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Reflection paper on antimicrobial resistance in the environment: considerations for current and future risk assessment of veterinary medicinal products

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1. Executive summary

This reflection paper on antimicrobial resistance (AMR) in the environment considers the impact(s) on ecosystems, animal and human health from the presence of antimicrobial residues (ARs) and/or antimicrobial resistance genes (ARGs) in the environment resulting from the use of veterinary medicinal products (VMPs). At the outset, we define AMR as the ability of microorganisms such as bacteria to become increasingly resistant to an antimicrobial to which they were previously susceptible.

It is recognised and acknowledged by the Committee for Medicinal Products for Veterinary Use (CVMP) that current guidelines on the environmental risk assessment (ERA) of VMPs for use in the European Union do not address how to assess the impact of antimicrobials, as veterinary pharmaceuticals, on the prevalence of AMR in the receiving environments e.g. soil, surface water, groundwater.

To produce this paper, an interdisciplinary team working across the antimicrobial working party (AWP) and the environmental risk assessment working party (ERAWP) of the CVMP has reviewed the current available data on antimicrobials in the environment and their role in the transmission of ARGs that may have clinical consequence for both human and animal health. This paper is quite unique in its remit and timely given the level of discussion within and across organisations such as the World Organisation for Animal Health (OIE), the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO). It focuses specifically, on information pertaining to veterinary medicines, particularly antimicrobials, the sources of potential resistance genes and their pathways in the environment, and the effects other pressures such as co-factors or contaminants have on the persistence of AMR. However, it is acknowledged that VMPs that are antimicrobial in nature act similarly to their human medicine counterparts and that many other pressures, including natural selection, drive the development of environmental AMR.

This paper has examined the key sources and identified the major exposure pathways and release scenarios and, subsequently, considered the likely extent of the accumulation and mobility of ARs and ARGs excreted from animals treated with VMPs. It has also considered the potential consequences of AMR in the environment on animals and human health.

The key findings from this paper highlight the significant gaps in our knowledge around the specific mechanisms and pathways of AMR. Further, there is little information on the potential impacts that ARs and ARGs, resulting from VMP use, can have on the functioning of the ecosystem and its key species. It is unknown whether putative changes induced in communities of bacteria, naturally present in the environment, may affect the emergence and spread of AMR in bacteria of clinical relevance for humans or animals (some of which have the ability to survive and grow in the environment) (ECDC/EFSA/EMA/SCENIHR, 2009; FAO, 2008).

There is, however, an increasing body of evidence that indicates that ARGs are transported through the environment. Further, the environment acts as a bridge to different compartments; animal to manure to soil to water to sediment, whilst simultaneously the environment acts like a reservoir or sink for the mixing of mobile genetic elements (MGEs) that interact and disperse to other compartments or to human and animal hosts. There is evidence that AMR pathogens have developed through these pathways and have impacted on human and animal wellbeing.

Gaps in our knowledge are identified and several suggestions are made to reduce or mitigate the impacts of 1) initiating/emergence of AMR in the first instance and 2) prolonging the longevity of existing or new antimicrobials going forward. This paper reflects on what is required to address the data gaps and to seek a better understanding of the factors that influence resistance emergence in the environment such as, movement of ARs and ARGs between different environmental compartments. Given the current state of knowledge, it is not considered appropriate or possible to recommend an

update of the current process of Marketing Authorisation Applications (MAAs), to evaluate AMR in the environment. In particular, it is noted that: (i) the relative contribution from the use of veterinary medicines to the overall burden of AMR in the environment is not known; and further (ii) uncertainty remains as to whether or not the presence of ARs and ARGs in the environment, resulting from veterinary medicinal use, is likely to result in a significant problem for the ecosystem and/or for animal/human health; and finally (iii) it is not currently possible to provide clear advice on what data/studies would be required to quantify and address the issue of AMR in the environment, from the use of veterinary medicines, for a new MAA with the potential of causing AMR, and how regulatory bodies could interpret such data. As a result, the paper highlights the need for appropriate tools and models.

Possible risk mitigation measures to reduce the incidence of AMR in the environment are identified. These measures tend to involve the implementation of best practices on disposal of manure and the implementation of education and training programmes for farmers and practitioners. Implementation of best practices on disposal of manure may help limit the emergence, spread or development of AMR at the farm level.

This reflection paper has also considered whether the risk assessment of VMPs, in EU member states, should be amended to include or address the risk from AMR in the environment arising from the use of VMPs containing antimicrobials. In response to this fundamental question, this paper concludes that the current ERA for VMPs cannot yet be amended to consider the risks posed by the accumulation of ARs and ARGs in the environment from the use of VMPs. In particular, at this point in time, it is not possible to provide clear advice on what data could or should be provided in the context of a MAA, and how regulatory bodies could interpret such data, to assess the AMR risk for the environment resulting from the use of VMPs. However, there is sufficient evidence to conclude that the environment is likely to play a role in the spread and/or persistence of AMR. Nevertheless, to evaluate the risks of AMR development appropriately, alternative tools (e.g. minimal selective concentration (MSC) assays) and models to understand the environment from the microbiological perspective are needed.

2. Aims of the reflection paper

This reflection paper aims to review the potential impact(s) on ecosystems, animal and human health from the possible presence of antimicrobial residues (ARs) and/or antimicrobial resistance genes (ARGs) in the environment arising from the use of VMPs. This paper will differentiate the key exposure pathways and subsequently, consider the likely extent of accumulation and mobility in the environment of ARs and ARGs excreted from animals treated with VMPs. In addition, the potential effects on the functioning of bacterial communities and the overall impacts on ecosystems, as a consequence of either AMR or by changing the microbial diversity without selecting for acquired antibiotic resistance, are considered. Moreover, an evaluation and understanding of the degree to which the environment is altered by VMP use, how it may contribute to the cycling of resistance genes between different ecosystem compartments (e.g. soil, water, animals and/or humans), and the effect or consequences of this on animal and human health is performed. Furthermore, as VMPs are not the only source of antimicrobials that enter the environment, the consideration of any potential impacts from VMP use needs to be done within the context of the global use of antimicrobials giving consideration to the 'One Health' approach.

This reflection paper also considers whether the current ERA for VMPs should or could be further developed to appropriately assess the potential risks posed by veterinary medicines, with antimicrobial properties, to drive environmental AMR.

3. Background

The European Commission has recognised, in its 2017 Action Plan against AMR (European Commission, 2017), that the problem of AMR cannot be successfully tackled by isolated, sectoral efforts. A holistic approach is needed which takes into consideration the different sectors committed to addressing AMR, in-line with the globally recognised "One Health" approach (the concept of One Health is used in the political declaration on AMR adopted during the high-level meeting of the UN General Assembly in 2016 (United Nations, 2016)); defined as "the integrative effort of multiple disciplines working locally, nationally and globally to attain optimal health for people, animals and the environment". More specifically, the term is used to describe a principle which acknowledges that human and animal health are interconnected, that diseases are transmitted from humans to animals and *vice versa* and must therefore be tackled in both. The four key elements of the "One Health" approach are considered to be (Calistri et al., 2013):

- geographical,
- ecological,
- human activities,
- livestock and other farming activities.

Therefore, the One Health approach also encompasses the environment, which is considered a significant link between humans and animals and, in the context of this reflection, a potential source of resistant microorganisms. As a result, the implications of using VMPs, within the four elements mentioned above needs consideration.

The CVMP strategy on antimicrobials 2016-2020 (EMA/CVMP, 2016) considers the interaction between humans, animals and the environment as sources of antimicrobial resistance genes in a One Health context, and states that: "The importance of the environment as a reservoir for antimicrobial resistance genes is now widely recognised. Use of antimicrobials in humans, animals (including in aquaculture) and plants leads to contamination of the environment both with antimicrobials and resistant bacteria. The presence of antimicrobials in the environment exerts a selective pressure for resistance genes in bacteria in a variety of ecosystems including animals, humans and plants. The cycling of these resistance genes between the different ecosystems is extremely complex and requires further research. The CVMP acknowledges that further consideration should be given to the contribution of veterinary antimicrobial use to the environmental resistome[1]."

Although the majority of AMR action plans and monitoring programmes currently focus on human and livestock activities, there has been growing concern that the natural environment may play a substantial role in the evolution, persistence and spread of AMR; and thus, may impact our ability to control and treat AMR-associated infections in both animals and humans. A review of the scientific literature on this issue has shown that the origin of many ARGs of clinical relevance can be traced back to bacteria that occur in the wider environment (Wright, 2007); hence indicating the environment to be an important reservoir of AMR. Yet, there is little information on the potential impacts that ARs and ARGs, resulting from VMP use, can have on the functioning of the ecosystem and its key species. Furthermore, it is still unknown whether putative changes induced in communities of bacteria, naturally present in the environment, may affect the emergence and spread of AMR in bacteria of clinical relevance for humans or animals (some of which have the ability to survive and grow in the environment) (ECDC/EFSA/EMA/SCENIHR, 2009; FAO, 2008).

[1] Considered as the pool of antimicrobial resistance genes within the natural environment

Knowledge gaps exist concerning the interplay between antimicrobial use in food producing species, resistance in the environment, potential adverse impacts on human and animal health, and other environmental side effects. Currently, it is not possible to analyse trends in AMR from environmental sources over time due to the absence of standardised or routine monitoring systems, safe thresholds for antimicrobials in the environment (in terms of impact on AMR), and standardised requirements and methods for susceptibility testing of bacteria from soil samples. An independent review on AMR (O'Neill, 2016) recommended that a coordinated effort should be taken to establish a global surveillance system to monitor the emergence and spread of drug-resistant infections. This review highlights the need to reduce unnecessary antimicrobial use in animals to mitigate any effects that could occur on animal health, ecosystems and public health from animal waste. Although out of the direct scope of this paper, it is noted that this review also recommends pharmaceutical companies to establish a systematic monitoring of waste products from their antibiotic manufacturing processes, and to support the installation of effective waste processing facilities to reduce or eliminate Active Pharmaceutical Ingredients (APIs) from being discharged into the environment.

In response to the rising threat from AMR, it is necessary for the CVMP, as part of the 'One Health' approach, to reflect on the current state of knowledge. There is a need to consider any interventions that could reduce the environmental drivers that enable the development of AMR, following use of antimicrobials in animal health, while maintaining the efficacy of the products. Such possible intervention measures under the remit of the CVMP include:

- promoting prudent use of antimicrobials, leading to a reduction of consumption of antimicrobials,
- any improvements in the risk assessment for VMPs containing antimicrobial agents, and
- the identification of practical and effective risk mitigation measures for the registration of new VMPs and maintenance of the longevity of existing VMPs.

4. Mechanisms of development of antimicrobial resistance

Antimicrobial resistance (AR) is the ability of a microorganism to survive and multiply in the presence of a compound with antimicrobial properties that would normally inhibit or kill this microorganism. AR is one of the adaptive traits that bacterial subpopulations may possess or acquire, enabling them to survive and overcome host strategies aimed against them. AR is a natural phenomenon that pre-dates the modern selective pressure of clinical antimicrobial use (D'Costa et al., 2011) because natural antimicrobials (antibiotics) are ubiquitously present in microbial and fungal communities. Several different mechanisms are involved in the development of resistance to antimicrobials (for more detail on the specific mechanisms see Annex I). The pool of ARGs within the environment, the so-called environmental resistome, is now widely recognised as a complex and diversified reservoir of resistance genes that can be transferred into and between environmental and clinically relevant bacteria (Cantas et al., 2013; Wellington et al., 2013). The recruitment of MGEs such as plasmids, transposons, integrons, insertion sequences, and integrative conjugative elements, including the genes they carry, will also occur. These MGEs enable the movement of DNA within and between genomes of prokaryotic species and the total collection of MGEs in a genome is known as the mobilome (Gillings, 2013).

AMR development occurs primarily because of selection pressures on microbial populations notably after the use of antimicrobial agents (Marshall and Levy, 2011). Concerning AMR, a distinction should be made between intrinsic resistance and acquired resistance (Holzbauer and Chiller, 2006). Intrinsic resistance occurs as a result of a structural or functional trait which allows tolerance to a particular substance or antimicrobial class by all members of a bacterial taxon. Acquired resistance results from a genetic change in the genome of formerly susceptible bacteria, which can be the consequence of a mutation (endogenous resistance) or following horizontal gene transfer (HGT) of foreign genetic

information (exogenous resistance)(Alekshun and Levy, 2007; Davies and Davies, 2010). The selection of bacteria with intrinsic or acquired resistance could result in a threat to human and/or animal health. For example, intrinsic resistant bacteria for many classes of antimicrobials, such as *Clostridium difficile*, can be selected during antimicrobial therapy and thereby cause harm including casualties in both human and veterinary medicine (Moono et al., 2016).

The environment receives inputs of ARs and ARGs as result of different anthropogenic activities, such as pharmaceutical manufacturing or the use of antimicrobials in human and veterinary medicines (Bengtsson-Palme et al., 2018). It is suggested that these activities increase environmental selection pressures and therefore the environmental resistome, notably by increasing the recruitment of MGEs and the genes they carry (Jechalke et al., 2014).

For a risk assessment on AMR, especially in the context of the environment where different bacterial populations may be exposed to different substances simultaneously, cross-resistance (bypass of same antimicrobial targets via the same resistance determinant) and co-resistance (bypass of different antimicrobial targets via linked resistance determinants) to antimicrobials and other substances should also be regarded carefully. Due to cross- and co-resistance, bacteria resistant to a certain antimicrobial substance can be selected by exposure to another antimicrobial or even another substance with antimicrobial properties. For example, biocides and heavy metals are known to have the potential to select for resistance to antimicrobial agents because the genes encoding resistance to various molecules often coexist on the same genetic elements (Cavaco et al., 2010; Singer et al., 2016; Soumet et al., 2012; Wales and Davies, 2015). This adds further complication to the already complex issue of resistance in the environment.

5. Consumption of veterinary antibiotics[2]

The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project, set up by the European Medicines Agency (EMA) following a request from the European Commission, publishes an annual report on sales of veterinary antibiotic active ingredients in EU/EEA countries (30 countries reported data for 2015, 25 of which provided data for the full 5-year period that the report covered (EMA/ESVAC, 2017)). The most recent report, published in October 2017, showed that sales of antibiotics for use in animals in Europe fell by 13.4% between 2011 and 2015 (EMA/ESVAC, 2017). It is noted that these sales data do not cover other antimicrobials such as antifungals and parasiticides. In addition, these sales data do not take into account wastage, imports or exports of veterinary antibiotics, but are considered the best currently available approximation of the quantity of antibiotics used in animals. Many EU/EEA countries have developed, or are developing, more robust systems which can collect and collate data on antibiotic use by animal species. Additionally, the European Centre for Disease Prevention and Control (ECDC) records human use of antibiotics based on population-normalised daily doses per year (ESAC-Net, website, last accessed 2018). However, it is noted that several countries have only recently set up monitoring systems to record these data, and these data are aggregated at a high level.

Significant issues are raised when considering the merit of using these sales/consumption data, in isolation, to give an accurate picture of the exposure of the environment and the prediction of likely AMR hotspots (which would be correlated with veterinary or human health concerns). It is overly simplistic to suggest that the likely excretion of ARs, antibiotic resistant bacteria (ARBs) and ARGs from treated animals into the environment is expressly related to the levels of antibiotics sold. For

[2] Note the distinction in terminology. The term 'antibiotics' is synonymous with anti-bacterials whereas, the term 'antimicrobials' is a general term for any compound with a direct action on micro-organisms used for treatment or prevention of infections. Antimicrobials are inclusive of anti-bacterials, anti-virals, anti-fungals and antiprotozoals. Therefore, in this case, reference is made to sales of VMPs that contain an active substance which is considered as an anti-bacterial.

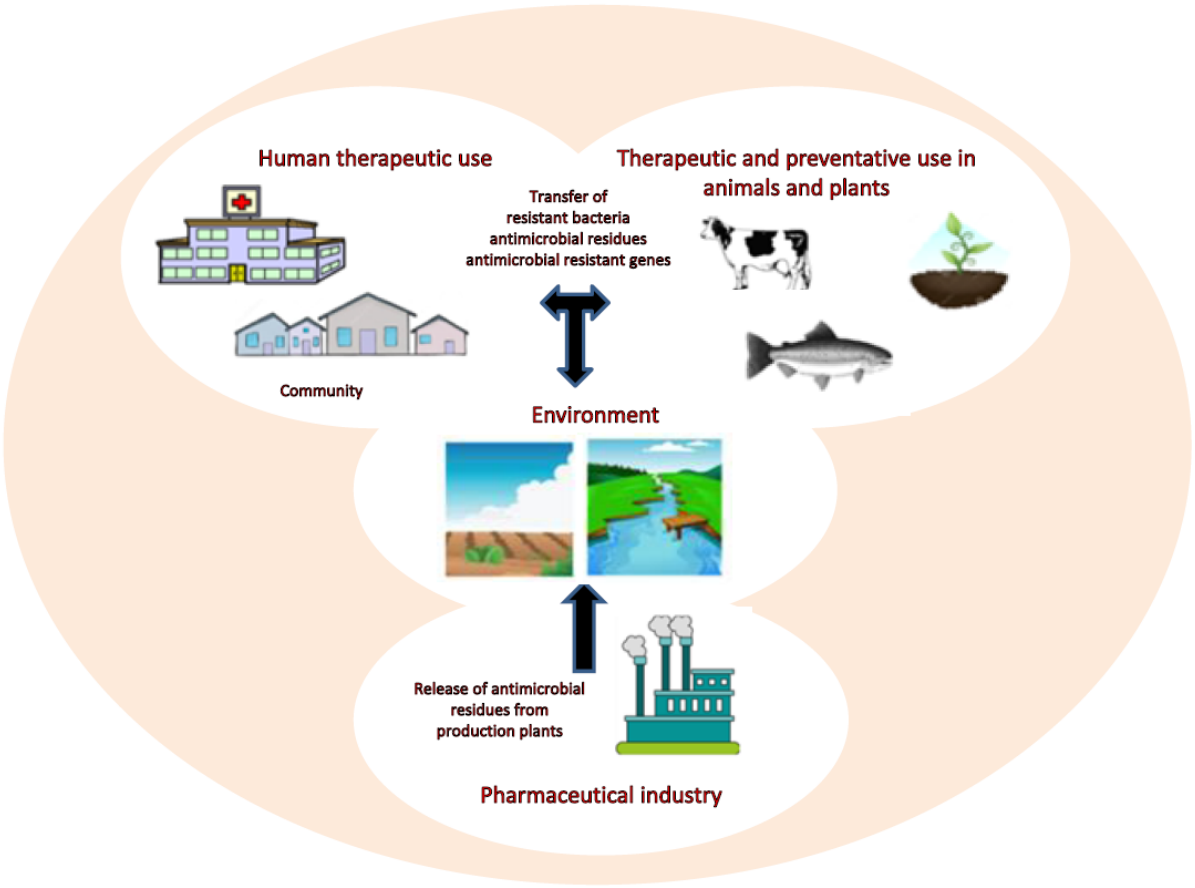
instance, several antibiotics (β-lactams, streptomycins, aminoglycosides, etc.) are produced by environmental bacteria, contributing to the natural background level of antibiotics in the environment. Further, besides consumption, production and manufacturing can also be important sources of antibiotics to the environment. Also, since antibiotic substances and AMR genes have different rates of depletion/degradation in the body of the treated animal and the environment, the AMR hot spots may not be those compartments where antibiotic substance consumption is the highest. Therefore, it is important to consider the physicochemical and environmental fate properties of antibiotics; especially in terms of their stability, sorption and persistence characteristics, partitioning to soil or water compartments, etc. Therefore, sales data alone should not be used to predict the extent of the occurrence and spread of AMR in the environment. The recommendations section, together with the emissions and fate sections, of this paper consider additional data that may be useful in determining the extent of exposure and persistence of ARs and ARGs in the environment.

6. Emissions and fate of VMPs as sources of antimicrobial substances to and within the environment

6.1. Emissions

Figure 1 provides a simple representation of how antibiotics are cycled between different environmental compartments, such as from medical sources (e.g. hospitals), agricultural settings, aquaculture, the pharmaceutical industry and the wider environment.

Figure 1. A simple schematic of the pathways for dispersion of AMR



In terms of the emission pathways of antimicrobials from animals treated with VMPs, a large fraction of the antimicrobials can be released into the environment in an active form, via excretion in urine and faeces (and other materials e.g. discarded milk, blood, etc.). As the activity of antimicrobial substances does not necessarily end when the bacterial infection has been treated in the animal, a widespread selective pressure on bacteria in the environment may be imposed. This in turn, may lead to the selection of resistant strains, which are also capable of moving between different environments, thereby creating the potential for the movement of ARGs and associated MGEs (further covered in Chapter 7 on the emissions and fate of ARB and their AMR genes to and within the environment).

Excretion rates of ARs depend on a number of factors including the antimicrobial itself, its mode of application, the animal (e.g., species and age) and the time elapsed since administration. Data on absorption, distribution, metabolism and excretion (ADME) are available in regulatory submissions relating to both Maximum Residue Limit (MRL) and MAAs. Such information, together with the exposure assessment as carried out for the ERA can provide useful information on the potential extent of a microbiologically active substance(s) passing into the environment. Exact figures of the rates of

excretion of an antimicrobial are not always available, but for some highly consumed antibiotic classes such as β-lactams, tetracyclines, phenicols and trimethoprim, excretion generally exceeds 50% of the administered dose (Alavi et al., 2015; Elizalde-Velázquez et al., 2016; EMA, 2015), depending on the route of administration. For sulfonamides, excretion is more variable, and for macrolides, the excreted fraction is generally lower. Amoxicillin is relatively inert in the body and will be excreted mainly as parental form (degradation rate of 10 - 20%), whilst sulfamethoxazole is extensively degraded (up to 85%) (Hirsch et al., 1999). Additionally, metabolites formed in the treated animal and subsequently excreted may retain their antibiotic activity. Therefore, a range of rates of excretion and degradation, and possible transformation events, are seen which are dependent on the individual active substance. It is noted that for VMPs containing antimicrobials where an extended (Phase II) environmental risk assessment is required, information on excretion, degradation, and transformation may already be available in the environmental safety section of these dossiers. Although relatively simplistic, assimilation of such data could be used to highlight those substances that can potentially enter the environment and their persistence in the environment.

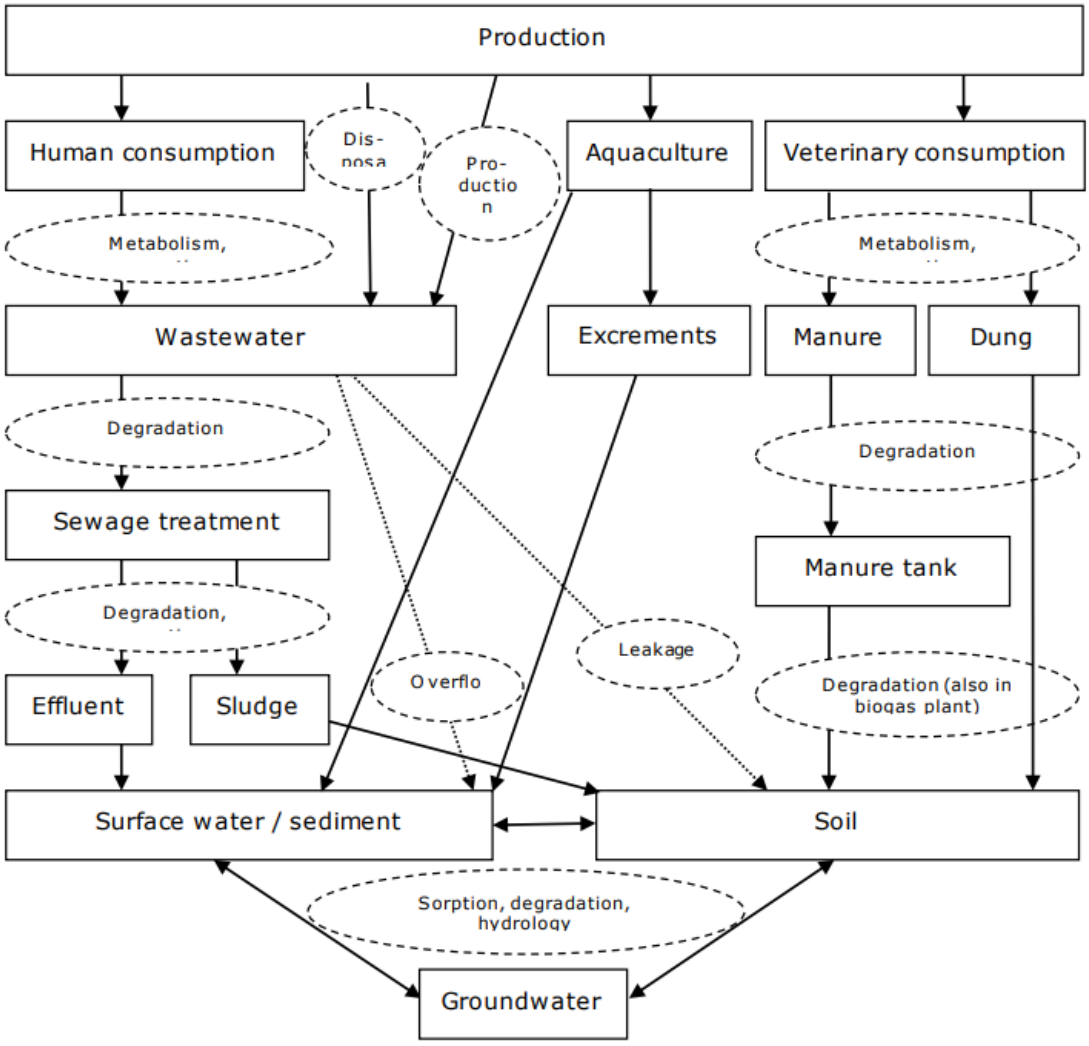
In addition to their indirect discharge, antimicrobials are also routinely used in aquaculture where they are generally used as infeed preparations. Ultimately, antimicrobials can reach various external environmental compartments such as rivers, lakes and soils (Kümmerer, 2009; Martinez, 2009; Sukul and Spiteller, 2007) where they can continue to exert their effects.

Based on the pattern of use of VMPs and the handling of the waste from treated animals, the main pathways by which antimicrobials used in veterinary medicine and their metabolites reach soils and water systems are considered to be the:

- direct excretion onto the land by pasture-reared animals,
- application of animal manure(s) or slurry to areas of agricultural use as fertilisers, and
- discharge of effluents from animal production units (husbandry and slaughter houses) to surface waters and soils, including aquaculture.

These pathways are highlighted in Figure 2.

Figure 2. Emissions and fate of antimicrobials in the environment following use of VMPs (Schmitt et al., 2017)



Antimicrobials partition into different environmental compartments according to their physicochemical properties and may be further transformed by abiotic or biological processes. Additional information on fate and behaviour in terrestrial and aquatic compartments is covered below, in Section 6.2 of this reflection paper.

6.2. Fate and behaviour of veterinary antimicrobials in the environment

As mentioned in Section 5, accurate consumption data for animals treated with antimicrobials does not give a representative picture of the environmental exposure to antimicrobials. Furthermore, although antibiotics from almost all substance classes have been detected in liquid manure at relevant concentrations (from µg to mg per kg, as discussed in section 6.2.1), there is presently no systematic monitoring of antibiotic compounds in environmental matrices such as water, soil, sediment or sewage sludge, and manure or residues from anaerobic digesters. Therefore, to assess the role of veterinary use of antimicrobials in the complex biological phenomena of the environmental resistome and mobilome, it is particularly important to understand and accurately model the fate and behaviour of veterinary antimicrobials in the environment, as well as the waste matrix which includes manure and slurry. Therefore, further research on this aspect is required.

The initial distribution and fate of antimicrobials in the environment is largely dependent on the pattern of use, the metabolism and transformation occurring within the animal and the excretion potential of such compounds from the animal. However, once released from the animal into the environment, the fate of the antibiotics will depend on their physicochemical properties (e.g., molecular structure, size, shape, solubility and hydrophobicity) and a variety of environmental effects (e.g., climatic conditions, soil types and hydrological effects). In addition, sorption properties of these antimicrobials together with transformation potential by abiotic or biological processes will also determine how they partition into different environmental compartments. In particular, antimicrobials released into the environment are likely to be naturally degraded by biodegradation processes (by bacteria and fungi) and non-biotic elimination processes such as hydrolysis, oxidation and reduction. Degradation processes are influenced (amongst others) by the temperature, moisture, pH and ionic strength of the environment and the composition on the local microbiota. It appears reasonable to suggest that factors that prolong the persistence of a compound in the environment could, by consequences, also enhance the potential of a substance to select for resistance in the environmental microbiome.

6.2.1. Terrestrial environment

For the terrestrial compartment, one of the main sources of antimicrobials comes from the spreading of manure from animals treated with antimicrobials (Hamscher et al., 2005). The spreading of unprocessed manure is recognised in the European legislation (Chapter III of Annex VIII of EC regulation 1774/2002 (Official Journal of the European Union, 2002)) as a way of fertilising the soil and disposing of manure, and is practiced if the manure does not represent a risk of spreading serious transmissible diseases (such as Newcastle disease or avian influenza, in the case of unprocessed poultry manure). The risk from AMR is not explicitly covered under this legislation.

Approximately 96 million tonnes of farm manures (both solid manures and slurries) are applied to agricultural land in the United Kingdom alone (Defra, 2010). Taking into consideration application rates, it is estimated that, as a result of application of manure to land, antimicrobials are being released into the environment in the region of kilograms per hectare and per year (Kemper, 2008). This represents an immense potential for environmental contamination from antibiotics used in livestock.

Knowledge of the concentrations of ARs in manure is important as it can give an indication as to the maintenance of bacterial resistance in the environment, as all bacteria replicating in the manure are still subject to selection processes there. A European study (Hölzel et al., 2010) investigating the association between ARs (of 24 antibiotics used in animal and/or human medicine) and bacterial AMR of *Escherichia coli*, *Enterococcus faecalis* and *Enterococcus faecium* in liquid pig manure used as fertiliser reported a range of antibiotic concentrations in manure from residual levels to commonly 1-10 mg/kg or mg/l, but also concentrations of more than 50 mg/kg (see Table 1).

386 **Table 1.** Detected antibiotics in pig manure (n = 305, adapted from Hölzel et al. (2010))

Antibiotic	Positive findings*		Concentration (mg/kg)	
	(n)	(%)	Median**	Range (min-max)
Chlortetracycline	113	37	0.33	0.1 - 50.8
Tetracycline	93	31	0.71	0.1 - 46.0
Oxytetracycline	18	6	0.14	0.1 - 0.9
Doxycycline	4	1	0.29	0.1 - 0.7
Σ TETs	166	54	0.73	0.1 - 52.6
Σ SULs	154	51	0.15	0.05 - 38.4

387 SULs, sulfonamides; TETs, tetracyclines.
388 *TETs >0·1 mg/kg; SUL >0·05 mg/kg.
389 **Positive findings.
390

391 The levels of antibiotics found in manure might seem generally low but European pigs and cows are
392 reported to jointly produce 1.27 billion tonnes of manure per year. Data on antibiotic consumption for
393 those two species is not available, but consolidated data from 30 EU/EEA countries shows that more
394 than 8 300 tonnes of active ingredients were sold for use in animals in 2015 (EMA/ESVAC, 2017).

395 Further data from the global perspective (Massé et al., 2014) also depict a wide range of reported
396 values of antibiotic concentrations in manure. A summary of the findings is presented in Table 2.

397 **Table 2.** Example of concentration of antibiotics in manure from global sources (adapted from Massé
398 et al. (2014)

Antibiotic	Matrix	Concentration	Reference
Chlortetracycline	Beef manure stockpile	6.6 mg/kg	Dolliver and Gupta (2008)
Chlortetracycline	Swine manure	764.4 mg/L	Pan et al. (2011)
Chlortetracycline	Swine manure	139 mg/L	Chen et al. (2012)
Chlortetracycline	Swine manure storage lagoon	1 mg/L	Campagnolo et al. (2002)
Doxycycline	Swine manure	37 mg/L	Chen et al. (2012)
Monensin	Beef manure stockpile	120 mg/kg	Dolliver and Gupta 2008)
Oxytetracycline	Manure	136 mg/L	Martínez-Carballo et al. (2007)
Oxytetracycline	Cow manure	0.5–200 mg/L	Ince et al. (2013)
Oxytetracycline	Fresh calf manure	10 mg/kg	De Liguoro et al. (2003)
Oxytetracycline	Swine manure	354 mg/L	Chen et al. (2012)
Sulfadiazine	Swine manure	7.1 mg/L	Chen et al. (2012)
Sulphonamides	Swine manure	2 mg/kg DM	Jacobsen and Halling-Sørensen (2006)
Tetracycline	Swine manure	98 mg/L	Chen et al. (2012)
Tylosin	Fresh calf manure	0.11 mg/kg	Jacobsen and Halling-Sørensen (2006)
Tylosin	Beef manure stockpile	8.1 mg/kg	Dolliver and Gupta 2008

The variation in antibiotic concentrations in manure can be attributed to differences in the total usage of the compounds but also in antibiotic metabolism, degradation processes or in the methods used for sampling and quantification of antibiotic concentrations.

Massé et al. (2014) highlighted that, of studies cited in their publication to determine concentrations of antibiotics in manure, the majority did not have sufficient description of the condition of the manure handling and management before sampling. Furthermore, there is a need for more reliable and standardised methods of quantification in complex matrices, such as soil and biological sludge. Without improvements in these areas, dependency on the results from such studies or the ability to make inter- and even intra-study comparisons is problematic.

Once in the environment, some antibiotics bind strongly to soil and sediments, which contributes to their persistence as they become inaccessible to degradation (these trapped compounds can persist in soil for many years). There is a significant amount of debate as to whether or not these non-extractable residues are bioavailable. Some studies suggest that sorption and fixation can reduce, but do not necessarily eliminate their antimicrobial activity (Sengeløv et al., 2003). For example, tetracycline and tylosin remain active even when tightly adsorbed by clay particles (Chander et al., 2005). These sorbed compounds might represent a reservoir of pollutants that can be mobilised to further contaminate other compartments (Pedersen et al., 2003). Persistence and accumulation of tetracycline in the environment has been reported (Hamscher et al., 2002). It is clear that additional research is needed to better understand the kinetics of biodegradation and the potencies of degraded products of various antibiotics in manures and the receiving soils.

Following application of manure to land, some of the antibiotic may become mobile as a result of the flow of water through the soil and subsequent leaching from it. This could result in a flow of the antibiotic (or mobile resistant genes) from soil into surrounding surface water and groundwater. The extent to which this will occur is dependent on the properties of the antibiotic, soil, and the hydrological effects. Further research is needed to gain a better understanding of the mobility and transport of antibiotics in the environment. From the available studies, it is possible to conclude that there are considerable differences in the environmental behaviour of the various antibiotics. Oxytetracycline, for example, is not transported into deeper soil segments as it is strongly adsorbed to soil, whilst olaquinox is only weakly adsorbed (Rabølle and Spliid, 2000). Distribution coefficients vary in different environments. In the case of oxytetracyclines and tylosin, the distribution coefficient is lower in manure than in soils (Loke et al., 2002).

It is acknowledged that the above considers the spreading of manure whereas another similar exposure route is that from recently treated pasture reared animals defecating directly onto land. It is assumed that this route will likely result in less extensive exposure, in comparison with exposure from the spreading of manure, but that this route of exposure nevertheless, could still produce localised impacts resulting in an increased selection pressure for AMR development.

6.2.2. Aquatic environment

As mentioned in section 6.2.1, following application of manure to land, some of the antimicrobials may leach/transport into aquatic compartments, including surrounding surface water and groundwater. Further, pasture animals that have been recently treated with an antimicrobial could in theory directly excrete, via faeces or urine, higher concentrations of antimicrobials than those expected via the spreading of manure. Little information is available on the relative contribution from the direct excretion exposure route.

In general, antibiotic concentrations reported in aquatic environments are less than 10 µg/L (sewage treatment plant effluents, receiving surface waters, groundwater) (Kümmerer, 2009). The relative

contribution of the use of antibiotics in veterinary medicine to the levels observed in environmental compartments is largely unknown, particularly considering that many of these antibiotics are also used in human medicine.

It should be noted that the treatment of water, sewage and other contaminated residues can reduce concentrations of certain classes of antibiotics but, invariably, a fraction of antibiotics remains after treatment (Watkinson et al., 2007). Water chlorination helps to degrade antibiotics such as β-lactams and trimethoprim (Dodd and Huang, 2007; Li et al., 2008). Traditional methods for wastewater treatment can eliminate up to 80% of fluoroquinolones and tetracyclines but they are less efficient in the removal of macrolides (Gulkowska et al., 2008; Shellie et al., 2002; Sukul and Spiteller, 2007).

An increase in the prevalence of ARB, including enterococci, *E. coli* and *Acinetobacter spp*., after wastewater treatment has been observed by several authors, despite a reduction of bacterial load in treated wastewater compared to the raw wastewater (Ferreira da Silva et al., 2007; Łuczkiewicz et al., 2010; Zhang et al., 2009b). Concerning ARGs, the situation is more complex given that they are not "degradable pollutants" but auto-replicative elements.

Antibiotics can also enter the aquatic environment directly from pharmaceutical production facilities. Emissions from industrial sites can be considerable, especially in developing countries (Larsson, 2014). Antibiotics are also used in culture medium for the production of biological pharmaceuticals. This exposure route is, however, not within the scope of this reflection paper.

6.2.2.1. Aquaculture

Figure 2 shows that antimicrobials have the propensity to reach the aquatic compartments directly from the faeces of fish treated with VMPs containing antimicrobials as infeed preparations. An additional waste stream of antimicrobials could result from that administered treated feed which remains uneaten.

According to an aquaculture sustainability briefing (European Commission, 2015), most VMPs used to manage finfish disease have been judged to have minimal negative environmental impacts if used correctly (IUCN, 2007), and VMP use is closely regulated and inspected in all EU Member States. Problems, such as risks to non-target species do occur where VMPs are used inappropriately. The use of antimicrobials is of particular concern in open marine aquaculture where they enter the surrounding marine environment via fish faeces and can persist for extended periods in sediments. In Europe, antibiotics are typically administered via medicated feed, but only a percentage is absorbed by the fish. Rigos et al. (2004) estimated that 60–73% of oxytetracycline administered to sea bass on Greek farms is released to the environment. Little is known regarding the significant impacts of antimicrobials on the marine environment. However, available studies indicate potential ecological risks. High concentrations of oxytetracycline and florfenicol, both active against *furunculosis* in salmon, have been shown to inhibit growth of algal species. This also highlights the need to understand the effects of 'real-world' chronic, low-level exposure to antimicrobials in wild species (Pittenger, 2007).

In the past, antimicrobials were used much more liberally in aquaculture. In response to growing awareness and stricter regulations on their use, they are now generally used as a last resort. Improvements in farming practices have led to improved animal health and have reduced the need for the use of antimicrobials (European Commission, 2015). Moreover, the development and use of vaccines is also a key factor in reducing antibiotic use. Nevertheless, aquaculture has the potential to contribute to the widespread pool of resistant bacteria in the environment. Taylor et al. (2011), suggest research is needed to understand its impacts in comparison to far more dominant sources of resistant bacteria, particularly from other animal sources such as manures.

6.2.3. Further considerations and recommendations on environmental fate

The fate of antimicrobials in the environment is depicted in Figure 2 and indicates those fate mechanisms at play and the dynamic relationship between relevant compartments (wastewater, manure, soil, surface water and groundwater). It is acknowledged that the existing MAA process for a VMP requires provision of certain data (including information on metabolism and excretion of a compound in the treated animal as well as on physicochemical properties, persistence and adsorption data in sediments and soils) that could, in some instances, be used to determine the significance of some of the key exposure and fate mechanisms in a simplistic manner. However, in order to produce a more robust evaluation of AMR in the environment, a better understanding of excretion, transformation and sorption of antimicrobial compounds would be required to quantify the environmental fate of antimicrobials.

An illustration of the complex fate of antimicrobials in the environment can be seen with the example of ciprofloxacin, a commonly prescribed fluoroquinolone in human medicine and a transformation product from enrofloxacin, which is used in veterinary medicine. Waste-water treatment removes up to 90% of ciprofloxacin by sorption to sewage sludge, but biological degradation is poor. As a result, ciprofloxacin accumulates in human sewage sludge and, if the sludge is used as fertiliser and subsequently applied to land, it can be found in the soil in concentrations in the low mg per kg range. In the soil, ciprofloxacin persists for more than 90 days with only minimal transformation. Although the strong absorption to soil might reduce its bioavailability, it still elicits effects on soil microorganisms for extended periods of time: the resistance gene qnrS was detected in soil treated with ciprofloxacin from day 14 onwards (Girardi et al., 2011).

In conclusion, a better understanding of excretion, transformation and sorption of antimicrobial compounds is required to accurately predict the fate of these substances and subsequently; to elucidate the role of the environment in the potential transfer of relevant AMR bacteria with associated risks to human and animal health.

7. Emissions and fate of ARB and ARGs to and within the environment

7.1. Excretion of ARB and ARGs from treated animals

Antimicrobial treatment is usually indicated against specific pathogenic bacteria responsible for the infection (EMA/CVMP, 2018). As the use of antimicrobials creates a selection pressure, any use of antimicrobials to treat diseased animals may have the potential to select or disseminate AMR within the pathogenic bacteria against which the antimicrobial is used (Aarestrup, 2005; Holzbauer and Chiller, 2006; Toutain et al., 2016). The potential to select for resistance is mainly correlated to the antimicrobial substance, the administered dose and the corresponding concentration that is reached in the target tissue (Toutain et al., 2016). In addition, antimicrobials (or corresponding microbiological active metabolites) administered to treat a specific pathogen exert a collateral selection pressure on the commensal microbiota (Baron et al., 2016; Beyer et al., 2015; Toutain et al., 2016). The importance of this “non-desired exposure” is dependent on the pharmacokinetic profile of the drugs and may also be driven by the route of administration of the VMPs (Bibbal et al., 2007; Bibbal et al., 2009; De Smet et al., 2017; Holman and Chénier, 2015). Exposure of the gastro-intestinal tract (GIT) microbial population could lead to major modifications in the microbial equilibrium and to some extent contribute, as demonstrated in the scientific literature, to increase the reservoir of resistance genes in the colon (Baron et al., 2016; Beyer et al., 2015; D’Costa et al., 2011a; Martínez et al., 2015; Toutain et al., 2016).

The high microbial load and the diversity of the bacterial population in the GIT, especially in the distal portion where the greatest population of established resident bacteria occurs, serves as a hot spot for AMR development (Toutain et al., 2016). Even if commensal bacteria are not considered as clinically relevant, they will harbour a range of resistance genes which may subsequently be directly excreted into the environment via the faeces of the treated animal (Bibbal et al., 2007; Fleury et al., 2015; Thames et al., 2012). Apart from the GIT, other reservoirs are also possible, including the skin and the upper respiratory tract (e.g. methicillin resistant *Staphylococcus* species (EMA/CVMP, 2015).

Product characteristics such as the dosage regimen and the route of administration will influence the pharmacokinetic profile, in particular the extent of exposure of the GIT to antimicrobials and their metabolites (Bibbal et al., 2007; Bibbal et al., 2009; Lees and Toutain, 2012; Zhang et al., 2013). For example, tetracyclines (tetracycline, chlortetracycline, oxytetracycline, and doxycycline) are the antimicrobial classes most commonly administered by the oral route in food-producing animals (EMA/ESVAC, 2017). They have a very low oral bioavailability in pigs, with values typically ranging between 5 to 15% of the total dose (Nielsen and Gyrd-Hansen, 1996; Toutain and Bousquet-Melou, 2004). The unabsorbed fraction (85 to 95%) remains in the GIT exposing the dense microbial environment for a duration that could exceed the treatment period, and subsequently unabsorbed fractions are released in the faeces of treated animals to the environment. Furthermore, tetracyclines can also trigger an enterohepatic cycle; meaning that microbial communities in the GIT might undergo consecutive selection pressures during one treatment (Toutain et al., 2016). Therefore, in terms of risk assessment, the following characteristics increase the risk to drive the selection and/or excretion of resistance determinants into the environment:

- antimicrobials administered orally that are poorly systemically bioavailable,
- antimicrobials administered parentally that are excreted into the GIT,
- and those above that are, additionally, associated with a collective treatment (herd/group treatment).

Indeed, in addition to ARs, waste from treated food animals may contain many pathogenic and commensal bacteria, some of them harbouring ARGs. For example, it is recognised that the spread of manure leads to a temporary increase in the occurrence of AMR in the manure-amended soil (Bengtsson-Palme et al., 2018; FAO, 2016; Kumar et al., 2018; Topp et al., 2018). Thus, the use of waste from treated animals for manure spreading contributes to the global dissemination of AMR in the environment (Heuer et al., 2011; Jensen et al., 2002; Sengeløv et al., 2003).

7.2. Selection of ARGs in environmental bacteria exposed to antimicrobial residues and ARGs from VMPs

Sources of antimicrobials contaminating different environmental compartments include food producing animals excreting active compounds (as parent form or as metabolites) in faeces and/or urine. Those residues have the potential for exerting a selective pressure on the bacterial populations in animal waste, sludge or manure and thereafter in the environmental compartments (Heuer et al., 2011). Once in the environment, several antimicrobials remain stable for several weeks or even months (Halling-Sørensen et al., 2003).

For non-environmental bacteria, survival seems more critical than growth in the environment. For those bacteria, which use the environment for dispersal only, the advantage of harbouring resistance genes even in the presence of antimicrobials is likely to be small (Bengtsson-Palme et al., 2018; Heuer et al., 2011); however, there is evidence that HGT still continues in the absence of growth; noting that even enteric bacteria can grow in the environment under some circumstances so the relationship is not

clear cut. Nonetheless, for bacteria that can grow outside the animal and use the environment as an alternative or main habitat, antimicrobial exposure would be more likely to contribute to the selection of resistant determinants during environmental dissemination. Bacteria from the latter include opportunistic and emerging pathogens such as *Aeromonas* spp; *Acinetobacter* spp; *Pseudomonas putida, Burkholderia cepacia, Stenotrophomonas maltophilia* and *Bacillus cereus* (D'Costa et al., 2006; Denet et al., 2017; Forsberg et al., 2016; Forsberg et al., 2012; Goñi-Urriza et al., 2000; Holmes et al., 1998; Raphael and Riley, 2017).

The selection pressure exerted by the concentration(s) of antimicrobial(s) found in contact with environmental bacteria might have a different impact on the development and spread of AMR. As a function of the exposure, the bacterial response could differ and result in different levels of genotypic and phenotypic adaptations (Andersson and Hughes, 2011; Andersson and Hughes, 2014; Gullberg et al., 2014; Gullberg et al., 2011; Rodríguez-Rojas et al., 2013). If a bacterial population is challenged by high concentrations of antimicrobial, e.g. concentrations higher than the minimum inhibitory concentration (MIC), the pre-existing resistant or less susceptible strains will be selected for, eventually establishing a highly resistant bacterial population. However, if a bacterial population encounters antimicrobial concentrations below the MICs, different mechanisms could increase their genetic variability. This could include an increase in the mutation rates, in genetic recombination, and also in HGT and finally lead to a greater heterogeneity in the resistance profile within the exposed bacterial community (Aminov, 2009; Andersson and Hughes, 2014; Gullberg et al., 2014; Gullberg et al., 2011; Jolivet-Gougeon et al., 2011; Rodríguez-Rojas et al., 2013; Sandegren, 2014). Nevertheless, at concentrations even well below the MIC of susceptible strains, resistant strains can maintain a selective advantage. This represents the concept of minimal selective concentration (MSC), as described by Gullberg et al. (2011). Therefore, clonal expansion (the relative increase in growth of ARB) is possible even at low concentrations. For example, Gullberg et al. (2011) demonstrated resistance at levels as low as ng/l for ciprofloxacin. Such *in vitro* studies indicate, in theory, that even at the lowest concentrations of antimicrobial (sub MIC concentrations) present in the environment, selection for some ARB/ARGs is still possible.

The consequences for the environment itself are currently difficult to estimate without specific knowledge on the direct impact on resident microbial communities and ecological process functions. However, all selection processes might occur and will increase, at least, the environmental resistome; subsequently increasing the potential risk of transfer of resistant bacteria or resistance genes to clinically relevant bacteria that could have an impact on public or animal health. However, it is acknowledged that the significance of this risk is yet to be elucidated. The fact that ARs and ARGs are introduced to soils together means that disentangling the selective effects of antibiotic residues from changes in ARB/ARG diversity and abundance associated with introduction of manure borne bacteria is challenging. However, one study by Cleary et al., (2016) on soil experimentally exposed to VMPs at levels close to the maximum recorded in animal faeces (i.e. 10 mg/kg of tylosin, sulfamethazine and chlortetracycline that commonly arise in commercial pig production) showed selection for AMR and changes in bacterial community structure including decreased relative abundance of key proteobacteria taxa including *Rhizobium* sp.

7.3. Exchange of ARGs between environmental bacteria and animal/human bacteria in the environment

The HGT of DNA plays a profound role in the evolution of prokaryotes. Acquisition of genes from other organisms provides an efficient way to acquire new traits and adapt to new or changing environments (Bobay and Ochman, 2017; Gillings, 2017). In the context of AMR, HGT contributes to the rapid dissemination of ARGs among commensal and/or pathogenic microbiota during a short period (Hiltunen

et al., 2017). This dissemination of ARGs from antimicrobial-producing organisms to clinically relevant species has occurred within the antibiotic era and is mediated by diverse MGEs (e.g. plasmids, transposons, genomic islands and integrons) (Perry and Wright, 2013).

Three main mechanisms of HGT exist and are well described in the scientific literature (conjugation, transduction and transformation). These are summarised in Annex I. Most of the demonstrations of transfer of DNA were realised *in vitro*; but the heterogeneity of bacterial communities *in vivo* might facilitate the spread of ARGs (Cooper et al., 2017). The ability, for example, of soil bacteria to transfer ARGs by conjugation has been demonstrated, especially in the rhizosphere (Schwaner and Kroer, 2001; Sengeløv et al., 2000; van Elsas et al., 1998). Furthermore, the presence of similar MGEs, harbouring ARGs, in pathogenic and environmental bacteria implies that exchange between those reservoirs has occurred and probably still takes place (Huijbers et al., 2015; Nesme and Simonet, 2015).

HGT might occur in all environments but has been most studied in soil where particular physical properties (temperature, pH, concentration of nutrients and oxygen, etc.) and a huge microbial diversity create favourable conditions for HGT (Aminov, 2011; Forsberg et al., 2015; Perry and Wright, 2013; van Elsas and Bailey, 2002). Currently, there is some evidence suggesting that aminoglycoside and vancomycin resistance enzymes, the extended-spectrum β-lactamase CTX-M as well as the quinolone resistance gene *qnr* originated in environmental bacteria (Perry and Wright, 2013; Poirel et al., 2005; Yan et al., 2017). Different examples of transfer of ARGs between environmental matrices and clinical isolates were described in the literature (Baquero et al., 2008; Benveniste and Davies, 1973; Forsberg et al., 2012; Humeniuk et al., 2002). For example, Poirel et al. (2005) screened a collection of 48 Gram-negative clinical and environmental bacterial species belonging to Enterobacteriaciae, Aeromonadaceae, Pseudomonadadeae, Xanthomonadaceae, Moraxellaceae and Shewanellaceae and identified that the the *qnr*A gene originated from the chromosome of *Shewanella algae*.

Therefore, a key consideration is the possibility that pathogenic or commensal bacteria that have acquired a resistance determinant from environmental bacteria can make it back to their human or animal host. Vice versa, transfer of ARGs to environmental bacteria is possible and has already been described. This enables human or animal associated bacteria to use strains of the environmental bacterial population as recipients for resistance genes that can later return to the human or animal associated resistome.

8. Risks to human and animal health from AMR and ARGs in the environment

8.1. Environmental transfer of ARGs and ARB and potential route of transfer to humans and animals from the use of VMPs

Environmental dissemination of ARBs is increasingly identified as a potential route for the spread of AMR (Marshall and Levy, 2011). Different environmental compartments might contribute to the dissemination of resistant pathogens and commensal bacteria associated with humans and animals alike. Opportunistic pathogens such as *Acinetobacter spp*. and *Pseudomonas spp*. are located within different environmental compartments. Thus, sewage, waste water treatment plants, agricultural and veterinary hospital effluents (Zhang et al., 2009a), drinking water (consumed either by humans or domesticated animals in close contact with humans), recreational water, air-borne aerosols, dust, wildlife fauna and contaminated food from agriculture or aquaculture are all vectors enabling the potential transmission of bacteria and ARGs between hosts through the environment (Singer et al., 2016).

The next section provides illustrative evidence from European studies to depict several of the many different routes that allow the transmission of ARGs and ARBs to humans and/or animals through the environment.

8.1.1. Food from crops

During cultivation in soil to which animal manure is applied, crops and irrigation water may be contaminated with resistant bacteria, for example Extended Spectrum Beta-Lactamases (ESBL) and AmpC Beta-Lactamases (AmpC) producing bacteria. A number of studies have investigated the prevalence of AMR bacteria in or on vegetables and fruits. Since fresh produce is often consumed raw, consumption may result in the ingestion of resistant bacteria that, depending on the bacterial species, are able to colonise the gut or pass through the intestine, thus posing a potential public health risk (FAO, 2016). For example, a study from the Netherlands revealed that third generation cephalosporin-resistant faecal Enterobacteriaceae were isolated from 2.7%, 1.3% and 1.1% of supermarket vegetables, iceberg lettuce from farms and agricultural soil, respectively. Comparison of fresh produce and its agricultural environment indicates that the Enterobacteriaceae population on fresh produce reflects that of the soil in which it is grown (Blaak et al., 2014).

8.1.2. Food from aquaculture

The occurrence and spread of ARB and ARGs in areas designated for fish farming (marine and freshwater) has exponentially increased during recent decades (Elbashir et al., 2018; Topp et al., 2018). The application of antimicrobials to the aquatic environment may select for ARGs not only in fish pathogens, but also in environmental bacteria (Muziasari et al., 2016). Most frequently resistance has been reported against oxytetracycline, tetracycline, ampicillin and florfenicol (Caruso, 2016), but some ARGs coding for resistance to quinolones and β-lactams can be found in fish pathogens, human pathogens and aquatic bacteria (Cabello et al., 2013). Furthermore, Cabello and colleagues suggested that the use of antimicrobials in aquaculture, notably the use of colistin in Asian aquaculture, could be correlated with the emergence of the plasmids encoded mobile colistin resistance (MCR) determinants (Cabello et al., 2017). That said, it is noted that colistin is not authorised as a VMP for use in aquaculture in the EU.

8.1.3. Contaminated drinking water

The prevalence and resistance patterns of various bacteria isolated from drinking water distribution systems have been recently reported. For example, in Romania, multiple AMR *E. coli* strains isolated from drinking water were found to harbour ARGs encoding resistance to aminoglycosides, beta lactams, tetracyclines and trimethoprim – sulfamethoxazole (Cernat et al., 2007). A strain of *E. coli* carrying the $bla_{CTX\text{-}M\text{-}1}$ IncI1/ST3 plasmid was isolated in France from drinking water. The plasmid was identical to those found in animals and humans (Madec et al., 2016). In another German study, the *van*A and *amp*C ARGs were detected in drinking water biofilms (Schwartz et al., 2003).

8.1.4. Contaminated recreational places

ARB have been detected in natural aquatic environments and direct ingestion of water from recreational locations (e.g. seawater, lakes) is a route by which the population could be directly exposed (Leonard et al., 2015). In England, Leonard and colleagues revealed that 0.12% of *E. coli* isolated from surface waters were resistant to third generation cephalosporins (3GCs) and could represent a human exposure risk for water users (Leonard et al., 2015). Leonard et al. also used a targeted metagenomic approach to estimate exposure to *E. coli* in UK coastal bathing waters carrying all known ARGs, concluding that all exposure events result in ingestion of at least one *E. coli* associated ARG (>100 million events per year) and 2.5 million events per year are likely to occur involving ingestion of 100 *E. coli* borne ARGs (Leonard et al., 2018a). The relationship between exposure, colonisation and infection with AMR opportunistic pathogens is uncertain and there are no dose-response data available for colonisation, infection or HGT of ARGs from ingested bacteria to those of the microbiome. A cross-sectional study of surfers and non-surfers in the UK found that surfers were 3 times as likely to be colonised by 3GC resistant *E. coli* and >4 times as likely to be colonised by $bla_{CTX\text{-}M}$ *E. coli* suggesting an association between coastal bathing water exposure and gut colonisation by ARB (Leonard et al., 2018b).

In the Netherlands, ESBL-producing *E. coli* were detected in four different recreational waters nearby waste water treatment plants (three recreational waters appointed under European Bathing Water Directive 2006/7/EC and one not appointed). *E. coli* were detected in all four recreational waters, with an average concentration of 1.3 colony forming units/100 ml, and in 62% of all samples (Blaak et al., 2014).

This section considered the sources and pathways of AMR transmission into humans and animals. The next section will elaborate on the potential consequences on health from these pathways being realised.

8.2. Consequences for the risk assessment of human and animal health

The contribution of VMPs to the environmental resistome and the subsequent impact on human and animal health is difficult to quantify at present. Antimicrobials, ARB and ARG may originate from various sources. In addition, the same classes of antimicrobials are used in both human and veterinary medicine and there is also the potential for co-selection. Consequently the presence of ARBs/ARGs in the environment cannot be clearly attributed to the use of antimicrobials in humans or to their use in animals and it is problematic when attempting to associate any consequences of the risk assessment to the use of VMPs only. However, the risk for human and animal health associated with the dissemination of resistance through the environment involves (i) the possibility of ARGs being transferred from non-pathogenic environmental bacteria to pathogenic or commensal bacteria (e.g. the environmental origin of *qnr* genes, as described above), (ii) the potential transfer of AMR pathogens directly from animal to human with the environment acting as a 'vehicle', and (iii) the possibility of

infection by resistant bacterial pathogens originating from environmental compartments (e.g. those represented by the ESKAPE family: *Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas, Escherichia*). Evidence of environmental transfer of AMR to humans or animals causing direct adverse health events is scarce.

A well described consequence for human health is the worldwide spread of New Delhi Metallo-beta-lactamase (NDM) carbapenemase. As carbapenems are not known to be authorised in veterinary medicine and off-label use is only possible in non-food producing animals, it is more likely that the development and spread of NDM carbapenemases can be traced back to the use of antimicrobials in humans rather than to that in animals. After contact with a contaminated environment, the resistance determinant can be maintained within the gut microbiota for several months, leading to dramatic health consequences when patients are hospitalised (Johnson and Woodford, 2013; Tängdén et al., 2010; Walsh et al., 2011).

AMR compromises our ability to deliver high quality medical care in the community and in the hospital environment. Effective antibiotic therapy is essential for many advanced medical procedures (e.g., heart transplants, hip replacements or any procedure associated with a high risk of bacterial infection), as well as for the treatment of people with suppressed immune systems.

Resistance to antibiotics among human and veterinary pathogens increases the risks of treatment failure, increases mortality by increasing the time from an initial diagnosis to an effective therapy, and can also lead to morbidity by increasing the use of more toxic antibiotics as replacements for those rendered ineffective due to pathogenic bacteria being resistant. This issue also imposes an additional healthcare cost and productivity loss that, in the EU, was estimated to be at least €1.5 billion in 2007 (ECDC/EMEA, 2009).

In 2014, a review commissioned by the United Kingdom government entitled “Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations” (O'Neill, 2014), suggested, that if left unchecked AMR will in the future, impose a financial burden on society in addition to leading to increased morbidity and mortality in human and animal health. The CVMP also recognises this and continues to build upon its efforts to gain a better understanding of the role of veterinary use of antimicrobials on the development of AMR, in this instance, relating to the reservoir in the environment. Importantly, the CVMP promotes responsible use of antimicrobials in animals, since eliminating unnecessary use in animals and humans is expected to have a beneficial impact on the occurrence of AMR (ECDC/EFSA/EMA, 2017).

9. Evaluation of the current risk assessment process for VMPs; consideration of AMR – knowledge gaps and research needs

9.1. Current risk assessment approach for VMPs

Directive 92/18/EC introduced the requirement for the assessment of environmental safety as part of the submission for a MA of a new VMP. The ERA is performed in two phases (as described in VICH guidelines GL6 (CVMP/VICH, 2000) and GL38 (CVMP/VICH, 2004) and in the supporting CVMP guideline (EMA/CVMP, 2008)) and aims to evaluate the potential harmful effects caused to the environment through the use of VMPs and to identify the risk from such effects. From the perspective of antimicrobials, soil bacteria and cyanobacteria are considered the most sensitive organisms that require assessment; however, certain antimicrobials have elicited adverse effects in other higher organisms tested (especially plants) during the evaluation process. It is noted that for human medicines, an additional study on inhibition of respiration activity in activated sludge also needs to be

determined (OECD, 2010). Therefore, in terms of the effects part of the assessment, few data are of significant relevance to AMR. With regard to the data requirements on the physicochemical and fate properties of compounds in soil (sometimes in manure) and aquatic compartments, greater relevance is seen. In particular, the findings from the existing studies on biodegradation and sorption of VMPs containing antibiotics could, in a simplistic manner, be used to evaluate the likely stability and persistence of an antimicrobial in the environment and its subsequent potential selection pressure for AMR. It is also noted that the current Phase II ERA process allows for the refinement of the exposure of a compound into the environment through the provision of data on metabolism and excretion. Such studies on metabolism and excretion may already be available as part of the MRL application or the safety part of the MA dossier and, could provide useful information on the potential extent of excretion of microbiologically active substance(s) into the environment.

The effects of antibiotics on environmental bacteria can range from simple parameters such as a decrease in biomass, respiration rate or denitrification rate, to more complex parameters like community shifts and the survival of new genetic information. The findings from the few studies available on the effects of some antimicrobials on nitrification and decomposition in soil indicated effects in standard studies at very high concentrations, compared to expected field concentrations (Jensen, 2001; Thiele-Bruhn, 2003). However, the guidelines mentioned above do not currently take into consideration any aspects of AMR. Consequently, the trigger value of 100 µg/kg soil in the Phase I assessment allows for high soil concentrations, and the safe concentrations based on traditional ecotoxicological endpoints (respiration rate, nitrification rate etc.) that fall short in avoiding minimal 'resistance-selective' concentrations (Berendonk et al., 2015; Montforts, 2005). Contamination with ARGs may change the genotype of autochthonous bacteria (Pruden et al., 2006; Schwartz et al., 2003). Acquiring resistance to antimicrobial substances, however, is part of the natural evolution of microbial species and which may not by definition result in a deleterious effect. It is evident that we need alternative tools and models to understand the environment from the microbiological perspective (e.g. applying tools from landscape ecology (Singer et al., 2006)), as well as different tools to assess the risk of ARGs in the environment (Midtvedt, 2004). For example, Hughes Martiny et al. (2006) chose to describe the biotic communities at the taxon level, not at the species level. The question as to whether taxa in communities, or at biocenoses as a whole should be considered is yet to be agreed. Studies that focus on the effects of ARGs on complete (autochthonous) microbial communities are currently not available. The ecological relevance of the introduction of ARG and the associated shifts in community composition have not been determined to date (McVey and Montforts, 2011; Mensink and Montforts, 2007)The ecological relevance of the introduction of ARG and the associated shifts in community composition have not been determined to date (McVey and Montforts; Mensink and Montforts, 2007).

In accordance with VICH GL27 (EMEA, 2004), in the pre-approval information for registration of new antimicrobial veterinary medicines for use in food-producing animals, an applicant must provide data addressing the potential for such products to select for resistant bacteria in the treated animal that might be of human health concern (zoonotic pathogens and commensals). Such ARB and ARGs once in the environment could equally be a risk to animal health. The required data, where available, may include information on the concentration of microbiologically active substance(s) within the animal's gastrointestinal tract, which could be used to inform on the possibility of selection of resistance in the organisms of concern. There is no specific requirement for studies to investigate directly the excretion of ARGs from treated animals, despite this hazard being well documented (section 8.1).

The CVMP's draft risk assessment guideline for VMPs containing antimicrobials (EMA/CVMP/AWP, 2015) identifies general environment contamination as a route of human exposure to ARGs resulting from the use of VMPs, but this route is not within scope of the guidance.

In summary, although the ERA process for VMPs does not currently take into consideration any aspects of AMR, there are data available from the existing process that could be useful in evaluating the significance of the fate mechanisms for certain antimicrobials, in terms of AMR. Of particular note, is the current data held on physicochemical and fate properties, as well as information on metabolism and excretion, of antimicrobials used as VMPs. In terms of the effects assessment for the current ERA process, none of the current data requirements are considered relevant to AMR. It is clear that to evaluate AMR in the environment appropriately, alternative tools (e.g. MSC assays) and models to understand the environment from the microbiological perspective would be required. Finally, any evaluation of AMR in the environment would have to consider the effects from the introduction of ARGs resulting from antimicrobial use as VMPs. Finally, it is important to note that the AMR/ERA assessment is hampered by the fact that, even if appropriate models were available to predict the magnitude of an AMR shift in an environmental compartment, from VMP uses, the relevance of such information and its subsequent consideration within the risk: benefit evaluation is problematic at best. In order to rectify this, it is of critical importance to know how and to what extent human/animal health and ecosystems will be impacted by certain environmental AMR "levels" or shifts, and how this relates to certain quantities of certain antimicrobial substances in the different environmental compartments. Therefore, the above highlights fundamental issues that prevent a straightforward amendment of the current ERA to account for AMR in the environment. In particular, these are:

- 'Safe levels' for antimicrobials (that will not adversely impact human or animal health, or the environment) have not been established for AMR.
- The current ERA is based on the active substance and its major transformation products. An assessment for AMR would need to evaluate these as well as the potential environmental contaminants of ARB and the ARGs.
- Absence of appropriate validated assays to model and quantify AMR.
- The extent that human/animal health and ecosystems will be impacted by certain environmental AMR "levels" or shifts need elucidation.

9.2. Knowledge gaps and research needs

Assessing the risks to human and animal health of AMR in the environment due to the use of antimicrobials in veterinary medicine is challenging given the complexity of the problem and the paucity of knowledge regarding the mechanisms and pathways involved at the genetic, cellular and population levels. In addition, the lack of understanding regarding the role that the receiving environment has on the fate of ARs, ARBs and ARG is a significant knowledge gap.

To properly assess the risks of AMR in the environment, it would be beneficial if the differential contributions of the environment, compared to the contribution from other sources, relating to the problem of AMR could be quantified. It is clear that ARGs have the potential to move from environmental bacteria to human and animal pathogens, and *vice versa*, as transfer of genes between bacteria can in theory occur anywhere (Bengtsson-Palme et al., 2018) and such a transfer is more likely to occur between phylogenetically closely related bacteria (Philippot et al., 2010). However, for the transfer of resistance to occur, the host and receiving bacteria need to share the same ecological niche, at least temporarily (Wiedenbeck and Cohan, 2011). Following this rationale, it is reasonable to suggest that the frequency of transfer of resistance would be higher between animal to animal, and human to human - associated bacteria (Porse et al., 2017; Salyers et al., 2004). The transfer of ARGs to animal and human bacteria from environmental bacteria, which are often less phylogenetically related, would therefore likely be less common, but not necessarily insignificant as environmental stressors may induce HGT to and from (opportunistic) human pathogens in environmental settings

(Bengtsson-Palme et al., 2018). The potential exposure pathways for environmental bacteria (whether only transient or not) to humans have already been discussed above, such as through recreational activities, interaction with farmed/wild animals, or eating/drinking contaminated food/water, respectively (Allen, 2014; Allen et al., 2010; Baquero et al., 2008; Ghaly et al., 2017; Lupo et al., 2012; Rolain, 2013). In theory, all of these could be linked to VMP use. However, the actual significance of any of these exposure scenarios remains uncertain due to the lack of knowledge of the factors triggering transfer of ARGs in environmental bacteria, and subsequent persistence/continued viability of the ARG and/or host bacteria once transferred under these scenarios. Furthermore, it is almost impossible to quantify the potential for opportunistic pathogens, which have been shown to thrive in soil (Johnning et al., 2013), to act as intermediary hosts of ARGs which they then transfer to human and veterinary pathogens at a later time. It is also recognised that VMPs select for AMR and that livestock production is associated with elevated environmental reservoirs of AMR (Magouras et al., 2017). Furthermore, environmental concentrations of several antibiotics used as VMPs are above the MSCs, and we are able to quantify the environmental exposure routes.

Recently, research papers have addressed the possibilities of conducting quantified assessments of exposure pathways for AMR in the environment (Ashbolt et al., 2013; Schmitt et al., 2017). Information to conduct such risk quantification is considered as currently lacking, given the number of knowledge gaps that need tackling to enable a proper risk assessment of AMR (Bengtsson-Palme, 2016). Some progress has, however, been made in addressing recognised knowledge gaps, as outlined in Table 3.

Table 3. Selected knowledge gaps hindering the assessment of risks associated with environmental AMR from VMP use (Adapted from Bengtsson-Palme, 2016)

OPEN QUESTION	SOME SUGGESTIONS
Where do horizontally transferrable resistance determinants emerge?	Polluted environments, sewage treatment plants, aquaculture, agriculture (Ashbolt et al., 2013; Berendonk et al., 2015). The spreading of manure is considered as most significant for VMPs.
What concentrations of antibiotics used in animals and other toxicants are selective for resistance?	Determination and predictions of minimal selective concentrations for antibiotics (Gullberg et al., 2014; Gullberg et al., 2011; Tello et al., 2012).
Which environments have the potential to drive resistance selection in bacterial communities?	Likely: animals given antibiotics, aquaculture, and application of manure to land. Possible: discharges from slaughter houses, sewage, sewage treatment plants, waste disposal, wastes from animal housing (Ashbolt et al., 2013; Larsson, 2014).
What roles do MGEs play in resistance development?	Transfer of resistance between bacteria, mobilisation of chromosomal resistance genes, rearrangement of existing resistance determinants (Stokes and Gillings, 2011).
What concentrations of antibiotics and other toxicants induce HGT?	Sub-inhibitory concentrations of antibiotics (Beaber et al., 2004; Prudhomme et al., 2006) Prudhomme et al., 2006), few minimal concentrations determined (Jutkina et al., 2016).
What are the dissemination routes for resistance	Water bodies (Lupo et al., 2012; Pruden et al.,

OPEN QUESTION	SOME SUGGESTIONS
genes to human and animal pathogens?	2013), agriculture and food trade (EFSA/ECDC, 2013; Rolain, 2013).
Which dissemination routes from selective environments connect to environments with human and animal pathogens?	Water bodies and agriculture have large potential.

The EU and member state national research councils are currently engaged in funding a range of activities to address the knowledge gaps in AMR in the environment. In particular, OIE, WHO, UK Natural Environment Research Council (NERC), the Biotechnology and Biological Sciences Research Council (BBSRC) and the Medical Research Council (MRC), as a cross-council initiative, have recently awarded large research grants and pump priming grants under the theme of 'understanding real world interactions'. This theme aims to address the need for a greater understanding of the role of the bacterial environment in influencing the evolution, acquisition and spread of AMR, and as a reservoir for resistance. The grants that have been awarded through this cross-council initiative include projects titled; 'Is AMR in the environment driven by dissemination of antibiotics or antibiotic resistance genes?' and 'Evaluating the threat of antimicrobial resistance in agricultural manures and slurry' (NERC, last accessed in 2018). A previous NERC Environmental Microbiology and Human Health call also funded a project on AMR, "Using next-generation sequencing to reveal human impact on aquatic reservoirs of antibiotic resistant bacteria at the catchment scale".

As well as local research grants, the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), is coordinating a partnership approach at EU level with the aim of pooling national research efforts to tackle the challenge of AMR more effectively. There are a number of grants that have been awarded that encompass the environment, examples of which include PREPARE (Predicting the Persistence of Resistance Across Environments), STARCS (Selection and Transmission of Antimicrobial Resistance in Complex Systems) and three separate projects concerning AMR in wastewater (JPIAMR, last accessed in 2018). As well as these commissioned projects, the JPIAMR has also provided funding to establish working groups to enhance alignment and maximise existing and future research efforts. One of these working groups is named 'Bridging the gap between exposure to AMR in the environment and impact to human health' and one of the outputs from this group will be to publish a defined toolbox of existing approaches, best practices for study protocols, and to identify research gaps.

Some authors have proposed the use of the concept of MSC as a quantitative indicator of the level of antibiotics necessary for the development of resistance. MSC is defined as the concentration at which the fitness cost of resistance is balanced by the antibiotic-conferred selection of the mutant and, according to in vitro data, usually corresponds to 1/4 to 1/230 of the MIC of the susceptible strains (depending on the antibiotic and type of mutation considered) (Gullberg et al., 2011). However, there are concerns, regarding the ability to extrapolate results from in vitro competition experiments to the complexity of microbial communities in the environment. In a recent study, Bengtsson-Palme and Larsson (2016) used a theoretical method for the assignment of MSCs for 111 antibiotics based on observed lowest MIC values for target organisms available on the EUCAST (European Committee on Antimicrobial Susceptibility Testing) database (EUCAST, last accessed in 2018). This framework relies on the assumption that an antibiotic concentration that inhibits growth of some bacteria will have selective effects at the community level and the values obtained correspond to the upper boundaries of the MSC for each compound. Subsequently, the predicted no effect concentrations (PNECs) for resistance selection in microbial communities were estimated by applying a flat assessment factor of 10 to each (to account for the difference between inhibitory concentration and selective concentration of antibiotics) for each calculated value of MSC. According to the authors, such values could be

considered as analogous to the lowest observed effect concentrations (LOECs) used in environmental risk assessment for different chemicals.

The overview of knowledge needs presented above, together with the UK Department of Health 'AMR systems map' presented in Annex II, highlight that there is a conceptual understanding of the role of the environment with respect to AMR. The systems map details the numerous different inputs and transmission pathways that are likely contributing to the potentially ever-growing burden of AMR in clinical, animal and environment settings. In addition, the AMR systems map comprehensively demonstrates how AMR is a highly complex problem that spans multiple sectors. Furthermore, as discussed in section 3.6 of the 2017 European Commission Action Plan against the rising threat from AMR (European Commission, 2017), there is a specific need to support research, develop tools and explore risk assessment methodologies to successfully understand and combat AMR.

10. Mitigation of AMR in the environment

Risk mitigation is an essential part of the evaluation of VMPs. Risk mitigation can be used to restrict the risks associated with a product to an acceptable level or even to completely remove such a risk. Further research is needed in order to estimate the exposures and risks associated with environmental pathways of antibiotics as VMPs that drive AMR in the environment. Nonetheless, certain management options might contribute to the reduction of these risks, acting synergistically with existing policies and goals. For example, Muurinen et al. (2017) studied the influence of manure application on the environmental resistome under Finnish agricultural practice with restricted antibiotic use. The paper reported that many genes spread from animals to the soil through manure application, but these genes did not appear to persist beyond 12 months in the soil environment. This study and others like it suggests that practices that minimise or control the frequency of repeat spreading of manures from treated animals, as followed in Finland, may lead to lower levels of clinically relevant ARGs in agricultural soils.

It is worth noting that an EU-wide ban on the use of antibiotics as growth promoters in animal feed was introduced in 2006 (European Commission, 2005; Official Journal of the European Union, 2003), which has impacted on the overall use of antibiotics in the EU. Furthermore, the EMA has produced recommendations regarding the use of critically important antibiotics for human medicine in animals (e.g., fluoroquinolones, colistin (EMA/AMEG, 2016) and 3rd- and 4th-generation cephalosporins (EMEA/CVMP/SAGAM, 2009)).

In this context, mitigation measures should aim to reduce the input of antibiotics into environmental compartments. For VMPs, this can be done by:

- reducing the quantities of antimicrobials prescribed/used (e.g., prudent use), and by;
- reducing the release of antimicrobials to the environment by establishing effective barriers (e.g., avoid the release of urine, faeces from antibiotic-treated animals into aquatic environments for a determined timespan).

The application of manure as a fertiliser to agricultural soils is expected to be a relevant vehicle for the dissemination of antibiotics and ARB into the environment and could, therefore, be a target for intervention. Manure is often stored prior to its land application. Degradation of residues occurs for some antibiotics (to different extents according to the bacterial species) but not others. Further work is needed to determine the best methods of storing manures such that we maximise the efficiency of manure treatment to reduce the levels of antibiotics and ARGs. This is particularly relevant as certain antibiotics have been shown to have long elimination half-lives in manure, for example 100 days for tetracyclines (Chee-Sanford et al., 2009). So, if the rate of application of contaminated manure

exceeds the degradation of the antibiotics, then net accumulation is expected. One possibility could be to optimise the process of anaerobic digestion of liquid manure, a process already used for waste management with associated methane/biogas production, with a focus on eliminating ARs. It should, however, be investigated if the digestion tanks themselves provide a novel source of AMR, since the conditions in the tanks are likely to support AMR development. Methods that improve nutrient reuse such as phosphate recycling from sewage sludge could also contribute to lessen the amount of antibiotics spread onto agricultural land.

Finally, there is clear scope to couple environmental management of manures to show the positive impact that interventions have already delivered in terms of reducing release of antimicrobials into the environment (Pruden et al., 2013). This is evidenced in Table 4 for optimising antibiotic use and minimising impacts from antimicrobials present in animal manures.

Table 4. Management options for reducing the release of antibiotics and ARGs from manures to the environment (based on recommendations by Pruden et al. (2013)

Optimising antibiotic use	**Maintaining good animal health**	**Alternatives to antibiotics**
Limiting the use of antimicrobials (especially critically important ones) Banning the use of antibiotics as growth promoters*	Optimising management practices by: - reducing animal density - improving nutritional status	Developing better vaccines and vaccination programmes
Management of manure containing antibiotics	**Biological treatment of ARGs in manure**	**Containment of ARGs in manure**
Composting can eliminate 50 to 70% of certain antibiotics Watering, aeration and turning of compost can accelerate decay of some antibiotics Fermentation is more effective at removing other antibiotics	Response to biological treatments varies greatly	Prevention of lagoon spills and seepage Control of surface runoff Improved manure collection Long-term manure storage Manure separation Limiting sediment erosion and transport from animal farms

* Growth promoters are banned in the EU, since 2006, on the grounds that such use potentially could lead to the unnecessary development of AMR.

11. Conclusions

This reflection paper aimed to consider how the presence of ARs and ARGs in the environment might impact ecosystems, as well as animal and human health.

On reflection, there is a growing body of work demonstrating that the use of antibiotics in veterinary (and human) medicine contributes to environmental reservoirs of ARB or ARGs; facilitating the transfer of MGE either directly or indirectly to humans and animals. It is important to note here, the difficultly in identifying the specific source of ARs or ARGs, as analogues of compounds exist between human and veterinary medicines. Therefore, it is problematic to attribute a single route as the major source of the

contamination (e.g. waste water versus manure discharge), and in particular, to disentangle the input from human versus veterinary antimicrobials.

In conclusion, the current ERA for VMPs cannot yet be amended to consider the risks posed by the accumulation of ARs and ARGs in the environment from the use of VMPs. However, the CVMP should continue to explore the development of improved or alternative risk assessment methodologies, with the support of other national and EU scientific agencies and bodies, to assess if improvements can be made. In particular, some areas for consideration are noted below:

A 'One Health' approach should be taken to minimising environmental contamination with ARs and ARB/Gs

In the EU, a greater volume of antimicrobials is sold to treat diseases in animal husbandry than for use in human medicine (ECDC/EFSA/EMA, 2017). Although some of those will be naturally degraded or transformed, there is a growing body of work demonstrating that the use of antibiotics in veterinary (and human) medicine contributes to environmental reservoirs of ARB that can directly or indirectly drive the transference of MGEs to humans and animals.

The WHO global action plan on AMR (WHO, 2015) included the environment in the One Health approach. The EU Joint Programming Initiative on AMR takes the starting point that the holistic assessment of the contributions of pollution on the environment with antibiotics, ARs and resistant bacteria is a necessity.

The development of strategies to minimise environmental contamination by antimicrobials and resistant bacteria is one of the priorities of the One Health approach. Therefore, any measure(s) taken should follow the One Health approach, in which the reduction of the exposure of the environment to substances with antimicrobial capabilities is not limited to one regulatory arena only, but all relevant sources of antimicrobial contamination are to be reduced as much as possible.

Assessment of the risk for the environment from the authorisation of antimicrobial VMPs

Consideration of AMR in the environment, in the context of a MAA for a VMP, might impact on the ecosystems, animal health, and human health. Due to the interdisciplinary nature of the problem, the CVMP acknowledges that any future changes to the risk assessment, if needed, would likely span Parts III and IV of the dossier assessment.

The CVMP should continue to monitor for new data/approaches/technologies which could be used to improve the current risk assessment process, especially in the identification of potential hazards, risks and risk management measures. Specifically, there is a need to monitor scientific developments and gain a better understanding of:

- The emerging area of using MSCs to determine risks posed by ARs. Additional data are due to be published on determining MSCs in complex microbial communities.
- The level and duration of excretion of ARB and ARGs from treated animals, and transfer of ARGs between animals via the environment.
- The fate of ARGs in manure, together with the fate of antibiotics. This gap in our knowledge concerns whether there is an increase or decrease of ARGs during manure storage, and what are the main factors affecting this. Risk mitigation measure(s) (e.g. relating to storage of manure) could subsequently be developed.
- The impact of environmental AMR on ecosystems, and human/animal health.

- The identification of relevant resistance determinants (e.g. on MGEs) and determining the concentrations of antimicrobials that select for them. It is noted that the generation of such data is already a requirement for new active substances undergoing the registration process for a new MA.
- The identification of environmental properties and environmental exposure profiles both for the antimicrobials and the ARGs.

As a result, it is acknowledged that there is a need to build expertise in the regulatory agencies on approaches to evaluating the AMR risks to the environment and the consequential risks to animal and public health.

Risk management measures to be applied in general to limit environmental contamination by ARs and ARGs

From a public and animal health perspective, it is a priority to use antimicrobials in an optimal way, to treat the disease effectively but also reduce any unnecessary consumption of antimicrobials, and their subsequent release into the environment. Measures taken and proposed that promote the prudent use of antibiotics will also reduce the amount of ARs entering the environment. The detailed EMA/EFSA recommendations on how to reduce the need to use antimicrobials in food producing animals (RONAFA) (EMA/EFSA, 2017) should be implemented as far as possible to reduce the use of antimicrobials in animals, and as a result, exposure of the environment to those antimicrobials.

In conclusion, the field of environmental AMR is a rapidly evolving scientific discipline and new insights and findings are published almost weekly. The CVMP will continue to monitor new evidence and consider new knowledge that addresses our current gaps in understanding.

Annex I

Mechanisms of AMR

Development of AMR

Some bacteria are innately resistant to certain types of antibiotics. However, bacteria may also develop antimicrobial resistant in two ways: either by a genetic mutation, or by the acquisition or resistance from another bacterium (which could be from the same or a different species). Most antimicrobial substances have an environmental origin, and are produced by microorganisms that protect themselves against threats by other organisms. In time, some of these prokaryotic organisms (certain types of bacteria) have developed a resistance against these antimicrobial substances by mutation of their genes. Resistance can appear spontaneously because of random mutations; or more commonly following gradual build up over time, and due to a presence of antimicrobials (Geenen et al., 2011). Thus, the presence of ARGs causes the bacteria to be resistant to antimicrobial substances. The transfer of these AMR genes may take place from parent to offspring bacteria, but may also take place from one bacterium species to another different species. When AMR genes are transferred from one bacterium to another bacterium that causes illness in humans or animals, the presence of these AMR genes may cause treatment failure in the patient, as the bacteria may have acquired resistance to the antimicrobial substance.

Selection and spread of AMR

Exposure of bacteria to antimicrobial substances is a known driver of AMR, and encourages selection of antimicrobial resistance genes. Antimicrobial substances include antibiotics used in human and veterinary health care as well as disinfectants used in cosmetics (e.g., triclosan) and in biocides (hand and surface disinfection). Other compounds, like metals (e.g., copper, zinc and silver) are also known to elicit co-selection for AMR genes and thus are attributed to play a role in the development and spread of AMR.

Figure A1. Possible effects of antimicrobials on resistance in the environment (Schmitt et al., 2017)

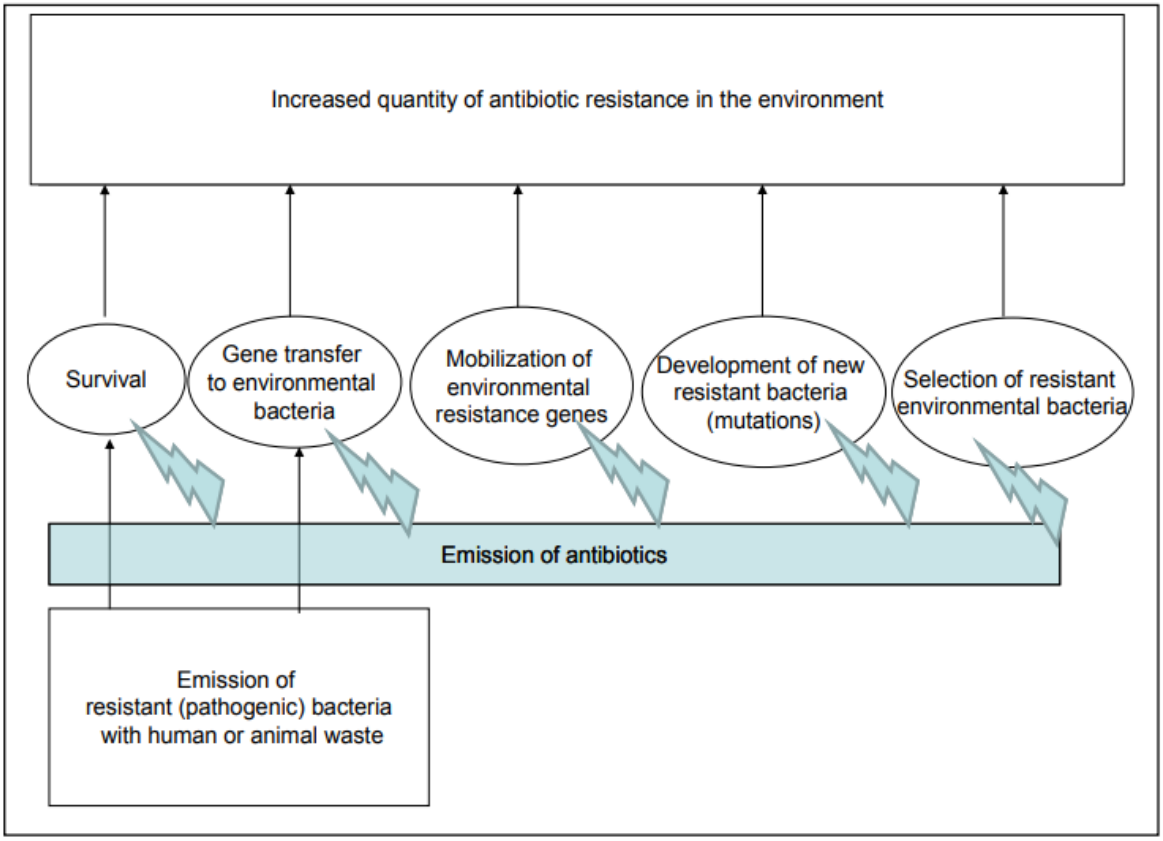


Figure A1 depicts the possible spread of resistant microorganisms following the release of ARB in the presence of an antimicrobial selective pressure. Potential releases may originate from manure or waste water treatment plants (WWTPs), Irrespective of the type of release, the presence of antimicrobials in the environment in biologically relevant concentrations can select for resistant microorganisms. Although there is a relatively clear picture of how AMR develops and spreads in hospitals, the pathways that act via environmental matrices are not well understood (Berkner et al., 2014).

Berkner et al. (2014) suggested that several environmental hot spots with the potential for the development and spread of AMR have been identified so far, such as biofilms, certain sediments, treated effluents and sewage sludge (human medicines), pharmaceutical production sites, aquaculture facilities, liquid manure tanks and soil repeatedly fertilised with manure. In particular, it was proposed that intestinal bacteria from livestock treated with antibiotics might survive in manure storage facilities only to be directly transmitted onto land. A further example involved locations, such as biogas production units, where large numbers of microorganisms under favourable nutrient conditions are exposed to antibiotic concentrations that can select for resistance. In addition, manure contains metal ions from animal feed and biocides from the disinfection of livestock housing that are implicated in co-selecting for resistance or that could enhance mutation frequencies that may lead to the development of resistance.

The influence of metals on the selection and spread of AMR

The environmental conditions in which a bacterium resides can have a significant effect on its potential to develop or acquire ARGs. The multitude of unique factors and stressors at play in the wider environment (soils, sediments, water) create a complex arena in which AMR can develop and persist. Heavy metals are one such set of stressors that are commonly found in the environment and have long

been implicated in the development, persistence, and spread of AMR. This is because of their ability to co-select for ARGs (Poole, 2017).

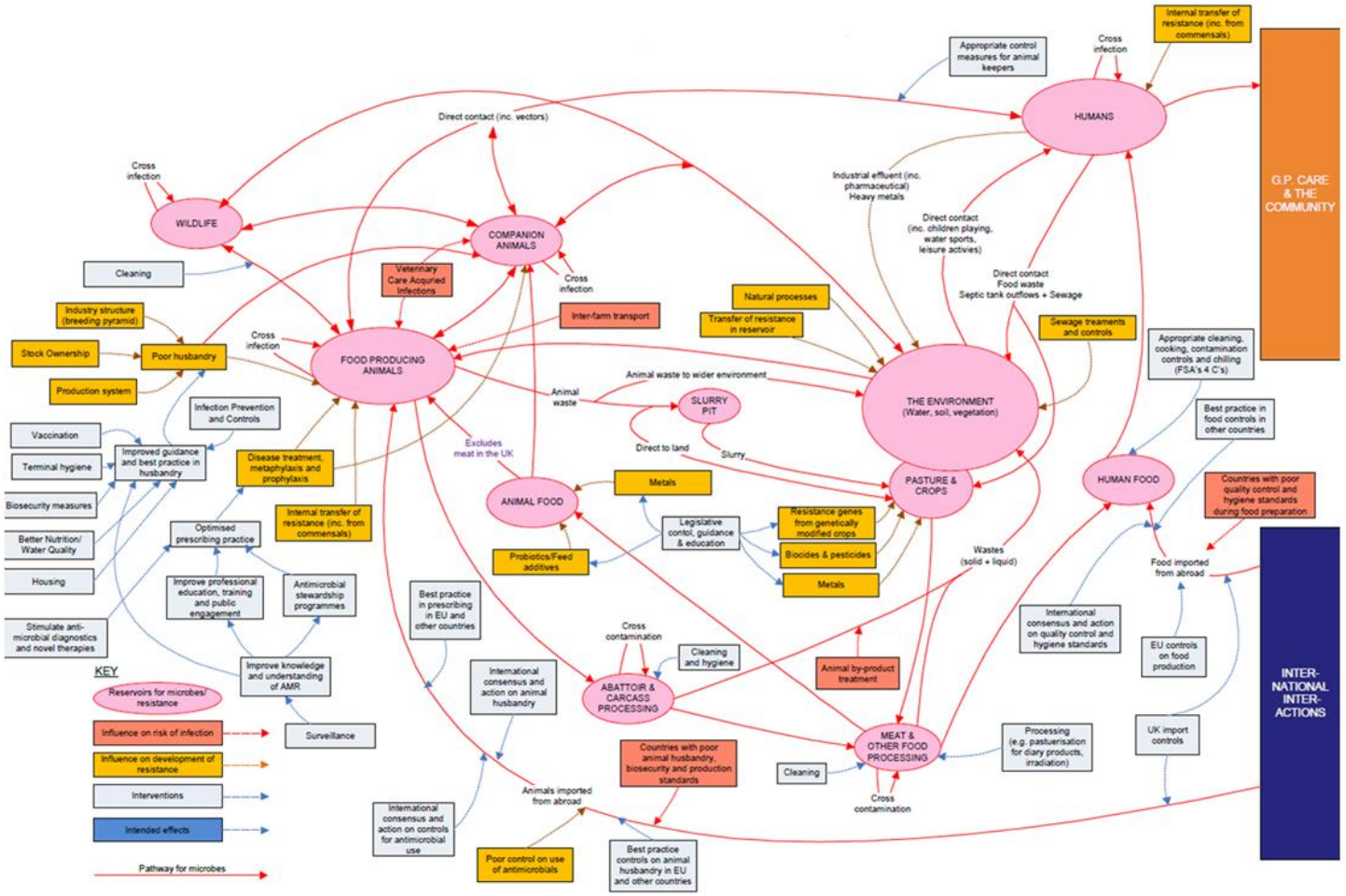
A recent review conducted by Poole summarises the knowledge base regarding the influence of zinc (Zn) and copper (Cu) on AMR development and spread:

- Metals provide a selective pressure for metal resistance which can, in turn, co-select for AMR due to physical genetic linkages between genes.
- Concentrations of Cu in the environment have been shown to correlate with an increased occurrence of ARGs and MGEs in environmental bacteria.
- The use of Zn and Cu in veterinary medicine has been linked to the development and persistence of resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and MDR *E. coli* and *Salmonella* spp.
- Cu and Zn can encourage biofilm formation in certain organisms and also promote the induction of dormant persistence states in a number of Gram negative bacteria.

Cu and Zn have been shown to inhibit as well as synergistically enhance a number of antibiotic compounds.

Annex II

UK Department of Health 'AMR Systems Map'[3]



This systems map shows the influences on the development of AMR in humans, animals and the environment.

[3] Dobra et al. 2014 https://www.gov.uk/government/publications/antimicrobial-resistance-amr-systems-map

12. Glossary

3GCs	Third generation cephalosporins
ADME	Absorption, distribution, metabolism and excretion
AMR	Antimicrobial resistance
API	Active pharmaceutical ingredient
ARBs	Antibiotic resistant bacteria
ARGs	Antimicrobial resistance genes
ARs	Antimicrobial residues
AWP	Antimicrobial working party
CVMP	Committee for Medicinal Products for Veterinary Use
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ERA	Environmental risk assessment
ERAWP	Environmental risk assessment working party
ESBL	Extended Spectrum Beta-Lactamases
ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
FAO	Food and Agriculture Organisation
GIT	Gastro-intestinal tract
GL	Guideline
HGT	Horizontal gene transfer
LOECs	Lowest observed effect concentrations
MA	Marketing authorisation
MAAs	Marketing Authorisation Applications
MGEs	Mobile genetic elements
MRL	Maximum Residue Limit
MRSA	Methicillin-resistant Staphylococcus aureus
MSC	Minimal selective concentration assays
NDM	New Delhi Metallo-beta-lactamase
OIE	World Organisation for Animal Health
PNECs	Predicted no effect concentrations
VICH	VICH is a trilateral (EU-Japan-USA) programme aimed at harmonising technical 2949 requirements for veterinary product registration. Its full title

	is the International 2950 Cooperation on Harmonisation of Technical Requirements for Registration of 2951 Veterinary Medicinal Products.
VMPs	Veterinary medicinal products
WHO	World Health Organisation
WWTPs	Waste water treatment plants

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