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Modes of action of three disinfectant active substances: A review

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ABSTRACT

This review deals with three categories of active substances for disinfectant products, their modes of action (MOA), and how MOA can help predict propensity for resistance in microorganisms. Within the European Union applications for approval of disinfectants of all kinds must be submitted in a few years, and documentation on MOA and resistance must be part of those applications. Peracetic acid is an unspecific, pervasive oxidizer of C–C double bonds and reduced atoms. This MOA would imply poor chance for development of resistance in microorganisms, as borne out by the absence of such reports in the literature. The quaternary ammonium compounds (QAC's) are much more specific in their antimicrobial mechanism. Even very low concentrations cause damage to the cytoplasmic membrane due to perturbation of the bilayers by the molecules' alkyl chains. Development of microbial resistance to QAC's, as well as cross-resistance to antibiotics, are particularly well documented. The polymer PHMB is antimicrobial because it disturbs the cell membrane's bilayer by interacting with it along the surface of the membrane. Resistance to the polymer appears not to develop despite many years of use in many fields. However, PHMB's toxicity to humans upon inhalation dictates great caution when deploying the substance.

1. Introduction

This review relates to disinfectants, to how they kill or inhibit unwanted microorganisms, and to the resistance that may arise as a consequence of use of disinfectants. In particular, this article reviews the scientific documentation that in the not-too distant future must be part of an application for approval of any disinfectant in any European Union (EU) member state (European Council & European Parliament, 1998; European Parliament & European Council, 2012). The past decades there have been legions of literature on the modes of action (MOA) of, and resistance to, disinfectants, and there has been rising scientific debate on the contribution of disinfectants to the increased frequency of antibiotic resistant microorganisms (White and McDermott, 2001; Maillard, 2002; Davies and Davies, 2010; Russell, 2003b; McDonnell and Russell, 1999; Bridier et al., 2011). Also the term *resistance* is being debated. In the present review resistance describes a situation where bacterial cells are not killed or inhibited by a concentration of antimicrobial substance that acts upon the majority of cells in that culture (European Commission, 2009). Thus, in this review, resistance is defined as a greater than 4-fold increase in the minimal inhibitory concentration (MIC), and tolerance covers increases in susceptibility less than 4-fold. This review will illustrate how knowledge of MOA can help predict propensity for either resistance or tolerance. The literature of the past three decades is summed up on the MOA's of peracetic acid (PAA), of the quaternary ammonium compounds (QAC's), and of poly(hexa methylenebiguanide) hydrochloride (PHMB). The result is an overview of the current understanding of the details of these MOA's that may be incorporated in applications for approval of disinfectant products containing these three active substances, as described in the next section.

1.1. Regulation of disinfectants by EU law

Disinfectant products are chemical mixtures that eliminate many or all undesirable microorganisms, except bacterial spores, on inanimate objects (Rutala et al., 2008). Disinfectants are commonly applied to biotic or abiotic surfaces such as directly on the skin, in bathrooms, kitchens, or in production facilities, but may also be added to for example drinking water or swimming pool water. In the EU, disinfectants are a subset of biocidal products (European Parliament & European Council, 2012). Other biocidal products include rat poison, mosquito repellent, antifouling paints for boats and slimicides, i.e., products not already covered by other, existing legislation.

When the EU biocides law is fully implemented, only specifically approved active substances will be allowed on the market, and only specifically approved products will be allowed (European Council & European Parliament, 1998; European Parliament & European Council, 2012). Active substances are evaluated and approved on behalf of the whole EU, whereas each product is

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evaluated and approved by the authority in the member state where the product is to be marketed. Product applications can only be submitted after their active substances have been approved. The dossiers for the disinfectant active substances were submitted in 2007, and as yet, only a few of these have been evaluated and approved. The active substance dossiers were compiled by companies or groups of companies with interest in the substances in question. These companies and groups will own the approvals of, and rights to, their active substances if and when approval is granted. New active substance dossiers may be submitted, but the time for evaluation is in the range of years. The dossiers both for the active substances and for future products must document their chemical identity, their efficacy as disinfectants, and their safety for humans and for the environment. Part of the efficacy documentation is a scientifically sound description of the substance's and the product's MOA. The gestalt of the MOA description for an active substance dossier is given in Box 1. The present review gives the data needed for the MOA description for PAA, the QAC's, and PHMB. Part of the safety documentation is considerations on the propensity of the active substance(s) for development of resistance. The present review also provides introductions to resistance issues for these three active substances.

1.2. Disinfectant risk and benefit

The overall technical objective of the EU law on biocides is to ensure that the benefits of a biocide (e.g., a disinfectant) outweigh the risks (European Parliament & European Council, 2012). According to the law, the risk–benefit balance must be conveyed to the user of the product via the product's label, as well as being part of the dossier kept by the approving authority (European Parliament & European Council, 2012). The benefit of a disinfectant is that it is efficacious at killing or inhibiting unwanted microorganisms. A risk of a disinfectant can be its toxicity to humans or its propensity for allowing development of resistance to the active substance.

The benefit of efficacy can be promoted or inhibited by many factors, and it is knowledge of the active substance's MOA on the targeted microorganism that allows predictions of efficacy to be made (European Commission, 2008b). For instance, knowledge of how the quaternary ammonium ion of benzalkonium chloride interacts with the bacterial cell membrane calls for caution when suggesting use of a viscose wipe to apply the disinfectant. The active substance is a cation, and viscose is anionic and a solid, which thus can adsorb and "inactivate" the active substance.

The risk of development of resistance to biocides has been a point of great concern for the European Commission for more than a decade (European Commission, 2001a, b). Indeed, the scientific literature abounds with reports of resistance to disinfectant active substances, and many of these reports have been reviewed (Davies and Davies, 2010; Russell, 2003a; McDonnell and Russell, 1999). Evaluation of the risk for the development of resistance is also helped by knowledge of the active substance's MOA (European Commission, 2008a). The MOA's for three disinfectant active substances are the primary focus of the rest of this review.

Box 1

Data required on mode of action in dossier for approval of a biocidal active substance

Excerpt of *Technical Notes for Guidance on Data Requirements*, Common core data set for active substances and biocidal products (European Commission, 2008c). Italicized text in brackets: reference to text in relevant annex of BPD (European Commission, 2008b; European Council & European Parliament, 1998).

5.4 Mode of action (including time delay) *[Ann. IIA, V.5.4.]*

- The mode of action in terms, where relevant, of the biochemical and physiological mechanism(s) and biochemical pathways involved should be stated. Where available, the results of experimental studies must be reported.
- Where it is known that in order to exert its intended effect the active substance must be converted into a metabolite or degradation product following application or use of a preparation containing it, justification should be submitted for why this metabolite or degradation product is not considered to be the active substance. In addition, available information relating to the formation of reactive metabolites or reaction products must be provided. This information must include:
  − the chemical name, empirical and structural formula, molecular mass, and CAS and EC (EINECS, ELINCS or No Longer Polymers list) numbers if available;
  − the processes, mechanisms and reactions involved;
  − kinetic and other data concerning the rate of conversion and if known the rate limiting step; and
  − environmental and other factors effecting the rate and extent of conversion.
- Indicate also if the actual active substance is the result of a combined action of different products (i.e., when such a combination is necessary to achieve the intended effect).

Table 1

Biocidal active substances in this review. See Fig. 1 for chemical structures. Section 3.2 presents differences among the antimicrobial efficacies of the three ADBAC's and compares these with that of the closely related DDAC.

Substance	Alkyl chain lengths	CAS No.	Product-type[a]	Relative production volume[b]
PAA	–	79-21-0	1–5	High
QAC				
ADBAC	C12–18[c]	68391-01-5	1–4	Low
ADBAC	C12–16[d]	68424-85-1	1–4	Low
ADBAC	C12–14[e]	85409-22-9	1–4	Low
DDAC	C10, C10[f]	7173-51-5	1–4	Low
PHMB	–		1–5	
Monomer	–	27083-27-8		n.i.
Polymer	–	32289-58-0		n.i.

[a] Product-types. Disinfectants for: 1, personal hygiene; 2, private and institutional use; 3, veterinary hygiene; 4, food and feed production; 5, drinking water (European Commission, 2013).

[b] Annual production volume within the EU: low, 10–1000 tons; high, >1000 tons; n.i., not in database (European Chemicals Agency, 2012; European Council, 1993). See database of production volumes: http://esis.jrc.ec.europa.eu/index.php?PGM=hpv.

[c] Stepan Company (2012c).

[d] (Stepan Company 2012a).

[e] (European Commission 2013).

[f] (Stepan Company 2012b).