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Microbial contamination of pharmaceutical preparations represents a serious risk to the health and safety of the receiving patient. Prevention of microbial contamination is necessary to minimize the risk of patient exposure to harmful microorganisms (especially in immunocompromised patients), harmful microbial metabolites or toxins, and from exposure to spoiled or degraded drug product.1 Protection against microbial growth is important not only for sterile compounded dosage forms, but also for nonsterile preparations. Upcoming USP <795> guideline changes put a greater emphasis on water activity and preservation status. Previous USP <795> guidelines official from 2014 to November 2023 did not have any stated preservative requirement for BUD extension, instead dividing aqueous dosage forms into categories based on oral vs non-oral (topical, mucosal etc.) routes of administration. Upcoming USP <795> guideline changes set to go into effect November 2023 now delineate the allowed BUD for aqueous dosage forms (in the absence of an acceptable study as outlined by the guideline) not based on route of administration but based on preservative status, allowing up to 14 days for unpreserved aqueous preparations and up to 35 days for preserved aqueous preparations.2 In light of a new emphasis on preservative status, it is essential to understand how preservatives work and which may be appropriate for a given preparation based on characteristics such as route of administration, excipients, and chemical characteristics such as a preparation's pH.

An appropriate preservative or preservative system must exert a wide spectrum of antimicrobial activity against a variety of organisms (gram positive, gram negative, molds, yeasts etc.), dissolve in the preparation vehicle, not have irritating properties, and not interact negatively with the active pharmaceutical ingredient (API) or other components.3 Antimicrobial Effectiveness Testing per USP <51>, a required element for BUD extension of aqueous preparations under new USP <795> guidelines official in November, requires challenges with escherichia coli, pseudomonas, staphylococcus, candida, and aspergillus species, encompassing gram

negative, gram positive, and fungal organisms.2 A preservative system must be effective against all three types of organisms to pass AET. Common preservative classes and their mechanisms of action are listed in the table below. In this blog post, we'll highlight a few of those classes and discuss their uses as well as important considerations for each preservative class.

Table 1: Summary of Common Preservatives and Preservative Classes[1]

Class	Typical Routes of Administration	Preservative	Mechanism of Action
Aryl or Alkyl Acids	Topical Oral Parenteral	Sodium Benzoate Benzoic Acid Sorbic Acid Potassium Sorbate	Cell wall and cytoplasm: Sorbic acid inhibits transport mechanisms across the cytoplasmic membrane. Benzoic acid acts through several mechanism, one being acidification of the cytoplasm resulting in inhibition of some enzymatic processes
Amino Aryl Acid Esters	Topical Oral Parenteral Nasal	Parabens	Increase membrane permeability leading to increased "leakage" and cell death. Also inhibits folic acid synthesis
Quaternary Ammonium Compounds	Topical Nasal Parenteral (benzethonium)	Benzalkonium Chloride Benzethonium Chloride	Bind to the cytoplasmic membrane causing damage and leakage
Biguanides	Topical	Chlorhexidine	Acts at the cytoplasmic membrane by inhibiting ATPase, thereby inhibiting cellular anaerobic activity. Also causes membrane permeability and leakage
Phenols	Topical Parenteral	Phenol m-cresol	Lysis of the cell wall due to enzyme inhibition. May also affect membrane permeability leading to cell "leakage" and death and cause protein denaturation
Formaldehyde Donators	Topical	Imidizolidinyl Urea Diazolidinyl Urea	Acts on the carboxylic and amino enzymes in the cytoplasm

Benzoic Acid Derivatives

Sodium benzoate and benzoic acid are especially common preservatives in oral aqueous preparations. Sodium benzoate is present in many cosmetics, foods, and pharmaceuticals in concentrations ranging from 0.02-0.5%. Sodium benzoate has greater solubility than benzoic acid and is sometimes used preferentially in oral aqueous preparations for that reason. Despite the solubility difference, the activity of sodium benzoate as a preservative relies on the formation of benzoic acid and so the preservative is most efficacious at acidic pH.4 Benzyl alcohol is commonly used in parenteral preparations at a range of 0.9-2%.4 Benzoic acid, benzyl alcohol, and other benzoate derivatives can be cause for concern in some populations. Benzoate derivatives have the ability to displace bilirubin from albumin. This is of particular concern in neonatal patients where,

given immaturity of metabolic enzymes until 8 weeks of age, accumulation of benzoic acid may occur. Given this, benzyl alcohol, benzoic acid, and sodium benzoate are not recommended for use as preservatives in neonatal patients.5 In addition to toxicity in neonatal patients, benzoate derivatives can also be of concern in cats. One study found that doses of benzoic acid greater than 0.2g/kg daily or single doses exceeding 0.45g/kg resulted in hyperaethesia, apprehension, and depression 48 to 72 hours post uptake eventually resulting in death in some cats.6 Other cases of toxicity following exposure to benzyl alcohol, benzyl benzoate, and even benzocaine have been reported. While some amount of benzoic acid in preparations for cats may be considered safe (such as usually in the case of metronidazole benzoate), some sources urge caution when working with cats and especially those with decreased liver function.7 Generally, for healthy adults, the World Health Organization (WHO) has set an acceptable daily intake of benzoic acid at 5mg/kg.4

Sorbic Acid/Potassium Sorbate

Potassium sorbate is a common preservative in foods, cosmetics, and pharmaceuticals. It is often used topically or orally at 0.1-0.2%. Much like the case with sodium benzoate and benzoic acid, potassium sorbate is a more soluble form of sorbic acid and is used more frequently in formulations for this reason. Sorbic acid and potassium sorbate exert their activity primarily at acidic pH.4 Generally, sorbic acid and potassium sorbate are considered to be safe orally even for pediatric patients.8 The WHO has set the total acceptable daily intake for potassium sorbate and sorbic acid at 25mg/kg as sorbic acid.4

Parabens

Parabens are a series of preservatives that share para-hydroxybenzoic acid (PHBA) as a structural component.9 They have good activity against yeasts, molds, as well as gram positive and negative bacteria. They are effective across a wide pH range and many are stable to autoclaving without decomposition. Their wide range of efficacy and stability make them popular options and they are often used in combination due to synergistic effects. However, parabens have been under increased scrutiny due to reports of potential toxicity including potential oxidative damage and estrogenic impacts. This increased scrutiny has culminated in the ban of propylparaben in oral products per California law, set to go into effect January 2027.10 The Joint Expert Committee on Food Additives (JECFA) recommends limiting the maximum daily intake for parabens to 10mg/kg of body weight, consistent with WHO recommendations, and also recommends that propylparaben be excluded from foods.4,9 Some parabens are associated with more toxicity than others due to length of the ester chain. Lipophilicity and stability increase with increasing ester chain length, unfortunately, this increasing lipophilicity is associated with increasing toxicity and increasing estrogenic effects.11 Methyl and ethyl parabens are generally smaller in size with shorter chain lengths, whereas butyl and propyl paraben have longer chain lengths.9 Currently EU Cosmetic Regulation has set the maximum concentration of methylparaben and ethylparaben to 0.4% and propyl and butylparaben to 0.14% with concentrations of no more than 0.8% being allowed for combination paraben products (assuming that if propyl or butyl paraben are also a part of these combinations, their concentration does not exceed 0.14%).12

Quaternary Ammonium Compounds

Quaternary ammonium compounds, such as benzalkonium chloride, have broad spectrum antimicrobial activity against bacteria, viruses, and fungi. They are often used as preservatives in nasal, ophthalmic, and other topical preparations. In addition to use as a preservative, benzalkonium chloride is sometimes used in topical antimicrobial rinses or soaps. Benzalkonium chloride antimicrobial activity is enhanced in the presence of edetate disodium (EDTA). Benzalkonium chloride and benzethonium chloride are not commonly used orally and benzalkonium chloride may be associated with bitter taste orally.4,14

Phenolic Derivatives

Phenol and phenolic derivatives such as m-cresol are commonly used as preservatives in parenteral products and are of particularly common use as preservatives in peptide and protein preparations.4,16 One study

evaluating single agent preservative systems in licensed peptide or protein products noted the most common preservative to be m-cresol, followed by phenol with benzyl alcohol and benzalkonium chloride in third and fourth place respectively.16 Phenol and m-cresol are active against gram positive and negative bacteria and also have antifungal activity. Phenol and m-cresol are more active in the acidic pH range but are used in some protein formulations with pH values up to 7.8.16

Summary

Preservatives must be carefully chosen based on route of administration, patient (human or veterinary) being treated, patient age, and chemical characteristics of the final preparation among other factors. For more information on characteristics, uses, concentrations, and incompatibilities of specific preservatives head to www.fagronacademy.us to view our FACTS member exclusive resource document on pharmaceutical preservatives!

References:

1. Microbial Quality Considerations in Non-sterile Drug Manufacturing: Guidance for Industry (Draft). Updated Sept. 2021. Accessed Oct 2023.
2. United States Pharmacopeia and National Formulary (USP 41-NF 36). Rockville, MD: United States Pharmacopeial Convention; 2019. https://online.uspnf.com/uspnf/document/GUID-98DCB48D-DC23-4A63-AD2E-01CA8979FB7E_3_en-US. Accessed August 13th, 2019.
3. Elder D, Crowley P. Antimicrobial Preservatives Part One: Choosing a Preservative System. American Pharmaceutical Review. Antimicrobial Preservatives Part One: Choosing a Preservative System | American Pharmaceutical Review - The Review of American Pharmaceutical Business & Technology. Published Jan 2012. Accessed Oct. 2023.
4. Rowe R, Sheskey P, Quinn M. Handbook of Pharmaceutical Excipients. 6th ed. Pharmaceutical Press. Italy. 2009
5. Questions and answers on benzoic acid and benzoates used as excipients in medicinal products for human use. European Medicines Agency. Published 10/9/2017. Accessed 10/10/2023.
6. Bedford P, Clarke E. Experimental benzoic acid poisoning in the cat. Veterinary Record. 1972; 90(3): 55-58
7. Davidson G. To benzoate or not to benzoate: cats are the question. International Journal of Pharmaceutical Compounding. 2001; 5(2): 89-90
8. Ogbonna JDN, Cunha E, Attama AA, Ofokansi KC, Ferreira H, Pinto S, Gomes J, Marx ÍMG, Peres AM, Lobo JMS, Almeida IF. Overcoming Challenges in Pediatric Formulation with a Patient-Centric Design Approach: A Proof-of-Concept Study on the Design of an Oral Solution of a Bitter Drug. Pharmaceuticals (Basel). 2022 Oct 27;15(11):1331. doi: 10.3390/ph15111331. PMID: 36355503; PMCID: PMC9694284.
9. Penrose MT, Cobb GP. Identifying potential paraben transformation products and evaluating changes in toxicity as a result of transformation. Water Environ Res. 2022;94(4):e10705. doi:10.1002/wer.10705
10. The California Food Safety Act, Cal. Assembly Bill No § 418 Chapter 328 et seq.
11. Lincho J, Martins RC, Gomes J. Paraben Compounds—Part I: An Overview of Their Characteristics, Detection, and Impacts. Applied Sciences. 2021; 11(5):2307. https://doi.org/10.3390/app11052307
12. Cherian P, Zhu J, Bergfeld WF, et al. Amended Safety Assessment of Parabens as Used in Cosmetics. International Journal of Toxicology. 2020;39(1_suppl):5S-97S. doi:10.1177/1091581820925001
13. Merchel Piovesan Pereira B, Tagkopoulos I. Benzalkonium Chlorides: Uses, Regulatory Status, and Microbial Resistance. Appl Environ Microbiol. 2019;85(13):e00377-19. Published 2019 Jun 17. doi:10.1128/AEM.00377-19
14. Allen L. Excipients Used in Nonsterile Compounding, Part 3: Preservatives. International Journal of Pharmaceutical Compounding. 2019: 23(6). 472-477.

15. Merchel Piovesan Pereira B, Tagkopoulos I. Benzalkonium Chlorides: Uses, Regulatory Status, and Microbial Resistance. Appl Environ Microbiol. 2019;85(13):e00377-19. Published 2019 Jun 17. doi:10.1128/AEM.00377-19
16. Stroppel L, Schultz-Fademrecht T, Cebulla M, Blech M, Marhöfer RJ, Selzer PM, Garidel P. Antimicrobial Preservatives for Protein and Peptide Formulations: An Overview. Pharmaceutics. 2023; 15(2):563. https://doi.org/10.3390/pharmaceutics15020563

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