi, W.; Bellili, S.; Jazi, S.; Bahloul, N.; Mnif, W. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. Medicines 2016, 3, 25.
34. Bach, H.; Bach, H. Antimicrobial and anti-inflammatory activities of commercial aromatizing fragrances. Future Sci. OA 2021, 7, FSO704.
35. Ding, T.; Li, W.; Cai, M.; Jia, X.; Yang, M.; Yang, B.; Li, J. Algal toxicity, accumulation and metabolic pathways of galaxolide. J. Hazard. Mater. 2020, 384, 121360.
36. Lozano, C.; Matallana-Surget, S.; Givens, J.; Nouet, S.; Arbuckle, L.; Lambert, Z.; Lebaron, P. Toxicity of UV filters on marine bacteria: Combined effects with damaging solar radiation. Sci. Total Environ. 2020, 722, 137803.
37. Lozano, C.; Lebaron, P.; Matallana-Surget, S. Shedding light on the bacterial resistance to toxic UV filters: A comparative genomic study. PeerJ 2021, 9, e12278.
38. Hora, P.I.; Pati, S.G.; McNamara, P.J.; Arnold, W.A. Increased Use of Quaternary Ammonium Compounds during the SARS-CoV-2 Pandemic and Beyond: Consideration of Environmental Implications. Environ. Sci. Technol. Lett. 2020, 7, 622–631.
39. Kucken, D.; Feucht, H.-H.; Kaulfers, P.-M. Association of qacE and qacE Î" 1 with multiple resistance to antibiotics and antiseptics in clinical isolates of Gram-negative bacteria. FEMS Microbiol. Lett. 2000, 183, 95–98.
40. Burata, O.E.; Yeh, T.J.; Macdonald, C.B.; Stockbridge, R.B. Still rocking in the structural era: A molecular overview of the small multidrug resistance (SMR) transporter family. J. Biol. Chem. 2022, 298, 102482.
41. Zou, L.; Meng, J.; McDermott, P.F.; Wang, F.; Yang, Q.; Cao, G.; Hoffmann, M.; Zhao, S. Presence of disinfectant resistance genes in Escherichia coli isolated from retail meats in the USA. J. Antimicrob. Chemother. 2014, 69, 2644–2649.

42. Hegstad, K.; Langsrud, S.; Lunestad, B.T.; Scheie, A.A.; Sunde, M.; Yazdankhah, S.P. Does the Wide Use of Quaternary Ammonium Compounds Enhance the Selection and Spread of Antimicrobial Resistance and Thus Threaten Our Health? Microb. Drug Resist. 2010, 16, 91–104.
43. Gaze, W.H.; Abdouslam, N.; Hawkey, P.M.; Wellington, E.M.H. Incidence of class 1 integrons in a quaternary ammonium compound-polluted environment. Antimicrob. Agents Chemother. 2005, 49, 1802–1807.
44. Barber, O.W.; Hartmann, E.M. Benzalkonium chloride: A systematic review of its environmental entry through wastewater treatment, potential impact, and mitigation strategies. Crit. Rev. Environ. Sci. Technol. 2022, 52, 2691–2719.
45. Kim, M.; Weigand, M.R.; Oh, S.; Hatt, J.K.; Krishnan, R.; Tezel, U.; Pavlostathis, S.G.; Konstantinidis, K.T. Widely Used Benzalkonium Chloride Disinfectants Can Promote Antibiotic Resistance. Appl. Environ. Microbiol. 2018, 84, e01201-18.
46. Guérin, A.; Bridier, A.; le Grandois, P.; Sévellec, Y.; Palma, F.; Félix, B.; Roussel, S.; Soumet, C.; Karpíšková, R.; Pomelio, F.; et al. Exposure to quaternary ammonium compounds selects resistance to ciprofloxacin in listeria monocytogenes. Pathogens 2021, 10, 220.
47. Jiang, X.; Yu, T.; Xu, P.; Xu, X.; Ji, S.; Gao, W.; Shi, L. Role of Efflux Pumps in the in vitro Development of Ciprofloxacin Resistance in Listeria monocytogenes. Front. Microbiol. 2018, 9, 2350.
48. Bay, D.C.; Stremick, C.A.; Slipski, C.J.; Turner, R.J. Secondary multidrug efflux pump mutants alter Escherichia coli biofilm growth in the presence of cationic antimicrobial compounds. Res. Microbiol. 2017, 168, 208–221.
49. Khan, R.; Lee, M.H.; Joo, H.; Jung, Y.H.; Ahmad, S.; Choi, J.; Lee, S.W. Triclosan resistance in a bacterial fish pathogen, Aeromonas salmonicida subsp. salmonicida, is mediated by an enoyl reductase, FabV. J. Microbiol. Biotechnol. 2015, 25, 511–520.
50. Carey, D.E.; McNamara, P.J. The impact of triclosan on the spread of antibiotic resistance in the environment. Front. Microbiol. 2014, 5, 780.
51. Condell, O.; Sheridan, A.; Power, K.A.; Bonilla-Santiago, R.; Sergeant, K.; Renaut, J.; Burgess, C.; Fanning, S.; Nally, J.E. Comparative proteomic analysis of Salmonella tolerance to the biocide active agent triclosan. J. Proteom. 2012, 75, 4505–4519.
52. Yazdankhah, S.P.; Scheie, A.A.; Høiby, E.A.; Lunestad, B.-T.; Heir, E.; Fotland, T.Ø.; Naterstad, K.; Kruse, H. Triclosan and Antimicrobial Resistance in Bacteria: An Overview. 2006. Available online: www.liebertpub.com (accessed on 12 January 2023).
53. Lozano, C.; Lee, C.; Wattiez, R.; Lebaron, P.; Matallana-Surget, S. Unraveling the molecular effects of oxybenzone on the proteome of an environmentally relevant marine bacterium. Sci. Total Environ. 2021, 793, 148431.

54. Bondurant, S.; McKinney, T.; Bondurant, L.; Fitzpatrick, L. Evaluation of a benzalkonium chloride hand sanitizer in reducing transient Staphylococcus aureus bacterial skin contamination in health care workers. Am. J. Infect. Control 2020, 48, 522–526.
55. Fazlara, A.; Ekhtelat, M. The Disinfectant Effects of Benzalkonium Chloride on Some Important Foodborne Pathogens. J. Agric. Environ. Sci. 2012, 12, 23–29.
56. Gravel, J.; Paradis-Bleau, C.; Schmitzer, A.R. Adaptation of a bacterial membrane permeabilization assay for quantitative evaluation of benzalkonium chloride as a membrane-disrupting agent. Medchemcomm 2017, 8, 1408–1413.
57. Tandukar, M.; Oh, S.; Tezel, U.; Konstantinidis, K.T.; Pavlostathis, S.G. Long-term exposure to benzalkonium chloride disinfectants results in change of microbial community structure and increased antimicrobial resistance. Environ. Sci. Technol. 2013, 47, 9730–9738.