Microbicides against emerging and new pathogens: a cause for concern

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Introduction
Examples of the use of microbicides can be traced back to ancient times when natural products were used to combat infection or to preserve mummies, and when metals such as silver were used to decontaminate potable water. Fumigation was introduced much later with, for example, the burning of juniper branches to decontaminate buildings where Black Death sufferers were housed, and later in the trenches during the First World War. The ‘modern’ use of microbicides to combat microbial infections probably dates from the 19th century, when pharmacists and medics experimented with hand hygiene and wound dressings with tremendous success. The 20th century witnessed an explosion in the diversity and use of microbicides as preservatives of pharmaceutical, medical and food products, as disinfectants and antiseptics, and in the plastics and textile industries (Figure 1). Today, public awareness of the role of microbial contamination in infection and spoilage has served as a springboard for the commercialisation of numerous products containing microbicides. As a result the market for microbicides is buoyant and competitive, although the European Scientific Committee for Emerging and Newly Identified Health Risks (SCENIHR 2009) recommended prudent use of microbicides. This is particularly relevant with the tightening control over microbicides allowed on the European market following the introduction and implementation of the Biocide Product Directive 1998 (BPD) and, more recently, the Registration, Evaluation and Authorisation of Chemicals Regulations 2006 (REACH).

Although the uses and applications of microbicides have increased tremendously over the last 10 years, their interactions with microbial cells remain poorly understood. This review addresses some of the issues in the light of emerging and re-emerging human pathogens.

Activity of microbicides
Empirically, microbicides have been described to have multiple targets against microbial cells; the number of targets damaged and the severity of damage produces either a lethal or a reversible static effect (Maillard 2002). It is now thought that such an effect is concentration dependent, whereby at a low concentration more specific interactions against defined bacterial targets might occur. This has been exemplified extensively with the bisphenol triclosan, which interacts specifically with an enoyl acyl reductase protein in bacteria at a low concentration, whereas at a higher concentration, non-specific membrane damage is likely to occur. Triclosan is unique at present as it is the most widely studied microbicide. With other microbicides such as chlorhexidine and isothiazolinones, specific targets have been reported, although there is an overall dearth of information on this subject (Maillard 2002).

The concentration of a microbicide is key to delivering a microbicidal effect (McDonnell and Russell 1999), but it is not the only factor that will affect the efficacy of a given microbicide (Maillard 2005). The nature of different microorganisms also needs to be taken into consideration. This was recognised in the Spaulding’s classification (Figure 2), which provide an indication of the intrinsic...
resistance of a microorganism to microbicides. Among the least susceptible are spores and bacterial endospores, while the least resistant are enveloped viruses. Although there are exceptions, this classification provides useful information about the susceptibility of different microorganisms and the types of microbicides that are required to kill them. It does not, however, take into consideration the response of a given microorganism to a microbicide in terms of decreased susceptibility and it does not cover activity against microbial biofilms (Maillard and Denyer 2009).

Levels of ‘germicidal’ action

![Figure 2](image)

<table>
<thead>
<tr>
<th>Level of 'germicidal' action</th>
<th>Description</th>
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<tbody>
<tr>
<td>High level</td>
<td>Complete destruction of microorganisms</td>
</tr>
<tr>
<td>Medium level</td>
<td>Reduction in numbers of microorganisms</td>
</tr>
<tr>
<td>Low level</td>
<td>Partial destruction of microorganisms</td>
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Intrinsinic mechanisms conferring resistance to microbicides

The intrinsic properties of microorganisms are interesting and deserve explanation. Apart from the spores, which are prominent in environmental strains and as such, not normally considered to be microorganisms but infectious agents that can self-replicate, the most resistant form of a microorganism is the bacterial endospore. Sporulation is a well-described mechanism by which certain bacteria, of the Clostridium and Bacillus genera, can survive detrimental conditions (for example, lack of nutrients) and detrimental physical and chemical factors, including exposure to microbicides. It is well recognised that the mechanisms of resistance of bacterial endospores reside in their structure, notably the presence of spore coats, cortex, a highly compressed inner membrane and the presence of small acid-soluble proteins that protect the spore nucleic acid in the core from oxidative damage. In addition, the spore is dehydrated and the concentration of Ca2+ ions is quite high, conferring resistance to heat (Leggett et al. 2012). It should be noted that there are differences in susceptibility/resistance of endospores to microbicides (Maillard 2011).

The other forms of microbial resistance to microbicides are not that dissimilar to the formation of endospores. For example, the formation of pseudomembranes confers resistance of amoeba to microbicides and their presence, notably in water treatment, might be responsible for the seasonal variation of a number of waterborne diseases (Thomas et al. 2010). Alternatively, the failure of disinfection treatment of contact lenses and their survival in cleaning and disinfecting solution is responsible for diseases such as amoebic keratitis (Thomas et al. 2010). There is little information on the process of encystation and the resulting decrease in susceptibility to microbicides. However, it is clear that cysts possess a cell wall and are highly dehydrated (Figure 3). Unfortunately, it has now been shown that protists can support the growth of a number of intracellular pathogens (Bacteria and viruses) and that protocysts can protect bacteria from a microbicide effect (Thomas et al. 2010).

Levels of resistance to microbicides are not available. The effect of microbicide on the microbial cell or in exerting a selective pressure might alter the classification (indicated with the green box and the red box with T). The microbial structures (endospores and cysts) that confer resistance to microbicides have been well documented and it might be bold but interesting to draw parallels to specific structures in vegetative bacteria. In Vibrio, the formation of rugose forms, which differ markedly from the usual bacterial cell appearance (Figure 3) results from a change in environmental conditions (nutrient availability). In V. cholerae, rugose variants have been associated with a marked decrease in susceptibility to dihydrin (Yildiz and Scholin 1996). The exact microbial mechanisms that confer such resistance to chlorex have not been studied, but the appearance of rugose variants, shorter, round cells, is a very useful diagnostic tool to note regular colony variants are also better biofilm producers (Yildiz and Scholin 1999).

The appearance of different colony morphotypes and bacterial cell structures following environmental changes is well described in other bacterial species such as Staphylococcus aureus, Rhizobium sp. and Clostridium spp. It is also worth noting that bacterial colony appearance following microbicide exposure sometimes changes; often small colonies are observed. This phenomenon has been associated with the presence of damaged bacterial cells that are recovering from microbicide challenge. In S. aureus, small colony variants have been associated with decreased susceptibility to triclosan (Bayston et al. 2007). Although it would provide more information about the mechanism of this phenomenon, we cannot draw conclusions on the association of different bacterial colony morphotypes with a change in microbicide susceptibility. A better understanding of these variants is needed, but the observations are interesting.

In addition to their intrinsic properties, microorganisms can acquire new properties enabling them to survive microbicide exposure. With the number of commercially available products or applications that make use of a low microbicide concentration, there is a concern that bacterial exposure to a product might promote the survival of microorganisms that have a reduced susceptibility to antimicrobials, including antibiotics (Scientific Committee on Emerging and Newly Identified Health Risks [SCENIHR], 2009, 2010). Scientific Committee on Consumer Safety (SCCS, 2010). The ability of a microorganism to survive microbicide exposure means that disinfection, preservative or antiseptic will fail. Persistence of microorganisms in controlled environments (food factories, healthcare facilities) is likely to occur (Maillard 2011).

The importance of microbial biofilms must be mentioned here. It is recognised that bacterial biofilms occur widely in the environment. Furthermore, it is well accepted that bacteria in a biofilm (i.e. sessile bacteria) are far less susceptible to antimicrobials than planktonic bacteria. A number of mechanisms have been proposed to explain this difference in susceptibility. These mechanisms include differential drug accumulation, a low metabolism, a ‘re-popping’ effect, expression of detoxifying enzymes and efflux (Maillard and Denyer 2009). It is worth noting that standard microbicide efficacy tests do not include the effect of microbial biofilms on the susceptibility of microorganisms (Maillard and Denyer 2009). It is worth noting that standard microbicide efficacy tests do not include the effect of microbial biofilms on the susceptibility of microorganisms (Maillard and Denyer 2009).

Bacterial resistance to microbicides is best explained by a combination of two different biological mechanisms: (i) low concentration of microbicide exposure resulting in outcomes or pseudo-outbreaks. A very useful paper is that of Rutala and Weber which reviews the scientific literature describing survival of bacteria following exposure mainly to antiseptics but also to disinfectants, and the development subsequently of infections (Weber and Rutala 2007). An earlier example of microbicide usage and survival of the target bacteria is provided with the use of silver nitrate (ionic silver) in wound dressings to combat Pseudomonas aeruginosa infections (Kasson et al. 1986). Although the incorporation of silver achieved the control of pseudomembrades, this paper provides information on emerging Ps. aeruginosa strains resistant to silver and the alteration in the commensal flora following the use of silver in the dressings. This paper provides an early example of the selective effect caused by microbicides, a concept that will be developed later. With the increasing use of silver and nano silver in numerous consumer products, such division requires refining, notably in consumer products, such division requires refining, together with the definition of resistance. Thus, in recent years the term ‘reduced susceptibility’, which refers to an increase in the minimum bactericidal concentration, has been used. It is also now accepted that the use of minimum inhibitory concentration can only provide limited information towards a susceptibility trend, but does not inform the resistance of a given bacterium to a microbicide. A useful practical definition of bacterial resistance to microbicides refers to the survival of bacteria in a product containing the in-use microbicide concentration.

Over the years a number of mechanisms that decrease the susceptibility of a bacterium to a microbicide have been described (Maillard and Denyer 2009). The mechanism that is thought to reduce the amount of a microbicide interacting with the cell and encompass a decrease in microbicide penetration, for example, with a change in outer cell membrane (lipopolysaccharide, peptidoglycan). A reduction of
In conclusion, microbicide interactions with micro-organisms remain to survive a microbicide insult. Mutations in bacteria (an associated with outbreaks and pseudo-outbreaks. However, examples of stricter regulations, at least in Europe. As with antibiotic misuse infection. They are becoming a limited resource following imposition.

The use of microbicides is essential to prevent contamination and maintenance of transfer of resistance genes between bacteria is costly for the bacterium, which reverts to being susceptible expressed. Some studies show that surviving microbicide exposure this selective effect of microbicides and its implication in practice.

Because of their nature, microbicides exert a selective pressure on microbial cells and hence will select for the least susceptible population. This is true when pure cultures or mixed bacterial populations are concerned. There is much debate at the moment on this specific effect of microbicides and its implication in practice. The fitness of less susceptible bacteria following microbicide exposure seems to depend upon the mechanisms of resistance expressed. Some studies show that surviving microbicide exposure is costly for the bacterium, which reverts to being susceptible in the absence of microbicide. Other studies demonstrated that the mechanisms expressed, with no detriment to bacterial competitiveness or virulence.

The real concern is the effect of microbicide exposure on the maintenance of transfer of resistance genes between bacteria (GEC4HR 2010). There is evidence that microbicides may be involved in horizontal gene transfer, but overall this has not been well studied and evidence is still lacking.

Conclusion
The use of microbicides is essential to prevent contamination and infection. They are becoming a limited resource following imposition of stricter regulations, at least in Europe. As with antibiotic misuse and abuse, there is a risk that improper and extensive use of microbicides may result in emerging resistance and cross-resistance in bacteria. There is already evidence emerging from the peer-reviewed literature that resistant bacteria have been isolated and associated with outbreaks and pseudo-outbreaks. However, examples remain anecdotal since the susceptibility of bacterial isolates is not regularly investigated. Indeed, to date, a call for a surveillance programme has been ignored, probably because of the financial resources that would be needed.

The application of microbicides at a sub-lethal concentration is of particular concern, since laboratory studies have demonstrated beyond doubt the ability of bacteria to express mechanisms enabling them to survive a microbicide insult. Mutations in bacteria (an acquired resistance mechanism) have also been under-studied over the years, although recently the association between expression of efflux and mutation rate has been reported.

In conclusion, microbicide interactions with micro-organisms remain a fascinating subject with practical applications. Although research activity has increased over the last 10 years, the subject warrants further investment considering the extensive use of microbicides today.
Engineered bioremediation approaches

As stated above, passive approaches such as MNA can be slow particularly for developer-driven remediation projects. As such, human intervention in the form of engineered approaches is more often than not required to accelerate bioremediation processes in a controlled manner. The two engineered approaches to remediate contaminated soil and groundwater are (i) ex situ and (ii) in situ remediation. In ex situ remediation, site soils or groundwater are collected, treated off-site to accelerate the biodegradation processes (e.g., biostimulation/bioaugmentation) and then returned to site. sequelae such as ecological, human health and property damage. For example, production of hydrocarbon fractions. Towards this end, significant research has been made for PAHs contaminant degraders of solvents, pesticides and explosives are included in such cases would enhance bioremediation. Some examples of contaminant degrader microorganisms

Table 4

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Contaminant type</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas sp. strain ADP</td>
<td>2,4,6-trinitrotoluene (TNT)</td>
<td>JMP134 (pJP4)</td>
</tr>
<tr>
<td>Dehalococcapsulifer sp. strain D2-1</td>
<td>Chlorinated solvent</td>
<td>TCE-DAE enirhodanine oxide</td>
</tr>
<tr>
<td>Bacillus sp. strain C15</td>
<td>Chlorinated hydrocarbon</td>
<td>1,2-Dichloroethene (DCE)</td>
</tr>
<tr>
<td>Phenacoea eutropha H16</td>
<td>Naphthalene</td>
<td>NAPD706</td>
</tr>
<tr>
<td>Paenibacillus sp. strain C15</td>
<td>2,4-Dichlorophenoxyacetic acid</td>
<td>2,4-Dichlorophenoxyacetic acid</td>
</tr>
</tbody>
</table>

Contaminant bioavailability in soils

Bioavailability is a term becoming increasingly important for bioremediation practitioners, as it relates to the ability to predict soil bioremediation endpoints. In short, if hydrocarbon contaminants are not bioavailable, microorganisms (whether indigenous or introduced) will not be able to degrade them. Therefore, the development of assays which can determine the bioavailable fraction of particular contaminants, rather than the total amount present (exhaustively extracted with harsh solvents) are potentially valuable. This is especially the case for polyaromatic hydrocarbons (PAHs) which often require their own separate human health risk assessment remediation criteria from petroleum hydrocarbon fractions. Toward this end, significant research has been made for PAHs contaminants in developing cyclodextrin based (non-exhaustive) extraction assays which have been shown to correlate with biodegradable fractions (Hickman et al., 2008). However, as PAH contaminated sites usually also require clean up of petroleum hydrocarbon contaminants, further work is needed in this area for remediation practitioners to be able to use such tests as an indicator of the suitability for using bioremediation at a particular site.

The ability of soils to provide ecosystem and social services can be affected by hydrocarbon contamination. As such, there is currently increasing interest in the remediation of contaminated soils to not only reduce pollution but also restore soil quality (function). Given the aggressive nature of many chemical and physical remediation techniques, this is another potential advantage of bioremediation.

In terms of monitoring soil quality, a number of biological indicators have been proposed and applied to agricultural, forested and to a lesser extent contaminated soils (Pottteau and Aspray, 2013). One popular biological indicator method is multiple substrate induced respiration (MSIR) where soil functional diversity is assessed by measuring the response of a soil to a number of individual carbon sources (substrates) and monitoring respiration response. In addition to assessing the restoration of contaminated soils, it has been suggested that MSIR assays may provide useful information prior to starting bioremediation projects (Shi et al., 2005). At this time, further research is needed on such assays for soil quality determination and treatability testing.

Conclusions

Although there remains a degree of uncertainty for remediation practitioners in the decision to adopt bioremediation over traditional dig and dump, fundamental research on hydrocarbon biodegradation and more recent development of bespoke assays (respiration and bioavailability) has helped in managing the risk. With alternatives requiring use of harsh and costly chemicals (chemical oxidation), energy (thermal desorption) or generating residual waste (biodegradation and washing) the future remains bright for bioremediation. The main challenge for bioremediation in the future will be to apply the processes to increasingly complex contaminants as well as higher concentrations and mixed contaminant scenarios.

About the author

Thomas has more than 10 years’ academic/research and industrial experience in the environmental sector. An environmental microbiologist by formal training he has expertise in general microbiology, molecular biology and analytical chemistry. Thomas
joined the School of Life Sciences at Heriot-Watt University in February 2012 after seven years working for a specialist soil and groundwater remediation contractor, where he was responsible for the design and delivery of full scale contaminated soil and groundwater treatment projects involving hydrocarbon, inorganic and/or heavy metal contaminants.

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References

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