



VKM Report 2016: 63

Antimicrobial resistance due to the use of biocides and heavy metals: a literature review

Opinion of the Panel on Microbial Ecology of the Norwegian Scientific Committee for Food Safety

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2016:63

Antimicrobial resistance due to the use of biocides and heavy metals: a literature review

Opinion of the Panel Panel on Microbial Ecology of the Norwegian Scientific Committee for Food Safety

09.12.2016

ISBN: 978-82-8259-253-6

Norwegian Scientific Committee for Food Safety (VKM)

Po 4404 Nydalen

N – 0403 Oslo

Norway

Phone: +47 21 62 28 00

Email: vkm@vkm.no

www.vkm.no

www.english.vkm.no

Suggested citation: VKM. (2016). Antimicrobial resistance due to the use of biocides and heavy metals: a literature review

Scientific Opinion on the Panel Panel on Microbial Ecology of the Norwegian Scientific Committee for Food Safety, ISBN: 978-82-8259-253-6, Oslo, Norway.

Antimicrobial resistance due to the use of biocides and heavy metals: a literature review

Authors preparing the draft opinion

Arne Tronsmo (chair), Tor GjØen, Henning SØrum, and Siamak Yazdankhah (VKM staff)

Assessed and approved

The opinion has been assessed and approved by the Panel on Microbial Ecology. Members of the panel are: Ida Skaar (chair), Tor GjØen, Jacques Godfroid, Anders Jelmert, JØrn Klein Arinze Okoli, Arne Tronsmo, og BjØrnar Ytrehus.

(Panel members in alphabetical order after chair of the panel)

Acknowledgment

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed a working group consisting of both VKM members and external experts to answer the request from the Norwegian Food Safety Authority/Norwegian Environment Agency. Project leader from the VKM secretariat has been Siamak Yazdankhah. The members of the working group Arne Tronsmo and Tor GjØen (Panel on Microbial Ecology), Henning SØrum (Norwegian University of Life Sciences) are acknowledged for their valuable work on this opinion. Kåre M. Nielsen member of the Panel on Genetically Modified Organisms is acknowledged for comments on this opinion.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

Table of Contents

Summary	6
Sammendrag på norsk	8
Abbreviations and/or glossary	10
Background as provided by the Norwegian Food Safety Authority/ Norwegian Environment Agency	15
Terms of reference:	15
1. Introduction	16
1.1 Literature	19
1.2 Search strategy	19
1.2.1 Inclusion criteria.....	19
1.2.2 Exclusion criteria	19
2. Hazard identification	20
3. Hazard characterisation	20
Theoretical background.....	20
3.1 Modes of action of biocides.....	20
3.2 Resistance mechanisms against biocides.....	20
3.3 Biocides with known antibacterial activity.....	21
3.4 Mode of action of heavy metals.....	25
3.5 Heavy metals with known antibacterial activity	26
3.6 Heavy metals: mechanisms of resistance	26
3.7 Horizontal gene transfer (HGT)	27
3.8 Drivers for AMR	28
Assessment	28
3.9 Literature	28
3.9.1 Biocides.....	30
3.9.2 Heavy metals	49
3.10 Use of biocides and heavy metals in Norway	63
3.10.1 Biocides	63
3.10.2 Heavy metals	65
3.11 Summary of hazard characterization	67
Discussion	67
3.12 Resistance and cross-resistance between biocides and antimicrobial agents.....	67
3.13 Cross-resistance between heavy metals and antimicrobial agents.....	68
Probability characterisation	69
4. Uncertainties	70

5. Conclusions (with answers to the terms of reference)	71
6. Data gaps	73
7. References	75
Appendix 1	88
Appendix 2	92

Summary

Antimicrobial resistance (AMR) in bacteria (often referred to as antibiotic resistance) is one of the major public health challenges of our time. AMR can be described as the ability of a bacterium to withstand the effects of an antimicrobial agent. In addition to antimicrobial agents used for treatment and prophylaxis in humans, animals, and plants, chemical substances, such as biocides and heavy metals, may also induce resistance in bacteria against antimicrobial agents used in human and veterinary medicine.

In June 2016, the Norwegian Environment Agency requested the Norwegian Scientific Committee for Food Safety (VKM) to conduct a literature review regarding development of bacterial resistance to biocides and heavy metals and cross-resistance to antimicrobial agents (e.g., antibiotics) in bacteria, with the following mandate:

1. List chemical substances that may contribute to increased antimicrobial resistance.
2. Describe the substances listed in question 1 that are used/in use in Norway and assess which fields of applications that have the potential to contribute to increased resistance.
3. Range the substances according to field of application that is assumed to have the strongest effect on development of resistance, based on characteristics and amount used.
4. Identify knowledge gaps according to the effect of these substances on development of resistance.

In order to answer the mandate, VKM appointed a working group consisting of two members of the Panel on Microbial Ecology, and one external expert, to prepare a draft opinion document and answer the questions. The Panel on Microbial Ecology has reviewed and revised the draft prepared by the working group and approved the opinion document "Antimicrobial resistance due to the use of biocides and heavy metals: a literature review".

A biocide is defined as an active chemical molecule that controls the growth of, or kills, bacteria and other microorganisms in a biocidal product. The biocides are classified into 4 main groups according to their application categories and further sub-divided into 23 product groups. In this assessment, we focus on biocides with potential antibacterial activity and their ability to induce antimicrobial (antibiotic) resistance in bacteria. These products belong mostly to main group 1; Disinfectants. Disinfectants include products used in human hygiene, veterinary hygiene, water treatment, and products used in the field of food and feed, but excludes cleaning products that are not intended to have a biocidal effect, including washing liquids, powders, and similar products.

Heavy metals are naturally occurring elements that have a high atomic weight and a density that is at least 5 times greater than that of water. Some heavy metals have been used as antimicrobial agents since antiquity, but often their modes of action have remained unclear. They are able to induce toxicity at low levels of exposure. In this report we have chosen to include the following elements in the category "heavy metals": silver (Ag), arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), and zinc (Zn). The selection is based on their area of use and on their ability to induce AMR.

Although most biocides and heavy metals are known to be high volume products, the working group was not able to obtain detailed data on the amounts used in Norway.

However, there is no doubt that the production and usage volumes and the area of application for several of these substances are several orders of magnitude higher than those of antimicrobial agents used in therapy and prophylaxis.

This Opinion document is not a traditional risk assessment, but a literature study that presents and compiles the available information regarding resistance development in bacteria due to use/misuse of biocides and heavy metals. Exposure assessment and risk characterization have therefore been excluded.

The following definitions regarding probability of biocides and heavy metals inducing AMR in bacteria are used:

- Highly likely - is expected to occur in most circumstances
- Likely - could occur in many circumstances
- Unlikely - could occur in some circumstances
- Highly unlikely (effectively zero) - may occur only in very rare circumstances

The most commonly used chemical substances with the potential to induce AMR and cross or co-resistance in bacteria are the heavy metals, copper, zinc, and cadmium ("highly likely"). Phenols, especially triclosan, surface-active agents, especially quaternary ammonium compounds (QACs), and the heavy metals arsenic and mercury are classified in the category "likely". The components in the "unlikely" group are aldehydes, biguanides, organic acids, inorganic acids, antimicrobial dyes, diamines, and silver.

It should be noted that a number of uncertainties are associated with assigning these substances to particular categories of probabilities/likelihoods (highly likely, likely, unlikely, and highly unlikely) in this assessment. Bacteria are living organisms that are continually changing their genetic compositions, and are able to adapt rapidly to altered living conditions. Furthermore, the concentrations of the substances to which the bacteria are exposed affects the probability of inducing resistance.

This report reviews the literature describing the current situation with regards to development of resistance in bacteria due to biocides and heavy metals. The current situation and genetic and phenotypic status may change as bacteria continue to adapt to exposure to biocides/heavy metals and antimicrobial agents at varying doses, durations, and combinations.

Data gaps

There is a lack of knowledge regarding the diverse reservoir of AMR in the environment including soil, sediment, water, air, wild plants, and animals that are impacted by biocides and heavy metals. We have not been able to gather sufficient data on the amount of the different biocides and heavy metals that end up, unintentionally, in the environment in Norway and the extent to which such exposure, alone or in combination with other antimicrobials, may result in development of AMR in microbial communities. Furthermore, limited data are available regarding use/misuse/presence of biocides and heavy metals in consumer products. Knowledge regarding development of resistance in bacteria due to use of biocides or heavy metals in cosmetic products is lacking.

Key words: VKM, assessment, literature study, Norwegian Scientific Committee for Food Safety, Norwegian Environment Agency, biocides, disinfectants, heavy metals, antimicrobial resistance, antibiotic resistance, antibiotics.

Sammendrag på norsk

Antimikrobiell resistens (vanligvis kalt antibiotikaresistens) er et raskt voksende problem i hele verden. Antimikrobiell resistens (AMR) kan beskrives som en bakteries evne til å motstå virkningen av et antimikrobielt middel (antibiotika). Antimikrobielle midler (antibiotika) brukes for å behandle og forebygge sykdom hos mennesker, dyr og planter. Bruk av slike midler kan føre til antimikrobiell resistens. Kjemiske forbindelser som biocider og tungmetaller kan også føre til utvikling av antimikrobiell resistens.

Miljødirektoratet ba Vitenskapskomiteen for mattrygghet (VKM) i juni 2016 om å gjennomføre et litteratursøk om utvikling av bakteriell resistens mot biocider og tungmetaller og kryssresistens mot antimikrobielle midler, med følgende mandat:

1. Liste opp hvilke kjemiske stoffer som kan ha resistensdrivende effekt hos mikroorganismer.
2. Beskrive hvilke av disse kjemiske stoffene som er brukt/brukes i Norge, og vurdere hvilke bruksområder som potensielt kan ha resistensdrivende effekt.
3. Foreta en rangering av hvilke kjemiske stoffer og bruksområder som antas å være mest resistensdrivende, basert på stoffenes egenskaper og bruksomfang i Norge.
4. Identifisere kunnskapshull vedrørende effekten av kjemiske stoffer på resistensutvikling.

For å svare på forespørselen, satte VKM ned en arbeidsgruppe bestående av to medlemmer fra faggruppen for mikrobiell økologi og en ekstern ekspert, til å forberede et utkast til rapport. Faggruppen i mikrobiell økologi har gjennomgått og revidert utkastet og godkjent rapporten.

Et biocid er definert som et aktivt kjemisk molekyl som kontrollerer veksten til eller dreper bakterier og andre mikroorganismer. Biocidene er klassifisert i fire hovedgrupper etter biocidenes bruksområder, og videre delt inn i 23 produktgrupper. I denne litteraturstudien har vi sett på biocider med potensiell antibakteriell aktivitet og deres evne til å utvikle antimikrobiell (antibiotika) resistens i bakterier. Vi har hovedsakelig omtalt produkter som tilhører hovedgruppe 1; desinfeksjonsmidler. Desinfeksjonsmidler inkluderer produkter som brukes til menneskelig hygiene, veterinær hygiene, vannbehandling og produkter som brukes i mat og fôr. Vi har ikke omtalt rengjøringsprodukter som ikke er ment å ha en biocid effekt, som vaskemidler og lignende produkter.

Tungmetaller er naturlig forekommende elementer med høy atomvekt, og med 5 ganger høyere egenvekt enn vann. Noen tungmetaller har blitt brukt som antimikrobielle midler siden antikken, men gjennom det meste av historien har tungmetallenes virkningsmekanisme vært ukjent. Tungmetaller er giftige i lave konsentrasjoner. I denne rapporten har vi valgt å inkludere og behandle følgende elementer i kategorien "tungmetaller": sølv (Ag), arsen (As), kadmium (Cd), kobber (Cu), kvikksølv (Hg) og sink (Zn). Disse er valgt på grunn av deres antimikrobielle effekt og evnen til å indusere antimikrobiell resistens (antibiotikaresistens).

De fleste biocider og tungmetaller tilføres naturen i større mengder, men arbeidsgruppen har ikke funnet gyldige data over hvilke mengder som brukes årlig i Norge. Det er imidlertid

ingen tvil om at produksjons- og bruksvolum for flere av disse produktene er flere titalls ganger høyere enn de antimikrobielle midlene som anvendes i behandling og forebygging.

Denne rapporten er ikke en tradisjonell risikovurdering, men en litteraturstudie som presenterer og sammenstiller tilgjengelige opplysninger om resistensutvikling hos bakterier som skyldes bruk eller misbruk av biocider og tungmetaller. Eksponeringsvurdering og risikokarakterisering er derfor ikke inkludert i rapporten.

Sannsynligheten for at biocider/tungmetaller kan føre til utvikling av resistens hos bakterier er definert på følgende måte:

- Svært sannsynlig - forventes å skje i de fleste tilfeller
- Sannsynlig - kan skje i mange tilfeller
- Usannsynlig - kan oppstå i enkelte tilfeller
- Svært lite sannsynlig - kan forekomme bare i svært sjeldne tilfeller

De vanligste anvendte kjemiske forbindelser med potensiale for å utvikle AMR og kryss eller ko-resistens hos bakterier er tungmetallene kobber, sink og kadmium. De er klassifisert i kategorien svært sannsynlig. Fenoler, spesielt triklosan, overflateaktive stoffer, spesielt kvartære ammoniumforbindelser, og tungmetallene arsen og kvikksølv, er klassifisert i kategorien sannsynlig. Komponentene i den usannsynlige gruppen er aldehyder, biguanider, organiske syrer, uorganiske syrer, fargestoffer, antimikrobielle diaminides og sølv.

Plasseringen av de ulike forbindelsene i en bestemt kategori av sannsynligheter er assosiert med usikkerhet. Bakterier er levende organismer som endrer sine gener kontinuerlig, og er derfor i stand til å tilpasse seg endrede levekår raskt. I tillegg vil konsentrasjonen av de kjemiske forbindelsene ha effekt på sannsynligheten for å føre til resistens.

I denne rapporten har vi gjennomgått litteratur som beskriver dagens situasjon med hensyn til utvikling av resistens i bakterier som blir eksponert for biocider og tungmetaller. Bakterienes genetiske og fenotypiske status kan imidlertid fortsette å endre seg. Bakteriene kan derfor fortsette å tilpasse seg eksponering mot av biocider og tungmetaller og andre antimikrobielle midler med variable doser, varighet og kombinasjoner.

Kunnskapshull

Det er mangel på kunnskap om utbredelsen av antimikrobiell resistens i naturlige miljøer som jord, sedimenter, vann, luft, ville planter og dyr som er påvirket av biocider og eller tungmetaller. Vi har ikke vært i stand til å samle tilstrekkelige data på mengden av de ulike biocider og tungmetaller som ender opp utilsiktet i miljøet i Norge, og i hvilken grad en slik eksponering alene eller i kombinasjon med andre antimikrobielle midler (antibiotika) kan føre til utvikling av antimikrobiell resistens i mikrobielle samfunn. Videre er det begrensede data tilgjengelig om bruk/misbruk/mengde biocider/tungmetaller i produkter til forbruker. VI mangler også kunnskap om utvikling av resistens hos bakterier som skyldes bruk av biocider eller tungmetaller i kosmetiske produkter.

Stikkord: VKM, risikovurdering, Vitenskapskomiteen for mattrygghet, Miljødirektoratet, biocider, desinfeksjonsmidler, tungmetaller, antimikrobiell resistens, antibiotika resistens.

Abbreviations and/or glossary

Abbreviations

ACP	Enoyl-Acyl carrier protein
AgNP	Silver nanoparticles
AMR	Antimicrobial resistance
ARB	Antimicrobial resistant bacteria
ARG	Antimicrobial resistance gene
BC	Benzylalkonium chloride
CoNS	Coagulase-negative staphylococci
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EPS	Extracellular polymeric substance
EUCAST	European Committee for Antimicrobial Susceptibility Testing
FAO	Food and Agricultural Organisation of the United Nations
HGT	Horizontal gene transfer
IUPAC	International Union of Pure and Applied Chemistry
MMC	Minimum metal co-selective concentration
MDR	Multidrug resistant
MIC	Minimum inhibitory concentration
MMC	Minimum metal co-selective concentration
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MRSE	Methicillin-resistant <i>Staphylococcus epidermidis</i>
MSSA	Methicillin-susceptible <i>Staphylococcus aureus</i>
NFSA	Norwegian Food Safety Authority

NORM	The Norwegian monitoring programme for AMR in human pathogens
OPA	<i>Ortho</i> -phthalaldehyde
PT	Product type
QACs	Quaternary ammonium compounds
RND	<u>Resistance-nodulation-cell division</u> protein family
ROS	Reactive oxygen species
SCCP	Scientific Committee on Consumer Products
SCENIHR	The Scientific Committee on Emerging and Newly Identified Health Risks
ToR	Terms of reference
VKM	Norwegian Scientific Committee for Food Safety
VRE	Vancomycin-resistant enterococci
WWTP	Wastewater treatment plant
WHO	World Health Organization

Glossary

Acquired resistance: Resistance to a particular antimicrobial agent to which the microorganism was previously susceptible. The change in resistance level is the result of genetic changes in a microorganism due to mutation(s), the acquisition of foreign genetic material, or a combination of both mechanisms.

Antibiotics: Traditionally refers to natural organic compounds produced by microorganisms that act in low concentrations against other microbial species, mostly bacteria. Today “antibiotics” also includes synthetic (chemotherapeutic) and semi-synthetic compounds (chemically modified antibiotics) with similar effects.

Antimicrobial agents: A general term for the drugs (antibiotics), chemicals, or other substances that either kill or inhibit the growth of microbes. The concept of antimicrobials applies to antibiotics, disinfectants, preservatives, sanitizing agents, and biocidal products in general.

Antimicrobial resistance: A property of microorganisms that confers the capacity to inactivate or exclude antimicrobials, or a mechanism that blocks the inhibitory or killing effects of antimicrobials.

Antiseptic agent: A chemical substance that kills or inhibits the growth and development of microorganisms, but has such low toxicity that it can safely be used on living tissue.

Bactericidal agent: An antimicrobial agent capable of killing bacteria.

Bacterostatic agent: An antimicrobial agent that inhibits the growth of bacteria.

Biocide/Biocidal products: Active substances and preparations containing one or more substances intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means.

Biocide resistance: When non-antibiotic antimicrobial agents (i.e., biocides) are considered, the word “resistance” is used in a similar way when a strain is not killed or inhibited by a concentration attained in practice (the in-use concentration) and in a situation where: 1) a strain is not killed or inhibited by a concentration to which the majority of strains of an organism are susceptible, or 2) bacterial cells are not killed or inhibited by a concentration acting upon the majority of cells in that culture (SCENHR, 2009).

Biofilm: Microbial biofilms are populations of microorganisms that are concentrated at an interface (usually solid/liquid) and typically surrounded by an extracellular polymeric slime matrix. Floccs are suspended aggregates of microorganisms surrounded by an extracellular polymeric slime matrix that is formed in liquid suspension.

Chemotherapeutics: Compounds with antimicrobial effect that are synthesized in the laboratory and that have no natural reserve in the environment. In modern popular literature chemotherapeutics and antibiotics are commonly referred to as “antibiotics”.

Clone (bacteria): Bacterial isolates that, although they may have been cultured independently from different sources in different locations and perhaps at different times,

still have so many identical phenotypic and genotypic traits that the most likely explanation for these similarities is a common origin within a relevant timespan.

Conjugation: Transfer of genetic material between different bacterial cells by direct cell-to-cell contact.

Co-resistance: Resistance occurring when the genes specifying different resistant phenotypes are genetically linked, for example by being located together on a mobile genetic element (e.g., a plasmid, transposon, or integron).

Cross-resistance: Resistance occurring when the same or similar mechanism(s) of resistance applies to different antimicrobials.

Disinfectant: Antimicrobial agents that are applied to non-living objects to destroy microorganisms that are living on the objects.

Disinfection: Use of physical procedures or chemical agents (disinfectants) to destroy most microbial forms (mainly on inanimate material, but also on skin surfaces). Disinfectants are often not effective against bacterial spores.

Fungicide: An agent that destroys fungi or inhibits their growth.

Germicide: An agent destroying many different microorganisms, also called disinfectant.

Heavy metal: Naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water.

Heavy metal resistance: Bacteria are considered to be resistant to heavy metals when: 1) a strain is not killed or inhibited by a concentration to which the majority of strains of a organism are susceptible, or 2) when bacterial cells that are not killed or inhibited by a concentration acting upon the majority of cells in that culture.

Indicator bacteria: Bacteria that are used to measure the hygienic conditions of food, water, processing environments etc. Indicator bacteria are not usually pathogenic, but their presence indicates that the product or environment tested may be contaminated with pathogenic bacteria, often originating from the same reservoirs as the indicator organisms.

Intrinsic resistance: A natural property of an organism resulting in decreased susceptibility to a particular antimicrobial agent.

Isolate (bacteria): A bacterial isolate is a single isolation in pure culture from a specimen.

Microbiota: Collective term for microflora (i.e., any type of microorganism) that may be found within a given environment.

Minimum Inhibitory Concentration (MIC): The lowest concentration of a given agent that inhibits growth of a microorganism under standard laboratory conditions. MIC data can provide information about the activity of antimicrobials (Seiler and Berendonk, 2012).

Normal flora: Indigenous microbial flora of human/animal external and internal surfaces like the skin, mouth, and gastrointestinal tract, and the upper respiratory tract. The normal flora contains numerous bacterial species, and numerous strains within each species.

Although it may contain opportunistic pathogens, the vast majority are symbiotic or commensals that contribute to general health, as well as to colonization resistance. However, some of these low-virulence bacteria of the normal flora may, under certain circumstances, become opportunistic pathogens.

Sanitizer: An agent that reduces microbiological contamination.

Selection (bacteria): A process by which some bacterial species or strains of bacteria in a population are selected for due to having a specific growth or survival advantage over other microorganisms. Antibacterial substances may provide a more resistant sub-population with such an advantage, enabling them to increase their relative prevalence.

Sterilization: The process of destroying all microorganisms (including spores).

Strain (bacteria): A subset of a bacterial species differing from other bacteria of the same species by some minor, but identifiable, difference.

Susceptibility: Describes the extent to which an antimicrobial agent affects a target microorganism.

Transduction: Transfer of genetic material from one bacterium to another via bacteriophages (viruses that infect bacteria and are integrated into the host genome).

Transformation: Direct uptake from the environment of fragments of naked DNA and their incorporation into the cell's own genome.

Tuberculocide / mycobacteriocide: Any agent that kills tubercle bacilli (*Mycobacterium tuberculosis*) or other mycobacteria.

Background as provided by the Norwegian Food Safety Authority/ Norwegian Environment Agency

The Norwegian Environment Agency refers to the assignment letter to VKM regarding risk assessment for 2016 and hereby ask VKM to perform a literature review on antimicrobial resistance due to the use of biocides and heavy metals.

Background

Development of antimicrobial resistance is a fast growing problem in the world. The national strategy against antibiotic resistance for 2015-2020 of the Norwegian government highlights that this problem must be considered in a holistic perspective, where human health and animal health and environment interact. Use of antibiotics may result in development of resistance, but other factors may also play a role. The presence of resistant bacteria in different environments, such as soil, water, sea, sediments and wild animals may all contribute to development of resistant bacteria of pathological relevance. Other substances such as biocides (disinfectants) and heavy metals may also play a role in the development of antibiotic resistance. However, more information is necessary about these factors. The strategy of the Norwegian government, which noted that increased knowledge on development of antibiotic resistance should be one goal, is based on the report "*Antibiotikaresistens – kunnskapshull og aktuelle tiltak (2014)*" prepared by an expert group. In this report, the presence of different substances in Norwegian environment and how these might contribute to the spread of antibiotic resistance are identified as areas where more information is necessary.

The goal of the current assignment is to compile available information on those substances that are most relevant to analyse further in relation to the presence and increase in resistance, with focus on the Norwegian environment. Available literature and relevant information on different substances (including naturally existing compounds) such as biocides and heavy metals, and their possible role in the development of antimicrobial resistance, should be assessed and included in the report.

Terms of reference:

- 1. List substances that may contribute to increased antimicrobial resistance.**
- 2. Describe the substances listed in question 1 that are in use in Norway and assess those fields of application that have the potential to contribute to increased resistance.**
- 3. Range the substances, according to field of application, that are assumed to have the greatest effect on the development of resistance, based on their characteristics and the amount used.**
- 4. Identify knowledge gaps regarding the effects of these substances on development of resistance.**

Studies that are relevant for understanding the effects of substances such as biocides and heavy metals on the development of AMR should be reviewed. The focus should particularly be on field of application and on substances that spread to the environment and are of relevance in Norway. Environments in this context include soil, sediment, water, air, wild plants, and animals. The assessment should not address antimicrobial agents alone, but their role in relation to these other substances may be discussed.

1. Introduction

Antimicrobial resistance (AMR) in bacteria (commonly referred to as antibiotic resistance) is now considered as one of the major public health challenges of our time (WHO, 2015). In Europe, the European Centre for Disease Prevention and Control (ECDC) and the European Medicines Agency (EMA) have estimated that more than 25 000 extra deaths annually are associated with AMR. The economic burden of this amounts to an estimated € 1.534.100.000 annually (ECDC/EMA, 2009).

The use of antimicrobial agents is intrinsically linked to the occurrence of bacterial resistance against these compounds. Antimicrobial agents are widely used for the treatment and prevention of diseases in humans, animals, and less commonly in crop plants. In addition to antimicrobial agents used for prophylaxis and treatment, heavy metals, used for example in animal farming and aquaculture, might promote the spread of AMR via co-selection. In this assessment, we focus on heavy metals with antibacterial activity and assess their capacity to promote AMR in bacteria.

According to the Directive 98/8/EC of the European Parliament and Council of the 16 February 1998, **biocidal products** are defined as active substances and preparations containing one or more active substances, intended to destroy, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organisms by chemical or biological means.

Biocides are composed of heterogeneous groups of natural and synthetic substances that can deter, render harmless, or exert a controlling effect on microorganisms by biological or chemical means (Barah, 2013). There are many biocidal substances in the market that act in different ways and sometimes several biocides are combined within a single product to increase the overall efficacy. Ideally, the combined action of all the biocides in a product should be greater than the sum of the individual actions (synergy). Biocides could be classified according to their chemical structure or according to their clinical and non-clinical application. According to the EC Product Directive 98/8/EC (BPD), which was adopted by the European parliament in 1998, biocides are classified into four main groups according to their application categories and further sub-divided into 23 product groups. For more information see Appendix 1.

Main group 1: Disinfectants

This group includes products used in human hygiene, veterinary hygiene, water treatment, and products used in the food and feed area, but excludes cleaning products that are not intended to have a biocidal effect, including washing liquids, powders, and similar products.

Main group 2: Preservatives

Unless otherwise stated these product types include only products such as preservatives for products during storage, film preservatives, wood preservatives, fibre, leather, rubber and polymerized materials preservatives, construction material preservatives, preservatives for liquid-cooling and processing systems, slimicides, and working or cutting fluid preservatives to prevent microbial and algal development.

Main group 3: Pest control.

Chemical pesticides that are used to control attacks by insects, parasites (nematodes), fungi, and bacteria on plants.

Main group 4: Other biocidal products: These include antifouling products, embalming products, and taxidermist fluids.

In this opinion, we focus on the biocides with potential antibacterial activity and their ability to induce AMR in bacteria. These are largely products that belong to main group 1; disinfectants.

Heavy metals are naturally occurring elements with a high atomic weight and a density at least 5 times greater than that of water. Some heavy metals have been used as antimicrobial agents since antiquity, but their modes of action are often unclear. They are able to induce toxicity at low levels of exposure. According to the International Union of Pure and Applied Chemistry (IUPAC), the term "heavy metal" may be a "meaningless term" because there is no standardized definition of a heavy metal (<https://www.iupac.org/publications/ci/2001/november/heavymetals.html>). Appendix 2 lists all the current definitions of the term "heavy metal" that the author (John H. Duffos) has been able to trace in scientific dictionaries or in other relevant literature. It should be noted that the term is frequently used without an associated definition, presumably by authors who thought that there was consensus about the meaning of the term. The table in Appendix II shows that this assumption is wrong and explains some of the confusion in the literature and in related policies and regulations. Some light metals or metalloids are toxic, but some high-density metals are not. For example, cadmium is generally considered a heavy metal, with an atomic number of 48 and specific gravity of 8.65, whereas gold is typically not toxic, but has an atomic number of 79 and a specific gravity of 18.88. For any given metal, the toxicity varies widely, depending on the allotrope or oxidation state of the metal. This example further illustrates the confusion that surrounds the term "heavy metals".

In addition to the use of heavy metals as biocides, there has been an increasing ecological and global public health concern in recent years associated with environmental contamination by heavy metals. Due to their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of considerable public health significance. They are all systemic toxicants that are known to induce multiple organ damage, even at relatively low levels of exposure. These metals are also classified as either "known" or "probable" human carcinogens based on epidemiological and experimental studies showing an association between exposure and cancer incidence in humans and animals (Tchounwou et al., 2012).

In environmental ecosystems, there is an intricate interaction between heavy-metal contaminants and native microorganisms. These organisms have developed unique resistance mechanisms that allow them to survive and, in some instances, remove/reduce the concentrations of contaminants in their environments. The use of natural microorganisms found in soil, water, and sludge was pioneering in the field of bioremediation, a treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substances (Monachese et al., 2012). Although important issues, both heavy metal toxicity and bioremediation are not mentioned in the ToR and are not covered in this report.

In this report we have chosen to include the following elements in the category "heavy metal": silver (Ag), arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), and Zinc (Zn), because of their usage area and their ability to induce AMR in bacteria.

Some studies suggest that metal contamination in natural environments could have an important role in the maintenance and proliferation of AMR (Alonso et al., 2001; Summers et

al., 1993). This is of particular concern considering that heavy metals of anthropogenic origin, like agricultural and aquacultural practices, are currently several orders of magnitude greater than levels of pharmaceutically-produced antimicrobial agents (Stepanauskas et al., 2005). Unlike antibiotics, metals are not subject to degradation and therefore represent a long-term selection pressure. Thus, there are concerns regarding the potential of metal contamination to maintain a pool of AMR genes in both natural and clinical settings.

After use, antimicrobials, including biocides residues and heavy metals, along with antimicrobial-resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) may be introduced to soil and water through sewage systems, direct excretion, land application of biosolids or animal manures as fertilizers, and irrigation with wastewater or treated effluents. The presence of active antimicrobial compounds and their metabolites in environmental compartments may also select for resistance in environmental bacterial communities or microbiota, which is defined as a collective term for microflora (i.e., any type of microorganism) that may be found within a given environment.

For the purpose of this report, **environment** is defined as the natural environment (or “outdoor” environment) for which exposure to the considered substances was not intended. Indoor environments, like hospitals and livestock housing, were excluded. Four environmental compartments were identified:

- Soil
- Water
- Air/dust, and
- Wildlife (animals and plants).

Wildlife (animals and plants) was categorized as an environmental compartment because these are not treated with antimicrobial agents, and their carriage of AMR bacteria is most likely explained by uptake of bacteria resistant to selective agents from the natural environment (Huijbers et al., 2015).

In this literature review, the panel focuses on the current body of knowledge regarding the role of substances such as biocides and heavy metals as components in the selection and co-selection of antimicrobial (e.g., antibiotic) resistance in the environment (Figure 1).

As this report is not a traditional risk assessment, but a literature review that presents and compiles the available information regarding resistance development in bacteria due to exposure to biocides and heavy metals, the risk assessment steps of exposure assessment and risk characterization have been excluded.

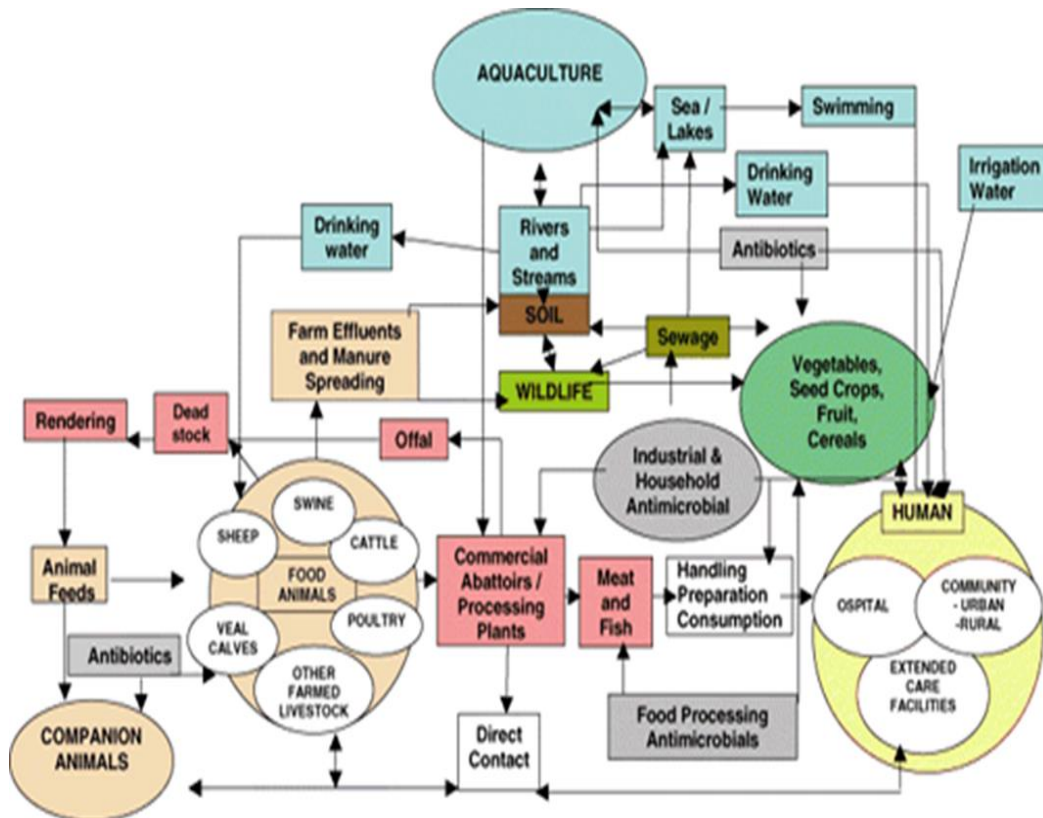


Figure 1. Schematic diagram of environmental compartments, contamination sources, exposure-relevant sites, and processes affecting survival and spread of bacteria, including antimicrobial (antibiotic) resistant bacteria (Davies and Davies, 2010), with permission from American Society for Microbiology.

1.1 Literature

1.2 Search strategy

The search was conducted in PubMed using the terms; different disinfectant agents (listed in Table 3), [Title/Abstract] AND Antimicrobial resistance [Title/Abstract] AND Review [Title/Abstract] using the Advanced Search Builder provided in PubMed (www.ncbi.nlm.nih.gov/pubmed) and resulted in 1588 citations (07. July 2016). Similar searches using the same terms, but different heavy metals listed in Table 3 resulted in 570 citations.

1.2.1 Inclusion criteria

We limited our search to review articles. Searches that included original articles resulted in several thousand papers. These could not be assessed during the time period available, but some studies were included in some special cases (See Table 3).

1.2.2 Exclusion criteria

Articles describing development of resistance in microorganisms other than bacteria, such as viruses, fungi, and parasites, were excluded as these were not part of the mandate. Articles that were not in English or a Scandinavian language (Swedish, Danish, and Norwegian) were excluded.

2. Hazard identification

Hazard identification is implicit in the title of this opinion and in the terms of reference (ToR). The issue of AMR in the environment is addressed either as a direct hazard or as an indirect hazard through resistance transfer.

- The direct hazard is an antimicrobial resistant pathogenic/apathogenic bacterium.
- The indirect hazard arises through resistance transfer. In this case, the hazard is the resistance gene.
- In some cases both hazards may occur; a resistant bacterium may transfer an additional genetic element to another resistant bacterium, enhancing the resistance level.

3. Hazard characterisation

Theoretical background

3.1 Modes of action of biocides

In contrast with chemotherapeutic agents, biocides have multiple target sites within the microbial cell and the overall damage to these target sites results in the bactericidal effect. Bacteriostatic effects, usually achieved by a lower concentration of a biocide, might correspond to a reversible activity on the cytoplasmic membrane and/or the temporal impairment of enzymatic activity. The bacteriostatic mechanism(s) of action of a biocide is less documented and a primary target site within the cell might be involved (Maillard, 2002).

The following factors may influence the efficacy of disinfectant agents:

- Innate resistance of microorganisms
- Number and location of microorganisms
- Concentration and potency of the disinfectant agent
- Physical and chemical factors (e.g., pH, temperature, salt)
- Organic and inorganic materials
- Duration of exposure
- Biofilms

3.2 Resistance mechanisms against biocides

Disease-causing bacteria can be described as being clinically resistant if they have a low probability of responding to a drug, even if the maximum dose of antimicrobial agent is administered (EUCAST, 2000). Degrees of susceptibility in bacteria are often defined in terms of the minimum inhibitory concentration (MIC) of an antimicrobial agent to prevent bacterial growth, and bacteria are defined as being resistant to an antimicrobial agent, when the MIC is significantly higher than that of its wild type counterpart. MIC determinations have been used in many studies as an indicator of the ability of bacteria to change their susceptibility to a biocide (Russell, 2002b; Walsh et al., 2003).

Bacteria can become resistant to biocides by using one or several of the pathways listed under. These pathways include both intrinsic and acquired resistance:

- a Biofilm formation
- b Change in the bacterial cell wall permeability (barrier)
- c Use of efflux pumps

- d Enzymatic degradation/inactivation of biocides
- e Target modification
- f Release of undamaged gene(s)
- g Alternative/unknown pathways.

As biocides have multiple target sites in a microbial cell, the emergence of general bacterial resistance is unlikely to be caused either (i) by a specific modification of a target site or (ii) by a by-pass of a metabolic process. Resistance generally emerges from a mechanism/process causing the decrease of the intracellular concentration of biocide under the threshold that is harmful to the bacterium. Several mechanisms based on this principle (mode of action) have been well-described, including change in cell envelope, alteration in permeability, efflux and degradation (SCENHR, 2009). Some of the resistance mechanisms are **intrinsic** (or **innate**) to the micro-organism, whereas others have been **acquired** (e.g., mutation, the acquisition of resistant determinants) through forced mutations or through the acquisition of mobile genetic elements (Poole, 2002). Innate mechanisms can confer high-level bacterial resistance («unsusceptibility») to biocides (**Table 1**).

Table 1. Bacterial mechanisms of resistance to biocides (SCENHR, 2009).

Mechanisms	Nature	Level of susceptibility to other biocides¹	Cross-resistance
Permeability	intrinsic (acquired)	no	yes
Efflux	intrinsic/acquired	reduced	yes
Degradation	acquired/intrinsic	reduced	no
Mutation (target site)	acquired	reduced	no ²
Phenotypic change	Following exposure	reduced	yes
Induction (stress response)	Following exposure	variable	yes

¹to other biocides - level of susceptibility defined according to the concentration of biocides

² not to other biocides, but cross-resistance with specific antibiotics.

3.3 Biocides with known antibacterial activity

In this opinion, only biocides belong to main group I (see Appendix 1), for which information about bacterial resistance is available in the public domain, will be discussed. The list of representative active substances classified on the basis of their chemical groups is presented in **Table 2**.

Table 2. List of active molecules in biocidal products classified on the basis of chemical groups (SCENHR, 2009). As this table is from 2009, we take the proviso that some of the active substances in this table may now be banned in EU.

Chemical Groups	Active molecules		CAS	Possible
			Registry Number	concentration range (%)
Phenols	Cresol	m-cresol ⁹	108-39-4	
		Isomeric mixtures	1319-77-3	
	Non-coal tar phenols	4-Tertiary octylphenol ¹⁰	140-66-9	
		2-Phenylphenol(2-phenylphenoxide)	90-43-7	
		4-Hexylresorcinol	136-77-6	
	Halo- and nitrophenols	2,4,6-Trichlorophenol	88-06-2	
		Pentachlorophenol (2-phenylphenoxide) [2 different substances, CAS N° refers to first]	87-86-5	Forbidden in EU
		4-Chloro-3-methylphenol (chlorocresol)	59-50-7	
		4-Chloro-3,5-dimethylphenol (chloroxylenol; para-chloro-meta-xylene; PCMX)	88-04-0	
		2,4-Dichloro-3,5-dimethylphenol (dichloroxylenol; dichloro-meta-xylene; DCMX)	133-53-9	
4-chloro-2-phenylphenol		607-12-5		
2-Benzyl-4-chlorophenol (chlorphen; ortho-benzyl-para-chlorophenol; OBPCP)		8013-49-8		
Nitrophenols Phenol		108-95-2		
Bis-phenols	Derivatives of dihydroxydiphenylmethane			
	Derivatives of hydroxydiphenylether		0.5	
	Derivatives of diphenylsulphide			
	Triclosan ¹¹ (2,4,4'-trichloro-2'-hydroxydiphenyl ether)	3380-34-5		
Organic and inorganic acids: esters and salts	Formic acid	64-18-6		
	Acetic acid (ethanoic acid)	64-19-7	0.4-52	
	Propionic acid	79-09-4		
	Undecanoic acid (undecylenic acid)	112-37-8		
	2,4-Hexadienoic acid (sorbic acid)	110-44-1		
	Lactic acid	598-82-3		
	Benzoic acid	65-85-0		
	Salicylic acid	69-72-7		
	Dehydroacetic acid (DHA, 3-acetyl-6-methylpyran-2,4[3H]-dione)	520-45-6		
	Sulphur dioxide, sulphites, bisulphites			
	Esters of p-hydroxybenzoic acid (parabens):			
	Methyl paraben	99-76-3		
	Ethyl paraben	120-47-8		

⁹ Estimated production in EU for m-cresol is greater than 1,000 tonnes per year (Dye et al. 2007).

¹⁰ USA: > 500 tonnes (Calafat et al. 2008).

¹¹ Estimated production in EU for triclosan is 10-1,000 tonnes per year (Dye et al. 2007).

Chemical Groups	Active molecules	CAS Registry Number	Possible concentration range (%)
	Propyl paraben Butyl paraben Vanillic acid esters	94-13-3 94-26-8	
Aromatic diamidines	Propamidine Dibromopropamidine	104-32-5 496-00-4	
Biguanides	Chlorhexidine Alexidine Polymeric biguanides	55-56-1 48110-46-8	0.43
Surface-active agents ¹²	Cationic agents (QACs) Anionic agents Nonionic agents Amphoteric (ampholytic) agents		0.03-50
Aldehydes	Glutaraldehyde (pentanedial) Formaldehyde (methanal) Ortho-phthalaldehyde Other aldehydes	111-30-8 50-00-0 643-79-8	2 0.03-15.7 0.5
Antimicrobial dyes	Acridines Triphenylmethane dyes Quinones		
Halogens	Iodine compounds	Free iodine Iodophors Iodoform	75-47-8
Chlorine compounds	Chlorine-releasing compounds Chloroform	67-66-3	0.02-22.4 Forbidden in EU by Directive 98/8/EC
Bromine	NH ₂ Br Alkaline bromine derivative	12124-97-9	10-25
Quinoline and isoquinoline derivatives	8-Hydroxyquinoline derivatives 4-Aminoquinaldinium derivatives Isoquinoline derivatives		
Alcohols	Ethyl alcohol (ethanol) Methyl alcohol (methanol) Isopropyl alcohol (isopropanol) Benzyl alcohol Phenylethanol (phenylethyl alcohol) Bronopol ¹³ (2-bromo-2-nitro-1,3-diol) Phenoxyethanol (phenoxyetol) Chlorbutanol (chlorbutol) 2,4-Dichlorobenzyl alcohol	64-17-5 67-56-1 67-63-0 100-51-6 60-12-8 52-51-7 122-99-6 57-15-8 1777-82-8	0.1-99.9 0.03-15 0.1-77.22

¹² Surface-active agents may not necessarily be used as active in a formulation, but as surfactants.

¹³ Bronopol tonnage is estimated from 10 to 1,000 tonnes per year in the EU (Dye et al. 2007).

Chemical Groups	Active molecules	CAS Registry Number	Possible concentration range (%)	
Peroxygens	Hydrogen peroxide	7722-84-1	0.5-29	
	Peracetic acid	79-21-0	0.008-0.23	
Heavy-metal derivatives	Copper compounds			
	Silver compounds			
	Mercury compounds	Mercurochrome (disodium-2,7-dibromo-4-hydroxymercurifluorescein)	129-16-8	
		Nitromersol (anhydro-2-hydroxymercuri-6-methyl-3-nitrophenol)		
		Thiomersal (merthiolate; sodium-o-(ethylmercurithio)-benzoate)	54-64-8	
Phenylmercuric nitrate (PMN) Phenylmercuric acetate (PMA)		55-68-5 62-38-4		
Tin and its compounds (organotins)				
Titanium				
Anilides	Salicylanilide	87-17-2		
	Diphenylureas (carbanilides)			
Derivatives of 1,3-dioxane	2,6-dimethyl-1,3-dioxan-4-ol acetate (isomeric mixture)(dimethoxane)	828-00-2		
	5-Bromo-5-nitro-1,3-dioxane (Bronidox)	30007-47-7		
Derivatives of imidazole	1,3-Di(hydroxymethyl)-5,5-dimethyl-2,4-dioximidazole; 1,3-Di-hydroxymethyl)-5,5-dimethylhydantoin (Dantoin)	6440-58-0	96-100	
	N,N''-methylene bis [5'[1-bydroxymethyl]-2,5-dioxo-4-imidazolidinyl urea] (Germall 115)	39236-46-9		
	Diazolidinyl Urea	78491-02-8		
Isothiazolones	5-Chloro-2-methyl-4-isothiazolin-3-one (CMIT) and 2-Methyl-4-isothiazolin-3-one (MIT) (mixture)	26172-55-4	0.00007-0.000141	
	2-Methyl-4-isothiazolin-3-one (MIT)	2682-20-4		
	2-n-Octyl-4-isothiazolin-3-one	26530-20-1		
	1,2-Benzisothiazolin-3-one (BIT)	2634-33-5		
Derivatives of hexamine	Triazines			
	Oxazolo-oxazoles			
	Sodium hydroxymethylglycinate	70161-44-3		
	Methylene bistiocyanate	6317-18-6		
	Captan	133-06-2		
1,2-dibromo-2,4-dicyanobutane (Tektamer 38)	35691-65-7			
Terpenes	Limonene (isomeric mixture)			
Vapour-phase disinfectants	Ethylene oxide	75-21-8		
	Formaldehyde-releasing agents			
	Propylene oxide	75-56-9		
	Methyl bromide	74-83-9		
	Ozone			

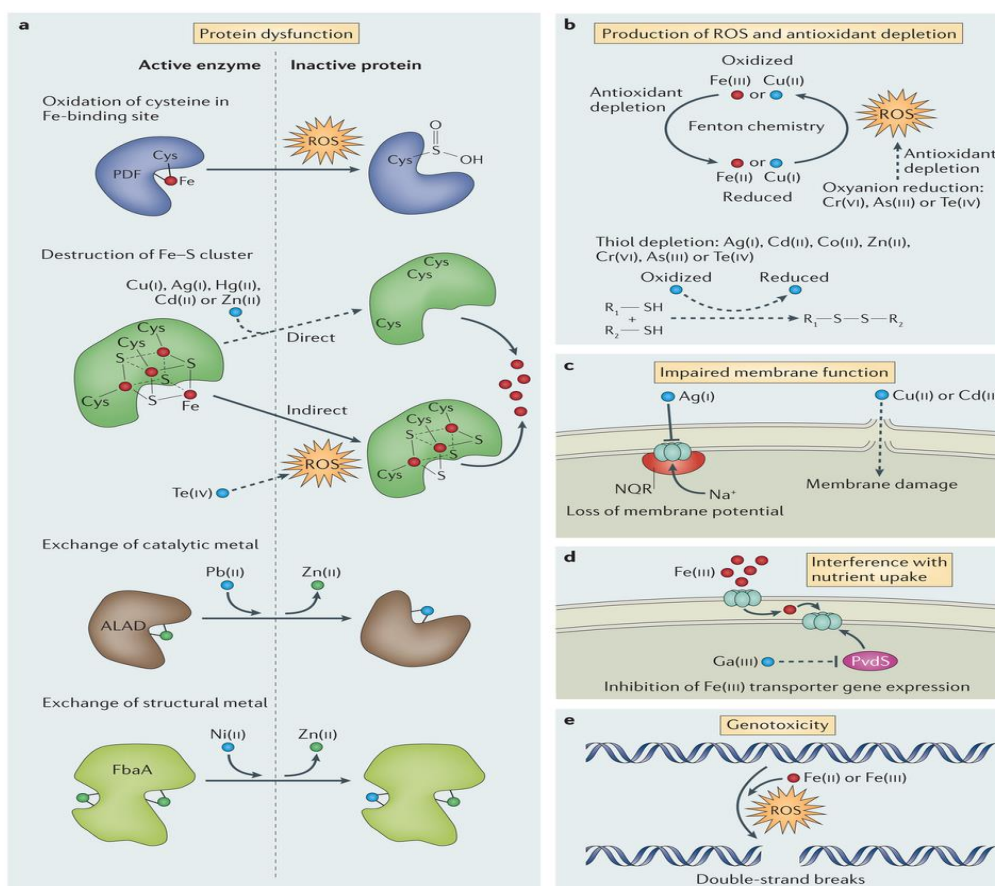
3.4 Mode of action of heavy metals

Metals are elements characterized as being good conductors of electricity and heat. They form ions and ionic bonds with non-metals. In a metal, atoms readily lose electrons to form cations that are surrounded by delocalized electrons. This behaviour is responsible for the conductivity and for the antimicrobial effects (Fraise et al., 2012). Heavy metals may be toxic to bacteria and this microbial toxicity may be due to their chemical affinity to thiol groups of macro-biomolecules, but also depends on the solubility of the metal compounds under physiological conditions (Lemire et al., 2013; Yazdankhah et al., 2014).

Several possible modes of action of heavy metals have been reported (Lemire et al., 2013):

- Protein dysfunction
- Production of reactive oxygen species (ROS) and antioxidant depletion
- Impaired membrane function
- Interference with nutrient uptake
- Genotoxicity

These mechanisms have been reviewed by Lemire et al. (2013) and are shown in **Figure 2**.



Nature Reviews | Microbiology

Figure 2. Exemplified mode of action of heavy metals (Lemire et al., 2013). These mechanisms of toxicity are specific to particular metal species. a | Metals can lead to protein dysfunction. b | They can also lead to the production of ROS and depletion of antioxidants. c | Certain metals have been shown to impair membrane function. d | Some can interfere with nutrient assimilation. e | They can also be genotoxic. Solid arrows represent pathways in which the underlying biochemistry has been elucidated, whereas dashed arrows represent a route of toxicity for which the underlying biochemical mechanism is unclear. ALAD, δ -aminolevulinic acid dehydratase; FbaA, fructose-1,6-bisphosphate aldolase; NQR, NADH:quinone oxidoreductase; PDF, peptide deformylase; PvdS, a σ -factor (σ 24) from *Pseudomonas aeruginosa*. With permission from Nature Publishing Group.

3.5 Heavy metals with known antibacterial activity

Probably the most commonly used toxic metals or metalloids in medicine and agriculture have been mercury (Hg), copper (Cu), silver (Ag), arsenic (As) and antimony (Sb), and zinc (Zn). Other inorganic or organic metal compounds, such as lead (Pb) (Trotter, 1990), tin (Sn) (Cooney and Wuertz, 1989), bismuth (Bi) (Ge and Sun, 2007; Mahony et al., 1999), gold (Au) (Novelli et al., 1999), cerium (Ce) (Garner and Heppell, 2005), palladium (Pd), (Cooney and Wuertz, 1989; Ray et al., 2007), tellurite (Te) (Taylor, 1999), thallium (Tl) (Kazantzis, 2000), and gallium (Ga) (Chitambar, 2010), have also been used because of their antimicrobial properties, although their areas of use have been limited. In addition, compounds containing gold (Au), platinum (Pt), palladium (Pd), vanadium (V), rhodium (Rh), titanium (Ti), iridium (Ir) and other rare metals have been used recently in medical diagnostics or imaging, as radiotherapeutics, or as anti-arthritis and anticancer therapeutics (Abrams and Murrer, 1993; Desoize, 2004; Zhang and Lippard, 2003).

In this assessment we focus only on the heavy metals Ag, As, Cd, Cu, Hg, and Zn, because of their broad usage, except Hg, and their ability to induce resistance in bacteria.

3.6 Heavy metals: mechanisms of resistance

In order to avoid cellular toxicity to elevated exposure to heavy metals, bacteria have evolved mechanisms of metal tolerance. Both the mechanisms of resistance and tolerance to heavy metals are discussed in the review article of Seiler and Berendonk (2012). The authors concluded that, like antimicrobial agents, heavy metals might promote the spread of AMR via co-selection.

There are three general mechanisms that may result in heavy metal resistance. These are illustrated in **Figure 3**:

- 1- The first mechanism is the complex formation or sequestration of toxic metals (Silver and Phung, 1996). Upon metal binding, the concentration of the free toxic ions in the cytoplasm is minimized. Biosorption of toxic metals is known from cell membranes, cell walls, and extracellular polymeric substance (EPS) of biofilms (Harrison et al., 2007). For example, the EPS matrix and the polysaccharides contained have been reported to bind heavy metals (Teitzel and Parsek, 2003). Thus, the metal tolerance of bacteria belonging to that biofilm was enhanced.
- 2- The second mechanism of resistance to toxic metals is detoxification through reduction of intracellular ions (Nies, 1999). A well-understood example is mercury reductase, encoded by the *merA* gene. This MerA protein reduces Hg^{2+} to the less toxic Hg^0 (Schiering et al., 1991). Hg^0 will then diffuse out of the cell, due to its low evaporation point (Nies, 1999).
- 3- Finally, excretion of toxic ions by efflux systems is the third mechanism of heavy metal resistance (Nies and Silver, 1995). The cation/proton antiporter Czc, known, for example, from *Alcaligenes eutrophus*, mediates resistance to the metal ions Cd^{2+} , Zn^{2+} , and Co^{2+} by removal of metals from the cytoplasm through the inner and outer membrane to the surrounding environment (Silver and Phung, 1996).

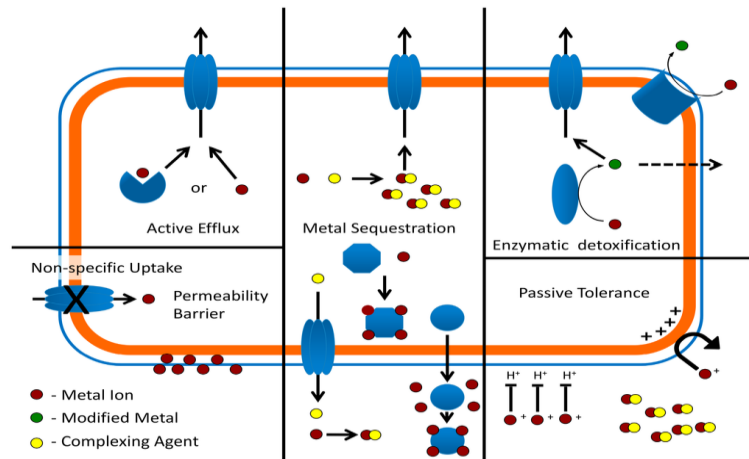


Figure 3. Overview of metal resistance mechanisms for acidophiles (Wheaton et al., 2015), Free access.

Most data regarding biocides/heavy metals resistance are collected from studies using planktonic phase microorganisms (unattached micro-organisms living freely in suspension) rather than microorganisms in more natural conditions, such as in a biofilm. Notably, gene expression in microorganisms living in a biofilm differs from that in planktonic cells, and the concentration of an agent needed to kill microorganisms in biofilms may be 10-500 times higher than in the planktonic phase.

3.7 Horizontal gene transfer (HGT)

While AMR properties in bacteria are transferred from one generation to the next by vertical gene transfer within the same bacterial species, horizontal gene transfer (HGT) may occur both within the same species and between different bacterial species, including unrelated bacterial species.

HGT may occur within and between bacterial species by conjugation, transformation, or transduction (see glossary), as has been described extensively in a review article by (Huddlestone, 2014) and illustrated in **Figure 4**.

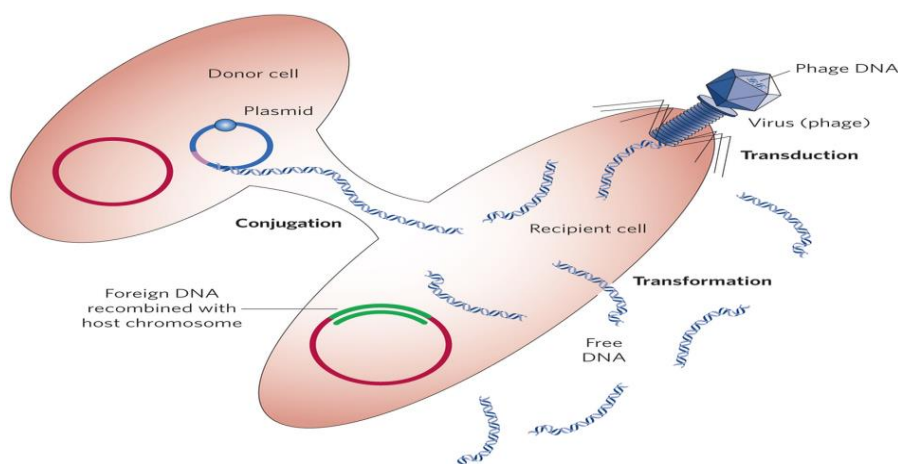


Figure 4. Different mechanisms involved in HGT. Bacteria and *Archaea* can acquire new genes, or variant copies of existing genes from genetically distant relatives through HGT. This can occur through the uptake of extracellular DNA (transformation), cell-to-cell transfer through surface appendages (conjugation), and viral import (transduction) (Stewart, 2013), with permission from Nature Publishing Group.

Multiple resistance in bacteria may occur either by co-resistance or cross-resistance (see glossary). The environment acts as a reservoir of bacteria of enormous density and species diversity, as well as being a reservoir for hundreds to thousands of known AMR genes with the mechanisms in place for HGT of any genes (Huijbers et al., 2015).

3.8 Drivers for AMR

All uses of antimicrobials, including biocides and heavy metals, in human and veterinary medicine, including aquaculture and agriculture, may be important drivers for the development of AMR in bacteria. The spread of AMR does not necessarily respect phylogenetic or ecological borders (Nielsen et al., 2014). Resistance to a certain antimicrobial agent can be selected, even by the use of other agents like antimicrobials, sanitizers, and some metal-containing compounds. The mobility of these AMR genes is attributed to their residence on mobile genetic elements – plasmids, transposons, and integrons (IFT, 2006).

Assessment

3.9 Literature

Titles and abstracts of all citations identified were screened and those that did not relate to the terms of reference were excluded. Of those of potential relevance, the full text was obtained and assessed whether it was of relevance to this Opinion. Review articles that focused on bacteria with reduced susceptibility against biocides and/or heavy metals with antibacterial activity were included in this assessment, except for biocide anilides and the heavy metals arsenic and cadmium as there was a lack of data in the review articles regarding the effects of these three compounds on resistance. **Table 3** shows the number of review articles included in this assessment.

We have not identified any reports of studies of the Norwegian environment in which the effects of biocides (listed in the **Table 3**) or heavy metals (**Table 3**) on microbiota in animals/humans or on microorganisms in the environment were investigated.

Table 3. Literature search in PubMed, using the terms name of the biocides or heavy metals AND Antimicrobial resistance AND Review.

Biocides or heavy metals	PubMed N= number	Excluded n	Included n	Comments
Phenols	202	194	18	Triclosan is reported as an important agent in this group that induces resistance in bacteria.
• Triclosan	47	32	15	
Alcohol	0	0	0	

Biocides or heavy metals	PubMed N= number	Excluded n	Included n	Comments
Aldehyde	42	37	5	New literature search using the terms: Aldehyde AND biocide AND Antimicrobial resistant bacteria. The search was limited to publications from 2000-2016
	98	63	35	
Anilides	24	24	0	New search was performed, using the terms "Antimicrobial" resistance" AND "Salicylanilide" and "Antimicrobial" resistance" AND "Carbanilides"
• Salicylanilide	21	21	0	
• Carbanilides	41	41	0	
Peroxygen				
• Hydrogen peroxide	101	101	0	
	7	7	0	
• Peracetic acid				
Biguanide				
• Chlorhexidine	85	74	9	
•				
• Alexidine	7	7	0	
•				
• Polymeric biguanides	7	7	0	
QACs (quaternary ammonium compounds)	83	65	18	
Organic acids	19	18	1	
Inorganic acids	2	2	0	

Biocides or heavy metals	PubMed N= number	Excluded n	Included n	Comments
Acridine	30	28	2	
Triphenylmethane	2	1	1	
Quinones	81	81	0	
Diaminides	16	16	0	
Fungicides*	41	40	1	Search words; "Antibiotic resistance + fungicides+Review"
	632	13	0	"Antibiotic resistance + fungicides"
Arsenic	35	32	3	Review articles
	200	153	47**	Search words; «Arsenic AND antimicrobial AND resistance AND bacteria
Copper	44	31	13	
Mercury	12	8	4	
Silver	65	61	4	
Zinc	81	76	5	
Cadmium	2	2	0	Review articles
	131	29	102	Original articles using the terms cadmium AND antimicrobial resistant bacteria
Totals	2158	1264	264	

*Using the terms "antibiotic resistance" AND "Fungicides" AND "Review" resulted in 41 articles. Using the terms "antibiotic resistance" AND "Fungicides" resulted in 632 articles. Only 14 articles fulfilled the inclusion criteria. None of these articles were relevant except for the articles on the so-called DMI fungicides (Gisi, 2014). These fungicides belong to the azole group, and are used to control pathogenic fungi in agriculture, veterinary medicine, and human medicine.

**30 articles from bacteria isolated from animal/human, 17 from bacteria from environmental origin

3.9.1 Biocides

The biocides market represents 10-11 billion euros in Europe and has been growing at 4-5% per annum for the last 15 years. This market is also predicted to continue expanding during the next years (for further information see <http://www.pan-europe.info/campaigns/biocides>). Whereas pesticides legislation plans for more sustainable use of pesticides in agriculture and up-to-date statistics on sales and use, no such demands have been proposed for biocides.

In this assessment, the chemical structure of biocides, and not the area for use, have been used for classification. We focus on biocides in category 1; disinfectant agents, as the products in this group are used because of their antimicrobial properties.

Below we discuss the mechanisms of antimicrobial action and mechanisms of resistance for a list of disinfectant agents, used in different settings.

Table 4. Alcohols

Chemical group	Alcohol R-OH (R: aliphatic/aromatic)
Active molecules	This group is composed of several active substances, listed in Table 2 . However, ethyl alcohol (ethanol), methyl alcohol (methanol), and isopropyl alcohol (isopropanol) are the most widely used as biocides (McDonnell and Russell, 1999). Based on their chemical structure, they can be divided into aliphatic (e.g., ethanol, isopropanol) and aromatic alcohols (e.g., benzyl alcohol).
Mechanisms of action	The exact mechanism of action for alcohols as biocides is still to be determined, but due to their increased efficacy in the presence of water, it is generally believed that alcohol causes membrane damage, thereby inducing inhibition of cell wall synthesis, rapid denaturation of proteins, and inhibition of DNA and RNA synthesis, with subsequent interference with metabolism and cell lysis (McDonnell and Russell, 1999).
Antimicrobial activity	Alcohols have generally rapid bactericidal activity. Their antimicrobial activity is optimal in concentration range of 60-90 %; within this range alcohols exhibit rapid broad spectrum activity against vegetative bacteria (including <i>Mycobacteria</i> spp.), some, but not all, viruses, and fungi. Although they are not sporicidal, alcohols are known to have reversible features of inhibiting sporulation and spore germination (Barah, 2013).
Uses	Most aliphatic alcohols are used as antiseptics and disinfectant agents and some can be used as preservatives (chlorbutanol and broponol). Aromatic alcohols are mainly used as as preservatives (benzyl alcohol, phenylethanol and phenoxy ethanol).
Antimicrobial resistance	Searches with the terms; alcohol AND Antimicrobial resistance in PUBMED did not identify any articles that provided information on bacterial species with reduced susceptibility/resistance against alcohols.
Resistance in environmental bacteria	See above; AMR

Conclusion	Alcohols have high and rapid bactericidal effects on both Gram-positive and Gram-negative bacteria. Despite the long history of use of alcohols as non-antibiotic biocides, no bacteria have been reported to have developed resistance towards alcohols.
-------------------	---

Table 5. Aldehydes

Chemical group	Aldehydes R-CHO
Active molecules	<ul style="list-style-type: none"> • Glutaraldehyde • Formaldehyde • <i>Ortho</i>-phthalaldehyde (OPA) • Other aldehydes
Mechanisms of action	Aldehydes act by alkylating various chemical groups associated with proteins and DNA/RNA, resulting in subsequent cross-linking of macromolecules (Rutala and Weber, 2008).
Antimicrobial activity	Most aldehydes currently used as biocides (see above), have both bactericidal and sporicidal activity (Rutala and Weber, 2008).
Uses	<p>Usage areas for aldehydes are reviewed in McDonnell and Russell (1999):</p> <p>Glutaraldehyde has been recommended for disinfection/sterilization of some medical equipment, notably cystoscopes and anaesthetic equipment. Due to the carcinogenic effects of glutaraldehyde, this use has been minimized.</p> <p>Formaldehyde is used as a disinfectant in both liquid and gaseous states. Vapour-phase formaldehyde is used in disinfection of sealed rooms and treatment of warts. It is also used to inactivate bacterial products in the process of toxoid vaccine production.</p> <p>Disinfection with OPA is indicated for semi-critical instruments that come into contact with mucous membranes or broken skin, such as specula, laryngeal mirrors, and internal ultrasound probes. OPA has been suggested as a replacement for glutaraldehyde in endoscope disinfection.</p>

<p>Antimicrobial resistance</p>	<p>Searches with the terms "Aldehyde" AND "Antimicrobial resistance" AND "Review" gave 42 papers. Three of these reviews indicate tolerance/resistance against aldehydes in bacteria (Russell, 2002a; Russell, 2002b; Savluchinske-Feio et al., 2006). A further literature search using the terms "Aldehyde" AND "Biocide" AND "Antimicrobial resistant bacteria", limited to the period 2000-2016 gave 98 articles, of which 35 reported reduced susceptibility/tolerance in different bacterial species such as <i>Pseudomonas</i> (Kampf et al., 2013; Selvaraju et al., 2011; Simoes et al., 2011; Tschudin-Sutter et al., 2011; Vikram et al., 2015), <i>Bacillus</i> (Herruzo Cebera, 2005; March et al., 2012; Moeller et al., 2012; Simoes et al., 2011), <i>Mycobacterium</i> (Lorena et al., 2010; Mitsui et al., 2005; Svetlikova et al., 2009; Wang et al., 2005), <i>Helicobacter</i> (Chiu et al., 2009), and <i>E. coli</i> (Dorsey and Actis, 2004).</p> <p>Phenotypic adaptation to glutaraldehyde in the bacterial species examined is generally associated with genetic change(s). In <i>E. coli</i> VU3695, the gene encoding resistance against aldehyde contains both a chromosomal copy and a plasmid copy of <i>adhC</i> actively expressed, with the latter involved in resistance to exogenous formaldehyde (Dorsey and Actis, 2004). Correlation between resistance against aldehyde and the MIC of the antibiotic classes was reported in many of the studies. Therefore, there is concern that widespread use of glutaraldehyde and OPA in clinical settings may select for drug-resistant bacteria (Svetlikova et al., 2009).</p> <p>Bacteria can survive aldehyde-based disinfection and may pose a cross-contamination risk to patients.</p>
<p>Resistance in environmental bacteria</p>	<p>A formaldehyde-tolerant bacterial strain designated as DM-2 strain has been used to biodegrade formaldehyde. The cells, precultivated in the presence of 400 ppm of formaldehyde, were able to degrade formaldehyde in a minimal medium supplemented with up to 400 ppm of formaldehyde in the presence of 3 % NaCl (Yamazaki et al., 2001). No other studies regarding development of resistance towards aldehyde in bacteria of environmental origin were identified.</p>
<p>Conclusion</p>	<p>Bacterial species that are exposed to aldehydes used for disinfection/sterilization of certain types of medical equipment may develop insusceptibility/adaptation against aldehydes. A possible link has been observed between resistance against aldehyde and clinically important antimicrobial agents. Due to the restricted use of aldehydes as disinfectants, resistance against aldehydes and cross-resistance against antimicrobial agents may not pose a major problem in the environment.</p>

Table 6. Anilides

Chemical group	Anilides General structure: C ₆ H ₅ NHCO-R
Active molecules	<ul style="list-style-type: none"> • Salicylanilide • Diphenylureas (carbanilides), including triclorcarban
Mechanisms of action	These compounds owe their bacteriostatic action to their ability to discharge part of the proton-motive force, thereby inhibiting processes dependent upon it (active transport and energy metabolism). They may also act by absorbing to, and destroying the semipermeability of, the cytoplasmic membrane (McDonnell and Russell, 1999).
Antimicrobial activity	Mainly active against Gram-positive bacteria and significantly less active against Gram-negative bacteria and fungi.
Uses	Anilides are rarely used clinically. Triclorcarban, the most studied anilide, is used mostly in antiseptic soaps, deodorants, and other household products. Triclorcarban is one of the most potent agents in the diphenylureas (carbanilides) family and inhibits growth of many Gram-positive bacteria, including MRSA and VRE (Walsh et al., 2003).
Antimicrobial resistance	Searches with the terms; Anilides AND Antimicrobial resistance/Salicylanilide AND Antimicrobial resistance / carbanilide AND Antimicrobial resistance did not identify any articles that provided information on bacterial species with reduced susceptibility/resistance against anilides.
Resistance in environmental bacteria	See above; AMR
Conclusion	Anilides are mostly active against Gram-positive bacteria, including MRSA and VRE, and also <i>Mycobacterium</i> , but have less/no activity against Gram-negative bacteria. No information was identified regarding reduced susceptibility or development of resistance against anilides and the most used active molecules (salicylanilide, diphenylureas (carbanilides) including triclorcarban).

Table 7. Phenols

Chemical group	Phenols (C₆H₆O) Phenols are mainly synthetic chemicals, but also occur naturally in algae and plants. The synthetic group is composed of cresols, non-coal tar phenols, halophenols, nitrophenols, and bisphenols.
Active molecules	See Table 3 , for active molecules belonging to this group. The most well known active molecule in this group with antibacterial activity is triclosan ^a .

Mechanisms of action	<p>At low concentrations, triclosan, which is a broad-spectrum antibacterial agent, inhibits bacterial fatty acid synthesis at the enoyl-acyl carrier protein (ACP) reductase (FabI) step.</p> <p>Low concentrations of triclosan discharges membrane potential in <i>E. faecalis</i>.</p> <p>Low concentrations of fenticlor and triclosan inhibit energy-dependent uptake of amino acids</p> <p>Dinitrophenol interferes with membrane energy (ATP synthesis).</p> <p>For more information see SCENHR (2009)</p>
Antimicrobial activity	<p>Reports on the antimicrobial efficacy of commonly used phenols show that they are bactericidal, fungicidal, virucidal, and tuberculocidal (Rutala and Weber, 2008).</p>
Uses	<p>Phenols are used for their antiseptic, disinfectant, or preservative properties, depending on compound (McDonnell and Russell 1999). The largest single use of phenol is to make plastics, but it is also used to make caprolactam (to make nylon 6 and other man-made fibres) and bisphenol A (used to make epoxy and other resins). It is also used as a slimicide (to kill bacteria and fungi in watery slimes), as a disinfectant, and in medical products (Agency for Toxic Substances and Disease Registry (ATSDR 1998) (https://www.atsdr.cdc.gov/ToxProfiles/Index.asp).</p>
Antimicrobial resistance	<p>Triclosan is the most active molecule with antibacterial activity and with the ability to induce antibacterial resistance in this chemical group. Although, the bactericidal activity of triclosan involves action on multiple cellular targets, several studies have demonstrated that at sub-lethal concentrations triclosan inhibits a specific bacterial target, ACP reductase (FabI enzyme) in <i>Escherichia coli</i>, <i>Pseudomonas aeruginosa</i>, and <i>Staphylococcus aureus</i>, or its homologue, the InhA gene in <i>Mycobacterium smegmatis</i> and <i>Mycobacterium tuberculosis</i> (Yazdankhah et al., 2006). This mode of action may allow triclosan to induce resistance and cross-resistance in bacterial cells (Saleh et al., 2011).</p>

<p>Resistance in environmental bacteria</p>	<p>In the risk assessment regarding the use of triclosan in cosmetics – development of AMR in bacteria (VKM, 2005), it was concluded that: “Little information exists regarding the incidence of triclosan resistance amongst environmental bacteria. Two soil isolates, <i>Pseudomonas putida</i> and <i>Alcaligenes xylosoxidans</i> spp. denitrificans, expressed high levels of resistance to triclosan due to production of an enzyme that degraded triclosan. The extensive use of triclosan in household, industrial and clinical settings results in widespread disposal, commonly into the wastewater system, which ultimately leads to environmental deposition; triclosan has been found in wastewater, environmental sediments, and aquatic biota. The presence of triclosan at sub-lethal concentrations in the environment may lead to the emergence of resistance amongst environmental bacteria. Much of the research regarding development of AMR involves pathogenic bacteria and is restricted to cultivable, facultative anaerobe bacteria. Knowledge about the impact of triclosan use on commensal, non-cultivable and obligate anaerobes, which are the predominant bacteria in the oral cavity, gut, and skin flora, is limited. These bacteria may constitute pools of resistance determinants potentially transferable to human pathogens.” We could not identify any new information in the literature regarding the effect of triclosan on development of AMR in bacteria of environmental origin.</p>
<p>Conclusion</p>	<p>Triclosan was identified as the substance in the phenol group that induces resistance in bacteria from different origins. The literature search in PubMed identified 47 review articles on triclosan, of which 14 discussed resistance of bacteria due to use of triclosan and possible cross-resistance to antibiotics. Two of these 14 articles concluded that development of resistance in bacteria due to use of triclosan is questionable (Gilbert and McBain, 2001; Sreenivasan and Gaffar, 2002) , however, the other 12 papers (Aiello and Larson, 2003; Carey and McNamara, 2014; Giuliano and Rybak, 2015; Heir et al., 2001; Leaper et al., 2011; Levy, 2002; McArthur et al., 2012; Poole, 2002; Saleh et al., 2011; Schweizer, 2001; Weber and Rutala, 2006; Yazdankhah et al., 2006), mostly published during the last ten years, are concerned with the use of triclosan in different settings. As has been stated by Giuliano and Rybak (2015), resistance to triclosan and cross-resistance to antimicrobials have been consistently demonstrated in laboratory settings, although overall resistance rates and cross-resistance rates in the community setting are low.</p>

^aNorwegian Scientific Committee for Food Safety performed a risk assessment on the use of triclosan in cosmetics – development of antimicrobial resistance in bacteria (VKM, 2005). An updated assessment was performed in 2007 after the Scientific Committee on Consumer Products (SCCP) of European Commission had discussed the assessment report from 2005 (VKM, 2007).

Table 8. Peroxygen compounds

Chemical group	Peroxygen compounds
Active molecules	<ul style="list-style-type: none"> • Hydrogen peroxide H₂O₂ • Peracetic acid (C₂H₄O₃) is a mixture of acetic acid (CH₃COOH) and hydrogen peroxide (H₂O₂) <p>Both hydrogen peroxide and peracetic acid are considered to be high level disinfectants due to their highly reactive hydroxyl radicals (Al-Adham et al., 2013).</p>
Mechanisms of action	<p>Hydrogen peroxide: Hydrogen peroxide acts as an oxidizing agent by producing hydroxyl free radicals that attack essential cell components, including lipids, proteins, and DNA (Linley et al., 2012).</p> <p>Peracetic acid: not fully understood, but may denature proteins, disrupt cell wall permeability, and oxidize sulphhydryl and sulphur bonds in proteins, enzymes, and other metabolites (Block, 2001).</p>
Antimicrobial activity	<p>Hydrogen peroxide: broad-spectrum efficacy against bacteria and their spores, viruses, and fungi. In general, greater activity is seen against Gram-positive bacteria than Gram-negative bacteria. However, the ability of bacteria to produce catalase can increase tolerance when lower concentrations are used.</p> <p>Peracetic acid: a more potent biocide than hydrogen peroxide, being sporicidal, bactericidal, virucidal, and fungicidal at low concentrations.</p>
Uses	<p>Hydrogen peroxide: Hydrogen peroxide is widely used for disinfection, sterilization, and antiseptic use, particularly in applications where its decomposition into non-toxic by-products is important. The use of hydrogen peroxide for treatment against sea lice infections in salmon farming has increased from zero in 2008 to more than 30000 tonnes in 2014 (https://www.fhi.no/hn/legemiddelbruk/fisk).</p> <p>Peracetic acid: Mainly used as a disinfectant for wastewater effluents. Its other application is as a low-temperature liquid sterilant for medical devices, flexible scopes, and haemodialyzers, but it is also used as an environmental surface sterilant (McDonnell and Russell, 1999).</p>

Antimicrobial resistance	Data demonstrating that bacteria can develop insusceptibility/resistance to peroxygen compounds are lacking. However, catalases and NADH peroxidase (Ahp) are primary scavengers in many bacteria, and their activities and physiological impacts have been demonstrated through phenotypic analysis and direct measurements of H ₂ O ₂ clearance <i>in vivo</i> . A wide variety of additional enzymes have been proposed to serve similar roles: thiol peroxidase, bacterioferritin comigratory protein, glutathione peroxidase, cytochrome c peroxidase, and rubrerythrins. Each of these enzymes can degrade H ₂ O ₂ <i>in vitro</i> , but their contributions <i>in vivo</i> remain unclear (Mishra and Imlay, 2012).
Resistance in environmental bacteria	See above; AMR
Conclusion	Both hydrogen peroxide and peracetic acid are considered to be high level disinfectants due to their highly reactive hydroxyl radicals. Bacterial species that have developed resistance to peroxygens have not been identified, probably because the active substances have several targets in bacterial cells. However, enzymes like catalases and NADH peroxidase (Ahp) are primary scavengers in many bacteria, but their role <i>in vivo</i> remains unclear.

Table 9. Biguanides

Chemical group	Biguanides
Active molecules	<ul style="list-style-type: none"> • Chlorhexidine • Alexidine • Polymeric biguanides
Mechanisms of action	<p>Various modes of action for different biguanide compounds have been identified (McDonnell and Russell, 1999):</p> <p>Chlorhexidine: both bactericidal and bacteriostatic mechanisms of action, mainly dependent on membrane disruption. In addition, some reports link its mechanism of action to the inhibition of membrane-bound ATPase</p> <p>Alexidine: a more rapid bactericide and produces a significantly faster alteration in membrane permeability than chlorhexidine.</p> <p>Polymeric biguanides: unlike chlorhexidine, but like alexidine, these cause domain formation of the acidic phospholipids of the cytoplasmic membrane.</p>

<p>Antimicrobial activity</p>	<p>Chlorhexidine: wide spectrum of activity against both Gram-positive and Gram-negative bacteria. Some bacteria, notably strains of <i>Proteus</i> and <i>Providencia</i> spp., may be highly resistant against biguanides. Chlorhexidine is not sporicidal, but prevents development and outgrowth of spores without inhibiting germination. It is not lethal against acid fast bacteria such as <i>Mycobacterium</i> (Al-Adham et al., 2013).</p> <p>Alexidine: more rapidly bactericidal and produces a significantly faster alteration in membrane permeability than chlorhexidine (McDonnell and Russell, 1999).</p> <p>Polymeric biguanides: polymeric biguanides, such as vantocil, are active against Gram-positive and most Gram-negative bacteria. However, they are not sporicidal (McDonnell and Russell, 1999).</p>
<p>Uses</p>	<p>Chlorhexidine: its common applications are attributed to its long-lasting broad-spectrum efficacy and substantivity for the skin. One of the most commonly used biocides in antiseptic products, particularly in handwashing and dental and oral products. In addition, it can be used as a disinfectant and preservative. It is usually used as an active ingredient in mouthwash designed to reduce plaque, gingival inflammation, and bleeding.</p> <p>Alexidine: applications of alexidine are similar to those of chlorhexidine.</p> <p>Polymeric biguanides (e.g., vantocil): used as general disinfection agents in the food industry and, very successfully, for disinfection of swimming pools.</p>

<p>Antimicrobial resistance</p>	<p>Chlorhexidine: Nine of 85 articles identified discussed development of resistance in bacteria due to use of chlorhexidine. The results concur with the conclusions from the VKM risk assessment from 2010 (VKM, 2010) in which development of resistance in bacteria due to use of chlorhexidine in cosmetic products was assessed. It was concluded that: "Intrinsic resistance mechanisms towards chlorhexidine are particularly characteristic of Gram-negative bacteria, but also of bacterial spores, mycobacteria, and, under certain conditions, staphylococci also display such mechanisms. There are limited published data on acquired chlorhexidine resistance in bacteria, but from those available, acquired resistance towards chlorhexidine has been described from members of the <i>Streptococcus</i> spp., <i>Staphylococcus</i> spp., and <i>Enterobacteriaceae</i>. This resistance may result from increased expression of chromosomally located efflux pumps, acquisition of plasmid-encoded efflux pumps, or changes in susceptibility by other presently unknown mechanisms".</p> <p>Alexidine: No articles were identified in the literature review. Due to mechanisms of action that are similar to those of chlorhexidine, information regarding development of resistance in bacteria to chlorhexidine are also applicable to alexidine.</p> <p>Polymeric biguanides: no articles were identified in the literature review, but due to mechanisms of action that are similar to those of chlorhexidine, information regarding development of resistance in bacteria to chlorhexidine are also applicable to polymeric biguanides.</p>
--	--

<p>Resistance in environmental bacteria</p>	<p>In 2010, VKM assessed development of resistance in bacteria due to use of chlorhexidine in cosmetic products. The bacteria were assessed from different niches, including environment (VKM, 2010) . It was concluded that:</p> <p>“As chlorhexidine compounds enter the environment via the sewage system they will inevitably act on microbes found here. In a study by Lawrence et al. (2008), the effects of chlorhexidine on microbial biofilms from river water were examined. The authors observed significant effects of chlorhexidine at a concentration of 100 µg/L on the protozoan, algal, cyanobacterial and bacterial biomass. At this concentration a virtual elimination of the protozoan community in the biofilms was observed, resulting in lowered grazing activity. Nuñez and Moretton (2007) examined the bacterial resistance pattern to several disinfectants, including chlorhexidine, in hospital sewage effluents in Buenos Aires. Between 10³ and 10⁶ chlorhexidine-resistant bacteria/100 mL were isolated from the samples. The bacterial population resistant to disinfectants consisted mainly of members of the <i>Enterobacteriaceae</i> family, <i>Staphylococcus</i> spp., and <i>Bacillus</i> spp. Bacterial isolates were tested for their resistance patterns by an agar dilution method, with chlorhexidine in increasing concentrations. The chlorhexidine MIC in the resistant bacteria isolated from the hospital sewage ranged from 50 to 150 mg/L, and included <i>Shigella dysenteriae</i>, <i>Shigella flexneri</i>, <i>Proteus vulgaris</i>, <i>Aeromonas hydrophila</i>, <i>Alcaligenes</i> sp., <i>Acinetobacter</i> sp. and <i>Pseudomonas aeruginosa</i>. The authors conclude that hospital effluents are of importance in the bacterial resistance selection process, particularly in the case of disinfectants.</p>
<p>Conclusion</p>	<p>There are limited published data on acquired resistance to biguanides, including chlorhexidine resistance in bacteria. However, from the information available, acquired resistance towards chlorhexidine has been described from members of <i>Streptococcus</i> spp., <i>Staphylococcus</i> spp., and <i>Enterobacteriaceae</i>. Resistance against chlorhexidine may be due to: a) increased expression of chromosomally located efflux pumps; b) acquisition of plasmid-encoded efflux pumps; or c) other presently unknown mechanisms. No cross-resistance or co-resistance between biguanides and other antimicrobial agents has been reported.</p>

Table 10. Surface-active agents

Chemical group	Surface-active agents
Active molecules	Surface-active agents (surfactants) have two regions in their molecular structure, one a hydrocarbon, water-repellent (hydrophobic) group, and the other a water-attracting (hydrophilic) group. Depending on the basis of the charge or absence of the hydrophilic group, surface-active agents are classified (McDonnell and Russell, 1999) into: cationic agents, for example quaternary ammonium compounds (QACs), anionic agents, nonionic agents, and amphoteric (ampholytic) agents. The cationic agents, as exemplified by QACs, are the most commonly used antiseptics and disinfectants.
Mechanisms of action	QACs predominantly act on the cytoplasmic membrane. The action involves association of positively charged quaternary nitrogen with the head group of acidic-phospholipids, before integration of the hydrophobic tail into the hydrophobic membrane core. At high concentrations, QACs solubilize hydrophobic cell membrane components by forming mixed micellar aggregates (Gilbert and Moore, 2005). Disruption and denaturation of structural proteins and enzymes has been suggested as other mechanisms behind antimicrobial activity (Fredell, 1994).
Antimicrobial activity	QACs have antimicrobial effects against a broad range of microorganisms including vegetative bacteria, yeasts, moulds, and algae, and can inhibit germination of bacterial spores and growth of vegetative bacteria, yeasts, moulds and algae. Growth inhibition by QACs is higher for Gram-positive bacteria and algae than for Gram-negative bacteria and moulds.
Uses	Commonly applied QACs include benzalkonium chloride (BC), also known as alkyl-benzyl-dimethyl-ammonium chloride, and cetrimonium. There is widespread use of QACs as disinfectants in ordinary environmental sanitation of surfaces such as floors, furniture, and walls. QACs are also used in food hygiene, food processing industries, hospitals fabric-softening agents, foam depressants, and antistatic agents in many consumer products.

Antimicrobial resistance

In 2009 VKM performed a risk assessment entitled "Quaternary ammonium compounds in cosmetic products" and concluded that: "It is evident that resistance towards QACs is widespread among a diverse range of microorganisms, and that microbial resistance to QACs is facilitated by several mechanisms. Currently available literature on development of resistance due to QACs in cosmetics is lacking. However, it is likely that QACs in such products will add to the selection pressure towards more QAC-resistant microorganisms among the skin and mouth flora. Furthermore, there is increasing evidence of co-resistance and cross-resistance between QACs and a range of other unrelated antibacterial agents, as antibiotics and disinfectants. Thus, the contribution to increased occurrence of resistance to clinically important antimicrobial agents by QACs in cosmetic products cannot be excluded" (VKM, 2009). In the current assessment, all 18 review articles that discussed development of resistance due to use of QACs confirmed a link between QAC resistance and resistance against clinically important antibiotics. Five of these review articles have supported/confirmed the publication of VKM's risk assessment from 2009.

<p>Resistance in environmental bacteria</p>	<p>As QACs enter the environment they may be expected to act on microbes occurring there. Kümmerer et al. (1997) found BC at concentrations of 4 to 5 mg/l⁻¹ in hospital effluent water, and QACs are reported to be effective against aquatic microorganisms at these concentrations (Tubbing and Admiraal, 1991). Sutterlin et al. (2008b) reported toxicity of BC alone, and in mixtures with other anionic compounds, to <i>Vibrio fisheri</i> and <i>Pseudomonas putida</i>. In a study by McBain et al. (2004), the effects of QACs on bacterial community dynamics and antimicrobial susceptibility in a drain microcosm with mixed cultures were examined. In this study, QAC exposure caused both increased susceptibility among some strains (<i>Pseudomonas</i> spp. and <i>Enterococcus saccharolyticus</i>), and decreased susceptibility to QACs among other strains (<i>Pseudomonas</i> spp., <i>Eubacterium</i> spp., <i>Chryseobacterium</i> spp., <i>Ralstonia</i> spp., and <i>Aranicola</i> spp.). The effects of QACs on microbes in biological wastewater treatment plants (WWTP) are well known; nitrifying bacteria used in biological filters in sewage treatment plants seem to be particularly affected, as QACs interfere with their normal uptake of ammonium (Sutterlin et al., 2008a). Several studies suggest that WWTP are reservoirs for diverse mobile antibiotic resistance elements. In an article regarding development of AMR in bacteria isolated from wastewater, the authors studied the occurrence of plasmids belonging to the IncP-a group. These are self-transmissible, and transfer to, and replicate in, a wide range of hosts. These elements carry determinants conferring resistance to nearly all clinically relevant antimicrobial drug classes, to heavy metals, and to QACs used as disinfectants against bacteria from animal, human and plant origin (Schluter et al., 2007).</p>
<p>Conclusion</p>	<p>Among the cationic agents, QACs are the most commonly used antiseptics and disinfectants. They are used as disinfectants in food hygiene, food processing industries, hospitals, fabric-softening agents, foam depressants, and antistatic agents in many consumer products.</p> <p>Many published reports describe development of resistance in bacteria from different niches, including the environment, due to use /misuse of QACs. These bacteria may develop resistance, not only against QACs, but also against other biocides and clinically relevant antimicrobial agents.</p>

Table 11. Organic and inorganic acids: esters and salts

Chemical group	Organic and inorganic acids: esters and salts
Active molecules	<ul style="list-style-type: none"> • Acetic acid, propionic acid, butyric acid, caprylic acid, formic acid, undecanoic acid, sorbic acid, benzoic acid, lactic acid, salicylic acid, dehydroacetic acid. • Sulphur dioxide, sulphites, and bisulphites, esters of p-hydroxybenzoic acid (parabens), vanillic acid esters.
Mechanisms of action	<p>The efficacy of acidic agents is linked to the concentration of hydrogen (H⁺) that destroys the amino acid bond in proteins, modifies the cytoplasmic pH, and precipitates proteins.</p> <p>Many acid preservatives act by preventing the uptake of substrate, which depends on a proton-motive force for entry into the cell; in other words, they act as uncoupling agents. Further details can be found in Al-Adham et al. (2013).</p>
Antimicrobial activity	<p>Organic acids: The efficacy of an organic acid as an antimicrobial agent depends on two factors – its hydrophobicity and how much completely undissociated acid is present. These molecules inhibit the outgrowth of both bacterial and fungal cells, and sorbic acid is also reported to inhibit the germination and outgrowth of bacterial spores (Brul and Coote, 1999).</p> <p>Organic acids also improve the digestibility of proteins and amino acids and the absorption of minerals (Omogbenigun et al., 2003), modulate endocrine and exocrine secretions, and influence mucosal morphology (Partanen, 1991). Whether these effects can be applied to all animal species is unresolved. In chickens fed <i>ad libitum</i>, the pH in the intestinal tract is not altered by the addition of formic or propionic acid (Thompson and Hinton, 1997), and the pH in the proventriculus and the gizzard is very acidic <i>per se</i>.</p> <p>The use of organic acids may reduce the lipopolysaccharides layers in <i>E. coli</i>, making it more susceptible to organic acids (Novoa-Garrido et al., 2009).</p> <p>Inorganic acids: The antimicrobial activity of inorganic acids may be due to low pH, which can denature cell walls and proteins, and destroy DNA/RNA.</p>

<p>Uses</p>	<p>Some organic acids, both aliphatic and aromatic, and one or two inorganic acids are used as preservatives, especially in the food industry. Some, for example benzoic acid, are also used in preservation of pharmaceutical products; others (salicylic, undecylenic and benzoic acids) have been used for topical treatment of fungal infections of the skin.</p> <p>Organic acids like formic acid, benzoic acid, citric acid, and fumaric acid, including compounds derived from plants, are also used in poultry and swine production, as additives to animal feed (Diarra and Malouin, 2014; Thormar, 2012).</p> <p>Vinegar, containing acetic acid, has been used for wound dressing for some bacterial infections caused by bacteria like <i>Pseudomonas</i> (Nagoba et al., 2013).</p> <p>Organic acids are used in high volume as feed additives in animal production. However, the quantities of these organic acids used in Norway are unknown.</p> <p>Hydrochloric acid and sulphuric acid are sometimes used in veterinary disinfection. Hydrochloric acid is sporicidal at low concentrations and has been used for disinfecting hides and skin contaminated with anthrax spores. Sulphuric acid is not sporicidal, even at high concentrations, but in some countries is used, usually with phenol, for decontamination of floors, feed boxes, and troughs.</p>
<p>Antimicrobial resistance</p>	<p>The literature search identified 19 review articles on organic acids and no articles on inorganic acids.</p> <p>Tolerance/resistance to organic acids was discussed in one review article, which is summarized here: Microbial resistance to weak organic acids can involve various mechanisms. For bacteria, significant knowledge exists on their intrinsic, non-inducible resistance mechanisms against these compounds. Gram-positive bacteria do not possess an outer membrane, hence preservatives can easily enter these cells and their intrinsic resistance is relatively low. In Gram-negative bacteria, resistance mechanisms are more complicated as these organisms possess an inner and an outer membrane. The latter membrane has a clear role in modulating the accessibility of a cell to preservatives and other small molecules; the lipopolysaccharide layer is of crucial importance in this respect (Brul and Coote, 1999).</p> <p>We are not aware of data that indicate development of resistance against inorganic acids in bacteria.</p>
<p>Resistance in environmental bacteria</p>	<p>See above; AMR</p>

Conclusion	<p>Acidic agents destroy the amino acid bond in proteins, modify cytoplasmic pH, and precipitate proteins. Both organic and inorganic acids may inhibit outgrowth of both bacterial and fungal cells, and sorbic acid is also reported to inhibit germination and outgrowth of bacterial spores.</p> <p>Some studies indicate that bacterial species may develop tolerance/resistance against organic acids. Genetic determinants have not been identified/characterized in bacteria with tolerance to organic acids. Development of resistance/tolerance and even intolerance towards organic acids may be transient and reversible.</p> <p>Other studies indicate that bacteria like <i>E. coli</i> may be more susceptible to organic acids, due to reduction of lipopolysaccharides.</p> <p>Development of resistance against inorganic acids has not been reported.</p>
-------------------	--

Table 12. Antimicrobial dyes

Chemical group	Antimicrobial dyes
Active molecules	<p>Three main groups with application as microbiocidal agents:</p> <ul style="list-style-type: none"> • Acridines (e.g., acridine orange) • Triphenylmethane (e.g., crystal violet) • Quinones (e.g., benzoquinone); natural dyes that give colour to many plants and animals.
Mode of action	<p>Acridines: High affinity interaction with DNA. The polycyclic and planar structure of acridines is inserted between base pairs of DNA. This intercalation will interfere with major metabolic processes (Al-Adham et al., 2013).</p> <p>Triphenylmethane: interference with cell wall structure (Vasanthakumari, 2007).</p> <p>Quinones: multiple effects on DNA (Lown, 1983)</p>
Antimicrobial activity	<p>Acridines: generally active against Gram-negative bacteria, but do not display a selective action against Gram-positive bacteria, nor are they inactivated by serum.</p> <p>Triphenylmethane: mostly active against Gram-positive bacteria and less against Gram-negative bacteria.</p> <p>Quinones: toxicity to bacteria, moulds, and yeasts.</p>

Uses	<p>Acridines: the major use has been treatment of wounds around 1940s. Some new derivatives with anticancer activity.</p> <p>Triphenylmethane: Triphenylmethane is the basic skeleton of many synthetic dyes called triarylmethane dyes, many of them are pH indicators.</p> <p>Quinones: one large-scale industrial application of quinones is for production of hydrogen peroxide.</p>
Antimicrobial resistance	MRSA and MRSE strains containing <i>qac</i> gene are more resistant against acridine and triphenylmethane. This is believed to be due to an efficient efflux system in the resistant strains (Leelaporn et al., 1994; Paulsen et al., 1996a; Paulsen et al., 1996b) .
Resistance in environmental bacteria	See above; AMR
Conclusion	<p>Antimicrobial dyes, such as acridines, triphenylmethane, and quinones, are used in different areas, although the areas of application are limited. The agents, mainly acridines and triphenylmethane, may induce resistance in bacteria due to activation of efflux systems. Resistance in bacteria of environmental origin was not identified.</p> <p>The clinical relevance of resistance against antimicrobial dyes in bacteria remains uncertain.</p>

Table 13. Halogen-releasing agents

Chemical group	Halogen-releasing agents
Active molecules	<ul style="list-style-type: none"> • Chlorine-releasing agent • Bromine-releasing agent: not commonly used, therefore not discussed further • Iodine-releasing agent
Mechanisms of action	<p>Chlorine-releasing agent: The exact mechanism by which chlorine destroys microbial cells is unknown, but it is believed that chlorine activity against bacteria may involve several targets, like oxidation of proteins.</p> <p>Iodine-releasing agent: Iodine derivatives rapidly penetrate into microorganisms and attack thiol groups of proteins, resulting in cell death (McDonnell and Russell, 1999).</p>

Antimicrobial activity	Chlorine-releasing agent: Bactericidal against both Gram-positive and Gram-negative bacteria, sporicidal (depends on pH). Iodine-releasing agent: Although less reactive than chlorine, iodine is bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal (Gottardi and Karl, 1991).
Uses	Halogen-releasing agents are traditionally used for both antiseptic and disinfectant purposes in swimming pools, public pools, drinking water, sewage/wastewater, and in fish farms. Bromine-related compounds, such as ammonium bromide and alkaline bromine derivatives, are rarely used.
Antimicrobial resistance	No information regarding development of resistance in bacteria due to use of halogen-releasing agents were identified.
Resistance in environmental bacteria	No information regarding development of resistance in bacteria of environmental origin due to use of halogen-releasing agents were identified.
Conclusion	Halogen-releasing compounds are the most significant microbiocidal halogens, which are traditionally used for both antiseptic and disinfectant purposes. No information regarding development of resistance in bacteria due to use of halogen-releasing agents were identified.

Diamidines

The isethionate salts of two aromatic diamidine compounds, propamidine and dibromopropamidine, have been used as antibacterial agents. Clinically, diamidines are used for the topical treatment of wounds. The exact mechanism of action is unknown, but believed to inhibit oxygen uptake and induce leakage of amino acids (McDonnell and Russell, 1999). No information regarding development of resistance in bacteria against diamidines was identified.

Other biocides

Other biocides, such as quinoline and isoquinoline derivatives, derivatives of 1,3-dioxane, derivatives of imidazole, isothiazolones, derivatives of hexamine, terpenes, and vapour-phase disinfectants listed in Table 1 have not been discussed in this assessment as reports on the development of resistance in bacteria against these agents were not identified in the literature.

3.9.2 Heavy metals

Heavy metals with the potential to induce AMR has little to do with density, but rather concerns chemical properties. Although heavy metals are naturally occurring elements that are found throughout the Earth's crust, most environmental contamination and human exposure result from anthropogenic activities, such as mining and smelting operations, industrial production and use, and domestic and agricultural/aquaculture use of metals and metal-containing compounds (He et al., 2005; Herawati et al., 2000).

This is of particular concern, given that the anthropogenic levels of heavy metals used are currently several orders of magnitude greater than the levels of antibiotics. Unlike antibiotics, metals are not subject to degradation and can therefore represent a long-term selection pressure (Stepanauskas et al., 2005). There are concerns regarding the potential of metal contamination to maintain a pool of antibiotic-resistance genes in both natural and clinical settings. In addition to metals, other toxicants are implicated in the co-selection of antibiotic resistance, including QACs and anti-fouling agents and detergents (Chapman, 2003; Sidhu et al., 2001).

In this assessment, the heavy metals themselves, rather than their areas of use, have been used for classification. The mechanisms of antimicrobial action and mechanisms of resistance of heavy metals are considered to be universal. This information has already been provided under Hazard characterization and discussed further for some heavy metals, if available.

In the following, we concentrate on a limited number of the most important and most widely used heavy metals in medicines and agriculture, including copper, zinc, silver, mercury, and arsenic, and microbial resistances to these metals. In addition, we discuss cadmium because of its high occurrence in animal feed and food. We also briefly discuss the current uses of these antimicrobial metals, and the importance of the genetic legacy and dissemination of bacterial resistance to antimicrobial metals in bacteria and the genetic elements carrying multiple AMRs, both to metals and to other antimicrobial agents.

Table 14. Arsenic

Chemical group	Arsenic (As)
	Not a true heavy metal, but semi metal
Mechanisms of action	The exact mechanism of action is not known. For general information, refer to section 3.5., Mechanisms of action of heavy metals.
Antimicrobial activity	Activity has been reported against several bacterial species, including pathogenic bacteria such as <i>Campylobacter</i> (Shen et al., 2014), <i>S. aureus</i> /CoNS (Argudin and Butaye, 2016; Argudin et al., 2016), <i>Listeria monocytogenes</i> (Lee et al., 2013), <i>Salmonella</i> (Joerger et al., 2010), <i>E. coli</i> (Chen et al., 2015), <i>Yersinia enterocolitica</i> (Mallik et al., 2012).
Uses	<p>Agricultural and non-medical uses of arsenic compounds have included wood preservatives (particularly chromated copper arsenate), herbicides, rodenticides, defoliants (Agent Blue used in the Vietnam war was a mixture of dimethylarsenic acid (cacodylic acid) and its sodium salt (Cooksey, 2012), and fungicides.</p> <p>Arsenic has variously been used in antispasmodics, sedatives, haematinics, for treating skin disorders, in eye and cancer treatments, and for the treatment of a wide range of ailments, including trichomoniasis, malaria, ulcers, and syphilis (Liu et al., 2008).</p> <p>Arsenic compound roxarsone (4-hydroxy-3-nitrobenzenearsonic acid), has been used as a feed additive in the poultry industry for growth promotion (Shen et al., 2014).</p>

<p>Antimicrobial resistance</p>	<p>Arsenic tolerance in bacteria is usually mediated by the gene products of the widespread <i>ars</i> operon that has been extensively studied (Carlin et al., 1995; Rosen, 2002). Although the organization of <i>ars</i> operons varies greatly between strains, there are some core genes that are almost always present: the simple gene set conferring basal resistance consists of the three-gene operon <i>arsRBC</i> as present in the <i>E. coli</i> genome (Carlin et al., 1995) and on <i>S. aureus</i> plasmid pI258 (Silver, 1998).</p> <p>Mechanisms of resistance against arsenic in bacterial species have been reviewed by (Kruger et al., 2013), and (Hobman and Crossman, 2015). The main cross-resistance between arsenic and antimicrobial agents may be activation of efflux pumps.</p>
<p>Resistance in environmental bacteria</p>	<p>Resistance against arsenic in bacterial species from different niches has been reported. Abbas et al. (2014) and Novo et al. (2013) isolated different bacterial species from seawater, and a novel bacterial strain belong to <i>Enterobacteriaceae</i> was isolated as part of a project aimed at screening metal-contaminated soils for arsenic-resistant bacteria in China (Su et al., 2012). Nine morphologically distinct potent arsenate-tolerant bacteria were shown to be related to <i>Micrococcus varians</i>, <i>Micrococcus roseus</i>, <i>Micrococcus luteus</i>, <i>Pseudomonas maltophilia</i>, <i>Pseudomonas</i> sp., <i>Vibrio parahaemolyticus</i>, <i>Bacillus cereus</i>, <i>Bacillus smithii</i>, and <i>Bacillus smithii</i> isolated from surfacewater and groundwater in Nepal (Shakya et al., 2012). Other arsenic-tolerant isolates were found in various niches: <i>Bacillus indicus</i> sp. nov., an arsenic-resistant bacterium was isolated from an aquifer (Suresh et al., 2004), <i>Bacillus arsenicus</i> sp. nov., an arsenic-resistant bacterium isolated from a siderite concretion (Shivaji et al., 2005), <i>Pseudomonas aeruginosa</i> isolated from natural waters (de Vicente et al., 1990), and <i>Listeria monocytogenes</i> isolated from the environment of turkey-processing plants (Mullapudi et al., 2008).</p>
<p>Conclusion</p>	<p>Arsenic has been used for thousands of years in various forms as an antimicrobials in medicine until recently. The high concentration of As in groundwater in many countries is a human health problem. Arsenic compounds were previously extensively used in impregnation of wood, but has been prohibited in Norway since 2002. The discovery of antibiotics and new organic antimicrobial compounds during the twentieth century resulted in a general decline in the clinical use of heavy metals like As. As is active against both Gram-positive and Gram-negative bacteria. Bacteria from different ecological niches may develop resistance against arsenic and the main cross-resistance between arsenic and antimicrobial agents may be activation of efflux pumps.</p>

Table 15. Copper

Chemical group	Copper (Cu)
Mechanisms of action	<p>Copper interacts readily with molecular oxygens. Its radical character makes copper very toxic. Copper toxicity is based on the production of superoxide radicals and on interaction with cell membranes (Nies, 1999).</p> <p>For general information, refer to section 3.5, Mechanisms of action of heavy metals.</p>
Antimicrobial activity	<p>Copper is toxic to prokaryotes and eukaryotes, even at high cellular concentrations (Gaetke and Chow, 2003), and copper involvement in phagosomal killing of bacteria engulfed by macrophages is now recognized as an important defence mechanism (German et al., 2013).</p>
Uses	<p>Copper compounds are used as wood preservatives, in antifouling paints, and as molluscicides (Borkow and Gabbay, 2009). In agriculture, copper compounds have been used as antimicrobial, algicidal, pesticidal, and antifungal agents, and as animal feed additives. Copper sulphate solutions were used for antifungal treatment of seed grains in the eighteenth century. In the late nineteenth century, Bordeaux mixture (copper sulphate and calcium hydroxide) and Burgundy mixture (copper sulphate and sodium carbonate) were widely used to control mildew on grape vines, and to control fungal and bacterial diseases of seeds or plants (Russell, 2005). These inorganic antifungal agents are still widely used in plant protection, even in 'organic' agriculture. Copper sulphate is allowed, alongside zinc chloride, oxide or sulphate, as an additive in animal and poultry feed.</p> <p>Farmed animals, such as pigs and poultry, receive additional Cu in their diets via supplements in their compound feed, and also as an alternative to in-feed antibiotics for growth promotion.</p>

<p>Antimicrobial resistance</p>	<p>Of 44 review articles identified in our search in PubMed search, 13 discussed development of resistance towards Cu in different bacterial species and most of these discussed development of resistance in bacteria due to use of Cu as feed additive in animals like poultry and pigs.</p> <p>In the review article of Yazdankhah et al. (2014), which was based on VKM's risk assessment entitled: "Zinc and copper in pig and poultry production: fate and effects in the food chain and the environment" (VKM, 2014), the development of resistance and cross-resistance between Cu and clinically used antibiotics in enterococci and <i>E. coli</i> were discussed. It was concluded that the development of resistance to Cu in enterococci is associated with the presence of a Cu-resistance gene (<i>tcrB</i>), which is often located on a plasmid. In enterococci, the Cu-resistance gene, <i>tcrB</i>, was shown to be associated with resistance to the macrolide antibiotic erythromycin (<i>ermB</i>) (Amachawadi et al., 2011; Freitas et al., 2011; Jacob et al., 2010). A conjugation study demonstrated co-transfer of <i>tcrB</i> and <i>ermB</i> genes between <i>E. faecium</i> and <i>E. faecalis</i> (Amachawadi et al., 2011). Transferable <i>tcrB</i> has been reported in enterococci isolated from piglets, calves, poultry, as well as humans, in Denmark. Several studies performed in Denmark show a link between resistance to Cu and resistance to macrolides and also to glycopeptides (vancomycin) in enterococcal isolates of pig origin (Aarestrup et al., 2002). The authors concluded that the Cu-resistance gene occurs frequently in these isolates, in geographical areas where Cu sulphate is being used in large amounts as feed additive. This may have contributed to co-selection of resistance against macrolides and glycopeptides. Macrolides, like erythromycin, are commonly used in veterinary and human medicine.</p>
--	--

<p>Resistance in environmental bacteria</p>	<p>In a risk assessment performed by VKM in 2014 entitled: "Zinc and copper in pig and poultry production: fate and effects in the food chain and the environment", the development of resistance in bacteria of environmental origin was assessed (VKM, 2014). VKM's Panel on animal feed concluded that: "The content of Cu in manure has been shown to be especially high from pigs and poultry, and from other farmed animals receiving a high portion of their diet from Cu added animal feed."</p> <p>In bacterial isolates isolated from environmental samples, elevated tolerance (higher MIC) values to Cu was detected in several species, like <i>Pseudomonas</i> and <i>E. coli</i>. In six studies, the bacteria showed higher tolerance to either Zn or Cu or both. A link between resistance to Cu in bacteria found in the environment and resistance to the examined antibiotics were observed in all studies. The combined expression of antibiotic and metal resistance in bacteria isolated from the environment may be caused by selection resulting from metals present in environments rich in Zn and Cu. The source of Cu is not always identified in environmental studies, but Cu is currently accumulating in many soils as a result of current agricultural practices, where this trace element is often present in animal manure and sewage sludge spread on agricultural soils. Whereas antibiotics present in animal manure and sewage sludge may be degraded rapidly, the metals are persistent and may accumulate in soil. Bacterial isolates of environmental origin, with resistance to Zn or Cu, are frequently resistant to more antibiotics than isolates of animal origin.</p>
<p>Conclusion</p>	<p>Copper compounds are widely used as wood preservatives, in antifouling paints, and as molluscicides, and in agriculture as antimicrobial, algicidal, pesticidal and antifungal agents, and as animal feed additives.</p> <p>Resistance to Cu has been reported, both in bacteria isolated from human and animals, and in bacteria of environmental origin. Resistance against Cu may be linked to resistance against erythromycin (<i>erm</i>) or vancomycin (<i>van</i>) in enterococci. Resistance towards copper is frequently encoded by genes located on plasmids and transposons and is often transferable between bacterial species. Such resistance genes may be transferred to other bacteria and co-selection may occur.</p>

Table 16. Mercury

Chemical group	Mercury (Hg)
Mechanisms of action	Mercury toxicity has been attributed to the inactivation of enzymes and interference with other protein functions by the tight binding of mercuric ions to thiol and imino nitrogen groups in these, or the displacement of other metal cofactors from enzymes. Mercuric ions also bind to nucleotides and lipids, interfering with DNA function and contributing to lipid peroxidation. Mercuric ions and organomercurials have the ability to pass rapidly through biological membranes, and organomercurials are highly lipid soluble (Clarkson and Magos, 2006).
Antimicrobial activity	Mercury is active (toxic), both against Gram-positive and Gram-negative bacteria.
Uses	<p>Organic and inorganic mercury compounds have been widely used in agriculture and medicine. Mercury was heavily used in the cellulose industry and fjord sediments are still heavily polluted from the use of mercury in the cellulose industry. Mercury was used to protect plant seeds in the soil from fungus attack, and organic compounds containing mercury were used in agriculture to control plant diseases from the late nineteenth century until the 1970s, with aryl, aloxyl, and alkyl organomercurials becoming widely used in the 1950s, particularly as antifungal seed dressings, but also as pesticides and fungicidal sprays (Huisingsh, 1974). The largest current use of mercury in a healthcare-associated role is in dental amalgam, which typically contains 43–54 % mercury, 20–35 % silver, 15 % tin, 10 % copper, and 2 % zinc, depending on the formulation (Franke, 2007). There has been debate about the safety of mercury amalgam fillings and whether their use has negative effects on human health or may select for mercuric ion-resistant bacteria.</p> <p>Mercury-containing antimicrobial usage is in decline and likely to be eliminated. The use of thiomersal/thimerosal as a vaccine preservative has been subject to vigorous debate and controversy, and it has been banned in some countries. Other mercury-containing disinfectants include merbromin (Mercurochrome) and nitromersol that have been superseded or withdrawn from use in the US or Europe (Hobman and Crossman, 2015).</p>

<p>Antimicrobial resistance</p>	<p>Resistance to mercuric ions is believed to be an ancient resistance mechanism, evolving after the biosphere became widely oxygenated, and has been found in <i>Bacteria</i> and Archaea (Barkay et al., 2010). The mechanism of mercuric ion resistance to inorganic mercuric ions (narrow-spectrum resistance) is unusual for a metal ion resistance mechanism and counterintuitive. Rather than direct efflux of the metal, the simplest inorganic mercuric ion resistance operon in Gram-negative bacteria from Tn 501, encodes proteins that chaperone divalent mercuric ions (Hg²⁺) in the periplasm. The mechanism of mercuric ion resistance in Gram-positive bacteria is broadly the same as that in Gram-negative bacteria, but details of the regulation and mercuric ion import systems differ slightly (Hobman and Crossman, 2015).</p> <p>In Gram-negative enteric bacterial species, mercury-resistance genes are often found on plasmids and are associated with transposons / integrons (Foster, 1987; Khesin and Karasyova, 1984; Silver and Phung le, 2005). Similar mobile units are found in <i>S. aureus</i> and enterococci (Foster, 1987; Zscheck and Murray, 1993). More recently, oral streptococci and other oral genera have been shown to have reduced susceptibility to mercury, although, in general, the mechanisms of resistance have not been identified (Summers et al., 1993). Summers et al. (1993) suggested that mercury released from amalgams might act as a selective agent for both mercury-resistant and antibiotic-resistant oral and intestinal bacteria because of the link between mercury-resistant and antibiotic-resistant genes. Given that amalgam is a common restorative material used worldwide, this mercury source has the potential to have a considerable effect on the composition of the normal human flora. Unlike antibiotics, which are usually taken for short periods separated by long intervals without use, once an amalgam filling is in place, low levels of mercury are released for extended time periods or the life of the filling (Roberts, 2002).</p>
<p>Resistance in environmental bacteria</p>	<p>We have not identified any review articles that report Hg resistance in bacteria of environmental origin. However, since mercury occurs naturally in soil and water, the presence of such bacteria cannot be excluded.</p>
<p>Conclusion</p>	<p>Organic and inorganic mercury compounds have been widely used in agriculture and medicine. Usage of antimicrobials containing mercury is in decline and likely to be eliminated. Plasmid/transposon-mediated resistance to inorganic and organic mercury compounds by hydrolases and reductases has been extensively studied, but its significance remains uncertain. As mercury is a natural material found in soil and water, it is highly likely that Hg-resistant bacteria will be identified in bacterial species of environmental origin. Resistance against Hg with co-resistance and cross-resistance against clinically relevant antimicrobial agents has been identified in bacteria.</p>

Table 17. Silver

Heavy metal	Silver (Ag)
Mechanisms of action	<p>Silver ions are highly toxic to all microorganisms, probably due to disruption of membrane respiratory electron transport chains and components of DNA replication (Feng et al., 2000).</p> <p>Silver ions cause inhibition of respiration, membrane damage, and destruction of the proton-motive force. The interaction of Ag⁺ with thiol groups in membrane proteins/enzymes is thought to be a major toxicity mechanism; data suggest that the key toxicity event is interactions between Ag⁺ and respiratory chain enzymes (Holt and Bard, 2005).</p>
Antimicrobial activity	<p>There is no known beneficial role for silver in metabolism and it is highly toxic to bacteria (Nies 1999). Silver is active against both Gram-positive and Gram-negative bacteria.</p>
Uses	<p>In agriculture, Ag may be used as silver iodide (AgI) for cloud seeding, but the first use of silver as an antibacterial is reported to have occurred over 2000 years ago in drinking water containers. Silver is still widely used in water filters and other treatments for potable water, or as an algicide for swimming pools Hobman and Crossman (2015).</p> <p>One noticeable increase in the use of antimicrobial metal products is that of silver in consumer and 'lifestyle' products. Over the past 20 years or so, silver-containing plasters, clothes, water filters, and personal hygiene and consumer products have been produced worldwide (Edwards-Jones, 2009; Silver, 2003; Silver and Phung le, 2005), and the use of antimicrobial silver nanoparticles (AgNPs) in products is also rising (Chaloupka et al., 2010), including examples where they have been integrated into household items, such as computer keyboards, washing machine drums, air conditioners, and refrigerators.</p> <p>AgNPs have received considerable attention due to their significant antimicrobial properties. There are many reports on the physical, chemical, and biological syntheses of colloidal AgNPs. As ecofriendly and sustainable methods are needed, biological systems like bacteria, fungi, and plants are being used to synthesize these AgNPs (Singh et al., 2015).</p>

Antimicrobial resistance	<p>Molecular and genetic evidence for silver resistance in <i>Enterobacter cloacae</i> isolated from skin wounds and medical devices has been reported. The same bacterial strain was isolated from leg ulcers of an elderly woman (Landsdown and Williams, 2007).</p> <p>Bacterial silver resistance, like that to other toxic metal ions, is frequently encoded by genes located on plasmids (Davis et al., 2005; Gupta et al., 2001), but also sometimes found encoded on the chromosome, reviewed by Silver and Phung (1996).</p> <p>Although bacterial silver resistance has been reported sporadically since the 1960s (see (Chopra, 2007; Clement and Jarrett, 1994)), the pMG101 <i>sil</i> system remains the only system characterized in any detail at the genetic level.</p>
Resistance in environmental bacteria	We did not identify any articles regarding development of resistance against Ag in bacteria of environmental origin. However, the occurrence of such bacterial isolates is not unlikely.
Conclusion	A literature search using the terms "Antimicrobial resistance" AND "silver" resulted in 65 review articles, most of which addressed use of silver and AgNPs for treatment/prevention of infection, and only 4 articles discussed development of Ag resistance. Silver is one of several topical antiseptics that are gaining popularity again, partly due to the rise of antibiotic-resistant genotypes. The clinical incidence of silver resistance remains low, and emergence of resistance can be minimized if the level of silver ions released from products is high and the bactericidal activity is rapid.

Table 18. Zinc

Heavy metal	Zinc (Zn)
Mechanisms of action	<p>Zinc ions are known to inhibit multiple activities in bacterial cells, such as glycolysis, transmembrane proton translocation, and acid tolerance (Phan et al., 2004). Although ZnO nanoparticles may be lethal to bacteria (bactericidal), zinc ions are likely only able to inhibit proliferation (bacteriostatic).</p> <p>Trace elements like Zn may be toxic to bacteria and this may be due to their chemical affinity to thiol groups of macro-biomolecules, but may also be dependent on the solubility of the metal compounds under physiological conditions (Yazdankhah et al., 2014).</p>
Antimicrobial activity	Zn ²⁺ has bactericidal effects on both Gram-positive and Gram-negative bacteria, as well as spores that are resistant to high temperature and high pressure (Azam et al., 2011).

Uses	<p>Farmed animals, such as pig and poultry, receive supplementary Zn (dietary ZnO and CuSO₄) via compound feed, as well as in medical remedies (Yazdankhah et al., 2014). Zn and Cu in animal feed are usually in concentrations in excess of their nutritional requirements and are intended for prevention of diarrhoeal disease, and also as an alternative to in-feed antibiotics for growth promotion (Amachawadi et al., 2011; Cavaco et al., 2011).</p> <p>Zinc has been used as nanoparticles as an alternative to antibiotics and disinfectants, especially in biomedical applications. Zinc oxide consumption has increased globally, and it is one of the most popular nanoparticles (Seil and Webster, 2011), with its photocatalytic activity under light illumination utilized in different industries, especially textiles and polymers (Montazer et al., 2013). The safety of ZnO and its compatibility with human skin make it a suitable additive for textiles and surfaces that come into contact with the human body (Liu et al., 2014).</p>
-------------	--

<p>Antimicrobial resistance</p>	<p>Of 81 review articles identified in our search in PubMed search, five articles discussed development of resistance in bacteria due to use of Zn as a feed additive for animals like poultry and pigs.</p> <p>In the review article of Yazdankhah et al. (2014), which was based on VKM's risk assessment entitled "Zinc and copper in pig and poultry production fate and effects in the food chain and the environment" (VKM, 2014), the development of resistance and cross-resistance between Zn and clinically used antibiotics against staphylococci, enterococci, and <i>E. coli</i> were discussed. An association in staphylococci between resistance to Zn and resistance to methicillin has been demonstrated. The study performed by Cavaco et al. (2011) found that MRSA strains from pigs from European countries, Canada, and China had a high prevalence of Zn resistance (mainly associated with the <i>czrC</i> gene), whereas the corresponding MSSA were susceptible. A similar association between resistance to Zn and resistance to methicillin was also observed in samples from veal farms from the Netherlands. However, in Norway and countries within EU methicillin is not the drug of choice for treatment of infection in veterinary medicine. Knowledge regarding the source of methicillin–Zn-resistant staphylococci in animals is lacking. It is not clear whether the methicillin-resistant staphylococci in animals are of human origin and have been resistant to Zn after exposure to feed, or whether the Zn-resistant staphylococci have been resistant to methicillin due to exposure to antibiotic(s).</p> <p>A publication from Germany (Bednorz et al., 2013) showed a higher diversity of <i>E. coli</i> clones in piglets fed with diets supplemented with Zn than in the background control group. The proportion of multi-resistant <i>E. coli</i> was significantly higher in the Zn group than in the control group. The authors suggested two possible mechanisms for their results: 1) co-selection via Zn resistance, as some of the isolates demonstrated both Zn-resistance and AMR; 2) enhanced plasmid uptake under the influence of Zn, as the authors detected several resistance plasmids in isolates from the Zn-feeding group. Identical clones were not present in the control group.</p>
--	---

<p>Resistance in environmental bacteria</p>	<p>In a risk assessment entitled: "Zinc and copper in pig and poultry production fate and effects in the food chain and the environment" (VKM, 2014), development of resistance in bacteria of environmental origin was assessed. VKM's Panel on animal feed concluded that: "The content of Zn in manure has been shown to be especially high from pigs and poultry and from other farmed animals receiving a high portion of their diet from compound feed."</p> <p>In bacterial isolates found in the environment, elevated tolerance (higher MIC values) to Zn was detected in several species like <i>Pseudomonas</i> sp., <i>E. coli</i>, and in culturable bacteria from sludge, soil, and water samples. The bacteria in five studies showed higher tolerance to either Zn or Cu or both. A link between resistance to Zn in bacteria from the environment and resistance to the antibiotics examined was noted in all studies. The combined expression of antibiotic and metal resistance in bacteria isolated from the environment may be due to selection resulting from metals present in environments rich in Zn and Cu. In the environmental studies, the source of Zn/Cu is not always identified, but Zn/Cu is accumulating in many soils as a result of current agricultural practices; these trace elements are often present in animal manure and sewage sludge that are spread on agricultural soils. Whereas antibiotics present in animal manure and sewage sludge may be degraded rapidly, the metals are persistent and may accumulate in soil. Bacterial isolates of environmental origin, with resistance to Zn or Cu, are frequently resistant to more antibiotics than isolates of animal origin.</p>
<p>Conclusion</p>	<p>Farmed animals, such as pigs and poultry, receive additional Zn via supplementary elements in their compound feed, as well as in medical remedies. In addition, Zn is used in humans in combination with some antibiotics, like bacitracin or erythromycin, for treatment of wound infections. Zinc has been used as nanoparticles as an alternative to antibiotics and disinfectants, especially in biomedical applications. Enteral bacteria, both commensal and pathogenic, in farmed animals have been shown to develop resistance to Zn and concomitant cross-resistance to antimicrobial agents. Such bacteria may be transferred to other animals and to humans. Resistance to Zn, which is mainly associated with the <i>czrC</i> gene has been reported, in bacteria isolated from humans, animals, and from the environment. Resistance against Zn may be linked to resistance against methicillin in <i>S. aureus</i> and Zn supplementation in animal feed may increase the proportion of multi-resistant <i>E. coli</i> in gut microbiota. Resistance genes against Zn may be transferred to other bacteria.</p>

Table 19. Cadmium

Heavy metal	Cadmium (Cd)
Mechanisms of action	Specific mechanisms of action have not been described. The effects may be summed up under the general headings: "thiol-binding and protein denaturation", "interaction with calcium metabolism and membrane damage", "interaction with zinc metabolism", and "loss of protective function". The <i>dsbA</i> encoding gene for a product required for disulphite formation, leads to Cd sensitivity in Gram-negative bacteria (Nies, 1999).
Antimicrobial activity	Cd is considered as the most toxic heavy metal, especially against microorganisms. The activity of Cd is against both Gram-positive and Gram-negative bacteria.
Uses	Cd has no known biological function in either animals or humans, but mimics the actions of other divalent metals that are essential to diverse biological functions (EFSA, 2009). Human exposure to cadmium is possible through a number of sources including employment in primary metal industries, consuming contaminated food, smoking cigarettes, and working in cadmium-contaminated workplaces, with smoking being a major contributor (Paschal et al., 2000).
Antimicrobial resistance	<p>Using the terms "cadmium" AND "Antimicrobial resistance" AND "Review" identified two articles, neither of which were relevant and are therefore not included here.</p> <p>Using the terms "cadmium" AND "Antimicrobial resistant bacteria" identified 131 articles, of which 102 were relevant and discussed development of resistance against Cd in bacteria. Resistance against Cd, with a possible link to other heavy metals, QACs, and clinically relevant antimicrobial agents has been observed and was discussed in many of these articles.</p> <p>Resistance against Cd in bacteria is based on Cd efflux. In Gram-negative bacteria, Cd seems to be detoxified by an RND-driven system like <i>Czc</i>, which is mainly a Zn exporter, and <i>Ncc</i>, which is mainly a nickel exporter. Resistance against Cd in <i>S. aureus</i> and other Gram-positive bacteria is associated with CdA pump or other CdA like proteins (Nies, 1999).</p>

Resistance in environmental bacteria	<p>Of the 102 articles identified (see above), 38 addressed development of resistance in bacteria of environmental origin and discussed development of resistance against Cd with possible links to other heavy metals, QACs, and clinically relevant antimicrobial agents.</p> <p>Cyanobacteria contain methallothionein. Amplification of the <i>smt</i> methallothionein locus has been reported to increase Cd resistance and deletion of the locus decrease Cd resistance (Nies, 1999).</p> <p>Several studies have reported Cd resistance in bacteria from wastewater (Anssour et al., 2016), and in heterotrophic aerobic bacteria from marine hydrothermal vent fields (Farias et al., 2015).</p>
Conclusion	<p>Cd has no known biological function in either animals or human. Its presence in animal feed and food for human consumption is undesirable, due to its toxicity and carcinogenicity. Several studies have shown development of resistance against Cd in bacteria and cross-resistance to other heavy metals, biocides like QACs, and also antimicrobial agents.</p>

3.10 Use of biocides and heavy metals in Norway

Although most biocides and heavy metals are known to be high volume products, the Working group was not able to obtain exact data, from Norway regarding use of different active substances within disinfectant agents and all heavy metals included in this report.

According to data provided by the Norwegian Environmental Agency, approximately 14000 tonnes of disinfectant agents (types 1, 2, 3, 4 or 5) were used in 2015 (see appendix 1 for definitions of types 1-5). The most commonly used in Norway, with the highest volume of disinfectant agents are: ethanol, sodium hypochlorite, propan-2-ol, propan-1-ol, QAC, H₂O₂, peracetic acid, pentapotassium bis(peroxymonosulphate) bis(sulphat), glutaral, and 2-phenoxyethanol (personal communication, Espen Wigaard).

3.10.1 Biocides

In the following we summarize the use of biocides and heavy metals in different areas (SCENHR, 2009):

- A- Biocides in health care:** Disinfectants (mainly group I) are used in the decontamination process of patient-care devices, environmental surfaces, and intact skin. Some of the most commonly used biocides in this area are: ethyl or isopropyl alcohol, iodophor solution, phenols, QACs, sodium hydrochlorite, glutaraldehyde, H₂O₂, hypochlorite, and peracetic acid.
- B- Biocides used on skin and mucosa:** Antiseptic agents differ from disinfectants, in that they may be used on non-intact skin and mucosa. Some of the biocides most commonly used as disinfectants and antiseptics are alcohol (ethanol, isopropanol, n-propanol), iodophores (povidone-iodine), QACs, and triclosan.

C- Biocides in consumer products:

- a. Cosmetics and personal care products:** Biocides in this group are regulated by the EU Directive 76/768/EEC. The main function of these compounds in cosmetics is protection of the products from microbial degradation. Some of the most commonly used in this area are: alcohol, QACs, chlorhexidine, and triclosan.
- b. Household products:** The use of biocides in household products is not regulated in the EU. Many detergents may contain cationic surfactants and QACs. Cleaning product formulations for private homes may be similar to those used in industry, and in public and private buildings. Surfaces coated with biocides like triclosan and metallic ions (e.g., Ag, Cu) have been used with the intention of preventing or reducing the growth of microorganisms.
- c. Triclosan in consumer products and textiles:** Triclosan is used in cosmetic products, toothpaste, cleaning products, paints, textiles, and plastic products.

D- Biocides in food production: Disinfectant agents, like QACs and alcohols, are widely used for the disinfection of production plants and food containers, and the control of bacterial growth in food and drinks. This is regulated by Directive 98/8/EC. In addition, biocides may be used as preservatives in foods with the intention of prolonging the shelf-life of food-stuffs by protecting them from deterioration caused by microorganisms.

E- Biocides in animal husbandry:

- a. Cleaning and disinfection of farm buildings, particularly between batches of animals.
- b. Creating barriers, such as in the use of footdips outside animal houses, and disinfecting vehicles and materials during outbreaks of infectious diseases.
- c. Direct application (e.g., teat dips)
- d. Preservation (e.g., eggs, semen)

Some of the most commonly used biocides in veterinary medicines are: H₂O₂, acetic acid, QACs, glutaraldehyde, formaldehyde, and isopropanol. In addition, biocides may be used as preservatives in feeds with the intention of prolonging the shelf-life of feedstuffs by protecting them from deterioration caused by microorganisms.

Biocide use in fish farming: For decontamination of fish farms, fish eggs, ponds, and equipment. These include iodophores, metallic salts, halogenic compounds, aldehydes, H₂O₂, QACs, and antimicrobial dyes (Directive 98/8/EC).

In Norway, the following biocides are used for disinfection of poultry farms:

- Virocid, which contains alkyldimethylbenzylammonium chloride, didecyldimethylammonium chloride, glutaraldehyde, and propan-2-ol.
- Neoprednisan, containing Preventol CMK (p-Chloro-m-cresol).
- CID2000, containing hydrogen peroxide, peracetic acid, and acetic acid.

The following disinfectant agents are approved for use in aquaculture in Norway: (http://www.mattilsynet.no/fisk_og_akvakultur/akvakultur/desinfeksjon/godkjente_desinfeksjonsmidler_i_akvakultur.802):

- Virocid, containing alkyldimethylbenzylammonium chloride, didecyldimethylammonium chloride, glutaraldehyde, and propan-2-ol.

- Kick-Start, which contains hydrogen peroxide, acetic acid, peracetic acid, stabilizer, and surfactant agent.
 - Aqua Des, which contains peracetic acids.
 - Perfectoxid, which contains hydrogen peroxide, acetic acid, and peracetic acid.
 - NORMEX Desinfecta: Ozone
 - Grotanol 3025 (changed name from Buraton 3025), (Mixture of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-Methyl-2H-isothiazol-3-one), Glutaral, 5-chloro-2-methyl-2H-isothiazol-3-one, and 2-Methyl-2H-isothiazol-3-one.
 - ADDI AQUA, peracetic acid.
 - Redoxzon, ozone.
 - AquaZone, ozone/biofiltration and hydrogen peroxide and UV.
 - Hygi-Des, hydrogen peroxide, acetic acid, and peracetic acid.
- F- **Biocides in food of animal origin:** Biocides may be used to remove surface bacterial contamination from products of animal origin such as poultry carcasses.
- G- **Biocides in the environment:** Biocides may be used for a variety of applications like water treatment and wastewater treatment.

3.10.2 Heavy metals

Heavy metals have multiple uses in industrial, domestic, agricultural, medical, and technological applications. This has led to their wide distribution in the environment, raising concerns over their potential effects on human health and the environment (Tchounwou et al., 2012). Heavy metals like lead, **arsenic**, **mercury**, aluminum, **zinc**, chromium, and iron are found in a wide variety of personal care products, including lipstick, whitening toothpaste, eyeliner, and nail color. Some metals are intentionally added as ingredients, whereas others are contaminants (<http://www.safecosmetics.org/get-the-facts/chemicals-of-concern/lead-and-other-heavy-metals/#sthash.PMwCk7le.dpuf>).

Heavy metals used in industrial, domestic, and technological products

According to data provided by the Norwegian Environmental Agency, heavy metals like Ag, Hg, Cd, Cu, As, and Zn may be used in different industrial, domestic, and technological products (**Table 20**). Based on these data, the amounts of heavy metals used in various products in Norway can be ranked by quantity as follows: Zn > Cu > Cd > As > Ag > Hg (personal communication, Espen Wigaard).

Table 20. Heavy metals used in different industrial, domestic, and technological products, but not agricultural products, in Norway.

Heavy metals	Derivatives	Amount	Usage area
Zn	Zinc oxide, Zinc sulphate, Zinc chloride, Zinc sulphide, Zinc octoate	>100.000 tonnes	Ship building, repair and maintenance of ships and boats, paints and varnishes, Antifoulants (PT21). Raw materials (industrial use).
Cu	Cu, Copper dioxide	100-100.000 tonnes	Antifoulants (PT21). Raw materials (industrial use).

Cd	Cadmium oxide, Cadmium sulphide, Cd	100-1000 tonnes	Repair and maintenance of ships and boats, paints and varnishes, antifoulants (PT21), jointless floors. Raw materials (industrial use).
As	Arsenic, Arsenic trioxide	10-100 tonnes	Paints and varnishes, jointless floors, raw materials (industrial use).
Ag	Silver, Silver nitrate, Silver cholride	1-10 tonnes	Biocidal products for human hygiene (PT1), biocidal products within public health and nursing (PT2).
Hg	Mercury	1-10 kg*	Raw materials (industrial use).

*Mostly as contaminant

Heavy metals (Zn, Cu) used in agriculture

Cu and Zn are the most commonly used heavy metals applied in large quantities in Norway, being mainly used as feed additive for pigs and poultry. The approximate total amount of complete compound feed produced for pigs in Norway in 2012, based on the reported production from the three main industry organisations was 469,000 tonnes. The corresponding total amount of compound feed for poultry in 2012 was 428,000 tonnes. The concentrations of Zn and Cu in the complete compound feed, as reported by the different producers, are fairly similar (VKM, 2014). In complete compound feed for pigs in Norway in 2012, the estimated total amounts of Zn and Cu were 66,733 kg and 10,886 kg, respectively. In complete compound feed for poultry in Norway in 2012, the corresponding total amounts of Zn and Cu were 48,369 kg and 7,980 kg, respectively. These figures are based on turnover of the various categories of compound feed and their concentrations of Zn and Cu reported from the industry organisations.

The estimated amount of Zn in medical remedies in 2012 was 4,130 kg (data for Normin Sink, only). This amount of Zn represents an addition of 67 % to the Zn in complete feed for piglets. No medical remedies based on Cu are registered in Norway. In addition, Zn and Cu can be found in drinking water, barn installations, and other environments.

In the last ten years, a number of nanoparticles like AgNPs and Zn nanoparticles have received considerable attention. Due to their significant antimicrobial properties they have been integrated into household items, such as computer keyboards, washing machine drums, air conditioners, and refrigerators.

Heavy metals from human activity in the environment

Environmental levels of heavy metals are closely monitored at selected sites. The general trend in Norway is decreasing leakage of Cu, Hg, Pb, and Cd (<http://www.norskeutslipp.no/no/Komponenter/Avfall/EE-produkter111/?ComponentType=avfall&WasteComponentPageID=109&SectorID=600>), but steady levels of Zn (measured in sewage). However, dietary supplementation of Zn in animal production and heavy use of Cu as net pen antifouling in aquaculture may have the potential to create local concentrations of these chemicals that are of concern for AMR development.

There is no doubt that the production volumes and the use area for several biocide products and heavy metals are several orders of magnitude higher than those of antimicrobial agents used in therapy and prophylaxis.

3.11 Summary of hazard characterization

The main hazards with respect to use of chemicals are related to development of AMR and their role as drivers of AMR (e.g., resistance against antibiotics) through co-resistance or cross-resistance. Thus, a central question is concerned current trends with respect to levels of release to the environment. Release of chemicals like biocides (in particular disinfectants) and heavy metals, together with antimicrobial agents from agriculture, hospitals, and industry, has the potential of to create local concentrations that promote spread of AMR.

Mechanisms of cross-resistance are well described in laboratory experiments for several chemicals used as disinfectants, in particular phenols, like triclosan, and cationic agents, like QACs. These mechanisms include both metabolic and genetic changes in the bacteria. Although the levels of disinfectant use in Norway are not certain, the international trends are clear. The “infection control market” shows a steady growth; infection control in food-producing animals, both terrestrial and aquatic, is also a driver. These trends will therefore amplify these hazards in the future.

In addition, some heavy metals (notably Zn and Cu) can drive AMR through co-selection, as described in section 3.4. Environmental levels of heavy metals are closely monitored at selected sites in Norway and the general trend is decreasing release of Cu, Hg, Pb, and Cd, but steady levels of Zn (measured in sewage). However, dietary supplementation of Zn in animal production and heavy use of Cu as net pen antifoulant in aquaculture may have the potential to create local concentrations of these chemicals of concern for AMR development.

Discussion

3.12 Resistance and cross-resistance between biocides and antimicrobial agents

Resistance to biocides in microorganisms resulting from the use of the same biocide is well documented. But whether one biocide will induce resistance to other biocides is less clear. One mechanism is a mutation in one gene resulting in the organism becoming resistant to another biocide from the same chemical group. Another mechanism is the resistance mechanism being an efflux pump that is also able to export other similar biocides so efficiently that the concentration inside the cell falls below the toxic level.

Laboratory studies with bacteria have shown that a biocide might also co-select for resistance to antibiotics, but whether this occurs in nature has not yet been resolved. There is very little research in this field, but a EU project: “Confronting the clinical relevance of biocide induced antibiotic resistance (BIOHYPO)” investigated whether use of biocides such as triclosan, QACs, chlorhexidine, and sodium hypochlorite, in the food-chain would result in a clinically relevant increase in AMR in human pathogens.

The conclusions from this project were that resistance is usually selected by the antibiotic itself, but that biocides might also co-select for resistance to antibiotics. Although resistance to biocides is poorly defined at the mechanistic level, different *in vitro* studies have shown that mutants presenting low susceptibility to biocides also have reduced susceptibility to antibiotics. However, regarding studies on natural bacterial isolates, this EU project claims that no clear conclusions can be reached as to whether use of biocides results in acquired

antibiotic resistance in bacteria. (cordis.europa.eu/result/rcn/56830_en.html) (Oggioni et al., 2013).

In 2005, an expert panel convened by the FDA concluded by a vote of 11-to-1 that use of topical antiseptics does not provide a measurable benefit to consumers (FDA, 2005). Studies focused on household settings, rather than the outdoor environment, showed that the development and proliferation of drug resistance is likely. One unexplored locale is sewage sludge, where an abundance of pathogens, multiple antimicrobials, and extended contact times creates a large and risky setting for the emergence of drug resistance (Halden, 2014).

We have not identified any publication that demonstrates a link between development of antimicrobial (antibiotic) resistance under natural conditions due to the use of fungicides in agriculture.

3.13 Cross-resistance between heavy metals and antimicrobial agents

The literature search using the terms "heavy metal" and "antimicrobial resistance" produced a high number of papers, as reviewed in section 2. The most important compounds linked to AMR in this group were Cu and Zn, but examples of AMR driven by Hg and Cd were also found (Seiler and Berendonk, 2012). Urbanization, combined with industrial and agricultural activity, are sources of heavy metal contamination of the environment, resulting in levels that exceed the concentrations reached by natural geochemical processes (Grecco, 2011). Atmospheric deposits of heavy metal contamination increased steadily in Europe from the industrial revolution (1850) until the 1970s, but have since declined. The same trend has been observed in Norway (NTNU, 2001). However, the input of heavy metals to the environment from the agricultural sector is still rising, resulting in increasing levels in topsoil and waters (Monteiro et al., 2010). In salmon farming, more than 1000 metric tonnes of Cu were used for antifouling of net pens in 2014 (Skarbøvik et al., 2014). In high concentrations, Cu can both cause damage to sensitive species and result in long-term adverse effects in the aquatic environment. The authorities in Norway expect breeders to utilise more environmentally friendly methods, so that the use of Cu can be reduced. Fish farmers are obliged to measure the levels of copper in sediments under and around fish farms (<http://www.miljostatus.no/tema/hav-og-kyst/fiskeoppdrett/kobber-og-andre-kjemikalier-i-fiskeoppdrett/>). Heavy metals, like Zn, Cu, Hg, and Cd, occur as additives or contaminants in animal feed and fertilizers, leading to a steady supply to agricultural environments, and to both land and water by runoff. These anthropogenically caused elevations in heavy metals constitute a selective pressure on the local microbiota, and may result in resistance development. This resistance can be co-transferred to resistance towards antibiotics of medical importance. In some environments, antibiotics from sewage or agricultural activities may expose bacteria to a co-selective environment, increasing the chances of AMR development. Reuse of water for agricultural irrigation and use of liquid sludge as fertilizer are areas where heavy metals and antibiotics may meet in a co-selective environment, with the potential to drive the development of AMR. Seiler and Berendonk (2012) tried to rank the most important drivers of metal-induced AMR by combining datasets for environmental heavy metal concentrations with data from co-selection experiments in both the laboratory and the field. Heavy metal levels that elevated antibiotic MIC levels were considered as minimum metal co-selective concentration (MMC). Studies where the heavy metal concentrations in environmental matrices (water, sediment, sewage, and manure) were \geq MMC were found for Cd, Cu, Ni, Hg, Pb, and Zn more frequently than studies with the opposite ratio.

Most studies indicate a MMC ratio >1 for Cu and Zn. This suggests that co-selective levels of several heavy metals can be found in different environments where bacteria occur in high numbers. Although compelling, these findings from combined datasets needs verification in controlled laboratory experiments in which the conditions used mimic, as far as possible, natural environmental conditions. Correlation does not equal causation, and false positives may exist. However, mechanisms of co-selection for the individual heavy metals, notably Cu and Zn, have been described, providing rational explanations for the observed effects. In order to monitor these developments, there is an urgent need for standardization of resistance testing in environmental samples, in the same way as has been developed for clinical samples.

In a perspective article summarizing the results from the EU COST action "Detecting evolutionary hotspots of antibiotic resistance in Europe, TD0803", the authors propose the development of global databases that collect and collate information about AMR at local, national, and global levels (Berendonk et al., 2015). Controlling the release of potentially resistance driven chemicals to evolutionarily environmental hotspots of antibiotic resistance, where bacteria, antibiotics, resistance genes, and co-selecting environmental factors (like heavy metals) meet, for example urban and agricultural sewage, and industrial (in particular pharmaceutical and food production) wastewater, is of great importance.

We did not identify any publications that demonstrated a link between resistance against heavy metals and concomitant resistance against heavy metals and fungicides in fungal species.

Probability characterisation

Although the risk characterization generated by a qualitative risk assessment should ideally be based on numerical data for exposure assessment and hazard characterization, it is more generally of a descriptive or categorical nature, that is not directly tied to a more precisely quantified measure of risk.

As this report is not a traditional risk assessment, but a literature survey that presents and compiles available information regarding resistance development in bacteria due to use/misuse of biocides and heavy metals, risk characterization has been excluded here. The following definitions are used, based upon the Biosafety Resource Book published by FAO in 2011 (Sensi et al., 2011):

- Highly likely - is expected to occur in most circumstances
- Likely - could occur in many circumstance
- Unlikely - could occur in some circumstances
- Highly unlikely (negligible or effectively zero) - may occur only in very rare circumstances

Table 21 illustrates the probability/likelihood of occurrence of resistance in bacteria in environments associated with the use of biocides and heavy metals. These definitions are not only based on the ability of biocides /heavy metals to induce resistance and co-resistance or cross-resistance in bacteria, but also based on their volume and applications, and their use in different setting. The concentrations can be of importance when considering the specific environments.

Table 21. Biocides and heavy metals and their potential to induce resistance and cross/co-resistance in bacteria against antimicrobial agents.

Probability/ likelihood	Chemical group	Examples
Highly likely	Copper (Cu), zinc (Zn), cadmium (Cd)	
Likely	Phenols, surface-active agents, arsenic (As), mercury (Hg ²⁺)	Triclosan, QACs
Unlikely	Aldehydes, biguanides, organic acids, inorganic acids, antimicrobial dyes, diamides, silver (Ag)	Glutaraldehyde, formaldehyde, chlorhexidine, acetic acid, formic acid, acridine, triphenylmethane, quinones
Highly unlikely	Alcohol, anilides, peroxygens, halogen-releasing agents, diamidines	Ethanol, salicylanilide, carbanilide, H ₂ O ₂ , chlorine, bromine, iodine

4. Uncertainties

The degree of confidence in the final estimation of risk depends on the variability, uncertainty, and assumptions identified in all the previous steps. According to EFSA's guidance regarding uncertainties, assessments must state clearly and unambiguously the uncertainties that have been identified and their impact on the overall assessment outcome (<https://www.efsa.europa.eu/sites/default/files/consultation/150618.pdf>).

In this assessment, a number of uncertainties have been identified related to the probability of formation of, and dissemination of, AMR due to the use of biocides and heavy metals to the environment. Many of these uncertainties are caused by data gaps and a lack of quantitative framework.

Detailed data on the current and future use of biocides in Norway, along with their environmental levels, are not readily available. Without these data, estimating the selective pressure that could potentially induce increased AMR is challenging. In contrast, environmental levels of heavy metals in soil, sewage, and sediments are analysed at regular intervals and may therefore be used for exposure considerations. However, the present methods for determination of AMR in environmental samples is primarily based on culture studies (\pm antibiotics) or on the presence of antimicrobial resistance genes (ARGs) (by qPCR or sequencing), methods that do not fully capture the potential for co-selection with biocides or heavy metals.

There are also uncertainties regarding the ability of, and extent of. Biocides and/or heavy metal-resistant bacterial strains to colonize humans or animals, and the ability of their

resistance genes to be transferred to resident bacterial species in the environment. The issue of AMR in the environment is addressed either as a direct hazard or as an indirect hazard through resistance transfer, as noted in hazard identification.

The uncertainties identified are as follows:

- AMR requires greater understanding of the genetic interactions and spread that occur in environmental bacteria.
- AMR is an evolving situation; those factors that may promote/reduce the transmission of bacteria resistant to antimicrobials, and their corresponding gene determinants, have not been identified.
- The complex chemistry of environmental heavy metals may pose a challenge to the applicability of controlled laboratory experiments investigating co-selection of resistance to a single heavy metal and antimicrobial compounds.
- For uncertainties regarding laboratory methods, see data gaps.
- Quantitative approaches are largely lacking.
- HGT events can rarely be traced back to enable identification of the specific conditions and circumstances that gave rise to the event

5. Conclusions (with answers to the terms of reference)

1. List substances that may contribute to increased antimicrobial resistance.

Table 21 gives an overview of biocides and heavy metals and their potential to induce resistance and cross-resistance and/or co-resistance against antimicrobial agents in bacteria. The most common chemical compounds with the potential to induce resistance and cross or co-resistance against antimicrobial agents in bacteria are the heavy metals copper, zinc, and cadmium (highly likely). Phenols, especially triclosan, and surface-active agents, especially QACs, arsenic, and mercury are categorised as likely. However, aldehydes, biguanides, organic acids, inorganic acids, antimicrobial dyes, diaminides, and silver are categorised as unlikely.

2. Describe the substances listed in question 1 that are in use in Norway and assess those fields of application that have the potential to contribute to increased resistance.
3. Rank the substances, according to field of application, that are assumed to have the greatest effect on development of resistance, based on their characteristics and the amount used.

All the chemicals listed in **Table 21** are used in Norway.

Zinc and copper are used as additives to animal feed. As rather high amounts are used, we regard these two elements as the most potent AMR driving substances in Norway, after the antimicrobials themselves.

QACs are used in large amounts as biocides to clean and disinfect equipment in the industry, and may have a resistance-driving effect in some locations. There is also some concern that that may drive AMR when used in cosmetics.

Following the significant reduction in the use of arsenic, and mercury in Norway, we regard the probability of development of antimicrobial cross-resistance by these substances as unlikely. In our opinion, all the chemicals categorised as being in the unlikely or highly unlikely group, do not represent a risk for driving the development of AMR in the environment.

4. Identify knowledge gaps regarding the effects of these substances on development of resistance.

We have been unable to obtain data on the amounts of the different biocides and heavy metals that may be released into the environment in Norway, and whether these amounts singly, or in combination, may promote development of AMR. There is a lack of data regarding use/misuse/presence of biocides and/or heavy metals in consumer products. Knowledge of the effects of the disinfectant agents, chlorhexidine and QACs, and heavy metals in cosmetic products on the selection of cross-resistance against antimicrobial agents is lacking.

Further information is provided in section 6 Data gaps.

6. Data gaps

General

- No validated and standardised methods for determination of resistance to biocides and heavy metal are available.
- Detailed data regarding the amounts of biocides and heavy metals spread across different usage areas are lacking.
- Data regarding the use/misuse/presence of biocides/heavy metals in consumer products are lacking.
- *In vitro* data indicate that sub-lethal concentrations of biocides and/or heavy metals may promote non-susceptibility/resistance in bacteria. However, epidemiological data regarding the public health relevance are lacking.
- Most data regarding biocides/heavy metals resistance are collected from studies using planktonic phase microorganisms (unattached micro-organisms living freely in suspension) rather than microorganisms in more natural conditions, such as in a biofilm.
- There is a lack of data regarding the role of biocides and/or heavy metals in different man-made matrices in inducing resistance in bacteria.
- Validated methods that can demonstrate a relationship between the dose response and the threshold triggering the emergence of resistance to biocides and/or heavy metals resistance are not available.
- Environmental monitoring programmes regarding undesirable effects, including resistance development in bacteria, are lacking.
- Data regarding the development of resistance in non-pathogenic and environmental bacteria are sparse.
- There is a lack of knowledge regarding the effects of biocides and/or heavy metals on wildlife and natural ecosystems.
- Data regarding the contribution of HGT to resistance in the environment are sparse.
- Data regarding a possible link between development of resistance against heavy metals to biocides, and vice versa, are sparse.
- Data regarding disinfectant agents and/or heavy metals in Norway, specifically their application and spread to the environment (including soil, sediment, water, air, wild plants and animals) are lacking.
- Although, there are some studies regarding resistance of environmental bacteria to biocides and/or heavy metals, there is a lack of monitoring information, and knowledge about the clinical impact in humans/animals/plants is lacking.

Biocides

- Data regarding the stability and fate of biocides in the environment are lacking.
- There is a lack of *in vivo* data regarding the development of resistance due to use of biocides.

Heavy metals

- The distributions of resistance to heavy metals in the different ecosystems are not known.
- Data regarding development of resistance due to natural amounts of heavy metals in the environment are lacking.
- Analysis of resistance in environmental microbial samples is not standardized as it is for MIC for clinical samples.

7. References

- Aarestrup F.M., Hasman H., Jensen L.B., Moreno M., Herrero I.A., Dominguez L., Finn M., Franklin A. (2002) Antimicrobial resistance among enterococci from pigs in three European countries. *Appl Environ Microbiol* 68:4127-9.
- Abbas S.Z., Riaz M., Ramzan N., Zahid M.T., Shakoori F.R., Rafatullah M. (2014) Isolation and characterization of arsenic resistant bacteria from wastewater. *Braz J Microbiol* 45:1309-15.
- Abrams M.J., Murrer B.A. (1993) Metal compounds in therapy and diagnosis. *Science* 261:725-30.
- Aiello A.E., Larson E. (2003) Antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance in the community. *Lancet Infect Dis* 3:501-6.
- Al-Adham I., Haddadin R., Collier P. (2013) Types of microbiocidal and microbiostatic agents, in: A. P. Fraise, et al. (Eds.), *Principles and practice of disinfection, preservation and sterilization*, Willy-Blackwell, UK.
- Alonso A., Sanchez P., Martinez J.L. (2001) Environmental selection of antibiotic resistance genes. *Environ Microbiol* 3:1-9.
- Amachawadi R.G., Shelton N.W., Shi X., Vinasco J., Dritz S.S., Tokach M.D., Nelssen J.L., Scott H.M., Nagaraja T.G. (2011) Selection of fecal enterococci exhibiting tcrB-mediated copper resistance in pigs fed diets supplemented with copper. *Appl Environ Microbiol* 77:5597-603. DOI: 10.1128/AEM.00364-11.
- Anssour L., Messai Y., Estepa V., Torres C., Bakour R. (2016) Characteristics of ciprofloxacin-resistant Enterobacteriaceae isolates recovered from wastewater of an Algerian hospital. *J Infect Dev Ctries* 10:728-34. DOI: 10.3855/jidc.6727.
- Argudin M.A., Butaye P. (2016) Dissemination of metal resistance genes among animal methicillin-resistant coagulase-negative Staphylococci. *Res Vet Sci* 105:192-4. DOI: 10.1016/j.rvsc.2016.02.009.
- Argudin M.A., Lauzat B., Kraushaar B., Alba P., Agerso Y., Cavaco L., Butaye P., Porrero M.C., Battisti A., Tenhagen B.A., Fetsch A., Guerra B. (2016) Heavy metal and disinfectant resistance genes among livestock-associated methicillin-resistant *Staphylococcus aureus* isolates. *Vet Microbiol* 191:88-95. DOI: 10.1016/j.vetmic.2016.06.004.
- Azam A., Ahmed A.S., Oves M., Khan M.S., Habib S.S., Memic A. (2011) Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. *Int. J. Nanomedicine*, 7:6003-6009.
- Barah F. (2013) Non-antibiotic biocides: An updated review. *Microbial Pathogens and Strategies for Combating them: Science, Technology and Education* Formatex Research Centre Spain.

Barkay T., Kritee K., Boyd E., Geesey G. (2010) A thermophilic bacterial origin and subsequent constraints by redox, light and salinity on the evolution of the microbial mercuric reductase. *Environ Microbiol* 12:2904-17. DOI: 10.1111/j.1462-2920.2010.02260.x.

Bednorz C., Oelgeschlager K., Kinnemann B., Hartmann S., Neumann K., Pieper R., Bethe A., Semmler T., Tedin K., Schierack P., Wieler L.H., Guenther S. (2013) The broader context of antibiotic resistance: zinc feed supplementation of piglets increases the proportion of multi-resistant *Escherichia coli* in vivo. *Int J Med Microbiol* 303:396-403. DOI: 10.1016/j.ijmm.2013.06.004.

Berendonk T.U., Manaia C.M., Merlin C., Fatta-Kassinos D., Cytryn E., Walsh F., Burgmann H., Sorum H., Norstrom M., Pons M.N., Kreuzinger N., Huovinen P., Stefani S., Schwartz T., Kisand V., Baquero F., Martinez J.L. (2015) Tackling antibiotic resistance: the environmental framework. *Nat Rev Microbiol* 13:310-7. DOI: 10.1038/nrmicro3439.

Block S. (2001) Peroxygen compounds Lippincott Williams & Wilkins, Philadelphia.
Borkow G., Gabbay J. (2009) Copper, An Ancient Remedy Returning to Fight Microbial, Fungal and Viral Infections. *Infections Current Chemical Biology* 3:271-278.

Brul S., Coote P. (1999) Preservative agents in foods. Mode of action and microbial resistance mechanisms. *Int J Food Microbiol* 50:1-17.

Carey D.E., McNamara P.J. (2014) The impact of triclosan on the spread of antibiotic resistance in the environment. *Front Microbiol* 5:780. DOI: 10.3389/fmicb.2014.00780.

Carlin A., Shi W., Dey S., Rosen B.P. (1995) The ars operon of *Escherichia coli* confers arsenical and antimonial resistance. *J Bacteriol* 177:981-6.

Cavaco L.M., Hasman H., Aarestrup F.M. (2011) Zinc resistance of *Staphylococcus aureus* of animal origin is strongly associated with methicillin resistance. *Vet Microbiol* 150:344-8. DOI: 10.1016/j.vetmic.2011.02.014.

Chaloupka K., Malam Y., Seifalian A.M. (2010) Nanosilver as a new generation of nanoparticle in biomedical applications. *Trends Biotechnol* 28:580-8. DOI: 10.1016/j.tibtech.2010.07.006.

Chapman J.S. (2003) Disinfectant resistance mechanisms, cross-resistance, and co-resistance. *Int Biodeterior Biodegradation*, 51:271-276.

Chen S., Li X., Sun G., Zhang Y., Su J., Ye J. (2015) Heavy Metal Induced Antibiotic Resistance in Bacterium LSJC7. *Int J Mol Sci* 16:23390-404. DOI: 10.3390/ijms161023390.

Chitambar C.R. (2010) Medical applications and toxicities of gallium compounds. *Int J Environ Res Public Health* 7:2337-61. DOI: 10.3390/ijerph7052337.

Chiu H.C., Lin T.L., Yang J.C., Wang J.T. (2009) Synergistic effect of *imp/ostA* and *msbA* in hydrophobic drug resistance of *Helicobacter pylori*. *BMC Microbiol* 9:136. DOI: 10.1186/1471-2180-9-136.

Chopra I. (2007) The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern? *J Antimicrob Chemother* 59:587-90. DOI: 10.1093/jac/dkm006.

Clarkson T.W., Magos L. (2006) The toxicology of mercury and its chemical compounds. *Crit Rev Toxicol* 36:609-62. DOI: 10.1080/10408440600845619.

Clement J.L., Jarrett P.S. (1994) Antibacterial silver. *Met Based Drugs* 1:467-82. DOI: 10.1155/MBD.1994.467.

Cooksey C. (2012) Health concerns of heavy metals and metalloids. *Sci Prog* 95:73-88.
Cooney J.J., Wuertz S.J. (1989) Toxic effects of tin compounds on microorganisms. *J Ind Microbiol* 4:375-402.

Davies J., Davies D. (2010) Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev* 74:417-33. DOI: 10.1128/MMBR.00016-10.

Davis I.J., Richards H., Mullany P. (2005) Isolation of silver- and antibiotic-resistant *Enterobacter cloacae* from teeth. *Oral Microbiol Immunol* 20:191-4. DOI: 10.1111/j.1399-302X.2005.00218.x.

de Vicente A., Aviles M., Codina J.C., Borrego J.J., Romero P. (1990) Resistance to antibiotics and heavy metals of *Pseudomonas aeruginosa* isolated from natural waters. *J Appl Bacteriol* 68:625-32.

Desoize B. (2004) Metals and metal compounds in cancer treatment. *Anticancer Res* 24:1529-44.

Diarra M.S., Malouin F. (2014) Antibiotics in Canadian poultry productions and anticipated alternatives. *Front Microbiol* 5:282. DOI: 10.3389/fmicb.2014.00282.

Dorsey C.W., Actis L.A. (2004) Analysis of pVU3695, a plasmid encoding glutathione-dependent formaldehyde dehydrogenase activity and formaldehyde resistance in the *Escherichia coli* VU3695 clinical strain. *Plasmid* 51:116-26. DOI: 10.1016/j.plasmid.2003.12.001.

ECDC/EMA. (2009) The bacterial challenge: time to react,, ECDC.

Edwards-Jones V. (2009) The benefits of silver in hygiene, personal care and healthcare. *Lett Appl Microbiol* 49:147-52. DOI: 10.1111/j.1472-765X.2009.02648.x.

EFSA. (2009) Cadmium in food, *The EFSA Journal*, Scientific opinion of the panel on contaminants in the food chain. . pp. 23-139.

EUCAST. (2000) Terminology related to the methods for the determination of susceptibility of bacteria to antimicrobial agents, *Clinical Microbiology and Infection* 6, The European Committee on Antimicrobial Susceptibility Testing.

Farias P., Espirito Santo C., Branco R., Francisco R., Santos S., Hansen L., Sorensen S., Morais P.V. (2015) Natural hot spots for gain of multiple resistances: arsenic and antibiotic resistances in heterotrophic, aerobic bacteria from marine hydrothermal vent fields. *Appl Environ Microbiol* 81:2534-43. DOI: 10.1128/aem.03240-14.

FDA. (2005) Meeting of the Nonprescription Drugs Advisory Committee., US Food and Drug Administration.

Feng Q.L., Wu J., Chen G.Q., Cui F.Z., Kim T.N., Kim J.O. (2000) A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J Biomed Mater Res* 52:662-8.

Foster T.J. (1987) The genetics and biochemistry of mercury resistance. *Crit Rev Microbiol* 15:117-40. DOI: 10.3109/10408418709104455.

Fraise A.P., Maillard J.-Y., Sattar S. (2012) *Principles and Practice of Disinfection, Preservation and Sterilization*. John Wiley & Sons.

Franke S. (2007) *Microbiology of the toxic noble metal silver*. Springer., Berlin.
Fredell D.L. (1994) *Biological properties and applications of cationic surfactants* Marcel Dekker, New York.

Freitas A.R., Coque T.M., Novais C., Hammerum A.M., Lester C.H., Zervos M.J., Donabedian S., Jensen L.B., Francia M.V., Baquero F., Peixe L. (2011) Human and swine hosts share vancomycin-resistant *Enterococcus faecium* CC17 and CC5 and *Enterococcus faecalis* CC2 clonal clusters harboring Tn1546 on indistinguishable plasmids. *J Clin Microbiol* 49:925-31. DOI: 10.1128/JCM.01750-10.

Gaetke L.M., Chow C.K. (2003) Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology* 189:147-63.

Garner J.P., Heppell P.S. (2005) Cerium nitrate in the management of burns. *Burns* 31:539-47. DOI: 10.1016/j.burns.2005.01.014.

Ge R., Sun H. (2007) Bioinorganic chemistry of bismuth and antimony: target sites of metallodrugs. *Acc Chem Res* 40:267-74. DOI: 10.1021/ar600001b.

German N., Doyscher D., Rensing C. (2013) Bacterial killing in macrophages and amoeba: do they all use a brass dagger? *Future Microbiol* 8:1257-64. DOI: 10.2217/fmb.13.100.

Gilbert P., McBain A.J. (2001) Biocide usage in the domestic setting and concern about antibacterial and antibiotic resistance. *J Infect* 43:85-91. DOI: 10.1053/jinf.2001.0853.

Gilbert P., Moore L.E. (2005) Cationic antiseptics: diversity of action under a common epithet. *J Appl Microbiol* 99:703-15. DOI: 10.1111/j.1365-2672.2005.02664.x.

Gisi U. (2014) Assessment of selection and resistance risk for demethylation inhibitor fungicides in *Aspergillus fumigatus* in agriculture and medicine: a critical review. *Pest Manag Sci* 70:352-64. DOI: 10.1002/ps.3664.

Giuliano C.A., Rybak M.J. (2015) Efficacy of triclosan as an antimicrobial hand soap and its potential impact on antimicrobial resistance: a focused review. *Pharmacotherapy* 35:328-36. DOI: 10.1002/phar.1553.

Gottardi W., Karl A. (1991) [Chlorine coatings on skin surfaces. II. Parameters influencing the coating strength]. *Zentralbl Hyg Umweltmed* 191:478-93.

- Grecco L.E., Gómez, E.A., Botté, S.E., Marcos, Á.O., Marcovecchio, J.E., Cuadrado, D.G. (2011) Natural and anthropogenic heavy metals in estuarine cohesive sediments: geochemistry and bioavailability. *Ocean Dynamics* 61:285-293.
- Gupta A., Phung L.T., Taylor D.E., Silver S. (2001) Diversity of silver resistance genes in IncH incompatibility group plasmids. *Microbiology* 147:3393-402. DOI: 10.1099/00221287-147-12-3393.
- Halden R.U. (2014) On the need and speed of regulating triclosan and triclocarban in the United States. *Environ Sci Technol* 48:3603-11. DOI: 10.1021/es500495p.
- Harrison J.J., Ceri H., Turner R.J. (2007) Multimetal resistance and tolerance in microbial biofilms. *Nat Rev Microbiol* 5:928-38. DOI: 10.1038/nrmicro1774.
- He Z.L., Yang X.E., Stoffella P.J. (2005) Trace elements in agroecosystems and impacts on the environment. *J Trace Elem Med Biol* 19:125-40. DOI: 10.1016/j.jtemb.2005.02.010.
- Heir E., Langsrud S., Sidhu M.S., Steinbakk M. (2001) [Can disinfectants contribute to antibiotic resistance?]. *Tidsskr Nor Laegeforen* 121:3201-6.
- Herawati N., Suzuki S., Hayashi K., Rivai I.F., Koyama H. (2000) Cadmium, copper, and zinc levels in rice and soil of Japan, Indonesia, and China by soil type. *Bull Environ Contam Toxicol* 64:33-9.
- Herruzo Cabrera R. (2005) [Early-onset post-surgical pneumonia]. *An R Acad Nac Med (Madr)* 122:741-53; discussion 753-5.
- Hobman J.L., Crossman L.C. (2015) Bacterial antimicrobial metal ion resistance. *J Med Microbiol* 64:471-97. DOI: 10.1099/jmm.0.023036-0.
- Holt K.B., Bard A.J. (2005) Interaction of silver(I) ions with the respiratory chain of *Escherichia coli*: an electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag⁺. *Biochemistry* 44:13214-23. DOI: 10.1021/bi0508542.
- Huddleston J.R. (2014) Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. *Infect Drug Resist* 7:167-76. DOI: 10.2147/IDR.S48820.
- Huijbers P.M., Blaak H., de Jong M.C., Graat E.A., Vandenbroucke-Grauls C.M., de Roda Husman A.M. (2015) Role of the Environment in the Transmission of Antimicrobial Resistance to Humans: A Review. *Environ Sci Technol* 49:11993-2004. DOI: 10.1021/acs.est.5b02566.
- Huisingh D. (1974) Heavy metals: implications for agriculture. *Annu Rev Phytopathol* 12:375-388.
- IFT. (2006) Antimicrobial resistance: Implications for the food system, Institute of Food Technologists.
- Jacob M.E., Fox J.T., Nagaraja T.G., Drouillard J.S., Amachawadi R.G., Narayanan S.K. (2010) Effects of feeding elevated concentrations of copper and zinc on the antimicrobial

susceptibilities of fecal bacteria in feedlot cattle. *Foodborne Pathog Dis* 7:643-8. DOI: 10.1089/fpd.2009.0401.

Joerger R.D., Hanning I.B., Ricke S.C. (2010) Presence of arsenic resistance in *Salmonella enterica* serovar Kentucky and other serovars isolated from poultry. *Avian Dis* 54:1178-82. DOI: 10.1637/9285-022210-Reg.1.

Kampf G., Ostermeyer C., Tschudin-Sutter S., Widmer A.F. (2013) Resistance or adaptation? How susceptible is a 'glutaraldehyde-resistant' *Pseudomonas aeruginosa* isolate in the absence of selection pressure? *J Hosp Infect* 84:316-8. DOI: 10.1016/j.jhin.2013.05.010.

Kazantzis G. (2000) Thallium in the environment and health effects. *Environ Geochem Health* 22:275-280.

Khesin R.B., Karasyova E.V. (1984) Mercury-resistant plasmids in bacteria from a mercury and antimony deposit area. *Mol Gen Genet* 197:280-5.

Kruger M.C., Bertin P.N., Heipieper H.J., Arsene-Ploetze F. (2013) Bacterial metabolism of environmental arsenic--mechanisms and biotechnological applications. *Appl Microbiol Biotechnol* 97:3827-41. DOI: 10.1007/s00253-013-4838-5.

Kümmerer K., Eitel A., Braun U., Hubner P., Daschner F., Mascart G., Milandri M., Reinthaler F., Verhuf J. (1997) "Analysis of benzalkonium chloride in the effluent from European hospitals by solid-phase extraction and HPLC with post-column ion-pairing for fluorescence detection", *J Chromatogra* 774,: 281-286.

Landsdown A.B., Williams A. (2007) Bacterial resistance to silver in wound care and medical devices. *J Wound Care* 16:15-9. DOI: 10.12968/jowc.2007.16.1.26983.

Lawrence J.R., Zhu B., Swerhone G.D., Topp E., Roy J., Wassenaar L.I., Rema T., Korber D.R. (2008) Community-level assessment of the effects of the broad-spectrum antimicrobial chlorhexidine on the outcome of river microbial biofilm development. *Appl Environ Microbiol* 74:3541-50. DOI: 10.1128/AEM.02879-07.

Leaper D., Assadian O., Hubner N.O., McBain A., Barbolt T., Rothenburger S., Wilson P. (2011) Antimicrobial sutures and prevention of surgical site infection: assessment of the safety of the antiseptic triclosan. *Int Wound J* 8:556-66. DOI: 10.1111/j.1742-481X.2011.00841.x.

Lee S., Rakic-Martinez M., Graves L.M., Ward T.J., Siletzky R.M., Kathariou S. (2013) Genetic determinants for cadmium and arsenic resistance among *Listeria monocytogenes* serotype 4b isolates from sporadic human listeriosis patients. *Appl Environ Microbiol* 79:2471-6. DOI: 10.1128/AEM.03551-12.

Leelaporn A., Paulsen I.T., Tennent J.M., Littlejohn T.G., Skurray R.A. (1994) Multidrug resistance to antiseptics and disinfectants in coagulase-negative staphylococci. *J Med Microbiol* 40:214-20. DOI: 10.1099/00222615-40-3-214.

Lemire J.A., Harrison J.J., Turner R.J. (2013) Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nat Rev Microbiol* 11:371-84. DOI: 10.1038/nrmicro3028.

Levy S.B. (2002) Active efflux, a common mechanism for biocide and antibiotic resistance. *J Appl Microbiol* 92 Suppl:65S-71S.

Linley E., Denyer S.P., McDonnell G., Simons C., Maillard J.Y. (2012) Use of hydrogen peroxide as a biocide: new consideration of its mechanisms of biocidal action. *J Antimicrob Chemother* 67:1589-96. DOI: 10.1093/jac/dks129.

Liu J., Lu Y., Wu Q., Goyer R.A., Waalkes M.P. (2008) Mineral arsenicals in traditional medicines: orpiment, realgar, and arsenolite. *J Pharmacol Exp Ther* 326:363-8. DOI: 10.1124/jpet.108.139543.

Liu Q., Zhang M., Fang Z.X., Rong X.H. (2014) Effects of ZnO nanoparticles and microwave heating on the sterilization and product quality of vacuum-packaged Caixin. *J Sci Food Agric* 94:2547-54. DOI: 10.1002/jsfa.6594.

Lorena N.S., Pitombo M.B., Cortes P.B., Maya M.C., Silva M.G., Carvalho A.C., Coelho F.S., Miyazaki N.H., Marques E.A., Chebabo A., Freitas A.D., Lupi O., Duarte R.S. (2010) *Mycobacterium massiliense* BRA100 strain recovered from postsurgical infections: resistance to high concentrations of glutaraldehyde and alternative solutions for high level disinfection. *Acta Cir Bras* 25:455-9.

Lown J.W. (1983) The mechanism of action of quinone antibiotics. *Mol Cell Biochem* 55:17-40.

Mahony D.E., Lim-Morrison S., Bryden L., Faulkner G., Hoffman P.S., Agocs L., Briand G.G., Burford N., Maguire H. (1999) Antimicrobial activities of synthetic bismuth compounds against *Clostridium difficile*. *Antimicrob Agents Chemother* 43:582-8.

Maillard J.Y. (2002) Bacterial target sites for biocide action. *Symp Ser Soc Appl Microbiol*:16S-27S.

Mallik S., Viridi J.S., Johri A.K. (2012) Proteomic analysis of arsenite - mediated multiple antibiotic resistance in *Yersinia enterocolitica* biovar 1A. *J Basic Microbiol* 52:306-13. DOI: 10.1002/jobm.201100109.

March J.K., Cohen M.N., Lindsey J.M., Millar D.A., Lowe C.W., Bunnell A.J., O'Neill K.L., Schaalje G.B., Robison R.A. (2012) The differential susceptibility of spores from virulent and attenuated *Bacillus anthracis* strains to aldehyde- and hypochlorite-based disinfectants. *Microbiologyopen* 1:407-14. DOI: 10.1002/mbo3.45.

McArthur J.V., Tuckfield R.C., Baker-Austin C. (2012) Antimicrobial textiles. *Handb Exp Pharmacol*:135-52. DOI: 10.1007/978-3-642-28951-4_9.

McBain A.J., Ledder R.G., Moore L.E., Catrenich C.E., Gilbert P. (2004) Effects of quaternary-ammonium-based formulations on bacterial community dynamics and antimicrobial susceptibility. *Appl Environ Microbiol* 70:3449-56. DOI: 10.1128/AEM.70.6.3449-3456.2004. McDonnell G., Russell A.D. (1999) Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 12:147-79.

Mishra S., Imlay J. (2012) Why do bacteria use so many enzymes to scavenge hydrogen peroxide? *Arch Biochem Biophys* 525:145-60. DOI: 10.1016/j.abb.2012.04.014.

Mitsui R., Omori M., Kitazawa H., Tanaka M. (2005) Formaldehyde-limited cultivation of a newly isolated methylotrophic bacterium, *Methylobacterium* sp. MF1: enzymatic analysis related to C1 metabolism. *J Biosci Bioeng* 99:18-22. DOI: 10.1263/jbb.99.018.

Moeller R., Vlastic I., Reitz G., Nicholson W.L. (2012) Role of altered *rpoB* alleles in *Bacillus subtilis* sporulation and spore resistance to heat, hydrogen peroxide, formaldehyde, and glutaraldehyde. *Arch Microbiol* 194:759-67. DOI: 10.1007/s00203-012-0811-4.

Monachese M., Burton J.P., Reid G. (2012) Bioremediation and tolerance of humans to heavy metals through microbial processes: a potential role for probiotics? *Appl Environ Microbiol* 78:6397-404. DOI: 10.1128/AEM.01665-12.

Montazer M., Amiri M.M., Mohammad Ali Malek R. (2013) In situ synthesis and characterization of nano ZnO on wool: influence of nano photo reactor on wool properties. *Photochem Photobiol* 89:1057-63. DOI: 10.1111/php.12090.

Monteiro S.C., Lofts S., Boxall A.B.A. (2010) Pre-assessment of environmental impact of zinc and copper used in animal nutrition EFSA Supporting Publication 2010; 7(9):EN-74, 325 pp. doi:10.2903/sp.efsa.2010.EN-74.

Mullapudi S., Siletzky R.M., Kathariou S. (2008) Heavy-metal and benzalkonium chloride resistance of *Listeria monocytogenes* isolates from the environment of turkey-processing plants. *Appl Environ Microbiol* 74:1464-8. DOI: 10.1128/AEM.02426-07.

Nagoba B.S., Selkar S.P., Wadher B.J., Gandhi R.C. (2013) Acetic acid treatment of pseudomonal wound infections--a review. *J Infect Public Health* 6:410-5. DOI: 10.1016/j.jiph.2013.05.005.

Nielsen K.M., Bøhn T., Townsend J.P. (2014) Detecting rare gene transfer events in bacterial populations. *Front. Microbiol.* 4:415.

Nies D.H. (1999) Microbial heavy-metal resistance. *Appl Microbiol Biotechnol* 51:730-50.

Nies D.H., Silver S. (1995) Ion efflux systems involved in bacterial metal resistances. *J Ind Microbiol* 14:186-99.

Novelli F., Recine M., Sparatore F., Juliano C. (1999) Gold(I) complexes as antimicrobial agents. *Farmaco* 54:232-6.

Novo A., Andre S., Viana P., Nunes O.C., Manaia C.M. (2013) Antibiotic resistance, antimicrobial residues and bacterial community composition in urban wastewater. *Water Res* 47:1875-87. DOI: 10.1016/j.watres.2013.01.010.

Novoa-Garrido M., Steinum T.M., Marolda C.L., Valvano M.A., Sørum H. (2009) Reduced lipopolysaccharide O antigen expression, increased acid susceptibility and multicellular behaviour in an *Escherichia coli* isolate after long-term in vitro exposure to formic acid. *Microbial Ecology in Health and Disease* 21:87-94.

NTNU. (2001) Atmosfærisk nedfall av tungmetaller i Norge Landsomfattende undersøkelse i 2000, Rapport: 838/01 TA-nummer: TA-1842/2001

Nuñez L., Moreton J. (2007) Disinfectant-resistant bacteria in Buenos Aires City hospital wastewater. *Braz.J.Microbiol.* 38:644-648.

- Oggioni M.R., Furi L., Coelho J.R., J.Y. M., J.L. M. (2013) Biocides: do they select for antimicrobial resistance? *Expert Rev Anti Infect Ther.* 11:363-366.
- Omogbenigun F.O., Nyachoti C.M., Slominski B.A. (2003) The effect of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early-weaned pigs. *J Anim Sci* 81:1806-13.
- Partanen K.H., and Z. Mroz. (1991) Organic acids for performance enhancement in pig diets. *Nutr Res Rev* 12:117-145.
- Paschal D.C., Burt V., Caudill S.P., Gunter E.W., Pirkle J.L., Sampson E.J., Miller D.T., Jackson R.J. (2000) Exposure of the U.S. population aged 6 years and older to cadmium: 1988-1994. *Arch Environ Contam Toxicol* 38:377-83.
- Paulsen I.T., Brown M.H., Littlejohn T.G., Mitchell B.A., Skurray R.A. (1996a) Multidrug resistance proteins QacA and QacB from *Staphylococcus aureus*: membrane topology and identification of residues involved in substrate specificity. *Proc Natl Acad Sci U S A* 93:3630-5.
- Paulsen I.T., Skurray R.A., Tam R., Saier M.H., Jr., Turner R.J., Weiner J.H., Goldberg E.B., Grinius L.L. (1996b) The SMR family: a novel family of multidrug efflux proteins involved with the efflux of lipophilic drugs. *Mol Microbiol* 19:1167-75.
- Phan T.N., Buckner T., Sheng J., Baldeck J.D., Marquis R.E. (2004) Physiologic actions of zinc related to inhibition of acid and alkali production by oral streptococci in suspensions and biofilms. *Oral Microbiol Immunol* 19:31-8.
- Poole K. (2002) Mechanisms of bacterial biocide and antibiotic resistance. *Symp Ser Soc Appl Microbiol*:55S-64S.
- Ray S., Mohan R., Singh J.K., Samantaray M.K., Shaikh M.M., Panda D., Ghosh P. (2007) Anticancer and antimicrobial metallopharmaceutical agents based on palladium, gold, and silver N-heterocyclic carbene complexes. *J Am Chem Soc* 129:15042-53. DOI: 10.1021/ja075889z.
- Roberts M.C. (2002) Antibiotic toxicity, interactions and resistance development. *Periodontol* 2000 28:280-97.
- Rosen B.P. (2002) Biochemistry of arsenic detoxification. *FEBS Lett* 529:86-92.
- Russell A.D. (2002a) Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. *Symp Ser Soc Appl Microbiol.* 31:121S-135S.
- Russell A.D. (2002b) Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. *J Appl Microbiol.* 92 Suppl:121S-35S.
- Russell P.E. (2005) A century of fungicide evolution. *J Agric Sci* 143:11-25.
- Rutala W.A., Weber D.J. (2008) Guideline for disinfection and sterilization in healthcare facilities, CDC, USA.

- Saleh S., Haddadin R.N., Baillie S., Collier P.J. (2011) Triclosan - an update. *Lett Appl Microbiol* 52:87-95. DOI: 10.1111/j.1472-765X.2010.02976.x.
- Savluchinske-Feio S., Curto M.J., Gigante B., Roseiro J.C. (2006) Antimicrobial activity of resin acid derivatives. *Appl Microbiol Biotechnol* 72:430-6. DOI: 10.1007/s00253-006-0517-0.
- SCENHR. (2009) Assessment of the Antibiotic Resistance Effects of Biocides Scientific Committee on Emerging and Newly Identified Health Risks, European Commission Brussels.
- Schiering N., Kabsch W., Moore M.J., Distefano M.D., Walsh C.T., Pai E.F. (1991) Structure of the detoxification catalyst mercuric ion reductase from *Bacillus* sp. strain RC607. *Nature* 352:168-72. DOI: 10.1038/352168a0.
- Schluter A., Szczepanowski R., Puhler A., Top E.M. (2007) Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. *FEMS Microbiol Rev* 31:449-77. DOI: 10.1111/j.1574-6976.2007.00074.x.
- Schweizer H.P. (2001) Triclosan: a widely used biocide and its link to antibiotics. *FEMS Microbiol Lett* 202:1-7.
- Seil J.T., Webster T.J. (2011) Reduced *Staphylococcus aureus* proliferation and biofilm formation on zinc oxide nanoparticle PVC composite surfaces. *Acta Biomater* 7:2579-84. DOI: 10.1016/j.actbio.2011.03.018.
- Seiler C., Berendonk T.U. (2012) Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Front Microbiol* 3:399. DOI: 10.3389/fmicb.2012.00399.
- Selvaraju S.B., Khan I.U., Yadav J.S. (2011) Susceptibility of *Mycobacterium immunogenum* and *Pseudomonas fluorescens* to formaldehyde and non-formaldehyde biocides in semi-synthetic metalworking fluids. *Int J Mol Sci* 12:725-41. DOI: 10.3390/ijms12010725.
- Sensi A., Brandenberg O., Ghosh K., Sonnino A. (2011) Modul C: Chapter 3: Risk Analysis," in Biosafety Resource Book. FAO.
- Shakya S., Pradhan B., Smith L., Shrestha J., Tuladhar S. (2012) Isolation and characterization of aerobic culturable arsenic-resistant bacteria from surfacewater and groundwater of Rautahat District, Nepal. *J Environ Manage* 95 Suppl:S250-5. DOI: 10.1016/j.jenvman.2011.08.001.
- Shen Z., Luangtongkum T., Qiang Z., Jeon B., Wang L., Zhang Q. (2014) Identification of a novel membrane transporter mediating resistance to organic arsenic in *Campylobacter jejuni*. *Antimicrob Agents Chemother* 58:2021-9. DOI: 10.1128/AAC.02137-13.
- Shivaji S., Suresh K., Chaturvedi P., Dube S., Sengupta S. (2005) *Bacillus arsenicus* sp. nov., an arsenic-resistant bacterium isolated from a siderite concretion in West Bengal, India. *Int J Syst Evol Microbiol* 55:1123-7. DOI: 10.1099/ijms.0.63476-0.
- Sidhu M.S., Langsrud S., Holck A. (2001) Disinfectant and antibiotic resistance of lactic acid bacteria isolated from the food industry. *Microb Drug Resist* 7:73-83. DOI: 10.1089/107662901750152846.

- Silver S. (1998) Genes for all metals--a bacterial view of the periodic table. The 1996 Thom Award Lecture. *J Ind Microbiol Biotechnol* 20:1-12.
- Silver S. (2003) Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. *FEMS Microbiol Rev* 27:341-53.
- Silver S., Phung le T. (2005) A bacterial view of the periodic table: genes and proteins for toxic inorganic ions. *J Ind Microbiol Biotechnol* 32:587-605. DOI: 10.1007/s10295-005-0019-6.
- Silver S., Phung L.T. (1996) Bacterial heavy metal resistance: new surprises. *Annu Rev Microbiol* 50:753-89. DOI: 10.1146/annurev.micro.50.1.753.
- Simoes L.C., Lemos M., Araujo P., Pereira A.M., Simoes M. (2011) The effects of glutaraldehyde on the control of single and dual biofilms of *Bacillus cereus* and *Pseudomonas fluorescens*. *Biofouling* 27:337-46. DOI: 10.1080/08927014.2011.575935.
- Singh R., Shedbalkar U.U., Wadhvani S.A., Chopade B.A. (2015) Bacteriogenic silver nanoparticles: synthesis, mechanism, and applications. *Appl Microbiol Biotechnol* 99:4579-93. DOI: 10.1007/s00253-015-6622-1.
- Skarbøvik E., Allan I., Stålnacke P., Hagen A.G., Greipsland I., Høgåsen T., Selvik J.R., Beldring S. (2014) Riverine inputs and direct discharges to Norwegian coastal waters., NIVA – Norwegian Institute for Water Research.
- Sreenivasan P., Gaffar A. (2002) Antiplaque biocides and bacterial resistance: a review. *J Clin Periodontol* 29:965-74.
- Stepanauskas R., Glenn T.C., Jagoe C.H., Tuckfield R.C., Lindell A.H., McArthur J.V. (2005) Elevated microbial tolerance to metals and antibiotics in metal-contaminated industrial environments. *Environ Sci Technol* 39:3671-8.
- Stewart F.J. (2013) Where the genes flow. *Nature Geoscience* 6:688-690.
- Su J., Ye J., Zhu Y. (2012) Draft genome sequence of a novel bacterial strain, LSJC7, belonging to the family Enterobacteriaceae with dual resistance to arsenic and tetracycline. *J Bacteriol* 194:7005-6. DOI: 10.1128/JB.01652-12.
- Summers A.O., Wireman J., Vimy M.J., Lorscheider F.L., Marshall B., Levy S.B., Bennett S., Billard L. (1993) Mercury released from dental "silver" fillings provokes an increase in mercury- and antibiotic-resistant bacteria in oral and intestinal floras of primates. *Antimicrob Agents Chemother* 37:825-34.
- Suresh K., Prabakaran S.R., Sengupta S., Shivaji S. (2004) *Bacillus indicus* sp. nov., an arsenic-resistant bacterium isolated from an aquifer in West Bengal, India. *Int J Syst Evol Microbiol* 54:1369-75. DOI: 10.1099/ijs.0.03047-0.
- Sutterlin H., Alexy R., Coker A., Kummerer K. (2008a) Mixtures of quaternary ammonium compounds and anionic organic compounds in the aquatic environment: Elimination and biodegradability in the closed bottle test monitored by LC-MS/MS. *Chemosphere* 72:479-84. DOI: 10.1016/j.chemosphere.2008.03.008.

Sutterlin H., Alexy R., Kummerer K. (2008b) The toxicity of the quaternary ammonium compound benzalkonium chloride alone and in mixtures with other anionic compounds to bacteria in test systems with *Vibrio fischeri* and *Pseudomonas putida*. *Ecotoxicol Environ Saf* 71:498-505. DOI: 10.1016/j.ecoenv.2007.12.015.

Svetlikova Z., Skovierova H., Niederweis M., Gaillard J.L., McDonnell G., Jackson M. (2009) Role of porins in the susceptibility of *Mycobacterium smegmatis* and *Mycobacterium chelonae* to aldehyde-based disinfectants and drugs. *Antimicrob Agents Chemother* 53:4015-8. DOI: 10.1128/AAC.00590-09.

Taylor D.E. (1999) Bacterial tellurite resistance. *Trends Microbiol* 7:111-5.

Tchounwou P.B., Yedjou C.G., Patlolla A.K., Sutton D.J. (2012) Heavy metal toxicity and the environment. *EXS* 101:133-64. DOI: 10.1007/978-3-7643-8340-4_6.

Teitzel G.M., Parsek M.R. (2003) Heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. *Appl Environ Microbiol* 69:2313-20.

Thompson J.L., Hinton M. (1997) Antibacterial activity of formic and propionic acids in the diet of hens on *Salmonellas* in the crop. *Br Poult Sci* 38:59-65. DOI: 10.1080/00071669708417941.

Thormar H. (2012) Patented non-antibiotic agents as animal feed additives. *Recent Pat Food Nutr Agric* 4:155-68.

Trotter R.T., 2nd. (1990) The cultural parameters of lead poisoning: a medical anthropologist's view of intervention in environmental lead exposure. *Environ Health Perspect* 89:79-84.

Tschudin-Sutter S., Frei R., Kampf G., Tamm M., Pflimlin E., Battegay M., Widmer A.F. (2011) Emergence of glutaraldehyde-resistant *Pseudomonas aeruginosa*. *Infect Control Hosp Epidemiol* 32:1173-8. DOI: 10.1086/662624.

Tubbing D.M., Admiraal W. (1991) Inhibition of bacterial and phytoplanktonic metabolic activity in the lower River Rhine by ditallowdimethylammonium chloride. *Appl Environ Microbiol* 57:3616-22.

Vasanthakumari R. (2007) Textbook of Microbiology BI Publications Pvt Ltd, New Dehli. ISBN-10: 81-7225-234-x, ISBN-13:978-81-7225-234-2.

Vikram A., Bomberger J.M., Bibby K.J. (2015) Efflux as a glutaraldehyde resistance mechanism in *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother* 59:3433-40. DOI: 10.1128/AAC.05152-14.

VKM. (2005) Risk assessment on the use of triclosan in cosmetics. Development of antimicrobial resistance in bacteria.

VKM. (2007) Risk assessment on the use of triclosan in cosmetics; Development of antimicrobial resistance in bacteria - II.

VKM. (2009) Quaternary ammonium compounds in cosmetic products Risk assessment of antimicrobial and antibiotic resistance development in microorganisms, Norwegian Scientific Committee for Food Safety. ISBN:978-82-8082-339-7.

VKM. (2010) Chlorhexidine compounds in cosmetic products. Risk assessment of antimicrobial and antibiotic resistance development in microorganisms Norwegian Scientific Committee for Food Safety. ISBN:978-82-8082-404-2.

VKM. (2014) Zinc and copper in pig and poultry production – fate and effects in the food chain and the environment, Norwegian Scientific Committee for Food Safety, ISBN 978-82-8259-093-8.

Walsh S.E., Maillard J.Y., Russell A.D., Catrenich C.E., Charbonneau D.L., Bartolo R.G. (2003) Activity and mechanisms of action of selected biocidal agents on Gram-positive and -negative bacteria. *J Appl Microbiol* 94:240-7.

Wang G.Q., Zhang C.W., Liu H.C., Chen Z.B. (2005) Comparison of susceptibilities of *M. tuberculosis* H37Ra and *M. chelonae* subsp. *abscessus* to disinfectants. *Biomed Environ Sci* 18:124-7.

Weber D.J., Rutala W.A. (2006) Use of germicides in the home and the healthcare setting: is there a relationship between germicide use and antibiotic resistance? *Infect Control Hosp Epidemiol* 27:1107-19. DOI: 10.1086/507964.

Wheaton G., Counts J., Mukherjee A., Kruh J., Kelly R. (2015) The confluence of heavy metal biooxidation and heavy metal resistance: implications for bioleaching by extreme thermoacidophiles. *Minerals* 5:397-451.

WHO. (2015) Worldwide country situation analysis: response to antimicrobial resistance, <http://www.who.int/drugresistance/documents/situationanalysis/en/>. Accessed 21 Oct 2015.

Yamazaki T., Tsugawa W., Sode K. (2001) Biodegradation of formaldehyde by a formaldehyde-resistant bacterium isolated from seawater. *Appl Biochem Biotechnol* 91-93:213-7.

Yazdankhah S., Rudi K., Bernhoft A. (2014) Zinc and copper in animal feed - development of resistance and co-resistance to antimicrobial agents in bacteria of animal origin. *Microb Ecol Health Dis* 25. DOI: 10.3402/mehd.v25.25862.

Yazdankhah S.P., Scheie A.A., Hoiby E.A., Lunestad B.T., Heir E., Fotland T.O., Naterstad K., Kruse H. (2006) Triclosan and antimicrobial resistance in bacteria: an overview. *Microb Drug Resist* 12:83-90. DOI: 10.1089/mdr.2006.12.83.

Zhang C.X., Lippard S.J. (2003) New metal complexes as potential therapeutics. *Curr Opin Chem Biol* 7:481-489.

Zscheck K.K., Murray B.E. (1993) Genes involved in the regulation of beta-lactamase production in enterococci and staphylococci. *Antimicrob Agents Chemother* 37:1966-70.

Appendix 1

ECHA (European Chemical Agency) ECHA > Regulations > Biocidal Products Regulation > Product-types

(<https://echa.europa.eu/regulations/biocidal-products-regulation/product-types>)

Product-types

In Annex V to the BPR the biocidal products are classified into 22 biocidal product-types, grouped in four main areas. As a result of excluding biocidal products used as preservatives for food and feedstock from the scope, there is one less product type compared to the previous directive.

Number	Product-type	Description
<p>Main group 1: Disinfectants These product types exclude cleaning products that are not intended to have a biocidal effect, including washing liquids, powders and similar products.</p>		
PT 1	Human hygiene	Products in this group are biocidal products used for human hygiene purposes, applied on or in contact with human skin or scalps for the primary purpose of disinfecting the skin or scalp.
PT 2	Disinfectants and algaecides not intended for direct application to humans or animals	<p>Used for the disinfection of surfaces, materials, equipment and furniture which are not used for direct contact with food or feeding stuffs. Usage areas include, inter alia, swimming pools, aquariums, bathing and other waters; air conditioning systems; and walls and floors in private, public, and industrial areas and in other areas for professional activities.</p> <p>Used for disinfection of air, water not used for human or animal consumption, chemical toilets, waste water, hospital waste and soil.</p> <p>Used as algaecides for treatment of swimming pools, aquariums and other waters and for remedial treatment of construction materials.</p> <p>Used to be incorporated in textiles, tissues, masks, paints and other articles or materials with the purpose of producing treated articles with disinfecting properties.</p>
PT 3	Veterinary hygiene	<p>Used for veterinary hygiene purposes such as disinfectants, disinfecting soaps, oral or corporal hygiene products or with anti-microbial function.</p> <p>Used to disinfect the materials and surfaces associated with the housing or transportation of animals.</p>
PT 4	Food and feed area	Used for the disinfection of equipment, containers, consumption utensils, surfaces or pipework associated with the production, transport, storage or consumption of food or feed (including drinking water) for humans and animals.

		Used to impregnate materials which may enter into contact with food.
PT 5	Drinking water	Used for the disinfection of drinking water for both humans and animals.

Number	Product-type	Description
--------	--------------	-------------

Main group 2: Preservatives

Unless otherwise stated these product-types include only products to prevent microbial and algal development.

PT 6	Preservatives for products during storage	Used for the preservation of manufactured products, other than foodstuffs, feeding stuffs, cosmetics or medicinal products or medical devices by the control of microbial deterioration to ensure their shelf life. Used as preservatives for the storage or use of rodenticide, insecticide or other baits.
PT 7	Film preservatives	Used for the preservation of films or coatings by the control of microbial deterioration or algal growth in order to protect the initial properties of the surface of materials or objects such as paints, plastics, sealants, wall adhesives, binders, papers, art works.
PT 8	Wood preservatives	Used for the preservation of wood, from and including the saw-mill stage, or wood products by the control of wood-destroying or wood-disfiguring organisms, including insects. This product type includes both preventive and curative products.
PT 9	Fibre, leather, rubber and polymerised materials preservatives	Used for the preservation of fibrous or polymerised materials, such as leather, rubber or paper or textile products by the control of microbiological deterioration. This product-type includes biocidal products which antagonise the settlement of micro-organisms on the surface of materials and therefore hamper or prevent the development of odour and/or offer other kinds of benefits.
PT 10	Construction material preservatives	Used for the preservation of masonry, composite materials, or other construction materials other than wood by the control of microbiological and algal attack.
PT 11	Preservatives for liquid-cooling and processing systems	Used for the preservation of water or other liquids used in cooling and processing systems by the control of harmful organisms such as microbes, algae and mussels. Products used for the disinfection of drinking water or of water for swimming pools are not included in this product-type.

PT 12	Slimicides	Used for the prevention or control of slime growth on materials, equipment and structures, used in industrial processes, e.g. on wood and paper pulp, porous sand strata in oil extraction.
PT 13	Working or cutting fluid preservatives	Products to control microbial deterioration in fluids used for working or cutting metal, glass or other materials.

Number	Product-type	Description
Main group 3: Pest control		
PT 14	Rodenticides	Used for the control of mice, rats or other rodents, by means other than repulsion or attraction.
PT 15	Avicides	Used for the control of birds, by means other than repulsion or attraction.
PT 16	Molluscicides, vermicides and products to control other invertebrates	Used for the control of molluscs, worms and invertebrates not covered by other product types, by means other than repulsion or attraction.
PT 17	Piscicides	Used for the control of fish, by means other than repulsion or attraction.
PT 18	Insecticides, acaricides and products to control other arthropods	Used for the control of arthropods (e.g. insects, arachnids and crustaceans), by means other than repulsion or attraction.
PT 19	Repellents and attractants	Used to control harmful organisms (invertebrates such as fleas, vertebrates such as birds, fish, rodents), by repelling or attracting, including those that are used for human or veterinary hygiene either directly on the skin or indirectly in the environment of humans or animals.
PT 20	Control of other vertebrates	Used for the control of vertebrates other than those already covered by the other product types of this main group, by means other than repulsion or attraction.

Number	Product-type	Description
Main group 4: Other biocidal products		

PT 21	Antifouling products	Used to control the growth and settlement of fouling organisms (microbes and higher forms of plant or animal species) on vessels, aquaculture equipment or other structures used in water.
PT 22	Embalming and taxidermist fluids	Used for the disinfection and preservation of human or animal corpses, or parts thereof.

Appendix 2

Chemistry International, Vol. 23, No. 6, **November 2001**

"Heavy Metals"- A Meaningless Term

(<https://www.iupac.org/publications/ci/2001/november/index.html>)

Definitions of heavy metal: Survey of current usage (April 2001).

Definitions in terms of density (specific gravity)

- metals fall naturally into two groups—the light metals with densities below 4, and the heavy metals with densities above 7 [3]
- metal having a specific gravity greater than 4 [5, 6]
- metal of high specific gravity, especially a metal having a specific gravity of 5.0 or greater [22]
- metal with a density greater than 5 [8, 23]
- metal with a density greater than 6 g/cm³ [24]
- metal with a density of 5.0 or greater [25]
- metal whose specific gravity is approximately 5.0 or higher [7]
- (in metallurgy) any metal or alloy of high specific gravity, especially one that has a density greater than 5 g/cm³ [9]
- metal with a density higher than 4.5 g/cm³ [10]
- metal with a density above 3.5-5 g/cm³ [12]
- element with a density exceeding 6 g/cm³ [11]

Definitions in terms of atomic weight (mass)

- metal with a high atomic weight [26]
 - metal of atomic weight greater than sodium [13]
 - metal of atomic weight greater than sodium (23) that forms soaps on reaction with fatty acids [14]
-

- metallic element with high atomic weight (e.g., mercury, chromium, cadmium, arsenic, and lead); can damage living things at low concentrations and tend to accumulate in the food chain [27]
- metallic element with an atomic weight greater than 40 (starting with scandium; atomic number 21); excluded are alkaline earth metals, alkali metals, lanthanides, and actinides [15]
- metal with a high atomic mass [28]
- heavy metals is a collective term for metals of high atomic mass, particularly those transition metals that are toxic and cannot be processed by living organisms, such as lead, mercury, and cadmium [29]
- metal such as mercury, lead, tin, and cadmium that has a relatively high atomic weight [30]
- rather vague term for any metal (in whatever chemical form) with a fairly high relative atomic mass, especially those that are significantly toxic (e.g., lead, cadmium, and mercury). They persist in the environment and can accumulate in plant and animal tissues. Mining and industrial wastes and sewage sludge are potential sources of heavy metal pollution [31].
- a metal such as cadmium, mercury, and lead that has a relatively high relative atomic mass. The term does not have a precise chemical meaning [32].
- metal with a high relative atomic mass. The term is usually applied to common transition metals such as copper, lead, or zinc [33].

Definitions in terms of atomic number

In biology:

- in electron microscopy, metal of high atomic number used to introduce electron density into a biological specimen by staining, negative staining, or shadowing [34]
- in plant nutrition, a metal of moderate to high atomic number, e.g., Cu, Zn, Ni, or Pb, present in soils owing to an outcrop or mine spoil, preventing growth except for a few tolerant species and ecotypes [34]

In chemistry:

- the rectangular block of elements in the Periodic Table flanked by titanium, hafnium, arsenic, and bismuth at its corners but including also selenium and tellurium. The specific gravities range from 4.5 to 22.5 [17].
- any metal with with an atomic number beyond that of calcium [35]

- any element with an atomic number greater than 20 [36]
- metal with an atomic number between 21 (scandium) and 92 (uranium) [16]
- term now often used to mean any metal with atomic number >20, but there is no general concurrence [20]

Definitions based on other chemical properties

- heavy metals is the name of a range of very dense alloys used for radiation screening or balancing purposes. Densities range from 14.5 for 76% W, 20% Cu, 4% Ni to 16.6 for 90% W, 7% Ni, 3% Cu [37].
- intermetallic compound of iron and tin (FeSn₂) formed in tinning pots that have become badly contaminated with iron. The compound tends to settle to the bottom of the pot as solid crystals and can be removed with a perforated ladle [38].
- lead, zinc, and alkaline earth metals that react with fatty acids to form soaps. "Heavy metal soaps" are used in lubricating greases, paint dryers, and fungicides [39].
- any of the metals that react readily with dithizone (C₆ H₅ N), e.g., zinc, copper, lead, etc. [40].
- metallic elements of relatively high molecular weight [41].

Definitions without a clear basis other than toxicity

- element commonly used in industry and generically toxic to animals and to aerobic and anaerobic processes, but not every one is dense or entirely metallic; includes As, Cd, Cr, Cu, Pb, Hg, Ni, Se, and Zn [42]
- outdated generic term referring to lead, cadmium, mercury, and some other elements that generally are relatively toxic in nature; recently, the term "toxic elements" has been used. The term also sometimes refers to compounds containing these elements [18].

Definitions preceding 1936

- guns or shot of large size [1]
- great ability [2]

References in Appendix 2

1. J. Ogilvie. *The Comprehensive English Dictionary*, Blackie, London (1884).
2. A. M. Williams. *The English Encyclopedic Dictionary*, Collins, London (1930).
3. N. Bjerrum. *Bjerrum's Inorganic Chemistry*, 3rd Danish ed., Heinemann, London (1936).
4. A. D. McNaught and A. Wilkinson. *Compendium of Chemical Terminology: IUPAC Recommendations*, 2nd ed., Blackwell Science, Oxford (1997).
5. *Van Nostrand International Encyclopedia of Chemical Science*, Van Nostrand, New Jersey (1964).
6. R. Grant and C. Grant (Eds.). *Grant and Hackh's Chemical Dictionary*, McGraw-Hill, New York (1987).

7. S. P. Parker (Ed.). *McGraw-Hill Dictionary of Scientific and Technical Terms*, 4th ed., McGraw-Hill, New York (1989).
8. J. Lozet and C. Mathieu. *Dictionary of Soil Science*, 2nd ed., A. A. Balkema, Rotterdam (1991).
9. C. Morris (Ed.). *Academic Press Dictionary of Science and Technology*, Academic Press, San Diego (1992).
10. B. Streit. *Lexikon der Okotoxikologie*, VCH, Weinheim (1994).
11. I. Thornton. *Metals in the Global Environment: Facts and Misconceptions*, ICME, Ottawa (1995).
12. J. Falbe and M. Regitz (Eds.). *Roempp Chemie-Lexikon*, George Thieme, Weinheim (1996).
13. H. Bennet (Ed.). *Concise Chemical and Technical Dictionary*, 4th enlarged ed., Edward Arnold, London (1986).
14. R. J. Lewis, Sr. (Ed.). *Hawley's Condensed Chemical Dictionary*, 12th ed., Van Nostrand Reinhold, New York (1993).
15. G. M. Rand, P. G. Wells, L. S. McCarty. "Introduction to aquatic toxicology", in *Fundamentals of Aquatic Toxicology*, G. M. Rand (Ed.), Taylor & Francis, Washington, DC (1995).
16. W. J. Lyman. "Transport and transformation processes", in *Fundamentals of Aquatic Toxicology*, G. M. Rand (Ed.), Taylor & Francis, Washington, DC (1995).
17. D. C. Burrell. *Atomic Spectrometric Analysis of Heavy Metal Pollutants in Water*, Ann Arbor Science Publishers, Ann Arbor, Michigan (1974).
18. E. Hodgson, R. B. Mailman, J. E. Chambers (Eds.), *Macmillan Dictionary of Toxicology*, Macmillan, London (1988).
19. C. D. Klaasen (Ed.). *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 5th ed., McGraw-Hill, New York (1996).
20. D. A. Phipps. "Chemistry and biochemistry of trace metals in biological systems", in *Effect of Heavy Metal Pollution on Plants*, N. W. Lepp (Ed.), Applied Science Publishers, Barking (1981).
21. G. W. VanLoon and S. J. Duffy. *Environmental Chemistry: A Global Perspective*, Oxford University Press, Oxford (2000).
22. *3rd New International Dictionary*, Merriam, Chicago (1976).
23. M. Brewer and T. Scott (Eds.). *Concise Encyclopedia of Biochemistry*, Walter de Gruyter, Berlin, New York (1983).
24. B. E. Davies. "Consequences of environmental contamination by lead mining in Wales", *Hydrobiologia* 49, 213 (1987).
25. S. B. Flexner (Ed.). *The Random House Dictionary of the English Language*, 2nd ed., Random House, New York (1987).
26. G. Holister and A. Porteous (Eds.). *The Environment: A Dictionary of the World Around Us*, Arrow, London (1976).
27. U.S. EPA. *EPA's Terms of Environment*, U.S. Environmental Protection Agency (2000).
28. A. Porteous. *Dictionary of Environmental Science and Technology*, 2nd ed., Wiley, Chichester (1996).
29. P. Harrison and G. Waites. *The Cassell Dictionary of Chemistry*, Cassell, London (1998).
30. D. D. Kemp. *The Environment Dictionary*, Routledge, London (1998).
31. E. Lawrence, A. R. W. Jackson, J. M. Jackson (Eds.). *Longman Dictionary of Environmental Science*, Addison Wesley Longman, Harlow (1998).
32. A. Hunt. *Dictionary of Chemistry*, Fitzroy Dearborn, London (1999).
33. *Oxford Dictionary of Science*, 4th ed., Oxford University Press, Oxford (1999).
34. P. M. B. Walker (Ed.). *Chambers Science and Technology Dictionary*, Chambers/Cambridge, Cambridge (1988).
35. B. Venugopal and T. P. Luckey. "Toxicology of nonradioactive heavy metals and their salts", in *Heavy Metal Toxicity, Safety, and Hormology*, T. D. Luckey, B. Venugopal, D. Hutcheson (Eds.), George Thieme, Stuttgart (1975).
36. W. G. Hale and J. P. Margham (Eds.). *Collins Dictionary of Biology*, Collins, Glasgow (1988).
37. D. Birchon. *Dictionary of Metallurgy*, Newnes, London (1965).
38. A. D. Merriman. *A Concise Encyclopedia of Metallurgy*, MacDonald and Evans, London (1965).
39. C. A. Hampel and G. G. Hawley. *Glossary of Chemical Terms*, Van Nostrand Reinhold, New York (1976).
40. R. L. Bates and J. A. Jackson (Eds.). *Glossary of Geology*, 3rd ed., American Geological Institute, Alexandria, VA (1987).
41. L. H. Stevenson and B. Wyman. *The Facts on File Dictionary of Environmental Science*, Facts on File, New York (1991).
42. J. S. Scott and P. G. Smith. *Dictionary of Waste and Water Treatment*, Butterworths, London (1981).
43. E. Nieboer and D. H. S. Richardson. "The replacement of the nondescript term 'heavy metal' by a biologically and chemically significant classification of metal ions", *Environmental Pollution (Series B)*, 1, 3 (1980).