Results of the Public Consultation
SCENIHR preliminary report on
"Effects of the Active Substances in Biocidal Products on Antibiotic Resistance"

Please find below the 5 questions posed by the Commission and the answers submitted. Some questions are split into sub-questions. These questions and answers are extracted from the SCENIHR preliminary opinion (Chapter 4).

SECTION 1 – QUESTIONS

QUESTION 1.a Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?

Yes, current scientific evidence (including bacteriological, biochemical and genetic data) does indicate that the use of certain active substances in biocidal products in various settings may contribute to the increased occurrence of antibiotic resistant bacteria, both in humans and in the environment.

QUESTION 1.b If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?¹

Some of the mechanisms involved are similar to those involved in resistance to antibiotics. In specific situations such as hospital and veterinary environments where both biocides and antibiotics are used, it is not possible to discriminate the origin of antimicrobial resistance. The current dearth of information means that it is difficult to quantify the impact of biocides on the selection, survival and spread of multi-resistant strains.

QUESTION 2.a If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

The most studied biocides, triclosan and quaternary ammonium compounds, are probably instrumental in maintaining a selective pressure favouring the presence of mobile genetic elements harbouring specific genes involved in the resistance to biocides and antibiotics (See section 3.4/3.9). However, the dearth of available data on the other biocidal compounds prevents reaching a definitive answer as to their role in selecting for or maintaining bacterial antibiotic resistance. With the presence of overlapping cascades of regulation that control resistance genes that are activated by external stresses, it is important to determine the capacity of biocides to trigger this process.

QUESTION 2.b If yes, which modes of action create the highest risks for increasing antibiotic resistance?

¹ The SCENIHR is asked to consider in particular the possible risk that exposure to biocides or active substances in biocidal products may favour the emergence or selection of cross resistance mechanisms (in bacterial species) that may decrease the efficacy of antibiotic molecules during therapy.
Some mechanisms of resistance are common to both biocides and antibiotics (e.g. efflux pumps, permeability changes, biofilms...). The selective pressure exerted by biocides may favour the expression of these mechanisms of resistance.

The existence of horizontal gene transfer, and in particular the presence of mobile genetic elements, creates the highest risks for increasing antibiotic resistance. The organisation of these mobile genetic elements (i.e. presence of multiple resistance genes) and their dissemination as a result of selective pressure represent the highest risks. The formation of biofilms could also contribute to a potential high risk for the development of cross resistance between antibiotics and biocides.

**QUESTION 2.c If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?**

Any application that encompasses the widespread regular use of biocides at sub-lethal concentrations maintains a continuous selective pressure and thus increases the risk of selecting resistant bacteria. This may occur in a number of uses including hospitals, food and cosmetics manufacturing etc.

**QUESTION 3. If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?**

Quantitative data on exposure and standard protocols (not available at present) are required to answer this question.

In order to determine the precise impact and prevalence of a given application, the dose, specific environment (e.g. water, level of soiling, etc), stability of compound activity or structure, potentiation or antagonism with other molecules (e.g. formulation components), must be obtained to measure the risk for each biocide for specific applications. This is a gigantic task which might not be practical. Prediction models through the use of standard protocols (see below) are a better alternative.

**QUESTION 4. How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?**

There are currently no accepted standard protocols for the evaluation of antimicrobial resistance induced or selected by biocide. Such standards must be developed to provide informative data for biocidal product development and usage, and for regulatory bodies.

The Committee strongly recommends the development of (a) standard protocol(s) for the quantitative assessment of biocide induced resistance and cross-resistance. Such protocol(s) should combine repeated biocide exposures at sub-lethal (including residual) concentrations with existing standardized antibiotics susceptibility tests.

The quantitative assessment can take the form of the new concept of "minimal selective concentration" which is the lowest concentration at which a biocide is able to select or induce the emergence/expression of a resistance mechanism concerning an antibiotic class in a defined bacterium for a specific duration of exposure. This protocol should be used together with a standardized efficacy test to assess sub-lethal concentrations on suboptimal contact times.

**QUESTION 5. Please identify relevant gaps in scientific knowledge and suggest major research needs.**
Additional studies are needed on the mechanisms of cross-resistance, emergence of biocide-induced antibiotic resistance in different fields of application (e.g. health care, veterinary uses, food production, cosmetics, consumer products).

Standardized methodologies for the evaluation of the capability of a biocide to induce/select for antibiotic resistance must also be developed.

Standardized methodologies for the surveillance of resistance and cross-resistance are also needed, in conjunction with data on the use of biocides.

Surveillance programmes must be developed to survey the level of resistance and cross-resistance of environmental isolates in all areas of biocide usage, in particular the healthcare setting, veterinary setting and food industry.

Exposure studies that encompass concentration, environmental conditions (e.g. water, soiling, exposure time, temperature, pH, etc.), change in microbial population and the dissemination of resistant determinants (horizontal transfer), are necessary to identify and measure the risks for emerging resistance and cross-resistance in bacteria following biocide exposure.
SECTION 2 - SUBMISSIONS

Submission: 1

Name
Vera Melichercikova and Vladimir Spelina, National Institute of Public Health, Czech Republic (Organisation)

QUESTION 1.a: Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?

Do you agree with the response given?
Mostly agree

QUESTION 1.b: If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?

Do you agree with the response given?
Agree

QUESTION 2.a: If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 2.b: If yes, which modes of action create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 2.c: If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Mostly agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
The use of biocide substances as preservatives in food production seems to be quite well controlled and regulated thus bringing very low risks in terms of antibiotic resistance of food-borne pathogenic bacteria. Similarly, substantial regulation exists in cosmetics. In addition to the purpose of preservation described in 3.3.2.2 more important goal is to prevent dissemination of possible contamination by pathogenic bacteria (S.aureus, P. aeruginosa, C.albicans) of a cosmetic product during the use by consumers. Effort to reevaluate the use of, sometimes probably too many, preservatives in cosmetic products in relation to potential microbial risks have recently been made, unfortunately without remarkable progress. On the other in cosmetics, unlike in foodstuffs, microbial contamination is rather low and infrequent. The use of biocide substances in household cleaning products, though similar as in cosmetics, is not regulated up to date, but
appropriate recommendation is not mentioned in cl. 3.13. or elsewhere. From practice it is well known, that when using disinfectants in hospital and health care settings and at domestic use, biocides are often applied in subinhibitory concentrations. In these applications, together with indiscriminate use of biocides in agricultural and veterinary practices during primary food production stage, it may thus be prevailing contribution to emergence or induction of resistance to biocides and cross resistance to antibiotics.

QUESTION 3: If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 4: How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?

Do you agree with the response given?
Agree

QUESTION 5: Please identify relevant gaps in scientific knowledge and suggest major research needs.

Do you agree with the response given?
Agree
Submission: 2

Withheld upon request of the author (Organisation).
Submission: 3

Name
Cathrine Pedersen, A.I.S.E., Belgium (Organisation)

Do you agree with publishing your contribution on SCENIHR’s website?
yes

QUESTION 1.a: Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?

Do you agree with the response given?
Disagree

If you chose the option ‘disagree’, explain why:
Unsatisfactory conclusion from the scientific point of view

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
In laboratory studies, some bacteria have been shown to have inducible resistance mechanisms to certain biocidal ingredients and, in some but not all instances, those mechanisms also confer resistance to antibiotics. The question is: does this occur with any frequency in the environments where these products are used? The few studies available have not shown widespread biocidal resistance in these settings – industrial (Lear et al. 2000, see Section 3.4.4.3), domestic (Cole et al. 2003), clinical (see Section 3.4.4.1) or agricultural (see Section 3.4.4.4). This topic has been extensively reviewed in the past 10 years. Both Russell and Gilbert, acknowledged leaders in the area, have concluded that while cross-resistance to biocides and antibiotics can be shown in the laboratory using pure cultures in optimal growth conditions, this does not equate to the development of such resistance in clinical or other use settings. In these settings, complex multi-species communities predominate and growth conditions are usually sub-optimal (Russell 2003, Russell 2004, Gilbert et al. 2002, Gilbert & McBain 2002) In their review, the SCENIHR expert panel noted in Section 3.4.4.1 that “issues relating to biocide resistance are considered to have a very low profile and priority”, and that evidence of biocide resistance in practice (rather than in the laboratory) is lacking. This has been the conclusion of three recent regulatory reviews on triclosan, one of the most studied biocides (Scientific Committee of Consumer Products 2006, the National Industrial Chemicals Notification and Assessment Scheme 2008, and the US EPA, 2008). Consequently we feel that while the development of biocidal-resistant strains of bacteria has been shown in the laboratory, the potential for similar strains to occur in the various settings where biocides are used has not been adequately demonstrated.

References
QUESTION 1.b : If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?

Do you agree with the response given?
Disagree

If you chose the option ‘disagree’, explain why:
Unsatisfactory conclusion from the scientific point of view

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)

As the SCENIHR committee points out in Section 3.10, antibiotic use is still the major cause of antibiotic resistance in clinical practice. Antibiotic resistance appeared following the use of antibiotics in farm animals (reviewed by EFSA 2007). Antibiotic resistance has not been found in areas that do not have direct exposure to antibiotics. Despite the widespread use of disinfectants and other biocides in healthcare and veterinary settings, acquired resistance to current disinfectants in bacteria isolated from clinical specimens or the environment has rarely been well characterized (see SCENIHR review 3.4.4.1 and 3.4.4.4). Looking at the data on triclosan, one of the most studied biocides, surveillance studies have failed to find natural isolates with Minimum Inhibitory Concentrations outside the normal range for triclosan in a wide variety of settings: the triclosan factory, other industrial settings, clinics, homes, on the skin or in the oral cavity of users of triclosan. While the surveillance studies can be criticized for being limited in scope, it is affirming that in all of the environmental studies, no evidence of increasing resistance to triclosan has been shown. While these findings do not preclude the possibility that resistance can develop outside the laboratory, they indicate that such a development does not commonly or readily occur. They also indicate that intrinsically resistant species do not out-compete susceptible strains in biocide-treated environments. So while the some of the mechanisms involved in biocide resistance may be shared with antibiotic resistance, there is no evidence that shows the impact of biocide resistance on antibiotic resistance outside the laboratory. What is shown in numerous studies is the benefit of biocidal products. We agree with the SCENIHR Committee (Section 3.11) that “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.”

references

QUESTION 2.a: If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Uncertain
**QUESTION 2.b:** If yes, which modes of action create the highest risks for increasing antibiotic resistance?

**Do you agree with the response given?**
Mostly agree

**Please provide the scientific/technical evidence to improve the overall assessment (with complete references)**
Some biocide resistance mechanisms have been shown in laboratory studies to confer increased resistance to some antibiotics. There are many different mechanisms by which this can occur (see SCENIHR report Section 3.4.3). One of these shared resistance mechanisms, horizontal gene transfer, has been shown to confer increasing antibiotic resistance in the laboratory. Whether this mechanism presents the highest risk for increasing antibiotic resistance has yet to be proven. It should be noted, however, that while these modes of action may be shared and demonstrated in laboratory studies, such changes do not necessarily occur within the complex microorganism communities commonly in the various settings of concern. Studies in the oral cavity (Bryskier 2002), consumer homes (Cole et al. 2003) and biocide factories (Lear et al. 2006) have shown that biocide resistance is not generally readily inducible, nor has it been shown to be transferred across bacterial species. In fact Bryskier concluded: “Data generated from this applied home environment study showed a lack of antibiotic and antibacterial agent cross-resistance in target bacteria from the homes of antibacterial product users and nonusers across different geographical locations. This information runs counter to recent speculative claim that antibacterial-containing antiseptics or disinfectants can contribute to the selection and propagation of drug-resistant bacteria in the home environment.” One of the most studied biocides, triclosan, is used to control oral biofilms i.e. dental plaque. In none of the studies conducted to date, has biocide or antibiotic cross-resistance been found. This type of study needs to be carried out on a wider range of naturally occurring biofilms.

**references**

**QUESTION 2.c:** If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?

**Do you agree with the response given?**
Disagree

**If you chose the option ‘disagree’, explain why:**
Unsatisfactory conclusion from the scientific point of view

**Please provide the scientific/technical evidence to improve the overall assessment (with complete references)**
The main mechanism for inducing antibiotic resistance is the use and abuse of antibiotics. Therefore areas where antibiotic use is high are the most likely to present the highest risk for increasing antibiotic resistance. There are no studies to date that demonstrate that biocides in any concentration lead to an increased risk in selecting antibiotic or
biocide resistant bacteria in environments where they have been used. Biocides are very valuable in promoting and maintaining public health. They are used to keep the levels of bacteria low, while allowing the natural ecosystem to exert the natural selective pressures that prevents the establishment of a more pathogenic population. They truly epitomize the phrase “an ounce of prevention is worth a pound of cure”. By reducing the numbers of bacteria they reduce the risk of infection and thereby reduce the potential prescription of antibiotics. Commercial biocides have been used for over 100 years in homes, hospitals, and manufacturing of all types including foods. Yet, all studies to date, looking in areas where there has been continued use have not found biocidal-resistant pathogens (Cole 2003, SDR p. 35; Lear et al. 2002, SDR p. 46). So while certain niches may occur where sub-lethal concentrations may increase the risk of selecting resistant bacteria, this is not the norm as evidenced by the data in hand. While the use of biocides may provide a theoretical risk of promoting antibiotic resistance, the evidence to date shows that they play an important role in public health (Bleasdale et al. 2007, Maillard, 2005) and provide an actual benefit in controlling antibiotic resistance outbreaks (Marshall et al. 1997, Johnson et al. 2005)

**REFERENCES**


**QUESTION 3:** If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?

**Do you agree with the response given?**

Mostly agree

**Please provide the scientific/technical evidence to improve the overall assessment (with complete references)**

We agree that quantitative data on exposure and standard protocols are not available. We also agree that determining the effect of all of the individual factors that impact the efficacy and potency of a biocide or an antibiotic would be a gigantic effort. Consequently we feel it is very important to develop protocols where the impacts of biocides on the ecology of specific settings are studied. Only in a more natural environment can the true impact of biocides be accurately assessed and prediction models developed. The development of a prediction protocol necessitates some ‘real world’ demonstration of the contribution of biocides to increasing antibiotic resistance. To date, that evidence has not been developed. In the studies where this type of data has been sought (farms, clinics, consumer households), the link between biocidal resistance and antibiotic resistance has not been found. In fact, biocide resistance has not been found. Examples cited in the SDR include: • Section 3.4.4.1 “Emerging bacterial resistance to biocides has been well described in vitro; but evidence in practice is lacking.” • Section 3.4.4.2 “Cole et al. 2003...showed a lack of cross-resistance to antibiotics and antibacterial agents in target bacteria” taken from the homes of users of antimicrobial products. • Section 3.4.4.6
“However, to date, no study seems to have focused on the emergence of biocide resistant bacteria in hospital environments”. Development of prediction models using controlled natural settings would be the most appropriate and practical ways of evaluating biocides. Any research conducted on biocides and their potential impact on antibiotic resistance should be cross referenced and benchmarked against the impact of over-usage of antibiotics.

**QUESTION 4:** How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?

**Do you agree with the response given?**
Mostly disagree

**If you chose the option ‘mostly disagree’ explain why:**
Unsatisfactory conclusion from the scientific point of view

**Please provide the scientific/technical evidence to improve the overall assessment (with complete references)**

We agree that standard methodologies and terminology would be useful. However, the draft SCENIHR opinion does not address the need for prospective studies in settings where antibiotic resistance would be most likely to develop. While elegant laboratory studies will help in understanding potential mechanisms of resistance, microcosm and field studies are needed to demonstrate the actual presence or absence of genetically stable biocidal resistance mechanisms which confer cross-resistance to antibiotics of medical importance. These types of experiments are difficult to conduct, but not impossible. We believe that they should have a higher priority than additional laboratory studies. The development of protocols with repeated biocide exposures at sub-lethal (including residual) concentrations have some inherent problems in that the repeated passage of isolates causes them to lose non-essential traits. For instance triclosan-insensitive E. coli strains were selected during routine and repeated passage against triclosan in monoculture, identifying ACP as a major target of this biocide (McMurray 1998a, SDR p. 32); such stable reductions in triclosan susceptibility have been repeated by other groups using E. coli (McBain et al. 2004, SDR p. 46; Braoudaki & Hilton 2004, SDR p. 44). In contrast, replication of these selection/training protocols, using over 40 fresh isolates from the mouth, skin and domestic drain, together with representative laboratory strains of typical oral flora showed that only the enteric species E. coli and Klebsiella oxytoca undergo selectable decreases in triclosan susceptibility (McBain et al. 2004, SDR p. 46; Ledder et al. 2006, SDR p. 46). Their susceptibilities to antibiotics were not significantly decreased. None of the remaining test isolates, including other enteric species such as salmonellae, were affected in terms of their susceptibility to triclosan or to any antibiotic representatives. This shows that the ability to select for triclosan resistance is not universal and might even be restricted to E. coli (Gilbert et al. 2007). So while this might be one standardized protocol to develop, the inherent problems of bacterial selection, maintenance, and identification must be addressed. The definition above of “minimal selective concentration” needs to be expanded to explain “a defined bacterium.” Species of bacteria can exhibit a wide range of responses to antibiotics—this was the origin of the concept of the MIC 50 or MIC 90 (Minimum Inhibitory Concentration), where the antibiotic is tested against a large number of isolates of a species and the MIC where 50 and 90 percent of the strains are inhibited is reported. So to define a bacterium as being of a specific genus and species, does not necessarily mean that that strain is representative of the majority of strains in that species. Culture collection type strains could be considered, but they are occasionally atypical of the range of strains within a species, and have the problem of extensive passage. We also feel that a baseline needs to be developed. As stated in the draft SCENIHR report, bacteria in the environment tend to be more robust than those in laboratories. Surveys
using fresh isolates should be conducted to develop more robust data for comparison purposes.

**references**

**QUESTION 5: Please identify relevant gaps in scientific knowledge and suggest major research needs.**

Do you agree with the response given?
Mostly agree

**Please provide the scientific/technical evidence to improve the overall assessment (with complete references)**

Epidemiological data indicating public health risk due to biocide resistance is lacking, as is evidence relating biocide resistance with antibiotic resistance outside the laboratory. The current study base is dominated by in vitro data. The current knowledge about resistance to biocides and antibiotics is reminiscent of the discovery phase of drug development. During this phase, potential drugs are scrutinized in laboratory tests to determine their ability to affect target genes or mechanism. Following this comes a phase where the best candidates are tested in animal models, and finally, successful candidates are tested in humans. Currently there are a number of mechanisms of resistance to biocides that have been identified in laboratory tests. The question now is: are these mechanisms relevant in more complex ecosystems than those found in the laboratory, i.e. in model systems such as McBain drain studies (2003, SDR p. 39) or in the actual clinical, veterinary or consumer sites where these products are used? A few of these studies have been already conducted on triclosan (Cole et al. 2003, Lear et al. 2002, McBain et al. 2004, SDR p. 35, 46, & 47). They have not shown any significant incidence of biocide resistance in areas where there has been repeated application of biocides, i.e. isolates from the homes of users of biocides, the factories where biocides are made, or model drains where there is a consistent dosing of biocide. Gilbert et al. (2007) reviewed the considerable number of microcosm studies (domestic drains and in dental plaque) performed to evaluate triclosan-containing products, as well as environmental surveillance studies to monitor effect on oral flora. The theoretical risks are not supported by either field or clinical studies, or by laboratory studies using bacterial microcosms. More of these studies need to be conducted for a wider range of biocidal products. We agree that standardized methodologies and surveillance programs are needed. In addition, any research program should be inclusive of all types of biocides and should not be limited to one particular biocide or type of biocide.

**references**
**Submission: 4**

**Name**
Marie-Hélène Boos, Ciba Inc., Switzerland (Organisation)

**QUESTION 1.a: Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?**

**Do you agree with the response given?**
Disagree

**If you chose the option 'disagree', explain why:**
Unsatisfactory conclusion from the scientific point of view; Relevant information missing from the analysis of the situation

**Please provide the scientific/technical evidence to improve the overall assessment (with complete references)**
We respectfully disagree with this opinion based on the numerous reviews of existing data, including the present SCENIHR Draft Review (referred to as SDR in the text below). In some laboratory studies, the data demonstrate the ability of some active ingredients in biocidal products to trigger the expressions of a resistance mechanism(s) for that biocide, and in some cases confer cross-resistance to some antibiotics of medical importance. Outside the laboratory, however, there is no evidence that biocides have contributed to the occurrence of antibiotic resistant bacteria in humans or the environment. As SCENIHR points out in section 3.4.4.1, “Despite the widespread use of disinfectant and antiseptic in healthcare setting, acquired resistance to current disinfectants in bacteria isolated from clinical specimens or the environment has rarely been well characterized. Emerging bacterial resistance to biocides has been well described in vitro; but evidence in practice is lacking.” Laboratory isolates with reduced susceptibility usually remain susceptible to clinically used concentrations of antiseptics and disinfectants (Lear et al. 2006, SDR p. 34). This finding contrasts with antibiotic resistance, which has emerged over time, rendering a number of antibiotics clinically unusable. There are no studies to indicate that biocides are linked to antibiotic resistance and or the emergence of pathogenic organisms outside the laboratory with the exception of the Cookson studies (1991 a,b, SDR p. 33 & 35), which have not been duplicated (Suller & Russell 2000, SDR p. 35; Suller & Russell 1999). Suller and Russell (2000, SDR p. 35) showed that the acquisition of mupiricin resistance through a plasmid is unassociated with any change in triclosan susceptibility. Stickler and Jones (2008, SDR p. 35) demonstrated that laboratory strains of Proteus mirabilis could be selected with reduced susceptibilities to triclosan, but these strains did not exhibit any increased resistance to antibiotics. Consequently, the body of evidence indicates a lack of linkage of biocide and antibiotic resistance outside the laboratory. The SCENIHR committee reviewed the effect of a wide range of biocides in a wide range of applications. In its October 2006 opinion, the Scientific Committee on Consumer Products (SCCP, 2006) of the European Commission reviewed the data specific to triclosan finding: “Recent scientific papers and European institution reports have expressed concerns about the indiscriminate use of biocides including TCS. These concerns have been based on experimental studies and the theoretical association between increased occurrence of antibiotic cross resistance and the use of biocides....On the basis of the available data, the SCCP is of the opinion that there is presently no evidence of clinical resistance and cross-resistance occurring from the use of triclosan in cosmetic products.” The SDR does not identify any new studies that should affect that opinion. The selective pressures on bacteria in the environment are myriad, e.g. temperature, nutrients, pH, water
availability, competition, etc. In laboratory studies, optimal conditions for growth of monocultures are maintained. This is not the case in nature. These are important forces that must be considered in the evaluation of the contribution, if any, of biocides to antibiotic resistance. Biocides can and do play an important role in the prevention of infection and the promotion of health in the complex environments where they are used. In summary, there are shared mechanisms of resistance to biocides and antibiotics in some bacteria, however, studies of antibiotic resistant bacteria in clinical, industrial and agricultural settings have failed to provide any evidence that links biocide use to the development of antibiotic resistance. This indicates that while there could be some contribution to antibiotic resistance, it appears that it does not readily occur, if ever, in these settings.

References

QUESTION 1.b : If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?
Do you agree with the response given?
Disagree
If you chose the option ‘disagree’, explain why:
Other

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
The overuse and misuse of antibiotics is the major cause of antibiotic resistance encountered in healthcare, veterinary and agricultural settings. To date, there have been no field studies which have shown the use of biocides to result in the development of either biocide resistance (see SDR Section 3.4.4.1) or antibiotic cross-resistance (Cole et al. 2003, SDR p. 35). Antibiotic use is still the major cause of antibiotic resistance in clinical practice (see SDR p.55). Since antibiotic resistance remains a major public health concern and decreases the ability to treat infections, appropriate infection control strategies are paramount and involve prevention through good hygiene which encompass the appropriate use of biocides (Anon 1999, SDR p. 55). Indirect epidemiological evidence shows a lack of correlation between the patterns of biocide use in clinical settings and the emergence of antibiotic resistance (Gilbert & McBain 2003, SDR p. 46). Numerous investigations address the appearance of antibiotic resistance following the use of antibiotics in farm animals (EFSA 2007, SDR p. 38). Data relating the occurrence of resistance to the use of disinfectants in field studies are limited. However, the findings to date do not support the in vitro data that demonstrates the potential for biocides selecting for antibiotic resistant strains. A number of studies have evaluated whether clinical and environmental isolates show any evidence of significant reductions in their susceptibility to biocides and whether this might be linked with antibiotic resistance. Most of the studies support the view that antiseptic use in hospitals does not contribute to decreased susceptibility to biocides or increase in antibiotic resistance (see Gilbert & McBain 2003, SDR p. 46). Lear et al. 2002 (SDR p. 46) surveyed isolates which would be expected to have the greatest tendency to develop resistance to biocides, those from factories where those biocides are synthesized. There was no evidence suggesting that the residual levels of biocides in factory environment had led to changes in susceptibility of these isolates. There are no published studies where a biocide has been removed from
a hospital or farm resulting in a change in the levels of antibiotic resistant strains present in that setting. There are studies that show the introduction of biocidal products has helped in reducing the spread of antibiotic resistant strains in hospital units. Marshall et al. (1997) reported that during an intensive policy of antiseptic handwashing involving a triclosan-based medicated soap, aimed at combating a methicillin-resistant S. aureus (MRSA) infection, not only did the incidence of MRSA decrease significantly, but the percentage of ciprofloxacin-sensitive isolates increased from 8.1% to 22.5% within the trial. Johnson et al. (2005) studied the introduction of a number of cultural interventions and an alcohol/chlorhexidine hand solution in a large hospital with a high rate of MRSA colonization. There were significant reductions in hospital-wide rates of total clinical MRSA isolates, patient-episodes of MRSA bacteraemia and clinical isolates of ESBL-producing E. coli and Klebsiella spp, thirty-six months post-intervention. Also there are a number of studies in clinical settings where good hygiene has contributed to reduced antibiotic resistance through reduced prescribing of antibiotics (Gilbert & McBain 2003, SDR p. 43) In conclusion: a) There are studies which show that the introduction of biocidal products has helped in reducing the spread of antibiotic resistant strains in hospital units. b) So while similar mechanisms occur in the development of resistance to both biocides and antibiotics, the current evidence indicates the primary pressure for mutation stems from antibiotic use, not biocide use. The lack of ecological studies means that it is difficult to quantify the impact (if any) of biocides in the settings where they are used.

references

QUESTION 2.a: If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Disagree

If you chose the option ‘disagree’, explain why:
Other

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
To rank biocidal substances, more data are needed particularly for biocides other than triclosan and quaternary ammonium compounds (QAC). Triclosan and QAC have become “tools” for investigating potential resistance mechanisms over a wide range of bacteria. Consequently there is more information regarding their molecular action, but this does not mean that they present a greater or lesser risk than other biocides. There are very little data available on biocides other than triclosan and QAC. Consequently, there is no way of predicting the ranking of biocides which present a risk (if any) for increasing antibiotic resistance. We believe SCENIHR places an undue emphasis on triclosan and QAC when they state above that they are “probably instrumental”, and on p.9 that they are “likely to contribute”. In Section 3.8.7 they state: “It is difficult to ascertain how wide spread the development of bacterial resistance to a biocide is in practice mainly due to the paucity of information available”. Those studies which have looked for resistance
in the environment have not found it (Cole et al. 2003 SDR p. 35, Lear et al. 2002 SDR p.46). We also disagree with the above statement because it implies that the presence of mobile genetic elements (MGE) is the primary mechanism by which antibiotic and biocidal resistance is conferred. For triclosan, the majority of studies do not demonstrate a role for MGE. For QAC, MGE have been found which carry the biocide resistance genes. Biofilms are another important mechanism for the failure of bacteria to respond to biocides or antibiotics. There are no studies on the relevance of these findings in the environment. While it may be important to determine the capacity of biocides to trigger the presence of overlapping cascades of regulation that control resistance genes, it is at least equally important to determine if these mechanisms are relevant in the stress-filled environments outside the laboratory. Three international agencies have recently reviewed the issue of triclosan and resistance, and all three found no evidence that the use of triclosan leads to an increase in triclosan- or antibiotic- resistance: • In its October 2006 opinion, the Scientific Committee on Consumer Products (SCCP, 2006) of the European Commission reviewed the data specific to triclosan finding: “Recent scientific papers and European institution reports have expressed concerns about the indiscriminate use of biocides including TCS. These concerns have been based on experimental studies and the theoretical association between increased occurrence of antibiotic cross resistance and the use of biocides….On the basis of the available data, the SCCP is of the opinion that there is presently no evidence of clinical resistance and cross-resistance occurring from the use of triclosan in cosmetic products.” • In 2008, the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) in Australia published their draft Priority Existing Chemical Assessment Report on triclosan, in which they concluded that “[o]n the basis of available data, there is also no evidence that the use of triclosan is leading to an increase in triclosan-resistant bacterial populations or that there is any increased risk to humans regarding antibiotic resistance”(NICNAS, 2008). • In 2008, the US Environmental Protection Agency published their Reregistration Eligibility Decision (RED) document on triclosan, in which they conclude that “[t]here is currently some research attempting to demonstrate a connection between antimicrobial resistance and antibiotic resistance in regard to triclosan, but the linkage has not been expressly proven” (US EPA, 2008). In addition, the SCCP is currently reviewing the status of triclosan as a preservative in cosmetic products. The European Cosmetic Toiletry and Perfumery Association (COLIPA) provided a dossier on antibacterial resistance and triclosan (Colipa, 2007) which we will submit herein including all references.

references

QUESTION 2.b: If yes, which modes of action create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Mostly agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
We agree that there are common resistance mechanisms to both biocides and antibiotics, and that horizontal gene transfer has been shown for increasing antibiotic resistance. It should be noted, however, that while these modes of action may be shared and demonstrated in laboratory studies, such changes do not necessarily occur within the complex microorganism communities commonly found in the environments of the home, the hospital, the farm etc. Studies in drain microcosms (McBain et al. 2004, SDR p. 39),
the oral cavity (Bonta et al. 1992; Fine et al. 1998; Walker et al. 1994; Zambon et al. 1990; Zambon et al. 1995), consumer homes (Cole et al. 2003, SDR p. 35) and biocide factories (Lear et al. 2002, SDR p. 46) have shown that biocide resistance is not generally readily inducible, nor has it been shown to be transferred across bacterial species. Biofilms are common in nature, and while they could contribute to a potential risk for the development of cross-resistance, no such role has been demonstrated to date either in biofilms models (e.g. drains) or in nature (oral plaque).

**references**


**QUESTION 2.c: If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?**

Do you agree with the response given?

*Disagree*

**If you chose the option ‘disagree’, explain why:** Relevant information missing from the analysis of the situation

**Please provide the scientific/technical evidence to improve the overall assessment (with complete references)**

Areas such as hospitals and certain farming operations where antibiotics are routinely used and misused are the most likely situations where antibiotic resistance will be encountered. Use of biocides in these environments might be perceived as areas where higher risks of biocide resistance may be incurred, however, there are no studies to support that view. Areas such as food and cosmetic manufacturing, where antibiotics are not used and where biocides are used, are less likely situations where antibiotic resistance may be encountered as the main selective pressure for antibiotic-resistance (antibiotics) is absent. It should be noted that unlike antibiotics, biocides are delivered in formulations that usually contain other ingredients or properties which impact bacterial cells, such as detergents, chelators and extreme pH. The potential for a biocidal agent to induce a resistance mechanism is most likely irrelevant to a bacterium whose cell wall or membrane has been damaged. The purpose of most biocide-containing formulations is to reduce the bioburden on the treated surface. It is not meant to sterilize, or to impact the resident commensal population in such a way as to allow the establishment of a pathogen population. Rather it acts as a means of population control, thereby reducing the risk of transfer or infection. Commercial biocides have been used for over 100 years in homes, hospitals, and manufacturing of all types including foods. Yet, all studies to date, looking in areas where there has been continued use have not found biocidal-resistant pathogens (Cole 2003, SDR p. 35; Lear et al. 2002, SDR p. 46). So while certain niches may occur where sub-lethal concentrations may increase the risk of selecting resistant bacteria, this is not the norm as evidenced by the data in hand. While the use of biocides may provide a theoretical risk of promoting antibiotic
resistance, the evidence to date shows that they play an important role in public health (Bleasdale et al. 2007, Maillard, 2005) and provide an actual benefit in controlling antibiotic resistance outbreaks (Marshall et al. 1997, Johnson et al. 2005)

references

QUESTION 3: If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?

Do you agree with the response given?
Mostly agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
We agree that quantitative data on exposure and standard protocols are not available. We also agree that determining the effect of all of the individual factors that impact the efficacy and potency of a biocide or an antibiotic would be a gigantic effort. Consequently we feel it is very important to develop protocols where the impacts of biocides on the ecology of specific settings are studied. Only in a more natural environment can the true impact of biocides be accurately assessed and prediction models developed. The development of a prediction protocol necessitates some ‘real world’ demonstration of the contribution of biocides to increasing antibiotic resistance. To date, that evidence has not been developed. In the studies where this type of data has been sought (farms, clinics, consumer households), the link between biocidal resistance and antibiotic resistance has not been found. In fact, biocide resistance has not been found. Examples cited in the SDR include: • Section 3.4.4.1 "Emerging bacterial resistance to biocides has been well described in vitro; but evidence in practice is lacking." • Section 3.4.4.2 "Cole et al. 2003...showed a lack of cross-resistance to antibiotics and antibacterial agents in target bacteria" taken from the homes of users of antimicrobial products. • Section 3.4.4.6 "However, to date, no study seems to have focused on the emergence of biocide resistant bacteria in hospital environments”... Development of prediction models using controlled natural settings would be the most appropriate and practical ways of evaluating biocides. Any research conducted on biocides and their potential impact on antibiotic resistance should be cross referenced and benchmarked against the impact of over-usage of antibiotics.

QUESTION 4: How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?

Do you agree with the response given?
Mostly disagree

If you chose the option ‘mostly disagree’ explain why:
Unsatisfactory conclusion from the scientific point of view

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
We agree that standard methodologies and terminology would be useful. However, the draft SCENIR opinion does not address the need for prospective studies in settings where antibiotic resistance would be most likely to develop. While elegant laboratory studies will help in understanding potential mechanisms of resistance, microcosm and field studies are needed to demonstrate the actual presence or absence of genetically stable biocidal resistance mechanisms which confer cross-resistance to antibiotics of medical importance. These types of experiments are difficult to conduct, but not impossible. We believe that they should have a higher priority than additional laboratory studies. The development of protocols with repeated biocide exposures at sub-lethal (including residual) concentrations have some inherent problems, in that the repeated passage of isolates causes them to lose non-essential traits. For instance triclosan-insensitive E. coli strains were selected during routine and repeated passage against triclosan in monoculture, identifying ACP as a major target of this biocide (McMurray 1998a, SDR p. 32); such stable reductions in triclosan susceptibility have been repeated by other groups using E. coli (McBain et al. 2004, SDR p. 46; Braoudaki & Hilton 2004, SDR p. 44). In contrast, replication of these selection/training protocols, using over 40 fresh isolates from the mouth, skin and domestic drain, together with representative laboratory strains of typical oral flora showed that only the enteric species E. coli and Klebsiella oxytoca undergo selectable decreases in triclosan susceptibility (McBain et al. 2004, SDR p. 46; Ledder et al. 2006, SDR p. 46). Their susceptibilities to antibiotics were not significantly decreased. None of the remaining test isolates, including other enteric species such as salmonellae, were affected in terms of their susceptibility to triclosan or to any antibiotic representatives. This shows that the ability to select for triclosan resistance is not universal and might even be restricted to E. coli (Gilbert et al. 2007). So while this might be one standardized protocol to develop, the inherent problems of bacterial selection, maintenance, and identification must be addressed. The definition above of “minimal selective concentration” needs to be expanded to explain “a defined bacterium.” Species of bacteria can exhibit a wide range of responses to antibiotics– this was the origin of the concept of the MIC 50 or MIC 90 (Minimum Inhibitory Concentration), where the antibiotic is tested against a large number of isolates of a species and the MIC where 50 and 90 percent of the strains are inhibited is reported. So to define a bacterium as being of a specific genus and species, does not necessarily mean that that strain is representative of the majority of strains in that species. Culture collection type strains could be considered, but they are occasionally atypical of the range of strains within a species, and have the problem of extensive passage. We also feel that a baseline needs to be developed. As stated in the draft SCENIHR report, bacteria in the environment tend to be more robust than those in laboratories. Surveys using fresh isolates should be conducted to develop more robust data for comparison purposes.

references

QUESTION 5: Please identify relevant gaps in scientific knowledge and suggest major research needs.
Do you agree with the response given?
Mostly disagree

If you chose the option ‘mostly disagree’ explain why:
Other

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
Epidemiological data indicating public health risk due to biocide resistance is lacking, as is evidence relating biocide resistance with antibiotic resistance outside the laboratory. The current study base is dominated by in vitro data. The current knowledge about resistance to biocides and antibiotics is reminiscent of the discovery phase of drug development. During this phase, potential drugs are scrutinized in laboratory tests to determine their ability to affect target genes or mechanism. Following this comes a phase where the best candidates are tested in animal models, and finally, successful candidates are tested in humans. Currently there are a number of mechanisms of resistance to biocides that have been identified in laboratory tests. The question now is: are these mechanisms relevant in more complex ecosystems than those found in the laboratory, i.e. in model systems such as McBain drain studies (2003, SDR p. 39) or in the actual clinical, veterinary or consumer sites where these products are used? A few of these studies have been already conducted on triclosan (Cole et al. 2003, Lear et al. 2002, McBain et al. 2004, SDR p. 35, 46, & 47). They have not shown any significant incidence of biocide resistance in areas where there has been repeated application of biocides, i.e. isolates from the homes of users of biocides, the factories where biocides are made, or model drains where there is a consistent dosing of biocide. Gilbert et al. (2007) reviewed the considerable number of microcosm studies (domestic drains and in dental plaque) performed to evaluate triclosan-containing products, as well as environmental surveillance studies to monitor effect on oral flora. The theoretical risks are not supported by either field or clinical studies, or by laboratory studies using bacterial microcosms. More of these studies need to be conducted for a wider range of biocidal products. We agree that standardized methodologies and surveillance programs are needed. In addition, any research program should be inclusive of all types of biocides and should not be limited to one particular biocide or type of biocide.

references
Submission: 5

Name
Jane Mani-Saada, Health Protection Agency HCAI and AMR Programme Board, UK (Organisation)

QUESTION 1.a: Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?

Do you agree with the response given?
Agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
As the report says there is potential for concern about biocides selecting antibiotic resistance either (a) because the same plasmids carry determinants affecting both classes of agent and (b) both may be substrates for broad spectrum RND efflux pumps. It is striking how little certainty there is with the word "may " repeated 100 times and might 23 times.

QUESTION 1.b: If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?

Do you agree with the response given?
Agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
Lack of information and control of use of biocides means that it is difficult to quantify the impact on the selection, survival and spread of multi-resistant strains.

QUESTION 2.a: If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 2.b: If yes, which modes of action create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
The authors could make more of the case of P.aeruginosa, where up-regulation of MexAB-OprM-mediated efflux is a common mode of clinical antibiotic resistance and is also active against biocides; the AcrAB pump of E.coli which is stressed far more in the document is rarely a source of clinical resistance. It is suggested that clinical input is required.
QUESTION 2.c: If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 3: If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 4: How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?

Do you agree with the response given?
Agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
What is needed is a cross-over trial using different biocides in different wards with similar antibiotic use and see if there are any related shifts in resistance/plasmid types.

QUESTION 5: Please identify relevant gaps in scientific knowledge and suggest major research needs.

Do you agree with the response given?
Agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
There is scope for concern but a lack of clear evidence of widespread harm done.
QUESTION 1.a: Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?

Do you agree with the response given?
Uncertain

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
The report “Effects of the Active Substances in Biocidal Products on Antibiotic Resistance” offers a broad perspective of the subject matter, resting on an abundant bibliography. With the agreement of the co-authors, I join the manuscript that we just submitted to Antimicrobial Agents and Chemotherapy, entitled ‘A reminder: in-use concentration tests of antimicrobial and biocidal agents are based on totally divergent concepts and “resistance” has different meanings’. It discusses the indiscriminate use of the word ‘resistance’ to describe quite different phenomena. It also proposes short expressions, aimed at avoiding ambiguity, that we think are relevant in the context of the SCENHIR report. I copy here the suggested expressions: • “C-resistance” to antimicrobials used in clinical in vivo conditions; • “NWT-resistance” of non wild-type populations exposed to antimicrobials in vitro; • “B-resistance” to biocides at concentrations intended at killing a significant proportion of bacterial populations; • “B-tolerance” to biocides at inhibitory/sub-lethal concentrations. General comments The section 3.2.1.1 on Definitions is quite useful. It indicates: “the word ‘resistance’ is used … where a strain is not killed or inhibited by a concentration attained in practice (the in-use concentration)” and “Tolerance denotes a reduced susceptibility to an antimicrobial molecule characterised by a raised minimum inhibitory concentration (MIC), or a situation in which a preservative system does no longer prevent microbial growth”. Therefore as regards biocides the authors did choose to include within the term ‘resistance’ two distinct meanings: inhibition and killing. The text perfectly explains that these are two quite different processes. Yet when the results of a number of publications are exposed, the report often does not distinguish if the commented articles deal with killing (at the in-use concentration determined according to international standards, such as CEN or AOAC ones) or inhibition (at concentrations close to or slightly higher than the MIC). And in the latter case, where the word ‘tolerance’ as defined above should be used, ‘resistance’ is employed. The resulting texts are confusing and the conclusions that are drawn explicitly, or only suggested, might be biased or could lead to misinterpretation. May I urge the authors to modify the text in order to make it crystal clear in this respect? Some biocides can induce raised MIC, but not all of them. A recapitulation of those of concern as regards raised MIC would be helpful. The question of the potential for biocide use to cause resistance to antibiotics was treated in different sections. Is not the proportion of articles demonstrating that this is a non-problem higher in the sections on biocides, while the reverse is true for the sections on antibiotics? May I suggest the question is revised with the aim of unifying the views? Specific remarks Table 7, page 30: What is the meaning of ‘no’ in front of MICs; that the methodology for MIC measurement does not measure the resistance to a biocide, according to the title of the column? This would be contrary to the above-given definition of resistance. Page 34, one line from the bottom, the date of the reference to Rutala and Weber is wrong. Page 35, third para. from the bottom: this is quite a strong statement. Please give the reference(s). Or is it a partial summary/conclusion? Page 36, second para., first line, unclear if the words between brackets are the equivalent of what is written just before. Are “resistant bacteria” the
same as “bacteria with increased tolerance”? If yes, please replace ‘or’ by ‘that is’, or better delete ‘biocide resistant bacteria’ and the brackets. The use of ‘tolerance’ here is not in line with the given definition: tolerance is a ‘reduced susceptibility’, that is an increased ability to grow at concentration of disinfectant higher than the MIC. This conclusive sentence illustrates the ambiguity that pervades all the report. The reader here will likely remind a vague but far-reaching ‘increased resistance’. Page 61, section 3.12, line 2: the sentence “biocides trigger the emergence of antibiotic resistance” is a condemnation of the biocides. Please modify, for example: “some biocides used at sublethal concentration may trigger”. Page 61, section 3.12, line 5: “epidemiological data indicating public health relevance are lacking”. Do the authors say that, because no epidemiological surveys have been done (‘the surveys are lacking’), the public health problem has not become visible? This is how I understand the wording. Could you accept one of these expressions: “there is no evidence of a public health problem” or, softer, “there is no published evidence of a public health problem”? Expressions similar to ‘are lacking’ are used throughout the report, and some readers could understand the same suggested meaning. May I request that such an ambiguity is avoided? Page 62, opinion 1.a, line 6, after ‘use’ could you please add ‘and/or misuse’? This addition could be done in many parts of the report.

QUESTION 1.b : If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?

Do you agree with the response given?
Mostly agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
The report “Effects of the Active Substances in Biocidal Products on Antibiotic Resistance” offers a broad perspective of the subject matter, resting on an abundant bibliography. With the agreement of the co-authors, I join the manuscript that we just submitted to Antimicrobial Agents and Chemotherapy, entitled ‘A reminder: in-use concentration tests of antimicrobial and biocidal agents are based on totally divergent concepts and “resistance” has different meanings’. It discusses the indiscriminate use of the word ‘resistance’ to describe quite different phenomena. It also proposes short expressions, aimed at avoiding ambiguity, that we think are relevant in the context of the SCENHIR report. I copy here the suggested expressions: • “C-resistance” to antimicrobials used in clinical in vivo conditions; • “NWT-resistance” of non wild-type populations exposed to antimicrobials in vitro; • “B-resistance” to biocides at concentrations intended at killing a significant proportion of bacterial populations; • “B-tolerance” to biocides at inhibitory/sub-lethal concentrations. General comments The section 3.2.1.1 on Definitions is quite useful. It indicates: “the word ‘resistance’ is used ... where a strain is not killed or inhibited by a concentration attained in practice (the in-use concentration)” and “Tolerance denotes a reduced susceptibility to an antimicrobial molecule characterised by a raised minimum inhibitory concentration (MIC), or a situation in which a preservative system does no longer prevent microbial growth”. Therefore as regards biocides the authors did choose to include within the term ‘resistance’ two distinct meanings: inhibition and killing. The text perfectly explains that these are two quite different processes. Yet when the results of a number of publications are exposed, the report often does not distinguish if the commented articles deal with killing (at the in-use concentration determined according to international standards, such as CEN or AOAC ones) or inhibition (at concentrations close to or slightly higher than the MIC). And in the latter case, where the word ‘tolerance’ as defined above should be used, ‘resistance’ is employed. The resulting texts are confusing and the conclusions that are drawn explicitly, or only suggested, might be biased or could lead to misinterpretation. May I urge the authors to modify the text in order to make it crystal clear in this respect? Some biocides
can induce raised MIC, but not all of them. A recapitulation of those of concern as regards raised MIC would be helpful. The question of the potential for biocide use to cause resistance to antibiotics was treated in different sections. Is not the proportion of articles demonstrating that this is a non-problem higher in the sections on biocides, while the reverse is true for the sections on antibiotics? May I suggest the question is revised with the aim of uniting the views? Specific remarks Table 7, page 30: What is the meaning of ‘no’ in front of MICs; that the methodology for MIC measurement does not measure the resistance to a biocide, according to the title of the column? This would be contrary to the above-given definition of resistance. Page 34, one line from the bottom, the date of the reference to Rutala and Weber is wrong. Page 35, third para. from the bottom: this is quite a strong statement. Please give the reference(s). Or is it a partial summary/conclusion? Page 36, second para., first line, unclear if the words between brackets are the equivalent of what is written just before. Are “resistant bacteria” the same as “bacteria with increased tolerance”? If yes, please replace ‘or’ by ‘that is’, or better delete ‘biocide resistant bacteria’ and the brackets. The use of ‘tolerance’ here is not in line with the given definition: tolerance is a ‘reduced susceptibility’, that is an increased ability to grow at concentration of disinfectant higher than the MIC. This conclusive sentence illustrates the ambiguity that pervades all the report. The reader here will likely remind a vague but far-reaching ‘increased resistance’. Page 61, section 3.12, line 2: the sentence “biocides trigger the emergence of antibiotic resistance” is a condemnation of the biocides. Please modify, for example: “some biocides used at sublethal concentration may trigger”. Page 61, section 3.12, line 5: “epidemiological data indicating public health relevance are lacking”. Do the authors say that, because no epidemiological surveys have been done (‘the surveys are lacking’), the public health problem has not become visible? This is how I understand the wording. Could you accept one of these expressions: “there is no evidence of a public health problem” or, softer, “there is no published evidence of a public health problem”? Expressions similar to ‘are lacking’ are used throughout the report, and some readers could understand the same suggested meaning. May I request that such an ambiguity is avoided? Page 62, opinion 1.a, line 6, after ‘use’ could you please add ‘and/or misuse’? This addition could be done in many parts of the report.

**QUESTION 2.a:** If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Uncertain

**QUESTION 2.b:** If yes, which modes of action create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Uncertain

**QUESTION 2.c:** If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Uncertain

**QUESTION 3:** If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?

Do you agree with the response given?
QUESTION 4: How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?

Do you agree with the response given?
Uncertain

QUESTION 5: Please identify relevant gaps in scientific knowledge and suggest major research needs.

Do you agree with the response given?
Uncertain
Submission: 7

Name
Ministry of Health of the Republic of Latvia (Organisation)

QUESTION 1.a: Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?

Do you agree with the response given?
Agree

QUESTION 1.b: If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?

Do you agree with the response given?
Agree

QUESTION 2.a: If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 2.b: If yes, which modes of action create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 2.c: If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 3: If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 4: How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?

Do you agree with the response given?
Agree

QUESTION 5: Please identify relevant gaps in scientific knowledge and suggest
major research needs.

Do you agree with the response given?
Agree
Submission: 8

Name
Florian Schellauf, Colipa, Belgium (Organisation)

QUESTION 1.a: Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?

Do you agree with the response given?
Disagree

If you chose the option ‘disagree’, explain why:
Unsatisfactory conclusion from the scientific point of view

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
We respectfully disagree with this opinion based on the numerous reviews of existing data, including the present SCENIHR Draft Review (referred to as SDR in the text below). In some laboratory studies, the data demonstrate the ability of some active ingredients in biocidal products to trigger the expressions of a resistance mechanism(s) for that biocide, and in some cases confer cross-resistance to some antibiotics of medical importance. Outside the laboratory, however, there is no evidence that biocides have contributed to the occurrence of antibiotic resistant bacteria in humans or the environment. As SCENIHR points out in section 3.4.4.1, “Despite the widespread use of disinfectant and antiseptic in healthcare setting, acquired resistance to current disinfectants in bacteria isolated from clinical specimens or the environment has rarely been well characterized. Emerging bacterial resistance to biocides has been well described in vitro; but evidence in practice is lacking.” Laboratory isolates with reduced susceptibility usually remain susceptible to clinically used concentrations of antiseptics and disinfectants (Lear et al. 2006, SDR p. 34). This finding contrasts with antibiotic resistance, which has emerged over time, rendering a number of antibiotics clinically unusable. There are no studies to indicate that biocides are linked to antibiotic resistance and or the emergence of pathogenic organisms outside the laboratory with the exception of the Cookson studies (1991 a,b, SDR p. 33 & 35), which have not been duplicated (Suller & Russell 2000, SDR p. 35; Suller & Russell 1999). Suller and Russell (2000, SDR p. 35) showed that the acquisition of mupiricin resistance through a plasmid is unassociated with any change in triclosan susceptibility. Stickler and Jones (2008, SDR p. 35) demonstrated that laboratory strains of Proteus mirabilis could be selected with reduced susceptibilities to triclosan, but these strains did not exhibit any increased resistance to antibiotics. Consequently, the body of evidence indicates a lack of linkage of biocide and antibiotic resistance outside the laboratory. The SCENIHR committee reviewed the effect of a wide range of biocides in a wide range of applications. In its October 2006 opinion, the Scientific Committee on Consumer Products (SCCP, 2006) of the European Commission reviewed the data specific to triclosan finding: “Recent scientific papers and European institution reports have expressed concerns about the indiscriminate use of biocides including TCS. These concerns have been based on experimental studies and the theoretical association between increased occurrence of antibiotic cross resistance and the use of biocides…On the basis of the available data, the SCCP is of the opinion that there is presently no evidence of clinical resistance and cross-resistance occurring from the use of triclosan in cosmetic products.” The SDR does not identify any new studies that should affect that opinion. The selective pressures on bacteria in the environment are myriad, e.g. temperature, nutrients, pH, water...
availability, competition, etc. In laboratory studies, optimal conditions for growth of monocultures are maintained. This is not the case in nature. These are important forces that must be considered in the evaluation of the contribution, if any, of biocides to antibiotic resistance. Biocides can and do play an important role in the prevention of infection and the promotion of health in the complex environments where they are used. In summary, there are shared mechanisms of resistance to biocides and antibiotics in some bacteria, however, studies of antibiotic resistant bacteria in clinical, industrial and agricultural settings have failed to provide any evidence that links biocide use to the development of antibiotic resistance. This indicates that while there could be some contribution to antibiotic resistance, it appears that it does not readily occur, if ever, in these settings.

References

QUESTION 1.b : If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?
Do you agree with the response given?
Disagree
If you chose the option ‘disagree’, explain why:
Unsatisfactory conclusion from the scientific point of view

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
The overuse and misuse of antibiotics is the major cause of antibiotic resistance encountered in healthcare, veterinary and agricultural settings. To date, there have been no field studies which have shown the use of biocides to result in the development of either biocide resistance (see SDR Section 3.4.4.1) or antibiotic cross-resistance (Cole et al. 2003, SDR p. 35). Antibiotic use is still the major cause of antibiotic resistance in clinical practice (see SDR p.55). Since antibiotic resistance remains a major public health concern and decreases the ability to treat infections, appropriate infection control strategies are paramount and involve prevention through good hygiene which encompass the appropriate use of biocides (Anon 1999, SDR p. 55). Indirect epidemiological evidence shows a lack of correlation between the patterns of biocide use in clinical settings and the emergence of antibiotic resistance (Gilbert & McBain 2003, SDR p. 46). Numerous investigations address the appearance of antibiotic resistance following the use of antibiotics in farm animals (EFSA 2007, SDR p. 38). Data relating the occurrence of resistance to the use of disinfectants in field studies are limited. However, the findings to date do not support the in vitro data that demonstrates the potential for biocides selecting for antibiotic resistant strains. A number of studies have evaluated whether clinical and environmental isolates show any evidence of significant reductions in their susceptibility to biocides and whether this might be linked with antibiotic resistance. Most of the studies support the view that antiseptic use in hospitals does not contribute to decreased susceptibility to biocides or increase in antibiotic resistance (see Gilbert & McBain 2003, SDR p. 46). Lear et al. 2002 (SDR p. 46) surveyed isolates which would be expected to have the greatest tendency to develop resistance to biocides, those from factories where those biocides are synthesized. There was no evidence suggesting that the residual levels of biocides in factory environment had led to changes in susceptibility of these isolates. There are no published studies where a biocide has been removed from
a hospital or farm resulting in a change in the levels of antibiotic resistant strains present in that setting. There are studies that show the introduction of biocidal products has helped in reducing the spread of antibiotic resistant strains in hospital units. Marshall et al. (1997) reported that during an intensive policy of antiseptic handwashing involving a triclosan-based medicated soap, aimed at combating a methicillin-resistant S. aureus (MRSA) infection, not only did the incidence of MRSA decrease significantly, but the percentage of ciprofloxacin-sensitive isolates increased from 8.1% to 22.5% within the trial. Johnson et al. (2005) studied the introduction of a number of cultural interventions and an alcohol/chlorhexidine hand solution in a large hospital with a high rate of MRSA colonization. There were significant reductions in hospital-wide rates of total clinical MRSA isolates, patient-episodes of MRSA bacteraemia and clinical isolates of ESBL-producing E. coli and Klebsiella spp, thirty-six months post-intervention. Also there are a number of studies in clinical settings where good hygiene has contributed to reduced antibiotic resistance through reduced prescribing of antibiotics (Gilbert & McBain 2003, SDR p. 43). In conclusion: a) There are studies which show that the introduction of biocidal products has helped in reducing the spread of antibiotic resistant strains in hospital units. b) So while similar mechanisms occur in the development of resistance to both biocides and antibiotics, the current evidence indicates the primary pressure for mutation stems from antibiotic use, not biocide use. The lack of ecological studies means that it is difficult to quantify the impact (if any) of biocides in the settings where they are used.

references

QUESTION 2.a: If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Disagree

If you chose the option ‘disagree’, explain why:
Relevant information missing from the analysis of the situation

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
To rank biocidal substances, more data are needed particularly for biocides other than triclosan and quaternary ammonium compounds (QAC). Triclosan and QAC have become “tools” for investigating potential resistance mechanisms over a wide range of bacteria. Consequently there is more information regarding their molecular action, but this does not mean that they present a greater or lesser risk than other biocides. There are very little data available on biocides other than triclosan and QAC. Consequently, there is no way of predicting the ranking of biocides which present a risk (if any) for increasing antibiotic resistance. We believe SCENIHR places an undue emphasis on triclosan and QAC when they state above that they are “probably instrumental”, and on p.9 that they are “likely to contribute”. In Section 3.8.7 they state: “It is difficult to ascertain how wide spread the development of bacterial resistance to a biocide is in practice mainly due to the paucity of information available”. Those studies which have looked for resistance
in the environment have not found it (Cole et al. 2003 SDR p. 35, Lear et al. 2002 SDR p.46). We also disagree with the above statement because it implies that the presence of mobile genetic elements (MGE) is the primary mechanism by which antibiotic and biocidal resistance is conferred. For triclosan, the majority of studies do not demonstrate a role for MGE. For QAC, MGE have been found which carry the biocide resistance genes. Biofilms are another important mechanism for the failure of bacteria to respond to biocides or antibiotics. There are no studies on the relevance of these findings in the environment. While it may be important to determine the capacity of biocides to trigger the presence of overlapping cascades of regulation that control resistance genes, it is at least equally important to determine if these mechanisms are relevant in the stress-filled environments outside the laboratory. Three international agencies have recently reviewed the issue of triclosan and resistance, and all three found no evidence that the use of triclosan leads to an increase in triclosan- or antibiotic- resistance: • In its October 2006 opinion, the Scientific Committee on Consumer Products (SCCP, 2006) of the European Commission reviewed the data specific to triclosan finding: “Recent scientific papers and European institution reports have expressed concerns about the indiscriminate use of biocides including TCS. These concerns have been based on experimental studies and the theoretical association between increased occurrence of antibiotic cross resistance and the use of biocides...On the basis of the available data, the SCCP is of the opinion that there is presently no evidence of clinical resistance and cross-resistance occurring from the use of triclosan in cosmetic products.” • In 2008, the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) in Australia published their draft Priority Existing Chemical Assessment Report on triclosan, in which they concluded that “[o]n the basis of available data, there is also no evidence that the use of triclosan is leading to an increase in triclosan-resistant bacterial populations or that there is any increased risk to humans regarding antibiotic resistance” (NICNAS, 2008). • In 2008, the US Environmental Protection Agency published their Reregistration Eligibility Decision (RED) document on triclosan, in which they conclude that “[t]here is currently some research attempting to demonstrate a connection between antimicrobial resistance and antibiotic resistance in regard to triclosan, but the linkage has not been expressly proven” (US EPA, 2008). In addition, the SCCP is currently reviewing the status of triclosan as a preservative in cosmetic products. The European Cosmetic Toiletry and Perfumery Association (COLIPA) provided a dossier on antibacterial resistance and triclosan (Colipa, 2007) which we will submit herein including all references.

references

QUESTION 2.b: If yes, which modes of action create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Mostly agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
We agree that there are common resistance mechanisms to both biocides and antibiotics, and that horizontal gene transfer has been shown for increasing antibiotic resistance. It should be noted, however, that while these modes of action may be shared and demonstrated in laboratory studies, such changes do not necessarily occur within the complex microorganism communities commonly found in the environments of the home, the hospital, the farm etc. Studies in drain microcosms (McBain et al. 2004, SDR p. 39),
the oral cavity (Bonta et al. 1992; Fine et al. 1998; Walker et al. 1994; Zambon et al. 1990; Zambon et al. 1995), consumer homes (Cole et al. 2003, SDR p. 35) and biocide factories (Lear et al. 2002, SDR p. 46) have shown that biocide resistance is not generally readily inducible, nor has it been shown to be transferred across bacterial species. Biofilms are common in nature, and while they could contribute to a potential risk for the development of cross-resistance, no such role has been demonstrated to date either in biofilms models (e.g. drains) or in nature (oral plaque).

**references**


**QUESTION 2.c:** If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?

Disagree

If you chose the option ‘disagree’, explain why:

Unsatisfactory conclusion from the scientific point of view

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)

Areas such as hospitals and certain farming operations where antibiotics are routinely used and misused are the most likely situations where antibiotic resistance will be encountered. Use of biocides in these environments might be perceived as areas where higher risks of biocide resistance may be incurred, however, there are no studies to support that view. Areas such as food and cosmetic manufacturing, where antibiotics are not used and where biocides are used, are less likely situations where antibiotic resistance may be encountered as the main selective pressure for antibiotic-resistance (antibiotics) is absent. It should be noted that unlike antibiotics, biocides are delivered in formulations that usually contain other ingredients or properties which impact bacterial cells, such as detergents, chelators and extreme pH. The potential for a biocidal agent to induce a resistance mechanism is most likely irrelevant to a bacterium whose cell wall or membrane has been damaged. The purpose of most biocide-containing formulations is to reduce the bioburden on the treated surface. It is not meant to sterilize, or to impact the resident commensal population in such a way as to allow the establishment of a pathogen population. Rather it acts as a means of population control, thereby reducing the risk of transfer or infection. Commercial biocides have been used for over 100 years in homes, hospitals, and manufacturing of all types including foods. Yet, all studies to date, looking in areas where there has been continued use have not found biocidal-resistant pathogens (Cole 2003, SDR p. 35; Lear et al. 2002, SDR p. 46). So while certain niches may occur where sub-lethal concentrations may increase the risk of selecting resistant bacteria, this is not the norm as evidenced by the data in hand. While the use of biocides may provide a theoretical risk of promoting antibiotic resistance, the evidence to date shows that they play an important role in public health (Bleasdale et al. 2007, Maillard, 2005) and provide an actual benefit in controlling antibiotic resistance outbreaks (Marshall et al. 1997, Johnson et al. 2005)
references

QUESTION 3: If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?

Do you agree with the response given?
Mostly agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
We agree that quantitative data on exposure and standard protocols are not available. We also agree that determining the effect of all of the individual factors that impact the efficacy and potency of a biocide or an antibiotic would be a gigantic effort. Consequently we feel it is very important to develop protocols where the impacts of biocides on the ecology of specific settings are studied. Only in a more natural environment can the true impact of biocides be accurately assessed and prediction models developed. The development of a prediction protocol necessitates some ‘real world’ demonstration of the contribution of biocides to increasing antibiotic resistance. To date, that evidence has not been developed. In the studies where this type of data has been sought (farms, clinics, consumer households), the link between biocidal resistance and antibiotic resistance has not been found. In fact, biocide resistance has not been found. Examples cited in the SDR include: • Section 3.4.4.1 “Emerging bacterial resistance to biocides has been well described in vitro; but evidence in practice is lacking.” • Section 3.4.4.2 “Cole et al. 2003...showed a lack of cross-resistance to antibiotics and antibacterial agents in target bacteria” taken from the homes of users of antimicrobial products. • Section 3.4.4.6 “However, to date, no study seems to have focused on the emergence of biocide resistant bacteria in hospital environments”... Development of prediction models using controlled natural settings would be the most appropriate and practical ways of evaluating biocides. Any research conducted on biocides and their potential impact on antibiotic resistance should be cross referenced and benchmarked against the impact of over-usage of antibiotics.

QUESTION 4: How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?

Do you agree with the response given?
Mostly disagree

If you chose the option ‘mostly disagree’ explain why:
Relevant information missing from the analysis of the situation

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
We agree that standard methodologies and terminology would be useful. However, the draft SCENIHR opinion does not address the need for prospective studies in settings where antibiotic resistance would be most likely to develop. While elegant laboratory studies will help in understanding potential mechanisms of resistance, microcosm and field studies are needed to demonstrate the actual presence or absence of genetically stable biocidal resistance mechanisms which confer cross-resistance to antibiotics of medical importance. These types of experiments are difficult to conduct, but not impossible. We believe that they should have a higher priority than additional laboratory studies. The development of protocols with repeated biocide exposures at sub-lethal (including residual) concentrations have some inherent problems, in that the repeated passage of isolates causes them to lose non-essential traits. For instance triclosan-insensitive E. coli strains were selected during routine and repeated passage against triclosan in monoculture, identifying ACP as a major target of this biocide (McMurray 1998a, SDR p. 32); such stable reductions in triclosan susceptibility have been repeated by other groups using E. coli (McBain et al. 2004, SDR p. 46; Braoudaki & Hilton 2004, SDR p. 44). In contrast, replication of these selection/training protocols, using over 40 fresh isolates from the mouth, skin and domestic drain, together with representative laboratory strains of typical oral flora showed that only the enteric species E. coli and Klebsiella oxytoca undergo selectable decreases in triclosan susceptibility (McBain et al. 2004, SDR p. 46; Ledder et al. 2006, SDR p. 46). Their susceptibilities to antibiotics were not significantly decreased. None of the remaining test isolates, including other enteric species such as salmonellae, were affected in terms of their susceptibility to triclosan or to any antibiotic representatives. This shows that the ability to select for triclosan resistance is not universal and might even be restricted to E. coli (Gilbert et al. 2007). So while this might be one standardized protocol to develop, the inherent problems of bacterial selection, maintenance, and identification must be addressed. The definition above of “minimal selective concentration” needs to be expanded to explain “a defined bacterium.” Species of bacteria can exhibit a wide range of responses to antibiotics– this was the origin of the concept of the MIC 50 or MIC 90 (Minimum Inhibitory Concentration), where the antibiotic is tested against a large number of isolates of a species and the MIC where 50 and 90 percent of the strains are inhibited is reported. So to define a bacterium as being of a specific genus and species, does not necessarily mean that that strain is representative of the majority of strains in that species. Culture collection type strains could be considered, but they are occasionally atypical of the range of strains within a species, and have the problem of extensive passage. We also feel that a baseline needs to be developed. As stated in the draft SCENIHR report, bacteria in the environment tend to be more robust than those in laboratories. Surveys using fresh isolates should be conducted to develop more robust data for comparison purposes.

references

QUESTION 5: Please identify relevant gaps in scientific knowledge and suggest major research needs.

Do you agree with the response given?
Mostly agree
Please provide the scientific/technical evidence to improve the overall assessment (with complete references)

Epidemiological data indicating public health risk due to biocide resistance is lacking, as is evidence relating biocide resistance with antibiotic resistance outside the laboratory. The current study base is dominated by in vitro data. The current knowledge about resistance to biocides and antibiotics is reminiscent of the discovery phase of drug development. During this phase, potential drugs are scrutinized in laboratory tests to determine their ability to affect target genes or mechanism. Following this comes a phase where the best candidates are tested in animal models, and finally, successful candidates are tested in humans. Currently there are a number of mechanisms of resistance to biocides that have been identified in laboratory tests. The question now is: are these mechanisms relevant in more complex ecosystems than those found in the laboratory, i.e. in model systems such as McBain drain studies (2003, SDR p. 39) or in the actual clinical, veterinary or consumer sites where these products are used? A few of these studies have been already conducted on triclosan (Cole et al. 2003, Lear et al. 2002, McBain et al. 2004, SDR p. 35, 46, & 47). They have not shown any significant incidence of biocide resistance in areas where there has been repeated application of biocides, i.e. isolates from the homes of users of biocides, the factories where biocides are made, or model drains where there is a consistent dosing of biocide. Gilbert et al. (2007) reviewed the considerable number of microcosm studies (domestic drains and in dental plaque) performed to evaluate triclosan-containing products, as well as environmental surveillance studies to monitor effect on oral flora. The theoretical risks are not supported by either field or clinical studies, or by laboratory studies using bacterial microcosms. More of these studies need to be conducted for a wider range of biocidal products. We agree that standardized methodologies and surveillance programs are needed. In addition, any research program should be inclusive of all types of biocides and should not be limited to one particular biocide or type of biocide.

references
Submission: 9

Name
Kersti Gustafsson, Swedish Chemicals Agency, Sweden (Organisation)

QUESTION 1.a: Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?

Do you agree with the response given?
Agree

QUESTION 1.b: If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?

Do you agree with the response given?
Agree

QUESTION 2.a: If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 2.b: If yes, which modes of action create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 2.c: If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 3: If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?

Do you agree with the response given?
Agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
One way to find quantitative data is to use statistics. In Sweden there is a demand for statistics on pesticides. There seems to be a coming demand within the EU as well. Statistics should be valuable when discussing quantities. For pesticides there is a publication on sold quantities in Sweden per year. The publication is only in Swedish but with and English summary. There are compilations for sold quantities of active substances per year. The publication will be sent. On the KemI–website there are different statistical tools. www.kemi.se. Under statistics in brief it is possible to search on
Disinfectants 2004. Under KemI-stat search tool it is possible to search further. The statistics in brief and the search-tool can only be used via the web-site.

**references**

**QUESTION 4: How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?**

Do you agree with the response given?
Agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
There exists some standardized efficacy protocols for biocidal product types 1-5. A compilation has been performed which will be sent.

**references**

**QUESTION 5: Please identify relevant gaps in scientific knowledge and suggest major research needs.**

Do you agree with the response given?
Agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
For biocidal products in general there exists emission scenario documents (ESD) for respective product type. Information on minimal selective concentration as a measure of development of resistance could be added in the scenarios. The European Union System for the Evaluation of Substances (EUSES) is a decision-support instrument which enables government authorities, research institutes and chemical companies to carry out rapid and efficient assessments of the general risks posed by chemical substances. With some further development it might be possible to model the risk for emerging resistance via EUSES. Both the ESDs and the EUSES model can be found via the website of The European Commission, Institute for Health and Consumer Protection, The Consumer Products Safety & Quality (CPS&Q) Unit http://ecb.jrc.ec.europa.eu/biocides/. The documents and the model are not easily attached to this response.
Submission: 10

Name
Hazel Uppington, Health Protection Agency Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infections, UK (Organisation)

QUESTION 1.a: Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?

Do you agree with the response given?
Agree

QUESTION 1.b: If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?

Do you agree with the response given?
Agree

QUESTION 2.a: If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 2.b: If yes, which modes of action create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 2.c: If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 3: If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 4: How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?

Do you agree with the response given?
Agree
QUESTION 5: Please identify relevant gaps in scientific knowledge and suggest major research needs.

Do you agree with the response given?
Agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
The committee considers that the concerns raised in this report are certainly worthy of further study. Future studies should focus on the in vivo / in situ potential for biocide use to select for antibiotic resistance in human pathogens. Clear distinctions will be needed to determine which biocide categories are implicated in this phenomenon, otherwise there is a danger that a headline message that some biocides could increase the risk of selecting for human pathogens that are resistant to antibiotics will be translated into all biocides do this. The latter interpretation could be damaging as appropriate use of biocides may then be compromised.
QUESTION 1.a: Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?

Do you agree with the response given?
Mostly agree

QUESTION 1.b: If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?

Do you agree with the response given?
Mostly agree

QUESTION 2.a: If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Mostly agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
It would be helpful to mention those biocides which have so far not been described to induce bacterial resistance such as ethanol, iso-propanol and n-propanol (1). The commonly used concentrations of all three alcohols (60% - 95%) and their volatility do usually not allow the development of acquired bacterial resistance.

references

QUESTION 2.b: If yes, which modes of action create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Mostly agree

QUESTION 2.c: If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?

Do you agree with the response given?
Mostly agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
The susceptibility of antibiotic-resistant bacteria to disinfectants is described. For alcohols
various studies were performed but are unfortunately not mentioned. Alcohols were described to be very effective against VRE and VSE (1), MRSA and MSSA (2, 3) and various other MDR bacteria both against ATCC strains and clinical isolates (4, 5). This piece of information may well be included for a broader evaluation. The volatility of a biocide (examples: ethanol, iso-propanol, n-propanol) is also of general relevance because it limits the exposure time automatically. I suggest to add this aspect, may be also in chapter 3.10.1.6 on page 57 and may be also in the paragraph on page 64.

references

QUESTION 3: If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?

Do you agree with the response given?
Uncertain

QUESTION 4: How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?

Do you agree with the response given?
Uncertain

QUESTION 5: Please identify relevant gaps in scientific knowledge and suggest major research needs.

Do you agree with the response given?
Mostly agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
The role of biofilms for a reduced efficacy of disinfectants for instruments and flexible endoscopes is described. Proper cleaning before disinfection is essential to ensure maximum efficacy of biocides (1). I suggest to add that during the cleaning process any biocide with the potential for surface fixation such as aldehydes and peracetic acid should in general be avoided (2) as indicated as a general statement in the last paragraph of the chapter on page 50. Surface fixation of organic material and biofilm formation are quite closely linked for both aldehydes and peracetic acid (3).

references
Submission: 12

Name
Marco Oggioni, University of Siena, Italy (Organisation)

QUESTION 1.a: Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?

Do you agree with the response given?
Disagree

If you chose the option 'disagree', explain why:
Unsatisfactory conclusion from the scientific point of view; Relevant information missing from the analysis of the situation

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
Despite the general concern on possible influence of biocide use on increase in antibiotic resistance the data sustaining such an alarming claim are exceptionally poor. So far environmental surveys have not demonstrated an association between biocide usage and antibiotic resistance (Cole et al. 2003, Aiello et al. 2005). We consider it therefore irresponsible to state that there is scientific evidence (bacteriological, biochemical and genetic data) for impact of biocidal products on antibiotic resistance. In order to state this, what is absolutely missing, is a correlation based on statistically sound epidemiologic data that answers the two main outstanding questions which are (i) do biocides select for increase in antibiotic resistance in microorganisms (ii) is antibiotic resistance due to biocides is of clinical relevance. The FP7 EC project BIOHYPO was especially designed to provide by high throughput phenotypic and molecular screening of bacteria and fungi a robust dataset of sufficient size for statistical analysis of the above hypothesis. Only such data would permit to state YES as answer to question 1a. We would suggest that the answer to question 1a should be reworded underlining the fact that there are indications of a possible correlation between biocide use and antibiotic resistance, but that scientific evidence for this correlation is so far lacking. This comment is provided on behalf of the BIOHYPO consortium (FP7 research project 227258 (under negotiation): Confronting the clinical relevance of biocide induced antibiotic resistance; Coordinator MR Oggioni; Scientific Director G Orefici, partners I Morrissey, L Baldassarri, J Almeida, U Yetis, HJ Rodger, P Visa, JL Martinez, A Kalkanci, D Mora, S Leib, and C Viti).

References
References Cole et al. 2003 and Aiello et al. 2005 and already cited by the SCENIHR report FP7 FOOD-2009-227258, BIOHYPO project (under negotiation), Confronting the clinical relevance of biocide induced antibiotic resistance.

QUESTION 1.b : If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?

Do you agree with the response given?
Disagree

If you chose the option ‘disagree’, explain why:
Unsatisfactory conclusion from the scientific point of view

**Please provide the scientific/technical evidence to improve the overall assessment (with complete references)**

We agree that at the state of art it is impossible to enucleate to contribution of biocide use on antibiotic resistance development in an environment in which both antibiotics and biocides are used, but this does not mean that it is impossible. If any biocide would select for any given resistance determinant, either biocide resistance or antibiotic resistance, it is expected that this should become obvious from high throughput epidemiological strain phenotyping and genotyping. Most importantly if this should not become obvious after extensive screening this would indicate that there isn’t such an event. This comment is provided on behalf of the BIOHYPO consortium (FP7 research project 227258 (under negotiation): Confronting the clinical relevance of biocide induced antibiotic resistance; Coordinator MR Oggioni; Scientific Director G Orefici, partners I Morrissey, L Baldassarri, J Almeida, U Yetis, HJ Rodger, P Visa, JL Martinez, A Kalkanci, D Mora, S Leib, and C Viti).

**QUESTION 2.a:** If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

**Do you agree with the response given?**

Mostly agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)

No mention is done throughout the report on resistance in fungi. In addition the focus of the whole report is very much oriented towards medical, veterinary or alimentary use of biocides, while this This comment is provided on behalf of the BIOHYPO consortium (FP7 research project 227258 (under negotiation): Confronting the clinical relevance of biocide induced antibiotic resistance; Coordinator MR Oggioni; Scientific Director G Orefici, partners I Morrissey, L Baldassarri, J Almeida, U Yetis, HJ Rodger, P Visa, JL Martinez, A Kalkanci, D Mora, S Leib, and C Viti).

**references**

Neale M., Pesticide Outlook, 2003, 14, 71 - 73

**QUESTION 2.b:** If yes, which modes of action create the highest risks for increasing antibiotic resistance?

**Do you agree with the response given?**

Agree

**QUESTION 2.c:** If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?

**Do you agree with the response given?**

Mostly disagree

If you chose the option ‘mostly disagree’ explain why:

Relevant information missing from the analysis of the situation

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
Throughout the document uses other than medical/veterinary/alimentary use of biocides is underreported (Neale Pest Outlook 2003). Also these situations, as for example wood preservatives and paintings, may present significant risk factors, especially for fungi, which also were neglected in the report. This comment is provided on behalf of the BIOHYPO consortium (FP7 research project 227258 (under negotiation): Confronting the clinical relevance of biocide induced antibiotic resistance; Coordinator MR Oggioni; Scientific Director G Orefici, partners I Morrissey, L Baldassarri, J Almeida, U Yetis, HJ Rodger, P Visa, JL Martinez, A Kalkanci, D Mora, S Leib, and C Viti).

references
Neale M., Pesticide Outlook, 2003, 14, 71 - 73

QUESTION 3: If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?

Do you agree with the response given?
Agree

QUESTION 4: How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?

Do you agree with the response given?
Mostly disagree

If you chose the option ‘mostly disagree’ explain why:
Relevant information missing from the analysis of the situation

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
There are currently no accepted standards protocols for the evaluation of reduced susceptibility to biocides induced or selected by biocides. This fact is very important since it is a prerequisite for any test evaluating relative risks of biocide induced or selected antibiotic resistance. This comment is provided on behalf of the BIOHYPO consortium (FP7 research project 227258 (under negotiation): Confronting the clinical relevance of biocide induced antibiotic resistance; Coordinator MR Oggioni; Scientific Director G Orefici, partners I Morrissey, L Baldassarri, J Almeida, U Yetis, HJ Rodger, P Visa, JL Martinez, A Kalkanci, D Mora, S Leib, and C Viti).

QUESTION 5: Please identify relevant gaps in scientific knowledge and suggest major research needs.

Do you agree with the response given?
Mostly disagree

If you chose the option ‘mostly disagree’ explain why:
Unsatisfactory conclusion from the scientific point of view; Relevant information missing from the analysis of the situation

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
All points raised are appropriate but are lacking, in the opinion of the BIOHYPO research
group, the two central questions that have to be addressed are missing and should be added. To evaluate if biocides select for increase in antibiotic resistance in microorganisms high throughput phenotypic screening on extensive strain collections and molecular screening for associated markers is needed to demonstrate correlation of biocide resistance and antibiotic resistance. This analysis should help to evaluate specific interventions. To evaluate if antibiotic resistance due to biocides is of clinical relevance data of biocide induced antibiotic resistance have to be modelled against parameters for evaluation of disease burden including duration and severity of illness, clinical outcome and deaths. This analysis should permit to evaluate costs of interventions. In addition to the above two point SCENIHR might also consider the need for careful evaluation of products with low –cidal activity which could be more prone to induce resistance. In this context also the indication given by the directive to use the lowest efficacious concentration of a given biocide should be critically evaluated with respect to risk of resistance generation. This comment is provided on behalf of the BIOHYPO consortium (FP7 research project 227258 (under negotiation): Confronting the clinical relevance of biocide induced antibiotic resistance; Coordinator MR Oggioni; Scientific Director G Orefici, partners I Morrissey, L Baldassarri, J Almeida, U Yetis, HJ Rodger, P Visa, JL Martinez, A Kalkanci, D Mora, S Leib, and C Viti).

references
FP7 FOOD-2009-227258, BIOHYPO project (under negotiation), Confronting the clinical relevance of biocide induced antibiotic resistance.
QUESTION 1.a: Does current scientific evidence indicate that the use of certain active substances in biocidal products in various settings as mentioned above can contribute to the occurrence of antibiotic resistant bacteria, both in humans and in the environment?

Do you agree with the response given?
Agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)

Few comments concerning this preliminary report: 1) Concerning the hasard/risk in relation with the dissemination and distribution of the genes: I think it could be important to cite other papers to confirm the importance of this phenomena and the necessity to develop investigation in this way: Norihisa Nogushi and al, 2005, 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. of Med. Micr., 54, 557-565. The results indicated that qacA/B is functionally the most important while the geographical distribution of smr is more limited. Bjorland and al, 2005, Widespread distribution of disinfectant resistance genes among Staphylococci of bovine and caprine origin in Norway, J. Clin Microb., 43, 9, 4363-4368. In conclusion, it seems that the widespread distribution of staphylococci carrying QAC resistance genes in Norwegian dairy cattle and goat herds is the result of both the intra- and interspecies spread of QAC resistant plasmids and the clonal spread of QAC-resistant strains. These conclusions are important because we don't have many experimentations in this field.

2) Comments concerning the table 7, p.30: I don't totally agree with the information concerning the MICs and "no" in the first column. In the paper of Randall (2007) 8 Salmonella typhimurium, including filds isolates and laboratory mutants, are exposed to phenol, oxidising, aldehyde, quaternary ammonium disinfectants. The strains with acrB,tolC inactivated were more susceptible to most disinfectants. 3 of the mutants recovered after disinfection required longer exposure time to generate 5 log kill. So there is a close relationship between the inactivation kinetics and the MICs. 3)Resistance to biocides used in animal husbandry (p. 3) In this field it is noted that the data relating the occurence of resistance to the use of disinfectants are limited. In my Laboratory we published two papers in the early 90s: Maris P., 1991, Resistance of 700 Gram negative bacterial strains to antiseptics and antibiotics, Ann. Rech. Vet., 22, 11-23. The sensitivity of this strains were tested towards 4 antiseptics (cetrimide, chlorhexidine, hexachlorophene, mercuric chloride) and 6 antibiotics (ampicillin, streptomycin, erythromycin, chloramphenicol, kanamycin and tetracycline). The statistical analysis of correlation showed high positive resistance links between antiseptics and between antiseptis and antibiotics specially for Serratia marcescens and Alcaligenes. Martin H. and Maris P., 1995, Resistance of 310 Gram-positive strains isolated from milking cow udders to antiseptics and antibiotics after use of post-milking teat germicides, Vet. Res., 26, 43-56. This analysis revealed positive links between chlorhexidine and 5 antibiotics (ampicillin, kanamycin, streptomycin, tetracycline, gentamycine for Streptococcus, and between hexachlorophene and oxacillin for Bacillus . Globally I totally agree with this document and the need to develop research in the main
areas (food industry, animal husbandry, hospital, consumer products) and the development of surveillance programmes.

**QUESTION 1.b**: If so, how does this effect compare to resistance due to application of medicinal products or veterinary medicinal products and other relevant applications?

*Do you agree with the response given?*

Agree

*Please provide the scientific/technical evidence to improve the overall assessment (with complete references)*

To discriminate the effect of biocides and antibiotics would be easier in the field of food industry because there is no use of antibiotics and the biocides are daily used.

**QUESTION 2.a**: If yes, which types of active substances create the highest risks for increasing antibiotic resistance?

*Do you agree with the response given?*

Agree

*Please provide the scientific/technical evidence to improve the overall assessment (with complete references)*

Yes for the main chosen active substances. But two remarks: -very often the quaternary ammonium are in association with other active substances, mainly in food industry and in veterinary area. -it is important to take into account the evolution of the european regulation: n°1451/2007 (4th december 2007) and the recent european decision (2008/809/CE - 14th october 2008) with the suppression of numerous active substances. The impact of these suppression have to be considered in the futur.

**QUESTION 2.b**: If yes, which modes of action create the highest risks for increasing antibiotic resistance?

*Do you agree with the response given?*

Agree

**QUESTION 2.c**: If yes, which types of areas of application create the highest risks for increasing antibiotic resistance?

*Do you agree with the response given?*

Agree

*Please provide the scientific/technical evidence to improve the overall assessment (with complete references)*

food industry and hospital: mainly in the aera where these biocides are daily used.

**QUESTION 3**: If yes, what is the extent of the resulting antibiotic resistance and the relative contribution of the different applications to the risk of increasing antibiotic resistance?

*Do you agree with the response given?*

Agree
Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
In my opinion we need first a close relationship with the representatives of the chemical industry, the hygiene product manufacturer to better know the diversiy of the application.

QUESTION 4: How can the development of antibiotic resistance due to the use of active substances in biocidal products be examined? Could the Committee advise on the methodologies?

Do you agree with the response given?
Agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
OK for this classical kind of test. But two comments: -it is necessary to adapt these repeated aplications with the field use of these products which could be different from an aera to another. -it is also necessary to study the stability of the emergence of this resistance.

QUESTION 5: Please identify relevant gaps in scientific knowledge and suggest major research needs.

Do you agree with the response given?
Agree

Please provide the scientific/technical evidence to improve the overall assessment (with complete references)
It would be necessary to develop experiments in the field of food industry with a surveillance programme. Research to study the impact of association of the main molecules, because very often the biocide products are associations of two to four molecules belonging to different chemical families.