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Scientific Committee on Emerging and Newly Identified Health Risks

SCENIHR

Research strategy to address the knowledge gaps on the antimicrobial resistance effects of biocides

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

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ABSTRACT

One aspect about the application of biocides that has not been fully appreciated is the induction of a selective pressure which may favour the selection of less-susceptible bacteria and the expression and dissemination of mechanisms of resistance. These aspects have not been particularly well studied, but scientific research has yielded some important information on the potential negative impact of biocides *in vitro* and *in situ.*

All of the research studies suggested in this opinion are important to gain a better understanding of the interaction between biocides and bacteria and to perform an appropriate risk assessment on the use of biocides and emerging resistance and cross-resistance in bacteria. One of the main objectives of these recommendations is to determine the resistance mechanisms against biocides and antibiotics following exposure to biocides. However, in order to take into account probable financial constraints, this opinion strongly suggests that two work packages should be considered in priority:

- WP1: Characterisation of mechanisms involved in cross-resistance against biocides and antibiotics, and

- WP2: Development of strategies to combat cross-resistance mechanisms.

Although these work packages will not be able to lead to a full risk assessment of the use of biocides and emerging resistance and cross-resistance, they are essential in providing a fundamental understanding of the bacterial mechanisms involved in resistance necessary for the development of measures that can be used by biocide manufacturers to decrease the development of resistance mechanisms in bacteria.

Keywords: biocides, resistance, cross-resistance, antibiotics, mechanisms of bacterial resistance

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

There are very many biocides, though information on their efficacy and bacterial resistance and cross-resistance is only available for a limited number of them. Triclosan is the most widely studied biocide with respect to bacterial resistance.

This document aims at addressing knowledge gaps about bacterial resistance and cross-resistance to biocides. It is acknowledged that the same lack of information on emerging resistance or selection for resistance may also concern other micro-organisms, notably fungi and protozoa.

This opinion updates a previous thorough review of the scientific literature on biocides by the SCENIHR (2009) and proposes research projects to address the scientific and technical gaps that have been identified.

All of the studies suggested in this opinion are important to gain a better understanding of the interaction between biocides and bacteria and to perform an appropriate risk assessment on the use of biocides and emerging resistance and cross-resistance in bacteria. One of the main objectives of these recommendations is to determine the resistance mechanisms against biocides and antibiotics following exposure to biocides. However, in order to take into account probable financial constraints, this opinion strongly suggests that two work packages should be considered in priority:

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1. BACKGROUND

Directive 98/8/EC of the European Parliament and of the Council on the placing on the market of biocidal products[1] not only aims to harmonise the European market for biocidal products and their active substances but also to provide a high level of protection for humans, animals and the environment. Active substances under this Directive are assessed at Community level, and if the outcome of the evaluation is positive, they are included in Annex I, IA or IB to the Directive. Member States then authorise biocidal products containing these active substances in accordance with harmonised criteria. The scope of the Directive is very wide, covering 23 different product types ranging from disinfectants, preservatives of products and materials, to substances for pest control in non-agricultural applications such as antifouling products used on hulls of vessels. The Directive does not apply to products already covered by other Community legislation such as plant protection products, medicines, food contact materials and cosmetics. Neither does the Directive apply to articles imported from the third countries (e.g. textiles, clothes, or other objects) treated with biocides. However, it should be noted that the Commission proposed to replace the Directive with a Regulation (COM(2009)267). Apart from improving the environmental and human health protection and streamlining the authorisation and mutual recognition procedures, this revision would also address the problem of imported treated articles. Furthermore, the revision highlights the importance of the prevention of resistance development.

In its opinion on Antimicrobial Resistance[2] adopted on 28 May 1999, the Scientific Steering Committee recommended, inter alia, "the prudent use of antimicrobials", "the reduction of the overall use of antimicrobials in a balanced way in all areas" and "the identification of major contributors to resistance". Furthermore, it recommended in its opinion on triclosan[3] adopted on 27/28 June 2002 that "the potential for biocides, in general, to induce antimicrobial resistance of importance to clinical medicine, or management of the wider environment be kept under continuous review. If new scientific evidence were to indicate a significant risk of biocides causing anti-microbial resistance to antibiotics used in human medicines, then appropriate action to manage these risks might be needed".

Recent scientific evidence suggests that during the last decade, antibiotic resistance by various mechanisms has increased worldwide in bacterial pathogens leading to treatment failures in human and animal infections. However, the bacterial resistance against different types of biocides (including disinfectants, antiseptics, preservatives and sterilants) has been studied only recently. Furthermore, research indicates that biocides and antibiotics may share some common behaviour and properties in their respective activity and in the resistance mechanisms developed by bacteria. One of the problems within Directive 98/8/EC and Directives dealing with similar kinds of substances is that cumulative risks and impacts resulting from the use of the active substance outside the scope of the Directive are not addressed in the evaluation process. This is especially problematic in view of the possibility of cross-resistance.

In 2008 the Commission therefore asked SCENIHR to assess the antibiotic resistance effects of biocides. The SCENIHR opinion delivered in January 2009[4] confirmed that at least some resistance mechanisms are common to both biocides and antibiotics. Scientific evidence from bacteriological, biochemical and genetic data does indicate that the use of active molecules in biocidal products may contribute to the increased occurrence of antibiotic resistant bacteria. In view of the large and increasing use of biocides and the continuous increase of bacterial resistance to antibiotics, the SCENIHR identified a number of data and knowledge gaps to be filled, especially:

[1] http://ec.europa.eu/environment/biocides/pdf/dir_98_8_biocides.pdf
[2] http://ec.europa.eu/food/fs/sc/ssc/out50_en.pdf
[3] http://ec.europa.eu/food/fs/sc/ssc/out269_en.pdf
[4] http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_021.pdf

a) Quantitative data on exposure to biocides;
b) Standards and methods to evaluate the ability of a biocide to induce/select for resistance against biocides and antibiotics; and
c) Environmental studies focusing on the identification and characterisation of resistance and cross-resistance to antibiotics following use and misuse of biocides.

In particular, the recommendation to develop standard protocols for the evaluation of antimicrobial resistance induced by biocides would be valuable in the review programme of the Biocides Directive where active substances used in biocidal products are currently being evaluated for their risks to human health and the environment. At present biocidal active substances are evaluated without taking account of this issue systematically in the testing and assessment under the review programme. However, steps should be undertaken to start developing these protocols in order to properly address the concern and recommendation stated in the above-mentioned opinion and to take account of antimicrobial resistance at the product authorisation stage (within 4-5 years) or at the first renewal of the biocidal active substances (within 10 years).

As the issue of the possible health effects of antimicrobial resistance (AMR) remains a very sensitive political subject, more research is needed to address the issues identified. The Commission, through the 7th Framework Programme for Research and Development (FP7), can finance such research through calls for proposals launched on a yearly basis.

2. TERMS OF REFERENCE

As a result, and in order for the Commission to be in a position to propose the most relevant research topics on this issue for future funding, the Committee is requested:

1. To develop, and if necessary expand the research recommendations presented in the *SCENIHR Opinion on the Assessment of the Antibiotic Resistance Effects of Biocides*. This would include the definition of the main scientific gaps addressed by each recommendation, in particular related to the development of standard protocols for the evaluation of antimicrobial resistance induced by biocides. The opinion should also include methodological guidance on the experimental design and on the requirements to ensure high quality and usability of the results for risk assessment.

2. To propose a pragmatic, stepwise research strategy based on studies which are feasible and able to deliver results within a reasonable time-frame (5-10 years).

3. SCIENTIFIC RATIONALE

3.1. Introduction

Bacterial resistance to antibiotics has increased worldwide, leading to treatment failure against pathogens responsible for human and animal infectious diseases (EARSS 2005, WHO 2007). The main reason for this increase is undeniably linked to the usage and abuse of antibiotics. However, *in vitro* evidence has shown that biocides can also play a role in the development, or selection and dissemination of bacterial pathogens showing resistance phenotypes to both biocides and antibiotics. This is a cause for concern that has been raised at both national and international levels and must be evaluated.

Biocides are invaluable compounds that provide society with numerous benefits. They play an important role in the control of bacteria in a variety of applications and are thus a precious resource that must be managed so as to be protected from loss of activity over time. In order to preserve the role of biocides in infection control and hygiene, it is paramount to prevent the emergence of bacterial resistance and cross-resistance through their appropriate and prudent use.

The indiscriminate use of biocides in an increasing number of applications across many fields (medicine, household, animals, foods etc.) goes against a prudent use of these antimicrobials. As biocides are also used in products not covered by the Biocides Directive, their use is less regulated than that of antibiotics. It is worth noting that the total amount of biocides used in Europe is largely unknown and only approximate estimates are available for some of them (SCENIHR 2009). Furthermore, the efficacy of biocides in many applications has not been established in practice, and is often measured *in vitro* with standard tests which rarely reflect the final application of a biocidal product (Maillard and Denyer 2009).

Biocides have been far less studied than antibiotics in terms of efficacy, mechanisms of action, and mechanisms and epidemiology of bacterial resistance. Despite the lack of information on many biocides, current knowledge indicates that bacteria possess a number of mechanisms to survive exposure to a biocide. Some of these mechanisms (e.g. efflux pumps, permeability changes and biofilms) also confer resistance to antibiotics (Bailey et al. 2009, Daniels and Ramos 2009, Maseda et al. 2009). Other mechanisms have also been described, such as mutation (Chen et al. 2009, Webber et al. 2008a) and by-pass of metabolism (Webber et al. 2008b), although their impact on cross-resistance is less evident.

It must also be recognised that some environmental factors contribute to a reduced susceptibility to biocides. Although the effect of microbial biofilms on biocide susceptibility has been well-documented (SCENIHR 2009), new factors such as bacterial swarming motility are emerging as contributing to cross-resistance (Lai et al. 2009).

One aspect of the application of biocides that has not been fully appreciated is the induction of a selective pressure which may favour the selection of less-susceptible bacteria and the expression and dissemination of mechanisms of resistance. These aspects have not been particularly well-studied, but have yielded some important information on the potential negative impact of biocides *in vitro* and *in situ*. A number of biocides such as phenolics, cationic biocides, and more reactive ones such as alkylating and oxidising biocides, may select less susceptible or resistant (i.e. to the in-use concentration) bacteria to biocides and antibiotics *in vitro* (Alonso-Hernando et al. 2009, SCENIHR 2009). However, such selection, demonstrated on numerous occasions, is not universal (Cottell et al. 2009). Recently, the failure of a high-level disinfectant to kill pathogenic bacteria led to an outbreak of *Mycobacterium massiliense* resistant to antimycobacterial antibiotics in 38 hospitals in Brazil (Duarte et al. 2009). This was the first *in situ* investigation linking failure of a biocide, cross-resistance to chemotherapeutic antibiotics and infectious outbreak. Other *in situ* investigations indicated a potential

relationship between the domestic use of certain quaternary ammonium compounds and the selection for antibiotic resistant pathogens (Carson et al. 2008). However, such results might not be universal for all biocides as demonstrated by a 6 month study on the effect of triclosan in the home environment (Cole et al. 2003).

The effect of biocides on maintaining extra-chromosomal elements (Birošová and Mikulášová 2009) and the horizontal transfer of resistance genes have been less-established because of a general paucity of information. The existence of horizontal gene transfer is the most likely mechanism for selecting and increasing antibiotic resistance. The dissemination of mobile genetic elements, their capacity to contain several resistance genes, and the presence of overlapping genetic cascades of regulation responding to selective pressures from chemicals on bacteria represent the highest risk factors.

Biocidal products are complex formulations that include components which may affect the bacterial cell and potentiate the activity of individual active ingredients. Biocidal activity is also affected by various other factors such as concentration, contact time, soiling, temperature, etc. The lack of understanding of these factors by manufacturers and users decreases biocide bactericidal efficacy, resulting in bacterial survival (Maillard and Denyer 2009). Other factors such as formulation (including stability), pH and inactivating materials are also important but are more of an issue for the manufacturers of biocidal products.

A recent study (Rajamohan et al. 2010) demonstrated the role of AdeB and AdeJ efflux pumps in *A. baumannii*; the deletion of respective genes generated an increase of susceptibility to various biocides. Transcriptome analyses (including microarray and RT-PCR experimental approaches) of *E. coli* and *S. enterica* cells exposed to triclosan (0.12 mg/L for 30 minutes) indicated an induction in the expression of various genes involved in drug efflux (e.g. *acrB*) and in the genetic control of resistance genes (e.g. *marA*), an effect in the control of oxidative and drug response (e.g. *soxS*), and in the control of membrane permeability (e.g. *ompR*). Despite some differences in the response between the two genera, triclosan induces a rapid and adaptive response including the activation of several genes (regulatory and structural) involved in antibiotic resistance (Bailey et al. 2009).

3.2. Preamble

This document aims at addressing knowledge gaps about bacterial resistance and cross-resistance to biocides. It is acknowledged that the same lack of information on emerging resistance or selection for resistance may also concern other micro-organisms, notably fungi and protozoa.

A thorough review of the scientific literature on biocides (SCENIHR 2009) led to the identification of serious gaps in knowledge. This precluded the development of a complete risk assessment of biocide capacity to develop or select for bacterial resistance to antibiotics. In particular, the SCENIHR recognised that:

"1. *Quantitative data on biocide exposure including concentrations, environmental conditions affecting activity (e.g. temperature, organic load, exposure time etc.), dissemination of resistance genes, change in bacterial population following exposure, and potential synergies with other molecules are required to formulate an appropriate risk assessment.*

2. *There are no accepted standard protocols for the evaluation of antimicrobial resistance induced or selected by a biocide. Such standards must be developed to provide informative data for biocidal product development and usage, and for regulatory bodies. In addition, surveillance programmes must be introduced to monitor the level of bacterial resistance and cross-resistance in all areas of biocide usage.*

3. *Environmental studies focusing on the identification and characterisation of resistance and cross-resistance to antibiotics following use and misuse of biocides. All suggestions and questions raised at the occasion of the public consultation on this opinion were taken into account and adequate responses were formulated in the final version."*

Additional shortcomings were also identified by the SCENIHR (2009) and by the joint opinion on AMR by ECDC, EFSA, EMEA, and SCENIHR (ECDC 2009):

1. The lack of harmonisation of prudent use guidelines for biocides.
2. The lack of surveillance programme on bacterial resistance to biocides.
3. The effect of the suppression of numerous active substances following recent European regulation: n°1451/2007 (4th December 2007) and European decision (2008/809/CE – 14th October 2008).
4. The lack of information on production and use of biocides.

This document does not aim to repeat the very extensive information provided in the SCENIHR (2009) opinion, but will provide up to date information and a complement of information on selected aspects of bacterial resistance and cross-resistance.

3.3. Strategic Framework

The large scale release of biocides by human activities has added a new stress to the bacterial environment and may contribute to selecting resistant bacteria. This may impact human health and the environment as shown in the scheme in Figure 1. The various aspects of bacterial changes and impacts on humans and the environment are described below.

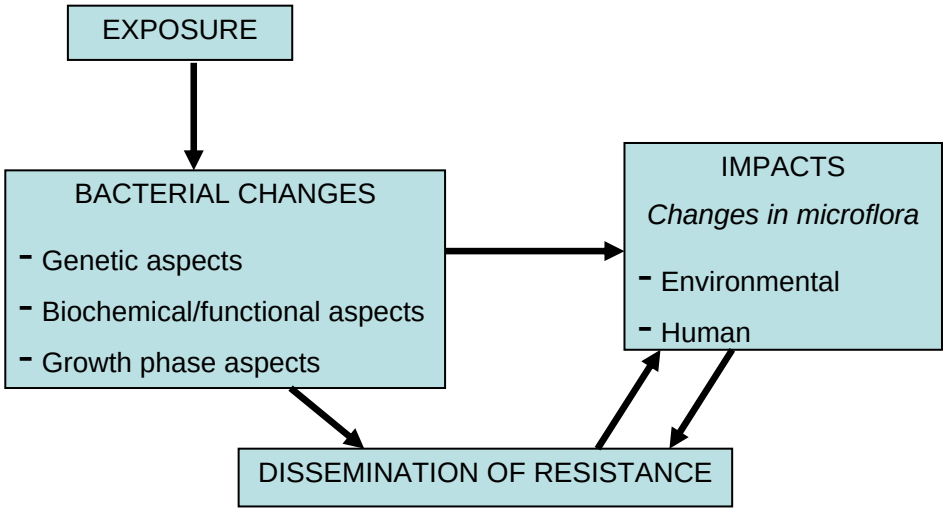

Figure 1: Global change in bacterial adaptation

The widespread and large scale use of biocides may create an additional external selective pressure[5] on the bacterial cells in a similar fashion to that previously reported for antibiotics. This type of external stress induces bacterial changes involving genetic regulation and other phenomena (genetic, biochemical, functional, physiological, etc.) that modify the capability and the fitness of the cell, or alternatively, select the well-adapted bacterium (e.g. intrinsically less susceptible to an aggressive compound) to the new environmental conditions. A direct consequence of this evolution may be a change in microflora that favours the emergence of bacteria exhibiting the required capability or, more drastically, the selection of new variants showing a high level of natural/acquired resistance to the stress exposure (from natural reservoir such as soil microflora).

The external pressure can also favour the dissemination, via genetic elements, of the mechanism(s) involved in this adaptation. This transmission of resistance genes can be vertical or horizontal (SCENIHR 2009).

3.3.1. Exposure

The increased use of biocides in human medicine, cosmetics, agriculture, livestock farming, food treatments, personal care and household use, etc. has resulted in a significant amount of biocidal compounds in wastewaters and a subsequent noticeable release into the environment. Recent studies have confirmed the presence of high concentrations of various biocides in river water and wastewater treatment effluents (Kumar et al. 2010, Pedrouzo et al. 2009, Wilson et al. 2009). The concentrations detected in some of these environmental locations are high enough to select for bacterial strains exhibiting a decreased susceptibility against antibacterial compounds or to trigger the expression of associated resistance mechanisms *in vitro*.

The use of the many biocides, listed in the 23 product categories defined by the Biocides Directive (98/8/EC), results in a permanent exposure of both humans and the environment. Within the framework of the Biocides Directive, procedures have been developed for the collection and reporting of production and use data for each biocide as well as for the use of biocides in the various product types. The use scenario data requirement is based on the concentration of individual biocides in each biocidal product type. Therefore, the use of biocides as preservatives, fragrances, surfactants, etc. in non-biocidal product categories such as cosmetics, personal care products, household products, medical devices, processing aids, toys, etc. are not included in the use scenarios. As a result, human and environmental exposures to biocides resulting from the use and discharge of "non-biocidal products" are not considered in biocide exposure assessments.

Both humans and animals are exposed to active substances through their surrounding indoor and outdoor environments. This exposure should be taken into account when evaluating possible development of bacterial resistance of micro-flora on humans and animals. Repeated exposure to an active substance, and cumulative exposure to an active substance from various products, and through different routes, should also be considered when assessing exposures to active substances of biocidal products.

Exposure of bacteria to biocides and/or their metabolites in various matrices could not be assessed due to the lack of information on production and use volumes.

Resistance to antimicrobials is a relatively common feature of natural microbial communities for a range of different habitats such as soils, aquatic systems, and animal- and human-associated habitats.

[5] Selective pressure: chemical, physical, or biological factors or constraints which select well-adapted bacteria or induce the expression of specific biological mechanisms involved in the bacterial response to external stresses (SCENIHR 2009).

Risk assessments of the effect of biocides on both target and non-target micro-organisms are scarce. Ideally, the knowledge required for the evaluation of possible development of bacterial resistance and cross-resistance in the environment should include both knowledge of microbial communities and exposure of biocides as described below:

- The state of microbial communities (complex biofilms) in various environmental matrices where exposure to biocides is high.
- Concentrations of the active substances in different environmental matrices (surface waters, ground water, air, soil), in influent, effluent and biosolids of wastewater treatment plants, in sludge and sediments.
- The fate and kinetics of the active substances under natural physical conditions (temperature, humidity, pH, sunlight/dark, etc.) and under transformed conditions, for example, sludge amended soil.
- The concentrations of the possible metabolites of the active biocides.
- The bioavailability of the active substance(s) and its metabolites; factors such as binding of active substance to the soil, degradation of the active substance in the environment, presence of other chemicals, bioaccumulation by other species, etc. may influence the bioavailability of the active substance or its metabolite.

In practice, gathering all this information might not be feasible.

A strategy for the collection of the above mentioned data, except for the knowledge of micro-organisms in the environment, is developed within the framework of the Biocides Directive 98/8/EC. However, to date, the data available for biocide exposure assessment are still scarce and it may take many years to get a global picture for all biocides. Therefore, a priority list for collecting data on biocides is necessary. Such a priority can be based on: a) the production and use data on biocides, such as developed by the REACH Regulation (EC 1907/2006); or b) the data on biocides which are intensely used where microbial pathogens may be present (e.g. healthcare, veterinary settings, food industry, etc.).

The European Commission has produced an assessment of human and environmental risks linked to the use of biocides (EC 2009). This overview is based on the minimum annual production/import volumes in the EU of biocides and proposes four main groups based on applications (see Table 1). For our purpose, Main Group 1 (disinfectants and general biocidal products) and Main Group 2 (preservatives) are probably the most relevant as they have high tonnages and high human exposure. Such a list may still be very large because they comprise by far the largest groups with almost 2/3 of the total tonnage. However, limited available data on the production of individual biocides restricts the number of biocides on a priority list.

Table 1: Overview, indication of significance of elements in the human and environmental risk assessments relating to the use phase of biocides (per product type) and overall assessment[1]. The specific exposure assessments do not include consideration of the overall tonnage (European Commission 2009).

Product-type	Tonnage (annual)	Human exposure, users	Human exposure, general	Env. Exposure, direct	Env. Exposure via WWTPs	Overall assessments of "risks"
Main Group 1: Disinfectants and general biocidal products						
1: Human Hygiene biocidal products	XXX	XXX	-	-/X	XX	X
2: Private area and public health area biocidal products	XXX	XX	X	X	XXX	XX
3: Veterinary and hygiene biocidal products	XXX	XX	-	X	XX	X
4: Food and feed area disinfectants	XXX	XX	-	-	XXX	X/XX
5: Drinking water disinfectants	XXX	X	X	X	X	X
Main Group 2: Preservatives						
6: In-can preservatives	XX	X	X	X	X	X
7: Film preservatives	XX	X	X	XX	X	X/XX
8: Wood preservatives	XXX	XX	X	XX/XXX	-	XX/XXX
9: Fibre, leather, rubber, and polymerised materials preservatives	XX	X	X	-	X	X
10: Masonry preservatives	XXX	XX	-	XX	-	XX
11: Preservatives for liquid cooling and processing systems	XXX	X	-	XX	XX	XX
12: Slimicides	XX	X	-	XX	XX	X/XX
13: Metal working fluid preservatives	XX	XX	-	-	X	X
Main Group 3: Pest control						
14: Rodenticides	-	XX	X	XX	X	XX
15: Avicides	-	X	-	XX	-	-/X
16: Molluscicides	-	X	-	XX	-	-/X
17: Piscicides	-	X	-	XXX	-	-/X
18: Insecticides and products to control other arthropods	XX	XXX	XX	XXX	-	XX/XXX
19: Repellents and attractants	XX	XX	X	XX	-	-/X
Main Group 4: Other biocidal products						
20: Preservatives for food and feedstock	X	X	X	-	-	-/X
21: Antifouling products	X	XX	X	XXX	-/X	XX
22: Embalming and taxidermist fluids	-	-	-	-	-	-
23: Control of other verterbrates	-	X	-/X	XX	-	-/X

[1]: XXX = major/high; XX = significant; X = moderate; - = minor/low

3.3.2. Bacterial changes

Bacterial changes refer to modifications that are induced or selected by (during or following) biocide exposure (see Table 2). These include changes in genetic regulation (e.g. triggering the expression of silent genes, repressing genes currently expressed, mutations, etc.), changes in bacterial metabolism (e.g. favouring new enzymatic pathways, modification of bacterial membrane components), changes in growth pattern (e.g. from planktonic to biofilm) and changes in the composition of the microflora.

Environmental studies

Although the number of studies investigating changes in environmental isolates where biocides are regularly used is low, in most instances an increase in biocide resistance was often observed together (but not always) with a change in antibiotic susceptibility profile (SCENIHR 2009). The most compelling evidence comes from the study of surviving bacteria following high-level disinfection in an endoscope washer disinfector. In 1997, Griffiths et al. isolated *Mycobacterium chelonae* showing resistance to the in-use concentration of glutaraldehyde (2%), but also cross-resistance to a number of other biocides such as sodium dichloroisocyanurate (1000 ppm), Virkon (1%) and Gigasept (10%). More recently, Duarte et al. (2009) isolated *Mycobacterium massiliense* resistant to glutaraldehyde (2%), ciprofloxacin (MIC_{90} ≥ 32 µg/ml), cefoxitin (MIC_{90} = 128 µg/ml) and doxycycline (MIC_{90} ≥ 64 µg/ml). Martin et al. (2008) isolated a number of Gram-positive vegetative bacteria including *Bacillus subtilis*, *Micrococcus luteus*, *Streptococcus mutans*, *Strep. sanguinis* and *Staphylococcus intermedius* resistant to the in use concentration of chlorine dioxide. In addition, *B. subtilis* showed cross-resistance to hydrogen peroxide (7.5%). Cheeseman et al. (2009) showed that genotypically distinct meticillin-resistant and -susceptible isolates of *Staphylococcus aureus* had different susceptibility to alcohol hand rubs. However, in these studies the mechanisms of resistance have not been identified.

There is evidence that the long-term exposure (several months) of a complex microcosm to a biocide contributes to a change in bacterial population, where the most susceptible bacteria disappear (McBain et al. 2003, McBain et al. 2004). There are too few *in situ* studies to show significant evidence for such events. Only a few studies investigated changes in microbial population following long-term exposure to a biocide, and they focused on a number of predominant bacteria and did not look at the entire community.

In vitro studies

In vitro studies highlighted important changes in the physiology of bacteria that have acquired resistance to certain biocides such as cationic compounds (Tattawasart et al. 2000) and oxidising agents (Martin et al. 2008). The change in bacterial hydrophobicity following exposure to biocides has also been well documented (SCENIHR 2009).

It has been well reported in studies *in vitro* that a sub-lethal (often sub-MIC) concentration of a biocide decreases the susceptibility of bacteria to that biocide and modifies the antibiotic susceptibility profile, but does not necessarily trigger clinical antibiotic resistance. In addition, there is no rule of thumb as to which antibiotic susceptibility would be affected following exposure to a given biocide in a given bacteria (SCENIHR 2009). A recent transcriptomic study highlighted the differences in bacterial response to triclosan between *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, concluding that there is no model bacteria to study response to biocides (Bailey et al. 2009). A recent study evaluated the selection potency of cetylpyridinium chloride, a quaternary ammonium compound widely used worldwide for disinfection in hospitals, on *Serratia marcescens* (Maseda et al. 2009). The authors demonstrated that a resistant strain was selected exhibiting noticeable resistance stability (over 60 generations) and a large MDR phenotype including resistance against cetylpyridinium

chloride, quinolones, tetracycline and chloramphenicol. This resistance level is associated with the overproduction of the SdeAB efflux pump that expels the toxic compounds, i.e. biocides and antibiotics, from the bacterial cells (Maseda et al. 2009). This is important since *S. marcescens*, is an opportunistic pathogen which has been associated with health-care acquired infections. Kastbjerg et al. (2010) observed that while a sub-lethal concentration of quaternary ammonium compounds (QAC) and triclosan increased virulence gene expression in *Listeria monocytogenes*, other disinfectants based on chlorine and peroxides decreased such expression.

Bacterial biofilms

It is widely accepted that bacterial biofilms offer a particular challenge to antimicrobials. *In situ*, bacteria are usually found in complex biofilms that provide protection to external factors. To mimic these complex biofilms is challenging and one approach has been to transfer environmental biofilm into a fermentor to sustain growth (McBain et al. 2003, McBain et al. 2004) and to investigate biofilm susceptibility to biocide exposure. Other approaches have been to study biofilms *in situ*. The use of triclosan and other biocides against the oral microbial flora has provided some useful information, although in most cases only a number of key bacteria within that oral flora were investigated (Jones et al. 1988, Walker et al. 1994).

The main challenge for studying biofilms is to select for the appropriate protocol to produce reproducible but realistic biofilms. This is particularly important when standardisation is the goal. From a practical point of view, increasing the concentration of a biocide might have a better activity against a bacterial biofilm, but this means potentially higher environmental toxicity and certainly higher product cost.

Occurrence of mechanisms involved in resistance

Among the most described mechanisms of resistance to biocides in bacteria are changes in the outer wall (impermeability) and expression of efflux mechanisms (Table 2). These mechanisms have been shown in laboratory studies to also confer resistance to unrelated antimicrobials including antibiotics when expressed or triggered (Table 3).

Table 2 Resistance mechanisms in bacteria

Mechanism[1]	Characteristics
Change in cell permeability	Decrease in concentration (that reaches the target sites) Spores (layers: cortex, spore envelope) Gram-negative (outer membrane) - Lipopolysaccharides - Proteins (porins) - Fatty acid - Phospholipids Mycobacteria mycoylarabinagalactan
Change in surface properties	Decrease binding and interaction between biocide and cell surfaces Surface charge
Efflux mechanisms	Decrease intracellular concentration of a biocide - Small multidrug resistance (SMR) family (now part of the drug/metabolite transporter (DMT) superfamily) - Major facilitator superfamily (MFS) - ATP-binding cassette (ABC) family - Resistance-nodulation-division (RND) family - Multidrug and toxic compound extrusion (MATE) family
Enzymatic modification	Decrease intracellular and exocellular concentration of a biocide
Target mutation	Fab1 mutation e.g. in *Mycobacterium smegmatis*
By-pass metabolic activity	Increase in pyruvate synthesis and fatty acid production via an altered metabolic pathway (expression of "triclosan resistance network")

Notes: [1] For references for specific mechanisms, refer to SCENIHR (2009).

Table 3 Bacterial mechanisms inducing potential cross-resistance

Mechanism	Nature	Level of susceptibility to other biocides[1]	Cross-resistance
Change in bacterial envelope	intrinsic (acquired)	no	yes
(over)Expression of efflux pumps	intrinsic/acquired	reduced	yes
Enzymatic modification	acquired/intrinsic	reduced	no[2]
Mutation (target site)	acquired	reduced	no[3]
Phenotypic change	Following exposure	reduced	yes

Notes: [1] To other biocides - level of susceptibility defined according to the concentration of biocides.

[2] In the case of acquired resistance, co-resistance has been described.

[3] Triclosan cross-resistance with specific antibiotics (e.g. isoniazid) acting against enoyl acyl carrier proteins (e.g. FabI) has been described.

One of the important aspects when dealing with emerging resistance following biocide exposure is not necessarily intrinsic resistance, but acquired resistance, and the mechanisms that will induce expression of biocide resistance mechanisms in bacteria (e.g. regulation of porins, efflux pump expression). The regulation of the regulators that

trigger expression of genes involved in other mechanisms such as degradation should also be considered. Webber et al. (2008b) identified a "triclosan resistance network" in *S. enterica* serovar Typhimurium, involving a set of proteins with commonly altered expression in all resistant strains. Some of these proteins are involved in production of pyruvate or fatty acid.

Genetic dissemination of resistance genes

Horizontal transfer of genes and genetic elements, between bacteria, has been shown to occur in a very wide variety of natural situations (Davison 1999). It may occur by transformation by naked DNA, by transduction by a bacteriophage intermediate, or by cell to cell conjugation. The latter is particularly efficient and is mediated by conjugative plasmids. Some plasmids (such as RP4) are self-conjugative, while others (such as RSF1010) are incapable of self-transfer but are easily transferred in the presence of conjugative plasmids. Some plasmids (such as those cited above) are capable of transfer, maintenance and replication in a very wide range of different bacteria, and are known as wide host range plasmids. Plasmids may, in turn, carry transposons which are naturally occurring mobile elements capable of chromosome integration (Davison 1999). Both plasmids and transposons may carry a variety of genes for conjugal transfer, bacteriocins, resistance to heavy metals, catabolism of xenobiotic carbon sources, and for the interest of the present study, resistance to antibiotics and to biocides. Such mobile genetic elements play an important role in the evolution of bacteria. They allow the rearrangement or exchange of DNA between species, thereby increasing genetic diversity and flexibility of genomes (Dobrindt et al. 2004, Ochman et al. 2000). Among the various types of mobile genetic elements, genomic islands take up a distinct position, because they are integrated in the chromosome of the bacterial host and thus potentially stably maintained. Those genomic islands that are mobile can excise from their genome location, can induce self-transfer and reintegrate into a new host cell chromosome. Genomic islands can carry large regions (50–400 kilobases) with variable auxiliary functions that potentially benefit the host, such as growth in the presence of antibiotics or heavy metals, invasion of eukaryotic tissues via virulence factors, and exclusive growth with aromatic compounds (Dobrindt et al. 2004, Gaillard et al. 2008).

Resistance to the quaternary ammonium compound (*qac*)-based disinfectant benzalkonium chloride correlated with plasmid-based antibiotic resistance was demonstrated in *Staphylococcus*, where some isolates harboured multi-resistance plasmids that contain *qac*, *bla* and *tet* resistant genes (Sidhu et al. 2002). The results are compatible with selective advantages of isolates carrying both disinfectant and antibiotic resistance genes and the data indicate that the presence of *qac* genes in staphylococci results in the selection of antibiotic-resistant bacteria (Paulsen et al. 1998). Previous investigators have also reported a genetic linkage between disinfectant (*qac*) and antibiotic resistance genes (*blaZ*, *aacA-aphD*, *dfrA*, and *ble*) on the same *staphylococcal* plasmids from clinics and food environments (Sidhu et al. 2001, Sidhu et al. 2002) as well as the geographical dissemination of resistance genes among staphylococci (Bjorland et al. 2001, Noguchi et al. 2005).

In *Escherichia coli* of porcine origin, a plasmid-encoded multi-drug efflux pump (*OqxAB*) was demonstrated to have a wide substrate specificity including animal growth promoters, antimicrobials, disinfectants and detergents (Hansen et al. 2005). The *OqxAB* pump can be transferred between *Enterobacteriaceae* (*Salmonella enterica* serovar Typhimurium*, Klebsiella pneumoniae, Kluyvera* sp. and *Enterobacter aerogenes*), conferring reduced susceptibility to various antibiotics including chloramphenicol, ciprofloxacin and olaquindox (Hansen et al. 2007). Similar mobile elements containing biocide and antibiotic resistance genes have been reported in clinical isolates of another major human pathogen, *Pseudomonas aeruginosa* (Laraki et al. 1999, Sekiguchi et al. 2005, Sekiguchi et al. 2007, Wang et al. 2007).

Consequently, the segregation/transfer of biocide and antibiotic resistance genes carried by mobile genetic elements is a significant hazard for the selection and dissemination of multi-drug resistant bacteria. The uncontrolled use of biocides may recruit bacteria containing this type of genetic element and favour the vertical and horizontal spreading of the mobile elements to other bacteria (intra- or inter-species) sharing the same ecological niches.

3.3.3. Dissemination and impact

Biocides are used extensively for a number of applications. The dissemination of antimicrobial-resistant bacteria is a key contributor to the widespread emergence of problems in the treatment of infectious diseases. It is important to consider the dissemination and transmission of a specific resistance gene within the same bacterial species and its horizontal transmission from one bacterium to another (or to another bacterial species by means of a mobile genetic element). Account also needs to be taken of the role of external factors (chemical-stress pressure) which can promote the selection of bacteria exhibiting these resistance mechanisms, maintain the presence of resistance genes or favour the expression of specific complexes responsible for antimicrobial resistance.

Exposure to some biocides (cationic biocides) has been shown to favour the dissemination and maintenance of genetic mobile elements in bacteria and subsequently may facilitate the exchange of key genes between bacterial species (Bjorland et al. 2001, Noguchi et al. 2002, Paulsen et al. 1998, Pearce et al. 1999, Sidhu et al. 2001, Sidhu et al. 2002). An outbreak of antibiotic resistant *Mycobacterium massiliense* in 38 hospitals in Brazil was linked to the failure of glutaraldehyde to eliminate this opportunistic pathogen (Duarte et al. 2009). This is the first time that an *in situ* investigation links failure of a biocide, cross-resistance to antibiotics and infectious outbreak.

The dissemination of resistance genes in bacteria following the use of antibiotics is better documented. For example, the case of the transmission of resistant *Campylobacter* sp. and *Salmonella* sp. from animals to humans was made by the joint opinion on AMR by ECDC, EFSA, EMEA and SCENIHR (ECDC 2009). The joint opinion stated that "*the evolution and dissemination of antimicrobial-resistant strains of food-borne bacterial pathogens in food animals and subsequently to humans, creates an increase in the "attributable fraction", the number of excess illnesses caused by antimicrobial-resistant zoonotic bacteria*. *This increases the risk of invasive infections, hospitalization and deaths associated with these bacteria*." This same opinion concluded that there was a *temporal* increase in quinolone resistance in both animal and human pathogens.

3.4. Knowledge gaps

In the course of the previous work of the SCENIHR (2009), several important gaps were noted. These can be divided into scientific and technical gaps:

Scientific gaps:

1. *Environmental studies focusing on the identification and characterisation of resistance and cross-resistance to antibiotics following use and misuse of biocides.*
2. *In vitro studies demonstrate that some biocides used at sub-lethal concentrations trigger the emergence of antibiotic resistance and/or select bacteria resistant to antibiotics. Despite this mechanistic evidence from in vitro data, epidemiological data indicating public health relevance are lacking.*

3. *Knowledge of (measured) concentrations of biocides in various environmental matrices is required to assess biocide exposure especially of the environment.*

Technical gaps:

1. *Exposure of bacteria to biocides and/or their metabolites in various matrices cannot be assessed due to lack of information on production and use volumes; lack of mechanistic studies at a small scale.*
2. *Despite the regulatory requirements to study the environmental stability of individual products, data on the fate and concentrations of biocides in the environment are sparse. No validated methodologies are available for the determination of the dose-response relationship and of the threshold triggering the emergence of antibiotic resistance and/or the selection of resistant bacteria*
3. *The role of bacterial biofilm in resistance to both biocides and antibiotics has been shown. Furthermore, bacterial biofilms are very common in the environment. Yet most laboratories are not using biofilm tests to assess the efficacy of biocides (Cookson 2005). There are currently no European standards for the testing of disinfectants against biofilms for health care applications."*

4. OPINION

4.1. ToR 1 – Research recommendations

The following list is not ordered according to importance.

4.1.1. Development of tools and standard protocols for measuring resistance and cross-resistance in bacteria

Study type:

In vitro studies testing the relevance/practicality/ability of various tools for testing resistance and cross-resistance in bacteria with the aim of developing validated standards.

Rationale/justification:

Concentration is central to the definition of bacterial resistance in practice. Therefore, the measurement of bacterial lethality rather than the measurement of bacterial growth inhibition is paramount. However the study of the bacterial response is often based on the determination of inhibitory concentration instead.

Although the study of individual biocides yields some important information in terms of bacterial ability to develop resistance, it does not provide a rule of thumb for the formulated product. In addition there is no model biocide that can be used. Likewise, there are no model bacteria to study, since proteomic investigations highlighted differences in response to the same biocide in bacteria. However, the methodology to study biocide resistance can be standardised. To date the majority of studies have relied on an increase in MIC to define an increase in resistance. Such a measurement might indicate a trend or emerging resistance to a biocide, but it does not reflect conditions in practice whereby the biocide might be used at a much higher concentration. It usually does not reflect activity of the biocidal product either. To develop a standard methodology to measure resistance, two criteria reflecting on the concentration of the biocide must be attained; 1) concentration of the biocide in the biocidal product and 2) the residual concentration after usage. The first concentration is important to test for intrinsic resistance. The second concentration reflects concentrations attained on a surface (residual concentration) or in the environment after application. In addition, the activity of the biocidal product should be tested to reflect the effect of the formulation and the conditions found in practice (temperature, type of surface, $\%CO_2$, etc.).

In terms of antibiotic resistance, many studies have described an increase in inhibition zones to an antibiotic, but have not reported resistance to a concentration used in practice. There are several national standard methodologies to measure antibiotic susceptibility in bacteria (e.g. British Society of Antimicrobial Therapy, Société Française de Microbiologie, etc.). Such methodologies and recommendations, reflecting on specific pathogens and therapeutic antibiotics in use and their concentration, should be used to determine the susceptibility of bacteria that survive biocide exposure to the concentrations mentioned in points 1) and 2) above. For example, comprehensive studies have looked at a decrease in antibiotic susceptibility that would be associated with treatment failure (Cottell et al. 2009, Lear et al. 2006).

The effect of biocides on antibiotic susceptibility in bacteria has been measured by treating a bacterial population first with a biocide. The surviving bacteria are then investigated for their susceptibility to antibiotics. However, there are currently no well referenced criteria or standard protocols for the evaluation of the capability of a biocide

to induce or select for resistance to antibiotics. Therefore, tools need to be developed to define for example the "*minimal selecting concentration*": the minimal concentration of a biocide which is able to select or trigger the emergence/expression of a resistance mechanism that will confer clinical resistance to an antibiotic class in a defined bacterium (SCENIHR 2009).

Minimum technical requirements to ensure usability of results

- Knowledge of test methodologies for evaluating biocide **AND** antibiotic activity is essential.
- Comparative studies using large enough sample sizes to demonstrate clearly the superiority of one method against another.
- Use of several bacterial genera and different biocides to ensure the application of the selected methods to a wide range of bacteria/biocide interactions.
- Training and dissemination of the standard protocol.

Expected impact of results (use for risk assessment)

Standardisation of the research tools to measure appropriately bacterial resistance and cross-resistance to a given biocide in a given bacteria following biocide exposure. The objective is for the standardised method to become the norm.

4.1.2. Effect of sub-lethal concentrations of biocides on emerging biocide and antibiotic resistance

Study type

a) Exposure studies of biocide concentrations following usage.

b) *In vitro* studies to determine the possible relationship between biocide concentrations and expression of resistance mechanisms.

Rationale/justification

Knowledge of the concentrations found in the environment is important. Some recent environmental studies highlighted the high concentrations of biocides found in rivers and lakes. Such concentrations can be used to monitor possible emerging resistance and cross-resistance in bacteria. Some manufacturers indicate that their biocidal products leave a "residual concentration" which is claimed to be part of the long-term efficacy of their products. However, there is no information available from manufacturers on this "residual concentration" and on the effect of such a concentration on bacteria. Some studies investigating this "residual concentration" highlighted the rapid selection of less susceptible bacteria (e.g. Thomas et al. 2005).

The misuse of biocides includes the development of inappropriate biocidal products and the inappropriate use of biocidal products. In the first case, biocidal products that contain a low (inhibitory) concentration of a biocide or for which bio-availability is low, risk promoting the emergence of resistance. The efficacy of such products can be determined *in vitro*, providing appropriate tests are used, i.e. tests that reflect conditions found in practice. An example of developing such methodology, where conditions in practice are used, is the study on antimicrobial wipes by Williams et al. (2009b). Surviving bacteria can then be assessed for the reason of their survival and possible cross-resistance to antibiotics and other biocides.

Our understanding of the mechanisms involved in bacterial resistance and cross-resistance has improved over the last 15 years. Advances in "omics" (i.e. genomics, proteomics, transcriptomics) have resulted in a better understanding of the control of gene expression following biocide exposure and provided evidence of the link between biocide exposure and expression of resistant and virulent determinants. Examples of such studies are given by Webber et al. (2008a) and Bailey et al. (2009).

Minimum technical requirements to ensure usability of results

- Knowledge and ability to measure/assess low concentrations of biocides in the environment is required.
- Knowledge of the biocide-bacteria interactions is essential.
- Knowledge of "omics" protocols is essential.
- High repeatability and reproducibility of the methods must be demonstrated.
- Training and dissemination of these methods.

Expected impact of results (use for risk assessment)

An understanding of the dose of biocides triggering a genetic response in bacteria which lead to the expression of resistance and cross-resistance mechanisms will provide an assessment of the risk associated with low concentrations of a biocide. A dose-dependent relationship between a biocide and a specific resistance mechanism is expected.

4.1.3. Resistance and cross-resistance to antibiotics following use of biocides in practice

Study type

a) *In situ* studies to test the efficacy of formulations containing biocides in practice, including testing for potential emergence of resistance and cross-resistance to biocides and antibiotics in bacteria.

b) Surveillance studies to observe the long-term effects of the use of formulations containing biocides on the targeted bacterial flora.

c) Identification of the mechanisms of resistance where resistant bacteria are isolated *in situ.*

Rationale/justification

There is a paucity of information on emerging bacterial resistance in the environment or *in situ*. Because of the increased usage of biocidal products and the potential for biocides to select for bacterial resistance and cross-resistance, it is important to provide data on bacteria and bacterial microcosms that are regularly exposed to biocides. Two different approaches can be considered:

***In situ* tests:** CEN TC/216 is now considering phase 3 tests (tests *in situ*). These are complex to put together and will be certainly costly. However, results from these tests will provide valuable information on the efficacy of biocidal products *in situ*. There is little information about the nature of these tests at present. In the published literature, comparison of biocide efficacy, or the efficacy of biocide regimen in practice have met some success with the design of double blind cross-over studies over a period of several months. However, these studies contain little information on the nature of the bacteria

isolated following biocide exposures, these trials reflecting mainly on a decrease in bioburden from surfaces or skin.

Surveillance: another approach is to sample the environment where biocides are extensively used. This approach has been used in a few studies that investigated the nature of the resistance mechanisms in the surviving bacteria. In some of these investigations, the environmental isolates were subjected to the biocide used in practice using some conditions found in practice (e.g. contact time). This involved an observation period at the place where the biocidal product(s) is/are used, to define the test conditions to be used *in vitro*. Examples of such studies focusing on a specific biocide are provided by Griffiths et al. (1997), Lear et al. (2002), Martin et al. (2008), and on biocidal products by Cheeseman et al. (2009) and Williams et al. (2009a).

Whichever approach is used, bacteria that survive repeated exposures to biocidal products should be investigated for the nature of their resistance: intrinsic or acquired. The mechanisms involved should be described, thus identifying the risk of expressing resistance mechanisms common to biocides and antibiotics. This implies that the tools to study expression of genes, and mechanisms of resistance, are in place.

The use of surveillance programmes should be recommended and expanded. Such programmes will ensure in the short-term that biocidal products used pose no risks for the selection of resistance and/or the development of acquired resistance and cross-resistance in bacteria.

Minimum technical requirements to ensure usability of results

- Knowledge of the conditions of use of biocides in practice is essential. The environment can vary to include the food production, healthcare, household applications, water, etc.
- Knowledge of planning and performing *in situ* studies is essential. The sample size must be large enough and duration of the study long enough to demonstrate clearly the effect of biocide usage in practice against environmental bacteria.
- Understanding of mechanisms of resistance expressed in bacteria is essential.
- Knowledge of "omics" protocols is essential.
- Reproducibility of the methods and low variability in results must be demonstrated.
- Training and dissemination of these methods.

Expected impact of results (use for risk assessment)

This study is essential to understand the risks associated with emerging bacterial resistance and cross-resistance following the use of biocides or biocidal products in practice. The scarcity of *in situ* evidence linking bacterial resistance with the use of biocides has been highlighted in this and other documents (ECDC 2009, SCCS 2010, SCENIHR 2009).

4.1.4. Role of bacterial biofilms in conferring resistance to biocides and antibiotics

Study type

a) *In vitro* studies to test the development of bacterial biofilms following exposure to biocides.

b) *In vitro* studies on the role of persister cells in biofilm resistance profile and biofilm regrowth.

c) *In vitro* studies testing bacterial susceptibility in regrown biofilms following biocide exposure.

Rationale/justification

Bacterial biofilms are very common in the environment. However, most laboratories are not using biofilm tests to assess the efficacy of biocides. There are currently no European standards for the testing of disinfectants against biofilms for health care applications. There are however several methods used to produce biofilms *in vitro*. These methods (e.g. CDFF, Robin device, sedimentation biofilm, etc.) produce biofilms with different structural characteristics. Furthermore, variability in results depends on the type of method used to produce the biofilm.

Biofilm resistance to antimicrobials results from a number of mechanisms (e.g. exopolysaccharides production, enzymatic modification, over-expression of efflux) that usually reduce the intracellular concentration of the biocide or antibiotic. The presence of persister (dormant) cells scattered through the biofilm has also been reported. These cells are believed to be highly resistant to biocides and antibiotics, and to be involved in biofilm regrowth following exposure to antimicrobials. However, their characteristics and role has been poorly described despite their potential importance in conferring resistance.

Bacterial biofilms are of concern as they have been shown to be less susceptible to biocides and antibiotics than planktonic cells. There are many methodologies available to produce biofilms and they all have advantages and drawbacks. It must be noted that testing biocides against a bacterial biofilm is challenging for the manufacturers and a decision should be made whether a manufacturer must obligatorily present data on biocide activity against biofilms. If the biocidal product is to be used against bacterial biofilms then efficacy data against biofilms should be available. In this case a biofilm protocol that provides reproducible data should be used and data should be generated on a biofilm similar to that encountered *in situ* for the intended application.

Studies of the long-term exposure of complex bacterial biofilms (e.g. environmental, oral) to a biocide should investigate changes in the biofilm population, as well as changes in the susceptibility of the biofilm to both the biocide and clinically relevant antibiotics. The use of complex microcosms in the laboratory for studying environmental biofilms (e.g. drain microcosm; McBain et al. 2003, McBain et al. 2004) was successful and the use of such methodology should be encouraged.

Minimum technical requirements to ensure usability of results

- Knowledge of biofilm growth methodologies is essential.
- Understanding of biofilm characteristics is required.
- Documentation of method reproducibility is essential.
- Training and dissemination of these methodologies.

Expected impact of results (use for risk assessment)

Biofilms are widespread in the environment and yet they are rarely used to study resistance and cross-resistance following biocide exposure. This study addresses the characteristics of biofilms, the role of persisters, and the characteristics of biofilm regrowth following exposure to biocides. In the industry, where biofilms are the source of bacterial contaminants, biofilm regrowth is often recurrent.

4.1.5. Gene transfer and maintenance following biocide exposure

Study type

a) *In vitro* study testing the dissemination of genes conferring cross-resistance following biocide exposure.

b) *In vitro* study testing the maintenance of genes conferring cross-resistance following biocide exposure.

Rationale/justification

One of the most important risks identified is the acquisition of resistance following biocide exposure. This can occur following mutation or the acquisition of new genetic determinants conferring a resistance mechanism (e.g. efflux, enzymatic degradation). Yet, the effect of biocides on the transfer of genes encoding for resistant determinants has rarely been measured.

There is some evidence that biocides, through selective pressure, maintain mobile genetic elements in bacteria. The impact of keeping a gene pool has not been assessed.

Bacteria with a large genomic content (e.g. *Burkholderia.* sp.) and with the capability to express resistant determinants following exposure to adverse conditions are of particular interest.

Minimum technical requirements to ensure usability of results

- Understanding of gene transfer mechanisms in bacteria is essential.
- High reproducibility of the method use to determine the transfer of genes is essential.
- Study of several biocides and bacterial genera is essential to ensure the usefulness of the results.

Expected impact of results (use for risk assessment)

For risk assessment, it is essential to have a clear understanding of the ability of biocides to promote/favour the transfer or/and maintenance of genes in several bacterial genera, especially as this would help understand the acquisition of new properties in a microcosm.

4.1.6. Exposure of bacteria to biocides and/or their metabolites in various matrices

Study type

a) Study of the bioavailability of biocides and their metabolites in various matrices.

b) Small scale field studies of the fate of biocides and population changes.

Rationale/justification

Despite the regulatory requirements to study the environmental stability of individual products, data on the fate and concentrations of biocides in the environment are sparse. A strategy should be developed at the European level for correct/adequate data reporting (data collection) for exposure assessment. Knowing total tonnage of each biocide

produced, together with import and export of the respective biocides, both as raw materials and in biocidal products, may give an indication of exposure to each biocide in Europe. Although this is covered by the framework of the Biocides Directive 98/8/EC, the data on production and use of biocides is lacking. Knowledge of the residual concentrations of biocides as well as their fate in the European environment is very limited.

The concentration of a biocide and its bioavailability are paramount for activity. There are a number of factors that can affect the efficacy of a biocide, although there is usually no information about these factors from the manufacturers, or understanding by end users. The lack of understanding of these factors will lead to misuse of a biocide in practice. Examples of factors are concentration (over-dilution of a disinfectant), contact time (hand washing in practice), temperature, soiling (lack of cleaning) and type of surfaces (porous surfaces are harder to disinfect).

A decrease in concentration is a key requirement for a change in expression of genes involved in the expression of resistant and virulent determinants. Such low concentrations are also implicated in the maintenance of genetic material.

Minimum technical requirements to ensure usability of results

- Understanding of factors affecting the efficacy of biocides.
- Ability to measure concentrations of specific biocides in the environment.
- Development of a protocol for small scale field studies and knowledge of its practicability and performance are required.
- Training and dissemination of this protocol.

Expected impact of results (use for risk assessment)

3-5 years are necessary to obtain data usable for risk assessment.

Table 4 provides an overview of all the research recommendations made under ToR1.

4.2. ToR 2 – Research strategy

There are many biocides. Information on the efficacy and bacterial resistance and cross-resistance is only available for a limited number of them, triclosan being the most widely studied biocide. All of the studies suggested in this opinion are important to gain a better understanding of the interaction between biocides and bacteria and to perform an appropriate evaluation of emerging resistance and cross-resistance in bacteria due to the use of biocides. One of the main objectives of these recommendations is to determine the resistance mechanisms against biocides and antibiotics following exposure to biocides. However, due to financial constraints, this opinion strongly suggests that two work packages (WP1, WP2) should be prioritised. Although these WPs will not be able to lead to a full risk assessment of the use of biocides and emerging resistance and cross-resistance, they are essential in providing a fundamental understanding of the bacterial mechanisms involved in resistance and the development of measures that can be used by biocide manufacturers to decrease the development of such resistance mechanisms in bacteria. These WPs cannot be expected to cover all bacteria and biocides in the suggested timeframe. These WPs are not covered by ongoing research projects and can deliver results within a reasonable time frame.

Table 4 Summary of all studies suggested under ToR 1

Study	Study type	Expected time until delivery[6]	Work Package
Development of tools and standard protocols for measuring resistance and cross-resistance	*In vitro*	3 years	WP1, WP2
Effect of sub-lethal concentrations of biocides on emerging biocide and antibiotic resistance	*In situ* and *in vitro*	> 5 years	WP1
Resistance and cross-resistance following use of biocides in practice	*In situ*	5 years	WP1, WP2
Role of bacterial biofilms in conferring resistance to biocides and antibiotics	*In vitro*	5 years	WP1, WP2
Gene transfer and maintenance following biocide exposure	*In vitro*	3 years	
Exposure of bacteria to biocides and/or their metabolites in various matrices	*In situ*	5-10 years	

WP1: Characterisation of mechanisms involved in cross-resistance against biocides and antibiotics

a) Development of tools to measure resistance and cross-resistance.

b) Understanding the resistance mechanisms expressed/triggered following biocide exposure.

Although there is no rule of thumb to predict the development of bacterial resistance and cross-resistance following biocide exposure, it is essential to get a fundamental understanding of the mechanisms involved in triggering such a response as well as the mechanisms responsible for cross-resistance in bacteria. To that end, research tools need to be developed to standardise the measurement of resistance and cross-resistance in bacteria, together with the development of a validated method measuring the "*minimal selecting concentration*".

These methods are necessary to define the public health implications of emerging bacterial resistance following biocide usage and to justify the in-depth study of the mechanisms involved using "omics" protocols. Understanding gene regulation enables identifying the changes in bacterial phenotypes associated with the expression of resistant and virulent determinants. Understanding the triggering mechanisms will provide the basis for assessing the risks associated with emerging resistance in bacteria following the use of biocides.

Following such an objective, the study of bacterial biofilms and their resistance to both biocides and antibiotics should also be evaluated, as biofilms constitute a widespread reservoir of potentially resistant bacteria.

[6] Depending on level of funding.

WP2 - Development of strategies to combat cross-resistance mechanisms

a) Increasing biocide research collaboration.

b) Education and training.

c) Developing new approaches.

Research on the antimicrobial activity of biocides is conducted by a small number of research groups and is fragmented in Europe. A concerted approach and better collaboration between these groups is needed to increase our understanding of biocide resistance and cross-resistance in bacteria. Increased collaborations between academics and end users, for example to conduct surveillance programmes, will provide important information for regulators and end users about the efficacy of the biocidal products and the risks associated with emerging bacterial resistance and cross-resistance. Integrated collaboration between academics and manufacturers will provide better biocidal products, using existing knowledge on biocide resistance, avoiding the design of underperforming biocidal products. Translational research evaluating the efficacy of biocides *in vitro* using conditions found in practice together with translational research on the risks associated with emerging bacterial resistance to specific biocidal products will improve biocide safety.

The inappropriate use of biocidal products is linked to poor education, training and control; education of the end users (e.g. about the factors affecting biocidal activity), training of the end users to the best practice and control that the end users are using the biocidal products according to manufacturers' instructions. This implies that the manufacturers' instructions are clear and easy to follow. Clear indications for maintaining the best hygienic standards should also be provided by the manufacturers.

Clear strategies to combat/stop the expression of known resistance mechanisms (e.g. quorum sensing, efflux pumps) need to be developed through a concerted approach. Rational synthesis of new compounds (biocides or antibiotics) should be approached taking into account the mechanisms expressed by resistant bacteria. For instance, the design of new molecules may integrate some chemical aspects which decrease efflux pump efficacy so as to maintain the level of antimicrobial activity.

The intelligent delivery of biocides might ensure that a lethal concentration is rapidly reached in applications and minimizes the potential for generating resistance.

Outcome (5-10 years):

New regulation and guidelines for antibacterial agents, diagnostic tools, new molecules to combat resistant bacteria, restoration of antibiotic and biocide activity.

5. MINORITY OPINION

None

6. LIST OF ABBREVIATIONS

ABC	ATP-binding
AMR	Antimicrobial resistance
CDFF	Constant depth film fermentor
DMT	Dry/metabolite transporter
DNA	Deoxyribonucleic acid
EARSS	The European Antimicrobial Resistance Surveillance System
EC	European Commission
ECDC	European Centre for Disease prevention and Control
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EU	European Union
FP7	7th Framework Programme for Research and Development
MATE	Multi-drug and toxic compound extrusion
MDR	Multi-drug resistance
MFS	Major facilitator superfamily
MIC	Minimal inhibitory concentration
QAC	Quaternary ammonium compounds
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RND	Resistance-nodulation-division
RT-PCR	Real-time polymerase chain reaction
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SMR	Small multidrug resistance
ToR(s)	Term(s) of reference
WHO	World Health Organization
WP	Work package
WWTP	Waste water treatment plant

7. REFERENCES

Alonso-Hernando A, Capita R, Prieto M, Alonso-Calleja C. Comparison of antibiotic resistance patterns in *Listeria monocytogenes* and *Salmonella enterica* strains pre-exposed and exposed to poultry decontaminants. Food Control 2009; 20:1108-11.

Bailey AM, Constantinidou C, Ivens A, Garvey MI, Webber MA, Coldham N, et al. Exposure of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium to triclosan induces a species-specific response, including drug detoxification. J Antimicrob Chemother 2009; 64:973-85.

Birošová L, Mikulášová M. Development of triclosan and antibiotic resistance in *Salmonella enterica* serovar Typhimurium. J Med Microbiol 2009; 58:436-41.

Bjorland J, Sunde M, Waage S. Plasmid-borne *smr* gene causes resistance to quaternary ammonium compounds in bovine *Staphylococcus aureus*. J Clin Microbiol 2001; 39:3999-4004.

Carson RT, Larson E, Levy SB, Marshall BM, Aiello AE. Use of antibacterial consumer products containing quaternary ammonium compounds and drug resistance in the community. J Antimicrob Chemother 2008; 62:1160-2.

Cheeseman KE, Denyer SP, Hosein IK, Williams GJ, Maillard J-Y. Evaluation of the bactericidal efficacy of three different alcohol hand rubs against 57 clinical isolates of *Staphylococcus aureus*. J Hosp Infect 2009; 72:319-25.

Chen Y, Pi B, Zhou H, Yu Y, Li L. Triclosan resistance in clinical isolates of *Acinetobacter baumannii*. J Med Microbiol 2009; 58:1086-91.

Cole EC, Addison RM, Rubino JR, Leese KE, Dulaney PD, Newell MS, et al. Investigation of antibiotic and antibacterial agent cross-resistance in target bacteria from homes of antibacterial product users and nonusers. J Appl Microbiol 2003; 95:664-76.

Cookson B. Clinical significance of emergence of bacterial antimicrobial resistance in the hospital environment. J Appl Microbiol 2005; 99:989-96.

Cottell A, Denyer SP, Hanlon GW, Ochs D, Maillard J-Y. Triclosan-tolerant bacteria: changes in susceptibility to antibiotics. J Hosp Infect 2009; 72:71-6.

Daniels C, Ramos JL. Adaptive drug resistance mediated by root-nodulation-cell division efflux pumps. Clin Microbiol Infect 2009; 15:32-6.

Davison J. Genetic exchange between bacteria in the environment. Plasmid 1999; 42:73-91.

Dobrindt U, Hochhut B, Hentschel U, Hacker J. Genomic islands in pathogenic and environmental microorganisms. Nat Rev Microbiol 2004; 2:414-24.

Duarte RS, Lourenço MCS, Fonseca LdS, Leão SC, Amorim EdLT, Rocha ILL, et al. Epidemic of postsurgical infections caused by *Mycobacterium massiliense*. J Clin Microbiol 2009; 47:2149-55.

EARSS (European Antimicrobial Resistance Surveillance System). The EARSS, funded by DG SANCO of the European Commission, is an international network of national surveillance systems which collects comparable and validated antimicrobial susceptibility data for public health action. Annual Report 2005. Available from: http://www.rivm.nl/earss/ (accessed 28 April 2010).

EC (European Commission). Assessment of different options to address risks from the use phase of biocides. European Commission, Environment Directorate-General. Final report. March 2009. Available from: http://ec.europa.eu/environment/biocides/pdf/report_use.pdf (accessed 18 February 2010).

ECDC (European Centre for Disease Prevention and Control), Stockholm, Sweden; European Food Safety Authority (EFSA), Parma, Italy; European Medicines Agency (EMA), London, United Kingdom; Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), Brussels, Belgium. Joint Opinion on antimicrobial resistance (AMR) focused on zoonotic infections. EFSA Journal 2009; 7(11):1372 Question N° EFSA-Q-2008-781. doi:10.2903/j.efsa.2009.1372; European Medicines Agency Reference EMEA/CVMP/447259/2009.

Gaillard M, Pernet N, Vogne C, Hagenbüchle O, van der Meer JR. Host and invader impact of transfer of the *clc* genomic island into *Pseudomonas aeruginosa* PAO1. Proc Natl Acad Sci USA. 2008; 105:7058-63.

Griffiths PA, Babb JR, Bradley CR, Fraise AP. Glutaraldehyde-resistant *Mycobacterium chelonae* from endoscope washer disinfectors. J Appl Microbiol 1997; 82:519-26.

Hansen LH, Jensen LB, Sørensen HI, Sørensen SJ. Substrate specificity of the OqxAB multidrug resistance pump in *Escherichia coli* and selected enteric bacteria. J Antimicrob Chemother 2007; 60:145-7.

Hansen LH, Sørensen SJ, Jørgensen HS, Jensen LB. The prevalence of the OqxAB multidrug efflux pump amongst olaquindox-resistant *Escherichia coli* in pigs. Microb Drug Resist 2005; 11:378-82.

Jones CL, Ritchie JA, Marsh PD, Van der Ouderaa F. The effect of long-term use of a dentifrice containing zinc citrate and a non-ionic agent on the oral flora. J Dent Res 1988; 67:46-50.

Kastbjerg VG, Lardsen MH, Gram L, Ingmer H. Influence of sublethal concentrations of common disinfectants on expression of virulence genes in *Listeria monocytogenes*. Appl Environ Microbiol 2010; 76:303-9.

Kumar KS, Priya SM, Peck AM, Sajwan KS. 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; 58:275-85.

Lai S, Tremblay J, Déziel E. Swarming motility: a multicellular behaviour conferring antimicrobial resistance. Environ Microbiol 2009; 11:126-36.

Laraki N, Galleni M, Thamm I, Riccio ML, Amicosante G, Frère JM, et al. Structure of In31, a bla_{IMP}-containing *Pseudomonas aeruginosa* integron phyletically related to In5, which carries an unusual array of gene cassettes. Antimicrob Agents Chemother 1999; 43:890-901.

Lear JC, Maillard J-Y, Dettmar PW, Goddard PA, Russell AD. Chloroxylenol- and triclosan-tolerant bacteria from industrial sources. J Ind Microbiol Biotechnol 2002; 29:238-42.

Lear JC, Maillard J-Y, Dettmar PW, Goddard PA, Russell AD. Chloroxylenol- and triclosan-tolerant bacteria from industrial sources - susceptibility to antibiotics and other biocides. Int Biodeter Biodegrad 2006; 57:51-6.

Maillard J-Y, Denyer SP. Emerging bacterial resistance following biocide exposure: should we be concerned? Chem Oggi 2009; 27:26-8.

Martin DJH, Denyer SP, McDonnell G, Maillard J-Y. Resistance and cross-resistance to oxidising agents of bacterial isolates from endoscope washer disinfectors. J Hosp Infect 2008; 69:377-83.

Maseda H, Hashida Y, Konaka R, Shirai A, Kourai H. Mutational upregulation of a resistance-nodulation-cell division-type multidrug efflux pump, SdeAB, upon exposure to a biocide, cetylpyridinium chloride, and antibiotic resistance in *Serratia marcescens*. Antimicrob Agents Chemother 2009; 53:5230-5.

McBain AJ, Bartolo RG, Catrenich CE, Charbonneau D, Ledder RG, Price BB, et al. Exposure of sink drain microcosms to triclosan: population dynamics and antimicrobial susceptibility. Appl Environ Microbiol 2003; 69:5433-42.

McBain AJ, Ledder RG, Moore LE, Catrenich CE and Gilbert P. Effects of quaternary-ammonium-based formulations on bacterial community dynamics and antimicrobial susceptibility. Appl Environ Microbiol 2004; 70:3449-56.

Noguchi N, Tamura M, Narui K, Wakasugi K, Sasatsu M. Frequency and genetic characterization of multidrug-resistant mutants of *Staphylococcus aureus* after selection with individual antiseptics and fluoroquinolones. Biol Pharm Bull 2002; 25:1129-32.

Noguchi N, Suwa J, Narui K, Sasatsu M, Ito T, Hiramatsu K, et al. Susceptibilities to antiseptic agents and distribution of antiseptic-resistance genes *qacA/B* and *smr* of methicillin-resistant *Staphylococcus aureus* isolated in Asia during 1998 and 1999. J Med Microbiol 2005; 54:557-65.

Ochman H, Lawrence JG, Groisman EA. Lateral gene transfer and the nature of bacterial innovation. Nature 2000; 405:299-304.

Pearce H, Messager S, Maillard J-Y. Effect of biocides commonly used in the hospital environment on the transfer of antibiotic-resistance genes in *Staphylococcus aureus*. J Hosp Infect 1999; 43:101-7.

Paulsen IT, Brown MH, Skurray RA. Characterization of the earliest known *Staphylococcus aureus* plasmid encoding a multidrug efflux system. J Bacteriol 1998; 180:3477-9.

Pedrouzo M, Borrull F, Marcé RM, Pocurull E. Ultra-high-performance liquid chromatography-tandem mass spectrometry for determining the presence of eleven personal care products in surface and wastewaters. J Chromatogr A. 2009; 1216:6994-7000.

Rajamohan G, Srinivasan VB, Gebreyes WA. Novel role of *Acinetobacter baumannii* RND efflux transporters in mediating decreased susceptibility to biocides. J Antimicrob Chemother 2010; 65:228-32.

SCCS (Scientific Committee on Consumer Safety). Preliminary opinion on triclosan (antimicrobial resistance), March 23rd, 2010.

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks). Assessment of the Antibiotic Resistance Effects of Biocides, 19 January 2009.

Sekiguchi J, Asagi T, Miyoshi-Akiyama T, Fujino T, Kobayashi I, Morita K, et al. Multidrug-resistant *Pseudomonas aeruginosa* strain that caused an outbreak in a neurosurgery ward and its *aac(6')-Iae* gene cassette encoding a novel aminoglycoside acetyltransferase. Antimicrob Agents Chemother 2005; 49:3734-42.

Sekiguchi J, Asagi T, Miyoshi-Akiyama T, Kasai A, Mizuguchi Y, Araake M, et al. Outbreaks of multidrug-resistant *Pseudomonas aeruginosa* in community hospitals in Japan. J Clin Microbiol 2007; 45:979-89.

Sidhu MS, Heir E, Leegaard T, Wiger K, Holck A. Frequency of disinfectant resistance genes and genetic linkage with β-lactamase transposon Tn*552* among clinical staphylococci. Antimicrob Agents Chemother 2002; 46:2797-803.

Sidhu MS, Heir E, Sørum H, Holck A. Genetic linkage between resistance to quaternary ammonium compounds and β-lactam antibiotics in food-related *Staphylococcus* spp. Microb Drug Resist 2001; 7:363-71.

Tattawasart U, Maillard J-Y, Furr JR, Russell AD. Membrane changes in *Pseudomonas stutzeri* strains resistant to chlorhexidine diacetate and cetylpyridinium chloride. Inter J Antimicrob Agents 2000; 16:233-8.

Thomas L, Russell AD, Maillard J-Y. Antimicrobial activity of chlorhexidine diacetate and benzalkonium chloride against *Pseudomonas aeruginosa* and its response to biocide residues. J Appl Microbiol 2005; 98:533-43.

Walker C, Borden LC, Zambon JJ, Bonta CY, DeVizio W, Volpe AR. The effects of 0.3% triclosan-containing dentiftrice on the microbial composition of supragingival plaque. J Clin Periodontol 1994; 21:334-41.

Wang C, Cai P, Guo Y, Mi Z. Distribution of the antiseptic-resistance genes qacEΔ1 in 331 clinical isolates of Pseudomonas aeruginosa in China. J Hosp Infect 2007; 66:93-5.

Webber MA, Coldham NG, Woodward MJ and Piddock LJV. Proteomic analysis of triclosan resistance in *Salmonella enterica* serovar Typhimurium. J Antimicrob Chemother 2008b; 62:92-7.

Webber MA, Randall LP, Cooles S, Woodward MJ, Piddock LJV. Triclosan resistance in *Salmonella enterica* serovar Typhimurium. J Antimicrob Chemother 2008a; 62:83-91.

WHO. The world health report 2007 – A safer future: global public health security in the 21st century. Available from: http://www.who.int/whr/2007/en/index.html (accessed 27 April 2010).

Williams GJ, Denyer SP, Hosein IK, Hill DW, Maillard J-Y. The use of sodium dichloroisocyanurate for floor disinfection. J Hosp Infect 2009a; 72:279-81.

Williams GJ, Denyer SP, Hosein IK, Hill DW, Maillard J-Y. Limitations of the efficacy of surface disinfection in the healthcare setting. Infect Control Hosp Epidemiol 2009b; 30:570-3.

Wilson B, Chen RF, Cantwell M, Gontz A, Zhu J, Olsen CR. The partitioning of Triclosan between aqueous and particulate bound phases in the Hudson River Estuary. Mar Pollut Bull 2009; 59:207-12.

8. GLOSSARY

The terms in the glossary were generally used as previously defined by EU legislation, with some adaptations as presented below.

Antimicrobial	a chemical substance which, at low concentrations, exerts an action against microbes and exhibits selective toxicity towards them
Antiseptic	a product, excluding antibiotics, that is used to bring about antisepsis (CEN/TC 216)
Antisepsis	application of an antiseptic on living tissues causing an action on the structure or metabolism of micro-organisms to a level judged to be appropriate to prevent and/or limit and/or treat an infection of those tissues (CEN/TC 216)
Bioavailability	the concentration of biocides or antibiotics in contact with the target organism
Biofilm	biofilms are communal structures of micro-organisms encased in an exopolymeric coat that form on both natural and abiotic surfaces
Chemical disinfection	the reduction of the number of micro-organisms in or on an inanimate matrix or intact skin, achieved by the irreversible action of a product on their structure or metabolism, to a level judged to be appropriate for a defined purpose (CEN/TC 216)
Disinfectant	product capable of chemical disinfection
Handrub	product used for post-contamination treatment that involves rubbing hands, without the addition of water, which is directed against transiently contaminating micro-organisms to prevent their transmission regardless of the resident skin flora (CEN/TC 216)
Health care	environment encompassing hospital, retirement-medicated home, general practitioner practices
Household	home environment
Microcosm	a community of micro-organisms
Molecule (active)	the active principle
Non-biocidal product	product not commercialised as a biocidal product, but nevertheless containing a biocide
Resistance	the capacity of an organism or a tissue to withstand the effects of a harmful environmental agent
Selective pressure	chemical, physical, or biological factors or constraints which select well-adapted bacteria or induce the expression of specific biological mechanisms involved in the bacterial response to external stresses
Surface disinfection	chemical disinfection of a solid surface, excluding those of certain medical and veterinary instruments, by the application of a product (CEN/TC 216)
Therapeutic use	use of antimicrobials to treat individual humans or animals (or groups of animals) suffering from a bacterial infection