



Effects of Biocides on antibiotic resistance

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Level 2 - Details on Effects of Biocides

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The answers to these questions are a faithful summary of the scientific opinion produced in 2009 by the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR):

"Assessment of the Antibiotic Resistance Effects of Biocides (2009)"

The full publication is available at: <http://copublications.greenfacts.org/en/biocides-antibiotic-resistance/>
and at: <http://ec.europa.eu/health/opinions/en/biocides-antibiotic-resistance/>

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1. What are biocides and how widely are they used?

1.1 How are biocides defined?

Bacteria can be killed or inhibited by different antimicrobial products, namely **antibiotics** that act against infections in humans or animals and **biocides** such as disinfectants, antiseptics and preservatives.

According to the Biocides Directive (98/8/EC), biocidal products are intended to destroy, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means. The 23 product types covered by the directive range from drinking water disinfectants, through wood preservatives and insecticides, to antifouling products (see full table [[see Annex 6, p. 24](#)]).

Table: 23 Biocidal products listed in Annex V of the Biocides Directive (98/8/EC) [[see Annex 6, p. 24](#)]

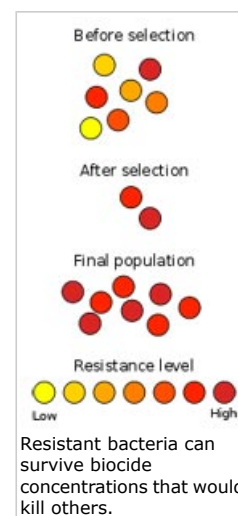
Only biocidal products that act against bacteria are the focus of this assessment and not biocides used to control other micro-organisms such as fungi, protozoans, plants or other animals.

1.2 When are bacteria considered resistant?

Although antimicrobial products are used in concentrations that are usually sufficient to inhibit or kill the bacteria treated, some strains of bacteria are able to survive and even grow at these concentrations; they are said to be "**resistant**".

Bacteria are considered resistant to antibiotics or biocides in any of the following situations:

- when a strain is not killed or inhibited by the antimicrobial concentration typically used in practice,
- when a strain is not killed or inhibited by a concentration at which the majority of strains of that micro-organism are affected
- when bacterial cells are not killed or inhibited by a concentration acting upon the majority of cells in that culture.



In some cases, resistance mechanisms against biocides can contribute to resistance to antibiotics.

Bacteria are called "**insusceptible**" when they have natural (innate) properties, such as a specific envelope structure, that impairs biocide penetration. Bacteria develop "**tolerance**" if they become less affected by a biocide concentration that is active on susceptible strains, so that higher concentrations of the biocide are needed to stop them multiplying.

Bacteria can transfer diverse bits of genetic material (plasmids, transposons, etc.) to other bacteria containing several associated genes. When genetic information coding for different antimicrobial resistance mechanisms is transferred to a new host it is referred to as "**co-resistance**".

“**Cross-resistant**” bacteria are those that have developed survival methods that are effective against different types of antimicrobial molecules having the same mechanism(s) of action.

The term **Multi-Drug Resistance** (MDR) is used when a bacterial strain is resistant to several different antimicrobial classes.

1.3 How do biocides act?

There are many biocidal substances in the market that act in different ways and sometimes several biocides are combined in a product to increase the overall effectiveness. Ideally, the combined action of all the biocides in a product should be greater than the sum of the individual actions (synergy). Biocidal products contain many different molecules and they can all affect how well the product works.

Moreover, some of the components that are added to many household products for a variety of purposes – such as surfactants or membrane permeabilisers - may increase the efficacy of biocides in killing bacteria.

This assessment focuses on the most commonly used biocides for which information on bacterial resistance is available.

Table 2: List of active substances in biocidal products and their mode of action
[see Annex 2, p. 21]

1.4 How widely are biocides used in Europe?

The use of antibiotics in human and animal health care is monitored regularly but the same is not true for biocide use.

Although most biocides are used in large quantities and the volumes produced are many orders of magnitude higher than those of antibiotics, there is no reliable information on the total amounts used in Europe.

The estimated EU market value of biocidal products was €10-11 billion in 2006, and market expansion is expected to continue.

In Europe, biocidal products need to be approved before they are released on the market. Their active ingredients must be safe for humans, animals and the environment. However, even if the products themselves are safe, the fact that they are used in huge volumes could have safety implications. If biocides kill all the bacteria that are reasonably easy to eradicate, the only bacteria left are resistant strains and these are free to grow with no competition from other bacterial populations. It is conceivable that the huge amount of biocides released into the environment alone may already pose a biological threat by applying a selective pressure on bacterial populations, leading to the selection and dissemination of resistant bacteria.

2. What are the main uses of biocides?

2.1 What are the main applications for biocides in health care?

The proper use of biocides is crucial in preventing and controlling the spread of infectious diseases in hospitals and other health facilities. Biocides are used to decontaminate the skin of patients and health professionals, any surfaces that could harbour bacteria, and any instruments in contact with patients. Biocides are also used as antiseptics to treat infections in mucous membranes and damaged skin.

Disinfectants are classified as low, medium or high-level disinfectants, depending on how many types of micro-organisms they kill. High-level disinfectants that are applied for long periods of time can inactivate all micro-organisms and are called chemical sterilants.

Table 3: Biocides approved by US-FDA for health care settings, or registered by the US-EPA [see Annex 3, p. 22]

The level of disinfection in medical facilities usually depends on the degree of infection risk involved:

1. Surgical instruments, needles, catheters and any other devices that enter the patient's tissues must be sterile. The best way to achieve this is to use steam under pressure but instruments that cannot be heated need to be treated with chemical sterilants instead.
2. The risk of infection from devices that come into contact with mucous membranes or damaged skin, such as endoscopes and tubes used in anaesthesia, is not as high but these should still be sterilized to provide the widest margin of safety.
3. Stethoscopes, bedpans, blood-pressure cuffs and similar devices pose little risk of transmitting infections and can be treated with low-level disinfectants. Biocides are used to disinfect these as well as surfaces that are near patients such as floors, walls, tables, bedrails and screens.

There is evidence that surfaces can act as a source of contamination and may contribute to the spread of infections such as MRSA and *Clostridium difficile*. However, the routine use of biocides to treat these surfaces is controversial. Antimicrobial wipes are increasingly common in hospitals but their inappropriate use, for instance cleaning several surfaces with the same wipe, can cause problems. There are new products such as shower curtains and trolleys that incorporate biocides in their surfaces. In some health facilities they have reintroduced the use of metals for surfaces that are touched frequently hoping to reduce the spread of infections, but it is difficult to evaluate precisely if these have had any effects.

Biocides are also used to kill or reduce the numbers of harmful micro-organisms on the skin of patients and medical staff. The most common method of disinfecting the hands of medical staff is the use of alcohol-based hand-rubs because they are easy to use and effective. In addition to alcohols, common disinfectants and antiseptics include quaternary ammonium compounds and triclosan, and some preparations combine several substances.

Table 4: Commonly used skin disinfectants and antiseptics [see Annex 4, p. 22]

2.2 In which consumer products are biocides used?

Many consumer products contain biocides but the major sources of exposure in homes are the regular use of cosmetics and wipes, cleaning products, some toothpastes, laundry detergents, pet disinfectants and general disinfectants.



Biocides are for instance included in cleaning products.
Credit: Sanja Gjenero

Biocides are added to cosmetics and personal care products to prevent micro-organisms from growing on them. In the EU, the use of 57 different chemicals is allowed for this purpose. Besides these chemicals, cosmetics often contain other non-regulated antimicrobials.

Many of the substances that are added to household products to improve their properties also kill bacteria. This is for instance the case of surfactants that are included in detergents to decrease the surface tension of water enabling the detergent to better penetrate and loosen dirt. Cleaning products and laundry detergents contain preservatives and disinfectants but the use of these substances in household products is not regulated. Surfaces coated with biocides have been developed recently. These biocide-treated surfaces include several active ingredients such as triclosan and metallic ions.

The biocide triclosan is used in consumer products and textiles, notably in cosmetics, toothpastes and products for dental hygiene, and in deodorants, but also in cleaning products, paints, plastic products and in clothes to avoid unpleasant odours produced by decomposition of sweat.

2.3 How are biocides used in the food industry?

Biocides are widely used in the food industry as disinfectants and food preservatives.

They treat production plants, processing areas and food containers to control the microbial growth in food and drinks. They are also commonly used to disinfect equipment, containers, surfaces or pipes associated with the production, transport and storage of food or drink, including drinking water. In the EU, the use of disinfectants in the food-processing industry and in the treatment of drinking water is regulated.

Drinking water is treated with biocides to eliminate any harmful micro-organisms at the water works and in the distribution system to ensure that the water that reaches the consumer is fit to drink. For the last century, chlorine has been added to the water before it enters the waterworks for treatment. Ozone and chlorine-dioxide are now more commonly used for that purpose to avoid the creation of unwanted by-products. In some countries, disinfection in the distribution system is always performed with chlorine or chloramines.

Biocides are added as preservatives to foodstuffs to prolong their shelf-life by protecting them against deterioration caused by micro-organisms. They are considered as food additives and their use in the EU is regulated.

2.4 How are biocides used in animal husbandry and in products of animal origin?

Proper cleaning and disinfection play a vital role in protecting food animals from diseases that they could pass on to humans. Although the use of biocides in breeding and raising livestock is regulated in each Member State, there are no exact data on the amounts of biocides used. It appears that each farm only uses few types of disinfectants and the same brand may be used for extended periods of time.



Biocides are used when breeding and raising livestock. Credit: Mark Foreman

Biocides have four main uses in animal husbandry:

- Cleaning and disinfecting farm buildings, particularly between batches of animals as well as decontaminating ponds and equipment in fish farming.
- Creating barriers against bacteria, such as foot dips outside animal houses, and disinfecting vehicles and materials during outbreaks of infectious diseases.
- Preventing infections through direct application to animal skin, for instance to clean the udders of animals used for milk production
- Preserving specific products such as eggs or semen.

Table 5: Major biocides used in veterinary medicine and animal husbandry
[see Annex 5, p. 23]

Moreover, biocides are used to protect animal feed from deterioration by micro-organisms. They are considered as food additives and they are not allowed without a safety assessment.

Chemicals used in animal production could leave residues in milk, meat or eggs. Therefore, before antimicrobials are allowed, they are tested to see if they are safe. This includes an assessment of the possible effects of these residues on the bacteria that naturally live in the human gut.

Biocides can be used to kill bacteria on the surface of animal products such as poultry and other carcasses. This practice is not authorized in the EU so far.

2.5 How are biocides used in water treatment and industrial applications?

Biocides are used in industry and in the treatment of drinking water and wastewater, but the quantities involved are not known.

Many wastewater treatment plants, especially those near the sea, include a final step of disinfection with chlorine. However, this practice is being increasingly questioned because the by-products are toxic to sea animals and because it can lead to false-negative tests, where water samples appear clean but in fact contain viruses and other micro-organisms that survive chlorine and may cause outbreaks that can affect swimmers or consumers of sea-food.

Disinfectants are intensively used in cooling towers since some harmful bacteria such as *Legionella* might otherwise thrive in the warm water and be spread through air by tiny water droplets (aerosols) released by the cooling tower. After use, these biocides may reach the environment either as aerosols or in the wastewater.

Biocides are increasingly added to building materials, antimicrobial surfaces and other products, to stop them becoming encrusted with moulds or other micro-organisms; but the quantities used are unknown. Some of these surfaces release small amounts of biocide

progressively into the environment and this could kill certain types of bacteria in the immediate vicinity, leaving only resistant-bacteria. Therefore, biocide aerosols could have a role in emerging resistance of bacteria to biocides or antibiotics, but this point has not yet been investigated.

3. Is there evidence that bacteria resistant to biocides are emerging?

3.1 How can bacterial resistance to biocides be determined?

Whether or not a biocide is effective depends to a large extent on the concentration of the active molecule in the product.

To measure resistance, bacteria are exposed to a biocide for a set period of time. Standard strains of bacteria are killed or stop growing while resistant strains are unaffected.

In many reports, bacterial resistance to biocides is determined by the minimum concentration of a biocide needed to stop the bacteria from growing (the minimum inhibitory concentration, or MIC). However, a better measure of resistance is the minimum bactericidal concentration (MBC) that would kill the micro-organisms after a certain period of exposure. Monitoring changes in the MIC is still useful to detect strains of bacteria that are beginning to develop tolerance to a biocide.

Bacterial resistance was already reported in the 1950s and, in many of the early cases, resistance developed because the biocides were used or stored incorrectly, so that the concentration of the biocide in the product was too low to be effective. Since then, the number of reports of resistance to biocides and to all known preservatives has increased.

In health care facilities, bacteria resistant to biocides have long been found. Some biocides currently used in hospitals were found to be ineffective against bacteria that grow as biofilms attached to surfaces, and this may have an important role in the transmission of hospital-acquired infections.

3.2 Has resistance to biocides been observed in health care applications?

Bacteria resistant to the biocides present in medical products have long been observed.

Silver has antibacterial properties and has traditionally been added to compresses applied to burn wounds to prevent infection. However, in the 1960s there were reports of bacteria resistant to silver. Over the years, different silver compounds were developed to overcome this problem, but bacteria developed resistance to those too. Today, resistant bacteria are reported for almost all biocides.

When a micro-organism becomes resistant to an **antibiotic**, the antibiotic can no longer be used to tackle it, and other antibiotics that are more expensive and might have more side effects need to be used.

However, unlike antibiotic resistance, the issues relating to biocide resistance are considered to have a very low profile and priority. Despite the widespread use of disinfectants and antiseptics in health care settings, emerging bacterial resistance has only been studied in the laboratory but not yet in practice.

Concentrations of biocide that are used in clinics and hospitals are so high that it is expected that they even kill bacteria that are less easily affected.

There is evidence of bacteria resistant to both biocides and antibiotics occurring in hospitals, and this resistance can be transferred to other bacterial strains. Therefore, further research is needed to see if the long-term use of biocides in hospitals has an effect on the emergence of resistance against antimicrobials including antibiotics and biocides.

3.3 Has resistance to biocides been observed in consumer products?

Biocides are added to cosmetics to prevent micro-organisms from growing on them and spoiling the product. Because only a few biocides are used extensively in many different products, bacteria are becoming resistant to them. For instance, many home and personal care products contain triclosan and the widespread use of this biocide may be associated with bacteria becoming resistant to it.

Resistant bacteria have been found in industrial plants where cosmetics are manufactured and in the cosmetic products themselves. Studies have focused on how these bacteria spoil the cosmetics and not on whether or not they can cause disease.

As a result to this resistance to specific preservatives, cosmetic products now contain a mixture of biocides to preserve them better, but this means that the consumer is exposed to larger amounts and more types of biocides.

There is accumulating evidence that biocide resistant bacteria can be found in consumer products, but to date there are no studies to indicate that they are linked to antibiotic resistance or the emergence of harmful micro-organisms.

3.4 Has resistance to biocides been observed in the food production chain?

Biocides are used widely in food production and there is evidence that some harmful bacteria found in food are becoming increasingly tolerant to biocides.

Bacteria can become resistant by biochemical (membrane changes) or genetic (new gene expression, mutations, etc) modifications. Genetic mutations or acquisition of external genetic materials (plasmids, transposons, etc.) that make bacteria resistant to biocides could also make them resistant to antibiotics. Given the increasing use of biocides in animal facilities, this issue is of growing concern.

There is a lot of research on whether using antibiotics in animals leads to the emergence of resistant bacteria. However, data on the role that disinfectant use may have in the emergence of bacterial resistance are scarce.

A study from 1998 compared different strains of bacteria found in a poultry farm. Those that were resistant to a specific biocide were also more resistant to several antibiotics, disinfectants and dyes. However, a study in 2005 from Denmark on the five most common disinfectants used in poultry farms did not find a link between biocide use and resistance in the Salmonella bacteria. Similar research in 2007 showed that using disinfectants stimulated bacteria to activate a defence mechanism that "pumps out" harmful chemicals from their cells. However, a single exposure to the disinfectant did not result in the selective survival of strains with resistance genes.

Laboratory studies show that biocide use could lead to antibiotic resistance, particularly when biocides are used improperly over a long period of time and at concentrations that are too low to be effective. However, to date this result has only been found in laboratory studies and not in working situations.

More research is needed to establish whether the current use of biocides in food production and in the disinfection and decontamination of foods of animal origin could lead to antibiotic resistance.

3.5 Has resistance to biocides due to discharges to the environment been observed?

Once biocides have been used, they are discharged into wastewater and they can be found throughout the environment in concentrations possibly leading to the selective survival of resistant bacteria.



Biocides are discharged with wastewater.
Credit: Carl-Fredrik Runqvist

Studies on bacteria that form biofilms in sink drains found that exposure to a biocide did not change the total number of bacteria present, but those that were naturally resistant to it grew at the expense of bacteria that are more easily affected by it.

Another study found resistant bacteria on the factory floor of biocide manufacturers. Even resistant bacteria could be killed by the concentrations of biocide used in practice for disinfection, but they became resistant to some unrelated antibiotics.

A number of studies have investigated whether hospital wastewater, in which high concentrations of disinfectants and antibiotics are found, contains resistant bacteria. However, there are no studies on the possible emergence of biocide resistant bacteria in other hospital environments.

4. How can bacteria become resistant to biocides or antibiotics?

4.1 How can bacteria become resistant to biocides?

Bacteria become resistant to biocide exposure when they are able to limit their internal concentration of active biocide to harmless levels. Bacteria can do this by a number of methods and may combine several.

For instance, some bacteria become resistant by changing the structure of their **cell envelope** so that it lets in smaller amounts of biocides. This is particularly the case of bacteria that grow as biofilms attached to surfaces. The outer layers of biofilms are considerably less permeable than those of free bacteria, and this could lead them to be much less easily affected by biocides and antibiotics.

Some bacteria become more tolerant to biocides by activating a system that “pumps out” toxic compounds generally termed **efflux pump**. This reduces the efficacy of biocides.

Some bacteria use enzymes to cause chemical changes in biocides and to **degrade** them so that they are less effective, but it is not clear whether this mechanism is relevant for the high concentrations of biocides used in practice. Some bacteria use enzymes to cause chemical changes in biocides and to degrade them so that they are less effective, but it is

not clear whether this mechanism is relevant for the high concentrations of biocides used in practice.

Bacteria can **modify the parts of their structure** that biocides attach to and attack. However, there are many different sites that biocides can target so modifying one of these does not have a large effect on increased resistance.

Bacteria that could previously be controlled by a biocide can develop resistance by **acquiring resistance genes** and this is a serious cause for concern. In some cases, exposure to a low biocide concentration leads to genetic changes that make the bacteria resistant to several unrelated compounds, but the mechanism for this is unknown.

Recent studies have demonstrated that some biocides are able to activate several genes that are involved in the control of resistance mechanisms affecting the activity of biocides and antibiotics.

Sometimes bacteria become resistant once they reach sufficiently high numbers. Bacteria secrete certain "signal" molecules that other bacteria can detect. Once bacteria detect enough of these from neighbouring bacteria, the whole colony activates specific genetic cascades involved in the formation of biofilms. This mechanism is involved in the development of resistance to biocides and antibiotics but more research is needed in this field.

4.2 How can bacteria become resistant to antibiotics?

Antibiotics work either by altering the bacterial envelope or by interfering with important physiological processes inside the bacteria as well as with their growth.

Table 10: Mechanisms of action of antibiotics [[see Annex 1, p. 20](#)]

Bacteria may be "insusceptible" or intrinsically resistant to an antibiotic because they have no sites that the molecule can attack, because the envelope does not let the antibiotic in, because some efflux pumps expel the antibiotic, or because the bacteria produce enzymes that destroy it.

An increasing and ongoing concern are bacterial strains that become resistant by mutation, by changing their gene expression or by transfer of resistance genes from other bacteria. The transfer of genes can take place in different ways but usually involves genes that can move between different parts of the genome. Some of these acquired genes enable the bacterium to destroy the antibiotic or to expel it and others change the parts of the bacteria that antibiotics attack. There are three possible mechanisms:

1. Bacteria can make their membrane less permeable to the antibiotic or "pump out" any antibiotic from the cell before it starts to act by producing an efflux pump.
2. Bacteria can attack the antibiotic (alter the structure) and make it ineffective by producing detoxifying enzymes.
3. Bacteria can protect or modify the parts of their structure that antibiotics attack (target mutation) or can produce decoys that antibiotics attack instead of the real target sites.

"Multi-drug resistant bacteria" that become simultaneously resistant to different classes of antibiotics are a cause for serious concern in hospitals, where they are commonly found. They mainly act by pumping out any compounds harmful to them so that their concentration inside the bacteria becomes harmless in addition to other resistance mechanisms including target mutation or detoxifying enzymes.

Once resistant bacteria emerge, using antibiotics can help resistant strains thrive by killing other strains so that bacteria with resistance genes can grow and reproduce without competition from other strains. These bacteria can also transfer their resistance genes to other bacteria of similar or different species.

4.3 Which resistance mechanisms are common to both biocides and antibiotics?

There are many similarities in the ways that biocides and antibiotics penetrate bacteria and work. Both diffuse into bacteria, they can modify or destroy the bacterial membrane, i.e. the layer that encloses the bacterium, and can disrupt key steps in bacterial chemical reactions. Therefore, some bacterial mechanisms of defence are effective against both antibiotics and biocides such as the decrease of membrane permeability that reduce the uptake of active molecules, or the production of efflux pumps that expel antibiotic and biocide molecules.

Genes that confer resistance to antibiotics can also be involved in biocide resistance such as efflux pump genes, so bacteria that acquire resistance genes sometimes become resistant to both types of antimicrobials at the same time. In other cases, genes that confer resistance to different antimicrobial products (such as beta-lactams and quaternary ammonium products) are very close to each other in a same genetic element (plasmid, transposon, etc) transferable from one bacterium to another. As a result, when this genetic element passes from one bacteria to another, both types of resistance genes (to antibiotics and to biocides) are transmitted together.

In yet other cases, exposing bacteria to some biocides can activate the genes responsible for resistance against both biocides and antibiotics.

This raises concerns over the indiscriminate and often inappropriate use of biocides in situations where they are unnecessary, because it can contribute to the development of resistance mechanisms. This is especially important in cases where potentially harmful bacteria, such as those found in hospitals, are exposed to biocides.

5. Does biocide use contribute to the development of antibiotic resistant bacteria?

There have been several laboratory studies that show a possible link between exposure to biocides and antibiotic resistance. However, other investigations have not found such a link.

Exposure to biocides can affect bacterial populations so that only the resistant strains survive, and this has been associated with increasing resistance to antibiotics. This could be relevant for biocides used in consumer goods because a small number of biocides are used widely in many different household and personal hygiene products. As a result, the bacteria on human skin and in homes are repeatedly exposed to certain antimicrobial products. However, it is not clear whether this type of bacterial exposure to biocides will lead to antibiotic resistance.

Data are scarce but there is some evidence of a link between using biocides in veterinary products, and increased resistance to antibiotics. This emphasizes the need to develop research and surveillance programmes in the area of animal husbandry.

Measuring the effect of biocides on the way that bacteria react to antibiotics is far from straightforward. In experiments, a group of bacteria are first treated with a biocide and

those that survive are then tested to see to what extent they are affected by antibiotics. This latter stage is complicated and the results are often hard to interpret because several mechanisms may modulate antibiotic susceptibility. Recent data clearly demonstrate that some biocides activate genetic controls that are involved in triggering resistance mechanisms that alter both biocide and antibiotic activity.

Some studies on bacteria that grow as biofilms have investigated whether using biocides can lead to the emergence of more resistant strains, and the results vary depending on the species of bacteria and on the biocide used. Some studies showed that biocide use had no effect on the development of resistance. Other studies found that using biocides made some strains of bacteria grow at the expense of others, and that successful bacteria were less easily affected by biocides.

6. What are the potential threats of biocide use in terms of bacterial resistance?

6.1 How might the use of biocides constitute a direct or indirect threat?

Using biocidal products could potentially lead to bacteria that are resistant to biocides, antibiotics or both. These successful bacteria would reproduce more than other strains and if they were transmitted to humans, could pose a direct health threat.

Bacteria can also pose an indirect threat if they develop genes that confer resistance to antibiotics and biocides. These resistance genes can move between different bacteria and can thus transfer to a bacterium that could previously be controlled by antimicrobials. Genetic material can also pass between bacteria that are resistant to different substances so that they achieve an even higher level of resistance. This process can take place anywhere: in the environment, in food, animals or in the human body.

6.2 What are potential threats of using biocides in veterinary settings?

The use of biocides in intensive, industrial-scale farming could potentially lead to the emergence of strains of bacteria that are resistant to the disinfectants used, particularly when the concentration of the biocide is lower than it should be. This can happen when large animal houses are washed and disinfected and some areas are not cleaned thoroughly enough, or in disinfectant baths outside of animal houses that can easily be diluted by rainfall. Some of these baths also contain other chemicals that could make the biocide less effective. According to the findings of laboratory studies, the use of common farm disinfectants can enable strains of bacteria that are resistant to one antibiotic or to several classes of antibiotics to become more common than susceptible bacteria. This could be a stepping stone to a higher level of antibiotic resistance. In addition, low concentrations of biocides can favour genes involved in resistance and their expression.

If the bacteria found in animals can also cause disease in humans, the emergence of resistant strains would compromise antibiotic treatments used in humans.

There is a need for further studies on whether the intensive, and in some cases long-term use of biocides in animal facilities, can lead to antimicrobial resistance. This is also important because modern, intensive animal husbandry relies increasingly on the use of antibiotics; and harmful resistant micro-organisms can pass between animals and humans by direct contact and via the food chain.

6.3 What are potential threats of using disinfectants in health care settings?

Biocide-resistant micro-organisms are increasingly found in hospitals and there is a possible link between the use of biocides and antibiotic resistance in health care settings. However, evidence of such a link has only been found in some cases of antibiotics. In addition, both bacteria that are resistant to antibiotics and those that are not are killed by disinfectants at the concentrations used in practice in health care facilities.



Biocides are used in hospitals to prevent and control infections.
Credit: Fernando Audibert

Some of these biocide-resistant bacteria cause serious concerns. A particularly challenging problem is the presence of resistant bacteria that grow as biofilms on surfaces such as walls or floors, and on medical devices; and which are the cause of most hospital-acquired infections. Even high-level disinfectants are less effective against bacteria when these grow as biofilms. Despite this, most laboratories do not use biofilms to test the efficacy of biocides and there are no European standards for such tests.

More research is needed to see if there is a link between the use of biocides and antibiotic resistance, and researchers need to agree on how best to measure resistance to biocides. There should be a surveillance system to detect any emerging resistance to biocides, particularly for important micro-organisms that become resistant to antibiotics or those that are already multi-drug-resistant.

6.4 What are potential environmental threats of using biocides?

Biocides are very widely used in consumer products and in health care settings so they find their way into wastewater from homes and hospitals. Some biocides are used in such large volumes that even though sewage treatment plants remove large proportions of biocide in wastewater, significant amounts are still discharged. There is concern that these environmental concentrations might lead to resistance in micro-organisms.

A laboratory study showed that bacteria exposed to a range of triclosan concentrations below the level that would kill them did not become resistant to it, even when another triclosan-resistant bacterial species was present. The researchers concluded that environmental concentrations did not induce resistance to triclosan and that resistance was not transferred between different species of bacteria. In contrast, a recent report described that the exposure to triclosan at the minimum inhibitory concentration (MIC) during a short period of time is sufficient to activate the expression of resistance genes in two bacterial species.

A similar study investigated the effects of detergent containing another common disinfectant, on biofilms that form in household sink drains. They found that long term use of the biocide did not result in significant changes in resistance. The researchers concluded that although exposure to the biocide can lead to resistance in isolated bacteria, the same is not necessarily true for large groups of bacteria growing as a colony.

7. What explains resistance to both biocides and antibiotics?

7.1 How can biocide exposure lead to antibiotic resistance?

In laboratory experiments, bacteria exposed to biocides were found to develop antibiotic resistance in five different ways:

- ⌘ **Cross-resistance:** Some bacteria have genes that make them resistant to a biocide, and which also happen to make them resistant to one or more antibiotics. There are several classes of antibiotics and all antibiotics in the same class work in the same way. Therefore, bacteria that are resistant to an antibiotic are also resistant to all the antibiotics in the same class. Some bacteria are resistant to both antibiotics and biocides, for instance because they have developed a mechanism that “pumps out” such unwanted chemicals from within their cells.
 - ⌘ **Change in the physiological response:** As a result of exposure to a biocide, some bacteria change the way they respond to it and that makes them less easily affected by either the biocide or antibiotics.
 - ⌘ **Co-resistance :** Some resistance genes can be transferred from one bacterium to another whether or not it is related. Some bacteria are simultaneously resistant to biocides and antibiotics because the genes that confer resistance to one are found near the genes that confer resistance to the other so that the two sets of genes are transferred together.
 - ⌘ **Indirect selection:** Exposing a population of bacteria to a biocide wipes out those which are easily affected (susceptible bacteria) and only resistant bacteria remain. These surviving bacteria are less easily controlled by either biocides or antibiotics and over time the resistant bacteria are more common in the population.
5. **DNA repair:** exposing bacteria to biocide can activate mechanisms of DNA repair.

Unfortunately, researchers have limited their investigations to one or two of these ways and not to all five at the same time, potentially missing some important information on a possible link between biocide and antibiotic resistance.

7.2 In practice, does resistance emerge in homes and the environment?

In homes, repeated exposures of bacteria to biocides in cleaning products, disinfection products and other relevant products could be considered to be a continuous selective pressure. This could lead to resistant bacteria surviving better than standard bacteria, and to the emergence of resistant strains. In natural environments, bacteria are continually exposed to low concentrations of biocides present in air, water and soil which might contribute to the emergence of resistance.

Laboratory studies show that bacteria exposed to non-lethal concentrations of biocide can develop resistance quickly. However, it is difficult to tell how widespread the development of bacterial resistance is in practice because there is very little information available.

One of the most important factors in the development of resistance is the concentration of the biocide so it is crucial to determine actual exposure. It has been impossible to get any information on the production volumes and uses of the various biocides. Exposures of bacteria to biocides in homes and the environment has to be estimated by other means, for instance based on the concentration and frequency of use of cleaning products and concentrations measured in the environment.

7.3 How can dissemination of resistance genes lead to resistance?

Bacteria can pass sections of DNA to each other via genetic mobile elements (plasmids, transposons, etc.), even among different species. These genetic mobile elements can confer useful properties to the bacteria receiving it such as the ability to grow in the presence of antibiotics, biocides or heavy metals.

A 2002 study investigated whether bacteria resistant to a particular biocide were also resistant to antibiotics. They found that some of the genes that conferred resistance to the biocide, also conferred resistance to antibiotics. Biocide-resistant strains were more likely to be antibiotic-resistant than standard strains and some strains were multi-resistant. This shows that resistance to antibiotics and to biocides were linked at the genetic level and that the presence of biocide-resistance genes led to the selective survival of antibiotic-resistant bacteria.

The transfer of resistance genes together with other useful functions has been observed in several bacteria species that can cause disease in humans. The uncontrolled use of biocides could therefore lead to the selective survival of bacterial strains with resistance genes. Bacteria could not only pass these resistance genes to their offspring but also to neighbouring bacteria of the same or of different species.

7.4 How can the formation of biofilms lead to resistance?

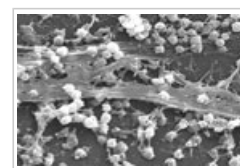
Bacteria can adapt to changes in nutrient availability, environmental stresses, and presence of toxic compounds. One particularly important example of bacterial adaptation is the ability for a group of bacteria to grow as a biofilm attached to a surface. It is now recognized that many bacterial diseases involve the formation of a biofilm in the affected part of the body or implant.

Bacteria living as a biofilm are able to resist to biocides and to antibiotics more effectively than those living as free organisms and they withstand considerably higher doses of antimicrobial products. This simultaneous resistance to both antibiotics and to biocides could be explained if the underlying mechanism is effective against both antimicrobials. For instance, biofilms are encased in polysaccharide layers that reduce the diffusion of antimicrobials. Compared to free cells, bacteria in biofilms are more concentrated, grow more slowly and are in a different physiological state; and all this could affect their susceptibility to antimicrobial products. However, there is very little information on the cross-resistance of bacteria in biofilms to antibiotics and biocides.

In one study, exposing biofilms of bacteria to biocides used commonly to treat drinking water, did not increase antibiotic resistance.

8. How can risks of resistance to both antibiotics and biocides be assessed?

Laboratory and field studies have shown that treating bacteria with low concentrations of biocide, leads to the preferred survival of resistant bacteria.



Antibiotic use is still the major cause of antibiotic resistance in clinical practice. Since antibiotic resistance decreases our ability to treat infections, it is crucial to prevent infection through good hygiene and the appropriate use of biocides.

8.1 What factors increase the risk of resistance to both biocides and antibiotics?

Bacteria have the ability to transfer genes not only to their offspring but also from one organism to another, be it a similar bacterium or a different bacterial species. This horizontal transfer of DNA plays a very important role in the spread of bacterial resistance. The transfer is most effective between bacteria sharing the same ecological niche. Bacteria can be classified according to their efficiency at transferring genes. Some bacteria have developed highly specialised mechanisms to transfer genes and there is a high risk that they will pass genetic material to unrelated species. Medium-risk bacteria can only pass DNA to related species and low-risk bacteria are those with no known mechanism of transferring DNA to other organisms.

The presence of biocides can lead to the expression of a number of genes in bacteria that enable them to develop general defence mechanisms. These, in turn, can increase the bacteria's resistance to antibiotics.

The risk of bringing about resistance also depends on the type of biocide used. Bacteria are unlikely to escape damage from highly reactive biocides so these do not readily lead to resistance unless they are used or stored incorrectly. Other biocides such as metallic salts and quaternary ammonium compounds are more prone to induce resistance because of the way they interact with bacteria. For many biocides used heavily in consumer products and in the food industry, there is little information on whether or not they can lead to resistance.

Growing as a community attached to a surface (biofilm) allows bacteria to survive under hostile conditions. Therefore, any circumstances that allow the formation of biofilms are a potential risk for developing resistance to both antibiotics and biocides. These include solid surfaces in the body such as prosthetics, catheters or implants, as well as surfaces in food and chemical factories, or in water treatment plants.

In water, environmental factors such as pH, the amount of oxygen and nutrients available, temperature and exposure time influence how bacteria grow and thus affect the way they respond and, sometimes, adapt to biocides. They also have an effect on how bacteria relate to other bacteria by transferring genetic mobile elements or by arranging themselves into biofilms. Large colonies of bacteria can act as one and activate resistance genes simultaneously by exchanging signal molecules. Environmental factors can affect this mechanism and therefore play a role in the development of resistance.

The large and indiscriminate use of biocides will increase the selection and dissemination of resistance genes among bacteria. As biocides used in homes and in industries come into contact with micro-organisms in animals and soil, they can lead to the emergence of resistance genes and the transfer of these between micro-organisms, including those in food. Therefore there is a risk that humans may be exposed to resistant bacteria through food, and this may concern a significant part of the population.

8.2 What (new) methods are required to effectively assess the risk of resistance?

There are no standard, internationally agreed methods of establishing the efficacy of biocides. Current tests vary from basic preliminary studies to sophisticated methods that aim to simulate the conditions found in practice.

Designing tests is complicated because many different factors have to be controlled. Results are often not reproducible, especially if the tests are carried out in real conditions outside

the laboratory. Then again, tests in the laboratory can be too rigid, time-consuming and not represent the real conditions found in practice.

Samples of bacteria are often tested separately for resistance to antibiotics and to biocides but there are no set methods to determine resistance to both at the same time. The information available on the effectiveness of different biocides is inconsistent because of the wide range of testing methods used and because tests are not always carried out properly. There is an urgent need to design a standard method of measuring both biocide and antibiotic resistance in bacterial samples.

Bacteria are often present in the environment as biofilms and these play an important role in bacterial resistance. Despite this, most laboratories do not measure the efficacy of biocides on biofilms and there are no European standards for such tests. This is an important shortcoming because biofilms are ever-present and notoriously difficult to remove, even after intense cleaning. Moreover, higher concentrations of a biocide will most probably have to be used to clear biofilms and this will increase the amounts of biocide released to the environment.

The presence of biocides in the environment also increases the likelihood of developing resistance, especially when the concentrations are too low to be effective. Two specific situations need particular consideration: biocides that can persist in the environment for a long time, and biocides used or released frequently in places where there are many micro-organisms.

9. Conclusions & recommendations

Biocides – including disinfectants, antiseptics, preservatives and sterilants – are invaluable chemicals that provide society with many benefits, keeping harmful micro-organisms at bay. They play an important role in the control of bacteria in many applications and are a precious resource that must be managed so that it remains effective for as long as possible.

There is scientific evidence that the use or misuse of biocides can contribute to the increased occurrence of bacteria that are resistant not only to biocides but also to antibiotics.

Any situations where biocides are used extensively and regularly at concentrations too low to kill bacteria, can lead to increased antimicrobial resistance. This may happen for instance in hospitals, in food production, in cosmetic manufacturing, etc.

Resistance genes can be transferred from one bacterium to another and using biocides can lead to the preferred survival of bacteria with resistance genes. Many well-studied biocides lead to resistance by this mechanism but the role that less studied biocides play in inducing or maintaining antibiotic resistance is not known.

Some biocides are more likely to lead to resistance than others but it is hard to quantify the risk of increasing antibiotic resistance for each biocide and each application. In some situations where both antibiotics and biocides are used, it is not possible to discriminate the origin of antimicrobial resistance at this moment.

Standard methods of measuring resistance brought about by biocide use are not available and should be developed. The tests should measure the lowest concentration of biocide that can lead to resistance if it is used repeatedly at concentrations too low to kill bacteria. Environmental monitoring programmes for undesirable substances should include biocides. To enable the assessment of exposure, and thus the likelihood of resistance emerging, companies should be encouraged to report the volumes of biocides they produce or use.

Additional studies are needed to:

1. understand the mechanisms of cross-resistance and the emergence of biocide-induced antibiotic resistance in different fields of application (e.g. health care, veterinary uses, food production, cosmetics and consumer products).
2. develop standard methods to evaluate the ability of a biocide to cause or maintain antibiotic resistance.
3. develop standard methods to identify/measure resistance, cross-resistance and multi-drug resistance.
4. develop surveillance programmes to monitor the level of resistance, cross-resistance and multi-drug resistance of micro-organisms found in health care facilities, veterinary settings and the food industry.
5. study exposures in order to identify and quantify the risks of resistance, cross-resistance and multi-drug resistance emerging in bacteria following biocide exposure.

To preserve the role of biocides in infection control and hygiene, it is paramount to prevent the emergence of bacterial resistance and cross-resistance through their appropriate use.

Annex

Annex 1:

Table 10 Mechanisms of action of antibiotics

Action	Alteration of bacterial envelope	Inhibition of protein synthesis	Inhibition of nucleic acid synthesis	Inhibition of metabolic pathway
Antibiotic family	β -lactam	MLS	Quinolone	Sulfamide
	Glycopeptide	Phenicol,	Rifamycine, Ansamycine	Folic acid
	Polymyxin, daptomycin	Oxazolidinone		Nitro-imidazole
	CAMP	Aminoglycoside		
		Cycline (tetracycline)		
MLS: macrolide, lincosamide, streptogramin CAMP: cationic antimicrobial peptide				

Source: SCENIHR, *Assessment of the Antibiotic Resistance Effects of Biocides (2009)* [see http://ec.europa.eu/health/ph_risk/committees/04_scenihhr/docs/scenihhr_o_021.pdf],
Section 3.5.1 Resistance mechanisms to antibiotics, p. 40

Annex 2:**Table 2 List of active substances in biocidal products and their mode of action**

Biocide	Usage/areas of applications	General mode of action
Quaternary ammonium compounds	Health care, household products, surface preservation (various application), food industry, pharmaceutical/cosmetic (preservation)	Membrane destabiliser, at a high concentration – produce cytoplasmic protein aggregation (loss of tertiary structure)
Biguanides	Health care, household products	Chlorhexidine specifically inhibits membrane-bound ATPase
Phenols/cresols	Health care, home care products, surface preservation (various applications)	Triclosan: enoyl acyl reductase at a low concentration Dinitrophenol collapses membrane energy (ATP synthesis) A low concentration of fenitichlor and triclosan inhibits energy-dependent uptake of amino acids A low concentration of triclosan discharges membrane potential in <i>E. faecalis</i>
Alcohols	Health care, pharmaceutical/cosmetic (preservation)	Inhibition of DNA and RNA synthesis, cell wall synthesis (secondary effect) Low concentration of phenoxyethanol induce proton translocation in <i>E. coli</i>
Aldehydes	Health care, pharmaceutical/cosmetic (preservation), industry (paper)	Alkylating agents
Ethylene oxide	Health care, single-used medical devices (e.g. catheter sterilisation)	Alkylating agent
Anionic agents	Household products, Pharmaceutical/cosmetic (preservation)	As part of a formulation (i.e. usually not the main active)
Organic acids	Pharmaceutical/cosmetic (preservation), food preservation	Dissipation of proton motive force; Inhibition of uptake of amino acids
Metallic salts	Health care, pharmaceutical preservation	Interactions with thiol-group (mercury, silver)
Isothiazolinones	Personal care products, Household products and Industrial products	BIT (benzisothiazolone) affects active transport and oxidation of glucose in <i>S. aureus</i> , activity of thiol-containing enzymes, ATPases, glyceraldehyde-3-phosphate dehydrogenase
Peroxides	Health care, personal care products and Industrial products	Oxidising agents
Chlorine compounds and halogens	Health care, Household products, Industrial products, water treatment (private and industrial use)	Oxidising agents
Amphoteric agents	Health care, household products	Unknown membrane interaction
Non-ionic agents	Health care, household products	Unknown membrane interaction
Limonene	Household and industrial products	Unknown membrane interaction
Antimicrobial dyes	Health care	DNA-intercalating agents
Iodophors	Health care products	Covalent binding to thiol groups
Pentamidine, isethinate of pentamidine, propamidine (dibromo derivatives)	Medical devices (e.g. catheters)	Inhibition of DNA synthesis

Source: SCENIHR, *Assessment of the Antibiotic Resistance Effects of Biocides (2009)* [see http://ec.europa.eu/health/ph_risk/committees/04_scenih/docs/scenih_o_021.pdf],
Section 3.3. Production, use and fate of biocides, p. 18

Annex 3:**Table 3 Biocides approved by US-FDA for health care settings, or registered by the US-EPA**

Disinfection level	Biocides
Low-level	Ethyl or isopropyl alcohol (70-90%)
	Iodophor solution (follow product label for use-dilution)
	Phenolic (follow product label for use-dilution)
	Quaternary ammonium detergent solution (follow product label for use-dilution)
	Sodium hypochlorite (5.25%-6.15% household bleach diluted 1:500, ≈100 ppm available chlorine)
Intermediate-level	Ethyl or isopropyl alcohol (70-90%)
	Phenolic (follow product label for use-dilution)
	Sodium hypochlorite (5.25%-6.15% household bleach diluted 1:100, ≈500 ppm available chlorine)
High-level	Glutaraldehyde ≥2%
	Glutaraldehyde (1.12%) and phenol/phenate (1.93%)
	Hydrogen peroxide (7.5%)
	Hydrogen peroxide (7.35%) and peracetic acid (0.23%)
	Hydrogen peroxide (1%) and peracetic acid (0.08%)
	Hypochlorite (single-use chlorine generated by electrolyzing saline containing >650-675 ppm of active free chlorine)
	Ortho-phthalaldehyde (0.55%)
	Peracetic acid (0.2%)

Source: SCENIHR, *Assessment of the Antibiotic Resistance Effects of Biocides (2009)* [see http://ec.europa.eu/health/ph_risk/committees/04_scenihhr/docs/scenihhr_o_021.pdf],
Section 3.3.1 *Biocides in health care*, p. 20

Annex 4:**Table 4 Commonly used skin disinfectants and antiseptics**

Biocides	Most commonly used dilution
Alcohols (ethanol, isopropanol, n-propanol)	60%-95%
Chlorhexidine gluconate	Aqueous or detergent preparations containing 0.5 or 0.75% chlorhexidine Alcohol preparations containing 4% chlorhexidine
Chloroxylenol (parachlorometaxyleneol: PCMX)	0.3%-3.75%
Hexachlorophene	3%
Iodophors (Povidone-iodine)	7.5%-10%
Quaternary ammonium compounds	
Triclosan	0.2-2%

Source: SCENIHR, *Assessment of the Antibiotic Resistance Effects of Biocides (2009)* [see http://ec.europa.eu/health/ph_risk/committees/04_scenihhr/docs/scenihhr_o_021.pdf],
Section 3.3.1 *Biocides in health care*, p. 22

Annex 5:**Table 5 Major biocides used in veterinary medicine and animal husbandry**

Veterinary use
Na-dichloro-isocyanurate
Na- p -toluene-sulfonchloramide (Halamid)
H ₂ O ₂
Acetic acid
Quarternary ammonium chlorides
Glutaraldehyde (in combinations)
Formaldehyde (in combinations)
Isopropanol (in combinations)
Disinfection of instruments and animal facilities/houses
Na-dichloro-isocyanurate
H ₂ O ₂
Acetic acid
QAC: Didecyl-dimethyl-ammonium Cl
QAC: Alkyl-demethylbenzyl-ammonium Cl
Glutaraldehyde (in combinations)
Formaldehyde (in combinations)
Isopropanol (in combinations)
Disinfection of transporters/trucks
Na-dichloro-isocyanurate
H ₂ O ₂
Acetic acid
Quarternary ammonium chlorides
QAC + KOH
Glutaraldehyde (in combinations)
Formaldehyde (in combinations)
Isopropanol (in combinations)
Disinfection of boots and tools
Na- p -toluene-sulfonchloramide (Halamid)
H ₂ O ₂ / acetic acid

Source: SCENIHR, *Assessment of the Antibiotic Resistance Effects of Biocides (2009)* [see http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_021.pdf],
Sections 3.4. Biocides in animal husbandry & 3.3.5. Biocides in food of animal origin, p. 25

Annex 6:**Table: 23 Biocidal products listed in Annex V of the Biocides Directive (98/8/EC)**

Main Group	Product-type
1: Disinfectants & general biocidal products	1: Human hygiene biocidal products
	2: Private area and public health area disinfectants and other biocidal products
	3: Veterinary hygiene biocidal products
	4: Food and feed area disinfectants
	5: Drinking water disinfectants
2: Preservatives	6: In-can preservatives
	7: Film preservatives
	8: Wood preservatives
	9: Fibre, leather, rubber and polymerised materials preservatives
	10: Masonry preservatives
	11: Preservatives for liquid-cooling and processing systems
	12: Slimecides (for organisms that produce slime)
	13: Metalworking-fluid preservatives
3: Pest control	14: Rodenticides (for mice, rats and other rodent)
	15: Avicides (for birds)
	16: Molluscicides (for molluscs)
	17: Piscicides (for fish)
	18: Insecticides, acaricides and products to control other arthropods
	19: Repellents and attractants
4: Other biocidal products	20: Preservatives for food or feedstocks
	21: Antifouling products
	22: Embalming and taxidermist fluids
	23: Control of other vertebrates

Source: http://ec.europa.eu/environment/biocides/pdf/98_8_ec_web.pdf#page=48 [see http://ec.europa.eu/environment/biocides/pdf/98_8_ec_web.pdf#page=48]

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