Health Council of the Netherlands

Careful use of disinfectants

Gezondheidsraad
Health Council of the Netherlands

To the Minister of Health, Welfare and Sport and
the Minister for the Environment

Subject : presentation of advisory report *Careful use of disinfectants*
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Dear Ministers,

In response to your request for advice of February 2015, please find enclosed the advisory report Careful use of disinfectants. It has been written by the Disinfectants Committee.

A draft of the advisory report was evaluated by the Standing Committees on Public Health and Health Care. The related background document (Resistance due to disinfectants) is available from the Health Council website.

A key motivation for the advisory report was your question of whether resistance issues related to these substances give sufficient reason to develop policy. The Committee recommends that you do so. The scientific evidence in this field indicates there are two aspects to disinfectant use. On the one hand, their use helps prevent or combat infections and thus limit antibiotic use and the development of antimicrobial resistance. On the other hand, disinfectants can also promote resistance, not only to the disinfectants themselves but also to antibiotics. The severity and scope of development of resistance to disinfectants continues to be insufficiently clear. Therefore, as a precaution, the Committee recommends stimulating careful use of these products, tightening admission policies, and initiating surveillance for use and resistance development.

I support the Committee's analysis and recommendations.

Yours sincerely,
(signed)
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Careful use of disinfectants

to:

the Minister of Health, Welfare and Sport

the Minister for the Environment

No. 2016/18E, The Hague, December 21, 2016

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research...” (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare and Sport, Infrastructure and the Environment, Social Affairs and Employment, and Economic Affairs. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

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Executive summary

Disinfectants can make a significant contribution to infection control in humans and animals. In this way, they help to reduce morbidity and mortality due to infections. They also help in the food industry to prevent spoilage and are used to remedy annoyances such as odour and mould or deposits of algae.

However, the excessive or improper use of disinfectants, can have adverse effects. For example, people may be poisoned or develop an allergy. In addition, by using disinfectants, resistance can develop to disinfectants themselves and also to antibiotics. Partly as a result of this kind of risk, there is European legislation to authorise disinfectants on the market (the so-called Biocidal Products Regulation; biocides are chemical or biological agents that kill living organisms). There are also directives for the use of disinfectants in specific sectors such as healthcare, agriculture and the food sector.

In 2001, the Health Council advised caution in using disinfectants in consumer products since there is no evidence that they confer a health benefit. The Ministers of Health, Welfare and Sport and Infrastructure and the Environment are now asking the Health Council whether this advice still applies taking the current state of scientific knowledge into account. They would also like to know whether restraint is also needed in other sectors and whether national policy must be developed for this. The ministers are also asking if there are indications that resistance to disinfectants is growing due to their increasing use. And finally, they would like to know the extent to which resistance to disinfectants has an impact on antibiotic resistance.

The Disinfectants Committee of the Health Council has examined the ministers' questions. Its answers are based on a detailed analysis of the scientific literature and a hearing with representatives from various sectors in which disinfectants are used.

Applications of disinfectants may or may not be proven effective

There is a wide variety of disinfectants on the market that are used for a range of -applications. Examples include hand disinfection with alcohol among medical staff, preoperative skin disinfection with chlorhexidine, disinfection of medical equipment (endoscopes) with glutaraldehyde, disinfection of surfaces and pipes in the food industry with quaternary ammonium compounds, disinfection of stalls with formaldehyde and disinfection of swimming pool water with chlorine compounds. Most of these agents are covered by biocidal legislation. But the active substances are also used in products that are covered by different legal regimes: pharmaceuticals, veterinary drugs, medical devices and cosmetics.

Sectors in which disinfectants are commonly used are healthcare, agriculture (especially livestock farming), the food industry and water treatment. Deployment of the agents is mostly preventive (intended to avoid contamination and infections), but sometimes also curative (aimed at combating infections).

The exact amount of disinfectants used in the Netherlands is not known, since this is not measured and monitored. Its use is gradually increasing in our neighbouring countries. It is estimated that the use of disinfectants (expressed in kilograms) is at least ten times higher than the use of antibiotics.

For human health, animal health and food safety, the proper use of disinfectants is indispensable in many cases. However, there are also applications whose effectiveness has not been proven or is under discussion. This applies, for example, to the daily washing of patients in intensive care with chlorhexidine and the routine disinfection of floors, walls and ceilings in hospitals.

Disinfectants are increasingly being offered to consumers, particularly for household use. Examples include antibacterial hand soap, disinfectant wipes and 'hygienic' cleaning products. However, there is no scientific evidence that the routine use of disinfectants by consumers at home is healthier than cleaning with 'ordinary' water and soap. The same applies to the use of disinfectants at the office. Disinfectants can be useful in situations where water and soap are not always available, such as on holiday. On certain medical indications, the use of disinfectants in the household also provides a health benefit, for example, in fighting MRSA bacteria.

Resistance can develop in various ways

Like antibiotics, disinfectants can cause resistance to develop. This not only occurs with improper use (e.g. applying too low concentrations or too short contact times) but it can also happen even if used properly. Bacteria can be naturally resistant or may become resistant to disinfectants, rendering these agents less effective or no longer effective at all. At places where disinfectants are used and elsewhere in the environment, bacterial populations inevitably come into contact with concentrations of the active substance that are not lethal to all bacteria. This activates resistance mechanisms and promotes the selection of resistant bacteria. Under these circumstances, bacteria with reduced susceptibility can oust their sensitive congeners and then spread. In addition, the resistance mechanisms themselves can spread since bacteria can exchange the genes that encode for this. Once resistance has developed, it has proved difficult to get rid of, even if the use of the disinfectants is stopped.

Resistance to disinfectants and to antibiotics frequently occurs together. Some resistance mechanisms work against both disinfectants and antibiotics (cross-resistance) and resistance genes against disinfectants and against antibiotics may be linked in the DNA (co-resistance). In this way, resistance to disinfectants also exacerbates the problem of antibiotic resistance. After all, resistant bacteria that survive exposure to disinfectants may spread. Because of their 'double resistance', this also increases the proportion of bacteria that is resistant to antibiotics.

The extent of the resistance problem is unclear

It is currently difficult to assess the extent of the problem of resistance to disinfectants in practice and the extent to which it is spreading. However, there are indications that resistance to disinfectants is expanding less vigorously than resistance to antibiotics. To date, high levels of resistance that threaten the efficacy of disinfectants have rarely been observed.

An important caveat to this finding is that sensitivity to disinfectants in practice is not often investigated – not even during outbreaks of pathogenic bacteria that are related to the use of disinfectants (proper or improper). Moreover, not only high but also low levels of resistance are important in healthcare. Even low levels of resistance can undermine the effectiveness of disinfectants and increase the likelihood that the desired results of disinfection (prevention of disease, spoilage and annoyances) are not achieved in practice.

The extent to which the use of disinfectants contributes to the development of antibiotic resistance is currently unknown. On the one hand, disinfectants can have a beneficial effect: if they help to prevent infection, antibiotics are needed less often. On the other hand, based on current knowledge of cross- and co-resistance, it is expected that the use of disinfectants may actually promote the development of antibiotic resistance. Laboratorytesting confirms this. However, research at places where disinfectants are used shows a variable picture on this issue. It is currently unclear whether the use of disinfectants plays a major or a limited role in antibiotic resistance. It is also unclear which agents and applications in practice are the main contributors to resistance development.

Authorisation procedures do not cover all risks

For products that contain disinfectants, European-based regulations apply. These must guarantee the efficacy and safety of products that appear on the market. Especially concerning biocides, pesticides and pharmaceuticals and veterinary medicines extremely wide-ranging authorisation procedures exist. Nevertheless, they do not cover all risks. For example, the procedures cannot take account of the use of disinfectants that deviates from the rules. However, in practice, this is rather common. Due to lack of knowledge and ignorance, disinfectants are sometimes used improperly, not applied where they are needed or applied where they are not needed. The authorisation procedures only take limited account of the extent of the use of disinfectants and of the effects of the accumulation of various biocide substances in the environment.

In addition, the authorisation procedures do not take into account the effect of disinfectants on the microorganisms that are naturally present in and on the human body (the natural microbiome). As a result, the use of personal care products in which disinfectants are found can have negative side effects. For example, there are indications that mouthwash with chlorhexidine, although effective against plaque and gum inflammation, also disturbs the natural role of the microbiome of the mouth on blood pressure regulation.

Recommendations

Develop policy for disinfectants

With a view to public health, it is desirable that additional policy should be developed to promote the careful use of disinfectants. The agents are indispensable for the prevention of infections and spoilage, but their use also gives rise to undesirable effects such as resistance development. ‘Use disinfectants properly and only when really necessary’ should be the motto of a forward-looking disinfection policy. This policy will need to be further specified by sector through close collaboration between government, professionals, users, manufacturers and experts. The committee confines its advice to global recommendations that apply in varying degrees to all sectors. Since this cooperation is difficult to organise in the consumer sector, the emphasis must partly lie on other measures there.

Where possible, methods should be encouraged to achieve health, cosmetic and aesthetic objectives without the use of disinfectants. This is consistent with the current policy in crop protection, where the government also promotes first trying non-chemical methods to prevent or control pests. The use of disinfectants must be limited to situations that demonstrate that they will be beneficial to health, for the shelf life or safety of products and for cosmetic purposes (e.g. odour control).

In professional sectors, the use of disinfectants should only be promoted where they have a clear added value in preventing or fighting infection or spoilage. The use of disinfectants in applications whose effectiveness (prevention of infections or spoilage) or efficacy has not been demonstrated in practice should be avoided as much as possible. It is advisable to record this in sectoral directives and protocols. For proper use, training and continuing education of professional users is very important.

The routine use of disinfectants at the office or by consumers at home for the purpose of protecting health should be discouraged. There is no proof that such use without medical indication results in health benefits. It is advisable to exclude products with such a purpose from the market. Through information, consumers can be shown that use of disinfectants in the daily routine and in the household is unnecessary and unwise. It can also be explained that many microorganisms are harmless and even useful.

Use and resistance development should be systematically registered

An integrated approach is needed to address the resistance issue, which includes all antimicrobial agents (disinfectants and antibiotics).

The committee recommends setting up a surveillance system to monitor the use of disinfectants and resistance development, similar to the surveillance systems that already exist for the use of and resistance to antibiotics. In this way, better understanding of the extent and severity of the resistance issue and the factors that play a role can be gained. The Netherlands can initiate it nationally, but it is also advisable to seek international cooperation in this field. After all, resistant microorganisms do not respect national borders.

Sharpen authorisation procedures

According to the committee, it is not possible in the short term to develop a test for the authorisation of a new disinfectant which can establish the resistance promoting ability of that product. In the longer term, the knowledge provided through surveillance may help in the development of such a predictive test. This is important since the European Biocidal Products Regulation determines that biocides may not cause unacceptable resistance in the bacteria they have to fight.

For the authorisation of disinfectants, it is advisable to use efficacy tests that better reflect the circumstances under which disinfectants must do their work in practice. This fits well with the new European regulations that also require a simulated practice test in many cases.

Encourage further research

Finally, it is advisable to encourage research on:

- the effectiveness of applications of disinfectants in practice and the added value with respect to other hygiene and prevention measures such as cleaning;
- methods to achieve health benefit and cosmetic objectives without the use of disinfectants whenever possible;
- the emergence of acquired resistance to disinfectants and antibiotics in relation to the use of disinfectants;
- the influence of disinfectants on the composition and functions of human and animal microbiomes – particularly in exposed professional users, patients and consumers – and of microbiomes in the environment.

Chapter 1

Introduction

1.1 Background

In 2001, the Health Council published an informative advisory report entitled *Disinfectants in consumer products*.[1] In this report, the Health Council recommended restraint in the marketing and use of consumer products with disinfectant, antiseptic or antibacterial properties. This is because there was no evidence that use of such products by laypersons at home provided additional health gains compared with soap and water hygiene, unless there were medical indications for use. In contrast with the lack of proven advantages, the Health Council did see potential disadvantages, particularly related to excessive and incorrect use. For example, the use of such products could create a false sense of security, potentially contributing to more infectious diseases. Other risks included intoxications and allergies, disruption of natural human microflora, development of resistance to disinfectants, and even development of resistance to antibiotics. The estimation of the latter risks was based mainly on theoretical considerations and laboratory studies. There were limited real-world indications of increased resistance due to disinfectant use by consumers.

Based in part on the Health Council advisory report, the government of The Netherlands attempted to limit and discourage the marketing and use of consumer products with disinfectant properties over the past decade. Some of these products fall under biocidal products legislation. Until recently, the 1998 Biocidal Products Directive (98/8/EC) applied within the European Union. This allowed member states some leeway in terms of implementation. On 1 September 2013, the Biocidal Products Regulation (528/2012/EG) came into effect, which immediately has the force of law in all member states.[2] An important part of the regulation is the possibility for central authorisation for the entire union. Instead of proceeding with national authorisation procedures, manufacturers can apply for a single European authorisation. Exceptions to Union authorisations at the individual member state level are only allowed based on specific national circumstances, but this is a complex procedure.

1.2 Request for advice

Due to these changes in legislation, the freedom to create national policy is decreasing. Therefore, the responsible ministers from the Ministries of Health, Welfare and Sport and of Infrastructure and the Environment asked the Health Council for advice about disinfectants on 4 February 2015. They wanted to know whether the 2001 Health Council's recommendation to observe restraint in adding disinfectants to consumer products still applies in the light of current scientific insights. The issue of resistance development was of particular interest. Among other things, the ministers want to know whether there are any indications of increased microbial resistance to disinfectants due to increased use of these substances, and whether this can lead to health damage. They also wanted to know whether resistance to antibiotics increases due to the (increased) use of disinfectants. The ministers suggested broadening the scope of the advisory report to uses of disinfectants other than consumer products. Should the Health Council conclude that there are still sufficient grounds for restraint, the ministers will ask what exposure routes, applications and products contribute most to the development and maintenance of microbial resistance, whether national policy is required, whether said policy should extend to the use of disinfectants by professionals, and whether said policy should also include similar substances from other legal frameworks (cosmetics, detergents). Additionally, the ministers requested prioritisation of possible European measures, such as methods or guidelines for use. Finally, they hope that the advisory report may contribute to the operationalisation of the requirement in the Biocidal Products Regulation that biocides may not cause any unacceptable resistance in the organisms to be combated. The full request for advice may be found in Annex A to this advisory report.

1.3 Committee and method

On 21 January 2015, Professor J.L. Severens, Vice President of the Health Council, appointed the Disinfectants Committee. The Committee is composed of experts from multiple relevant fields, expressly selected for diverse views on the topic to be studied. Annex B contains a list of committee members.

1.3.1 Task definition and demarcation

As suggested in the request for advice, the Committee did not limit itself to uses for and by consumers. Significant quantities of disinfectants are also used in other societal sectors, including the medical field, the agricultural sector, the food industry and water treatment, often by professionals. The same antimicrobial substances are often involved. An adequate analysis of resistance requires a coherent, connected view of all applications.

The Committee also considered a second expansion necessary, which was also part of the 2001 advisory report. Whether a product is considered a disinfectant (within European Union definitions) depends solely on the purpose for which the product is marketed: combating bacteria and viruses on surfaces. Chemical composition is irrelevant. The same or similar antibacterial substances that form the active ingredients in disinfectants are also used for other purposes, such as combating unpleasant odours (in cosmetics and personal hygiene products), preventing spoilage (preservatives in any number of products) or preventing and combating infections (in medical devices, such as silver compresses). Such uses and products are not covered by disinfectants legislation. However, they are relevant to the question of resistance development. Therefore, the Committee did not take the disinfectant itself as a point of departure, but rather the active ingredients (antibacterial substances) contained therein.

Considering the scope of its assignment, the Committee focused solely on bacteria where micro-organisms were considered, as resistance development and high disease burden go together in this group of organisms. Viruses and fungi are not considered. For a broad overview of resistance development in pathogens and plague organisms in response to exposure to biocides, the Committee refers to a report published by the National Institute for Public Health and the Environment (RIVM).[3]

The Committee has limited its focus to the health aspects of the issue. It is aware that there are also ecological, agricultural and economic aspects to consider in the decision-making process.

1.3.2 Approach

On 1 January 2016, disinfectants based on a total of 127 active ingredients (antibacterial substances) were available in the European Union. Discussing each in detail is beyond the scope of this report. The Committee also does not believe this is necessary to answer the questions asked by the ministers. In order to limit the amount of literature to be reviewed, the Committee focused primarily on a detailed analysis of resistance development in five (groups of) disinfectants: chlorhexidine, quaternary ammonium compounds, triclosan, silver compounds and chlorine-releasing compounds. These case studies are presented in the background document to this advisory report, entitled Resistance due to disinfectants.[369] Together, these substances are a good representation of the wide variety of chemical compounds used for disinfection. Furthermore, this selection is a fair reflection of usage in key sectors in society. Finally, a relatively large amount of scientific literature is available on the groups listed. Applying these limits in no way prevents the questions asked by the ministers from being answered. Where necessary, the Committee also comments on other substances in its discussion in the main report. As the search string 'disinfectant name AND resistance' yielded hundreds of articles per substance (781 for chlorhexidine, for example), the Committee obtained a great deal of its information from countless reviews, editorials and opinion articles published in authoritative journals in recent years. The Committee examined original research where this was deemed necessary. It also consulted recent reports by leading organisations, such as the National institute for Public Health and the Environment (RIVM), and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). The Committee expressly focused not only on determining what scientific evidence is present, but also on where the knowledge gaps and uncertainties lie. It did not perform any laboratory, clinical or field research, or any measurements.

In order to meet the information needs of all societal stakeholders with interests in the advisory report, and to take note of their perspectives and practical experience, the Committee held a hearing in Utrecht on 25 January 2016. The names of the participants are listed in Annex C. One of the Committee's scientific secretaries participated in a study day on the professional use of disinfectants on 15 March 2016 in Brussels, organised by the International Association for Soap, Detergents and maintenance Products (A.I.S.E.).

The draft advisory report was submitted to the Standing Committees on Public Health and Health Care for review.

1.4 Advisory report outline

In Chapter 2, the Committee describes what disinfection with chemical substances entails, what substances are used, and how they work. They also address the differences between disinfectants and antibiotics. The legal frameworks are also addressed, and the Committee discusses the sectors in which these substances are used, and what is known about the scope of their use. Chapter 3 is about resistance: what is resistance, how does it develop, and what health consequences does this have? The Committee also addresses the degree to which resistance to disinfectant substances and resistance to antibiotics can affect each other, and examines the potential for a predictive test for determining in advance the degree to which a newly marketed antimicrobial product will cause resistance. In Chapter 4, the Committee discusses other potential objections to the use of disinfectants: the toxicological risks and other harmful health effects. In the final Chapter, the Committee answers the questions asked by the ministers.

Chapter 2

Disinfection with chemical substances

In this Chapter, the Committee discusses what disinfection entails, which chemical substances can be used and how they work. The Committee also addresses the differences between disinfectants and antibiotics. The use of disinfectants is also examined: the legal frameworks, use in various sectors, and what is known about the extent of their use.

2.1 Terminology

Micro-organisms are ubiquitous in our environment. They are part of ecosystems and living communities, and fulfil functions essential to their maintenance. Micro-organisms can also transfer from their natural environments to man-made objects and products. Under certain circumstances, they can cause disease, spoilage or nuisance. In such cases, combating them may be required, for example via disinfection.

According to the definition of the Working Group Infection Prevention (WIP), disinfection is "the irreversible inactivation of micro-organisms (vegetative bacteria and/or fungi and/or viruses and/or bacterial spores) on lifeless surfaces, as well as on intact skin and mucous membranes".[4] Disinfection aims to minimise the risk of micro-organism transmission, without necessarily inactivating all micro-organisms.[4] Skin or mucous membrane disinfection is also called antisepsis. These descriptions are from the medical field. In the food industry, disinfection must reduce the number of micro-organisms to a level that does not affect food safety or suitability for consumption.[5] The Board for the Authorisation of Plant Protection Products and Biocides (Ctgb) applies harmonised, EU-wide pass criteria for marketing authorisation for all disinfectants. A disinfectant must be able to reduce the number of viable bacteria on a surface to be treated by a certain factor within a predetermined period of time. This factor varies between 10^3 and 10^7, depending on the type of product and type of test it is subjected to.[6]

A more rigorous approach to combating micro-organisms is sterilisation. This entails that even more difficult to combat bacterial forms are killed or inactivated (the odds of a live organism being present per sterilised unit is smaller than 1 in 10^{-6}).[4] A less stringent form of protection is cleaning. The purpose of cleaning is to remove dirt and organic material in order to prevent micro-organisms from being sustained and multiplying and spreading.[4] Cleaning will generally also lead to a reduction in the number of micro-organisms present. A final process related to disinfection is preservation. Preservation is applied in situations where the growth of micro-organisms must be prevented because they pose a contamination risk.[4] In addition to preventing contamination, preventing product spoilage due to micro-organisms or undesirable chemical reactions is an important aspect of preservation.

Disinfection may be performed with hot water, steam or chemical substances. Chemical products used for this purpose are labelled disinfectants. In general, these are mixtures of multiple chemicals, namely one or more active ingredients (the substances that inactivate or kill the micro-organisms) and one or more adjuvants (solvents, carriers, stabilisers, absorption promoters, potentiators). For the sake of simplicity, the active ingredients themselves are often also labelled as disinfectants. The Committee does this as well.

Although the distinction is not always clear, disinfectants differ from another well-known group of antimicrobial substances – antibiotics – in a number of ways.[7-9] Antibiotics are organic compounds that are generally produced by the micro-organisms themselves, or that are derived from compounds produced by micro-organisms. Disinfectants are synthetic organic compounds, simple inorganic compounds or pure chemical elements. Antibiotics are only effective against prokaryotic cells (cells without nuclei, bacterial cells in particular). Disinfectants are less selective, and are also effective against eukaryotic cells (cells with a nucleus, i.e. fungi, plants, and animals, including humans). Antibiotics are effective even when highly diluted, disinfectants require much higher concentrations. Antibiotics must generally achieve their task within a few days, sometimes longer. In contrast, disinfectants are usually only given a few minutes. Finally, the mechanism of action of antibiotics is very specific: they attack one specific process or structure on or in the cell, disrupting its function. Disinfectants attack multiple cellular structures. The fact that antibiotics are effective against bacterial cells at low concentrations and do not attack eukaryotic cells makes them ideal for combating bacteria on and particularly inside the bodies of humans and animals. They are supported by the host's immune system. Disinfectants are effective in concentrations that are generally too toxic for internal use.

2.2 Substances and mechanisms of action

Disinfectants based on a total of 127 active, antimicrobial ingredients were available in the European Union (count on 1 January 2016). The Committee presents an overview of the most important groups of disinfectants in Table 1, along with common representatives per group, mechanisms of action and applications. Classification is based on chemical relatedness of the substances. The mechanism of action differs per substance[10-21] and is dependent on concentration[13,19,20]. In practice, disinfectants are often used in high concentrations (grams/L) and have a very broad, fairly non-specific effect. If a product is used on skin or mucous membranes (antisepsis), its toxicity will limit in-use concentrations. If the product is used on lifeless surfaces, the upper limit to the in-use concentration is only determined by solubility, costs and potential corrosive properties.[8] Lower concentrations are used as preservatives compared with disinfection.[13,22] Quality requirements that apply to products generally limit the amount of preservative that may be added. The same chemical substance can often be used for a variety of purposes, with the concentration used being the most important difference. For example, chlorhexidine is used as a disinfectant in concentrations of 0.5-4 percent (5-40 g/L), as an antiseptic in concentrations of 0.02-4 percent (0.2-40 g/L) and as a preservative in concentrations of 0.0025-0.01 percent (25-100 mg/L).[23]

The efficacy of an antimicrobial substance depends primarily on the nature, number and form of the micro-organism that needs to be combated.[23] Due to the high in-use concentrations and multiple points of attack, disinfectants are effective against a broad range of micro-organisms. However, some substances are more effective against one group of micro-organisms, others against other groups.

The efficacy of disinfectants also depends on other factors.[23] The most important of these are the concentration of the substance used and the exposure or contact time. These are listed on the instructions for use issued by the manufacturer of the disinfectant. Short exposures or excessive dilution are

Table 1 Disinfectant substances, their mechanisms of action and applications[12,19]; the substances examined in greater detail by the Committee in the case studies are displayed in boldface type.

substance group	substance	mechanism of action	application
alcohols	ethanol isopropanol	protein denaturation in cytoplasm and membrane	antisepsis, disinfection, preservation
aldehydes	glutaraldehyde formaldehyde o-phtalaldehyde (OPA)	alkylation of amino groups in proteins and nucleic acids	disinfection, sterilisation, preservation
anilides	triclocarban	inhibits fatty acid synthesis, membrane damage at high concentrations	antisepsis, deodorant
biguanides	**chlorhexidine** alexidine polymeric biguanides	bonding to phosphate groups and phospholipid fatty acid chains in cell membranes	antisepsis, anti-dental plaque substance, preservation, disinfection
bisphenols	**triclosan**	inhibits enzyme in fatty acid synthesis, at high concentrations non-specific	antisepsis, anti-dental plaque substance, deodorant, preservation
	hexachlorophene	membrane damage, inhibits electron transport chain in membrane	
diamidines	propamidine dibromopropamidine	membrane damage, amino acid leakage	antisepsis, preservation
halogen-releasing compounds	**chlorine compounds** iodine compounds	oxidation of thiol groups, halogenation of aromatic amino acids in proteins	disinfection, cleaning, antisepsis
halophenols	chloroxylenol (PCMX)	probably membrane damage	antisepsis, disinfection, preservation
heavy metals	**silver compounds** mercury compounds copper compounds zinc compounds	interaction with protein thiol groups	antisepsis, preservation, disinfection
peroxides	hydrogen peroxide ozone peracetic acid	oxidation of thiol groups and double bonds	disinfection, sterilisation, antisepsis
phenols and cresols	phenol cresol	protein denaturation in cytoplasm and membrane	disinfection, preservation
quaternary ammonium compounds	**cetrimonium chloride** **benzalkonium chloride** **cetylpyridinium chloride**	bonding to phosphate groups and phospholipid fatty acid chains in cell membranes	disinfection, antisepsis, preservation, cleaning, deodorant
gas-forming substances	ethylene oxide formaldehyde	alkylation of amino groups in proteins and nucleic acids	sterilisation, disinfection

common causes of failed disinfection. Additionally, external factors also play a role, such as temperature, acidity, water hardness and most importantly the presence of organic material. Contact with this material can drastically reduce the effective concentration of the active substance. Some disinfectants are particularly susceptible to this effect. Therefore, cleaning is always recommended prior to disinfection. An additional advantage is that some of the micro-organisms are removed by cleaning.

Some disinfectants, such as certain quaternary ammonium compounds, chlorhexidine, triclosan and heavy metals leave residues behind on the treated surfaces, and have long-acting antimicrobial effects. In contrast, other substances, such as alcohols, chlorine compounds and ozone, evaporate or disintegrate quickly. Their antimicrobial effect is short-lasting.

2.3 Current use of disinfectants

2.3.1 Legal frameworks

Hundreds of chemical products with disinfecting properties are available on the Dutch market. Depending on the intended use and manufacturer claims, they are subject to different legislation. Most disinfectants fall under the Biocidal Products Regulation. On 1 January 2016, 760 different disinfectants classified as biocide based on a total of 45 active ingredients (antimicrobial substances) were available in the Netherlands. Based on the nature of their use, these products may be divided into five types:

- PT01: products for human hygiene
- PT02: disinfectants not used directly on humans or animals, but intended for the disinfection of surfaces, materials, equipment, furniture, swimming and waste water
- PT03: products for animal hygiene
- PT04: products for disinfection of equipment, retainers, eating and drinking utensils, surfaces or pipelines for the production, transportation, storage or consumption of food or animal feed
- PT05: products for drinking water disinfection.

Additionally, there are disinfectants that are legally classified as (veterinary) medicines. This applies to disinfectants applied to damaged skin (wound disinfection) or to skin that will subsequently be opened (prior to surgery or placement of piercings or tattoos).[24] Other items, such as bandages containing disinfectants, are subject to medical devices legislation. This also applies to products intended for use in endoscope disinfectors.[25] Cosmetics legislation applies to products with antimicrobial properties that serve a cosmetic rather than a medical purpose, such as (some) deodorants, toothpastes and mouthwashes. For antimicrobial soap products focused primarily on (hygienic) cleaning, detergents legislation may apply.

Depending on the applicable legal framework, products may or may not be required to complete an authorisation process before marketing, and different requirements apply for the required evidence for product efficacy and safety for humans and the environment. The borders between various legal frameworks are not sharply defined. Therefore, various guidelines have been developed to help determine which framework a product is subject to and thus the requirements it must meet.[24,26,27] In order to provide an impression of the immense variety of applications of disinfectants, the Committee will briefly touch on disinfectant use in a number of key societal sectors in the following paragraphs. It makes a distinction between professional and private use.

2.3.2 *Professional use in various sectors*

Medical sector (biocides PT01 and PT02, medicines, medical devices)

Patients with (transmissible) infectious diseases and patients with reduced immunity due to injury, disease or treatment come together in the hospital. Invasive procedures are also performed. This means there is a constant risk of hospital-acquired infections. In 2015, 4.6 percent of hospital patients in the Netherlands had one or more healthcare-associated infections.[28] Hospital hygiene policy is therefore focused on minimising infection risk. Crucial measures taken to achieve this are the isolation of patients with transmissible diseases or multi-resistant pathogens, patient skin disinfection prior to invasive procedures, hand hygiene among medical staff, and cleaning, disinfection or sterilisation of medical equipment and surfaces in the hospital environment.[29] The WIP* has drafted a large number of guidelines for hospitals, long-term care facilities, rehabilitation facilities and home care, precisely describing the hygiene measures that need to be taken in a certain situation, and how to implement them. They are updated regularly based on new scientific insights. Medical microbiologists and infection prevention experts shape hygiene policy, supervise correct implementation and investigate and deploy measures in the event of infection outbreaks. The Health Care Inspectorate is responsible for oversight. This institution recently determined that infection prevention in hospitals requires improvement.[30]

The domain of healthcare hygiene is an active field of research: new ways to further reduce healthcare-associated infections are continuously being investigated. This includes both technical developments and new procedures. For

* http://www.rivm.nl/Onderwerpen/W/Werkgroep_Infectie_Preventie_WIP

example, studies are being conducted involving surfaces and materials on or in rooms, furniture, instruments and textiles that have been made self-disinfecting using coatings or impregnation with disinfectants (including metals such as silver, copper and titanium oxide, triclosan).[31,32] A specific example is suturing material with an antibacterial coating to reduce infection of a sutured wound.[33] Innovations of a more procedural nature include daily washing of patients admitted to intensive care wards with chlorhexidine[34,35] and the disinfection of non-critical surfaces (such as walls, floors, instrument panels) that the patient does not personally come into contact with[36,37]. For many of these innovations, their efficacy in reducing hospital-acquired infections still requires further investigation before broad implementation in daily practice. Others have already been implemented in daily practice to a greater or lesser degree, despite the fact that their efficacy or cost/benefit balance is still a topic of scientific debate.

Agricultural sector (PT03 and veterinary medicines) and the food industry (PT04)

Good cleaning and disinfection are also important in the agricultural sector. This is particularly true in animal husbandry.[38] Good hygiene should primarily benefit the health and welfare of the animals, but is indirectly also important to the health of humans, because it reduces the risks of outbreaks of zoonoses and food poisoning due to pathogens such as *Salmonella enteritidis* or *Campylobacter*. Common practices differ significantly per sub-sector. Use of disinfectants may be related to cleaning and disinfection of stables, particularly between flocks. Disinfectants are also used to prevent the spread of infectious diseases. Examples include decontamination of cattle trucks and bins with disinfectant solution for boot decontamination at the stable door. Disinfectants can also be applied to the animals themselves. This may be done preventively, for example, on the udders of dairy cows prior to milking to prevent contamination of the milk with manure bacteria, or after milking to protect milk ducts from invasive pathogens. Products used curatively, for example, to treat mastitis or claw diseases, are subject to the Veterinary Medicines Act. Extensive cleaning and disinfection is performed daily at slaughterhouses.

On a smaller scale, disinfectants are also used in other sub-sectors. The farming of sprouting vegetables, such as bean sprouts, alfalfa, and cress, decontamination of the primary material is performed because growing conditions promote the growth of bacteria that can easily result in food-borne infections with, among others, *salmonella* or *coli* bacteria.[39] Other sectors where disinfection is common are mushroom growing and fish farming.

Cleaning and disinfection are also extremely important for the food industry in order to control the quality of the product, increase shelf-life and guarantee food safety. A broad range of cleaning and disinfection products is used every day to decontaminate surfaces and ductwork that comes into contact with food and drink during production, transportation and storage processes. Cleaning and disinfection steps and quality control are often fully integrated into the manufacturing process, particularly in large companies.

The manufacture of food must meet the Commodities Act, which states that the manufacturer must ensure the safety of its products. To do so, the manufacturer must implement a so-called *Hazard Analysis and Critical Control Points* (HACCP) procedure. This is often achieved by application of industry or sector specific hygiene codes that dictate where, when and how certain hygiene measures must be taken. This includes the use of chemical disinfectants.

In large food companies, cleaning and disinfection are often outsourced to specialised companies. Expertise is likely more limited in smaller companies and in the primary production sector. The design of stables and cattle trucks, and the presence of ample quantities of organic material also complicate good cleaning and disinfection. The overseeing body for food safety is the Netherlands Food and Consumer Product Safety Authority (NVWA).

Other sectors (PT02, PT05, PT11, PT12)

Professional cleaning and disinfection is common in a large number of other societal sectors. Water treatment is one of the most important. This includes disinfection of swimming water (PT02), coolant water (PT11) and waste water. In the past, chlorine was used for the primary disinfection of drinking water (PT05), but this form of disinfection was discontinued in 2005 due to the formation of by-products harmful to health.[40] Disinfection is also common in the sports and recreation sector, hospitality and childcare sectors, often following sector-specific hygiene codes.

2.3.3 *Private use in the household and for personal hygiene (PT01, PT02, (veterinary) medicines, cosmetics)*

Good household hygiene, particularly during food preparation, is very important in preventing infectious diseases.[41] In addition to water and regular soap, consumers also have access to countless products that have antibacterial properties thanks to the addition of disinfectant substances. This includes hand soap, hand alcohol, wipes and cleaning products. According to manufacturers, they offer users additional security. The use of such products is controversial. In 2001, the Health Council concluded that there was no evidence that the use of such products (without a medical indication) by private individuals made an additional contribution in reducing the disease burden due to infectious diseases compared to good water and soap hygiene.[1] The Committee is of the opinion that more recent research has not changed this conclusion. Although a later meta-analysis did find that antibacterial soap results in a slightly stronger reduction (0.5 log) in the number of bacteria found on contaminated hands than regular soap[42], there is still no scientific evidence that use of antibacterial soap or gel at home, school or work results in health benefits.[41,43-54] Due in part to this fact, the US Food and Drug Administration recently terminated marketing authorisation for a number of consumer antibacterial soap products.[55] Use of alcohol-containing hand gel is recommended in situations where water and 'regular' soap are unavailable, for example, on holiday.* In cases where a medical indication exists - where there is an elevated risk of infections – use of antibacterial products at home is useful, but only in consultation with and after receiving instructions from a doctor or other expert. This may be the case when combating MRSA[56] or the Norovirus[57] or in patients with home haemodialysis or peritoneal dialysis[58].

The evidence that antimicrobial substances in toothpaste provide health benefits seems stronger. Triclosan in toothpaste reduces the amount of dental plaque, gingivitis, gingival bleeding and tooth crown caries by between a few and a few dozen percentage points.[59] Mouthwash with disinfectant substances (chlorhexidine in particular) appears to be effective against dental plaque and gingivitis.[60] However, these health gains are also associated with health risks (see Chapter 4).

Disinfectants are also used by private individuals to treat nuisances. Examples are use of disinfectants to treat algae, mould on damp walls, and smells in car air conditioning.

There is also a large variety of different products for consumers with added antimicrobial substances (often silver or triclosan).[61,62] These include textiles, building materials, kitchen utensils, bathroom accessories, cleaning items, office supplies and articles for the care of babies and young children. Manufacturers and importers promote their positive effects on hygiene and their contributions to preventing illness. However, research into their efficacy is limited, never mind their contributions to reducing disease burden due to infections. Research into the usefulness of articles and surfaces treated with disinfectants in food

* See the US CDC, for example: http://www.cdc.gov/handwashing/when-how-handwashing.html.

preparation has not demonstrated any health benefits.[63-65] Products may also contain antimicrobial substances to prevent the formation of unpleasant odours. This includes both personal hygiene products (deodorants, washing gels) and textiles (e.g. socks with silver).

Finally, many consumer products, such as cosmetics, contain low concentrations of antimicrobial substances to preserve the product itself from decay.

2.4 Extent of use and trends over time

2.4.1 Extent of use

Data on the extent of disinfectant use are extremely scarce.[38] The Committee found almost no useful data on use in our own country. Disinfectant use in hospitals in the Netherlands was estimated in 1998.[66] Consumption was estimated at 12 tonnes per year for the product Sekusept and 165 tonnes per year for bleach. The combination of chlorhexidine and cetrimide was estimated at 16 tonnes per year. However, it is unclear whether these numbers applied to the formulated product or the active ingredients. The use of disinfectants in the food industry at the time was estimated to be 14,000 tonnes of active ingredients per year (Van Haelst 1996; referenced in[66]).

More information about the extent of use is available in some of the countries around us, particularly in Belgium. Since 2010, marketing authorisation holders for biocides must indicate how much of each biocide they market in a given year. In 2011, over 10,000 tonnes of active ingredients were marketed.[67] About 6,000 tonnes of this was processed into disinfectants. The largest amounts were used in PT02 (non-living surfaces and water treatment) and PT04 (foodstuffs). The most used active ingredients are hypochlorite and hydrogen peroxide. In the entire European Union, total annual production and import of biocides around the turn of the century was about 400,000 tonnes of active ingredients, two-thirds being disinfectants.[68]

Note that the above figures do not include usage figures for antimicrobial substances in products subject to other legal frameworks, medicines, veterinary medicines, medical devices, cosmetics, detergents and food additives. The Committee has no overview of the amounts of active antimicrobial ingredients in these products, but it is clear that larger quantities than classified as biocides are involved in some cases. For example, about 350 tonnes of triclosan is used each year in Europe, for example in cosmetics and personal hygiene products.[69]

2.4.2 *Trends over time*

The European biocides market is valued at around EUR 10-11 billion. This has grown by four to five percent annually over the past fifteen years.* The global market for biocides and disinfectants is expected to grow by over ten percent per year in the coming years. These figures are based on expected growth in South-East Asia and China. A growth rate of between one and two percent is expected for Europe and the U.S.**

The trends for individual antimicrobial substances and applications may diverge significantly from this overall trend. For example, the number of patent publications on nanosilver in consumer products has increased from one or two per year in the 1980s to 162 in 2010 (Lem, 2014). According to RIVM, the number of consumer products with nanosilver increased from 47 to 235 between 2006 and 2008 (Wijnhoven, RIVM 2009).

2.5 Conclusions

Disinfectants are often of major importance, particularly in healthcare, the food industry and the agricultural sector. The efficacy of disinfectants is often clear-cut, but there are applications for which efficacy in practice is the topic of ongoing scientific debate, such as daily washing of patients with chlorhexidine on hospital intensive care wards, or routine hospital floor, wall and ceiling disinfection. The health impact of use in consumer products has barely been researched and is mostly unproven. The amounts of disinfectants used are not exactly known, but based on weight are at least ten times higher than those of antibiotics.

* http://www.pan-europe.info/campaigns/biocides (consulted 5-10-2015).
** http://www.european-coatings.com/Markets-companies/Raw-materials-market/Study-analysis-emerging-markets-and-opportunities-of-specialty-biocides (consulted 5-10-2015).

Chapter 3

Resistance to disinfectants

Resistance to antibiotics is currently considered to be one of the greatest threats to public health.[70,71] Experts believe there is a very real risk that antibiotics as medication will (largely) be lost, and that medical practice will essentially return to the times before antibiotics.[72-80] This would not only have serious consequences for treating infections, but also for all kinds of common medical procedures, which can only be performed safely due to the prophylactic use of antibiotics.[70,81] In this Chapter, the Committee examines to what degree resistance is also a problem when it comes to disinfectants. First, it discusses what resistance entails, how it develops, and how it spreads. Second, the extent of the phenomenon and its health significance and the interaction with resistance to antibiotics are examined. Finally, the Committee discusses a potential test that may allow evaluation of the degree to which a new disinfectant contributes to resistance development.

3.1 What is resistance?

3.1.1 Clinical definition for antibiotics

For antibiotics, ‘resistance’ is often defined on clinical grounds. A bacterial strain is resistant to a specific antibiotic if the strain is no longer killed or its growth prevented by antibiotic concentrations achievable in medical/veterinary practice at the site of a bacterial infection. The Minimum Inhibitory

Concentration (MIC) for the specific antibiotic is determined for each bacterial strain isolated from a patient, i.e. the lowest concentration that can still prevent the growth of the bacterial strain. This MIC is compared to internationally determined reference values, so-called breakpoints. Bacterial strains with a MIC above the highest breakpoint are considered clinically resistant. Even maximum dosage will not achieve the required concentration at the infection site.

3.1.2 *Broader definition for disinfectants*

This clinical definition of resistance is not very useful for disinfectants. This is because there are no defined breakpoints that correlate with the outcomes of clinical treatment for these substances in practice. This means that scientists and professionals sometimes use the term 'resistance' to mean different things when discussing disinfectants.[82] Sometimes the term is used to indicate that common in-use concentrations of a disinfectant are unable to reduce the number of bacteria of the studied strain in a standard test set-up sufficiently quickly (e.g. by a factor of 100,000 within five minutes). Sometimes the term is used for bacterial strains that have a higher MIC or MBC (Minimum Bactericidal Concentration, minimum concentration required to kill a bacterial strain) for a certain disinfectant than most (e.g. 95%) other studied strains of the same species. Finally, the term is often used for a bacterial strain that has developed a higher MIC or MBC than the parent strain for a certain disinfectant due to obtaining a new genetic property.

The Committee uses the term resistance to mean 'reduced susceptibility'. It thus encompasses all three of the above definitions. Defined like this, resistance is not an absolute, but a relative concept; it only indicates that one bacterial strain is less susceptible than another, or has become less susceptible than it previously was due to a change. In practice, such a bacterial strain will only show growth inhibition or death after higher levels of exposure or longer contact times. An advantage of this definition is that smaller, gradual changes and trends in susceptibility are covered, which may presage greater resistance in future. The Committee realises that, under this definition, not all forms of resistance to disinfectants can be translated directly to limited efficacy of these substances in daily practice. This is in contrast with the clinical definition of resistance used for antibiotics.

3.2 How does resistance develop?

3.2.1 Mechanisms

There are four major mechanisms that bacteria can use to reduce their susceptibility to antimicrobial substances:

- reducing permeability of their cell wall, preventing the antimicrobial substances from penetrating the bacterial cell
- removing antimicrobial substances that have entered the cell (using so-called efflux pumps) before they can cause damage
- making targets for the antimicrobial substances less susceptible by changing their structure, replacing them with alternatives, or producing them in excess
- producing enzymes that degrade or modify the antimicrobial substances in such a way that they lose their efficacy.

All four mechanisms play a role in resistance to antibiotics, with changing of targets or modification of antibiotics having the potential for high levels of resistance. The genetic information for these resistance mechanisms has always been present within populations of micro-organisms; antibiotics and resistance to antibiotics are natural phenomena that play a role in the ecology of microbial populations. Unlike antibiotics, disinfectants – at least at higher concentrations – do not have a single target in or on the cell, but have many.[8,19,20,83] Therefore, modification of targets is usually not a feasible option for bacteria.[84] Degradation or inactivation of the disinfectant will generally also be of subordinate importance due to the high in-use concentrations.[14,84] Therefore, protection of the cell using a difficult to penetrate cell wall and efflux pumps are the two most important defensive tactics used by bacteria to counter disinfectants.[85]

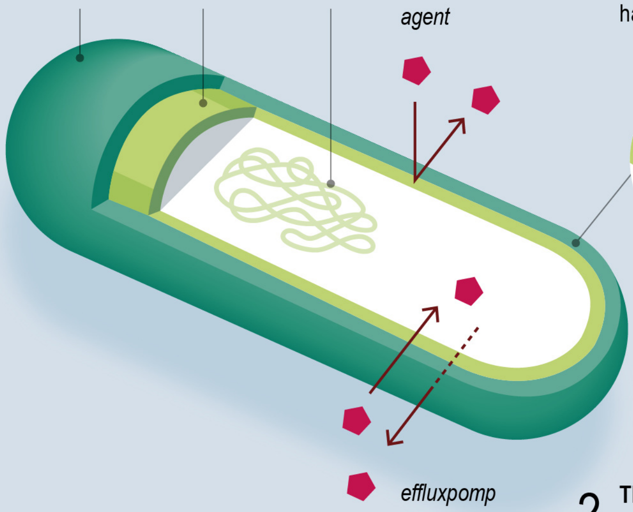
3.2.2 How do bacteria obtain their resistance mechanisms?

Low susceptibility – or resistance to – antimicrobial substances may be intrinsic, adaptive or acquired.[86] Bacterial spores are intrinsically, i.e. by their very nature, resistant to (most) disinfectants.[16] Their lack of susceptibility is due primarily to their impenetrable spore wall. Mycobacteria (such as the tuberculosis bacterium) have the unique composition of their cell wall to thank for their high intrinsic resistance to disinfectants. Bacteria with a double membrane in their cell walls (so-called Gram-negative bacteria, such as Escherichia coli and Salmonella enteritidis) are naturally less susceptible to disinfectants than bacteria with a

Four resistance mechanisms

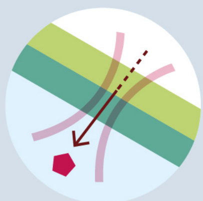
Some bacteria are less susceptible to antimicrobial agents due to:

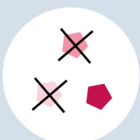
1 **A less permeable cell wall,** making it more difficult for the agent to enter the cell.



For example, Gram-negative bacteria have **a double membrane.**

2 **The presence of efflux pumps** that can remove the antimicrobial agents from the cell before it can cause harm.





3 **Degradation or modification** of antimicrobial agents using enzymes.

4 **Modifying target sites** of antimicrobial agents.

Mechanisms 1 and 2 are the most important defence mechanisms against disinfectants, 3 and 4 also against antibiotics.

single membrane (so-called Gram-positive bacteria, such as staphylococci).[16] The presence of efflux pumps can also contribute to some bacteria being naturally less susceptible to (certain) disinfectants.[87]

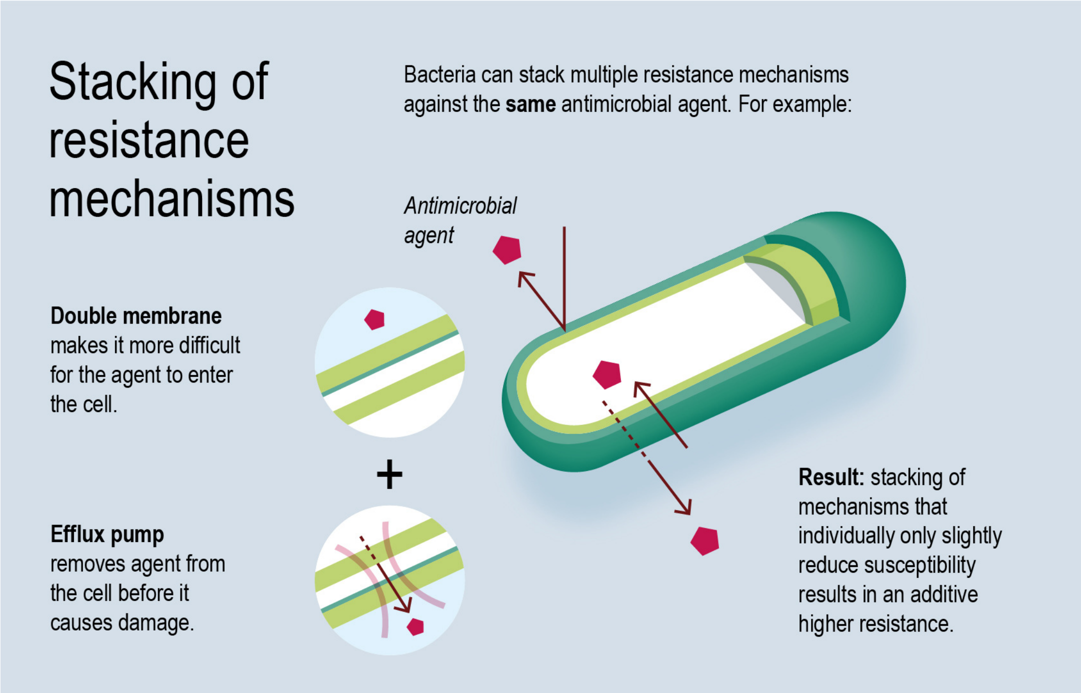
Exposure to non-lethal (sub-lethal) concentrations of disinfectants can trigger stress reactions in susceptible bacteria that induce temporary changes in the activities of genes. This can result in changes to the composition and permeability of the cell wall[88] or in increased activity of the efflux pumps[89-91]. Experts consider this to be an adaptive reduction in susceptibility. Adaptive changes are generally reversible; they disappear once exposure ends. However, there are some indications that susceptibility may not always fully return to initial levels.[86,92]

Finally, susceptibility to disinfectants may be reduced permanently via acquisition of new genetic properties, either via mutations in existing genes or by obtaining new genes from other bacteria. Bacteria can exchange genetic material, even across species, a phenomenon known as horizontal gene transfer (HGT). These new genetic properties may render the cell wall permanently less permeable to a disinfectant, permanently activate efflux pumps that are already present, or provide entirely new pumps. Resistance mechanisms against antimicrobial substances likely arise by modification of natural structures or processes within the cell.[93,94]

Exposure to sub-lethal concentrations of disinfectants can also stimulate bacteria to form biofilms.[95-107] Bacteria move out of a solution and on to a surface, attach to it, and surround themselves with a protective organic polymer layer.[108] The concentrations of antimicrobial substances required to prevent the growth of bacterial biofilms may be up to ten to a thousand times higher than those required to inhibit the growth of their planktonic compatriots.[109] Biofilms are also an optimal environment for HGT[110,111] and potentially for the development of mutations[110]. Exposure to sub-lethal concentrations of antimicrobial substances also increases the frequency of mutations and HGT.[112-120] This increases the probability of a few individuals in a stressed population of billions of bacteria undergoing a beneficial genetic modification by chance that makes them less susceptible to the stressor in question.

3.2.3 *Stacking of resistance mechanisms*

Over time, bacteria can acquire multiple resistance genes and mechanisms against the same disinfectant.[121] For example, the presence of efflux pumps in Gram-negative bacteria is particularly worrying, because their double membrane is already an obstacle to entry for various antimicrobial substances.[122,123] Resistance



mechanisms that result in a slight reduction in susceptibility may pave the way to acquiring additional mechanisms.[124-127] Intrinsic and adaptive resistance contribute to the development of acquired resistance.[86,128-132] Stacking of mechanisms that individually only have a limited effect on reducing susceptibility can thus result in high levels of clinically relevant resistance. In many cases, the development of resistance is a gradual, multi-step process.[129,133-135]

3.3 Selection and spread of resistant bacteria

3.3.1 Survival of the fittest

Correctly performed disinfection will result in a significant reduction in the total number of bacteria present (ideally by a factor of at least 100,000 in five minutes). However, live bacteria (almost) always remain. Generally, a heterogeneous population of bacteria is present at the site to be disinfected, consisting of more and less susceptible strains and species. It may contain individuals that are resistant enough to survive disinfection. Additionally, bacteria may seek shelter in biofilms, traces of difficult to remove organic material, and nooks, crannies and seams. There, and at the margins of the disinfected areas, bacteria may be exposed to lower concentrations or during shorter times, allowing them to survive disinfection. Inexpert use of disinfectants can further exacerbate the situation. Sub-lethal exposure can promote the development or acquisition of resistance mechanisms. Repeated disinfection will result in the elimination of susceptible bacteria and the selection of strains and species with reduced susceptibility. The latter can then multiply, filling the space left after the disappearance of more susceptible bacteria. This is about survival of the fittest. Resistant bacteria can spread via water, air and product streams, or via humans and animals. Bacteria can also transmit resistance genes to each other via HGT, further promoting the spread of resistance.

3.3.2 *Cross-resistance, co-resistance and co-selection*

As indicated previously, bacteria can stack multiple resistance mechanisms against the same antimicrobial substance and thus achieve higher levels of resistance. However, bacteria can also collect resistance mechanisms against different antimicrobial substances. Such bacteria are called multi-resistant. A resistance mechanism against a particular disinfectant or antibiotic can also provide (some) protection against one or more other substances.[136] For example, some efflux pumps can remove both chlorhexidine and quaternary ammonium compounds from the cell. Experts call this cross-resistance.

Additionally, bacteria may have multiple resistance mechanisms at their disposal that each protect against another substance. If these bacteria multiply, all of these resistance mechanisms are transmitted to the daughter cells. If the genetic information for various resistance mechanisms is physically linked on the same piece of DNA, for example a chromosome or a plasmid, it can also be transmitted to other bacteria via HGT. This transmissible form of linked resistance mechanisms against multiple antimicrobial substances is called co-resistance.

Cross-resistance and co-resistance can result in exposure to one substance being sufficient to select the bacterium in question. If this bacterium subsequently multiplies and spreads its genetic information to other bacteria via HGT, this automatically also promotes resistance to the other antimicrobial substances. This phenomenon is known as co-selection.[137] It explains why one antimicrobial agent can contribute to resistance to another agent.

Selection

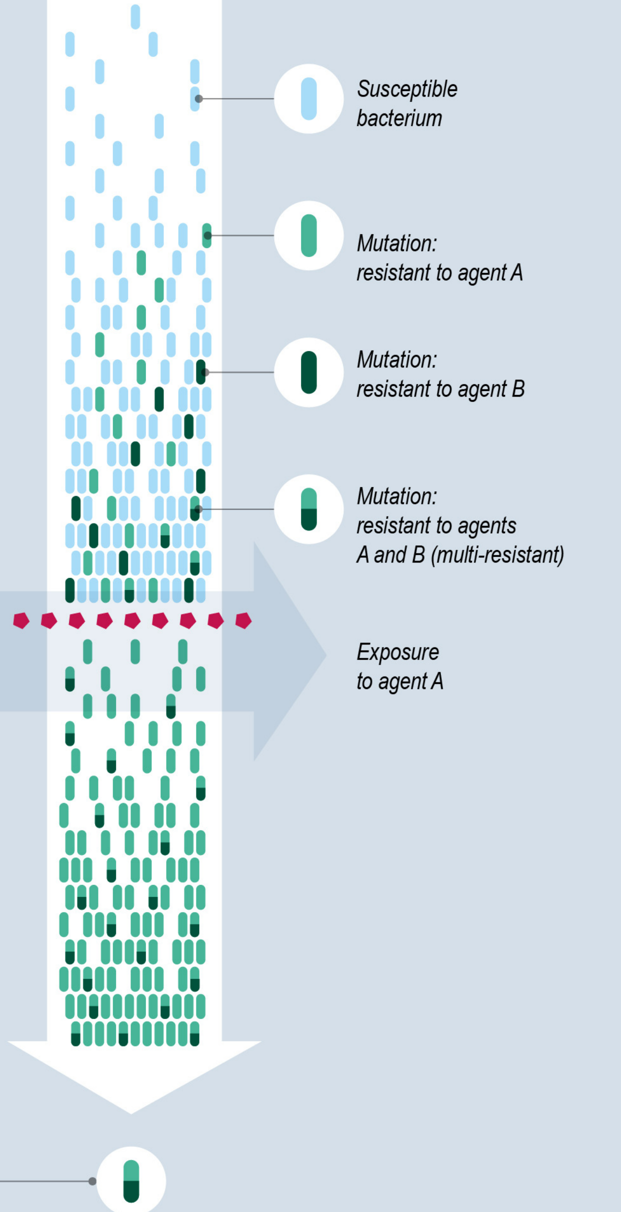
Exposing bacteria to antimicrobial agents (disinfectants and antibiotics) makes them less susceptible.

1 Bacteria multiply continuously. Spontaneous DNA mutations occur during this process.

2 Some mutations provide greater survival advantages against antimicrobial agents.

3 When exposed to antimicrobial agent A, all bacteria resistant to agent A survive.

4 Bacteria resistant to agent A and multi-resistant bacteria (resistant to A and B) survive and have room to multiply.



Co-selection

Exposure to one of these anti-microbial agents (agent A, in this case) is enough to select bacteria resistant to both agents (A and B).

3.3.3 Selection in the environment

Some disinfectants, such as hydrogen peroxide, ethanol and chlorine, quickly degrade or evaporate after use. Others, such as quaternary ammonium compounds, triclosan and triclocarban, end up in the sewer and, via water purification plants, in surface water. Disinfectants can also enter the soil via spread manure or slurry. Dilution and degradation result in environmental levels that are significantly lower than those used in practice.[138-146] However, some disinfectants are present in the environment in higher concentrations than any antibiotic.[145,147] Degradation and transportation processes also result in continuously fluctuating concentrations. Bacterial populations along these transport routes will be exposed to sub-lethal concentrations that may result in the induction, selection and spread of less susceptible bacteria. Competitiveness experiments with antibiotics and disinfectants in the laboratory have shown that concentrations far below the MIC enable less susceptible bacteria to gradually supplant their more susceptible kin.[135,148-150] In the laboratory, this already occurs at concentrations near the highest concentrations found in the outdoor environment. If multiple antimicrobial substances are present at the same time, this supplanting process in the laboratory occurs at even lower concentrations.[150] This may indicate that selection of less susceptible bacteria may not remain limited to the site of application, but may also occur elsewhere in the (outdoor) environment.[151] However, no research has been conducted to discover whether this actually occurs, and on what scale.

3.3.4 Resistance is tenacious

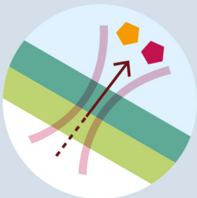
Bacteria often pay a price for their resistance mechanisms against antimicrobial substances, often in the form of reduced growth capacity. These so-called fitness costs may be due to the consumption of large amounts of raw materials and energy required for the production and continued operation of efflux pumps. Energy and materials that could otherwise have been used for growth. Reduced membrane permeability can make obtaining certain nutrients more difficult, and thus slow growth. The scale of these fitness costs varies by resistance mechanism and bacterium.

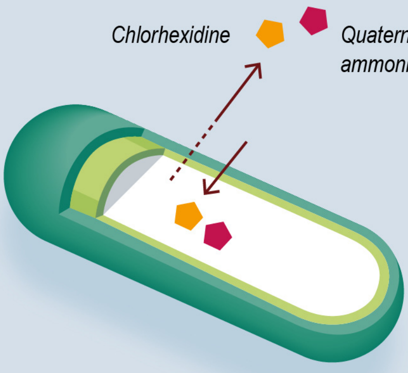
These fitness costs imply that resistant bacteria, at least in theory, are at a disadvantage compared with their susceptible kin, and will be out-competed as soon as the antimicrobial substance they are resistant to has disappeared from their environment. This too is all about *survival of the fittest.* This would mean

Cross-resistance

A resistance mechanism against a specific antimicrobial agent may also protect against one or more other antimicrobial agents.

Some efflux pumps can remove different antimicrobial agents from the cell, such as:





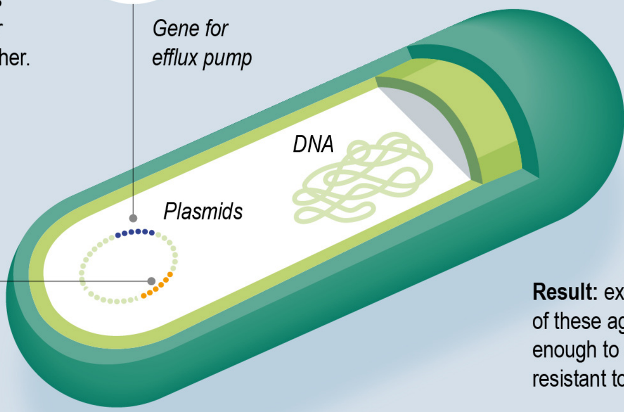
Result: exposure to one of these agents is enough to select bacteria resistant to both agents.

Co-resistance

Resistance mechanisms against various anti-microbial agents are linked because their genes are located on the same piece of DNA. They are always passed on to progeny or neighbouring cells together.

In addition to regular DNA, a bacterial cell can contain plasmids, independent circular segments of DNA.

Bacteria can exchange resistance mechanisms using **plasmids**, even crossing species lines.



Result: exposure to one of these agents is enough to select bacteria resistant to both agents.

that resistance problems would quickly disappear entirely if the antimicrobial substance in question were no longer used. However, experience has shown otherwise.[121,134,152-157] The explanation for this is that resistant bacteria can succeed in suppressing these fitness costs by acquiring additional mutations.[158] Furthermore, very low exposure to antimicrobial substances has been shown to select mutants with low fitness costs that can persist easily once the substance has disappeared from the environment.[159] This does not mean that reducing use is futile. This is demonstrated by the recent drop in antibiotic use in Dutch cattle farming: the result has been a significant drop in the share of resistant bacteria.[160]

3.4 Scope of the resistance problem and significance for health

3.4.1 Extent of the spread of resistance

Reduced susceptibility to disinfectants – like resistance to antibiotics – is a global phenomenon. Resistance has been observed in one or more bacterial species for all disinfectants studied.[83] In the case studies (see background document to this report, entitled Resistance due to disinfectants[369]), the Committee provides a detailed overview of resistance developments against a number of commonly used disinfectants. The Committee will focus on the main trends in this Section.

Bacteria with reduced susceptibility to disinfectants are found regularly in places where these products are frequently used. This is true for all societal sectors where these products are used, but has been studied best in clinical settings (hospitals)[12,16-18,83,161,162], the food industry[20,136,163] and in water treatment.[164-167] Chlorhexidine and quaternary ammonium compounds may top the list, with, depending on the country, up to 80% of clinical staphylococci strains examined in various studies having resistance genes for efflux pumps.[168] This percentage hovered around 42% in Europe in the late 1990s. Comparing the susceptibility of (or presence of resistance genes in) recently isolated bacterial strains with those of old isolates from microbiological collections pre-dating (large-scale) use of disinfectants often reveals a clear increase in resistance.[170-174]

3.4.2 Level of resistance

The scientific literature regularly indicates that disinfectants almost always induce a limited reduction in susceptibility. The Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of less susceptible strains are generally only two to eight times higher.[175] The concentrations of disinfectants used in practice are almost always 100-1000 times higher than the MICs or MBCs for susceptible species. According to many experts, the efficacy of the substances when used as prescribed is not or hardly affected by the slight increase in MIC or MBC.[175-179] High resistance levels, where commonly used concentrations of disinfectants are no longer effective, are rarely observed despite over half a century of use for many substances.[114,179-181]

According to the Committee, there are some problems with this viewpoint. The scientific literature regularly features reports of outbreaks of healthcare-associated contaminations and infections related to the use of disinfectants. This includes disinfection solutions that have themselves become contaminated with bacteria, and failing disinfection procedures for reusable medical devices such as endoscopes. Investigations into such incidents work intensively to track down sources and transmission routes of micro-organisms that have caused the incident, and on scrutinizing the disinfection procedures applied. However, potential resistance to disinfectants is often not examined as a potential cause of the outbreak. The Committee draws this conclusion based on an analysis of 138 publications listed in three review articles.[29,182,183] The Committee reviewed these articles to determine whether the bacteria responsible for the outbreak were screened for susceptibility to disinfectants. In 108 of the 138[*] publications cited, pathogens were not tested for susceptibility to disinfectants (but often were tested for susceptibility to antibiotics). In 13 publications, they were indeed tested for their susceptibility to the disinfectant used. Only in one of these cases, the pathogen was susceptible to the disinfectant used.[184] In the other 12 articles, the pathogen was found to be resistant to in-use concentrations of the disinfectant used (see Annex D).

Thus, resistance to disinfectants likely does play an important role in incidents involving disinfection failure in daily practice, but this role currently remains largely unexamined due to the focus on tracking down sources, transmission routes and procedural errors during outbreaks, thus failing to consider resistance to disinfectants as a potential determinant. The Committee suspects this is likely also true in other sectors. For example, a recent publication by the Dutch Safety Board into the causes of a salmonellosis epidemic due to smoked salmon did not investigate the potential role of resistance development.[185]

* Eleven articles could not be reviewed due to their age; no pathogen was isolated in six publications.

3.4.3 Health significance of resistance

Due to the relatively low levels of resistance observed and the lack of examples of high resistance, the health significance of resistance development is regularly doubted or challenged in the scientific literature.[177,186-190] The Committee agrees that the development of resistance to disinfectant substances appears to be less severe than for antibiotics. This may be explained by the high in-use concentrations used in practice and by the multitude of targets on and in the cell. Additionally, reduced susceptibility to disinfectants is based mainly on (changes in) the composition of the cell wall of micro-organisms and the presence, acquisition or expression of efflux pumps. These changes generally result in lower levels of resistance than modification of specific targets for antibiotics or degradation of antibiotics by enzymes.[126] Conversely, the Committee has the strong impression, based on the analysis of outbreaks of infections in hospitals described above, that the role of resistance often remains unexamined, and that clinically relevant levels of resistance may well be less rare than often believed. Resistance to disinfectants has also been found to contribute to disease and mortality in the treatment of burns and in skin disinfection.[191-194] Frequent skin disinfection in hospital intensive care wards has been found to cause shifts in pathogenic bacteria towards strains with resistance genes against the disinfectants used.[195] This is clinically relevant because such strains are more difficult to combat.

The development of resistance can also have health significance outside of healthcare settings. Recent Dutch research found that for food pathogen *Listeria monocytogenes*, which can cause meningitis in humans, resistance to quaternary ammonium compounds is associated with reduced susceptibility to certain antibiotics, and more severe meningitis, reflected by higher mortality and more patients with severe sequelae. Investigators suggested that use of quaternary ammonium compounds in the food industry may result in increased virulence.[196]

Additionally, biofilm formation is an issue in all sectors where disinfectants are used[197-208] and contributes to the development of hospital acquired infections, food-borne infections, legionellosis and ear infections in swimmers.

In addition to high levels of resistance and biofilm formation, the Committee believes limited reductions in susceptibility to disinfectant substances to be relevant to health as well. Although the concentrations of disinfectants used in daily practice are hundreds to thousands of times higher than the often only slightly elevated MIC or MBC values for bacteria in the field, the Committee is of the opinion that such a comparison – unlike for antibiotics – says little about the success of a scheduled treatment. MIC/MBC determinations are performed in the laboratory on bacteria isolated from the field (for example from a patient). In general, longer exposure times are used (a whole night) and contact between the bacteria and the disinfectant is optimal. In practice, disinfectants – unlike antibiotics – must do their work in only a few minutes. Furthermore, contact between the disinfectant and the bacteria is often far from optimal in practice. Micro-organisms are generally attached to a surface, hidden in cracks, crannies or seams of the material, or embedded in tissue, sometimes under dried-up dirt or in a biofilm, where the disinfectant can barely reach. The concentration bacteria are actually exposed to in practice is often much lower than the prescribed in-use concentration.[209] Thus, the reduction in the number of bacteria achieved by a disinfectant in practice is much lower than in a laboratory test.[209] Reduction of bacterial susceptibility due to acquired resistance mechanisms will further reduce the disinfection result, and increase the risk of sufficient bacteria surviving to cause disease and decay. Some experts suggest that a limited reduction in susceptibility only increases the risk of infections if disinfectants are used inexpertly[210, 211], but the Committee believes this is also the case if disinfection is performed correctly.

3.5 Interaction with resistance to antibiotics

3.5.1 Interaction mechanisms

In Section 3.3.2, the Committee briefly described how cross-resistance and co-resistance lead to co-selection. These phenomena explain how one antimicrobial substance can lead to resistance to another. It is also the main way in which disinfectant use can influence the susceptibility of bacteria to antibiotics.[9,212, 213]

Cross-resistance often involves chemically closely related substances, but may also involve very different substances. Efflux pumps that can expel both certain disinfectants and certain antibiotics are the most common cause of cross-resistance.[89,90,122,126,214-225] Changes to cell wall permeability, which prevent both certain disinfectants and certain antibiotics from entering the cell easily, is another frequently described cause of cross-resistance between disinfectants and antibiotics.[226-229] Finally, there is one known example in which the change to a target inside the bacterial cell results in concurrent decreased susceptibility to both a certain disinfectant and a certain antibiotic.[230,231]

A decreased susceptibility to disinfectants can also contribute to resistance to antibiotics via co-resistance. Resistance genes against disinfectants and antibiotics are often located on different pieces of DNA, but plasmids containing both resistance genes against disinfectants and against antibiotics have been observed.[213] The scientific literature contains a number of examples.[192,232-240] A well-known example of co-resistance are the class 1 integrons. These are pieces of DNA commonly found on plasmids of Gram-negative pathogens, such as coli and *salmonella* bacteria.[241,242] In addition to antibiotic resistance genes, they almost always also contain a gene that makes bacteria less susceptible to quaternary ammonium compounds and a gene that protects against sulphonamides. Scientists suspect that use of quaternary ammonium compounds and sulphonamides, for example in hospitals, since the 1930s has played an important facilitating role in the spread of these integrons, and thus in the evolution of antibiotic resistance in this clinically relevant group of pathogenic bacteria.[243-246] Mercury may have played a similar promoting role.[246-248]

Thus, disinfectant use can contribute to the selection and spread of bacteria resistant to antibiotics via cross-resistance and co-resistance. As indicated previously, disinfectants can trigger stress reactions that result in biofilm formation.[208] Infections with bacteria in biofilms are more difficult to treat with antibiotics.

There is some debate in the scientific literature about the question whether, conversely, antibiotic use can contribute to resistance to disinfectants. Some experts believe this is not the case, due to the high in-use concentrations and multiple targets of disinfectants.[162,249] Other researchers believe this is indeed the case due to the existence of efflux pumps that can remove substances from both groups.[148,250,251] The reduced susceptibility to disinfectants induced by antibiotics is not so significant that bacteria are resistant to normal in-use concentrations. However, in Section 3.4, the Committee argued that limited reduction in susceptibility can increase the chances of bacteria surviving disinfection in practice.

3.5.2 *Contribution of disinfectant use to antibiotic resistance in practice*

Based on current knowledge on cross-resistance, co-resistance, biofilm formation and co-selection, it is likely that disinfectants contribute to the selection and spread of antibiotic-resistant bacteria. Repeated exposure to increasing concentrations of disinfectants in the laboratory has often been shown to result in the selection of bacteria with reduced susceptibility to antibiotics.[107,252,253] Field research in hospitals, animal farms, slaughterhouses, food factories and other environments where disinfectants are commonly used, however, shows highly variable results.[9] In some cases, an association has been observed between the use of (and resistance to) a disinfectant and antibiotic resistance[193,194,254], but not in others[255-258]. It should be noted that there is no good surveillance data on resistance to disinfectants, which is available for antibiotics. Standardised and validated methods for measuring resistance to disinfectants are not yet available.

Differences in research methods have undoubtedly contributed to the heterogeneity of findings in field research.[9] This also applies to the conditions under which disinfectants are used. An additional explanation lies in the element of chance that characterises every resistance development. After all, mutations are random. Bacteria with relevant changes to their hereditary material will be selected by exposure to a disinfectant (survival of the fittest). Different resistance mechanisms may be acquired under similar conditions. Comparable levels of resistance to a disinfectant can thus be based on (combinations of) different resistance mechanisms.[259] This may explain why only moderate correlations are frequently found between susceptibilities for various antimicrobial substances.[260]

A surveillance system for the use of antibiotics and the incidence of resistance to these substances has already been in place for over ten years in the Netherlands, covering both human and veterinary sectors.[261] Surveillance involves the continuous and systematic collection of data, analysis of said data, and periodic reporting to persons or institutions that require such information. However, no such surveillance system exists for disinfectants. Thus, the degree to which disinfectants contribute to antibiotic resistance remains unclear. Some experts believe that the contribution is limited, compared to the contribution of antibiotic use itself.[262,263] Others are less certain of this.[121,248,264,265] The Committee believes it is impossible to estimate the contribution of disinfectant use on overall resistance issues based on available data. It is suspected that disinfectants that leave behind lasting residues at the application sites and in the environment (quaternary ammonium compounds, chlorhexidine, triclosan, silver) make a greater contribution to resistance than substances that degrade or evaporate quickly (peroxides, chlorine-releasing compounds, alcohol). However, recent research suggests that disinfection of drinking, swimming, cooling and waste water using chlorine can indirectly contribute to the development of antibiotic resistance due to the creation of mutagenic organochlorine compounds (capable of causing changes to DNA).[265-267] Additionally, the frequency and scale of use also play a role, as do the circumstances in which use occurs. If disinfectants are used in places where antibiotics are also used (healthcare, animal husbandry, in the home), the risk of promoting antibiotic resistance is greatest.

3.6 Testing for the risk of resistance development due to biocides

The Biocides Regulation requires that biocides do not cause unacceptable resistance or cross-resistance in target organisms (article 19, first paragraph, under b, sub ii).[2] This also applies to the disinfectants subject to this legislation. This aspect must be assessed before a product may be marketed. However, this is currently barely done, as there are no validated methods for assessing the risk of resistance development in advance.[268,269]

An understanding of the mechanism of action of disinfectants may provide tools to predict their resistance-inducing capacity.[21] According to SCENIHR, some disinfectants induce resistance more easily than others due to the nature of their interaction with bacteria.[38] This institution includes the following in the high-risk category: quaternary ammonium compounds, biguanides (such as chlorhexidine), phenols (such as triclosan) and metals (including silver). The low-risk group includes highly reactive, oxidising and alkylating biocides. According to SCENIHR, the moderate-risk category includes alcohols, inorganic acids and their esthers, anilides, isothiazolones and diamidines. Despite its utility for initial risk assessment, the Committee feels this classification is too coarse for use in authorisation procedures.

RIVM recently examined the feasibility of developing test methods for disinfectants intended for consumer use (non-professional products in PT01 and PT02).[269] The institute concluded that creating a protocol that would allow assessment of the risk of resistance development as part of the marketing authorisation procedure is currently a bridge too far. The development of predictive resistance tests using model organisms and use scenarios will take years. Furthermore, RIVM notes that a number of relevant factors are difficult to account for in the assessment method: the effect of adjuvants in disinfectants (formulation), the dissemination of the substance in the environment after use, the presence of the same antimicrobial substance in numerous products, use that deviates from instructions, and the difficult to estimate scale on which products will be used following marketing.

Participants in a recent international workshop of experts believe that a (semi)quantitative assessment is also still beyond reach due to a lack of insight into the factors responsible for causing resistance, a lack of relevant data, and a lack of consensus on a definition of resistance.[270] What would constitute (un)acceptable test results for marketing authorisation also remains unclear. So far, the development of a test predicting resistance to antibiotics has failed as well. According to the American Academy of Microbiology, resistance develops more frequently and via different mechanisms than previously observed in the laboratory once a product is used in practice.[121] According to this organisation, the complexities of daily practice cannot currently be captured in an experimental laboratory system. Despite this, initial attempts to develop a protocol for disinfectants have been made.[268,270,271]

Considering the above, the Committee believes a focus on reliable surveillance of use of and resistance to disinfectants is the better option. Such surveillance is also recommended by SCENIHR[38,272] and RIVM[269] and is consistent with the Health Council's advisory report on the application of the precautionary principle to issues characterised by significant uncertainty.[273] The methodology required for this can be developed more swiftly. Surveillance is necessary to gain insight into the scope and severity of the issue, and provides information to guide potential management measures and to monitor the effects thereof. Furthermore, such surveillance will provide information about which products, applications and conditions promote the development of resistance. According to the Committee, this will require combining surveillance of resistance development against disinfectants with a systematic registry of the extent of the use of disinfectants. The knowledge obtained can later be useful for the development and validation of risk assessment methods for marketing authorisation procedures.

The Committee recommends initially limiting surveillance to sectors where the risk of resistance development appears to be the greatest, with the greatest potential for relevant public health effects, and where good research facilities are already more or less available. The Committee is of the opinion that human and veterinary healthcare qualify. If the results in these sectors suggest a need, surveillance can later be expanded to other sectors such as the food industry and consumer products. Institutions tasked with surveillance of use and resistance to antibiotics could also be tasked with this surveillance.

Implementation of a national surveillance scheme for the use of disinfectants and the development of resistance to these substances is recommended. Additionally, the Committee recommends placing these items on the international agenda, and striving for international cooperation similar to what is already present for antibiotic resistance surveillance at the European level (ECDC).

3.7 Conclusions

Bacteria can deploy resistance mechanisms against disinfectants, and thus reduce their susceptibility to these substances. Once resistance has developed it is slow to disappear, even if use of the substances is discontinued.

Resistance development has been demonstrated for all disinfectants studied. Field research has shown that resistance to disinfectants is a worldwide phenomenon that occurs in all sectors where these substances are commonly used. To date, high levels are resistance that threaten the efficacy of these substances have only rarely been observed. Thus, resistance to disinfectants appears to be progressing less swiftly than resistance to antibiotics. However, the Committee notes that resistance to disinfectants remains underexposed, as susceptibility to disinfectants is not routinely tested in practice, not even in investigations into outbreaks of infections related to the use of disinfectants. High levels of resistance are likely more common in daily practice than currently believed. Additionally, the Committee is of the opinion that low levels of resistance are also significant for health. This is because low levels of resistance increase the risk of bacteria surviving disinfection.

Based on current knowledge about cross-resistance, co-resistance, biofilm formation and co-selection, disinfectant use may be expected to contribute to antibiotic resistance, and vice-versa. Laboratory studies confirm this. However, field research in areas where disinfectants are used in practice shows a mixed picture. According to the Committee, it is therefore impossible to conclude whether disinfectant use contributes substantially to the development of antibiotic resistance, or is merely a subordinate factor.

The Committee does not believe the development of a (semi)quantitative test protocol to determine the resistance promoting capacity of new disinfectants in advance for the purposes of marketing authorisation is feasible in the short term. It recommends first deploying reliable surveillance systems for the use of disinfectants and for resistance against these substances, as already exists for antibiotics. The information gathered will provide insight into the scope and severity of resistance (due) to disinfectants and into factors that play a role in the development of resistance.

Chapter 4

Other risks

In order to answer the ministers' question about the need for restraint in the use of disinfectants, the advantages and disadvantages need to be identified. The health (and other) benefits were presented in Chapter 2. Chapter 3 presented the potential for resistance developing against disinfectants themselves and to antibiotics. This Chapter addresses other concerns, particularly toxicological concerns. Special attention is given to the influence on the human microbiome (the collection of all micro-organisms on and inside the human body) and on the health significance thereof.

4.1 Toxicological risks

Disinfectants are subject to different legal frameworks, depending on their intended use (see Chapter 2). The procedures for limiting toxicological risks differ depending on the legal framework that applies to a specific product.

Legally speaking, most disinfectants are classified as biocides. Biocides are subject to a marketing authorisation procedure handled by the Board for the Authorisation of Plant Protection Products and Biocides (Ctgb), an independent government body that ensures that only those products are marketed that allow effective and safe (to humans, animals and the environment) combating of plague organisms. The marketing authorisation procedure for biocides (and for plant protection products) is the most extensive legally prescribed substance and product evaluation framework. The extensive dossier that the manufacturer must submit for evaluation includes, in addition to research data on the physical and chemical properties and efficacy, the results of extensive environmental and toxicological animal studies. Efficacy and risks are evaluated based on these data and the prescribed use by the manufacturer. Calamities and inexpert use are not taken into consideration in the evaluation. Inspectorates such as the Human Environment and Transport Inspectorate (ILT), the Healthcare Inspectorate (IGZ) and the Netherlands Food and Consumer Product Safety Authority (NVWA) are responsible for overseeing correct use. Additionally, the authorisation evaluation generally does not take the scale of use in society into account, nor the effects on the greater environment; such data are generally not available at the time of authorisation. Furthermore, each product is evaluated individually. The fact that a person may use several products (e.g. toothpaste and hand soap) with the same or closely related active substances is not taken into consideration, for example. This weakness has been acknowledged, and international efforts are underway to develop methods for this so-called aggregate and cumulative risk assessment.[274-276]

There are also marketing authorisation procedures for (veterinary) medicines in which potential adverse effects for the patient are examined alongside efficacy.

For medical devices, safety evaluation depends on the risk class the product falls in (Medical Devices Directive 93/42/EEC). For products in higher risk classes, safety is assessed by an independent government body, and more or less extensive marketing authorisation procedures apply. For products in the lowest class, safety assessment is left to the manufacturer. Products found to be safe receive a so-called CE mark. Post-marketing surveillance is conducted for both (veterinary) medicines and medical devices in order to identify potential long-term harmful side effects. The attention for the environmental risks of these products during the safety evaluation is more limited than for biocides.

There is no marketing authorisation procedure for cosmetics. However, manufacturers are responsible (under the Regulation on Cosmetics 1223/2009/EC) for the efficacy and safety of their products, and must be able to provide evidence thereof upon request. Positive and negative lists in the appendices to the legislation further define which ingredients (including antimicrobial ingredients) may be used in cosmetics (and in what concentrations) and which may not.

The above-mentioned legislation largely covers toxicological risks to humans, animals and the environment. The most important remaining cause for health damage is failure to strictly adhere to instructions for use. This is particularly important for products that are intrinsically highly toxic. Good instruction and education for users of these products is therefore necessary to ensure both optimal efficacy and safety.[277] This is more easily achieved for professional users than for private individuals. This is an additional reason for observing restraint in making disinfectants available to private individuals.

Where possible, the most dangerous products should be replaced with less dangerous alternatives. For example, glutaraldehyde has been replaced with orthophthaldehyde or mixtures of hydrogen peroxide and peracetic acid for the disinfection of endoscopes in some (foreign) hospitals. However, these substances are also not without risk.[278,279]

The marketing authorisation procedure is a work in progress, and continuously subject to improvements. As a result of this, products authorised in the past may no longer pass the new, stricter evaluation procedure. Triclosan is an example. The safety of the substance has increasingly been questioned in recent years[50,53,280,281], among other things due to its hormone-disrupting properties.[282-286] The European Committee recently decided no longer to authorise biocides based on this substance.[287,288] Use in soap intended for consumers was recently banned in the U.S as well.[55]

4.2 Effect on the human microbiome and potential health consequences

4.2.1 The human microbiome

According to the most recent insights, the human body contains roughly as many bacterial cells as own cells.[289] However, the genetic diversity in the microbial population is significantly greater than in the human genome. This provides the human host with functions it cannot fulfil itself.[290,291] The largest numbers of bacteria reside in the gastrointestinal tract, particularly the large intestine. The diversity in species (many thousands) is also greatest there.[291] Micro-organisms are also naturally found in large numbers in the mouth and on the skin.[292,293] Hundreds of species have been identified. Micro-organisms also reside in the vagina[294] and potentially also in places that until recently were considered more-or-less sterile under health conditions, such as the lungs[295], male ureters[294], the eye[296-298] and the placenta.[299-301]

Scientific insights into the significance of this microflora to the function of the human body is still limited. The importance of gut flora for the digestion of food, production of vitamins and other essential nutrients, prevention of infections and maturation of the immune system has been confirmed.[302] This also applies to the role that lactic acid bacteria play in preventing vaginal infections.[294] It was recently discovered that bacterial oral flora play a role in nitrogen monoxide (NO) metabolism.[303,304] NO is an important signalling substance in the human body, involved in processes such as blood pressure regulation.

Towards the end of the last century, the idea arose that infections contracted at a very young age, for example due to unhygienic contact with older brothers and sisters, may protect from allergic conditions such as hay fever, eczema and asthma later in life.[305] This concept is often referred to as the 'hygiene hypothesis'. Recently, it was suggested that 'biome depletion theory'* may be a more general and suitable term[306], but what the responsible missing components are remains unclear. Growing numbers of diseases are being associated with disruptions to the microbiome (dysbiosis). However, questions remain about whether these changes are cause or effect.[291,307]

4.2.2 *Effects of disinfectants on the microbiome*

Antibiotics are known to have effects on the natural microflora in humans, particularly on gut flora.[308-312] The unpleasant, acute adverse effects of antibiotics, such as diarrhoea, are a result of this. Additionally, there are indications from epidemiological research that prenatal exposure or exposure to antibiotics at a very young age is associated with an increased risk of immune disorders[313,314] and obesity[312,315-323]. Various researchers have drawn parallels with (past) use of antimicrobial growth promoters in animal husbandry.[316,320,323] However, some studies suggest that infections in early childhood, rather than antibiotic use, increase the risk of these conditions.[324,325]

Little is known about the influence of disinfectants and antibacterial components in consumer products and personal hygiene products on the composition and function of the human microbiome, let alone about the significance of any changes to human health. Because these products are applied to the skin and in the mouth, potential effects are expected there first.

Most research has been conducted into the influence of antibacterial toothpastes and mouthwashes. Outcomes are not clear-cut. Some researchers report (in part based on in-vitro research) that disinfectants can cause a shift in the composition of oral bacterial flora, although the health significance remains unclear.[326-329] Others observe no such shifts.[330-333]

Recently, multiple studies in volunteers found that use of chlorhexidine or cetylpyridinium mouthwash increases blood pressure by disrupting oral flora and

* The term 'biome' is used instead of the term 'microbiome' to indicate that the human ecosystem can include higher organisms such as worms in addition to micro-organisms.

NO metabolism.[334-339] Several researchers have suggested long-term use of antibacterial mouthwash may increase the risk of cardiovascular disease. However, all studies focused on short-term effects. Longer term research is required to answer these questions. This effect was not observed in one study with triclosan-containing toothpaste, possibly due to insufficient contact between the disinfectant and bacteria.[340] Good oral hygiene is crucial to reduce the risk of caries and parodontitis. However, this can often also be realised with a combination of tooth brushing and use of toothpicks, interdental brushes or dental floss. These simple interventions have a significantly lower overall impact on oral flora.[335]

Little research appears to have been conducted into the effects of professional exposure to skin disinfectants on the skin flora of medical staff. Research used to support hand hygiene guidelines in healthcare is focused on individual micro-organisms present temporarily, rather than on the overall community of temporary and permanent micro-organisms present.[341] A study among medical staff indicates that the diversity of hand flora decreases due to frequent disinfection using an alcohol-containing hand gel.[341] In another study, the hand flora of nurses in a hospital was compared to that of women in the general population. There were clear differences in flora, but it remains unclear whether this is due to intensive hand disinfection, different bacterial populations in hospitals, or both.[342]

The influence of chlorhexidine on patients' skin flora remains unclear. In one study, it was reported that frequent skin disinfection with chlorhexidine can result in a shift in skin flora dominance from Gram-positive to Gram-negative bacteria.[194] Other researchers found no such shift.[343] Chlorhexidine baths, mostly used in ICUs to reduce the number of bacteria on a patient's skin, appear to be most effective against Gram-positive bacteria; efficacy against Gram-negative bacteria is contested.[344]

Use of deodorant or antiperspirant may also affect the skin flora of the armpit. In a Belgian study, the diversity of flora was found to increase due to the use of these products, particularly the use of antiperspirants. In particular, the number of *Actinobacteria* increased, which is unfavourable for odour development.[345] In another study, a single dose of silver-containing deodorant resulted in an immediate, significant reduction in the number of micro-organisms on the skin. However, skin flora was restored within twenty-four hours. The composition of species remained unchanged.[346] Wearing a silver-containing T-shirt for four weeks, eight hours per day had no significant effect on the microflora of healthy skin.[346]

Because substances can enter the body orally or via the skin, effects on the microbiome elsewhere in the body cannot be ruled out entirely. Research into the relationship between triclosan in urine and the composition and function of gut flora did not yield any indications for negative effects of the antibacterial substance.[310,333] As effects of triclosan on the microbiome were seen in animal testing, experts recommend additional research.[347,348]

4.2.3 *Increased virulence*

Exposure to sub-lethal concentrations of disinfectants not only affects the susceptibility of bacteria. It can also have a demonstrable impact on their virulence. The exact influence differs per type of disinfectant and species of bacteria.[349] Exposure to sub-lethal concentrations of peracetic acid stimulates Staphylococcus aureus to increased exotoxin production.[350] Salmonella enterica produces higher concentrations of a protein (AvrA) that undermines the host's immunity when exposed to triclosan, benzalkonium chloride or hydrogen peroxide.[351] Sub-lethal concentrations of quaternary ammonium compounds may promote the expression of virulence genes in food pathogen Listeria monocytogenes. Among other things, these genes code for proteins involved in bonding to and entry of the bacteria into human host cells.[349] Sub-lethal concentrations of benzalkonium chloride have also been shown to promote the survival and proliferation of Listeria monocytogenes in human host cells.[352] The percentage of patients in the Netherlands that dies of Listeria-related meningitis or has permanent sequelae has gradually increased from 27% to 61% in recent years.[353] This is due to the rise of a new, more virulent bacterial strain (ST6). This strain has a plasmid with a qacH gene coding for an efflux pump which makes the bacteria less susceptible to benzalkonium chloride.[196] The exact relationship between this resistance and increased virulence still needs to be elucidated. Experts have expressed worries that use of quaternary ammonium compounds in the food industry may result in selection of bacterial strains with increased virulence.[196]

4.3 Conclusions

Disinfectants have intrinsic dangerous properties and may be harmful to humans, animals and the environment under certain circumstances. This is one of the reasons for a marketing authorisation procedure. The fact that health damage and environmental damage sometimes occur is primarily associated with the fact that products are not always used as prescribed. Additionally, imperfections in the marketing authorisation procedure may play a role.

The human body is naturally populated by countless micro-organisms – the human microbiome. Scientific insight into the composition of this microflora is quickly growing, but its relationship with health and disease remains largely unknown. Research into the effects of disinfectants or antibacterial products for personal hygiene on the composition of skin and oral microflora shows that besides positive also negative effects are possible. For example, there are consistent indications that mouthwash containing chlorhexidine or cetylpyridinium disrupts blood pressure regulation via effects on oral flora.

Chapter 5

Answers to the questions asked by the ministers

In this final Chapter, the Committee answers the questions asked by the ministers and makes a number of recommendations.

Are there indications that a possible increasing use of disinfectants has resulted in an increase in microbial resistance, with the potential for health harm?

It is clear that resistance to disinfectants exists and the resistance negatively affects the efficacy of disinfection, with harmful consequences for users and patients. Whether the use of disinfectants is actually increasing in the Netherlands could not be determined with certainty, as use is not registered. A gradual increase is visible in Belgium. It is also unknown how often resistance (high or low level) actually occurs, and what trends may exist. This is because the susceptibility of micro-organisms to disinfectants is almost never determined in practice, not even in incidents and disastrous events where there is a clear failure of disinfection procedures. Furthermore, there is no surveillance system for resistance to disinfectants. The Committee recommends setting up such a system in order to monitor the use of disinfectants and the development of resistance, similar to the systems already in place for antibiotics. This can provide insights into the scope and severity of the resistance problem and contributory factors.

Are there indications that resistance to antibiotics is increasing due to the use of disinfectants?

Based on current knowledge of the underlying mechanisms and outcomes of laboratory tests, it is likely that resistance to antibiotics increases due to use of disinfectants, and vice-versa. Whether the contribution of disinfectant use to overall antibiotic resistance is minimal or substantial cannot be determined based on current data. Use of disinfectants can contribute to management of resistance problems with antibiotics by preventing infections and thus preventing curative use of antibiotics. On the other hand, disinfectants can also promote resistance to antibiotics via cross-resistance and co-resistance.

Is the 2001 Health Council recommendation to observe restraint in adding disinfectants to consumer products still applicable?

Yes, the 2001 recommendation is still in full force and effect. To date, there are no data supporting any health gains for most consumer products with added disinfectants, except in cases where a medical indication exists. There are concrete indications that short-term use of the disinfectant chlorhexidine in mouthwash can deregulate a key function of the human oral microbiome, which can result in a (temporary) blood pressure increase. Although there are no data on the consequences of long-term use, these findings support the need for restraint.

Is there a need for national policy on restraint regarding use of disinfectants? If so, what uses should such a policy apply to?

Yes, the Committee is of the opinion that national policy on careful use of disinfectants should be formulated. On the one hand, this should be aimed at promoting restraint if no health need exists for the application, there are no clear benefits, or if there are adequate, less harmful alternatives available. It should also promote use of disinfectants where there is a clear medical/veterinary need, and their use results in clear advantages.

The policy should apply to all applications, including consumer products and professional uses, along with use of the same antimicrobial substances in products that fall under other legal frameworks (medicines, veterinary medicines, medical devices, cosmetics). After all, the same or very similar substances are involved in all cases. The issue of resistance cannot be tackled effectively if policy only applies to part of the use of disinfectants.

Where possible, methods for achieving health and cosmetic goals without the use of disinfectants should be stimulated. This is consistent with plant protection policies, where the government also promotes the use of non-chemical methods for preventing and combating plagues.[354] The Minister for the Environment recently indicated that this same approach would be followed for biocides policy.[355] According to the Committee, the use of disinfectants should be limited to situations where a beneficial effect on health, shelf-life or product safety or cosmetic objectives has been demonstrated.

In professional sectors, the use of disinfectants should only be promoted where they have a clear added value in preventing or fighting infection or damage, e.g. spoilage of food. Use of disinfectants in applications with no evidence for efficacy (prevention of infections or damage) or cost-effectiveness should be avoided or discouraged wherever possible. Training and education for professional users are essential for promoting correct use.

For consumers, it is important to prevent use of disinfectants without demonstrated health benefits. Disinfectants for the prevention of infections that are covered by biocides legislation should only be used by private individuals for medical indications, i.e. if prescribed by and under the supervision of a doctor or other expert. The Committee recommends regulating this via changes to marketing authorisation policy for biocides. The recommendation to ban disinfectants intended for routine use in offices or at home by consumers from the (European) market is consistent with the earlier Health Council advisory report on the precautionary principle.[273] In that report, the Health Council writes that a ban becomes a more attractive option with the availability of alternatives that provide the same benefits but with fewer (uncertain) risks. This is certainly the case here, as cleaning with water and soap at home or in the office is almost always sufficient.

Coordinating disinfectant and antibiotic policies is also recommended in order to create an integrated approach to antimicrobial products. This is important because the two groups affect each other’s use and because resistance mechanisms are partially shared or connected.

The Committee also recommends stimulating research into the efficacy of practical applications of disinfectants, into the added value over other hygiene and prevention measures such as cleaning, and into methods for achieving health gains, cosmetic goals, and preventing decay without use of disinfectants wherever possible. The development of acquired resistance to disinfectants in relation to use of these substances is also deserving of further study. This also applies to the influence of disinfectants on the composition and functions of human and animal microbiomes, particularly in users with professional exposure, patients, consumers and in the environment.

What measures could be taken in order to comply with the requirement from the Biocides Regulation that biocides may not cause unacceptable resistance?

The Committee does not believe that developing a test to predict the resistance promoting potential of new biocides is a realistic goal in the short or middle term. The required knowledge is lacking. The Committee recommends focusing on systematic surveillance of disinfectant use and the development of resistance to disinfectants first. Methods for this can be developed more quickly, and results will provide insight into the scope and severity of the issue, and contributory conditions. This knowledge may eventually be used to develop a predictive test. The Committee recommends starting such a programme at the national level, as was done for antibiotic resistance, but to put the issue of resistance to disinfectants on the EU agenda and to strive for international cooperation, as is the case for antibiotic resistance. Resistance development in disinfectants has little respect for national borders, and is thus best tackled internationally.

For the authorisation of disinfectants, it is advisable to use efficacy tests that better reflect the circumstances under which disinfectants must do their work in practice. This fits well with the new European regulations that also require a simulated practice test in many cases.

Guidelines for prudent use are necessary, and in part already exist. Unlike antibiotics, it is difficult to make these guidelines fully covering at the national level, as there are products containing antimicrobial substances that are not subject to an authorisation procedure. Therefore, authorising bodies, manufacturers and authorisation holders must be consulted about requirements for package leaflets, legal instructions for use, advertising, awareness raising and education. Information and insights obtained from the previously mentioned surveillance can help draw the attention of manufacturers and marketing authorisation holders to their corporate social responsibility in terms of product stewardship, and jointly work towards suitable solutions.

Which exposure routes and which products are most relevant to the development of microbial resistance to disinfectants? Is it possible to *determine which uses contribute most to the development and maintenance of microbial resistance to disinfectants?*

Which exposure routes, products or applications are most relevant to the development of microbial resistance to disinfectants cannot be determined. The required knowledge is lacking. Because resistance is primarily a consequence of use, the Committee expects that resistance appears and occurs in places where disinfectants are used in significant amounts on a daily basis (human and veterinary healthcare, animal husbandry, slaughterhouses and the food industry). Systematic surveillance of resistance development and disinfectant use can provide insights into these issues. Disinfectants that leave behind long-acting residues in the environment (quaternary ammonium compounds, chlorhexidine, bisphenols, silver) may make a greater contribution to resistance than substances that degrade or evaporate quickly (peroxides, chlorine-releasing compounds, alcohol). Frequency and scale of use also play a role. This likely also applies to the application site. If disinfectants are used in places where antibiotics are also used (human and veterinary healthcare, animal husbandry), the risk of promoting antibiotic resistance is likely the greatest.

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A The request for advice

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Annexes

Annex

A

The request for advice

On 4 February 2015, the President of the Health Council received the following request for advice on the development of microbial resistance due to disinfectants (ref no. 702964-13 136 1-VGP).

The development of microbial resistance to disinfectants due to use of these products in the household was included as a topic in your working programme for 2015. With this letter, I wish to further specify the questions on this subject, jointly on behalf of the Minister for the Environment.

Disinfectant use in the household has been increasing in recent years. In 2001, the Health Council recommended observing restraint in adding disinfectants to consumer products in its advisory report ‘Disinfectants in consumer products’. The reasons for this recommendation were the lack of health gains and the potential risks associated with use. A potential risk is that broad application of disinfectants in the household may lead to the development of antimicrobial resistance to disinfectants. This may lead to disinfectants losing their efficacy in situations where they are actually needed. As an example, the Health Council cited a study in which strains of the MRSA bacteria that were not susceptible to the disinfectant triclosan were identified in hospitals. This substance may be used in the treatment of patients contaminated with MRSA. The Health Council also indicated that there were growing concerns that increased use of disinfectants might result in increased resistance to antibiotics (cross-resistance).

Disinfectants are subject to biocides legislation, and may only be marketed after being authorised by the Board for the Authorisation of Plant Protection Products and Biocides (Ctgb). To date, a small number of new disinfectants for use in the household have been authorised. However, there are growing numbers of consumer products containing disinfectants on the market that are not subject to biocides legislation, but rather to cosmetics and detergents legislation. Additionally, use of disinfectants by professionals appears to be increasing, for example by sports facilities, cleaning companies and hospitals. Humans and the environment may therefore be exposed to the same disinfectants via a variety of sources, and exposure to these substances appears to be increasing.

Europe is increasingly leading the way in biocides evaluation, and the Netherlands is striving to apply European evaluation frameworks wherever possible. The enactment of the European Biocides Regulation 528/2012 in September 2013 will result in further harmonisation of evaluation methods and marketing authorisation decisions. In other member states, there is less attention for resistance development to disinfectants and relatively higher numbers of disinfectants for home use are available in the marketplace. These products may be marketed in the Netherlands as well via a simplified mutual recognition authorisation procedure, which may result in an increase in the number of disinfectants that are on the market for home use. Although the biocides regulation allows for national limitation of mutual recognition and authorisation of biocides based on a number of closely defined conditions, this is a major procedure. In article 19, first paragraph, under b, sub ii, the Biocides Regulation states that biocides may not cause unacceptable cross-resistance in target organisms. However, there are currently no validated tests for evaluating this potential effect of biocides. In 2013, RIVM concluded that developing such tests must take place in an international context, and that it will take several years.

Considering the developments described above, I want to ask you to assess available data and determine whether there are indications that possible increasing use of disinfectants has resulted in an increase in microbial resistance, and possibly health damage. I would also like to know whether there are indications that resistance to antibiotics is increasing due to the use of disinfectants. Finally, I would like to know whether the 2001 Health Council recommendation to observe restraint in adding disinfectants to consumer products is still applicable. Uses of disinfectants other than consumer products could also be involved in this assessment.

If the conclusion remains that there are still sufficient grounds for restraint in the use of disinfectants, I wish to ask your attention for the following questions:

- Is there a need for national policy on restraint regarding use of disinfectants? If so, should this policy be limited to use in consumer products, or apply to all uses, including professional use? Should such a policy be limited to biocides, or should it also address other regulations, such as cosmetics and detergents.
- Although the European biocides regulation does not permit biocides to cause resistance or cross-resistance in target organisms, this requirement is currently not assessed in practice. Further assessment of this issue is desirable in order to allow more specific implementation of this

requirement at the European level. The requested advice may contribute to this. What potential measures could be deployed at the European level, and can these measures be prioritised? Do potential measures include development of methods or guidelines for use?

- Which exposure routes and which consumer products or products from other legal frameworks are most relevant to the development of microbial resistance to disinfectants?
- Is it possible to determine which uses contribute most to the development and maintenance of microbial resistance to disinfectants?

Please inform me of how much time you believe will be necessary to draft the advisory report. You may make appeals on an observer or advisor on behalf of my Ministry, the Ministry of Infrastructure and the Environment, or RIVM.

Sincerely,

Minister of Health, Welfare and Sport
(w.g.)
E.I. Schippers, MSc

Annex B

The Committee

- Prof. H.A. Verbrugh, *Chairman*
  emeritus professor of medical microbiology, Erasmus UMC, Rotterdam
- Prof. T. Abee,
  professor of food microbiology, Wageningen UR
- Prof. D.J. Mevius,
  senior investigator, Wageningen Bioveterinary Research, Lelystad;
  Professor of antimicrobial resistance, Utrecht University
- Dr. W. van Schaik,
  microbiologist, UMC Utrecht
- Prof. H. Smidt,
  professor of microbial ecology, Wageningen UR
- Prof. J.W. Veening,
  professor of molecular genetics of prokaryotes, Groningen University and Lausanne University
- J.W. Andriessen, *structurally consulted expert*
  biocides policy advisor, Ctgb, Wageningen
- Prof. B.H. ter Kuile, *structurally consulted expert*
  microbiologist, NVWA, Utrecht and special professor of microbial food safety and antibiotic resistance in the food chain, UvA, Amsterdam
- Dr. M.H.M.M. Montforts, *structurally consulted expert*
  risk assessor for substances in the environment, RIVM, Bilthoven

- Dr. H. Schmitt, *structurally consulted expert*
  environmental microbiologist, IRAS, Utrecht University and RIVM, Bilthoven
- Prof. A. Voss, *structurally consulted expert*
  medical microbiologist, CWZ and professor of infection prevention, Radboud UMC, Nijmegen
- Dr. D.A. Heemsbergen, *observer*
  Ministry of Infrastructure and the Environment, The Hague (as of 29 March 2016)
- M.J. Martena, *observer*
  Ministry for Health, Welfare and Sport, The Hague
- Dr. M.N.E. Nelemans, *observer*
  Ministry of Infrastructure and the Environment, The Hague, (up to 1 September 2015)
- Dr. H.F.G. van Dijk, *scientific secretary*
  Health Council, The Hague
- J.W. Dogger, *scientific secretary*
  Health Council, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex C

Hearing participants

The hearing was held on 25 January 2016 in the Beatrix Building, Jaarbeurs, Utrecht.

- Mrs. E. van Ammers, Netherlands Association of Soap Manufacturers (NVZ), *speaker*
- Mr. H. Bloemen, RIVM, *audience member*
- Mr. G. Counotte, Animal Health Services (GD), *speaker*
- Mrs. I. van Geijlswijk, Royal Dutch Society for Veterinary Medicine, *speaker*
- Mr. P.C. Vesseur, Dutch Poultry Processing Industry Association (Nepluvi), *speaker*
- Mrs. L. Veldhuis, Netherlands Nutrition Centre, *audience member*
- Mrs. A. van Vliet, Working Group Infection Prevention, *speaker*
- Mrs. W.P. van der Vossen, Netherlands Nutrition Centre, *speaker*
- Mr. C. Yu, Netherlands Association of Soap Manufacturers (NVZ), *audience member*

The presentations by the speakers listed above are available on the Health Council website.

The following persons were present on behalf of the Committee: Mr. Van Dijk, Mr. Dogger, Mr. Martena, Mr. Mevius, Mr. Montforts, Mr. Van Schaik, Mrs. Schmitt, Mr. Verbrugh.

The following persons were present on behalf of the Health Council: Mr. Severens (Health Council Vice President), Mrs. Kerkhof (Health Council Executive director), Mrs. Schaule-Jullens, Mrs. Moekoet.

Annex **D**

Outbreaks of bacterial infections in hospitals

Outbreaks of bacterial infections in hospitals where the causative pathogen was resistant to in-use concentrations of the disinfectant

The scientific literature regularly features reports of healthcare-associated contamination and infections related to the use of disinfectants. Both intrinsically and extrinsically contaminated disinfectant solutions, as well as failure of disinfection procedures for reusable medical devices such as endoscopes have been reported. Investigations into such incidents work intensively to track down sources and transmission routes of micro-organisms that have caused the incident, and on finding errors in the disinfection procedures applied. Potential sources, transmission routes and procedural errors are therefore often mentioned in publications. Resistance to disinfectants rarely appears to play a role in these incidents, but the question arises whether resistance of pathogens involved in these outbreaks to the disinfectant used has been investigated. In order to answer this question, all publications referenced in three review articles[29,182,183] were examined to determine whether the pathogen was examined for susceptibility to the disinfectant. After de-doubling, this resulted in 138 publications referenced in the three review articles, with:

- No data available for 11 (the articles were no longer accessible via the Erasmus MC E-library due to their age).
- No pathogen was isolated in 6 publications (often procedural articles and questionnaires).

- In 108 of 138 publications, the pathogen(s) were not tested for susceptibility to the disinfectant used (but often were tested for antibiotics).
- The pathogen was tested for susceptibility to the disinfectant in 13 publications. In only one of these cases, the pathogen, a *Klebsiella pneumoniae*, was susceptible to the disinfectant peracetic acid.[184] In the other 12 articles, the pathogen was found to be resistant to in-use concentrations of the disinfectant used; the following species were involved:

Bacterial species	Resistant to	Reference
Salmonella sp.	1% Savlon (cetrimide + chlorhexidine)	356
Mycobacterium tuberculosis	iodofors	357
Mycobacterium fortuitum	2% glutaraldehyde	358
Pseudomonas aeruginosa	0.1% chlorhexidine	359
Serratia marcescens	20 g/L chlorhexidine	360
Burkholderia cepacia	0.2% chlorhexidine	361
Achromobacter xylosoxidans	5 g/L chlorhexidine	362
Pseudomonas multivorans	Savlon 1:30	363
Pseudomonas sp.	0.4% benzalkonium chloride	364
Pseudomonas sp.	0.1% benzalkonium chloride	365
Serratia marcescens	In-use solution of benzalkonium chloride in water 1:750 (0.13%) (<5 ^{10}log reduction in 5 minutes)	366
Mycobacterium abscessus	In-use solution of benzalkonium chloride in water 1:750 (0.13%)	367

In 2008, Romero-Gomez et al. cultured *B. cepacia* involved in an outbreak of bacteraemia on a haemodialysis ward directly from the in-use solution of 2.5% chlorhexidine.[368] The solution was used for skin disinfection, and was prepared weekly by diluting a commercial 5% chlorhexidine stock solution 1:1 with local demineralised water. The demineralised water was found to be the source, but the bacteria were apparently resistant to high concentrations of chlorhexidine. However, actual susceptibility was not determined.

In summary:
In ± 90% of outbreaks in which a pathogen was isolated, the susceptibility of the pathogen for the disinfectant used was not determined. In cases where such a determination was performed, it was found to be resistant to the disinfectant in question in ± 9/10 cases.

Conclusion:
Thus, resistance to disinfectants likely does play an important role in incidents involving disinfection failure in daily practice, but this role currently remains

largely unexamined due to the focus on tracking down sources, transmission routes and procedural errors during outbreaks, thus failing to consider resistance to disinfectants as a potential determinant. The inexperience of clinical laboratories in determining susceptibility for disinfectants may play a role.