WHO Guidance on waste and wastewater management in pharmaceutical manufacturing with emphasis on antibiotic production

DRAFT

FOR PUBLIC CONSULTATION

December 2023

Note to public reviewers:

- This is a **Draft** prepared by and with listed contributors – WHO is seek feedback on the technical content and considerations for implementation by the target audience.
- The document is **partially edited** – there is no need for minor editorial corrections. A later draft will be professionally edited.
- Please **provide your written feedback via in the (Qualtrics feedback form) no later than 26 January 2024.**
- WHO anticipates **submitter will also have an opportunity to provide verbal summary of written feedback** to drafting contributors at a meeting prior to finalization of the document.

Contents

Contributors

- Diana Aga - University of Buffalo, USA
- Stefan Berggren - Swedish Medical Products Agency, Sweden
- Stephanie Croft – Inspection services, World Health Organization, Switzerland
- Washington Dengu – Consultant GMP inspectors trainer, WHO AFRO
- Sabiha Yusuf Essack -University of KwaZulu-Natal, South Africa
- Walter Fuller -World Health Organization, Africa Regional Office
- Martijn van Gerven - Access to Medicine Foundation, The Netherlands
- Valeria Gigante - AMR secretariat, World Health Organization, Switzerland
- Bruce Gordon, Water, sanitation hygiene and health Unit Head, WHO, Switzerland
- Dinabandhu Gouda - Central Pollution Control Board, India
- Olivier Gross - European Commission, Belgium
- Amit Khurana - Centre for science and environment (CSE), India
- Paschalia Koufokotsiou -European Commission, Belgium
- Sigrun Kullik -Public Health Agency, Canada
- Joakim Larsson Göteborgs Universitet, Sweden
- Stephanie Laruelle - United Nations Environment Programme (UNEP), Switzerland
- Ramanan Laxminarayan - University of Washington, USA
- Alison Macintyre -Nossal Institute for Global Health, University of Melbourne, Australia
- Kate Medlicott – Water sanitation hygiene and health, World Health Organization, Switzerland
- Caroline Moermond - Rijksinstituut voor Volksgezondheid en Milieu (RIVM), The Netherlands
- Wayne Muller - Health Products Regulatory Authority, South Africa
- Iris Panorel - Stockholm International Water Institute, Sweden
- Fatema Rafiqi - Access to Medicine Foundation, The Netherlands
- Nicolai Schaaf - Independent, Sweden
- Narendar Sharma - Central Pollution Control Board, India
- Elizabeth Wellington - University of Warwick, UK
- Min Yang - Research centre for eco-environmental sciences (RCEES), China
- Yu Zhang -Research centre for eco-environmental sciences (RCEES), China

Acronyms and abbreviations

AMR	Antimicrobial resistance
API	Active pharmaceutical ingredient
BOD	Biological oxygen demand
COD	Chemical oxygen demand
CETP	Common effluent treatment plant
EC_M	Effluent concentration (estimated by mass balance)
EC_A	Effluent concentration (estimated by chemical analyses)
EMA	European Medicines Agency
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
ISO	International Organization for Standardization
LOQ	Limit of Quantification
OECD	Organisation for Economic Cooperation and Development
PEC	Predicted environmental concentration
PNEC	Predicted no-effect concentration
PNECres	Predicted no-effect concentration for resistance selection
PNECeco	Predicted no-effect concentration for ecological effects
RQ	Risk quotient
WASH	Water supply, sanitation and hygiene
WWTP	Wastewater treatment plant
ZLD	Zero liquid discharge (Note: disposal to land sometimes referred to as ZLD is assessed in the same way as disposal to water bodies)

Executive summary

[To be added in final version]

1 Introduction

1.1 Background

Pharmaceuticals provide great value to humanity by providing effective means to prevent and treat disease. As pharmaceuticals are biologically active, often highly potent molecules with conserved targets across species (Gunnarsson et al. 2008), environmental emission may cause unwanted effects on other organisms than those the pharmaceuticals are intended to affect. While the overall largest volume of active pharmaceutical ingredients (APIs) that reach the environment most likely originates from use by patients, the highest environmental concentrations found are the result of pollution from manufacturing (Larsson 2014) that are localized in certain industrial areas. Pollution with antimicrobials provides a case of special concern. In addition to direct ecological effects (Brandt et al. 2015), environmental pollution with antimicrobials may also contribute to the development of resistance, in both non-pathogenic and pathogenic microbes, thereby threatening the effectiveness of antimicrobials as therapeutic agents in humans, farmed and domestic animals and crops (Larsson et al. 2023; United Nations Environment Programme 2023; Larsson and Flach 2022). Such effects are not restricted to the site of the emissions, as microorganisms have the ability to propagate and eventually spread world-wide. Therefore, there is a recognised need for international evidence-based guidance and tools on the management of industrial waste containing antimicrobials to guide the target audience of this document (Review on Antimicrobial Resistance 2015; WHO 2020a; European Parliament 2020; United Nations Environment Programme 2023; Ifpma 2016; AMR Alliance 2022).

Since 1998, there have been several World Health Assembly (WHA) resolutions on AMR that lead to the endorsement of a global action plan to tackle AMR by the Sixty eighth WHA in May 2015 (WHO 2015). Shortly after, on 30 November 2018, the World Health Organization's (WHO's) Executive Board meeting asked WHO to provide technical input from the good manufacturing practices (GMP) guidance perspective on waste and wastewater management from the production of critically important antimicrobials (Executive Board 114 2018, WHO 2019). As a follow-up action to that decision a WHO "Points to consider for manufacturers and inspectors: environmental aspects of manufacturing for the prevention of antimicrobial resistance" was recommended for adoption by the Fifty-fourth Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP) (WHO 2020b).

In addition to the WHO hosted GMP, this document is intended to guide improvement in waste management to combat AMR by other entities such as regulators, procurers and industry directly as described in section 1.3.

1.2 Purpose

The purpose of this guideline is to establish independent, scientifically derived framework for applying targets for managing discharges from antibiotic manufacturing facilities, with the intent to limit antibiotic resistance development and ecological effects caused by discharges of antibacterial agents into the environment. The guidance complements other guidance in the area of the assurance of the quality and safety of pharmaceuticals, such as

the WHO Good Manufacturing Practices (GMP) and the TRS 1025 - Annex 6: Points to consider for manufacturers and inspectors: environmental aspects of manufacturing for the prevention of antimicrobial resistance.

While *health* first and foremost refers to human health, this guidance adopts a One-Health approach and incorporates ecosystem health. This document provides guidance on risk assessment, management and surveillance to ensure targets are consistently met. The guidance informs adoption of such targets, risk management processes and surveillance by the various target audiences.

1.3 Target audience

At least six key end users of the guidance are foreseen, (Nijsingh, Munthe, and Larsson 2019) including:

1) industrial actors in all stages of the production chain, including associations or other collective organizations;
2) waste and wastewater management services that handle antimicrobial waste and/or process effluents from the pharmaceutical industry;
3) environmental regulators (national or regional) in countries or regions that manufacture antibiotics;
4) regulatory bodies (national or regional) responsible for the regulation of pharmaceutical product manufacturing (e.g. Inspectorates from national or regional regulatory authorities,
5) procurers of antibiotics, including retail companies, hospitals, regional and national procuring bodies, including also the private sector;
6) governmental bodies or insurance companies responsible for generic substitution schemes and reimbursement decisions;
7) third party inspection schemes and auditors (e.g., ISO certification providers).

The needs, mandates, opportunities and risks are specific to different target audiences of the guidance and the different measures they may implement. While some may implement interventions or regulations to reduce antibiotic levels in industrial emissions, other stakeholders such as the procurers can apply the guidance to incentivize responsible manufacturing and waste management. The guidance is advisory in nature; hence it is the target audiences' responsibility to adapt and adopt it into various binding instruments.

1.4 Scope

The scope of this guidance covers:

- Human health-based targets for risk reduction for both emergence and spread of antibiotic resistance through selection, and ecotoxicological risks for aquatic life caused by antibiotics or active intermediates. The guidance defines the system for risk assessment and risk management, including directions on how both exposure and effect levels are generated.

- All antibiotics used as therapeutic agents for human, animal or plant use.[1]
- Emission of APIs as well as API intermediates and degradation products with known antimicrobial activity (MIC exceeding 5% of the final API). From hereafter, the term antibiotics also refers to such active intermediaries and degradation products[2].
- All steps from the manufacturing of biologically active intermediates, intermediates, to the production of the finished API and formulation into finished products.
- Liquid and solid (and air?) emissions with a focus on liquid emission and general procedures on management of solid waste contaminated by antibiotic agents.
- Release of resistant bacteria, selected for and enriched before the release of the waste.
- Separate assessment of production processes in any manufacturing site producing several antibiotics (intermediates, APIs or finished products), sequentially or in parallel.

The following aspects are not covered by this guidance:

- Other antimicrobials of concern (UNEP2023) including antifungal, antiviral and antiparasitic agents because the currently methods for health risk assessment are currently not sufficiently scientifically mature. Future updates could cover antifungals and possibly other pharmaceuticals.
- Antimicrobial biocides since their risk to human health is primarily related to co-selection of antibiotic resistance, which would require additional considerations.
- Other non-antimicrobial chemicals (e.g., heavy metals known to play a role in AMR through co-selection) present in manufacturing waste, still acknowledging that numerous other constituents could be important polluting agents.
- Other emissions covered under local or national regulations and control (such as Biological and Chemical Oxygen Demand (BOD, COD) and Total Suspended Solids (TSS)) are also not covered here.
- Potential direct (toxicological) effects on humans resulting from exposure to antibiotic residues in the environment
- Water use, energy use and greenhouse gas emissions, which may be included in broader environmental assessments.
- Emissions of antimicrobials to air (with potential health effects including allergies) since effects are considered minor compared to discharges through liquid or solid waste.

[1] Scope is not limited to critically important antibiotics since preservation of the efficacy of all antibiotics is needed and because there are risks for co-selection within and between classes of antibiotics.

[2] This document refers to antibiotics, antibacterials and antimicrobials. Antimicrobial agents cover substances that are intentionally used to kill or prevents growth of microorganisms, whether bacteria, fungi, viruses or eucaryotic parasites. Antibacterial is a narrower term, referring to compounds used to kill or prevent growth of bacteria. Antibiotics refer specifically to those antibacterials that are used as therapeutic agents (i.e. it does not include disinfectants, preservatives etc). This guidance, only apply to antibiotics and their active intermediaries and degradation products.

Adherence to this guidance does not replace other regulatory demands. This guidance should be applied observing existing provisions for manufacturing safe and effective antibiotics (e.g., GMP).

Box 1: Antimicrobial emissions from manufacturing in the context of other emissions to the environment

Antibiotic resistance leads to the loss of efficacy among available therapeutic options, in turn leading to increased morbidity, mortality as well as socioeconomic costs. Current estimates predict that over 1 million deaths globally could be attributed to antibiotic resistance in 2019 (Murray et al. 2022). There are several drivers behind increased antibiotic resistance. The use of antibiotics in both the human and animal sector (including, but not restricted to inappropriate use and overuse) causes selection pressures that strongly favours both the emergence and spread of resistance. Insufficient hygiene and sanitation can boost the effect of such selection processes, allowing favoured resistant strains to spread further (Collignon et al. 2018; WHO 2020a).

Selection pressure from antibiotics in the environment is also expected to drive resistance development and spread (Larsson and Flach 2022; United Nations Environment Programme 2023). Quantitative, reliable estimates of the contribution from different drivers to outcomes of ultimate concern (morbidity, mortality, socioeconomic costs) are, however, very difficult to acquire. The recognition that selection pressures can drive both the emergence of new forms of resistance (events that are probably rare, difficult to predict, but may have vast consequences) as well as increasing transmission opportunities for already established forms of antibiotic resistance (common events, in principle quantifiable, but where each individual transmission event has a much more limited impact) (Larsson and Flach, 2022) makes it even more challenging to quantitatively attribute consequences to different drivers.

The parallel processes of evolution and transmission, influences from how resistance is managed in other geographical areas and settings, as well as delays between preventive actions and measurable effects on ultimate health outcomes, calls for the use of more proximate targets in developing strategies to manage antibiotic resistance development. Indeed, targeting to reduce selection pressures by reducing antibiotic use in humans and animals, often combined with sanitation and hygiene measures, has become the main strategy to limit resistance development and subsequent impact on health. Over time, such measures have paid off greatly, as countries with well-developed antibiotic stewardship programmes, access to therapeutics and diagnostics and good sanitation and hygiene conditions, in general, carry a much lower burden of antibiotic resistance (Murray et al. 2022; European Centre for Disease Prevention and Control and World Health Organization 2023).

Similarly, the need to reduce environmental emissions of antibiotics are recognized widely (Review on Antimicrobial Resistance 2015; United Nations Environment Programme 2023; European Parliament 2020). The levels of antibiotics released from different types of point sources vary by several orders of magnitude, but the highest levels recorded come from antibiotic manufacturing (Larsson, 2014; Larsson and Flach, 2022). It is unknown to what extent different concentrations and different types of pollution sources contribute to selection and eventually development of resistance in pathogens circulating in humans. While emissions of low to moderate levels of antibiotics through use and excretion are exceptionally widespread, discharges (of sometimes very high concentrations) from manufacturing are considerably less widespread, and in that sense, easier to manage.

As such, there is a priority to start managing risks from environmental antibiotic pollution from sources potentially providing the highest selection pressures, and where the number of point sources is more easily manageable such as manufacturing (Review on Antimicrobial Resistance 2015). This, however, does by no means exclude that there are risks associated with other types of discharges and also with lower emission levels.

2 Conceptual framework

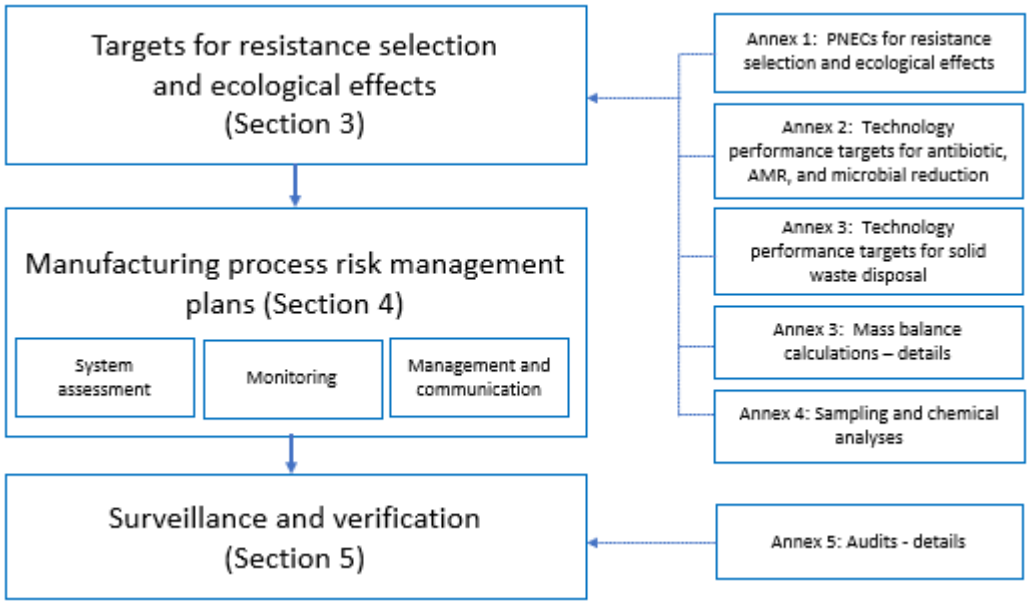
This guidance adopts a common conceptual framework used in WHO water safety guidance documents covering three core elements: define health-based targets based on exposure and risk assessment; establish risk management processes to reach those targets using recognized risk management tools (such as the principles of hazard analysis and critical control points (HACCPs)) and, perform independent surveillance including audits to verify targets are being met (Figure 1).

The following sections will address each of these elements in more detail.

A key guiding principle is the concept of *progressive improvement*. This enables users to enter at the appropriate level and work stepwise to achieve compliance with health-based targets so that application of stringent criteria without sufficient time to adapt does not jeopardize access to antibiotics (see also section 4). For this reason, different levels for progressive improvement are presented for targets in the guidance. Each target audience user needs to weigh potential impacts on access to, and costs of, medicines when adapting and adopting this guidance into binding instruments. When applied (e.g. in a regulatory context) where failure to pass would lead to market exclusion, there can be reasons to advance at a slower pace compared to applications where criteria are only linked to rewards (e.g. in procurement).

A second guiding principle is the *precautionary approach* which has been applied where scientific evidence is lacking or inconclusive.

Figure 1: Conceptual framework



3 Assessment against targets

This guidance covers protection of both human health (AMR development and spread) and ecological health caused by the emissions of antibiotics, including active intermediates and degradation products from the manufacturing chain.

To assess if a manufacturing process meets these protection goals, a risk assessment (section 3.3) is needed to compare emission/exposure levels (section 3.2) to relevant targets expressed as effect concentrations (for resistance selection and ecological effects) or performance of treatment technologies (section 3.1).

This section describes the grounds for such risk assessments. The methods to derive and apply ecological and human health-based targets differ and are dealt with separately.

To enable assessment of progression and to enable adoption of the guidance to different uses, two levels (BASIC and STRINGENT) are outlined and explained in more detail under Section 3.3. Overall, to meet the level of STRINGENT, waste must be treated in-house or by a dedicated industrial CETP (not municipal WWTP), exposure must be assessed by chemical analyses of wastewater, not only mass-balance estimates (see 3.2.2 and 3.3); and the risk assessment for resistance selection must be based on concentrations in the final effluent, not in the recipient effluent after dilution (see 3.2.3).

Figure 2 summarizes the assessment of solid waste and liquid waste streams covering: without treatment treated in-house; treated at a common effluent treatment plants (CETP); disposal to land (sometime referred to as 'ZLD'); and, treatment in municipal wastewater treatment plants (WWTP)).

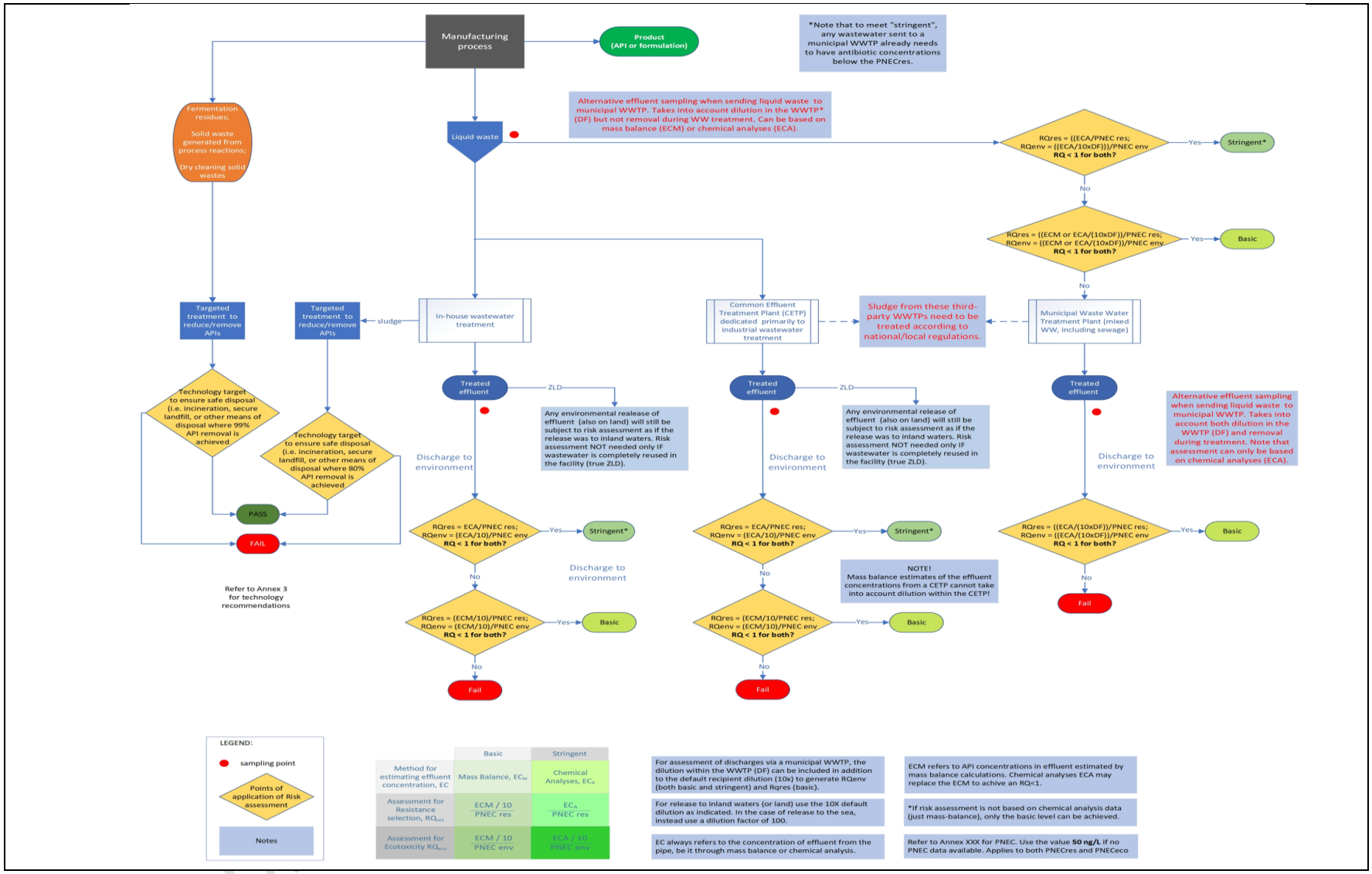


Figure 2: Process flow summarizing assessment against targets

[presentation for readability to be improved after technical aspects are finalized]

3.1 Targets

Effluent water quality, performance and specified technology targets (Table 1) to be applied for liquid effluent, solid waste and zero liquid discharge are outlined in the sections 3.1.1-3.1.4 below.

Table 1: Types of targets

Type of target	Nature of target	Typical application	Note
Health outcome Ecological outcome	Defined tolerable burden of disease Healthy ecosystems No adverse effect or negligible risk	High-level policy target set at national level, used to inform derivation other target type below where possible	No established method of assessment
Effluent water quality	PNEC values for resistance selection PNEC values for ecological effects	Chemical hazards	Used in this guideline for antibiotics and active intermediates/degradation products (section 3.1.1) and discharge to land ('ZLD') (section 3.1.4)
Performance	Specified removal of hazards	Microbial hazards (expressed as log reductions) Chemical hazards (expressed as percentage removal)	Used in this guideline for removal of antibiotic resistant bacteria (section 3.1.2) and for removal of antibiotics from solid waste (3.1.3)
Specified technology	Defined technologies	Control of microbial and chemical hazards Underpinned by established or validated performance of the specified technology	Used in this guideline for treatment of solid waste (section 3.1.3)

Source: Adapted from Table 3.2 of the WHO Guidelines for drinking-water quality: fourth edition incorporating the first and second, 2022.

3.1.1 Liquid effluent – PNECs for antibiotics, active intermediates and degradation products

Effluent water quality targets expressed as Predicted No Effect Concentrations (PNECs) for resistance selection and PNECs for ecological effects are outlined below with supporting information on derivation of the PNEC values presented in Annex 1.

PNECs for resistance selection

Concentration of antibiotics (including active intermediates and active degradation products) that are not likely to select for resistance (expressed as PNECs for resistance selection (annex 1)), are used as indicators for the ultimate goals of AMR prevention. This includes resistance development in pathogens, morbidity and mortality.

Box 2: Derivation of PNECs for resistance selection

Numerous approaches have been applied to assess selective concentrations of antibiotics in the environment, all with different pros and cons (Larsson and Flach 2022) (There is no formalized standard for assessing PNECs for resistance selection.

An approach is to derive PNECs from publicly available, standardized, experimentally derived data on a large range of bacterial MICs (minimal inhibitory concentrations) extracted from the European Committee on Antimicrobial Susceptibility Testing - EUCAST database (Bengtsson-Palme and Larsson 2016). The PNECs listed in Annex 1 need to be l periodically reviewed and updated, potentially by a WHO expert group). The overarching principle should be to use the lowest PNEC reported that is considered sufficiently reliable. When an appropriately derived PNEC for resistance selection for a given API or active intermediate is lacking, a default value of 50 ng/L should be applied (Vestel et al. 2022).

When a PNEC for resistance selection for a given active degradation product is lacking, the PNEC for the corresponding APIs should be used. When a PNEC for an active intermediate is lacking, the PNEC for the most potent API (with the lowest PNEC) that can be derived from that intermediate is to be used, alternatively the default PNEC (50ng/L), whatever is lowest.

A detailed description of derivation of PNECs for resistance selection is included in the attached background document.

PNECs for ecological effects

Ecological risk assessment and management most often have the aim to protect populations rather than individuals, and in the case of microbial ecosystems, the protection targets are primarily preserved ecosystem function and services. Each ecosystem provides an (often unique) range of functions and services, and assessing risk for all processes individually in different ecosystems would be an insurmountable task. Ecological risk assessment is therefore often highly simplified, as is environmental regulation. The aquatic environmental risk assessment for antibiotics within the EMA guidelines (EMA 2006), use a surrogate endpoint - the Predicted No Effect Concentration on growth for an aquatic bacterium (cyanobacteria), as bacteria, in general, are considerably more sensitive to antibiotics than are plants or animals. PNECs for growth in aquatic bacteria is also the target used here, used as a proxy for the potential to disturb ecosystem functions and services (see annex 1).

Box 3: Derivation of PNECs for ecological effects

PNECs for ecological effects should be based on growth inhibition tests of aquatic bacteria, primarily cyanobacteria, according to the OECD 201 standard. If data from several tests/bacterial species are available, the lowest PNEC should be used. If PNEC data is available from other aquatic organisms than bacteria (e.g. green algae using for example ISO 8692) and this value is lower than the PNEC for bacteria, the lowest PNEC should apply. The PNECs listed in Annex 1 need to be l periodically reviewed and updated, potentially by a WHO expert group).

When PNEC for ecological effects for a given API/active intermediate/active degradation product is lacking, a default value of 50 ng/L should be applied (Vestel et al. 2022). If a manufacturer can provide transparent and relevant PNEC data, derived according to the OECD 201 standard, it can replace the default value.

3.1.2 Liquid effluent - antibiotic resistant bacteria

Discharges of antibiotic-resistant bacteria in the wastewater from manufacturing also poses a health risk. Antimicrobials present in the (untreated) wastewater or, in the case of fermentation-based processes already in the microbial culture, have the potential to select for and drive the evolution of resistance before any wastewater is released to the environment. Hence, removing antimicrobials at the end of the process to safe levels does not necessarily completely alleviate risks if resistant bacteria, selected for earlier in the process, are released (Li et al. 2008; Li et al. 2011; Larsson and Flach 2022). While there are standards with regards to bacterial contamination of different kinds in water resources, it is currently difficult to assess what emission levels of resistant bacteria that should be considered safe or unsafe. Hence, this is a risk that is currently best managed by performance indicators (Annex 2).

Technology challenges, risk and solutions differ between fermentation-based production and production based on chemical synthesis. The former often involve very large volumes of waste that is high in organic content, and where there are few alternatives other than microbial treatment for reducing the organic content of the liquid waste. In such cases, final treatment tailored to reduce the cultivable bacterial load (e.g. oxidative treatment, UV-light, chlorination, sterile filtration, thermal treatment) by at least 100-fold compared to the biological treatment alone, should be applied before release to the environment or before sending waste to a municipal WWTP for further treatment.

For chemical production, processes that involve microbial treatment of waste streams with concentrations that strongly exceeds PNECs for resistance selection should be avoided, as this will likely stimulate the evolution of resistant bacteria. If microbial treatment is applied to remove antibiotics from wastewater, disinfection prior to release is encouraged.

All such treatments must be developed in line with local environmental regulations.

3.1.3 Solid waste - antimicrobials and antibiotic resistant bacteria

Antibiotics and antibiotic-resistant bacteria in solid waste also pose a potential health risk, unless managed appropriately. Our understanding of safe levels of antibiotics in solid systems (such as soils) is considerably less mature than in liquid systems, hence what levels of residues or bacteria that are safe/unsafe in solid waste is difficult to assess. Risks for solid waste are therefore currently best managed by performance targets to be achieved by treatment technologies (Annex 3).

Solid waste (excluding fermentation residue) from the manufacturing site or from a third-party, industrial common effluent treatment plant should be incinerated or deposited in secure landfills where infiltration of rainwater and runoff is prevented (approved of these sites as such should be obtained by local authorities). If measures (such as hydrothermal treatment) are taken to reduce the concentrations of active antimicrobials in the solid waste by at least 80 %, the solid waste may then be deposited in different ways (e.g. on land) given that it is in line with local regulations.

In the case of fermentation-based production, very large quantities of solid or semisolid waste (fermentation residue) is produced which often also contains very high antibiotic concentrations (Han et al 2022). For such waste, demands are higher (99% removal through e.g. hydrothermal treatment) to allow other disposal alternatives than incineration or secure landfill. In case wastewater is sent to a third party municipal WWTP treating primarily sewage, the solid waste (sludge) generated at the WWTP only needs be treated according to local regulations.

3.1.4 Zero liquid discharges

Zero liquid discharge is a water treatment system that is designed to remove impurities from the water so that it is clean enough to be suitable for use in facilities (i.e. boilers, cooling towers). These ZLD systems usually include pre-treatment and advanced wastewater treatment technologies, and conventionally uses distillation or evaporation processes separating behind the solid residue for further solid wastes handling while the condensed water vapour reused in some processes. Many manufacturers claim to apply zero liquid discharge. However, a production plant applying ZLD can still produce and discharge liquid waste that is not emitted to waterways but rather reused in other ways, e.g. water for process facilities, horticulture, etc. Unless the wastewater is contained and treated to a point where concentration levels do not exceed PNECs or API is removed (e.g. evaporated and solid waste taken care of appropriately, see section 3.1.3), there are still risks for environmental effects and selection of resistant bacteria. Adding wastewater with high concentrations of antibiotic residues to soils will in many cases select for resistance. Despite difficulties generating a quantitative risk assessment for emissions to soil/land without requesting generation of new PNEC data, this is not an appropriate way to eliminate risks. Therefore, based on a precautionary approach, unless all liquid waste is contained until all the antibiotic is removed, a risk assessment for an aquatic recipient described in section 3.1.1 also applies for liquid waste discharged to land.

3.2 Exposure assessment – estimations of concentrations in wastewater and recipients

Concentrations of antibiotics in effluents can be estimated either through mass balance calculation (estimated losses during production) or through chemical analysis of wastewater samples. While the former does not require established analytical protocols and can be done in-house, it may not provide the precision needed to ensure PNECs are met, and it is generally less transparent than chemical analyses. Chemical analysis is therefore preferred, but it also has limitations. Chemical analysis only reflects concentrations at the time point of sampling and doesn't reveal where lack of control in the process has caused an exceedance – hence section 4 outlines auditable process risk management procedures to ensure targets can be consistently met and that weak points leading to exceedances can be identified and remedied.

Active dilution of wastewater before discharge with the main intent to ensure PNECs are met is not allowed.

3.2.1 Mass-balance calculation of effluent concentration (ECM)

Emission of both APIs and active intermediates and degradation products may be estimated through mass-balance calculations of losses during production estimated at output points shown on Figure 2. Annex 4 provides details of mass balance calculation.

Theoretical removal during wastewater treatment (whether internal or external) is not allowed as an approach to reduce the EC_M. The way to take into account such reductions is to perform chemical analyses of residues in treated wastewater (see section 3.2.3).

In the case of batch production, to calculate effluent emission concentration (EC_M), estimated losses to wastewater over the entire batch production should be used. Note that solid API waste that has been collected/segregated during i.e. dry cleaning of a reactor or spills will not be included to estimate for API losses to wastewater since these will be handled as solid waste and should NOT go to the process wastewater. The API mass lost to process wastewater would be divided by the total wastewater volume (in litres) from the facility during the entire batch process period. If the facility has an onsite WWTP, the % removal of API can be subtracted if known, otherwise 0% removal is assumed.

If the wastewater is treated by a common CETP, mass-balance estimates of concentration in effluent cannot take into account dilution within the CETP. The strategy to take into account dilution (and removal) within the CETP is to apply chemical analyses of the wastewater leaving the CETP (optional).

If the wastewater is treated by a municipal WWTP, mass-balance estimates of concentration in effluent can take into account dilution within the WWTP (24h flow). To also take into account removal within the WWTP (optional), chemical analysis is required.

When estimating losses through mass balance calculations, the sensitivity/precision in the calculations in relation to the PNECs that are to be met, also needs to be taken into account. In particular, in cases where either wastewater volumes are low or PNECs are low, demands on precision in the mass balance calculation become particularly high. Therefore, an analysis needs to be presented on how small losses can be quantified with certainty, including estimates of potential errors. If losses down to the amounts that would equal the PNEC in the relevant exposure media (wastewater and/or recipient) cannot be determined with certainty, mass balance calculation alone is not considered sufficient and needs to be complemented with chemical analyses.

If losses corresponding to concentrations below the PNEC may be detected through a mass-balance approach, a risk assessment can be performed based on such estimates (Annex 4). A mass balance calculation of the EC_M should, regardless of its precision/sensitivity, always be carried out, as it can provide indication of how far above the PNECs emissions might be and reveal where and when in the process losses are likely to occur. This in turn defines when samples for sampling and chemical analyses in wastewaters should occur (see section 3.2.2).

3.2.2 Chemical analyses of effluent concentration (ECA)

Figure 2 shows five possible sampling locations for chemical analysis of liquid effluent depending on the treatment systems for the manufacturing site:

1. Untreated wastewater at the outlet to a municipal sewer or water body
2. Treated wastewater sampled at the outlet of in-house wastewater treatment processes
3. Treated wastewater at the outlet of common effluent treatment plant (CEPT) dedicated to the treatment of industrial wastewater (e.g., at an industrial park)
4. Treated wastewater from a municipal wastewater treatment plant (WWTP)
5. Treated wastewater from in-house treatment for application to land (ZLD)
6. Treated wastewater from CEPT treatment for application to land (ZLD)

Samples for chemical analyses should be taken at the point of discharge from the factory during active production, including the time when the release of antibiotics are expected to be highest, taking into account residence time of the wastewater.

Note that sampling and analyses should always be done on undiluted wastewater, not within the recipient as this includes many more sources of variability (as well as uncertainty with regards to accountability). Detailed sampling considerations, including the application of composite sampling strategies and storage and method for chemical analysis are provided in annex 5.

Chemical analyses should be done with a method that has sufficient sensitivity (Limit of Quantification, LOQ) to meet the targeted PNECs. Sensitivity should be evaluated using wastewater spiked with the analytical target in question. Measured concentrations in the wastewater as well as the LOQ of the method should be publicly disclosed (see section 6.3.3 on public transparency). Further information on the validation of the analytical method should be available on request.

When it is expected that concentrations of microbially active intermediates or specific degradation products (Lourenço et al. 2022) in the waste could exceed those of the active ingredient (the API) as based on mass-balance estimates, chemical analyses (and subsequent risk assessment) should include such agents.

3.2.3 Applying dilution factors to estimate exposure in recipients

As explained under 3.3, ecological risk assessments are always performed on estimated recipient concentrations, while risk assessments for resistance selection can be performed on either effluent concentrations or recipient water concentrations. To estimate exposure in the recipient, a fixed dilution factor should be applied. For discharges to inland waters, this dilution factor should be set to 10, whereas for discharges to the sea, it should be set to 100, in line with e.g. the environmental risk assessment procedures for pharmaceuticals in the EU by EMA (EMA 2006).

3.3 Assessment against targets (risk assessment)

A risks assessment is needed for all parts of the manufacturing chain of a product where liquid emissions of antibiotics or active intermediates/degradation products may occur, e.g. during production of active intermediates, APIs or during formulation. For emissions via solid waste, no formalised risk assessment is required. Instead, adequate measures for disposal must be taken and documented or performance targets need to be met or specified technologies used (see section 3.1.3). It is important to note that emissions always need to comply with local/regional/national standards/legislation/permits.

An unacceptable risk level is if the exposure/PNEC ratio is above 1.

As clarified under section 2 a key guiding principle is progressive improvement, and adaptability to different uses. Progressive improvement acknowledges that users may have reasons to apply criteria with different levels and advance to higher levels of stringency over time. Hence, Table 3 allows two levels: BASIC and STRINGENT.

Table 3: Pathway for progressive improvement

	Basic	**Stringent**
Method for estimating effluent concentration, EC	Mass balance (EC_M) sufficient to assess RQs but chemical analyses can replace mass balance estimates	Chemical analyses (EC_A) required to assess RQs but mass balance should be available as a complement
Risk assessment for selection, RQ_{res} applied to concentrations in wastewater or recipient	RQ res = $\frac{ECm/\,10}{PNEC\ res}$	RQ res = $\frac{ECa}{PNEC\ res}$
Site of waste water treatment	Release through municipal WWTP will disqualify possibilities for STRINGENT unless PEC/PNEC ratios are already met based on concentrations in wastewater sent to external WWTP	In-house or industrial CETP required (unless PEC/PNEC ratios are already met based on concentrations in wastewater sent to external WWTP)

The dilution depends on the recipient water body: 10 for inland water bodies and 100 for sea/ocean.

To meet the level of STRINGENT, the risk assessment must be based on chemical analysis, effluent concentrations (rather than recipient concentrations) must be applied to assess risks for resistance selection, and the wastewater must be treated in-house or at a dedicated industrial CETP. The only exception where treatment at a municipal WWTP can qualify for STRINGENT is if the RQs are already met based on measured concentrations in the wastewater leaving the industrial facility (for ecological risks, actual dilution within the municipal WWTP and the fixed recipient dilution factor can still be taken into account). Risk quotients for STRINGENT needs to be met for both ecological risk assessment and resistance selection in order to meet an overall level of STRINGENT. To reach BASIC, both need to meet the level of BASIC. For a final product to meet the criteria of STRINGENT, all facilities in the

production chain where risks for emissions of liquid waste containing antimicrobials need to meet the level of STRINGENT. Correspondingly, all facilities need to meet the level of BASIC in order for a final product to qualify for BASIC. With regards to solid waste streams, there are no separation of BASIC or STRINGENT, but simply pass or fail. Management of solid waste need to pass the requirements stipulated under 3.1.2 in order for the production process to qualify for BASIC or STRINGENT.

To meet BASIC	To meet STRINGENT
☑ RQres and RQeco for basic needs to be met ☑ risk assessment using mass balance is sufficient, but could be replaced by risk assessment based on chemical analyses	☑ risk assessment based on chemical analyses ☑ effluent concentrations (not recipient) applied to calculate RQres ☑ wastewater treatment in-house or at a dedicated CETP ☑ wastewater treatment at a municipal treatment can qualify if the RQres is met based on ECa from the pipe. RQeco allows taking into account the dilution factor of the MWTP and dilution factor of recipient water ☑ RQres and RQeco for stringent needs to be met

In the case where a PEC is generated not only for an API but also for active intermediates and/or active degradation products of that API in the same wastewater (see section 3.1.1), the sum of the concentrations should be compared with the PNEC for the API, unless there are separate PNECs available for the active intermediates/degradation products and each of these PNECs are at least 10 times lower than the PNEC for the API. In such cases, risk assessment should be done separately for each active compound.

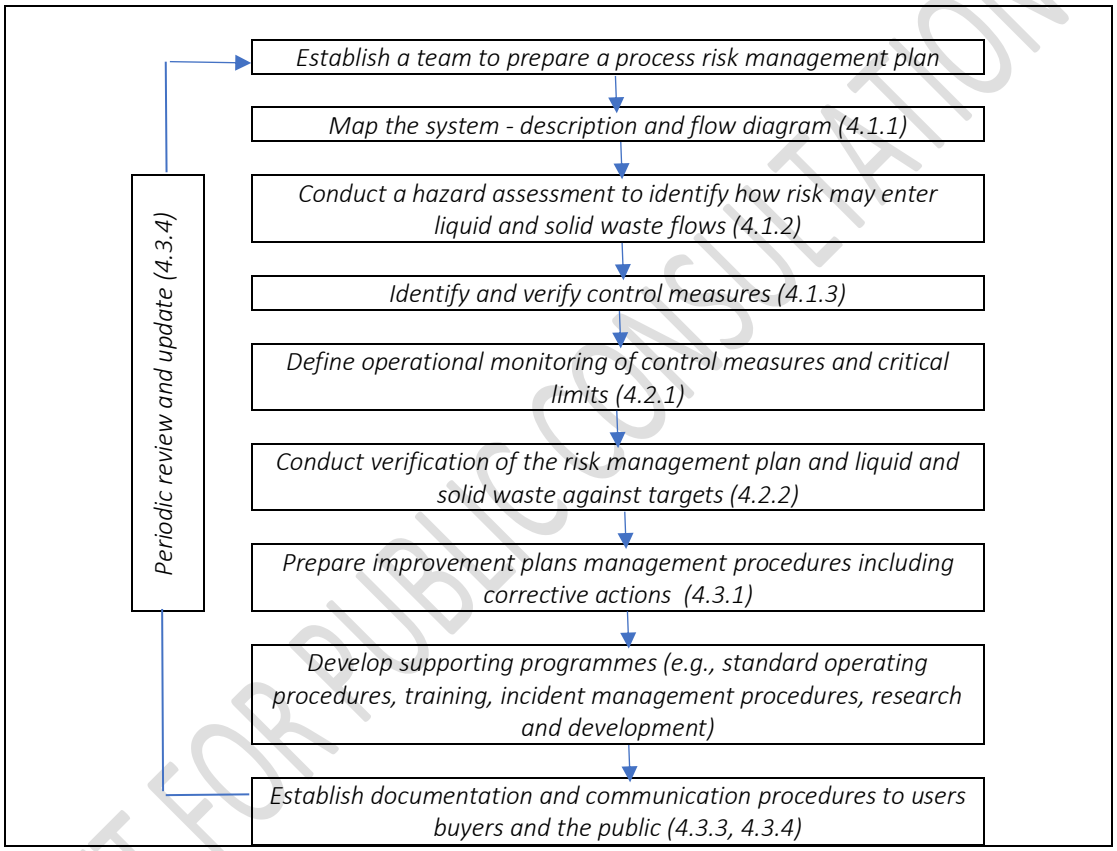
Presented here are different levels of uncertainty-reduction in the assessments of risks that could be used internally as levels of progression, and by external parties (e.g. procurers) as multi-level criteria with e.g. different levels of “rewards” coupled to meeting different levels. The guidance has a level of flexibility in that a procurer of final products may, if desired, introduce more levels of rewards by assessing and rewarding different production steps separately. Alternatively, the different levels presented may not be applied to stimulate step-wise progression, but rather as criteria that balances the needs for the given application. For example, as there are strong reasons to preserve access to medicines, the basic criteria presented are likely more suitable for applications that potentially could lead to market exclusion (such as GMP). Progress pathways should be defined through engagement with suppliers to mitigate the risk of excluding significant proportions of manufacturers from the market.

In order to facilitate progressive improvement, it is essential to implement a performance measurement system to track and improve different aspects of the manufacturing process and ensure that the company continuously aligns and advances with its objectives. Some examples that could be considered for instance is to identify key performance indicators (KPIs) such as production efficiency or optimized resource utilization. Measurement, data collection, monitoring, analysis and reporting provides insights on the performance of relevant processes that could contribute to unwanted release of antibiotics and helps facility managers to understand and put countermeasures if necessary.

4 Process risk management plans

This section describes the management process manufacturers should follow to identify and manage risks such that antimicrobial emissions in wastewater and solid waste meet targets described in the section 3. The risk assessment and management process follow the Hazard and Critical Control Point (HACCP) approach and steps (Figure 3) common across many WHO guidance documents. The contextualized risk management plans developed under this section of the guidance should be subject to verification by audits as described in section 5.

Figure 3: Overview of steps of a risk management plan



4.1 System assessment

4.1.1 Map the production system

For a comprehensive understanding of the manufacturing process, mapping of the production system for each site is important. This assessment will be applied for antibiotics and/or active intermediates based on their distinctive production techniques (fermentation, semi-synthetic, and synthetic processes) and on the finished product. The mapping process should involve the following steps:

- Identification of the different stages involved in the production of antibiotics from the active intermediate. In the case of the manufacture of a finished product, the

mapping starts from the active ingredient (API) to the finished product in its primary packaging, to identify which of the stages involved has any risk of release of such compounds.
- Documentation of the equipment, infrastructure, and operations involved at each stage, focusing on the areas where potential risks of antibiotic release into the effluent may arise (i.e. washing processes, tablet compression, capsule filling, etc.).
- Identification of the raw material inputs and outputs of each process (chemical reagents, solvents, fermentation production wastewater, chemical synthesis production wastewater, washing wastewater, antibiotic fermentation residue and sludge streams).
- Evaluation of the flow of materials and waste streams throughout the production process, including any potential cross-contamination or spill points.

4.1.2 Identify hazards that may release antibiotics into waste streams

With the production flow mapped, key processes that contribute to the release of antibiotics and their active intermediates into the effluent stream need to be identified and should include both process-related and equipment-related risk factors. This can involve quantification using mass-balance calculations. The following steps should be considered:

- Identification of stages in the process where loss of the active ingredient or active intermediate cannot be prevented due to the inherent nature of the process (e.g., Losses that occur in the mother liquors during crystallisation and recrystallisation or losses that occur as solid waste/powders during tabletting or capsule filling)
- Identification of potential sources of accidental antibiotic or antibiotic intermediate contamination and release to the effluent stream in each stage of the production process. This may include leaks in storage and pipes, spills, improper handling or storage of chemicals, as well as inadequate containment measures.
- Identification of stages where there is a potential for the escape or leakage of antibiotics, such as during transfers between vessels or equipment, cleaning procedures, or waste disposal practices.

4.1.3 Verify effectiveness of existing controls and introduce new controls where risks are not well managed

When hazards have been identified, it is important to verify how effective the existing process controls being applied are. The main types of waste from pharmaceutical manufacturing include antibiotic production wastewater and antibiotic fermentation residues. Controlling emission sources is the priority for deterring the transmission of antibiotic resistance in the environment (Zhang et al., 2022; Han et al., 2023). This includes preventive actions such as process improvements, loss minimization, additional dry-cleaning steps, and corrective actions in the end of pipe such as implementing wastewater pre-treatment, advanced oxidation techniques or other interventions to prevent the release of antibiotics in the final treated discharge. Interventions that were applied to not exceed the risk quotient also need to be verified. This process involves the following actions:

- Review of the existing control measures and mitigation strategies in place for each identified emission pathway for potential loss of antibiotic. This includes process/engineering controls, operating procedures, and manufacturing work instructions, and maintenance protocols.

- Thoroughly evaluate the effectiveness of these controls in minimizing antibiotic release into the effluent. This will involve conducting a new risk assessment (through mass balance or chemical analyses, depending on the desired stringency level (see 6.4)), and may also involve analyses of historical data.

- Areas where existing controls are not effectively addressing the risks should be identified and new control measures to address these gaps need to be developed and implemented. These measures may include process improvements, additional cleaning steps, targeted adsorbance of antimicrobials (such as separation to solids), pre-treatment technologies, and advanced oxidation methods.

- A monitoring and verification system to ensure the effectiveness of the implemented controls needs to be established. This should include records of inspections, sampling and analysis of effluent samples, and documented review of control measures to adapt to changes in production processes or emerging risks, at least once a year.

If these measures are followed, the manufacturing site can assess the production process thoroughly, identify potential hazards, and implement effective controls to prevent antimicrobial release into the waste.

4.2 Monitoring

4.2.1 Operational monitoring

Regular operational monitoring is necessary to assess the overall performance of the manufacturing system and ensure that the controls in place are working effectively. This should include the collection of basic data on correct performance of process controls.

- Monitor controls along the system: Establish a monitoring program to collect data on the basic key parameters such as flow rates, batch times, and cleaning frequency. Data on process mass intensity could be provided as a convenient benchmark to assess the efficiency of a process. This information helps track the operational efficiency of the system and allows for the identification of any deviations or anomalies that may impact antibiotic release into the effluent.
- Estimate API losses: Conduct a desktop analysis of the mass flows through recent batch records, applying conservative factors. This approach will however only give an estimate of the order of magnitude and not the actual emissions to the environment. From these calculations, a comparison between the PEC and PNEC (RQ) is determined. If PEC exceeds PNEC for the antibiotic being assessed, further evaluation needs to be conducted. This can include wastewater sampling and analysis of a representative sample from the final water output. Take appropriate action to reduce the release of the API.

4.2.2 Verification monitoring

Verification monitoring conform actual system performance against targets described in Section 3.

- Conduct laboratory analysis of the effluent sample to confirm if it meets the required standards and regulatory guidelines of relevant parameters. This analysis should include testing for antibiotic and active intermediate residues to check if RQ < 1. Verifying the system performance regularly ensures that the implemented controls are effective and provides an early warning system for any potential issues or deviations.

4.3 Management and communication

Effective management and communication practices are essential to ensure the sustainability of antibiotic manufacturing operations and maintain a responsible approach to environmental stewardship.

4.3.1 Identify and implement system improvements

Continuous assessment and improvement of processes and management systems are pivotal to ensure that environmental impact from antibiotics manufacturing are mitigated. Data and results from system assessment and operational monitoring (Sections 4.1. and 4.2) should be used to identify areas where improvements can be made. Strategies to be implemented should always aim to optimize resource utilization, minimize waste generation and reduce overall environmental footprint. A structured feedback loop involving different stages of the manufacturing process and across departments should be established and practiced for smooth integration of suggestions for improvement.

4.3.2 Internal training and communication

Internal communication is crucial in promoting a culture of environmental responsibility among the employees and facilitating the sharing of knowledge and experience throughout the production chain. The following aspects should be considered:

- Provide training and educational programs to employees at all levels regarding the importance of environmental protection, responsible antibiotic manufacturing practices, and the role they play within their work responsibilities in minimizing antimicrobial release. This training should include information on best practices, standard operating procedures to minimize risk in the context of regulatory requirements, and the potential impacts of antimicrobial contamination on human health and the environment.
- Establish effective communication channels within the organization to facilitate the exchange of information and experiences between different steps in the production chain. This can vary for each site depending on the normal practices, but should

include regular meetings, workshops, information boards, newsletters, or digital platforms where employees can share observations and ideas, raise concerns related to their work that may impact release of antimicrobials to the effluent stream or to the environment, and collaborate on finding solutions to improve overall environmental performance.

- Regular discussions in small groups should be encouraged for employees in the manufacturing line to participate in continuous quality improvement initiatives by sharing their insights, innovative ideas, and lessons learned. Teams that contribute to improvement and implementation of more effective environmental management practices to minimize antimicrobials in the effluent discharge stream should be recognized and rewarded to foster continuous improvements.
- Documentation of best practices and successful interventions in minimizing antimicrobial release should be communicated within the organization. This can be done through internal reports, case studies, or internal knowledge-sharing platforms, to ensure that valuable lessons learned are accessible to all relevant stakeholders.

Having an active internal communication and knowledge-sharing enhances the overall environmental awareness within the organization and promotes a stronger sense of collective responsibility to minimize release of antibiotics or intermediates in the different stages of the entire production process at the site.

4.3.3 External communication to users/buyers

It is important to establish effective communication channels with the procurers of the antibiotics to ensure transparency and build trust in the manufacturing process of each site. This can be through the following actions:

1. A dedicated platform in the company website should be available where procurers and other relevant stakeholders are able to access detailed information about relevant parts of the manufacturing process, environmental performance, and any ongoing improvement initiatives. It should include a means for providing feedback or asking questions regarding the environmental impact of the site's manufacturing operations.
2. Engaging in dialogues with relevant stakeholders, such as healthcare professionals, regulatory authorities, procurers and environmental organizations, opens a channel to gather feedback and address concerns related to antibiotic production, and at the same time build trust. Actively seeking opportunities for collaborations and partnerships to apply recent scientific developments will help drive continuous improvement in the manufacturing process.

4.3.4 Public transparency

Transparency is key for accountability and for incentivizing measures to reduce pollution through different parallel means. Transparency on who (what company) is responsible for the different steps in a production chain, where exactly each production step takes place, and how pollution is managed and that relevant targets are met in each of these sites,

allows a broad range of actors to respond in a way to stimulate positive change (Schaaf et al., 2020). Reciprocally, lack of transparency creates an uncertainty that can be viewed as an external cost and ultimately a global health risk (Nijsingh, Munthe, and Larsson 2019). To fulfil the criteria of this guideline, the manufacturer should, e.g. through a publicly accessible company website, make data available on emission levels (concentrations of antibiotics in wastewaters and recipient), method of estimating such concentrations (mass balance or chemical analyses), and for a final product, indicate the exact sites and manufacturer of each production step (active intermediate, API, formulation, packaging), similar to what is done in New Zealand (Årdal et al. 2021).

Environmental reporting is a key component for public transparency. It serves to inform stakeholders (regulatory authorities, other industries, public, etc.) on the efforts of the manufacturer to protect the environment. This provides valuable information on the impact of their activities in their immediate vicinity such as the state of the receiving water body, and report on the progress and improvements made in addressing the environmental challenges especially in achieving environmental concentrations of antibiotics below PNEC values. Reliable and timely reporting encourages and allows space for the scientific community, the public and policy makers to have a thorough understanding of the challenges and the limitations of the manufacturers in achieving targets, and encourages participation of the local community in discussions of issues that potentially affect them. This also allows regulators to get accurate information and helps them to develop environmental policies and strategies that are fact-based, implementable and verifiable.

Sustainability certifications also play an important role in showing public transparency as these processes usually provide a framework and set of standards that manufacturers can follow. The consistency of these certification standards makes it easier for stakeholders to understand and compare the adherence and efforts of different manufacturers from different regions on a global scale. To obtain these certification standards (i.e. ISO certifications), manufacturing companies undergo thorough audits and assessments by a third party, providing independent validation that sustainability claims from for example their environmental reporting are not merely greenwashing. Manufacturers with these certifications may be required to release some relevant environmental information to the public thereby promoting transparency.

4.3.5 Review and update

The management plan developed above should be regularly reviewed and updated to identify any new hazards, reflect improved controls and incorporate system improvement identified in audits with the objective of progressive and continuous improvement (section 5 below). Risk management plans should be reviewed at least annually and after any incident that lead to an exceedance of targets.

5 Surveillance and verification

There are two types of approaches to surveillance to independently verify performance against targets (Section 3). These are:

- Audits of risk management plans and verification monitoring results or
- Direct assessment of effluent.

Approaches may include a mix of both. However, audits of the quality of risk management plans (section 4) and operational and verification monitoring results (section 3 and 4) are the first option and may be sufficient without direct assessment if the audit does not reveal system weaknesses or exceedance of targets.

5.1 Audit

In the audit approach to surveillance, assessment activities, including verification testing, are undertaken largely by the manufacturer as described in section 4 with third-party auditing to verify compliance. The third party surveillance agency may be any of the target audience described in section 1.3.

Audits require expertise and capacity within third party surveillance agency or contracted audit service provider to:

- prepare an audit strategy detailing the selection and frequency of manufacturing facilities to be audited
- undertake or oversee auditing of the risk management plans for selected manufacturing sites as a programmed routine activity;
- respond to, investigate and provide advice on receipt of reports on significant incidents

The implementation of an audit-based approach places responsibility on the manufacturers to provide the surveillance agency with risk management plan documentation and information on performance against targets. Auditors may inspect via announced and unannounced visits for assurance of true independent verification of the activities of the manufacturer.

Periodic audit would normally include the following elements:

- examination of records to ensure that system management is being carried out as described in the risk management plan
- checking if operational monitoring parameters are kept within operational limits and that compliance is being maintained
- ensuring that verification programmes are carried out and review results against targets
- assessment of supporting programmes and of strategies for improvement of the risk management plan
- provide a summary assessment of performance according to STRINGENT, BASIC, or FAIL classification (Section 3)
- Provide recommendation for improvement of the risk management plan where needed

The surveillance agency will normally retain the authority to undertake some analysis of effluent quality to verify performance or enter into a third-party arrangement for such analysis.

5.2 Direct assessment

It may be appropriate for the surveillance agency to carry out independent testing of effluent if the audit reveals shortcoming in the risk management plan or verified system performance. Direct assessments requires access to analytical facilities (e.g., accredited laboratories) with staff trained to carry out sampling at the appropriate moment during production.

Direct assessment may lead to the identification of requirements to amend or update the risk management plan (Section 4) and the summary assessment of the facilities as having met STRINGENT, BASIC, or FAIL classification (Section 3.) General guidance on assessment against targets which is also applicable to surveillance through direct assessment is provided in Section 3.

6 Implementation considerations

[To be inserted]

7 Guidance development process

7.1 Search strategy and evidence review and quality appraisal

[To be inserted from background document]

7.2 Evidence to decision-making process

Evidence was synthesized into guidance text based on quality assessment and evidence to decision criteria and presented to the expert group for decision by consensus via online meetings and email exchange of draft text. A public consultation process was completed with written and verbal feedback for public submitters taken into account. Decision criteria used were: feasibility for immediate implementation, resources requirements, intervention/option acceptable to all stakeholders, balance between benefits and harms, impact on equity. The revised draft was then circulated for external review and feedback compiled into the final document.

7.3 Plans for updates

WHO will monitor uptake and implementation by stated target audiences and also new scientific literature with a view to providing updated implementation guidance and revised targets (i.e. PNECs and technology targets Annex 1-3) within approximately 5 years.

7.4 Selection and declaration of interests

Expert group members were selected via research and practitioner networks working on environmental dimensions of AMR globally. Selection aimed for a balance of research and implementation experience, gender and regional representation. All members of the expert group signed declarations of interest, which was reviewed in accordance with WHO principles and policies and assessed for any conflicts of interest. No conflicts of interest were identified that required individuals to abstain from consensus decision making.

Annex 1: PNECs for resistance selection and ecological effects

List of PNECs for resistance selection

Refer to Background Document *Evidence synthesis for deriving PNECs for resistance selection* for details of derivation of PNEC values listed and list of references

Active Pharmaceutical Ingredient	**PNEC for resistance selection (µg/L)**	**Reference**
Amikacin	16.000	Bengtsson-Palme and Larsson, 2016
Amoxicillin	0.250	Bengtsson-Palme and Larsson, 2016
Ampicillin	0.250	Bengtsson-Palme and Larsson, 2016
Avilamycin	8.000	Bengtsson-Palme and Larsson, 2016
Azithromycin	0.250	Bengtsson-Palme and Larsson, 2016
Aztreonam	0.500	Bengtsson-Palme and Larsson, 2016
Bacitracin	8.000	Bengtsson-Palme and Larsson, 2016
Benzylpenicillin	0.250	Bengtsson-Palme and Larsson, 2016
Capreomycin	2.000	Bengtsson-Palme and Larsson, 2016
Cefaclor	0.500	Bengtsson-Palme and Larsson, 2016
Cefadroxil	2.000	Bengtsson-Palme and Larsson, 2016
Cefaloridine	4.000	Bengtsson-Palme and Larsson, 2016
Cefalothin	2.000	Bengtsson-Palme and Larsson, 2016
Cefazolin	1.000	Bengtsson-Palme and Larsson, 2016
Cefdinir	0.250	Bengtsson-Palme and Larsson, 2016
Cefepime	0.500	Bengtsson-Palme and Larsson, 2016
Cefixime	0.060	Bengtsson-Palme and Larsson, 2016
Cefoperazone	0.500	Bengtsson-Palme and Larsson, 2016
Cefotaxime	0.130	Bengtsson-Palme and Larsson, 2016
Cefoxitin	8.000	Bengtsson-Palme and Larsson, 2016
Cefpirome	0.060	Bengtsson-Palme and Larsson, 2016
Cefpodoxime	0.250	Bengtsson-Palme and Larsson, 2016
Ceftaroline	0.060	Bengtsson-Palme and Larsson, 2016
Ceftazidime	0.500	Bengtsson-Palme and Larsson, 2016
Ceftibuten	0.250	Bengtsson-Palme and Larsson, 2016
Ceftiofur	0.060	Bengtsson-Palme and Larsson, 2016
Ceftobiprole	0.250	Bengtsson-Palme and Larsson, 2016
Ceftriaxone	0.030	Bengtsson-Palme and Larsson, 2016
Cefuroxime	0.500	Bengtsson-Palme and Larsson, 2016
Cephalexin	4.000	Bengtsson-Palme and Larsson, 2016
Chloramphenicol	8.000	Bengtsson-Palme and Larsson, 2016
Ciprofloxacin	0.064	Bengtsson-Palme and Larsson, 2016
Clarithromycin	0.250	Bengtsson-Palme and Larsson, 2016
Clinafloxacin	0.500	Bengtsson-Palme and Larsson, 2016
Clindamycin	1.000	Bengtsson-Palme and Larsson, 2016

Cloxacillin	0.130	Bengtsson-Palme and Larsson, 2016
Colistin (Polymyxin E)	2.000	Bengtsson-Palme and Larsson, 2016
Daptomycin	1.000	Bengtsson-Palme and Larsson, 2016
Doripenem	0.130	Bengtsson-Palme and Larsson, 2016
Doxycycline	2.000	Bengtsson-Palme and Larsson, 2016
Enrofloxacin	0.060	Bengtsson-Palme and Larsson, 2016
Ertapenem	0.130	Bengtsson-Palme and Larsson, 2016
Erythromycin	1.000	Bengtsson-Palme and Larsson, 2016
Ethambutol	2.000	Bengtsson-Palme and Larsson, 2016
Faropenem	0.020	Bengtsson-Palme and Larsson, 2016
Fidaxomicin	0.020	Bengtsson-Palme and Larsson, 2016
Florfenicol	2.000	Bengtsson-Palme and Larsson, 2016
Flumequine	0.250	Bengtsson-Palme and Larsson, 2016
Fosfomycin	2.000	Bengtsson-Palme and Larsson, 2016
Fusidic acid	0.500	Bengtsson-Palme and Larsson, 2016
Gatifloxacin	0.130	Bengtsson-Palme and Larsson, 2016
Gemifloxacin	0.060	Bengtsson-Palme and Larsson, 2016
Gentamicin	1.000	Bengtsson-Palme and Larsson, 2016
Imipenem	0.130	Bengtsson-Palme and Larsson, 2016
Isoniazid	0.130	Bengtsson-Palme and Larsson, 2016
Kanamycin	2.000	Bengtsson-Palme and Larsson, 2016
Levofloxacin	0.250	Bengtsson-Palme and Larsson, 2016
Lincomycin	2.000	Bengtsson-Palme and Larsson, 2016
Linezolid	8.000	Bengtsson-Palme and Larsson, 2016
Loracarbef	2.000	Bengtsson-Palme and Larsson, 2016
Mecillinam	1.000	Bengtsson-Palme and Larsson, 2016
Meropenem	0.060	Bengtsson-Palme and Larsson, 2016
Metronidazole	0.130	Bengtsson-Palme and Larsson, 2016
Minocycline	1.000	Bengtsson-Palme and Larsson, 2016
Moxifloxacin	0.130	Bengtsson-Palme and Larsson, 2016
Mupirocin	0.250	Bengtsson-Palme and Larsson, 2016
Nalidixic acid	16.000	Bengtsson-Palme and Larsson, 2016
Narasin	0.500	Bengtsson-Palme and Larsson, 2016
Neomycin	2.000	Bengtsson-Palme and Larsson, 2016
Netilmicin	0.500	Bengtsson-Palme and Larsson, 2016
Nitrofurantoin	64.000	Bengtsson-Palme and Larsson, 2016
Norfloxacin	0.500	Bengtsson-Palme and Larsson, 2016
Ofloxacin	0.500	Bengtsson-Palme and Larsson, 2016
Oxacillin	1.000	Bengtsson-Palme and Larsson, 2016
Oxytetracycline	0.500	Bengtsson-Palme and Larsson, 2016
Pefloxacin	8.000	Bengtsson-Palme and Larsson, 2016
Phenoxymethylpenicillin	0.060	Bengtsson-Palme and Larsson, 2016
Piperacillin	0.500	Bengtsson-Palme and Larsson, 2016
Retapamulin	0.060	Bengtsson-Palme and Larsson, 2016

Rifampicin	0.060	Bengtsson-Palme and Larsson, 2016
Roxithromycin	1.000	Bengtsson-Palme and Larsson, 2016
Secnidazole	1.000	Bengtsson-Palme and Larsson, 2016
Sparfloxacin	0.060	Bengtsson-Palme and Larsson, 2016
Spectinomycin	32.000	Bengtsson-Palme and Larsson, 2016
Spiramycin	0.500	Bengtsson-Palme and Larsson, 2016
Streptomycin	16.000	Bengtsson-Palme and Larsson, 2016
Sulfamethoxazole	16.000	Bengtsson-Palme and Larsson, 2016
Teicoplanin	0.500	Bengtsson-Palme and Larsson, 2016
Telithromycin	0.060	Bengtsson-Palme and Larsson, 2016
Tetracycline	0.100	Stanton et al, 2020; Lundström et al, 2016[3]
Thiamphenicol	1.000	Bengtsson-Palme and Larsson, 2016
Tiamulin	1.000	Bengtsson-Palme and Larsson, 2016
Ticarcillin	8.000	Bengtsson-Palme and Larsson, 2016
Tigecycline	1.000	Bengtsson-Palme and Larsson, 2016
Tilmicosin	1.000	Bengtsson-Palme and Larsson, 2016
Tobramycin	1.000	Bengtsson-Palme and Larsson, 2016
Trimethoprim	0.500	Bengtsson-Palme and Larsson, 2016
Trovafloxacin	0.030	Bengtsson-Palme and Larsson, 2016
Tylosin	4.000	Bengtsson-Palme and Larsson, 2016
Vancomycin	8.000	Bengtsson-Palme and Larsson, 2016
Viomycin	2.000	Bengtsson-Palme and Larsson, 2016
Virginiamycin	2.000	Bengtsson-Palme and Larsson, 2016

References: [reference formatting to be completed]

Bengtsson-Palme, J. and D.G.J. Larsson, *Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation.* Environment International, 2016. **86**: p. 140–149.

Lundstrom, S.V., et al., *Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms.* Sci Total Environ, 2016. **553**: p. 587-95

Stanton, I.C., et al., *Evolution of antibiotic resistance at low antibiotic concentrations including selection below the minimal selective concentration.* Communications Biology, 2020. **3**(1).

[3] The PNECs are based on the LOECs (rather than on the NOECs) with an assessment factor of 10, given the extensive data on selection by tetracycline. See also main text.

List of PNECs for ecological effects

The PNEC values for ecological risk below are for proposed inclusion in Annex 1 of "WHO Guidance on waste and wastewater management in pharmaceutical manufacturing with emphasis on antibiotic production" in the absence of other scientifically derived values. The approach for PNEC derivation is described in Vestel et al., 2021.

Active Pharmaceutical Ingredient	**ATC Drug Class**	**PNEC eco (µg/L)**	**PNEC eco rationale**	**Test Guideline/ Reference**	**Reference**
Amikacin	Aminoglycoside	-	-	-	No data
Amoxicillin	Penicillin	0.57	Anabaena flos-aquae EC10 ÷ 10[a]	OECD 201	Industry data
Ampicillin	Penicillin	0.60	Cyanobium gracile EC10 ÷ 10	OECD 201	Le Page et al., 2019
Avilamycin	Orthosomycin	125	Synechococcus leopolensis NOEC ÷ 10	OECD 201	Industry data
Azithromycin	Macrolide	0.03	Microcystis aeruginosa EC10 ÷ 10	EPA 1002.0	Industry data
Aztreonam	Monobactam	-	-	-	No data
Bacitracin	Cyclic peptide	114.59	Geomean of Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Bedaquiline	Diarylquinolines	0.08	Anabaena flos-aquae NOEC ÷ 10	OECD 201	Industry data
Benzylpenicillin	Penicillin	-	-	-	No data
Capreomycin	Antituberculosis Agent	-	-	-	No data
Cefaclor	Cephalosporin	-	-	-	No data
Cefadroxil	Cephalosporin	0.14	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Cefalonium	Cephalosporin	21.1	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Cefaloridine	Cephalosporin	-	-	-	No data
Cefalothin	Cephalosporin	-	-	-	No data
Cefazolin	Cephalosporin	-	-	-	No data
Cefdinir	Cephalosporin	-	-	-	No data
Cefepime	Cephalosporin	1.30	*Anabaena flos-aquae* EC_{10} ÷ 10	OECD 201	Industry data
Cefixime	Cephalosporin	0.60	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Cefoperazone	Cephalosporin	-	-	-	No data
Cefotaxime	Cephalosporin	0.12	Anabaena cylindrica EC10 ÷ 10	OECD 201	Le Page et al., 2019
Cefoxitin	Cephalosporin	-	-	-	No data
Cefpirome	Cephalosporin	-	-	-	No data
Cefpodoxime proxetil	Cephalosporin	1.76	Anabaena flos-aquae EC10 ÷ 10a	OECD 201	Industry data

Cefquinome	Cephalosporin	1.60	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Ceftaroline	Cephalosporin	0.12	Anabaena flos-aquae NOEC ÷ 10	OECD 201	Industry data
Ceftazidime	Cephalosporin	1.30	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Ceftibuten	Cephalosporin	-	-	-	No data
Ceftiofur	Cephalosporin	-	-	-	No data
Ceftobiprole	Cephalosporin	0.23	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Ceftolozane	Cephalosporin	1.90	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Ceftriaxone	Cephalosporin	0.33	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Cefuroxime	Cephalosporin	1.70	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Cephalexin	Cephalosporin	0.21	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Cephradine	Cephalosporin	0.19	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Chloramphenicol	Amphenicol	-	-	-	No data
Chlortetracycline	Tetracycline	5.00	Raphidocelis subcapitata EC10 ÷ 10	OECD 201	Industry data
Ciprofloxacin	Fluoroquinolone	0.45	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Ebert et al., 2011
Clarithromycin	Macrolide	0.25	Raphidocelis subcapitata NOEC ÷ 10	OECD 201	Watanabe et al. 2016
Clinafloxacin	Fluoroquinolone	-	-	-	No data
Clindamycin	Lincomycin	0.10	Raphidocelis subcapitata EC10 ÷ 10	OECD 201	Industry data
Cloxacillin	Penicillin	20.00	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Colistin (Polymyxin E)	Polymixin	9.00	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Daptomycin	Cyclic lipopeptide	510	Pimephales promelas NOEC ÷ 10	OECD 210	Industry data
Delamanid	Nitroimidazole	0.03	Raphidocelis subcapitata NOEC ÷ 10	OECD 201	Industry data
Doripenem	Carbapenem	0.46	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Doxycycline	Tetracycline	25.10	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Enramycin	Polypeptide	4.80	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Enrofloxacin	Fluoroquinolone	1.91	Anabaena flos-aquae NOEC ÷ 10	OECD 201	Ebert et al., 2011
Ertapenem	Carbapenem	14.00	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data

Erythromycin	Macrolide	0.50	Anabaena sp. CPB4337 EC10 ÷ 10	OECD 201	Gonzalez-Pleiter, et al., 2013
Ethambutol	Antituberculosis Agent	-	-	-	No data
Faropenem	Penem	-	-	-	No data
Fidaxomicin	Macrolide	891	Pimephales promelas NOEC ÷ 10	OECD 210	Industry data
Florfenicol	Phenicol	38.0	Anabaena flos-aquae EC10 ÷ 10[f]	OECD 201	Industry data
Flucloxacillin	Penicillin	26.8	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Flumequine	Fluoroquinolone	-	-	-	No data
Fosfomycin	Phosphonic	52.4	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Fusidic acid	Steroid Antibacterial	-	-	-	No data
Framycetine	Aminoglycoside	-	-	-	No data
Gatifloxacin	Fluoroquinolone	-	-	-	No data
Gamithromycin	Macrolide	0.24	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Gemifloxacin	Fluoroquinolone	-	-	-	No data
Gentamicin	Aminoglycoside	0.15	Raphidocelis subcapitata EC10 ÷ 10	OECD 201	Industry data
Imipenem	Carbapenem	0.41	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Isoniazid	Hydrazide	-	-	-	No data
Kanamycin	Aminoglycoside	1.05	Synechococcus leopoliensis EC10 ÷ 10	OECD 201	Industry data
Levofloxacin	Fluoroquinolone	1.52	Anabaena flos-aquae EC10 ÷ 10a	OECD 201	Industry data
Lincomycin	Lincosamide	0.81	Synechococcus leopoliensis EC10 ÷ 10	OECD 201	Guo et al., 2016
Linezolid	Oxazolidinone	3.50	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Loracarbef	Cephalosporin	-	-	-	No data
Mecillinam	Penicillin	-	-	-	No data
Meropenem	Carbapenem	1.50	Anabaena flos-aquae NOEC ÷ 10	OECD 201	Industry data
Metronidazole	Imidazole	-	-	-	No data
Minocycline	Tetracycline	1.10	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Moxifloxacin	Fluoroquinolone	-	-	-	No data
Mupirocin	Carboxylic acid	-	-	-	No data
Nalidixic acid	Quinolone	-	-	-	No data
Narasin	Ionophore	-	-	-	No data

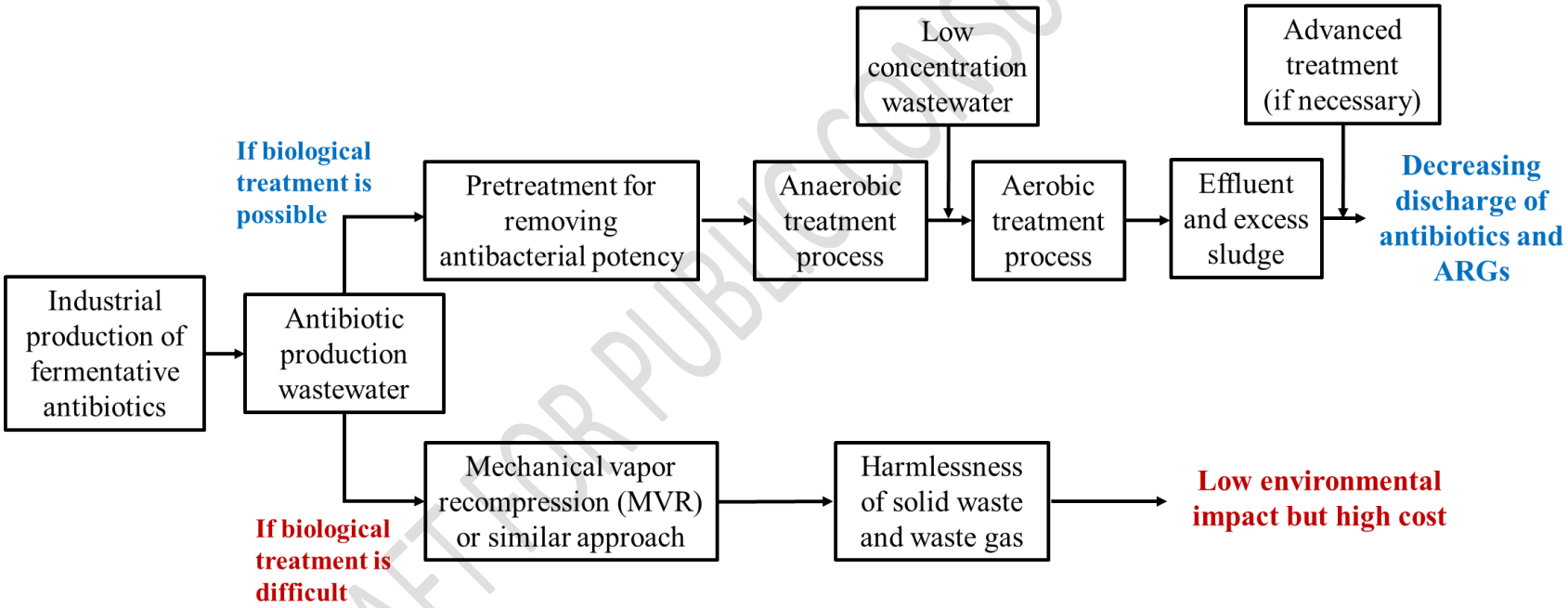
Natamycin	Antiseptic	210	Synechococcus leopoliensis EC10 ÷ 10	OECD 201	Industry data
Neomycin	Aminoglycoside	0.03	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Netilmicin	Aminoglycoside	-	-	-	No data
Nitrofurantoin	Nitrofuran	-	-	-	No data
Norfloxacin	Fluoroquinolone	120	Anabaena sp. CPB4337 EC10 ÷ 10	OECD 201	Gonzalez-Pleiter, et al., 2013
Ofloxacin	Fluoroquinolone	10.0	Anabaena flos-aquae NOEC ÷ 10	OECD 201	Industry data
Oxacillin	Penicillin	-	-	-	No data
Oxytetracycline	Tetracycline	47.0	Raphidocelis subcapitata EC10 ÷ 10	OECD 201	Kolar et al., 2014
Pefloxacin	Fluoroquinolone	-	-	-	No data
Penicillin G Procaine	Penicillin	16.0	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Phenoxymethylpenicillin	Penicillin	-	-	-	No data
Piperacillin	Penicillin	4.30	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Polymixin B	Polymixin	0.06	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Pristinamycin	Streptogramin	71.1	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Puromycin	Aminonucleoside	31.0	Daphnia magna EC10 ÷ 10	OECD 211	Industry data
Retapamulin	Pleuromutilin	-	-	-	No data
Rifampicin	Antituberculosis Agent	4.06	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Rifamycin	Antituberculosis Agent	1.00	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Rifaximin	Macrolactam	0.11	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Roxithromycin	Macrolide	6.80	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Secnidazole	Nitroimidazole	-	-	-	No data
Sparfloxacin	Fluoroquinolone	-	-	-	No data
Spectinomycin	Aminocyclitol	-	-	-	No data
Spiramycin	Macrolide	1.09	Synechococcus leopoliensis EC10 ÷ 10	OECD 201	Industry data
Streptomycin	Aminoglycoside	-	-	-	No data
Sulfadiazine	Sulfonamide	11.21	Geomean Raphidocelis subcapitata EC10 ÷ 10	OECD 201	Industry data
Sulfamethoxazole	Sulfonamide	0.60	Synechococcus leopoliensis NOEC ÷ 10	ISO 8692	Ferrari et al., 2004

Tedizolid	Oxazolidinone	3.20	Pimephales promelas EC10 ÷ 10	OECD 210	Industry data
Teicoplanin	Glycopeptide	12.90	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Telithromycin	Macrolide	-	-	-	No data
Tetracycline	Tetracycline	3.20	Raphidocelis subcapitata EC10 ÷ 10	OECD 201	Gonzalez-Pleiter, et al., 2013
Thiamphenicol	Amphenicol	-	-	-	No data
Tiamulin	Pleuromutilin	-	-	-	No data
Ticarcillin	Penicillin	-	-	-	No data
Tigecycline	Tetracycline	0.10	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Tildipirosin	Macrolide	0.42	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Tilmicosin	Macrolide	0.80	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Tobramycin	Aminoglycoside	4.30	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Trimethoprim	Trimethoprim	312.45	Geomean of Anabaena flos-aquae EC10 ÷ 10[a]	OECD 201	Industry data, Kolar et al., 2014, Guo et al., 2016
Trovafloxacin	Fluoroquinolone	-	-	-	No data
Tulathromycin	Macrolide	0.04	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Tylosin	Macrolide	0.98	Geomean of Synechococcus leopoliensis EC10 ÷ 10a	OECD 201	Industry data, Guo et al., 2016
Vancomycin	Glycopeptide	-	-	-	No data
Viomycin	Antituberculosis Agent	-	-	-	No data
Virginiamycin	Streptogramin	-	-	-	No data

[a] Geomean of most sensitive species used if EC10 or NOEC values were within one (1) order of magnitude; otherwise, lowest value used preferentially.

Annex 2: Technology performance targets for antibiotic, AMR, and microbial reduction

Information provided in this annex supports technology targets for microbial reductions (3.1.2) and technology information to support process risk management (Section 4). To note, it is important to understand that these are not intended to be a comprehensive list of technologies. In order to meet performance targets, the technology must be adequately functioning and monitored to ensure performance targets are met.



The table below provides an overview of API Manufacturing wastewater management options.

Types	Technologies	Scope of application	Performance obtained in the application cases or references	Evidence summary (Full-scale application cases or references)
Antibiotic production wastewater (Reference:1,2,3,4,5)	**Pre-treatment techniques**			
	Enhanced hydrolysis based techniques for removing antibacterial potency	Pre-treatment method used for removing high concentration fermentative antibiotics (e.g., tetracyclines, macrolides, and aminoglycosides) from production wastewater.	Removal of antibiotic could reach above 99%. Selective hydrolysis of functional groups of antibiotics with low cost and decrease of inhibition on biological treatment and dissemination of ARGs in environment. Different kinds of antibiotics require different treatment conditions (temperature, pH such as alkaline hydrolysis, catalysts). Some hard-to-hydrolyze antibiotics, such as aminoglycosides, should be treated at over 100℃.	Full-scale application cases: 1,2,3 Reference:3,6,7,8,9
	Biological technique using yeast	Pre-treatment method used for oil-containing antibiotic production wastewater. Oil is the substrate for the fermentation production of some kinds of antibiotic.	For example, in full-scale paromomycin or ribostamycin production wastewater (high oil residue from the fermentation production) treatment system using yeast, oil residue removal rate was 61.4%–74.2%, and. No ARGs from bacteria produced since yeast play the role in the biological treatment.	Full-scale application case:4 Reference:10
	Oxidation-based techniques for removing antibacterial potency, e.g., ozone oxidation, Fenton oxidation	Pre-treatment method used for removing antibiotics from production wastewater.	For example, ozone oxidation and Fenton oxidation. Doses of 1.2 mg O_3 per mg of initial OTC permitted 92% OTC removal from OTC production wastewater (OTC, 702 mg/L). In most case, oxidation-based approach is high cost and low selectivity for antibacterial potency removal.	Reference:11
	Advanced treatment techniques			
	Oxidation-based techniques, e.g., synchronized oxidation-adsorption (SOA), ozone oxidation, Fenton oxidation, electrochemical oxidation	Advanced treatment used for removing hardly biodegradable organic pollutants from biological treatment effluent.	It is suitable for the polishing purpose to remove limited amount of organic pollutants including antibiotics and microbial from wastewater before discharge, which is the end protection to meet discharge standard with high cost. SOA could selectively remove the residual antibiotics by adsorption with low cost and gentle reaction conditions comparing with Fenton oxidation.	Full-scale application cases: 5,6 Reference:3,12
	Technique integration processes for different application scopes			
	Pre-treatment + biological treatment	The effluent needs to be further treated in centralized	With the pre-treatment such as enhanced hydrolysis, the AMR development and discharge could be substantially	Full-scale application cases: 1,2,5,6

		or industrial park wastewater treatment plant.	reduced. When biological treatment uses up-flow anaerobic sludge bed (UASB), the discharge of ARGs from effluent and excess sludge could decrease 80%-95%. Following enhanced hydrolysis, the removal of denatured protein particles is necessary for improving UASB treatment performance of some antibiotic (such as oxytetracycline) production wastewater. When biological treatment uses anaerobic membrane bioreactor (AnMBR) by upgrade, ARGs in the effluent is close to zero, while the discharge of ARGs from excess sludge could decrease 80%-95%. AnMBR together with appropriate pre-treatment shows great potential for controlling AMR discharge from antibiotic production wastewater.	Reference:13,14,15,16 ,17,18
	Pre-treatment + biological treatment + advanced treatment	Effluent is most likely able to meet the standards for discharge to environment.	With the pre-treatment such as enhanced hydrolysis, the AMR development and discharge could be substantially reduced. Advanced treatment could use synchronized oxidation-adsorption (SOA), oxidation process, membrane filtration, reverse osmosis, etc.	
	Reverse osmosis (RO), subsequent to normal treatment.	Allows for water recycling.	It exhibits strong efficacy in mitigating antibacterial potency and ARGs in treated effluent, while it is cost-prohibitive	
	Multi-effect evaporator (MEE), mechanical vapor recompression (MVR) * (*relevant for wastewater with high salinity)	Treatment of process waste from reactor washings & product separation processes that usually have high dissolved solids content.	Distillate could be recycled for use as utilities water when feasible which allows for no wastewater discharge. Solid waste needs to be disposed of in a proper way that is applicable locally to ensure that it does not cause harm to the environment.	Full-scale application case: 8
Spent solvents	Solvent strippers	Recovery or removal of solvents from reaction processes	In-process recovery of purified solvents and recycling these reduce waste generation, minimizes cost for both disposal and fresh chemical purchases.	

Annex 2 References: [reference formatting to be completed]

1. Zhang Y, Walsh TR, Wang Y, Shen J, Yang M. Minimizing Risks of Antimicrobial Resistance Development in the Environment from a Public One Health Perspective. China CDC Weekly. 2022, 4(49):1105-1109.
2. Han Z, Feng H, Wang C, Wang X, Yang M, Zhang Y. Mitigating Antibiotic Resistance Emissions in the Pharmaceutical Industry: Global Governance and Available Techniques. China CDC Weekly. 2023 (accepted, doi:10.46234/ccdcw2023.195)
3. Ministry of Ecology and Environment of China. Guideline on available techniques of pollution prevention and control for pharmaceutical industry - Active pharmaceutical ingredients (fermentation, chemical synthesis, extraction) and preparation categories. Beijing 2023.
4. Access to Medicine Foundation. Methods matter: What steps are companies taking to help curb AMR by manufacturing responsibly? https://accesstomedicinefoundation.org/resource/what-are-pharmaceutical-companies-doing-to-limit-amr-risk-in-the-production-of-antibiotics.
5. China Pharmaceutical Enterprises Association (CPEA), Pharmaceutical Industry Environment, Health, and Safety (EHS) Guide (2020). Beijing 2020.
6. Yi Q, Gao Y, Zhang H, Zhang H, Zhang Y, Yang M. Establishment of a pretreatment method for tetracycline production wastewater using enhanced hydrolysis. Chemical Engineering Journal. 2016, 300:139-145.
7. Tang M, Dou X, Tian Z, Yang M, Zhang Y. Enhanced hydrolysis of streptomycin from production wastewater using CaO/MgO solid base catalysts. Chemical Engineering Journal. 2019, 355:586-593.
8. Tang M, Gu Y, Wei D, Tian Z, Tian Y, Yang M, et al. Enhanced hydrolysis of fermentative antibiotics in production wastewater: Hydrolysis potential prediction and engineering application. Chemical Engineering Journal. 2020, 391:123626.
9. Tang M, Li F, Yang M, Zhang Y. Degradation of kanamycin from production wastewater with high-concentration organic matrices by hydrothermal treatment. Journal of Environmental Sciences. 2020, 97:11-18.
10. Wang C, Ding R, Gao Y, Yang M, Zhang Y. Performance and Yeast Tracking in A Full-Scale Oil-Containing Paromomycin Production Wastewater Treatment System Using Yeast. Water. 2017, 9(4):295.
11. Liu M, Zhang Y, Zhang H, Zhang H, Li K, Tian Z, et al. Ozonation as an effective pretreatment for reducing antibiotic resistance selection potency in oxytetracycline production wastewater. Desalination and Water Treatment. 2017, 74:155-162.
12. Sun G, Zhang Y, Gao Y, Han X, Yang M. Removal of hard COD from biological effluent of coking wastewater using synchronized oxidation-adsorption technology: Performance, mechanism, and full-scale application. Water Research. 2020, 173:115517.
13. Tang L, Feng H, Luan X, Han Z, Yang M, Zhang Y. Occurrence, distribution, and behaviors of erythromycin A, production byproducts, transformation products, and resistance genes in a full-scale erythromycin A production wastewater treatment system. Water Research. 2023, 245:120640.
14. Yi Q, Zhang Y, Gao Y, Tian Z, Yang M. Anaerobic treatment of antibiotic production wastewater pretreated with enhanced hydrolysis: Simultaneous reduction of COD and ARGs. Water Research. 2017, 110:211-217.

15. He Y, Tian Z, Luan X, Han Z, Zhang Y, Yang M. Recovery of biological wastewater treatment system inhibited by oxytetracycline: rebound of functional bacterial population and the impact of adsorbed oxytetracycline on antibiotic resistance. Chemical Engineering Journal. 2021, 418:129364.
16. He Y, Tian Z, Yi Q, Zhang Y, Yang M. Impact of oxytetracycline on anaerobic wastewater treatment and mitigation using enhanced hydrolysis pretreatment. Water Research. 2020, 187:116408.
17. Tian Y, Tian Z, Feng H, Luan X, Han Z, Zhang Y, et al. Unveiling the threshold values of organic and oxytetracycline loadings for nitrification recovery of a full-scale pharmaceutical wastewater treatment system. Chemical Engineering Journal. 2023, 463:142487.
18. Tian Y, Tian Z, He Y, Sun G, Zhang Y, Yang M. Removal of denatured protein particles enhanced UASB treatment of oxytetracycline production wastewater. Science of the Total Environment. 2022, 816:151549.
19. Han Z, Feng H, Luan X, Shen Y, Ren L, Deng L, et al. Three-Year Consecutive Field Application of Erythromycin Fermentation Residue Following Hydrothermal Treatment: Cumulative Effect on Soil Antibiotic Resistance Genes. Engineering. 2022, 15(8):78-88.
20. Feng H, Tang M, Han Z, Luan X, Ma C, Yang M, et al. Simultaneous determination of erythromycin and its transformation products in treated erythromycin fermentation residue and amended soil. Chemosphere. 2023, 313:137414.
21. Awad M, Tian Z, Han Z, Luan X, Zhang Y, Yang M, et al. Application of the hydrothermally treated oxytetracycline fermentation residue in agriculture: concentrations of antibiotic and resistance genes in soil and plant. Journal of Soils and Sediments. 2022, 22(4):1095-1104.
22. Awad M, Tian Z, Gao Y, Yang M, Zhang Y. Pretreatment of spiramycin fermentation residue using hyperthermophilic digestion: quick startup and performance. Water Science and Technology. 2018, 78(9):1823-1832.
23. Xue G., Diao R.Q., Jiang M.J., et al. Significant effect of pH on tetracycline resistance genes reduction during sludge thermal hydrolysis treatment. Waste Management, 2021, 124: 36-45.
24. Zhao Z.M., Wang T., Xu Z.H., et al. The fate of antibiotic resistance genes and their influential factors during excess sludge composting in a full-scale plant. Bioresource Technology. 2021, 342: 126049.
25. Wong Q., Zhang Z., Xu G., Li G. Pyrolysis of penicillin fermentation residue and sludge to produce biochar: Antibiotic
26. Points to consider for manufacturers and inspectors: environmental aspects of manufacturing for the prevention of antimicrobial resistance. WHO Expert Committee on Specifications for Pharmaceutical Preparations. Fifty-Fourth Report Geneva, World Health Organization, 2020 (WHO Technical Report Series, No. 1025), Annex 6. https://www.who.int/publications/m/item/trs-1025-annex-6

Annex 3: Solid waste disposal – performance targets for technologies

The table below provides an overview of API Manufacturing solid waste management options. NOTE: Dewatering is essential to any sludge treatment process. Please refer to refence list in Annex 2 for relevant references noted in the table below.

[Table of solid waste disposal technology (incineration, secure landfills, hydrothermal treatment) to be completed]

Types	Technologies	Scope of application	Performance obtained in the application cases or references	Evidence summary (Full-scale application cases or references)
Antibiotic fermentation residue (Reference:1)	Enhanced hydrolysis-based techniques	• Hydrothermal treatment application could selectively hydrolyse functional groups of antibiotics (e.g., tetracyclines and macrolides) and decrease the antibiotic discharge to environment by antibiotic fermentation residues. • Some technologies application such as disc drying could decrease the easy-to-hydrolysis antibiotics such as penicillin and water content of antibiotic fermentation residues. • Some pilot studies including the hyperthermophilic pre-treatment method used for removing high concentration antibiotic and decreasing AMR development.	• Removal of antibiotic could reach 99%-100% (lower than the detection limit of UPLC-MS/MS). When the antibiotic potency is removed/reduced antibiotic fermentation residue was used as soil amendment, and fertilizers for planting industrial raw materials recycling to the fermentation process, and ornamental plant. • It is beneficial for resources recovery from antibiotic fermentation residue by making sure it cannot enter food chain.	Full-scale application cases: 9,10,11 Reference:19,20,21,22
	Alternative fuel	The antibiotic fermentation residue could be utilized as alternative fuel for power plants.	It is beneficial for resources and energy recovery from antibiotic fermentation residue.	Full-scale application case:12
	Incineration	Hazardous waste disposal using incineration.	It is suitable for antibiotic fermentation residues with small output and high toxicity.	Full-scale application case:13
Organic solid waste /Sludge	Ultra-high temperature aerobic fermentation	Utilizing aerobic fermentation bacteria capable of withstanding a temperature of at least 80 °C for fermentation for at least	Capable of degrading antibiotics and antibiotic-resistant microorganisms. Organic matter in solid waste is converted into stable humus.	WIPO PCT #2019100579

		5 to 7 days so as to carry out ultra-high temperature aerobic fermentation of organic solid waste.		
	Anaerobic digestion, AD; pre-treatment + AD; two-stage AD	Stabilization and decomposition of organic matter in anoxic environment	Reduction of antibiotic resistant bacteria and antibiotics in the sludge phase and improvement of energy recovery (methane production)	Reference: 23
	Static active oxygenated composting	Conversion of excess sludge to humus-like form.	Reduction of antibiotic resistant bacteria BUT land application of composting product still contains risks of spreading ARGs.	Reference: 24
	Pyrolysis with energy recovery	Conversion of antibiotic fermentation residues and sludge into biochar.	Pyrolysis temperatures higher than 600°C should guarantee no antibiotic nor resistance genes residues.	Reference: 25

Annex 4: Mass balance calculations

Mass balance is an evaluation and accounting of materials coming in and out of a physical process. By mass conservation, the mass entering a system should also leave the system while allowing for generation or depletion of chemical species in the presence of a chemical reaction. In the case of evaluating Effluent Concentrations (ECm) in an antibiotics manufacturing facility, it would include the whole process starting from raw material input to final product output and waste streams, accounting for the losses in between processes and the cleaning steps, and can be in solid, semi-solid, liquid or to some extent in gaseous form.

Mass = Flowrate x Concentration

Mass in = Mass out (product) + Mass (known losses) + **Mass (waste)** + **Mass (effluent)**

Knowing the mass of API in the product and the mass of API from the process, batch, and cleaning records to approximate for the known API losses, the API loss to wastewater effluent can be estimated.

where Mass (waste) and Mass (effluent) are the accumulated unaccountable losses of API that most likely end up in the environment if untreated.

The Responsible Manufacturing Effluent Management Technical Guidance Document published by EFPIA outlines the steps to estimate the API loss/year and from this calculate an average daily API loss for calculating ECm.

This guideline however wants to capture the API losses from the peak releases. The average daily loss over the entire year of manufacturing will NOT be the basis for the mass balance calculations. Mass (kg) of API losses to the wastewater should be estimated every batch.

i. Estimate or measure the mass of API lost during an entire batch (account for known loss to solid waste to estimate for the loss to process wastewater)
ii. Account for other wastewater sources in the facility that also goes to the same onsite WWTP
iii. If data is available for the removal rate of the onsite WWTP, subtract this from the mass of API in the effluent stream, otherwise assume 0% removal.
iv. The concentration of API coming out from the pipe in a batch will be:

$$\frac{\text{Mass of API lost to process water} - \text{Mass of API removed from onsite WWTP}}{\text{Total volume of wastewater from the facility during the batch period}}$$

Annex 5: Sampling and chemical analyses

[Details to be completed]

Annex 6: Audits

[Details to be completed]

References

[reference formatting to be completed]

AMR Alliance. 2022. Antibiotic Manufacturing Standard: Minimizing risk of developing antibiotic resistance and aquatic ecotoxicity in the environment resulting from the manufacturing of human antibiotics. In. Geneva: AMR Alliance.

Årdal, Christine, Enrico Baraldi, Peter Beyer, Yohann Lacotte, Dg Joakim Larsson, Marie-Cécile Ploy, John-Arne Røttingen, and Ingrid Smith. 2021. 'Supply chain transparency and the availability of essential medicines', *Bulletin of the World Health Organization*, 99: 319-20.

Bengtsson-Palme, Johan, and Dan Göran Joakim Larsson. 2016. 'Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation', *Environment International*, 86: 140–49.

Brandt, Kristian K., Alejandro Amézquita, Thomas Backhaus, Alistair Boxall, Anja Coors, Thomas Heberer, John R. Lawrence, James Lazorchak, Jens Schönfeld, Jason R. Snape, Yong-Guan Zhu, and Edward Topp. 2015. 'Ecotoxicological assessment of antibiotics: A call for improved consideration of microorganisms', *Environment International*, 85: 189-205.

Collignon, P., J. J. Beggs, T. R. Walsh, S. Gandra, and R. Laxminarayan. 2018. 'Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis', *Lancet Planet Health*, 2: e398-e405.

European Centre for Disease Prevention and Control and World Health Organization. 2023. "Antimicrobial resistance surveillance in Europe 2023 - 2021 data." In. Stockholm.

European Medicines Agency. 2006. "Guideline on the environmental risk assessment of medicinal products for human use." In.

European Parliament. 2020. 'Strategic approach to pharmaceuticals in the environment'. https://www.europarl.europa.eu/doceo/document/TA-9-2020-0226_EN.pdf.

Executive Board, 144. (2018). Follow-up to the high-level meetings of the United Nations General Assembly on health-related issues: antimicrobial resistance: report by the Director-General. World Health Organization

Gunnarsson, Lina, Alexandra Jauhiainen, Erik Kristiansson, Olle Nerman, and D. G. Joakim Larsson. 2008. 'Evolutionary Conservation of Human Drug Targets in Organisms used for Environmental Risk Assessments', *Environmental Science & Technology*, 42: 5807-13.

Han, Ziming, Haodi Feng, Xiao Luan, Yunpeng Shen, Liren Ren, Liujie Deng, D. G. Joakim Larsson, Michael Gillings, Yu Zhang, and Min Yang. 2022. 'Three-Year Consecutive Field Application of Erythromycin Fermentation Residue Following Hydrothermal Treatment: Cumulative Effect on Soil Antibiotic Resistance Genes', *Engineering*, 15: 78-88.

Ifpma. 2016. "Industry Roadmap for Progress on Combating Antimicrobial Resistance – September 2016." In.

Larsson, D. G. J., W. H. Gaze, R. Laxminarayan, and E. Topp. 2023. 'AMR, One Health and the environment', *Nature Microbiology*, 8: 754-55.

Larsson, D. G. Joakim. 2014. 'Pollution from drug manufacturing: review and perspectives', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369: 20130571.

Larsson, D. G. Joakim, and Carl-Fredrik Flach. 2022. 'Antibiotic resistance in the environment', *Nature Reviews Microbiology*, 20: 257-69.

Li, Dong, Rong Qi, Min Yang, Yu Zhang, and Tao Yu. 2011. 'Bacterial community characteristics under long-term antibiotic selection pressures', *Water Research*, 45: 6063-73.

Li, Dong, Min Yang, Jianying Hu, Yu Zhang, Hong Chang, and Fen Jin. 2008. 'Determination of penicillin G and its degradation products in a penicillin production wastewater treatment plant and the receiving river', *Water Research*, 42: 307-17.

Lourenço, Cecília, Louise E. A. Souza, André Valêncio, Marcelo F. M. Machado, Antonio Miranda, and Emerson R. Silva. 2022. 'Molecular Structure and Antibacterial Activity of Degradation Products from Cephalexin Solutions Submitted to Thermal and Photolytic Stress', 7: e202203032.

Murray, Christopher Jl, Kevin Shunji Ikuta, Fablina Sharara, Lucien Swetschinski, Gisela Robles Aguilar, Authia Gray, Chieh Han, Catherine Bisignano, Puja Rao, Eve Wool, Sarah C. Johnson, Annie J. Browne, Michael Give Chipeta, Frederick Fell, Sean Hackett, Georgina Haines-Woodhouse, Bahar H. Kashef Hamadani, Emmanuelle A. P. Kumaran, Barney McManigal, Ramesh Agarwal, Samuel Akech, Samuel Albertson, John Amuasi, Jason Andrews, Aleskandr Aravkin, Elizabeth Ashley, Freddie Bailey, Stephen Baker, Buddha Basnyat, Adrie Bekker, Rose Bender, Adhisivam Bethou, Julia Bielicki, Suppawat Boonkasidecha, James Bukosia, Cristina Carvalheiro, Carlos Årdal, Christine, Enrico Baraldi, Peter Beyer, Yohann Lacotte, Dg Joakim Larsson, Marie-Cécile Ploy, John-Arne Røttingen, and Ingrid Smith. 2021. 'Supply chain transparency and the availability of essential medicines', *Bulletin of the World Health Organization*, 99: 319-20.

Castañeda-Orjuela, Vilada Chansamouth, Suman Chaurasia, Sara Chiurchiù, Fazle Chowdhury, Aislinn J. Cook, Ben Cooper, Tim R. Cressey, Elia Criollo-Mora, Matthew Cunningham, Saffiatou Darboe, Nicholas P. J. Day, Maia De Luca, Klara Dokova, Angela Dramowski, Susanna J. Dunachie, Tim Eckmanns, Daniel Eibach, Amir Emami, Nicholas Feasey, Natasha Fisher-Pearson, Karen Forrest, Denise Garrett, Petra Gastmeier, Ababi Zergaw Giref, Rachel Claire Greer, Vikas Gupta, Sebastian Haller, Andrea Haselbeck, Simon I. Hay, Marianne Holm, Susan Hopkins, Kenneth C. Iregbu, Jan Jacobs, Daniel Jarovsky, Fatemeh Javanmardi, Meera Khorana, Niranjan Kissoon, Elsa Kobeissi, Tomislav Kostyanev, Fiorella Krapp, Ralf Krumkamp, Ajay Kumar, Hmwe Hmwe Kyu, Cherry Lim, Direk Limmathurotsakul, Michael James Loftus, Miles Lunn, Jianing Ma, Neema Mturi, Tatiana Munera-Huertas, Patrick Musicha, Marisa Marcia Mussi-Pinhata, Tomoka Nakamura, Ruchi Nanavati, Sushma Nangia, Paul Newton, Chanpheaktra Ngoun, Amanda Novotney, Davis Nwakanma, Christina W. Obiero, Antonio Olivas-Martinez, Piero Olliaro, Ednah Ooko, Edgar Ortiz-Brizuela,

Anton Yariv Peleg, Carlo Perrone, Nishad Plakkal, Alfredo Ponce-De-Leon, Mathieu Raad, Tanusha Ramdin, Amy Riddell, Tamalee Roberts, Julie Victoria Robotham, Anna Roca, Kristina E. Rudd, Neal Russell, Jesse Schnall, John Anthony Gerard Scott, Madhusudhan Shivamallappa, Jose Sifuentes-Osornio, Nicolas Steenkeste, Andrew James Stewardson, Temenuga Stoeva, Nidanuch Tasak, Areerat Thaiprakong, Guy Thwaites, Claudia Turner, Paul Turner, H. Rogier Van Doorn, Sithembiso Velaphi, Avina Vongpradith, Huong Vu, Timothy Walsh, Seymour Waner, Tri Wangrangsimakul, Teresa Wozniak, Peng Zheng, Benn Sartorius, Alan D. Lopez, Andy Stergachis, Catrin Moore, Christiane Dolecek, and Mohsen Naghavi. 2022. 'Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis', *The Lancet*, 399: 629-55.

Nijsingh, N., C. Munthe, and D. G. J. Larsson. 2019. 'Managing pollution from antibiotics manufacturing: charting actors, incentives and disincentives', *Environ Health*, 18: 95.

Schaaf, N., Panorel I., Caputo A., Prakash S., Shaw B., Verma N., and Veem K. 2020. Reducing Emissions from Antibiotics Production, SIWI.

Review on Antimicrobial Resistance. 2015. 'Antimicrobials in agriculture and the environment: reducing unnecessary waste', Review on Antimicrobial Resistance. https://amr-review.org/Publications.html.

United Nations Environment Programme. 2023. "Bracing for Superbugs: Strengthening environmental action in the One Health response to antimicrobial resistance." In. Geneva.

J. Vestel , D. J. Caldwell , J. Tell , L. Constantine , A. Häner and J. Hellstern , et al., Default predicted no-effect target concentrations for antibiotics in the absence of data for the protection against antibiotic resistance and environmental toxicity, Integr. Environ. Assess. Manag., 2021, 18 , 863 —867.

Vestel, Jessica, Daniel J. Caldwell, Joan Tell, Lisa Constantine, Andreas Häner, Jutta Hellstern, Romain Journel, Jim J. Ryan, Tim Swenson, and Wei Xei. 2022. 'Default predicted no-effect target concentrations for antibiotics in the absence of data for the protection against antibiotic resistance and environmental toxicity', *Integrated Environmental Assessment and Management*, 18: 863-67.

WHO. (2015). Global action plan on antimicrobial resistance. World Health Organization.

WHO. 2020a. 'Technical brief on water, sanitation, hygiene (WASH) and wastewater management to prevent infections and reduce the spread of antimicrobial resistance (AMR). '. https://www.who.int/water_sanitation_health/publications/wash-wastewater-management-to-prevent-infections-and-reduce-amr/en/.

WHO. (2020b). WHO Expert Committee on Specifications for Pharmaceutical Preparations: fifty-fourth report. World Health Organization.

WHO. (2019). WHO list of critically important antimicrobials for human medicine (WHO CIA list). World Health Organization

Zhang, Y., Walsh, T. R., Wang, Y., Shen, J., & Yang, M. (2022). Minimizing risks of antimicrobial resistance development in the environment from a public one health perspective. China CDC Weekly, 4(49), 1105–1109.