Guidance on wastewater and solid waste management for manufacturing of antibiotics

UN environment programme

World Health Organization

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Contents

List of tables, figures and boxes

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Acronyms and abbreviations

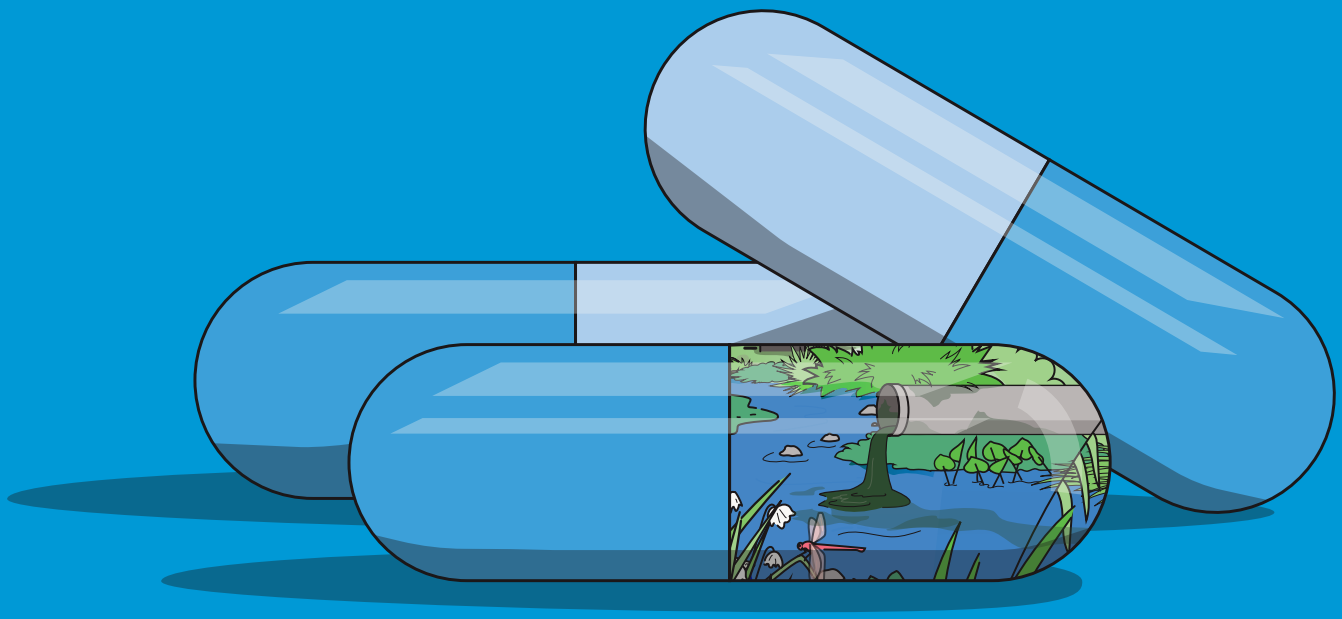
AMR	antimicrobial resistance
API	active pharmaceutical ingredient
CETP	common effluent treatment plant
EC(s)	effluent concentration(s)
EC_M	effluent concentration (estimated by mass balance)
EC_A	effluent concentration (measured by chemical analyses)[1]
$EC_{M/A}$	effluent concentration (measured mass balance or by chemical analyses)[2]
EHS	environment, health and safety
EMA	European Medicines Agency
ERP(s)	emergency response plan(s)
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GMP	good manufacturing practices
H_2O_2	hydrogen peroxide
ISO	International Organization for Standardization
LOQ	limit of quantification
OECD	Organisation for Economic Co-operation and Development
PNEC(s)	predicted no-effect concentration(s)
PNECeco	predicted no-effect concentration for ecological effects
PNECres	predicted no-effect concentration for resistance selection
QA/QC	quality assurance/quality control
SOP(s)	standard operating procedure(s)
SPE	solid-phase extraction
UV	ultraviolet
WHO	World Health Organization
WWTP	wastewater treatment plant
ZLD	zero liquid discharge[3]

1 Measured in wastewater and estimated using dilution factors for concentration in receiving water bodies.

2 For chemical synthesis process (if < 100% reaction efficiency is used), fermentation-based processes and discharge to land.

3 Note: Disposal to land that is not strictly ZLD is assessed in the same way as disposal to water bodies.

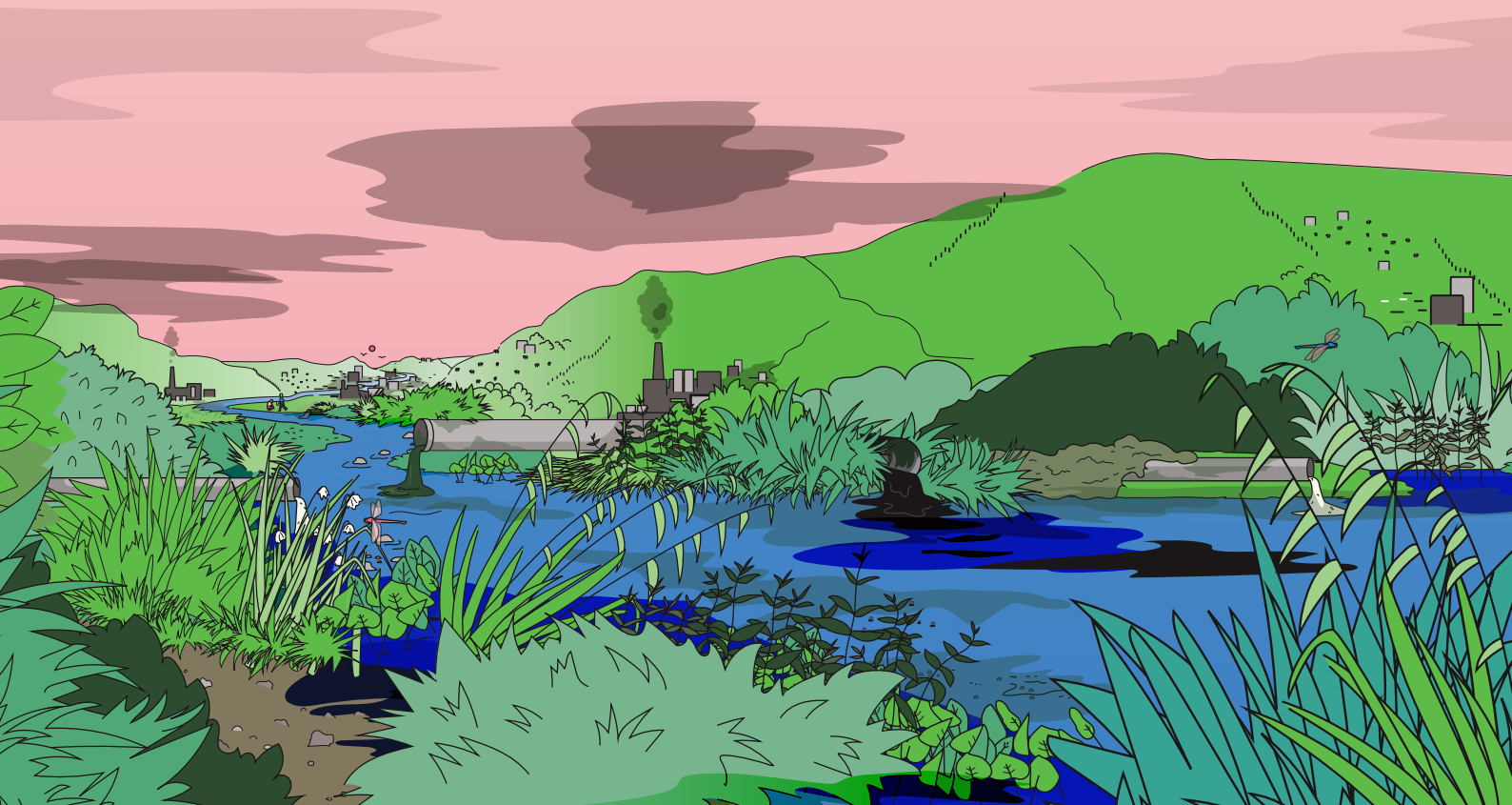
Executive summary



Control of pollution from antibiotic manufacturing is a key part of safeguarding the longevity of antibiotics for all.

Pollution contributes to antibiotic resistance, potentially undermining the effectiveness of medicines manufactured at the very same site. Yet, high levels of antibiotics in water bodies downstream of manufacturing sites have been widely documented *(1)*. Currently, antibiotic pollution from manufacturing is largely unregulated and quality assurance criteria typically do not address environmental emissions.

This guidance has been called for by a myriad of international bodies, strategies and reports, including several World Health Assembly resolutions that led to the *Global action plan to tackle antimicrobial resistance (2)*, a United Nations Environment Programme report *(3)*, a WHO Executive Board request on good manufacturing practices (GMP) (2019) and GMP points to consider (2020), the *G7 Health Ministers Meeting communiqué (4)*, the AMR Global Leaders Group call to action *(5)*, and technical reports on AMR and environment led by the quadripartite partners *(3, 6).*



Section 1

Purpose

The purpose of this guidance is to provide an independent scientific basis for the determination and inclusion of targets in the binding instruments of different target audiences to prevent the emergence and spread of antibiotic resistance. While this guidance is not binding in itself, it provides a foundation for coherence in any applicable policy or market instrument, binding or non-binding, to improve transparency and prevent fragmented or insufficient approaches.

Target audiences

The **target audiences** for this guidance are: **regulatory bodies** (national or regional) responsible for the regulation of pharmaceutical product manufacturing or wastewater and solid waste (in countries or regions that manufacture); **procurement** teams or agencies of antibiotics for human, animal and plant use; entities responsible for **generic substitution schemes and reimbursement** decisions; third-party **audit and inspection bodies**; **industrial actors** in all stages of the antibiotic production chain and their collective organizations and initiatives; **investors** in the sector; and **waste and wastewater management services** that handle antibiotic wastes.

Scope

The **scope covers** human health-based targets to reduce the risk of emergence and spread of antibiotic resistance as well as targets for ecotoxicological risks for aquatic life caused by all antibiotics intended for human, animal or plant use. It covers all steps from the manufacturing of active pharmaceutical ingredients (APIs) and formulation into finished products, including primary packaging. Guidance applies to both liquid and solid waste with a focus on liquid effluent, run-off and discharges to land. Assessment covers risks for selection of resistance by antibiotics before and after dilution in recipient water bodies and also release of resistant bacteria. Separate assessments are needed for manufacturing sites producing more than one API or finished product.

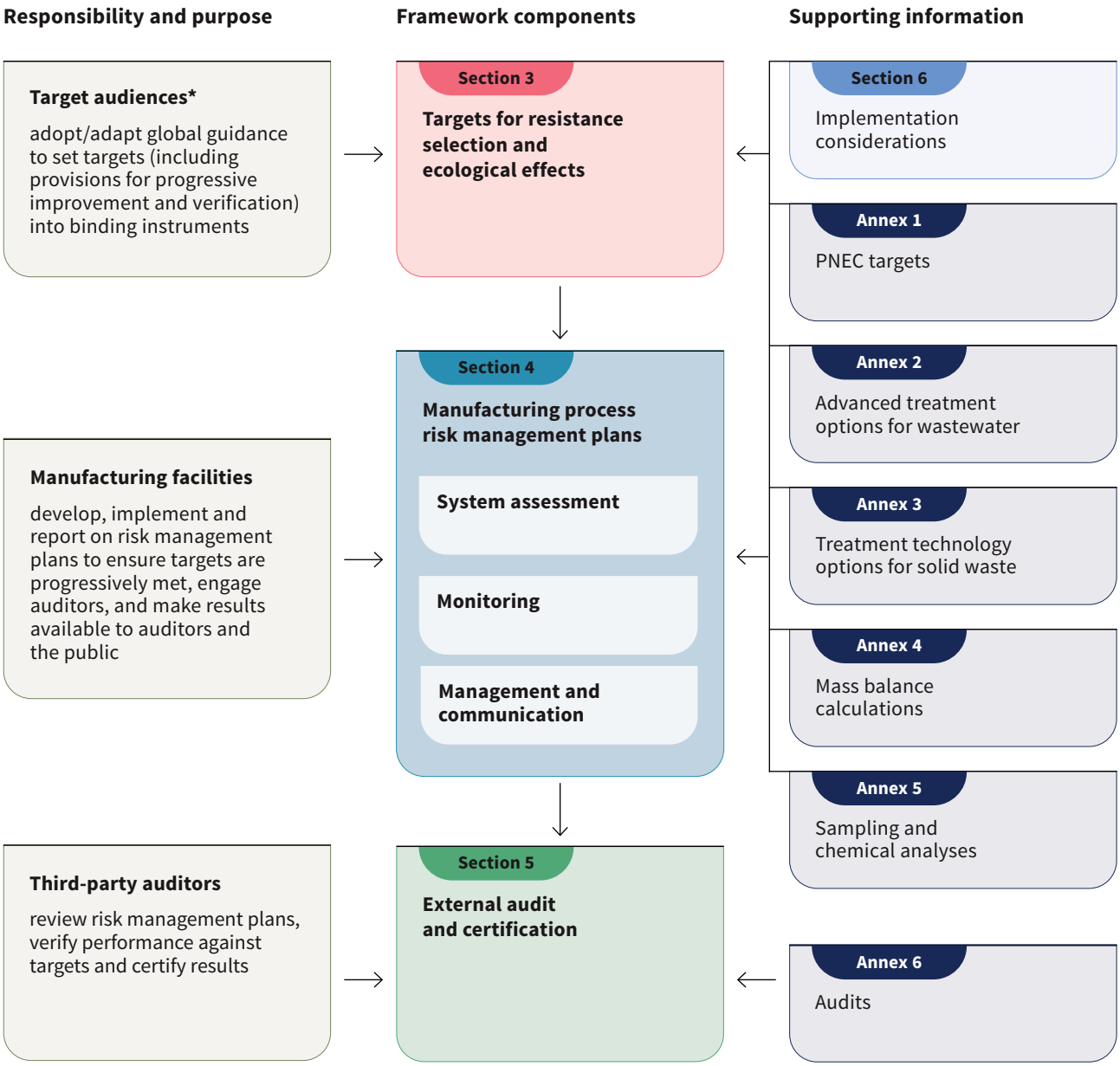
Principles

The guidance also includes **best practices for risk management plans, including internal and external audit and public transparency**, based on examples proven effective in other sectors (e.g. food and water safety) and industry-led initiatives *(7)*. Crucially, this guidance includes considerations for **progressive implementation and improvement when needed, together with a stepwise approach**. The approach recognizes **the need to protect and strengthen the global supply, and to ensure appropriate, affordable and equitable access to quality-assured antibiotics**. To mitigate risks to access to antibiotics, different approaches can complement each other, including realistic and context-specific time frames for compliance, and providing incentives such as reimbursements and subsidies.

Conceptual framework

The **conceptual framework (Figure 1) for implementation is organized according to the three separate roles of the respective actors to ensure that targets are independently set, met and reviewed with periodic monitoring and re-evaluation.** The framework is accompanied by supporting technical annexes that, taken together, enable implementation that is science-based, verifiable and transparent, technically achievable and universally applicable.

Figure 1: Conceptual framework



* Section 1.3: **Regulatory bodies** (national or regional) responsible for pharmaceutical products or for wastewater and solid waste where antibiotics are manufactured, **procurers** of antibiotics, **generic substitution schemes and reimbursements**, **third-party inspection schemes and auditors**, **industrial actors**, **investors**, and **waste and wastewater management services**.

Section 3

Assessment against targets

Targets, expressed as predicted no-effect concentrations (PNECs) for liquid effluent and as technology performance targets for solid waste, are detailed in **Annex 1** and **Annex 3**. **Two levels for liquid effluent are proposed; both can be considered to satisfactorily meet the PNEC targets (Table 3).** The two levels enable progressive improvement to methods that provide a greater degree of certainty that discharges are not leading to harmful effects.

The sections on targets and assessment methods in this guidance should be read in conjunction with the process flow diagram in **Figure 2**.

Table 2: Assessment levels for antibiotics in liquid effluent

Effluent water quality targets	Assessment methods		
See **Annex 1**: • Table A1.1: PNECs for resistance selection (PNECres) and PNECs for ecological effects (PNECeco)		***Good***	***Stringent***
	Method for estimating effluent concentration (EC)	• Formulation processes: mass balance (EC_M)[a, b] • Chemical synthesis processes: mass balance (EC_M) with assumption of 100% reaction efficiency,[c] or chemical analyses (EC_A)[d, e] • Fermentation-based processes and discharge to land: chemical analyses (EC_A)[c, d]	• All processes: chemical analyses (EC_A)[d, e]
		[a] See **Annex 4** for information on mass balance calculation. [b] Or chemical analyses (EC_A). [c] Or transparent data on accurate reaction efficiencies. [d] See **Annex 5** for information on sampling and chemical analysis. [e] With mass balance (EC_M) available as a complement.	
	Assessment for resistance selection and **Assessment for ecological effects**	$EC_{M/A}/10 < PNECres_{g}$ and $EC_{M/A}/10 < PNECeco_{g}$	$EC_A < PNECres_{f}$ and $EC_A/10 < PNECeco_{g}$
		[f] Applied to concentration in wastewater. [g] Applied to concentration in receiving water body. Dilution of 10 for inland water bodies and 100 for sea/ocean (see **Section 3.2.3** and *(8, 9)*).	
	Sites of wastewater treatment	• In-house treatment, industrial CETP or municipal WWTP	• In-house treatment or industrial CETP • Municipal WWTP only if EC_A and $EC_A/10 < PNECres$ and $PNECeco$ respectively before sending effluent to WWTP
	Sample collection site	• Treated wastewater outlet(s) (see **Figure 2**)	• Treated wastewater outlet(s) (see **Figure 2**)

Table 3: Assessment of antibiotic-resistant bacteria in liquid effluent

Technology targets	Assessment methods
• For fermentation-based processes: tertiary or advanced treatment processes known to effectively reduce heterotrophic bacteria. • For other processes: avoid microbial treatment of wastewater with antibiotic concentrations far in excess of PNECs (see **Annex 1**) if possible. If applied, disinfection is encouraged.	Assessment of specified technology installation and correct operation verified though operational monitoring and internal audit (see **Section 4.2**).

Table 4: Assessment of solid waste

Provisional[a] technology and performance targets	Assessment methods
Incineration or disposal to secure landfill. If disposed elsewhere, validated hydrothermal, chemical or enzymatic treatment to remove antibiotic residues should be applied meeting performance criteria: • For fermentation-based processes: ≥ 99% removal of API. • For other processes: ≥ 80% removal of API.	Assessment of technology type (e.g. hydrothermal, chemical or enzymatic treatment with capacity meeting performance target reductions, incineration or secure landfill) and performance verified though operational monitoring and internal audit (see **Section 4.2**)

[a] Provisional indicates significant scientific uncertainties regarding derivation of health-based value.

Table 5: Assessment of *zero liquid discharge*

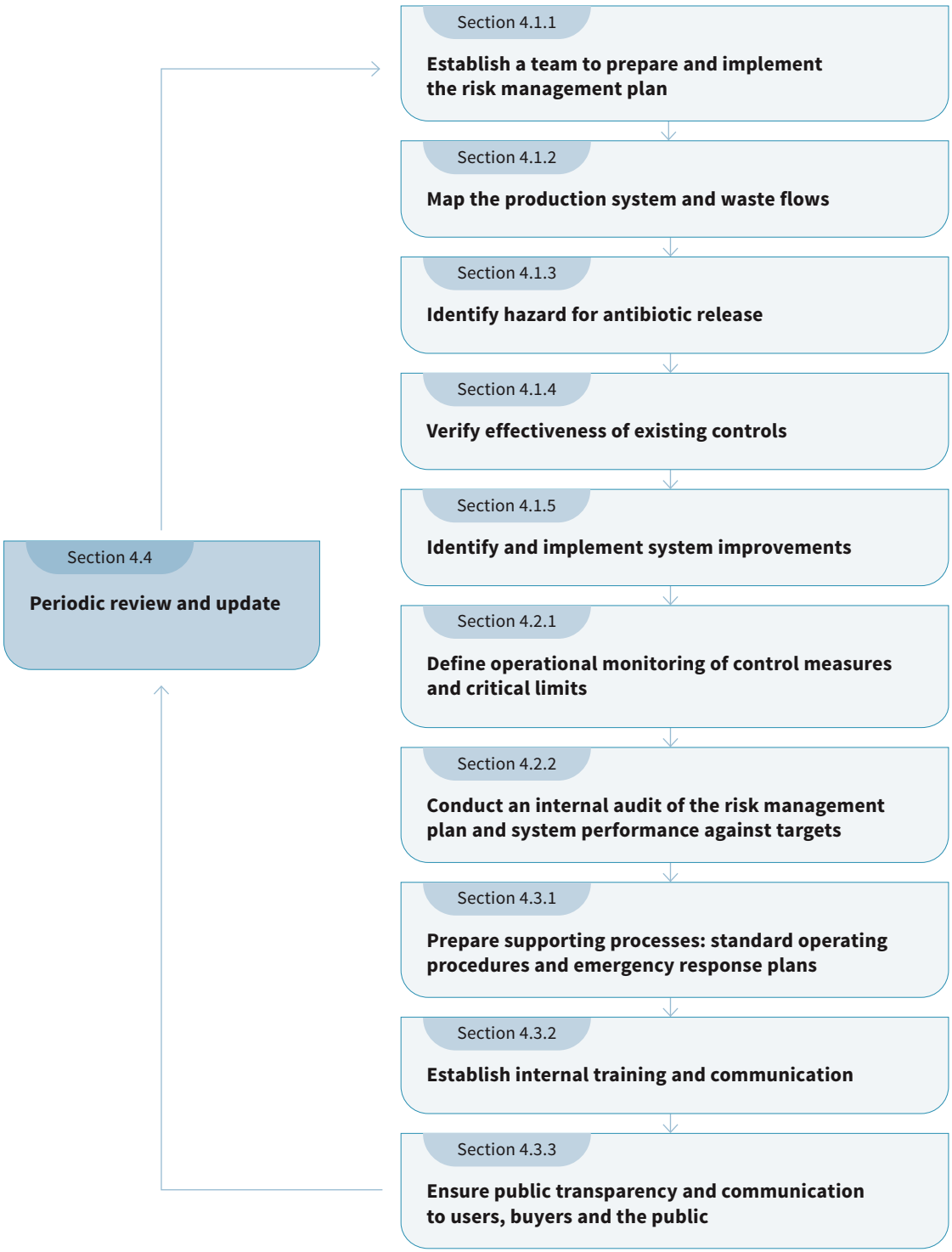
Target	Assessment methods
• ZLD: all liquid waste is contained until all of the antibiotic is removed. • Discharge to land (not strictly ZLD): as for effluent water quality targets for antibiotics (see **Section 3.1.1**).	Assessment of ZLD technology type and performance verified though operational monitoring and internal audit (see **Section 4.2**). As for effluent water quality targets for antibiotics (see **Section 3.1.1**).

Section 4

Risk management plans

Manufacturing facilities should implement risk management plans, including internal audits to ensure targets are progressively and consistently met. The risk management process described in Figure 3 follows the hazard and critical control point approach common across many WHO guidance documents. Plans may be either stand-alone or identifiably incorporated into other risk management exercises and documentation. Manufacturers should also engage and make results available to auditors and the public.

Figure 3: Overview of steps of a risk management plan



Section 5

External audit and certification

The purpose of external audit and certification is to ensure robust implementation of risk management plans and to verify performance against targets. Manufacturers engage and provide risk management plans and internal audit results to third-party auditors. Auditors use a consistent framework that allows global comparison of results by all target audiences for quality assurance purposes and public transparency. Audits include desk-based review and primarily announced site visits. An audit checklist, including information on content and frequency, is provided in **Annex 6**.

The number and capacity of auditors need to increase to implement this guidance. Auditors require expertise on industry-specific environmental risk management and competence within a third-party surveillance agency or contracted audit service provider to prepare, undertake and advise on audit results.

The third-party auditor may retain the authority to undertake direct sampling and analysis of effluent quality if the audit reveals shortcomings or inconsistencies in the risk management plan or internal audit results.

Section 6

Implementation considerations

While targets are fixed through best-available science, the approach to meeting them needs to follow a transition pathway that allows sufficient time for responsible entities to phase in, in a way that protects supply. Implementation considerations include adaptation to specific contexts including: clarification of context-specific roles and responsibilities; resources and capacity of manufacturers; availability of technologies and waste management services; time frames for scaling of capacity for target audiences (e.g. regulators and procurers), manufacturers (including differentiated capacities of generic and brand producer) and auditors; selection of levels for progressive improvement, pace of progression and cost of implementation by all parties with consideration of rewards or incentives for early adopters; and maturity of chemical analysis methods.

Section 7

Future updates

Learning from implementation and new research will inform future updates to this guidance, including possible future inclusion of out-of-scope aspects such as other antimicrobials.

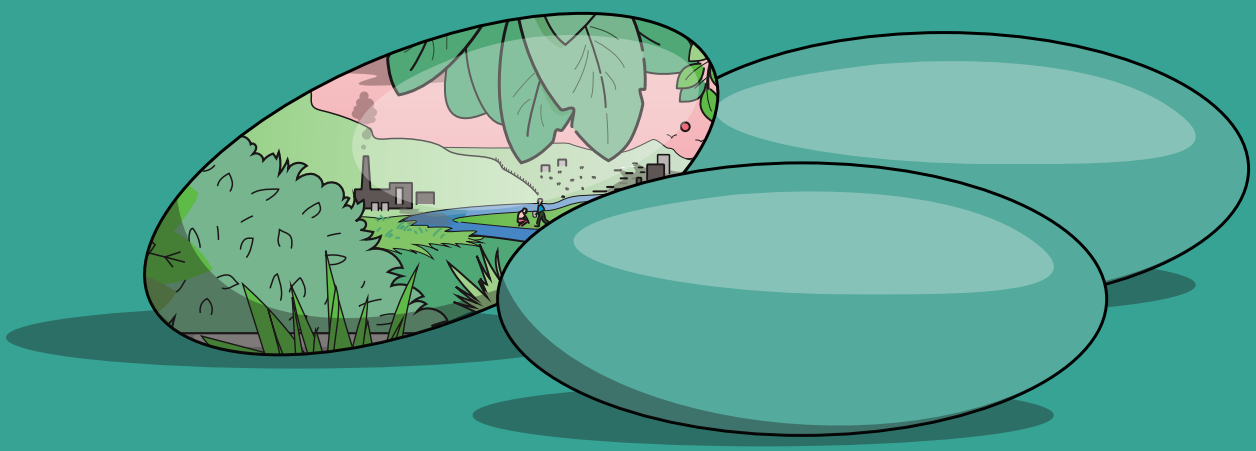
Section 8

Guidance development

This guidance was developed under the supervision of the WHO Steering Committee with a group of experts that was screened for conflicts of interest. The guidance underwent four rounds of expert review as well as a public consultation and hearing with industry and other interested stakeholders who responded to the public consultation.

1

Introduction



1 Introduction

1.1 Background

Pharmaceuticals provide great value to humanity by providing effective means to prevent and treat disease. Pharmaceuticals are biologically active and often highly potent molecules with conserved targets across species *(10)*. However, discharges to the environment primarily via wastewater may cause unwanted effects on other organisms. The overall largest volume of active pharmaceutical ingredients (APIs) that reach the environment comes from waste carrying excreta from animals and humans treated with pharmaceuticals. However, the highest environmental concentrations found are the result of pollution from manufacturing *(1)*.

Pollution with antimicrobials is a special concern. In addition to direct ecological effects *(11)*, environmental pollution with antimicrobials may also contribute to the development of resistance in both non-pathogenic and pathogenic microbes.[4] The antibiotic residues in pharmaceutical manufacturing wastewater could exert sufficient selection pressures for the antibiotic resistance development *(12)* with the ability to propagate and eventually spread worldwide. Therefore, waste from manufacturing threatens the effectiveness and longevity of antibiotics as therapeutic agents in humans, livestock, and companion animals and crops *(3, 13, 14)*.

The need for international evidence-based guidance on the management of wastewater and solid waste from antimicrobial manufacturing to guide the target audiences of this document has been raised by many international bodies and reports including but not limited to:

- Several World Health Assembly resolutions since 1998 on antimicrobial resistance (AMR) that led to the endorsement of the *Global action plan to tackle antimicrobial resistance* by the Sixty-eighth World Health Assembly *(2)*.
- *Frontiers reports 2016 and 2017: emerging issues of environmental concern* by the United Nations Environment Programme.
- The 2018 World Health Organization (WHO) Executive Board meeting request to provide technical input from the good manufacturing practices (GMP) guidance on waste and wastewater management from the production of critically important antimicrobials *(15)*.
- *Annex 6: points to consider for manufacturers and inspectors: environmental aspects of manufacturing for the prevention of antimicrobial resistance* adopted by the Fifty-fourth Expert Committee on Specifications for Pharmaceutical Preparations *(16)*.
- The 2021 G7 Health Ministers' Meeting communique *(4)*.
- The AMR Global Leaders Group call to action: *Reducing antimicrobial discharges from food systems, manufacturing facilities and human health systems into the environment (5)*.
- *Antibiotic manufacturing standard: minimizing risk of developing antibiotic resistance and aquatic ecotoxicity in the environment resulting from the manufacturing of human antibiotics (7)* and *Progress report (17)*.
- The European Parliament's *Strategic approach to pharmaceuticals in the environment (18)*.
- *Industry roadmap for progress on combating antimicrobial resistance (19)*.
- *Antimicrobials in agriculture and the environment: reducing unnecessary waste (20)*.
- *Technical brief on water, sanitation, hygiene and wastewater management to prevent infections and reduce the spread of antimicrobial resistance (6)*.
- *Bracing for superbugs: strengthening environmental action in the One Health response to antimicrobial resistance (3)*.
- *Methods matter: what steps are companies taking to help curb AMR by manufacturing responsibly? (21)*.
- *Research and innovation objectives of the European Partnership on One Health AMR (22)*.

4 Antimicrobial agents cover substances that are intentionally used to kill or prevent the growth of microorganisms, whether bacteria, fungi, viruses or eukaryotic parasites. Antibacterial is a narrower term, referring to compounds used to kill or prevent the 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 currently only apply to antibiotics.

1.2
Purpose

The purpose of this guidance is to establish an independent and scientifically derived framework for applying targets for managing liquid and solid waste from antibiotic manufacturing facilities to limit antibiotic resistance development and ecological effects. This guidance adopts a One Health approach and, in addition to human health, incorporates animal health and the wider environment, including ecosystem health.

The guidance complements other guidance on the assurance of the quality and safety of pharmaceuticals, such as the WHO GMP and *Annex 6: points to consider for manufacturers and inspectors: environmental aspects of manufacturing for the prevention of antimicrobial resistance (23)*.

This guidance provides scientifically derived targets and best practice guidance on process risk assessment, management, internal audits and transparency to ensure targets are consistently met and checked by external audits. The guidance informs adoption of such targets, risk management processes and surveillance by the various target audiences.

Crucially, this guidance recognizes the need to protect supply of and affordable access to antibiotics, especially for vulnerable populations. As such, it includes measures for progressive improvement and stepwise phase-in (such as realistic context-specific time frames for compliance) as well as incentives (such as reimbursements and subsidies)

1.3
Target audiences

At least eight key primary audiences are foreseen to enact this guidance into binding instruments *(24)* including:

1. **regulatory bodies** (national or regional) responsible for **environmental or public health aspects of wastewater and solid waste management** in countries or regions that manufacture antibiotics;
2. **regulatory bodies** (national or regional) responsible for the **regulation of pharmaceutical product manufacturing** (e.g. inspectorates from national or regional regulatory authorities);
3. **procurers** of antibiotics for human, animal and plant use (including retail companies), hospitals, regional and national procuring bodies (including the private sector);
4. governmental bodies or insurance companies responsible for **generic substitution schemes and reimbursement decisions**;
5. **third-party inspection schemes and auditors**;
6. all **industrial actors** in all stages of the production chain of antibiotics intended for use in humans, animals or plants, including associations or other collective organizations and industry-led initiatives, as part of their quality management systems as well as corporate environmental policies and commitments;
7. **investors** in the pharmaceutical sector; and
8. **waste and wastewater management services** that handle antibiotic waste and/or process effluents from the pharmaceutical industry.

This guidance also contains information that may be of use to researchers, physicians, veterinarians and the general public as a matter of enhanced public transparency (see **Section 4** and **Section 5**).

The needs, mandates, opportunities and risks for implementation of this guidance are specific to each target audience. While some may implement regulations to reduce antibiotic levels in industrial emissions, other stakeholders (such as procurers) can apply the guidance to stimulate responsible manufacturing (e.g. through various economic incentives). Informed and concerned prescribers and users of antibiotics are also included as the ultimate audience as a matter of public transparency and accountability.

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:

- all antibiotics intended for all human, animal or plant use;[5]
- all steps from the manufacturing of API and formulation into finished products, including primary packaging;
- human health-based targets for reduction of emergence and spread of antibiotic resistance as well as ecotoxicological targets to reduce risks for aquatic life caused by antibiotics, including a description of how exposure and effect levels are generated (see **Section 3.1** and **Table 1**);
- a system for risk management to meet targets and verification by external audits;
- a focus on liquid effluent, including piped effluent, surface run-off (e.g. from storage sites of solid waste) and discharges to land;
- risks for selection of resistance before (Stringent) and after (Good) dilution in recipient water bodies (applies to *Stringent* level only, see **Section 3**);
- release of resistant bacteria particularly for fermentation-based processes, selected for and enriched before release to recipient water bodies (see **Section 3.1.2**);
- solid waste performance targets and general procedures on management of solid waste contaminated by antibiotic agents; and
- separate assessment of production processes in any manufacturing site producing several antibiotics (APIs or finished products), sequentially or in parallel.

The following aspects are not covered by this guidance:

- Other antimicrobials of concern *(3)*, including antifungal, antiviral and antiparasitic agents, since risk assessment methods are less mature for these agents. Concentrations likely to cause selection are less known and, unlike bacteria, these agents do not engage in horizontal gene transfer, with implications for the role of selection pressures to non-pathogenic species. Evidence for manufacturing emissions of these agents is less mature. Future updates to this guidance may cover antifungals and possibly other pharmaceuticals if new evidence becomes available.
- Antimicrobial biocides, including heavy metals, because their risk to human health is primarily related to co-selection of antibiotic resistance, which requires additional considerations.
- Other non-antimicrobial chemicals present in manufacturing waste, still acknowledging that numerous other constituents could be important polluting agents.
- Other liquid effluent and solid waste parameters covered under local or national regulations and control (e.g. biological and chemical oxygen demand, and total suspended solids).
- Active intermediates and active degradation products of antibiotics since predicted no-effect concentrations (PNECs) are largely lacking, in particular for resistance selection. This guidance may be extended when such PNECs become available (see **Section 7**).
- Potential direct toxicological effects on humans resulting from exposure to antibiotic residues in the environment.
- Combined effects caused by mixtures of antibiotics or in conjunction with other substances.
- Water use, energy use and greenhouse gas emissions, which may be included in broader environmental assessments of manufacturing processes.
- Emissions of antibiotics to air (with potential health effects including anaphylactic or other types of allergic reactions), since risks are considered minor compared with discharges through liquid or solid waste.
- Secondary and tertiary packaging sites, and all later steps in the life-cycle of the antibiotics (see **Section 7**).
- Emissions in solid and liquid waste from disposal of unused medicine covered in other WHO guidance (in press).

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

5 This document uses the terms antibiotics, antibacterials and antimicrobials. Antimicrobial agents cover substances that are intentionally used to kill or prevent the growth of microorganisms, whether bacteria, fungi, viruses or eukaryotic parasites. Antibacterial is a narrower term, referring to compounds used to kill or prevent the growth of bacteria. Antibiotics refer specifically to those antibacterials that are used as therapeutic agents (e.g. not disinfectants, preservatives). This guidance only apply to antibiotics.

Box 1: Antibiotic pollution as a driver for antibiotic resistance development

Two key reports by the quadripartite organizations outline the wider context of environmental drivers of AMR *(3, 6)* in which emissions from antimicrobial manufacturing is considered alongside the other drivers of wastewater, sludge, manure and solid waste from communities, health care facilities, and plant and animal (livestock and fish) raising.

Antibiotic resistance leads to the loss of efficacy among available therapeutic options, in turn leading to increased morbidity, mortality and socioeconomic costs. Current estimates predict that over 1 million deaths globally could be attributed to antibiotic-resistant infections in 2019 *(25)* and 4.95 million deaths (including those directly attributable to AMR) were associated with bacterial AMR. 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 *(6, 26)*.

Selection pressure from antibiotics in the environment is also expected to drive resistance development and spread *(3, 14)*. Quantitative and 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 the emergence of new forms of resistance (events that are probably rare and difficult to predict, but may have vast consequences) as well as increasing transmission opportunities for already established forms of antibiotic resistance (common events and, in principle, quantifiable, but where each individual transmission event has a much more limited impact) *(14)* makes it even more challenging to quantitatively attribute consequences to different drivers.

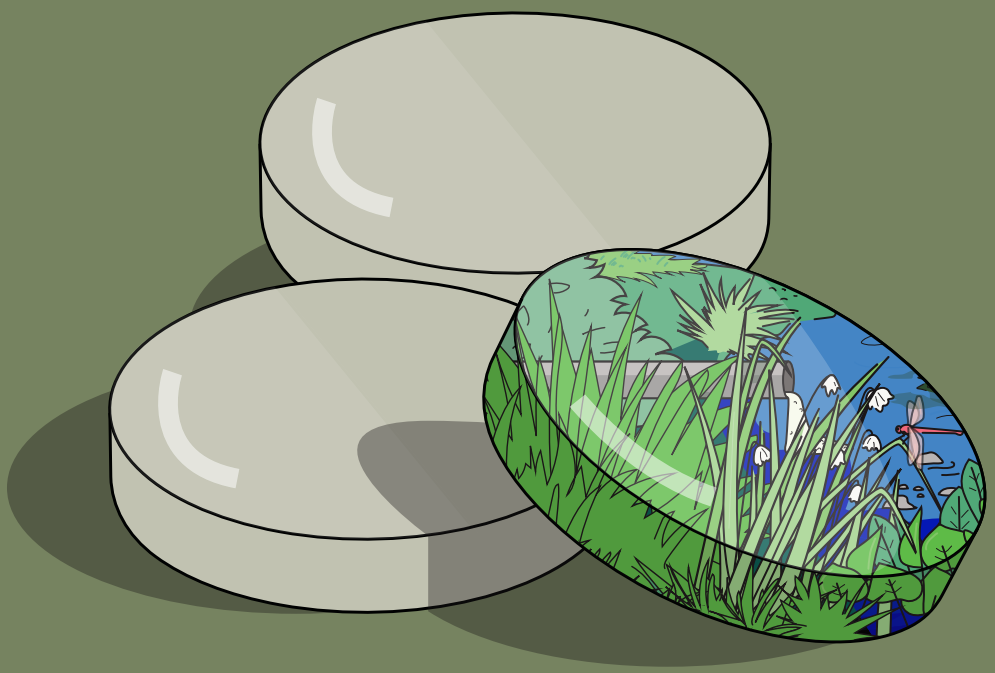
The parallel processes of evolution and transmission, influences from how resistance is managed in other geographical areas and settings, as well as delays between preventive actions and measurable effects on ultimate health outcomes calls for the use of more proximate targets in developing strategies to prevent and manage antibiotic resistance development. Indeed, targeting to reduce selection pressures by reducing antibiotic use in humans, animals and plants, often combined with sanitation and hygiene measures biosecurity and biosafety as well as integrated pest management, among others, 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 *(25, 27)*. These interventions need to be combined with prevention and management at other key sources, such as agriculture, health care facilities, and waste and wastewater in municipal systems *(3)*.

Similarly, the need to reduce environmental emissions of antibiotics are recognized widely *(3, 18, 20)*. 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 *(1, 14)*. 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 in manufacturing *(20)*. This, however, does not exclude risks associated with other types of discharges and lower emission levels.

2

Conceptual framework



2

Conceptual framework

This guidance adopts a common conceptual framework used in WHO water safety guidance documents covering three core elements and who is responsible for implementing each element (**Figure 1**):

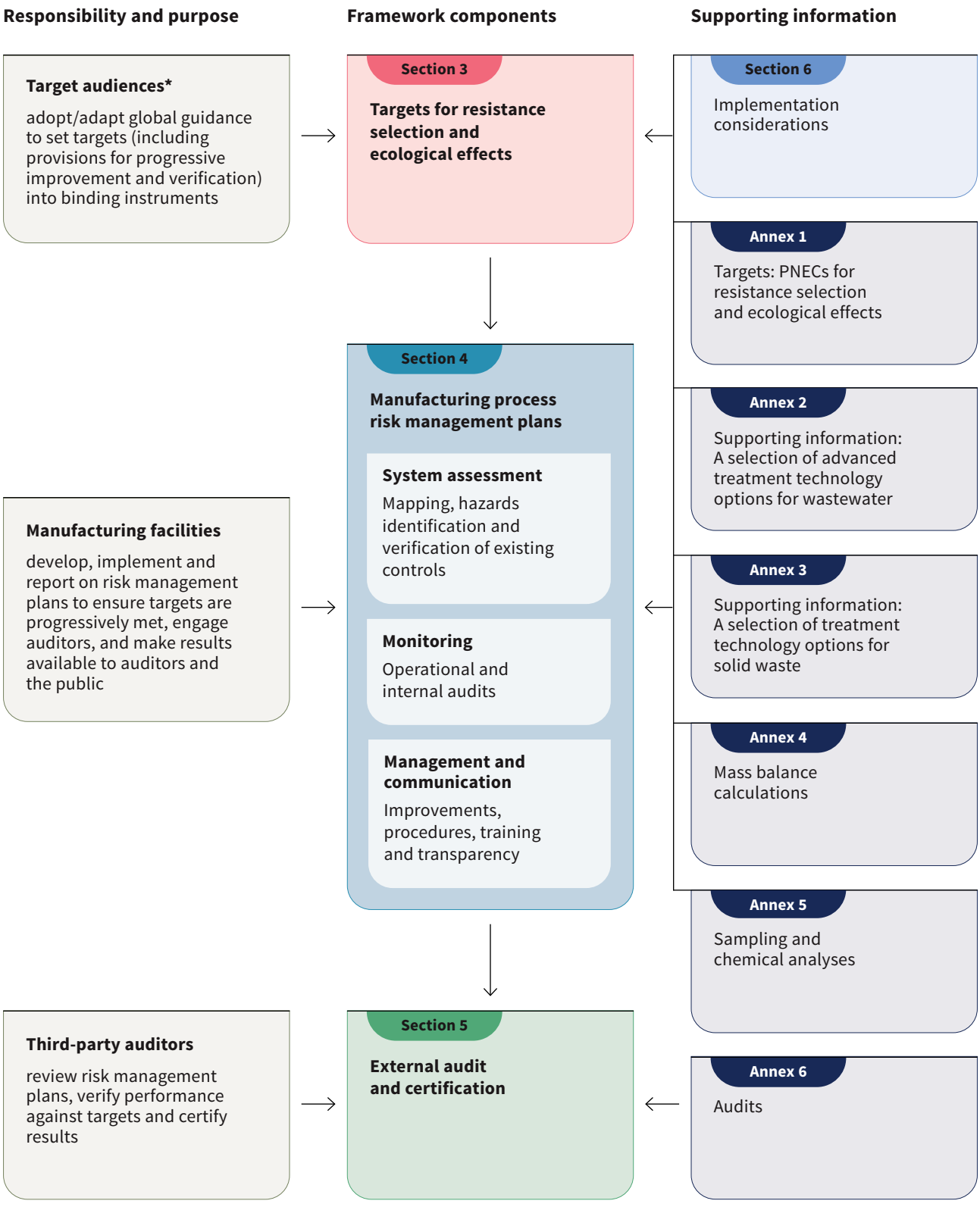
1. **Define targets** for resistance selection and ecological effects based on exposure and risk assessment.
2. Establish **risk management processes** to reach those targets using recognized risk management tools (such as the principles of hazard analysis and critical control points) with accompanying internal audits and public communications.
3. Perform **independent audits** to verify targets are being met.

The following sections will address each of these elements in more detail. Collectively, the conceptual framework set out in Figure 1 responds to the key factors for sustainability for antibiotic manufacturing i.e. science-based, verifiable, technically achievable and universally applicable *(28)*.

A first guiding principle is the **precautionary approach** for derivation of targets which has been applied where scientific evidence is lacking or inconclusive (**Box 3** and **Box 4**).

A second key guiding principle is the concept of **progressive improvement** to meet targets. This enables users to enter at the appropriate level and work stepwise to achieve compliance with based targets so that application of stringent criteria without sufficient time to adapt does not jeopardize access to antibiotics (see also **Section 4** and **Section 6**). For this reason, two levels for progressive improvement are presented, representing increasing certainty that liquid effluent is not leading to harmful effects. **Each target audience user needs to weigh potential impacts on access to and costs of medicines when adapting and adopting this guidance into different binding instruments, including regulatory frameworks.** When applied in a context where not meeting targets would lead to market exclusion, there can be reasons to advance at a slower pace compared to applications where criteria may be linked only to rewards (e.g. in procurement and subsidy decisions).

Figure 1: Conceptual framework



* Section 1.3: **Regulatory bodies** (national or regional) responsible for pharmaceutical products or for wastewater and solid waste where antibiotics are manufactured, **procurers** of antibiotics, **generic substitution schemes and reimbursements**, **third-party inspection schemes and auditors**, **industrial actors**, **investors**, and **waste and wastewater management services**.

Box 2: How can liquid effluent and solid waste from antibiotic manufacturing contribute to emergence and spread of AMR?

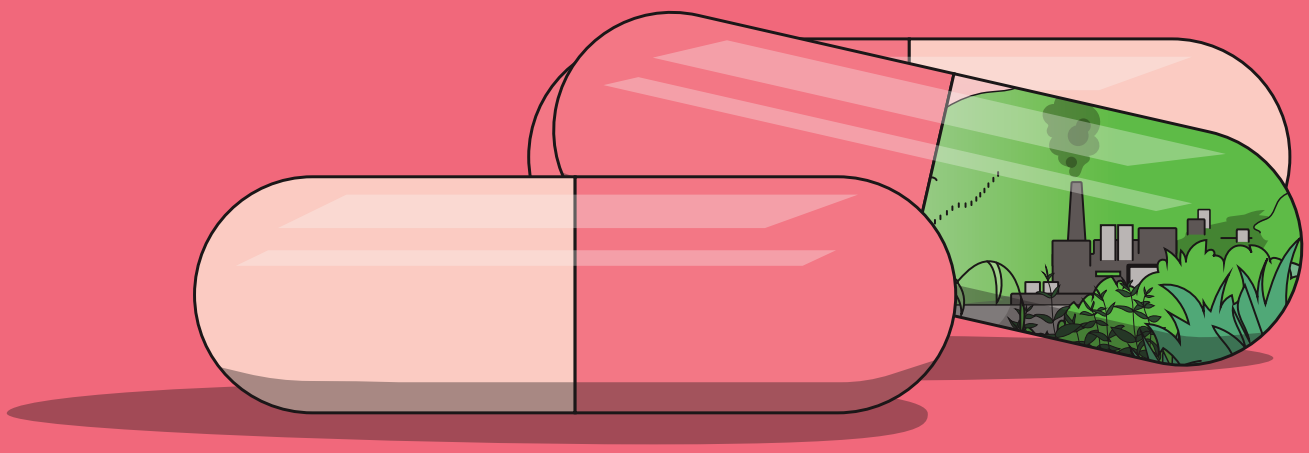
Pollution from antibiotic manufacturing is unlikely to contribute significantly to the transmission of bacterial resistance already circulating widely in society. The main concern from manufacturing emissions is the emergence of new and successful resistance genotypes. Such events are rarer than transmission events, but the consequences of emergence and subsequent spread of a new and successful resistance genotype may be vast and global, with the potential to undermine the effectiveness of antibiotics produced in those very same manufacturing facilities.

Liquid effluent and solid waste from manufacturing and downstream water bodies can sometimes contain very high concentrations of antibiotic residues *(1)*, higher than those found in wastewater carrying excreta from humans and animals being treated with antibiotics. Exposing bacterial communities to selective concentrations of antibiotics create risks for resistance evolution. It is difficult to prevent exposure during necessary use in humans or animals. However, it is, and it should be, possible to avoid exposure through liquid effluent and solid waste from antibiotic manufacturing effluent and waste.

Resistance may develop first in non-pathogenic bacteria present in liquid effluent, solid waste or the downstream water body, and then it may spread through horizontal gene transfer to pathogenic bacteria. Risks of emergence and spread can be reduced by limiting contact between selective concentrations of antibiotics (i.e. above PNEC values) and microbial communities, thereby lowering or removing the selection pressure and then limiting release of bacteria that may have acquired resistance.

3

Assessment against targets



3

Assessment against targets

The action of setting targets described in this section should be the responsibility of the six primary target audiences described in **Section 1.3** by adopting or adapting this guidance into their respective binding instruments. Targets in such instruments should be accompanied by mechanisms for progressive improvement (e.g. *Good* or *Stringent* levels, time frames and incentives for implementation) and the approach for verification by external audit.

This section describes: targets (see **Section 3.1**) for liquid effluent, solid waste and zero liquid discharge (ZLD); how they were derived; the methods to estimate or measure antibiotic concentrations in liquid effluent (see **Section 3.2**); and how to assess if all targets are met (see **Section 3.3**).

The methods to derive and apply human health-based and ecological targets expressed as PNECs for liquid effluent differ and are dealt with separately. Performance and specified technology targets are applied for antibiotic-resistant bacteria in liquid effluent and for solid waste.

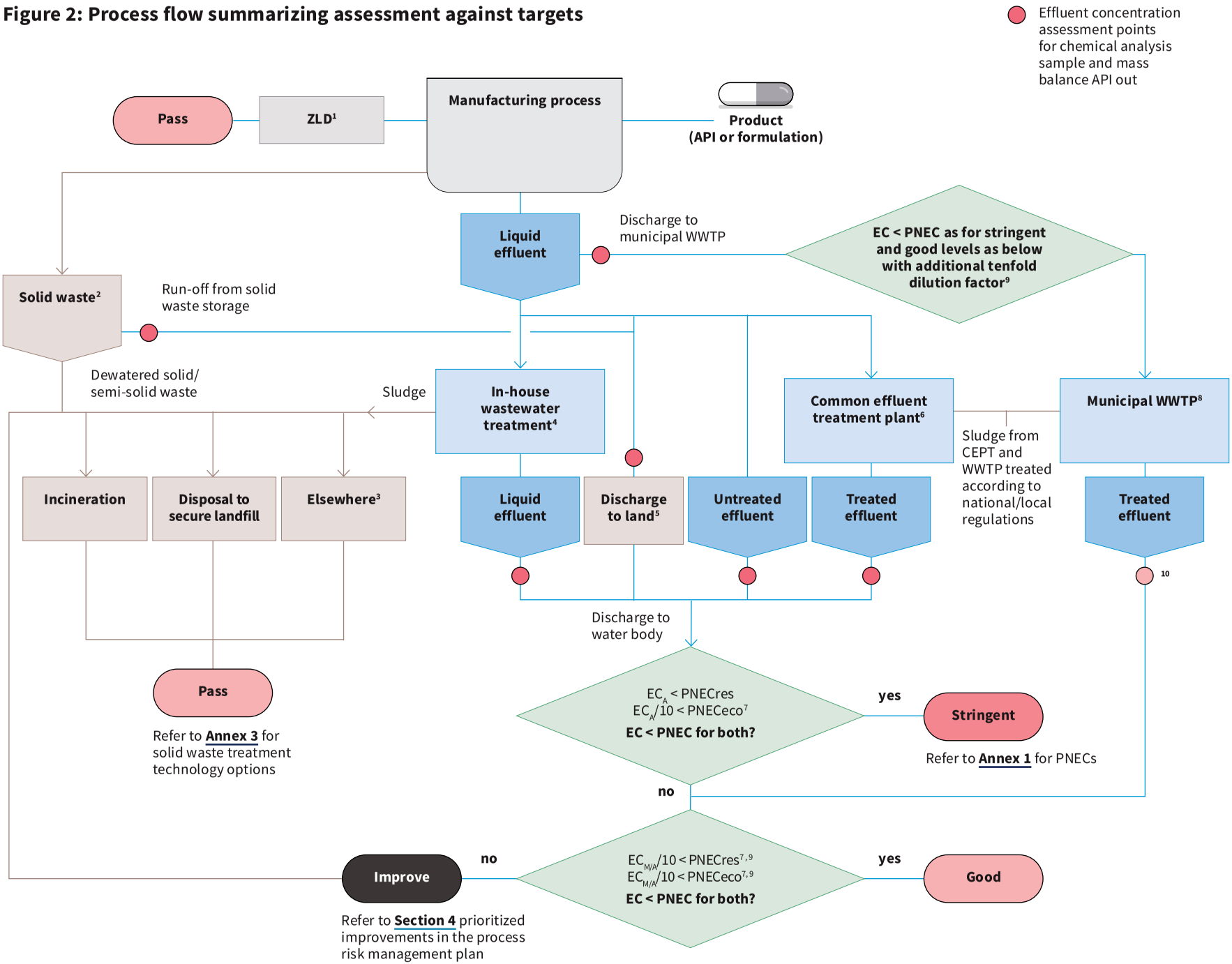
Figure 2 summarizes the assessment of all wastes:

- Liquid waste from the manufacturing process and potential run-off from solid waste storage: treated; without treatment; in-house; at a common effluent treatment plant (CETP); at a municipal wastewater treatment plant (WWTP); and disposed to land.[6]
- Solid waste: as fermentation residue and sludge treated by incineration or secure landfill, with performance targets if alternative disposal methods are used.

This guidance recognizes the need for stepwise progression allowing time for capacity and system improvement such that access to antibiotics is not jeopardized (see **Section 6**). Two acceptable levels (*Good* and *Stringent*) are outlined for liquid effluent to enable progressive adoption of the guidance by the different primary target audiences (see **Section 3.3**).

6 Not strictly ZLD (see **Section 3.1.4**).

Figure 2: Process flow summarizing assessment against targets



Notes:

1 Risk assessment not needed only if wastewater is completely reused in the facility. Otherwise, follow discharge to land.

2 Solid waste: fermentation residues, solid waste generated from process reactions, dry cleaning solid wastes, unsold or unsellable APIs and products containing APIs.

3 Technology achieving:

- 99% API removal for fermentation residues.
- 80% API removal for other solid wastes.

4 For fermentation-based processes: tertiary or advanced treatment processes known to effectively reduce heterotrophic bacteria. For other processes: avoid microbial treatment of wastewater with antibiotic concentrations far in excess of PNECs (see **Annex 1**) if possible. If applied, disinfection is encouraged. See **Annex 2** for treatment technology options.

5 Not strictly ZLD.

6 Note: Mass balance estimates of the effluent concentrations from a CETP cannot take into account dilution within the CETP.

7 Applied to concentration in receiving water body. Dilution of 10 for inland water bodies and 100 for sea/ocean.

8 To meet stringent level, any wastewater sent to a municipal WWTP already needs to have antibiotic concentrations below the PNECres.

9 For assessment of discharges to municipal WWTP, a 10 times dilution factor can be included in addition to the default recipient 10 times dilution factor for for ECres and ECeco.

10 Alternative sampling point for WWTP effluent. Accounts for both actual dilution in the WWTP and removal during treatment. Assessment by chemical analyses only.

3.1
Targets

Different types of targets are applied to liquid effluent, solid waste and ZLD as outlined in Table 1 and elaborated in **Section 3.1.1** to **Section 3**.

Table 1: Types of targets

Type of target	Nature of target	Typical application	Application in this guidance
Health outcome	Tolerable burden of disease in humans and domestic animals attributable to antibiotic resistance	High-level policy target set at national level, used to inform derivation of other target type below where possible	No established method of assessment for attributing emergence of new forms of resistance to different environments or processes. Risks for selection of resistant bacteria below is used as a surrogate end point for risks for resistance development, with ultimate consequences for the burden of infections attributable to antibiotic resistance.
Ecological outcome	No or negligible adverse effect on ecological health		
Effluent water quality	PNEC values for resistance selection PNEC values for ecological effects	Chemical hazards	Used in this guidance for liquid effluent and discharge to land (ZLD) (see **Section 3.1.1** and **Section 3.1.4**) as proxies for: • human health risks expressed as PNECs for resistance selection (PNECres); and • ecological outcomes expressed as PNECeco for ecological effects.
Performance	Specified removal of hazards	Microbial hazards (expressed as log reductions) Chemical hazards (expressed as percentage removal)	Used in this guidance for: • removal of antibiotics from solid waste disposed elsewhere than incineration or secure landfill (see **Section 3.1.3**).
Specified technology	Defined process or treatment technologies	Control of microbial and chemical hazards Underpinned by established or validated performance of the specified technology	Used in this guidance for: • removal of antibiotic-resistant bacteria in liquid effluent (see **Section 3.1.2**); and • treatment or disposal of solid waste (see **Section 3.1.3**).

Source: Adapted from Table 3.2 *(29)*.

3.1.1
Liquid effluent: PNECs for antibiotics

Effluent water quality targets expressed as PNECres and PNECeco are outlined in this section, with supporting information on derivation of the PNEC values presented in **Annex 1**.

PNECs for resistance selection

Concentrations of antibiotics that are not likely to select for resistance (PNECres) are used as indicators for the ultimate goal of AMR prevention. This includes resistance development in pathogens, and subsequently morbidity and mortality attributed to resistance. A full list of PNECres targets is provided in **Table A1.1** of Annex 1.

PNECs for ecological effects

PNEC targets for growth in aquatic bacteria (PNECeco) is used in this guidance as a proxy for the potential to disturb ecosystem functions and services. A full list of PNECeco targets is provided in **Annex 1**.

The targets are based on the aquatic environmental risk assessment for antibiotics within the European Medicines Agency (EMA) guidelines *(8, 9)*, which use a surrogate end point: the PNEC on growth for an aquatic bacterium (cyanobacteria), since bacteria are generally considerably more sensitive to antibiotics than plants or animals. Such ecological risk assessments are simplified because individually assessing risk for all processes in different ecosystems would be an insurmountable task in pursuits of the aim of protecting an often unique range of ecosystem functions and services.

3.1.2
Liquid effluent: antibiotic-resistant bacteria

Discharges of antibiotic-resistant bacteria and resistance genes in the wastewater also poses a health risk. Antibiotics present at concentrations above the PNECres in untreated wastewater or in the microbial culture of fermentation-based processes may select for and drive resistance before wastewater is released. Hence, removal of resistant bacteria prior to release in addition to meeting PNECs for antibiotics (see **Section 3.1.1**) should be part of risk management *(14, 32, 33)*.

For fermentation-based processes, there is often no feasible alternative to microbiological waste treatment. Pretreatment of fermentative antibiotic production wastewater to remove antimicrobials, e.g. through enhanced hydrolysis, is the best way to reduce risks for resistance development.

Such manufacturing facilities should have tertiary or advanced treatment processes (e.g. oxidative treatment, ultraviolet [UV] light, chlorination, sterile filtration, thermal treatment) capable of efficient reduction of heterotrophic bacteria (in addition to the removal achieved through biological treatment) prior to release of liquid effluent into sewers or water bodies.

For chemical synthesis processes, microbial treatment of wastewater with antibiotic concentrations far in excess of PNECs (see **Annex 1**) should be avoided if possible. If microbial treatment is applied, disinfection prior to release is encouraged.

Annex 2 provides information on some available advanced treatment technology options for antibiotic production wastewater.

Box 3: 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 *(14)*. There is no formalized agreed standard for assessing PNECres. A detailed description of derivation of PNECres used in this guidance is included in a separate background document *Evidence synthesis for deriving PNECs for resistance selection*.

The precautionary principle is to use the lowest PNEC reported that is considered sufficiently reliable. Applying fixed PNECs is a simplification, as many other factors can influence what concentrations are selective in a given exposure situation.

The majority of PNECs applied in this guidance have been derived from publicly available, standardized and experimental data on a large range of bacterial minimal inhibitory concentrations extracted from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) database *(30)*. When an appropriately derived PNEC for resistance selection for a given API is lacking, a default value of 50 ng/L has been applied *(31)*. The default value for PNECres can be replaced if a manufacturer can provide a PNEC derived according to the methodology by Bengtsson-Palme & Joakim Larsson *(30)*, which would require that standardized MIC data is available in the EUCAST database.

The list of PNECres in **Annex 1** needs to be periodically reviewed and updated, potentially by a WHO or independent expert group, with revisions announced sufficiently ahead of time to allow manufacturers and auditors to adapt.

Box 4: Derivation of PNECs for ecological effects

PNECeco are based on growth inhibition tests of aquatic bacteria, primarily cyanobacteria, according to the Organisation for Economic Co-operation and Development (OECD) 201 standard.

If data from several tests and bacterial species are available, the lowest PNEC should be used in accordance with a precautionary approach. If PNEC data is available from aquatic organisms other than bacteria (e.g. green algae using, for example, International Organization for Standardization [ISO] 8692) and this value is lower than the PNEC for bacteria, the lowest PNEC should apply. When a PNECeco for an API is lacking, use PNECs for resistance selection if available; otherwise, a default value of 50 ng/L should be applied *(31)*. The default value can be replaced if a manufacturer can provide transparent and relevant PNEC data, derived according to the OECD 201 standard.

The PNECs listed in **Annex 1** need to be periodically reviewed and updated, potentially by a WHO or independent expert group.

3.1.3
Solid waste: antibiotics and antibiotic-resistant bacteria

Antibiotics and antibiotic-resistant bacteria in solid waste pose a potential health risk unless managed appropriately. Evidence on safe levels of antibiotics in solid media (such as soils) is considerably less mature than in liquid systems; hence, safe levels of antibiotics or resistant bacteria in solid waste is difficult to assess. Therefore, targets for risks associated with solid waste are expressed as specified technologies and performance targets for treatment technologies.

All solid waste should be treated and disposed by specified technologies, and if incineration or secure landfill are used,[7] this should be with approval by local authorities. Any unsold or unsellable APIs, finished formulated products or process by-products containing antibiotic APIs (including API intermediates with antibiotic activity) that need to be disposed of should be either thermally, chemically or enzymatically treated/deactivated through validated methods, and/or incinerated.

Or, if disposal by alternatives methods is desired (e.g. disposal to land as a soil conditioner, raw material for cement manufacturing), performance targets should be applied with differentiated targets for fermentation-based processes and other manufacturing processes. Alternative disposal methods should also be approved by local regulations and authorities.

- For fermentation-based processes, treatment achieving ≥ 99% API removal (e.g. through enhanced hydrolysis, chemical or enzymatic treatment), since very large quantities of solid or semi-solid waste (fermentation residue) is produced, which often also contains very high antibiotic concentrations *(34)*.
- For other solid waste, from the manufacturing site or from a third-party CETP, treatment achieving ≥ 80% API removal.

Annex 3 provides supporting information on available treatment technologies for solid waste.

If wastewater is sent to a third-party municipal WWTP treating primarily sewage, the solid waste (sludge) generated at the WWTP should be treated according to local regulations.

Any transboundary transport and disposal of solid waste must conform to the articles of the Basel Convention *(35)*.

3.1.4
Zero liquid discharges

ZLD requires a water treatment system capable of producing water suitable for use within the manufacturing facility processes (e.g. in boilers and cooling towers). ZLD systems usually include pretreatment and advanced wastewater treatment technologies, and conventionally use distillation or evaporation processes separating solid residue and condensing water vapour for reuse in processes. Targets for solid waste (see **Section 3.1.3**) apply for solid residue from ZLD systems.

A production plant that is not discharging liquid effluent to water bodies or sewers, but is reusing or disposing liquid waste in other ways (e.g. disposal to soil, horticultural use), is not strictly ZLD. Therefore, PNECres and PNECeco targets described in **Section 3.1.1** apply with assessment by chemical analyses (EC_A) of the discharged liquid.

The precautionary approach is the rationale for applying the same targets for discharge to land as for water bodies. Application to land also pose risks for selection of resistant bacteria and environmental effects. Despite lack of quantitative risk assessment for emissions to soil, adding wastewater with high concentrations of antibiotic residues directly or via surface run-off will in many cases select for resistance, and is therefore not an appropriate way to eliminate risks.

7 A secure landfill prevents infiltration of rainwater and leaching to surface or groundwater.

3.2
Estimation and measurement of antibiotic concentrations

Concentrations of antibiotics in effluents can be estimated either through mass balance calculation to estimated losses during production (see **Annex 4**) or measured through chemical analysis of wastewater samples (see **Annex 5**).

Mass balance methods are generally less reliable and transparent than chemical analyses because they are estimated using built-in assumptions rather than measured, and are more challenging to assess via audit.[8] Mass balance methods also provide a lower level of precision to assess if PNECs are met, particularly when both wastewater volumes and PNECs are low. Furthermore, while mass balance methods can often be applied to formulation processes, they are less reliably applied to chemical synthesis processes[9] and fermentation-based processes.[10] Mass balance estimates cannot be applied for liquid run-off since it is not a closed system.

Chemical analysis is therefore preferred, but it also has limitations. Chemical analysis only reflects concentrations at the time point of sampling (which may not reflect the peak pollution period), and chemical analysis methods may not be available or readily developed at the required level of quantification for all antibiotics (for instance, several aminoglycosides). If sufficiently sensitive methods cannot be provided, then products may be certified to the *Good* level if: all other criteria are fulfilled; evidence is provided that sufficiently sensitive methods of analysis do not exist and that such methods are currently highly challenging to develop; and information is provided on efforts to develop methods (e.g. biological assays).

Neither method reveals where lack of control in the process has caused an exceedance. Hence, **Section 4** outlines an auditable risk management process to ensure targets can be consistently met, and that weak points leading to exceedances can be identified and remedied.

3.2.1
Mass balance estimation of effluent concentration (EC_M)

Emissions of APIs may be estimated through mass balance calculations of losses from the production process at wastewater output points shown on **Figure 2**. The mass balance approach calculates the mass flows of the API in compared with the API out and assigns any unaccounted losses to liquid waste. The estimate of potential losses of API is highly dependent on accurate data on the mass of API or ingredients entering the manufacturing process as well as of known losses. An underestimation of incoming API or reaction efficiency will lead to underestimation of losses through liquid waste.

Mass balance methods are more or less challenging depending on the antibiotic production process:

- **For formulation processes: mass balance**
  During formulation processes, there is usually a well-defined mass of API entering the manufacturing process, allowing a relatively straightforward calculation of API in minus API out minus other accounted losses to estimate losses to liquid waste.
- **For chemical synthesis processes: mass balance with assumed 100% reaction efficiency or transparent data on accurate reaction efficiencies**
  For synthetic API production, reactants entering the process are converted into API. Commonly reported yield does not include the unknown losses that the mass balance aims to estimate. Therefore, reaction efficiencies based on stoichiometric masses of reactants are needed. In practice, reaction efficiencies are less than 100%. The manufacturer should

8 Unless the assessor has a detailed understanding of the specific manufacturing processes.

9 Due to inability to assess reaction efficiencies.

10 Because of difficulties in assessing the produced mass of API by the fermenting microorganisms, and because of unknown losses to the fermentation residue, acknowledging that all unknown losses should be assigned to the liquid waste.

provide transparent data to ensure accurate reaction efficiencies are used to avoid underestimations of losses to liquid effluent. Otherwise, a conservative approach of 100% reaction efficiency should be assumed. Noting that yields often vary from batch to batch and that reliable data on reaction efficiencies can be challenging to provide, 100% reaction efficiency may lead to overestimates exceeding PNECs and chemical analyses of wastewater may therefore be preferable in many cases.

- **For fermentation-based processes: chemical analysis needed**
  Mass balance should not be applied to fermentation-based processes because of: the challenges to accurately and conservatively estimate the mass of API generated by the microorganisms (or the mass of an intermediate used as a reactant in subsequent steps); and often large, unknown losses of API to the fermentation residue (noting that all unknown losses should be assigned to the liquid waste stream).
- **For discharge to land (not strictly ZLD and solid waste run-off): chemical analysis needed**
  For assessments of concentrations of antibiotics in liquid run-off from potential storage sites of solid waste, mass balance calculations cannot be used, but chemical analyses are needed. The same applies for discharges to land from reused water (such as the water produced from ZLD) as it is wastewater that has normally undergone extensive processing. In the case that the manufacturer can convincingly argue that methodology for the chemical analysis of a given antibiotic is not available with a sensitivity down to the listed PNECs, no analysis or risk assessment is required for such discharges. Note that for regular wastewater, a risk assessment will still be needed even if chemical analyses methods are not available, but are then based on mass balance calculations.

General considerations for mass balance calculations:

- Historical data on yields cannot replace data on API entering the process, as yield data does not include unknown losses.
- For batch production, estimated losses to wastewater over the entire batch production should be used to calculate EC (EC_M).
- The API mass lost to process wastewater should be divided by the total wastewater volume (in litres) from the facility for one day (24 hours) of the batch process period to estimate the EC_M.
- Solid API waste collected and segregated (e.g. during the dry cleaning of a reactor) or spilled should not be included in losses to wastewater, since these are handled as solid waste and should not be disposed in the process wastewater.
- Theoretical removal during wastewater treatment (whether internal or external) is not suitable as an approach to reduce the EC_M. To also consider such removal (optional), chemical analysis is required.
- If the wastewater is treated by a common CETP, mass balance estimates of concentration in effluent should not consider dilution within the CETP. Dilution (and removal) within the CETP can be considered with optional chemical analyses of CETP effluent.
- If the wastewater is treated by a municipal WWTP, mass balance estimates of concentration in effluent can take into account additional tenfold dilution within the WWTP. Optional chemical analysis can account for removal within the WWTP.
- The sensitivity/precision in mass balance calculations in relation to PNECs also needs to be taken into account. In particular, demands for precision of mass balance calculation is high in cases where either wastewater volumes are low or PNECs are low. Therefore, details on how small losses can be quantified with certainty, including estimates of potential errors, need to be presented. A conservative approach should focus on not underestimating incoming masses of API or reactants, and not overestimating known losses.

A detailed description of mass balance calculation is provided in **Annex 4**.

3.2.2
Chemical analyses of effluent concentration (ECA)

Figure 2 shows six 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 WWTP or point of direct discharge to a water body.
2. Treated wastewater sampled at the outlet of in-house wastewater treatment processes.
3. Treated wastewater at the outlet of a CETP dedicated to the treatment of industrial wastewater (e.g. at an industrial park).
4. Treated or untreated wastewater from in-house treatment or CEPT for application to land (not strictly ZLD).
5. Potential liquid run-off from storage sites of solid waste.
6. Alternative sampling of treated wastewater from a municipal WWTP.

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.

Sampling and analyses should always be done on undiluted wastewater and not within the recipient water body, which introduces uncertainty of flow and mixing with other possible pollution sources, meaning less certainty with regard to assessing emission levels and measured antibiotics cannot be clearly attributed to a specific source.

Chemical analyses should be done using a method that can achieve the limit of quantification (LOQ)[11] necessary to meet the targeted PNECs. The LOQ of the method should be evaluated using wastewater spiked with the analytical target and should be demonstrated to have a signal that is at least 10 times greater than the noise or background (signal-to-noise ratio > 10).

Measured concentrations in the wastewater as well as the LOQ of the method should be publicly disclosed (see **Section 4.3.3**). Further information on the validation of the analytical method should be available to auditors on request.

Detailed sampling and chemical analysis considerations, including the application of composite sampling strategies and storage as well as the method for chemical analysis are provided in **Annex 5**.

3.2.3
Applying dilution factors

Ecological risk assessments are always performed on estimated concentrations in receiving water bodies, while risk assessments for resistance selection can be performed on either liquid ECs[12] or recipient water concentrations (see *Good* and *Stringent* levels in **Section 3.3**).

To estimate exposure in the recipient, a fixed dilution factor should be applied.

- For discharges to inland waters: a dilution factor of 10 should be applied.
- For discharges to the sea: a dilution factor of 100 should be applied in line with the environmental risk assessment procedures for pharmaceuticals in the European Union by EMA *(8, 9)*.
- For discharge to a municipal (not industrial) WWTP prior to discharge to inland water or sea: an additional fixed tenfold factor should be applied to account for dilution within the WWTP.
- No additional factors are allowed to take into account dilution within a CETP.

Active dilution of wastewater before discharge is not allowed as means to ensure PNEC targets are met.

11 LOQ is the smallest amount of the analyte that can be measured with reasonable accuracy (see also **Annex 5**).

12 Thereby also providing protection for resistance selection in bacteria within the wastewater.

3.3
Assessment against targets

A risk assessment is needed for all parts of the manufacturing chain of a product, starting from API synthesis/fermentation, where liquid emissions of antibiotics may occur. In addition to the criteria listed in this section, liquid effluent and solid waste discharges need to comply with local, regional and national standards, legislation and permits.

This section should be read in conjunction with the process flow in **Figure 2**.

Preparation and implementation of process risk management plans (see **Section 4**) are needed to facilitate progressive improvement of different aspects of the manufacturing process and ensure that manufacturers continuously advance towards targets.

3.3.1
Liquid effluent

For liquid effluent, an unacceptable risk level is when the exposure is greater than the PNEC for either resistance selection or ecological effects indicating exceedance of safe antibiotic concentrations.

Table 2 sets out two levels: *Good* and *Stringent*. For a final product to meet the criteria of *Good* or *Stringent*, all facilities in the production chain starting from API synthesis or fermentation to primary packaging need to meet at least the same level.

Two levels are presented to enact the principle of progressive improvement (see **Section 2** and **Section 6**) and enable different users to phase in implementation to avoid restrictions that may jeopardize access to antibiotics. Progressive improvement acknowledges that users may have different criteria and will need to select a workable level and advance over time. The levels represent different levels of uncertainty in the assessments of risks that can be used by manufacturers and primary target audiences (e.g. procurers), coupled with different incentives to meet each level (see **Section 6**).

Table 2: Assessment levels for antibiotics in liquid effluent

Effluent water quality targets	Assessment methods	*Good*	*Stringent*
See **Annex 1**: • Table A1.1: PNECs for resistance selection (PNECres) and PNECs for ecological effects (PNECeco)	**Method for estimating effluent concentration (EC)**	• Formulation processes: mass balance (EC_M)[a, b] • Chemical synthesis processes: mass balance (EC_M) with assumption of 100% reaction efficiency,[c] or chemical analyses (EC_A)[d, e] • Fermentation-based processes and discharge to land: chemical analyses (EC_A)[c, d]	• All processes: chemical analyses (EC_A)[d, e]
	Assessment for resistance selection and **Assessment for ecological effects**	$EC_{M/A}/10 < PNECres_{g}$ and $EC_{M/A}/10 < PNECeco_{g}$	$EC_A < PNECres_{f}$ and $EC_A/10 < PNECeco_{g}$
	Sites of wastewater treatment	• In-house treatment, industrial CETP or municipal WWTP	• In-house treatment or industrial CETP • Municipal WWTP only if EC_A and $EC_A/10 <$ PNECres and PNECeco respectively before sending effluent to WWTP
	Sample collection site	• Treated wastewater outlet(s) (see **Figure 2**)	• Treated wastewater outlet(s) (see **Figure 2**)

[a] See **Annex 4** for information on mass balance calculation.
[b] Or chemical analyses (EC_A).
[c] Or transparent data on accurate reaction efficiencies.
[d] See **Annex 5** for information on sampling and chemical analysis.
[e] With mass balance (EC_M) available as a complement.
[f] Applied to concentration in wastewater.
[g] Applied to concentration in receiving water body. Dilution of 10 for inland water bodies and 100 for sea/ocean (see **Section 3.2.3** and *(8, 9)*).

For antibiotic-resistant bacteria in liquid effluent, pass or improve is assessed via verification of specified technologies verified as installed and operating correctly though operational monitoring and internal audits made available to external auditors (Table 3).

Table 3: Assessment of antibiotic-resistant bacteria in liquid effluent

Technology targets	Assessment methods
• For fermentation-based processes: tertiary or advanced treatment processes known to effectively reduce heterotrophic bacteria. • For other processes: avoid microbial treatment of wastewater with antibiotic concentrations far in excess of PNECs (see **Annex 1**) if possible. If applied, disinfection is encouraged.	Assessment of specified technology installation and correct operation verified though operational monitoring and internal audit (see **Section 4.2**).

Box 5: Comparison with industry-led initiatives

The criteria presented in this independently derived guidance have many similarities with the AMR Industry Alliance standard *(7)*. The core of both is a comparison of estimated exposures to aquatic bacteria with targets aimed to protect ecosystems and reduce risks for resistance development through selection. The PNECs applied are identical for nearly all antibiotics. Both frameworks provide options to assess exposure through either mass balance estimates or chemical analyses, and both engage third-party auditors leading to certification. This guidance goes beyond the requirement of the industry standard in some aspects. These include:

- a two-level approach (*Good* and *Stringent*),
- a strong emphasis on public transparency;
- limitations of when and how mass balance calculations can be used;
- refinement on how to assess risks with peak emissions;
- a requirement of chemical analyses to take into account removal during wastewater treatment;
- the application of fixed rather than site-specific dilution factors;
- the assessment of risks of selection for resistance in the wastewater before dilution (for *Stringen*t level only);
- specifying how risks associated with emissions to land from not strictly ZLD facilities should be assessed;
- specifying how risks associated with solid waste including run-off from external storage sites should be assessed; and
- management of risks associated with release of resistant bacteria.

3.3.2
Solid waste

For solid waste streams, pass or improve is assessed via verification of installed and operated technologies verified though operational monitoring and internal audits made available to external auditors (**Table 4**).

Table 4: Assessment of solid waste

Provisional[a] technology and performance targets	Assessment methods
Incineration or disposal to secure landfill. If disposed elsewhere, validated hydrothermal, chemical or enzymatic treatment to remove antibiotic residues should be applied meeting performance criteria: • For fermentation-based processes: ≥ 99% removal of API. • For other processes: ≥ 80% removal of API.	Assessment of technology type (e.g. hydrothermal, chemical or enzymatic treatment with capacity meeting performance target reductions, incineration or secure landfill) and performance verified though operational monitoring and internal audit (see **Section 4.2**).

[a] Provisional indicates significant scientific uncertainties regarding derivation of health-based value.

3.3.3
Zero liquid discharge

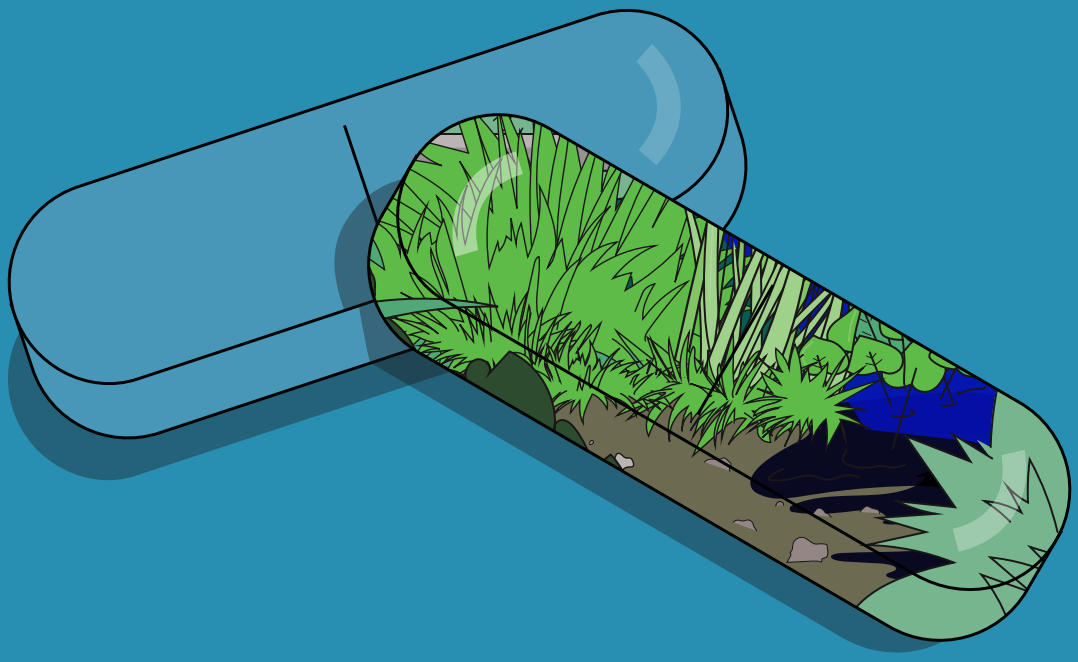
For ZLD, pass or improve is assessed via verification of installed and operated technologies verified though operational monitoring and internal audits made available to external auditors. Not strictly ZLD with disposal to land is treated the same way as liquid effluent as described in **Section 3.1.1**.

Table 5: Assessment of zero liquid discharge

Target	Assessment methods
• ZLD: all liquid waste is contained until all of the antibiotic is removed. • Discharge to land (not strictly ZLD): as for effluent water quality targets for antibiotics (see **Section 3.1.1**).	Assessment of ZLD technology type and performance verified though operational monitoring and internal audit (see **Section 4.2**). As for effluent water quality targets for antibiotics (see **Section 3.1.1**).

4

Process risk management plans



4
Process risk management plans

The action of developing, implementing and reporting on risk management plans described in this section should be the responsibility of manufacturing facilities to ensure the targets set (see **Section 3**) are progressively and consistently met. Manufacturing facilities are also responsible for making results available to auditors and the public (see **Section 4.3**), and for engaging auditors (see **Section 5**).

This section describes the management process manufacturers should follow to identify and manage risks. Steps to assess, 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.

The risk assessment and management process follow the hazard and critical control point approach and steps (Figure 3) common across many WHO guidance documents. The internally audited risk management plans developed under this section of the guidance should be subject to external audits described in **Section 5**.

At the facility level, risk management plans described in this section may be incorporated into other risk assessment exercises as long as risk and management responses associated with liquid effluent and solid waste are clearly identifiable within the wider plan.

4.1
System assessment

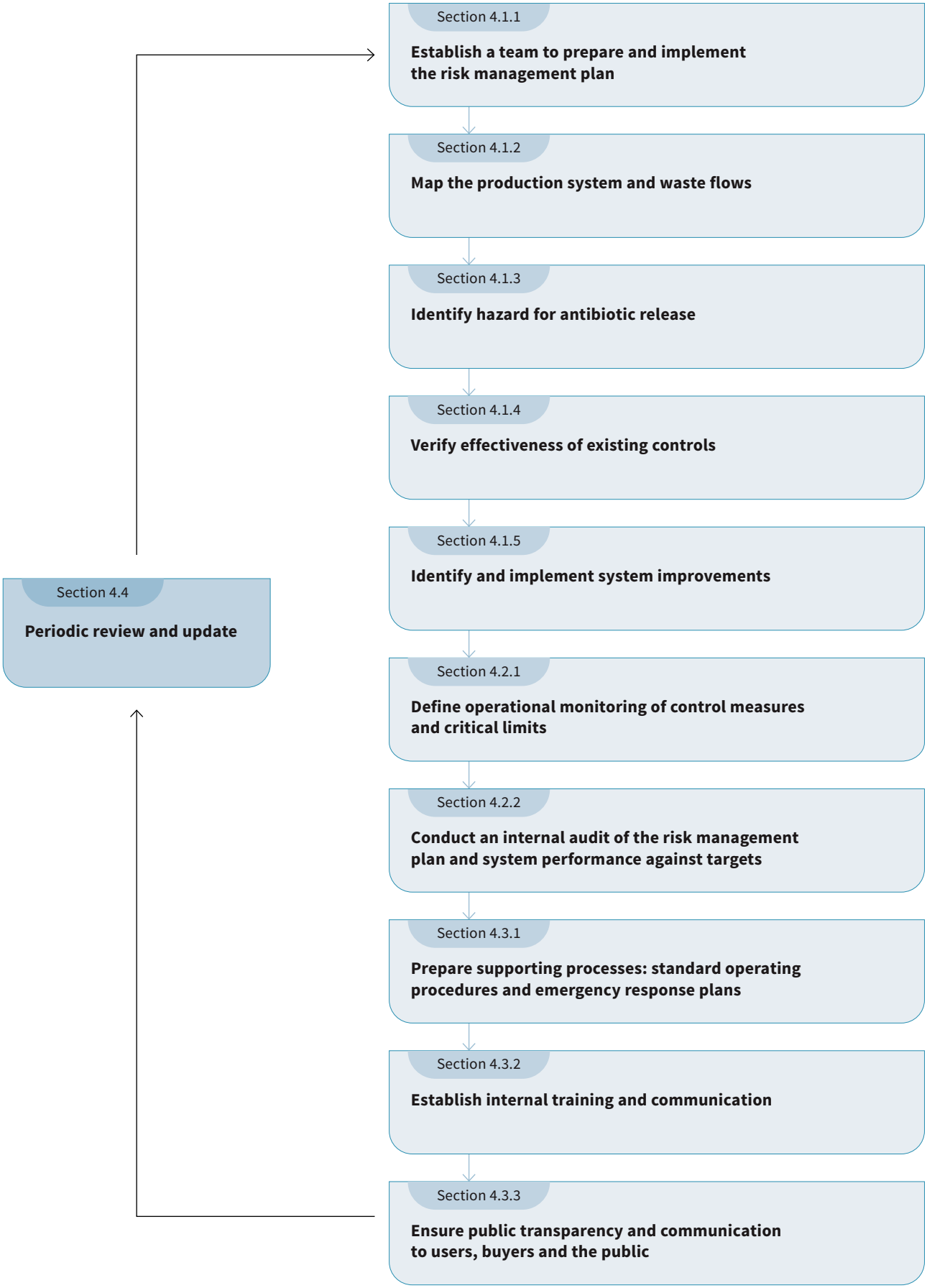
4.1.1
Establish a team

A quality assurance team is needed to conduct the prepared risk management plans through regular assessments, together with production managers who are directly involved in maintaining system performance, efficient production and personnel adherence to operating procedures. The environment, health and safety (EHS) team also assesses adherence and compliance to safety protocols, waste management and environmental impact to ensure regulatory compliance. It is also important to include operators in this process, as they have valuable insights into the actual operations and would be the first-hand people able to identify issues or opportunities for improvement.

The quality assurance team should strive for continuous improvement methodologies and bring in expertise from process engineers in identifying comprehensive solutions for improvement.

The quality assurance team is responsible for completing all steps shown in **Figure 3** and elaborated in this section.

Figure 3: Overview of steps of a risk management plan



4.1.2
Map the production system and waste flows

For a comprehensive understanding of the manufacturing process, the manufacturer needs to map the production system and waste flows for each site. This is expected to vary significantly between production techniques (fermentation, semi-synthetic and synthetic processes, formulation and primary packaging). The mapping process should involve the following steps:

- Identification of the different stages involved in the production of antibiotics. In the case of the manufacture of a finished product, the mapping includes production of APIs and formulation into the finished product, including primary packaging, to enable identification of which part of each stage 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, also including potential off-site storage of solid waste and off-site wastewater treatment. Attention should also be given to any potential cross-contamination or spill points as well as areas that are prone to potential dust/particle accumulation of APIs within the production areas and on-site storage units, which can be risk points for surface run-off.

System mapping should be updated in circumstances described in **Section 4.4**.

4.1.3
Identify hazards for antibiotic release

With the production and waste flow mapped, key processes that could contribute to the release of antibiotics into liquid effluent and solid waste 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 cannot be fully prevented due to the inherent nature of the process (e.g. losses that occur in the mother liquors during crystallization and recrystallization, or losses that occur as solid waste or powders during tabletting or capsule filling).
- Identification of potential sources of accidental antibiotic 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.

4.1.4
Verify effectiveness of existing controls

When hazards have been identified, it is important that the manufacturer verifies the effectiveness of the existing process controls being applied. This includes preventive measures, such as loss minimization, dry cleaning steps, wastewater pretreatment, advanced oxidation or other measures that reduce release of antibiotics in the final treated liquid effluent or solid waste. 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, manufacturing work instructions and maintenance protocols.
- Thoroughly evaluate the effectiveness of these controls in minimizing antibiotic release into the effluent using current and historical data. Verification to check if combined controls meet targets for liquid effluent and solid waste is covered in **Section 4.2**.
- 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 (see **Section 4.1.5**). These measures may include process improvements, additional cleaning steps, targeted adsorbance of antimicrobials (such as separation to solids), pretreatment technologies and advanced oxidation methods.
- A monitoring and verification system to ensure the effectiveness of existing controls needs to be established (see **Section 4.2**).

If all controls are verified as effective, no additional investments in improvements (as described in **Section 4.1.5**) may be needed.

4.1.5
Identify and implement system improvements

Where existing controls assessed in **Section 4.1.4** are found to be insufficient, investment in new controls is needed to reduce hazards identified in **Section 4.1.3**. Improvement measures may include process improvements (e.g. additional cleaning steps, targeted absorbance of antimicrobials [such as separation to solids], pretreatment technologies and advanced oxidation methods) or improved operating procedures (e.g. manufacturing work instructions and maintenance protocols).

The team needs to define:

- the specific improvement action to control identified hazards, considering the technical effectiveness to meet targets (see **Section 3.1**) and the reliability of the control measure;
- the person(s) or party(ies) responsible for the improvements;
- the estimated cost of the improvement (or indicative costs, such as low, medium or high cost);
- the proposed source of funding (e.g. internal budgets, stakeholder budgets, regional/ national funds);
- the due date for completing the improvement; and
- the status of the improvement (e.g. not yet started, delayed, in progress, completed).

The implementation phase should be monitored by the quality assurance team at regular intervals to assess the progress and completion of actions.

Financial constraints may hinder the implementation of the improvement measures. Nevertheless, measures should be recorded to orient budgets and secure funds for improvements. Records can also indicate to an auditor or regulatory authority that risks have been identified and there is a will to tackle them.

Improvement interventions may take a significant amount of time to implement. In such a situation, it is crucial to identify shorter-term low-cost measures to be implemented first in a stepwise approach to reduce risks within available resources (e.g. updating operating protocol and organizing a refresher training for staff).

Improvement options should also consider sustainability as well as climate adaptation and mitigation aspects (e.g. resilience to water scarcity, droughts and floods, selection of energy-efficient and green technologies).

4.2
Monitoring

4.2.1
Operational monitoring

Regular operational monitoring by the manufacturing facility 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 and recording of basic data on correct performance of process controls against operational limits and records of inspections, sampling and analysis of effluent samples.

The team should establish a monitoring programme for critical control points in the system to monitor operational key parameters (e.g. flow rates, batch times, cleaning frequency, etc.) with performance limits for each. The frequency of operational monitoring depends on the type of production process and conditions (batch, semi-batch, continuous systems).

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 risks for antibiotic release into the liquid effluent or solid waste.

Any exceedance of operational monitoring performance limits should result in an improvement action as described in **Section 4.1.5**.

4.2.2
Internal audits

Internal audits by the manufacturing facility verifies actual system performance against targets described in **Section 3**. Reports from the internal audits are assessed during external audits, and hence are part of the certification process (see **Section 5**). It is recommended that this is conducted by a quality control or assurance team in the organization, using assessment methods for liquid and solid waste described in **Section 3.3**:

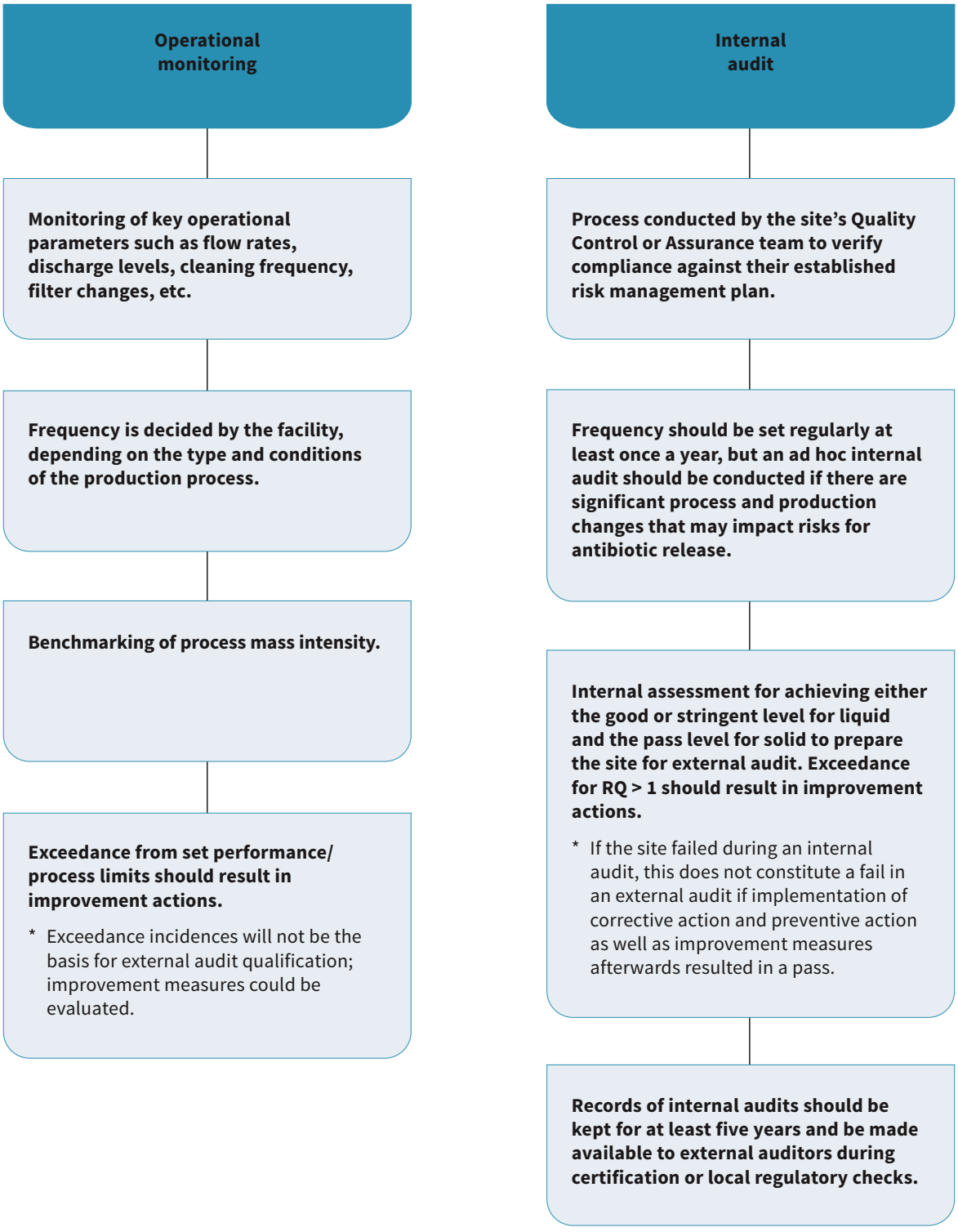
- antibiotics in liquid effluent: Table 2 supported by **Annex 1**, **Annex 4** and **Annex 5**
- antibiotic-resistant bacteria: Table 3 supported by **Annex 2**
- solid waste: Table 4 supported by **Annex 3**
- Zero Liquid discharge Table 5.

Frequency of verification monitoring should be at least once a year, but could be more frequent depending on process and production changes as well as the resources and capacity of the internal monitoring team.

Records of verification monitoring should be kept for at least five years and made available to external auditors during audit certification or local regulatory checks.

Any exceedance of verification monitoring resulting (i.e. EC > PNEC for antibiotics in liquid effluent) should result in an improvement action as described in **Section 4.3.1**.

Figure 4: Internal process verification



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.

This should be a collaborative effort across different areas within the organization from senior management to establish the targets, production line engineers/ operators to implement the actions, and EHS engineers and the quality assurance group to ensure system improvements and compliance as well as preparedness for emergencies.

4.3.1
Standard operating procedures (SOPs) and emergency response plans (ERPs)

SOPs and ERPs should be prepared or updated to support implementation of the risk management plan, and are essential components containing step-by-step procedures for routine operations and for responding effectively to emergencies (e.g. spills).

Adherence to SOPs ensures that there is consistency, quality and compliance throughout the processes of raw material handling, equipment operation, quality control procedures, monitoring, product packaging and storage, as well as waste management and pollution prevention.

ERPs provide clear guidance on immediate actions to mitigate risks of API releases, and also clear communication protocols for alerting the right personnel, management and local regulatory agencies in the event of emergencies. These procedures need to be regularly updated and communicated as part of employee training.

4.3.2
Internal training and communication

Internal communication is crucial to promote a culture of environmental responsibility among the employees, ensure staff consistently manage the process to meet operational performance limits, and to facilitate sharing of knowledge and experience throughout the production chain. The following aspects should be considered:

- Identify training and knowledge transfer needs within the organization and designate training coordinators. Seek and leverage subject matter experts (e.g. EHS engineers).
- Provide training to employees at all levels on responsible antibiotic manufacturing practices and its links to antibiotic resistance, and their roles and responsibilities to minimize antibiotic release.
- Ensure employees are regularly trained and evaluated on SOPs and ERPs.
- Establish communication channels to ensure that employees take up training opportunities and facilitate the exchange between different steps in the production chain.
- Enable regular discussions among employees for continuous quality improvement, and recognize or reward high performers.
- Document and communicate best practices within the organization.

4.3.3
Public transparency and external communication

Manufacturers should establish effective communication with auditors, procurers, regulators, investors, local communities and other relevant stakeholders (such as health care professionals, researchers and environmental organizations) to ensure transparency and build trust in the manufacturing process of each site.

Environmental reporting is a key component for public transparency, which informs stakeholders on the progress of efforts by the manufacturer to protect local water bodies and to combat AMR.

Transparency is key for accountability and for incentivizing measures to reduce pollution through different means. Transparency also allows a broad range of actors to respond in a way to stimulate positive change *(36)*. Lack of transparency creates an uncertainty that can be viewed as an external cost and ultimately a global health risk *(24)*.

Manufacturers should make the following information publicly available on an annual basis through a publicly accessible channel (e.g. a website):

- Which company is responsible for the different steps in a production chain for each antibiotic.
- Where each production step takes place. For a final product, the exact sites and manufacturer of each production step (active intermediate, API synthesis, formulation, primary packaging) *(37)*.
- How pollution is managed, and if targets are met in each of these sites, including:
  - emission levels in the wastewater, reported in mg/L;
  - method of estimating such concentrations (mass balance or chemical analyses);
  - exposure above or below PNECres and PNECeco to conclude if the targets are met in wastewater and/or recipient; and
  - summary results of audits and certification (see **Section 5**).

These channels or other dialogues can allow stakeholders to provide feedback, ask questions and build trust. Reliable and timely reporting also allow space for stakeholders to have a thorough understanding of the challenges and limitations of the manufacturers in achieving targets, and provide accurate and verifiable implementation information to inform updates to regulatory and procurement criteria. It can encourage participation of the local community in discussions of issues that potentially affect them.

4.4
Review and update

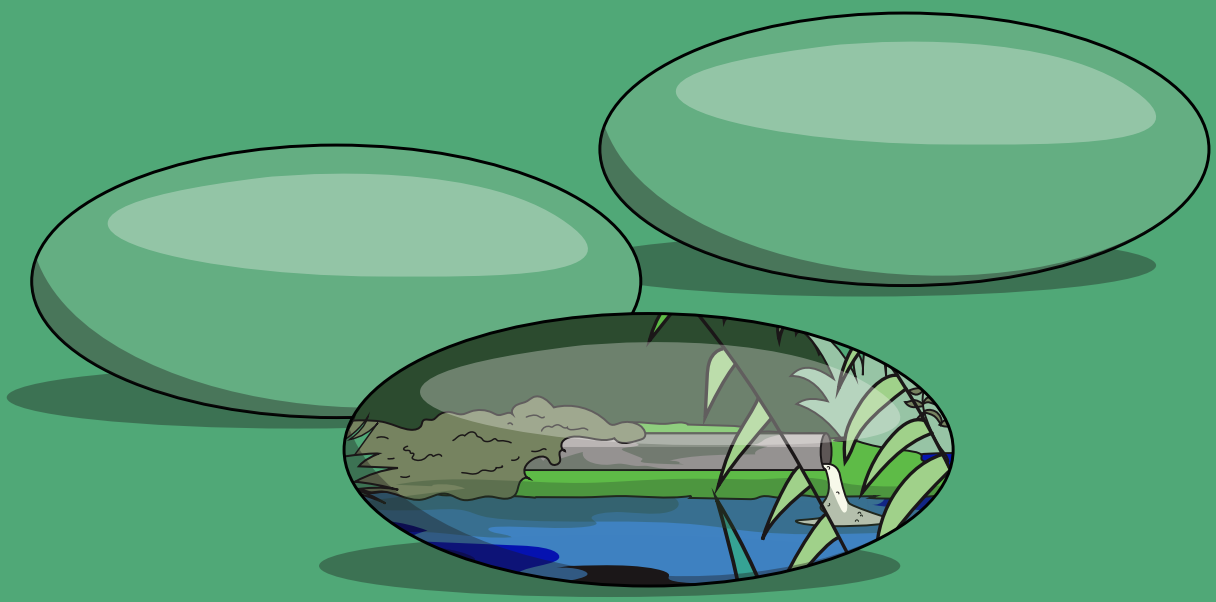
The management plan developed should be reviewed and updated at least annually and after any incident leading to an exceedance of targets. Reviews identify any new hazards, reflect improved controls and incorporate system improvement identified in audits with the objective of progressive and continuous improvement. A structured feedback loop involving different stages of the manufacturing process and across departments should be established and practised for smooth integration of suggestions for continuous improvement.

Reviews are needed because a risk management plan can quickly become out-of-date, for example through:

- changes in manufacturing processes (e.g. size or formulation of batches);
- changes in the implementation of improvement plans (e.g. addition of a new treatment unit);
- a significant incident or near miss;
- changes in regulatory requirements;
- changes in management processes and procedures (e.g. SOPs); and
- organizational changes within the manufacturing entity.

5

External audit and certification



5

External audit and certification

The action of verification by external audit and certification described in this section should be the responsibility of private or public third-party auditors and inspectors who are independent from the manufacturer (see **Section 4**). Their purpose is to ensure that manufacturers' risk management plans and implementation of the plans are robust, and to verify performance against targets to ensure compliance (see **Section 3**). Auditors also issue certificates for use by all target audiences (see **Section 1.3**) for quality assurance purposes.

Audits and certifications demonstrate public transparency by providing a framework that manufacturers can follow. Consistency of these certification standards allows stakeholders to understand and compare the performance on management of liquid effluent and solid waste across manufacturers on a global scale. Manufacturers engage third-party auditors and undergo thorough assessments to independently validate risk management plans and internal audits (see **Section 4**) to receive certification. Manufacturers can share and publicize certifications as part of public transparency as described in **Section 4.3**.

An audit-based approach places responsibility on the manufacturers to provide the auditor with their risk management plan and internal audit results relevant to the target audiences.

Audits require expertise on environmental risk management, with focus on an industry setting and competence of a third-party surveillance agency or contracted audit service provider to:

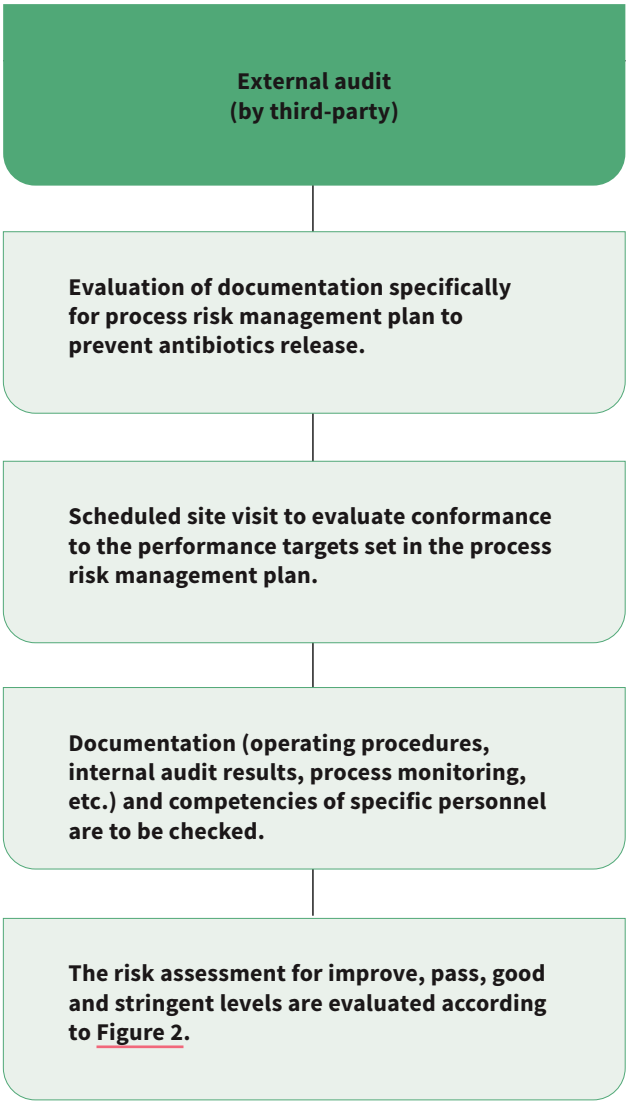
- prepare an audit strategy detailing the selection and frequency of manufacturing facilities to be audited;
- undertake or oversee auditing of the risk management plans for selected manufacturing sites as a programmed routine activity; and
- respond to, investigate and provide advice on receipt of reports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

The global number of auditors and their capacity needs to increase to fulfil the role described in this guidance (see **Section 6**).

Periodic audits would normally include the following elements gathered by review of documentation and site visit:

- A desktop exercise to assess if the site has understood the requirements, prepared the necessary documentation and the logistics for an on-site audit.
- A site visit with examination of records to ensure that system management is being carried out as described in the risk management plan and risk assessment points (EC < PNEC) are evaluated according to **Figure 2**.
- Checking if operational monitoring parameters are kept within operational limits and that compliance is being maintained.
- Ensuring system improvements to manage identified risks are being implemented.
- Ensuring that internal audits are carried out and review results against targets.
- Assessment of supporting programmes (e.g. SOPs and staff training) and strategies for improvement of the risk management plan.
- Provide a summary assessment of performance according to stringent, good or improve classification with accompanying certificate (see **Section 3**).
- Provide recommendation for improvement of the risk management plan where needed.

Figure 5: External audit and certification



An exceedance of PNECs at the applicable risk assessment points for the site constitutes a major non-conformity, meaning a corrective action plan needs to be presented to the auditor. A follow-up visit is necessary to ensure effectiveness of corrective actions.

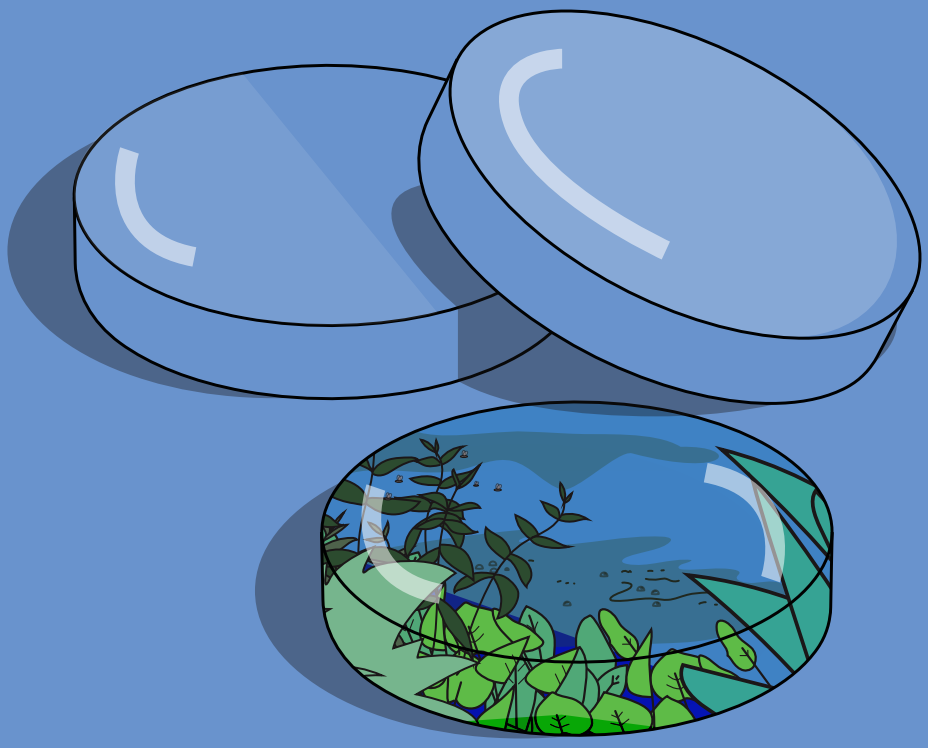
Lapses in adherence to process risk management and operational monitoring from the internal audit results that did not have adequate improvement measures and are still present at the time of external audit constitute a minor non-conformity. The manufacturer has to present acceptable corrective action and preventive action to the auditor. No follow-up visit is necessary, but the implementation will be evaluated at the next external audit.

Auditors may inspect via announced and unannounced visits for assurance of true and independent verification of the activities of the manufacturer. Since the audit process would normally be initiated by the facility, this implies that the assessment process will be scheduled. Unannounced visits are not coordinated in advance and could happen as a follow-up to a recent audit.

A more detailed audit checklist, including information on content and frequency, is provided in **Annex 6**.

6

Implementation considerations



6
Implementation considerations

Change is clearly needed from the current situation of largely unregulated pollution from antibiotic manufacturing. Achieving this in a coherent way is the goal of the criteria outlined in this guidance.

However, implementation at scale is a challenge that requires careful phase-in by each of the target audiences to contextualize and minimize impact on antibiotic supply and price.

Harmonization and coherence along various possible policy and market instruments are assumed to support scaled implementation and create synergies that limit the burden of reporting and verification. Ultimately, this may require governance through a global expert body or organization.

This section focuses on implementation considerations, including the various roles, mandates and responsibilities of the involved stakeholder.

6.1
Adaptation to specific contexts

Target audiences will need to consider various contextual factors when adopting and adapting this guidance, such as:

- A market analysis of available manufacturers, including their production capacity and current certification status or probability to become certified, to assess risks that added criteria could disturb supply chains or lead to unacceptably increasing prices.
- An analysis of how the nature of the instrument in question could disturb supply chains or lead to unacceptably increasing prices; noting that risks are minimal for strictly award-based approaches (e.g. procurement, subsidy decisions) and overall greater for approaches that potentially could lead to market exclusion of some manufacturers (e.g. GMP, national or regional environmental regulations).
- An assessment of which antibiotic to focus on initially, with a view to scaling to broad application, based on factors such as branded versus generic and level of demand.
- Resources of the manufacturers as well as capacity for pace and complexity of implementation (e.g. for generic versus branded antibiotics).
- Appropriate incentives to hasten the pace and quality of implementation.
- Availability of technologies and waste management services (i.e. secure landfills, well-functioning municipal WWTPs) in the local context as well as capacity of the manufacturers' workforce and auditors to implement and check.

6.2
Roles and responsibilities

Target audiences (see **Section 1.3**) of this guidance as well as roles and responsibilities for implementation of the three elements are set out in **Figure 1** and at the beginning of **Section 3** to **Section 5**.

However, each target audience will need to contextualize and specify which entities will perform each role described. In doing so, care should be taken to ensure appropriate separation of roles and equal application to antibiotics for human and animal use, noting that, globally, over two thirds of antibiotics are used for food-producing animals.

6.3
Capacity

Implementation needs to be supported by training and new capacity in all of the three framework elements, based on an assessment by the responsible entities:

- Target audiences to adopt and adapt global guidance into binding instruments. This requires individuals with specific expertise on waste and environmental management in addition to any existing capacities for quality assurance.
- Manufacturers need additional training of staff and subcontractors to develop, implement and report on risk management plans to ensure targets are progressively met, as well as lab capacities, data analysis and interpretation, and commensurate resources.
- Auditors to verify risk management plans and performance against targets as well as issues certificates are currently very limited in number. Therefore, both the quantity auditors with ability to service manufacturers in all locations as well as their capacity combining industry experience and environmental risk management experience need to increase to carry out widespread and high-quality audits.

6.4
Progressive implementation

This guidance deploys a two-tier system of good and stringent specifically to allow for progressive implementation towards more rigorous and reliable assessment methods so that risk of reducing supply or market access is minimized. Target audiences (see **Section 1.3**) should consider pathways and incentives towards stringent that balances these two objectives.

The principle of progressive improvement also applies in the circumstance when a facility/production process does not achieve the good level of certification. It is the decision of each primary target audience how lack of certification should be treated.

For certain situations, short-term consequences that limit market access or risks shutdown of a facility should be considered unacceptable. Emphasis should be placed on a time-bound stepwise process to reach certification, to implement the improvements identified in the process risk management plan (see **Section 4.4**), and in the external audit and follow-up audit, to address the aspects not fulfilled.

The improvement process to reach certification may be linked to incentives where applicable as described in this section.

Progressive implementation also applies to scaling up of capacity for risk management plans and their implementation by manufacturers as well as growth in availability of competent auditors, also described in this section.

6.5
Incentives

Some stand-alone initiatives (e.g. public procurement of antibiotics) already include criteria to limit antibiotic pollution. A range of further mechanisms may be deployed by target audiences to incentivize improvements in processes to meet targets set out in this guidance. For example:

- **Regulatory bodies** may introduce certification or selected parts of the guidance in relevant environmental regulatory frameworks, or as part of regulation related to registration, import or sales of antibiotics. The nature of such instruments are often that they are mandatory, meaning that good may be a pragmatic level to balance access risks, quite plausibly also combined with providing manufacturers a sufficiently long time frame for implementation.
- **Procurers** at any level may similarly use the guidance, either with certification as a criterion that need to be fulfilled or as an award-based criteria where the seller do not need to be certified for selling, but has the option to use their certification (to either *Good* or *Stringent*) together with price as means to win the procurement. When used strictly as an award-based criterion, the risk for market exclusion is negligible and applying both levels connected to different levels of rewards is recommended to stimulate stepwise progression. The size of rewards must be evaluated in each specific context.
- In **generic substitution schemes and reimbursement decisions**, the situation is similar to that of procurement and award-based criteria in that there is a value in applying both levels of achievement and that risks for market exclusion are negligible.
- **Investors** may also use the guidance, including certification, as part of their decisions to invest in companies or as means to act towards sustainability from within the manufacturing companies as shareholders and potentially board members.

Other mechanisms that relate to manufacturers' responsibility after sale and use (such as extended producer responsibility) are not applicable for manufacturing pollution since they relate to treatment of wastewater carrying excreted antibiotic, resistant bacteria and genes, and safe disposal of unused medicines, which is the subject of other WHO guidance (in press).

6.6
Transparency

Needs for transparency are listed in **Section 4.3.3**.

Overall, implementation needs to consider improvements in transparency in several domains:

- transparency of compliance against effluent water quality and solid waste targets
- transparency regarding cost of compliance for manufacturers and regulatory authorities
- transparency in how this information is utilized in the different policy or market instruments.

Achieving transparency, especially regarding costs, is a precondition for designing adequate incentives or adopting this guidance in any other policy or market instruments without creating risks (e.g. for access) (see also **Section 6.2**).

6.7
Cost

Development of this guidance did not include a detailed financial and economic analysis of implementation though the modes of the various target audiences.

Each target audience should: do an economic analysis for uptake of this guidance in their specific application, primarily to quantify the impact on cost of and access to medicines (especially for vulnerable groups); select the level (good or stringent); the pace of phase-in; and the consequences of improve-level results to allow producers and markets to adapt so that supply is not jeopardized, while also including rewards or incentives for early adopters.

Cost categories to consider for all components include: set up regulatory mechanism; process risk management; investment in improvements in technologies; staff capacity; and cost of audits, noting risk of costs being passed on to consumers, but also likely market rewards for early adopters.

These cost assessments should also be weighed against risks of inaction, as ecological deterioration and increased resistance development also represent costs to society. Although more ambiguous to assess than implementation costs, any target audience adopting this guidance should reflect on the objectives and risk of business as usual.

6.8
Analytical methods for chemical analysis

Currently, for some antibiotics, analytical methods of chemical detection (see **Annex 5**) are not available or not sufficiently sensitive to demonstrate the exposure less than PNECs (see **Annex 1**) are met.

Therefore, in some circumstances, application of the *Stringent* level may not be possible for some antibiotics and therefore should not prevent certification. Instead, the approach should prioritize access with a strong incentive to develop methods.

Target audiences (see **Section 1.3**) should allow for the *Good* level in such circumstances. In the case where no sufficiently sensitive analyses methods exist for an API produced through fermentation processes, products may still be certified to the *Good* level if: all other criteria are fulfilled; evidence is provided that sufficiently sensitive methods of analysis do not exist and that such methods are currently highly challenging to develop; and information is provided on efforts to develop methods (including, e.g. biological assays).

Further, there is a need for improvement in laboratory capacity and quality assurance/quality control (QA/QC) to ensure consistent and validated methods of sampling and chemical analysis (see **Annex 5**) to achieve reliable results.

Box 6: Examples of application of incentives and transparency for pollution control

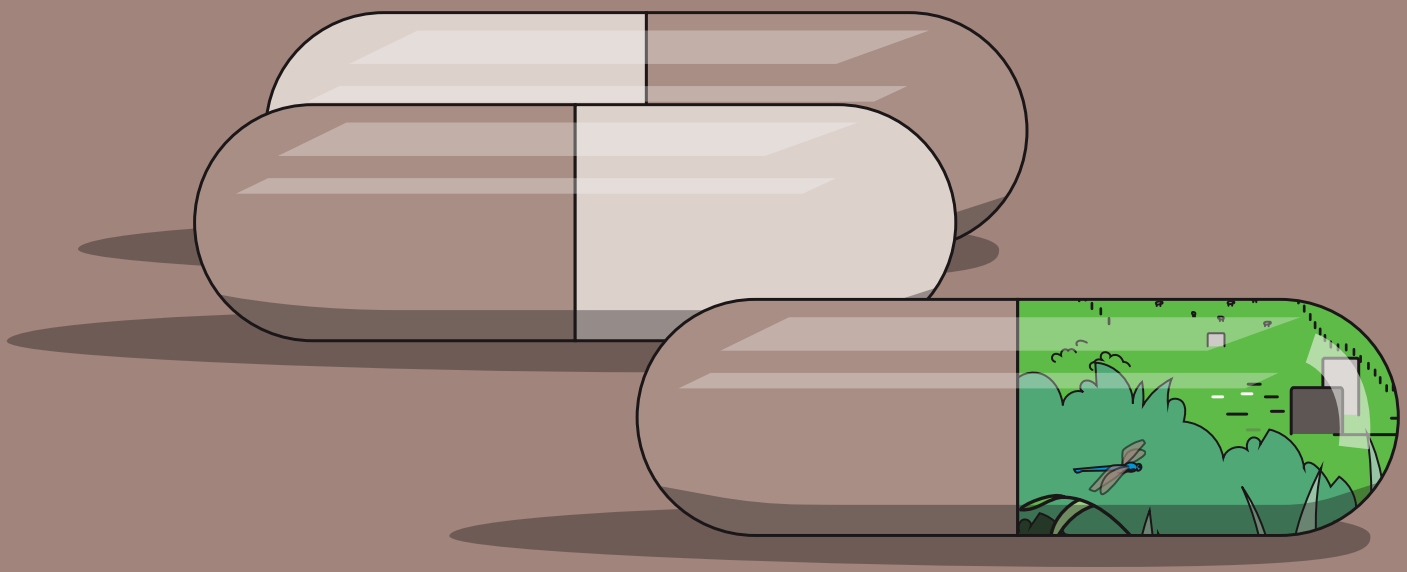
There are a number of recent examples where entities have included or proposed inclusion of environmental pollution criteria using each of the instruments discussed. These include:

- **Regulation:** In 2020, the Indian Government proposed a bill on pollution limits for antibiotics at manufacturing sites. The proposed limits, based on measured concentrations in treated effluents, were lower than in this guidance, with the rationale that concentrations might be even higher in untreated effluents. However, the bill passed without antibiotic discharge limits due to critiques primarily from manufacturers.
- **Procurement:** In Sweden and Norway, many regional hospital organizations have started asking for pollution control information during procurement and providing rewards for companies that meet the criteria. Formalized guidelines have been developed by the Swedish procurement agency *(38)* and some regions use a modified version. In Norway, several companies have won the procurement as a result of meeting the environmental criteria.
- **Generic substitution and reimbursement decisions:** In 2013, the Swedish Government reported on how to revise the generic substitution and reimbursement system for pharmaceuticals to reward not only low price but also pollution control at manufacturing sites. Later, the Swedish Medical Products Agency led work to develop and pilot such a system for antibiotics and some other pharmaceuticals to be launched in 2025 *(39)*.
- **Investors:** Some years ago, the investment bank Nordea commissioned a report on pollution from drug manufacturing in India *(40)* and presented it to the leads of several of the world's largest pharmaceutical companies in order to incentivize actions.
- **Transparency:** New Zealand authorities publish detailed data on supply chain (API production and formulation) for pharmaceutical products, including the company's name, exact location and contact details for both API production and formulation demonstrating the feasibility of supply chain transparency *(37)*. The Access to Medicines Foundation has in several reports highlighted the lack of sufficient transparency by large antibiotic manufacturers, but also acknowledged some progress taken in recent times *(4)*.
- **Voluntary actions:** The initiative by the AMR Industry Alliance resulting in a standard *(7)*. Although a voluntary standard, one of the objectives with its development was that it could be used in instruments such as procurement.

Note that criteria applied in the above examples are not all the same as the guidance, both stricter and less strict criteria. Harmonization between pilot approaches and instruments is also lacking. Hence, one of the aims of this guidance is to contribute to improved coherence and comparability, and reduce the burden of compliance with varying or overlapping criteria.

7

Research needs and future updates



7

Research needs and future updates

There are a number of scientific and implementation knowledge gaps that need to be filled to support full and up to date implementation of this guidance and extension in future updates to aspects currently out of scope. Those highlighted though the development of this guidance include:

Scientific studies:

- A robust process (potentially by a WHO or independent expert group) for periodically reviewing and updating PNEC values listed in **Annex 1** with revisions announced sufficiently ahead of time to allow manufacturers and auditors to adapt. This includes broadening of the literature base for PNECres and PNECeco noting the small number of reference studies.
- Improving chemical analysis methods and limits of quantification for compounds with PNECs values lower than current limits of quantification so that chemical analysis methods can be performed for all antibiotics in **Annex 1**.
- Scientific studies on the selective potential of intermediates and active degradation products, to enable guidance to be extending to cover those as well.
- Scientific studies on manufacturing risk and associated PNECs for other types of antimicrobials to enable guidance to be extending to those as well, if warranted.
- Scientific research and development of methods to assess risks associated with mixture exposures.

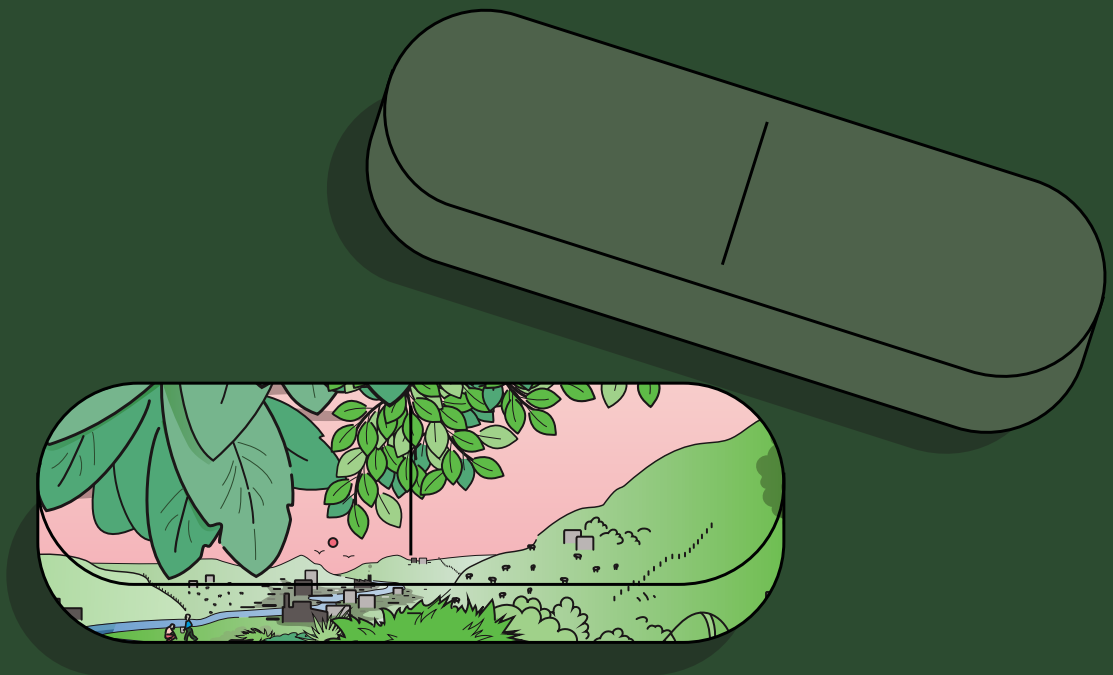
Operational research:

- Implementation review of the critical control points for typical largest risk along risks at steps of the manufacturing process and effective controls.
- Implementation review of the quality of risk management plans.
- Economical/financial analysis of cost of guidance implementation to manufactures (regulatory compliance process and report as well as investment system upgrades).
- Study of equity considerations between brand and generic manufacturers.

Future updates to this guidance will be needed to remain up to date with scientific advances and operation experiences. This is likely to involve periodic update scientific and technical specifications of the guidance, and also improvements in the platforms for collaboration, dialogue, coherent implementation and support for capacity-building.

8

Guidance development process



8
Guidance development process

8.1
Evidence review and quality appraisal

Multiple lines of evidence were used to inform this guidance:

- Review of scientific literature and methods on PNEC derivation and values summarized in the accompanying background document *Evidence synthesis for deriving PNECs for resistance selection.* The background document informs **Annex 1** and **Section 3**.
- Review of wastewater and solid waste treatment technologies used in antimicrobial manufacturing to inform technologies available for treatment to meet PNEC values and reduction of resistant bacteria for liquid effluent in **Section 3.1.2** and **Annex 2**. The treatment technology review also informs performance targets for solid waste described in **Section 3.1.3** and **Annex 3**.
- Reports, methods and policies from implementation including: the AMR Industry Alliance; British Standards Institution; Global Antibiotic Research and Development Partnership; and national and regional implementation, including in China, Europe, India, New Zealand, Norway, Sweden and the United Kingdom.
- Expert opinion of expert group members on inconclusive aspects in the above points.

Data were extracted from the scientific and grey literature and public consultation responses by consultants to prepare drafts for sharing and review ahead of expert group meetings and a hearing with public consultation respondents prior to finalization and publication.

8.2
Evidence to decision-making process

Evidence was synthesized into the guidance text based on quality assessment and evidence for decision criteria, and presented to the expert group for decision by consensus via four online meetings and email exchanges of draft text.

A public consultation process was completed with written and verbal feedback for public submitters taken into account, followed by a public hearing to present to submitters how feedback had been incorporated and to hear final verbal submissions. Decision criteria used were: feasibility for immediate or staged implementation; interventions/options acceptable to all stakeholders; balance between benefits and harms; and impact on equity. The revised draft was then circulated for review by the expert group and feedback compiled into the final document.

8.3
Plans for updates

WHO will monitor uptake and implementation by the 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 in **Annex 1** and **Annex 3**) within approximately five years.

8.4
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 academic (e.g. environmental pharmacology, chemical process engineering and technology), implementation (e.g. regulation and auditing, civil society), experience, gender and regional representation. All members of the expert group signed declarations of interest, which were 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.

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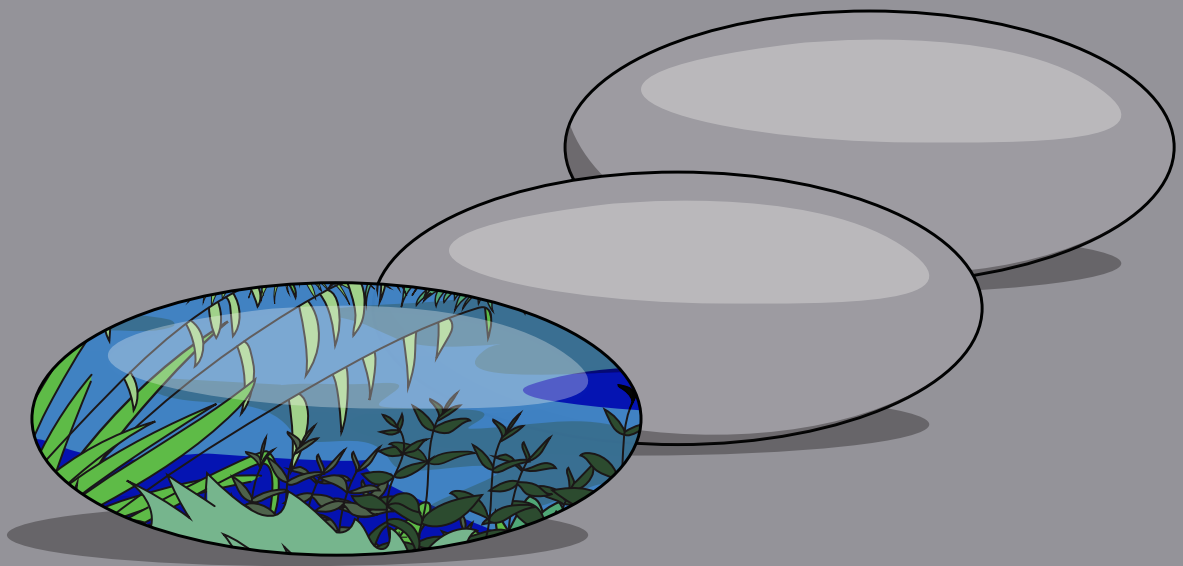

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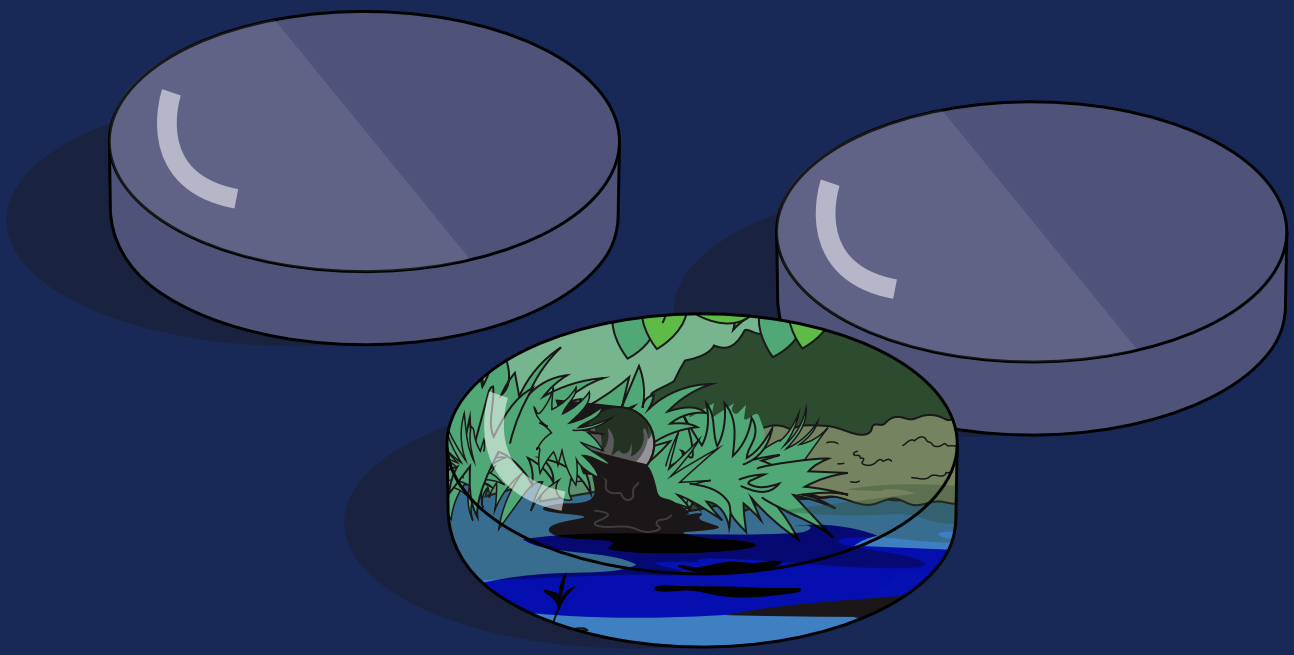
Annexes



Annex 1

Targets: PNECs for resistance selection and ecological effects

The list of PNECres is derived from Bengtsson-Palme & Larsson *(1)*, Gullberg et al. *(2)* and Stanton et al. *(3)*. A separate background document *Evidence synthesis for deriving PNECs for resistance selection* describes details of derivation of PNEC values listed and includes an additional list of references. The list of PNECeco risk are based on the best available and standardized data. The approach for PNEC derivation is described in Vestel et al. *(4)*.

PNEC values may be periodically updated to align with new scientific data as described in **Section 7**.

Table A1.1 List of PNECs for resistance selection (PNECres) and PNECs for ecological effects (PNECeco)

API	Anatomical therapeutic chemical drug class	PNECres (µg/L)	Reference	PNECeco (µg/L)	PNECeco rationale	Test guideline/ reference	Reference
Amikacin	Aminoglycoside	16	*(1)*	-	-	-	No data
Amoxicillin	Penicillin	0.25	*(1)*	0.57	Anabaena flos-aquae EC10 ÷ 10 [a]	OECD 201	Industry data
Ampicillin	Penicillin	0.25	*(1)*	0.6	Cyanobium gracile EC10 ÷ 10	OECD 201	*(5)*
Avilamycin	Orthosomycin	8	*(1)*	125	Synechococcus leopoliensis NOEC ÷ 10	OECD 201	Industry data
Azithromycin	Macrolide	0.25	*(1)*	0.03	Microcystis aeruginosa EC10 ÷ 10	EPA 1002.0	Industry data
Aztreonam	Monobactam	0.5	*(1)*	-	-	-	No data
Bacitracin	Cyclic peptide	8	*(1)*	114.59	Geometric mean (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	0.25	*(1)*	-	-	-	No data
Capreomycin	Antituberculosis agent	2	*(1)*	-	-	-	No data
Cefaclor	Cephalosporin	0.5	*(1)*	-	-	-	No data
Cefadroxil	Cephalosporin	2	*(1)*	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	4	*(1)*	-	-	-	No data
Cefalotin	Cephalosporin	2	*(1)*	-	-	-	No data
Cefazolin	Cephalosporin	1	*(1)*	-	-	-	No data
Cefdinir	Cephalosporin	0.25	*(1)*	-	-	-	No data
Cefepime	Cephalosporin	0.5	*(1)*	1.3	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data

API	Anatomical therapeutic chemical drug class	PNECres (µg/L)	Reference	PNECeco (µg/L)	PNECeco rationale	Test guideline/ reference	Reference
Cefixime	Cephalosporin	0.06	*(1)*	0.6	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Cefoperazone	Cephalosporin	0.5	*(1)*	-	-	-	No data
Cefotaxime	Cephalosporin	0.13	*(1)*	0.12	Anabaena cylindrica EC10 ÷ 10	OECD 201	*(5)*
Cefoxitin	Cephalosporin	8	*(1)*	-	-	-	No data
Cefpirome	Cephalosporin	0.06	*(1)*	-	-	-	No data
Cefpodoxime [a]	Cephalosporin	0.25	*(1)*	-	-	-	-
Cefpodoxime proxetil [a]	Cephalosporin	-	-	1.76	Anabaena flos-aquae EC10 ÷ 10 [a]	OECD 201	Industry data
Cefquinome	Cephalosporin	-	-	1.6	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Ceftaroline	Cephalosporin	0.06	*(1)*	0.12	Anabaena flos-aquae NOEC ÷ 10	OECD 201	Industry data
Ceftazidime	Cephalosporin	0.5	*(1)*	1.3	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Ceftibuten	Cephalosporin	0.25	*(1)*	-	-	-	No data
Ceftiofur	Cephalosporin	0.06	*(1)*	-	-	-	No data
Ceftobiprole	Cephalosporin	0.25	*(1)*	0.23	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Ceftolozane	Cephalosporin	-	-	1.9	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Ceftriaxone	Cephalosporin	0.03	*(1)*	0.33	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Cefuroxime	Cephalosporin	0.5	*(1)*	1.7	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Cephalexin	Cephalosporin	4	*(1)*	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	8	*(1)*	-	-	-	No data
Chlortetracycline	Tetracycline	-	-	5	Raphidocelis subcapitata EC10 ÷ 10	OECD 201	Industry data
Ciprofloxacin	Fluoroquinolone	0.023	*(2)*	0.45	Anabaena flos-aquae EC10 ÷ 10	OECD 201	*(6)*
Clarithromycin	Macrolide	0.25	*(1)*	0.25	Raphidocelis subcapitata NOEC ÷ 10	OECD 201	*(7)*
Clinafloxacin	Fluoroquinolone	0.5	*(1)*	-	-	-	No data
Clindamycin	Lincomycin	1	*(1)*	0.1	Raphidocelis subcapitata EC10 ÷ 10	OECD 201	Industry data
Cloxacillin	Penicillin	0.13	*(1)*	20	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Colistin (polymyxin E)	Polymixin	2	*(1)*	9	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data

API	Anatomical therapeutic chemical drug class	PNECres (µg/L)	Reference	PNECeco (µg/L)	PNECeco rationale	Test guideline/ reference	Reference
Daptomycin	Cyclic lipopeptide	1	*(1)*	510	Pimephales promelas NOEC ÷ 10	OECD 210	Industry data
Delamanid	Nitroimidazole	-	-	0.03	Raphidocelis subcapitata NOEC ÷ 10	OECD 201	Industry data
Doripenem	Carbapenem	0.13	*(1)*	0.46	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Doxycycline	Tetracycline	2	*(1)*	25.1	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Enramycin	Polypeptide	-	-	4.8	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Enrofloxacin	Fluoroquinolone	0.06	*(1)*	1.91	Anabaena flos-aquae NOEC ÷ 10	OECD 201	*(6)*
Ertapenem	Carbapenem	0.13	*(1)*	14	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Erythromycin	Macrolide	1	*(1)*	0.5	Anabaena sp. CPB4337 EC10 ÷ 10	OECD 201	*(8)*
Ethambutol	Antituberculosis agent	2	*(1)*	-	-	-	No data
Faropenem	Penem	0.02	*(1)*	-	-	-	No data
Fidaxomicin	Macrolide	0.02	*(1)*	891	Pimephales promelas NOEC ÷ 10	OECD 210	Industry data
Florfenicol	Phenicol	2	*(1)*	38	Anabaena flos-aquae EC10 ÷ 10 [b]	OECD 201	Industry data
Flucloxacillin	Penicillin	-	-	26.8	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Flumequine	Fluoroquinolone	0.25	*(1)*	-	-	-	No data
Fosfomycin	Phosphonic	2	*(1)*	52.4	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Fusidic acid	Steroid antibacterial	0.5	*(1)*	-	-	-	No data
Framycetin	Aminoglycoside	-	-	-	-	-	No data
Gatifloxacin	Fluoroquinolone	0.13	*(1)*	-	-	-	No data
Gamithromycin	Macrolide	-	-	0.24	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Gemifloxacin	Fluoroquinolone	0.06	*(1)*	-	-	-	No data
Gentamicin	Aminoglycoside	1	*(1)*	0.15	Raphidocelis subcapitata EC10 ÷ 10	OECD 201	Industry data
Imipenem	Carbapenem	0.13	*(1)*	0.41	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Isoniazid	Hydrazide	0.13	*(1)*	-	-	-	No data
Kanamycin	Aminoglycoside	2	*(1)*	1.05	Synechococcus leopoliensis EC10 ÷ 10	OECD 201	Industry data

API	Anatomical therapeutic chemical drug class	PNECres (µg/L)	Reference	PNECeco (µg/L)	PNECeco rationale	Test guideline/ reference	Reference
Levofloxacin	Fluoroquinolone	0.25	*(1)*	1.52	Anabaena flos-aquae EC10 ÷ 10 [a]	OECD 201	Industry data
Lincomycin	Lincosamide	2	*(1)*	0.81	Synechococcus leopoliensis EC10 ÷ 10	OECD 201	*(9)*
Linezolid	Oxazolidinone	8	*(1)*	3.5	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Loracarbef	Cephalosporin	2	*(1)*	-	-	-	No data
Mecillinam	Penicillin	1	*(1)*	-	-	-	No data
Meropenem	Carbapenem	0.06	*(1)*	1.5	Anabaena flos-aquae NOEC ÷ 10	OECD 201	Industry data
Metronidazole	Imidazole	0.13	*(1)*	-	-	-	No data
Minocycline	Tetracycline	1	*(1)*	1.1	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Moxifloxacin	Fluoroquinolone	0.13	*(1)*	-	-	-	No data
Mupirocin	Carboxylic acid	0.25	*(1)*	-	-	-	No data
Nalidixic acid	Quinolone	16	*(1)*	-	-	-	No data
Narasin	Ionophore	0.5	*(1)*	-	-	-	No data
Natamycin	Antiseptic	-	-	210	Synechococcus leopoliensis EC10 ÷ 10	OECD 201	Industry data
Neomycin	Aminoglycoside	2	*(1)*	0.03	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Netilmicin	Aminoglycoside	0.5	*(1)*	-	-	-	No data
Nitrofurantoin	Nitrofuran	64	*(1)*	-	-	-	No data
Norfloxacin	Fluoroquinolone	0.5	*(1)*	120	Anabaena sp. CPB4337 EC10 ÷ 10	OECD 201	*(8)*
Ofloxacin	Fluoroquinolone	0.5	*(1)*	10	Anabaena flos-aquae NOEC ÷ 10	OECD 201	Industry data
Oxacillin	Penicillin	1	*(1)*	-	-	-	No data
Oxytetracycline	Tetracycline	0.5	*(1)*	47	Raphidocelis subcapitata EC10 ÷ 10	OECD 201	*(10)*
Pefloxacin	Fluoroquinolone	8	*(1)*	-	-	-	No data
Penicillin G procaine	Penicillin	-	-	16	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Phenoxymethylpenicillin	Penicillin	0.06	*(1)*	-	-	-	No data
Piperacillin	Penicillin	0.5	*(1)*	4.3	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Polymyxin B	Polymyxin	-	-	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	Daphnia magna EC10 ÷ 10	OECD 211	Industry data
Retapamulin	Pleuromutilin	0.06	*(1)*	-	-	-	No data

API	Anatomical therapeutic chemical drug class	PNECres (µg/L)	Reference	PNECeco (µg/L)	PNECeco rationale	Test guideline/ reference	Reference
Rifampicin	Antituberculosis agent	0.06	*(1)*	4.06	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Rifamycin	Antituberculosis agent	-	-	1	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Rifaximin	Macrolactam	-	-	0.11	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Roxithromycin	Macrolide	1	*(1)*	6.8	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Secnidazole	Nitroimidazole	1	*(1)*	-	-	-	No data
Sparfloxacin	Fluoroquinolone	0.06	*(1)*	-	-	-	No data
Spectinomycin	Aminocyclitol	32	*(1)*	-	-	-	No data
Spiramycin	Macrolide	0.5	*(1)*	1.09	Synechococcus leopoliensis EC10 ÷ 10	OECD 201	Industry data
Streptomycin	Aminoglycoside	16	*(1)*	-	-	-	No data
Sulfadiazine	Sulfonamide	-	-	11.21	Geomean of Raphidocelis subcapitata EC10 ÷ 10	OECD 201	Industry data
Sulfamethoxazole	Sulfonamide	16	*(1)*	0.6	Synechococcus leopoliensis NOEC ÷ 10	ISO 8692	*(11)*
Tedizolid	Oxazolidinone	-	-	3.2	Pimephales promelas EC10 ÷ 10	OECD 210	Industry data
Teicoplanin	Glycopeptide	0.5	*(1)*	12.9	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Telithromycin	Macrolide	0.06	*(1)*	-	-	-	No data
Tetracycline	Tetracycline	0.1	*(3)*	3.2	Raphidocelis subcapitata EC10 ÷ 10	OECD 201	*(8)*
Thiamphenicol	Amphenicol	1	*(1)*	-	-	-	No data
Tiamulin	Pleuromutilin	1	*(1)*	-	-	-	No data
Ticarcillin	Penicillin	8	*(1)*	-	-	-	No data
Tigecycline	Tetracycline	1	*(1)*	0.1	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Tildipirosin	Macrolide	-	-	0.42	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Tilmicosin	Macrolide	1	*(1)*	0.8	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Tobramycin	Aminoglycoside	1	*(1)*	4.3	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data
Trimethoprim	Trimethoprim	0.5	*(1)*	312.45	Geomean of Anabaena flos-aquae EC10 ÷ 10 [a]	OECD 201	Industry data, *(10)*, *(9)*
Trovafloxacin	Fluoroquinolone	0.03	*(1)*	-	-	-	No data
Tulathromycin	Macrolide	-	-	0.04	Anabaena flos-aquae EC10 ÷ 10	OECD 201	Industry data

API	Anatomical therapeutic chemical drug class	PNECres (µg/L)	Reference	PNECeco (µg/L)	PNECeco rationale	Test guideline/ reference	Reference
Tylosin	Macrolide	4	*(1)*	0.98	Geomean of Synechococcus leopoliensis EC10 ÷ 10 [a]	OECD 201	Industry data, *(9)*
Vancomycin	Glycopeptide	8	*(1)*	-	-	-	No data
Viomycin	Antituberculosis agent	2	*(1)*	-	-	-	No data
Virginiamycin	Streptogramin	2	*(1)*	-	-	-	No data

[a] when PNEC data is available for both a prodrug and the corresponding active substance as in the case of cefpodoxime and cefpodoxime proxetil, risk assessment should be performed on both.

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

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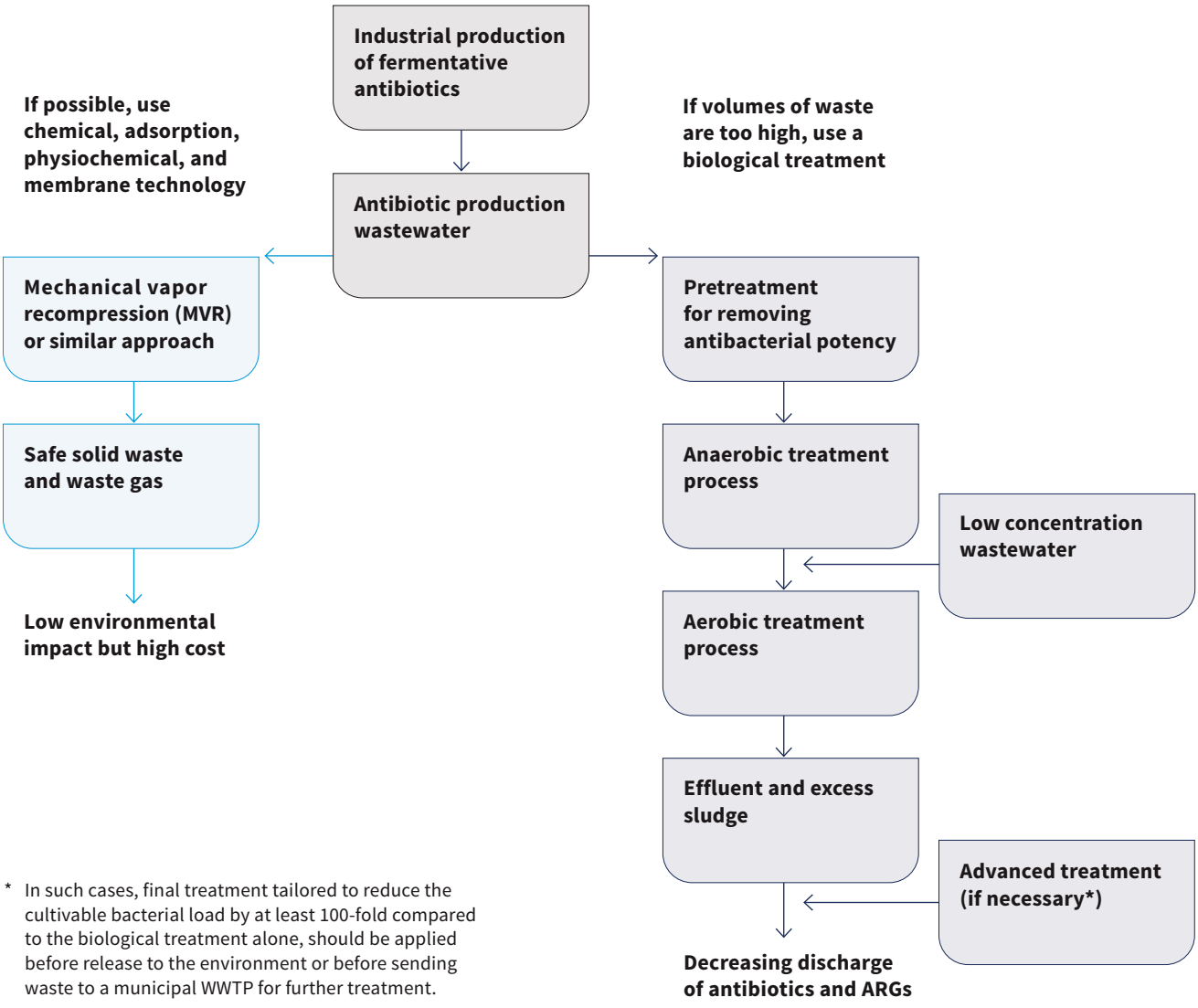
Annex 2

Supporting information: A selection of advanced treatment technology options for wastewater from antibiotic manufacturing

This annex includes wastewater treatment technology options that may be considered to meet PNECs and microbial reduction (described in **Section 3.1.1** and **Section 3.1.2**) and technology improvements, identified as part of the process risk management plan (see **Section 4**). The list of technologies is not exhaustive, nor does it imply technologies will meet targets in every context. All technology must be properly operated, maintained and monitored to ensure the intended performance is met. Users should consult with different solution providers' technologies to tailor and optimize performance to meet targets.

Note that in many cases, upstream control measures that prevents the antibiotic reaching wastewater can be more cost-effective than end-of-pipe solutions. Separately, more advanced treatment of waste streams with high antibiotic content can also be a cost-effective strategy.

Figure A2.1: Schematic of treatment steps and processes



* In such cases, final treatment tailored to reduce the cultivable bacterial load 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.

Table A2.1: Selection of advanced treatment technology options for wastewater from antibiotic manufacturing

Types	Technologies	Scope of application	Performance obtained in the application cases or references	Evidence summary (published literature) (see Annex 2 references)
Antibiotic production wastewater ***(1), (2), (3), (4), (5)***	**Pretreatment techniques**			
	Enhanced hydrolysis-based techniques for removing antibacterial potency.	Pretreatment method used for removal of high concentration fermentative antibiotics (e.g. tetracyclines, macrolides and aminoglycosides) from production wastewater.	Removal of antibiotics may reach > 99%. Low-cost selective hydrolysis of functional groups of antibiotics. Some hard-to-hydrolyse antibiotics, such as aminoglycosides, should be treated at over 100°C.	*(3)*, *(6)*, *(7)*, *(8)*, *(9)*
	Biological technique using yeast.	Pretreatment method used for oil-containing antibiotic production wastewater where oil is the fermentation substrate for some antibiotic.	In full-scale paromomycin or ribostamycin production wastewater (high oil residue from the fermentation production) treatment system using yeast, oil residue removal rate of 61.4%–74.2% has been achieved.	*(10)*
	Oxidation-based techniques (e.g. ozone oxidation, Fenton oxidation).	Pretreatment method used for removing antibiotics from production wastewater.	Examples for ozone oxidation and Fenton oxidation show doses of 1.2 mg O_3 per mg of initial oxytetracycline permitted 92% oxytetracycline removal from production wastewater (oxytetracycline, 702 mg/L).	*(11)*, *(26)*, *(29)*, *(30)*
	Coagulation-flocculation, dissolved air flotation.	Treatment for removal of antibiotics in wastewater.	Can be used in pretreatment of industrial effluents before entering municipal sewers. Studies indicate coagulation-flocculation average removal efficiency of 92% of total suspended solids.	*(19)*, *(20)*, *(22)*

Types	Technologies	Scope of application	Performance obtained in the application cases or references	Evidence summary (published literature) (see Annex 2 references)
Antibiotic production wastewater *(1), (2), (3), (4), (5)*	**Advanced treatment techniques**			
	Oxidation-based techniques (e.g. synchronized oxidation-adsorption, ozone oxidation, Fenton oxidation, electrochemical oxidation).	Advanced treatment used for removing refractory biodegradable organic pollutants from biological treatment effluent.	Suitable for effectively removing many antibiotics and other pharmaceuticals from wastewater before discharge, but requires that the total organic load is reduced first through other technologies. Synchronized oxidation-adsorption can often selectively remove the residual antibiotics by adsorption with lower cost and more gentle reaction conditions compared to Fenton oxidation.	*(3)*, *(12)*, *(27)*, *(29)*, *(30)*
	Activated carbon, powdered activated carbon, granular activated carbon.	Treatment method used for removing antibiotics from wastewater.	Both powdered activated carbon and granular activated carbon are broadly applied to municipal wastewater for removal of pharmaceuticals; for many substances, achieving well over 90% removal, for some, less. Notably, as with oxidation technologies, efficient removal of antibiotics with activated carbon requires prior reduction of the organic load.	*(21)*, *(23)*, *(43)*
	Cellulose membranes, zeolites, aluminium oxide, iron hydroxide.	Treatment method used for removing antibiotics from wastewater.	A study with cellulose membranes showed elimination of 27% of ciprofloxacin. Another study achieved 90% and 97% elimination of ciprofloxacin in using zeolites.	*(24)*, *(25)*
	UV and hydrogen peroxide (UV/H_2O_2).	Treatment method to eliminate antibiotics in wastewater.	A study using photo-Fenton ($Fe^{2+,3+}/H_2O_2$) with UV254, 50 mg L^{-1} of H_2O2, with and without adding iron (5 mg L^{-1} of Fe^{2+} added or 1.48 mg L^{-1} of total iron already present), removed 98% and 97% of micropollutants, respectively, after 30 minutes of treatments. Degradation increases with increasing concentrations of H_2O_2. Photo-Fenton under simulated sunlight provided lower percentages of removal. Another study demonstrated that ozone-based advanced oxidation processes are more energy-efficient than the UV/H_2O_2 process at all H_2O_2 levels, and that adding H_2O_2 in equimolar concentration translated in 35% greater energy consumption over the ozone only process.	*(28)*, *(29)*, *(30)*

Types	Technologies	Scope of application	Performance obtained in the application cases or references	Evidence summary (published literature) (see Annex 2 references)
Antibiotic production wastewater ***(1), (2), (3), (4), (5)***	Microfiltration, ultrafiltration, nanofiltration, reverse osmosis.	Treatment to intercept antibiotics as the wastewater passes through small pores in the membrane.	A study demonstrated that ultrafiltration and flocculation had similar removal of colloids. Ultrafiltration retained 93% ± 4% of pharmaceuticals while no pharmaceutical removal was observed with flocculation. Another study found that microfiltration and reverse osmosis had an average removal rate of antibiotics from the liquid phase of 92% with tests on cephalexin, ciprofloxacin, cefalclor, sulphamethoxazole and trimethoprim. A third study, using nanofiltration and reverse osmosis, reduced sulfamethoxazole by more than 85%.	*(22)*, *(31)*, *(32)*, *(33)*
	Hydrothermal treatment.	Treatment to remove antibiotics and antibiotic resistance genes.	A study found that a hydrothermal treatment of 180°C combined with anaerobic digestion decreased antibiotic resistance genes by 5.2 logs. Another study used hydrothermal treatment to remove erythromycin from erythromycin fermentation residue and had a removal ratio of 97.7%. Hydrothermal technology is also promising for recycling erythromycin fermentation residue as bioenergy.	*(34)*, *(35)*, *(36)*
Antibiotic production wastewater ***(1), (2), (3), (4), (5)***	**Combination of processes**			
	Pretreatment + biological treatment.	Depending on the antibiotic concentrations, effluent needs to be further treated in centralized or industrial park WWTP.	Using combined processes such as enhanced hydrolysis pretreatment and biological treatment, AMR released could be reduced substantially. Biological treatment using up-flow anaerobic sludge bed can reduce antibiotic resistance genes from effluent and sludge by 80%–95%. Anaerobic membrane bioreactors together with appropriate pretreatment also shows potential.	*(13)*, *(14)*, *(15)*, *(16)*, *(17)*, *(18)*, *(37)*
	Pretreatment + biological treatment + advanced treatment.	Likely able to meet targets and standards for liquid effluent discharge to environment.	With pretreatment such as enhanced hydrolysis, advanced treatment including synchronized oxidation-adsorption, oxidation process, membrane filtration and reverse osmosis can substantially reduce AMR development and release.	*(13)*, *(14)*, *(15)*, *(16)*, *(17)*, *(18)*, *(37)*
	Reverse osmosis, subsequent to normal treatment.	Allows for water recycling.	Exhibits strong efficacy in mitigating antibacterial potency and antibiotic resistance genes in treated effluent; however, it can be cost-prohibitive.	

Types	Technologies	Scope of application	Performance obtained in the application cases or references	Evidence summary (published literature) (see Annex 2 references)
Antibiotic production wastewater ***(1), (2), (3), (4), (5)***	Multi-effect evaporator, mechanical vapour recompression*. (*Relevant for wastewater with high salinity.)	Treatment of process waste from reactor washings and product separation processes that usually have high dissolved solids content.	Distillate can be recycled for use as water supply when feasible, which allows for no wastewater discharge. Solid waste needs to be disposed of in accordance with local environmental regulations.	*(4)*, *(38)*, *(39)*, *(40)*, *(41)*
Spent solvents	Solvent strippers.	Recovery or removal of solvents from reaction processes.	In-process recovery of purified solvents and recycling these reduce waste generation as well as minimizes cost for both disposal and fresh chemical purchases.	*(4)*, *(41)*, *(42)*, *(43)*

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Annex 3

Supporting information: A selection of advanced treatment technology options for solid waste from antibiotic manufacturing

Table A3.1 provides an overview of management options for treating solid waste from API manufacturing. This is not a comprehensive list of technologies, nor does it imply that a given technology will meet targets in every context. Users should consult with different local solution providers' technologies to tailor and optimize performance to meet targets and comply with local regulations for solid waste disposal. Any technology must be properly operated and monitored to ensure the intended performance is met. Dewatering is essential to all sludge treatment processes.

Table A3.1: Selection of advanced treatment technology options for solid waste from antibiotic manufacturing

Types	Technologies	Scope of application	Performance obtained in the application cases or references	Evidence summary (published literature (see Annex 3 references)
Antibiotic fermentation residue	Enhanced hydrolysis-based techniques	• Hydrothermal treatment application can selectively hydrolyse functional groups of antibiotics (e.g. tetracyclines and macrolides) and decrease the antibiotic discharge to the environment from antibiotic fermentation residues. Other types of chemical reactions can also deactivate the active moiety • Some technologies application, such as disc drying, can decrease the easy-to-hydrolyse antibiotics, such as penicillin, and the water content of antibiotic fermentation residues • Some pilot studies have included hyperthermophilic pretreatment to remove high concentration antibiotic	• Removal of antibiotic can reach 99%– 100% (lower than the detection limit of ultra-performance liquid chromatography mass spectrometry). When the antibiotic potency is removed, antibiotic fermentation residue was used as soil conditioner and fertilizers • Beneficial resource recovery from antibiotic fermentation residue treated by enhanced hydrolysis-based techniques prevents antibiotics entering the food-chain	*(1)*, *(2)*, *(3)*, *(4)*
	Alternative fuel	The antibiotic fermentation residue can be utilized as alternative fuel for power plants	Beneficial for energy recovery from antibiotic fermentation residue	*(8)*, *(12)*

Types	Technologies	Scope of application	Performance obtained in the application cases or references	Evidence summary (published literature (see Annex 3 references)
Antibiotic fermentation residue	Secure landfill	Disposal of sludge generated from industrial effluent treatment plants	Impermeable, horizontal and vertical liner to prevent leakage of leachate from landfill	
	Incineration	Hazardous waste disposal using incineration	Suitable for antibiotic fermentation residues, particularly if volumes are limited	*(11)*, *(12)*
Other organic solid wastes including 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 5 to 7 days so as to carry out ultra-high temperature aerobic fermentation of organic solid waste	Capable of degrading some antibiotics and antibiotic-resistant microorganisms. Organic matter in solid waste is converted into stable humus	*(13)*, *(14)*, *(15)*, *(16)*
	Anaerobic digestion, pretreatment + anaerobic digestion, two-stage anaerobic digestion	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)	*(5)*, *(17)*, *(18)*
	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	*(6)*, *(19)*
	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	*(7)*, *(9)*, *(10)*
	Management of packaging	Packaging containing traces of intermediates and APIs	Sending them to approved recyclers or a controlled landfill	

Annex 3 references

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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 (EC_M) of APIs 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. Losses can be in solid, semi-solid, liquid or, to some extent, in gaseous form.

Masses may be assessed by weighing or volumetric assessments (of reactants or pure API). In solutions, masses may be estimated by chemical analyses multiplied by volumes. In liquid streams, the mass can be assessed as the flow times measured concentration. **Mass in = Mass out (product) + Mass (known losses) + Mass (unknown losses)**

The general principle is to account all unknown losses to the liquid waste (conservative approach).

Mass (liquid waste) = Mass in – Mass out (product) – Mass (known losses)

In the mass balance calculation, the mass of other waste streams than the liquid waste can be accounted for as a known loss (mass subtracted from incoming mass) as long as it can be accurately assessed. Otherwise, such losses should be considered as unaccounted. Weighing can be a suitable approach if the entire waste stream is made up by only reactants and/or API (which may be the case in e.g. vacuum cleaning of reaction tanks during synthetic API production). The potential existence of impurities, contaminants, different water content, excipients, etc. should be considered when estimating masses by weighing. An alternative to assess the mass flow of API (and reactants optionally) in the waste stream is by, for example, chemical analysis. Note, however, that precision of mass estimates based on chemical analyses is often much lower than when based on weighing the pure compound.

As outlined in the main text, the estimate of potential losses of API is highly dependent on accurate data on the mass of API entering the process (and of known losses), as an underestimation of the incoming API mass (or overestimation of known losses) will lead to underestimation of losses/emissions through liquid waste. A conservative approach should therefore be taken (based on the precision of the mass estimates) where it ensured that incoming masses are not underestimated and known losses are not overestimated, which together will safeguard that unaccounted losses (assigned to liquid waste) are not underestimated.

During formulation and primary packaging, there is usually a well-defined mass of API entering the manufacturing process. For synthetic API production, however, there are rather reactants that are fed into the process. Using a typical or historical yield of API is not appropriate to estimate the mass of API entering the process. The term “yield” normally refers to the amount of product that can be collected at the end of the process. It may include known losses, but not unknown losses. Using yield data as a surrogate for the mass of API entering the process reaction efficiency is therefore inappropriate as it will systematically underestimate the amount of actually synthesized API. Consequently, it would also lead to underestimation of unaccounted losses to waste.

The conservative approach for a mass balance calculation for synthetic API production is to assume 100% reaction efficiency (on a stochiometric basis) of well-defined masses of reactants. However, most often reaction efficiencies are lower than 100%. To take into account lower than 100% (the default), the manufacturer should provide transparent data on reaction efficiencies, ensuring that these are not underestimated.

It is foreseen that the mass balance calculations can be quite complex, including multiple mass flows and assessments associated with different levels of precision. Calculations and considerations therefore need to be documented in sufficient detail and presented pedagogically to enable external audit and certification (see also **Annex 6**).

For fermentation-based manufacturing steps, mass flows cannot simply be based on reactants and expected stochiometric relationships, as microorganisms and nutrients are added to the process, in turn generating the API (or an intermediate used as a reactant in subsequent steps). The API mass generated by the microorganisms, corresponding to the “Mass in”, is therefore difficult to assess accurately. Furthermore, unknown losses to the fermentation residues are typically high, noting that all unknown losses should be assigned to the liquid waste when applying a mass balance approach. Chemical mass determination of APIs generated by fermentation is therefore needed.

The *Responsible manufacturing effluent management technical guidance document* published by the European Federation of Pharmaceutical Industries and Associations, Association of the European Self-Care Industry and Medicines for Europe *(1)* outlines the steps to estimate exposure by the API loss per year, and from this, calculate an average daily API loss over a year for calculating EC_M. This estimate is used to assess risks with "chronic exposure". The same guidance provides an opportunity to calculate short-term (24-hour average) exposure concentrations and state that risks may need to be assessed using either short- or long-term exposures (or both), depending on the nature of the process. However, when short-term exposure estimates are used, they are compared with acute, not chronic, PNECs for ecotoxicity. These acute PNECs are higher than the chronic PNECs for ecotoxicity listed in the AMR Industry Alliance standard and in this guidance. Emissions from drug manufacturing can often be highly variable *(2)*. Furthermore, given the often very short life-cycle of bacteria (sometime less than an hour), even short exposures can be considered chronic from a bacterial perspective. This guideline therefore has an ambition to capture API losses from peak releases. The average daily loss over the entire year of manufacturing will therefore not be the basis for the mass balance calculations here. Instead, mass (kg) of API losses to the wastewater should be estimated for every batch and a maximum loss per day estimated (assuming that all losses over a batch is lost in one day, unless unaccounted losses can be assigned to different days with certainty). The procedure to estimate emissions should therefore be to:

i. Estimate or measure the mass of API lost during an entire batch (account for known losses and assign any unaccounted loss to process wastewater).

ii. Account for other wastewater sources within the same industrial facility that contributes to the total flow.

iii. Chemical analyses are needed to allow for the removal of API during wastewater treatment, whether it is in-house, at a CETP or a municipal WWTP (and then the risk assessment should be based on these analyses rather than the mass balance calculations); otherwise, assume 0% removal.

iv. The EC_M is the unaccounted losses during a batch production cycle divided by the total volume of wastewater from the facility during one day of the batch period, unless unaccounted losses can be assigned to different days with certainty.

Note that this approach still does not capture peak releases within a batch cycle, in particular if high releases are associated with certain cleaning steps.

Another difference between how mass balance calculations are estimated here compared with the guidance document published by European Federation of Pharmaceutical Industries and Associations, Association of the European Self-Care Industry and Medicines for Europe *(1)* is that the latter contains no further elaboration of how to assess the accuracy of "Mass in" as applied to API production (see above with regard to reaction efficiencies as well as the need for chemical analyses of fermentation products).

Annex 4 references

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Annex 5
Sampling and chemical analyses

Laboratory analyses of antibiotics in effluent samples serves the purpose to enable comparisons of exposure with PNECs. Verifying the system performance through assessing concentrations in wastewater periodically ensures that the implemented controls are effective and provides an early warning system for any potential issues or deviations.

Monitoring the risks for discharging high levels of APIs in the effluent from manufacturing through mass balance calculations (as described in **Annex 4**) needs to be complemented by actual sampling and chemical analyses of wastewater to meet the *Stringent* level as well as for fermentation-based production. Chemical analyses can also replace mass balance estimates for reaching the *Good* level. This section is intended to provide some general guidance when sampling and analysing antibiotic levels in the effluent as well as record-keeping of the results, but will not cover specific requirements from different regulators.

Sample plan

Sample points

Sample points are described in Figure 2, depending on if the manufacturer treats the wastewater in-house, at a CETP or at a municipal WWTP. Normally, the sampling should be at the end of the pipe, just before the treated effluent is released to the environment. Measures to minimize API losses to the wastewater should always be the first action when possible (see **Section 4.1**). If there is liquid run-off from storage of solid waste, this should be analysed in addition to the main wastewater stream.

Sampling time, sample type and handling

Sampling should be conducted when concentrations at the wastewater sampling point is expected to be at its peak. For typical batch processing, consider a timing when other activities that generate discharges containing API (i.e. cleaning of equipment) and the residence time of wastewater treatment processes are factored in. To capture the peak discharge, a composite sample should be taken; that is, a series of pooled grab samples collected at defined regular time intervals over a period when peak discharge of API is expected to take place). If the peak release is expected to be defined and short (e.g. less than five hours), the sampling period could be limited to extend a few hours both before and after (in total perhaps 12 hours). In case there are uncertainties about when peak discharge occurs, particularly if high discharges could be expected to occur over several days within the same batch production cycle, daily composite samples should be taken over those days. Composite samples from each day of collection should be filtered using 0.45 micron glass filter and then stored frozen at -20°C or lower until they are ready for analysis, and each daily composite sample should be analysed separately. The frequency of sampling is site-specific, depending on the objective of the sampling, and may be performed manually (pooled grab samples) or using automatic samplers for easier sample collection. When pooling grab samples, each subsample should be combined in equal volumes to form the composite sample. If an automated sampler is used, the sample container should be cooled to a maximum of 4°C during the entire sampling period. Grab samples should also be cooled after taking each grab sample. Samples should ideally be filtered using 0.45 micron glass filter prior to storage at -20°C or lower until they are ready for analysis, but filtering after thawing just prior to analysis is also acceptable. Samples should be refrigerated (maximum 4°C) for no longer than 24 hours after completion of sampling and before chemical analyses to preserve and minimize changes in the chemical composition. If longer time is expected until chemical analyses can be initiated, the sample should be frozen (at least -20°C) as soon as possible and kept frozen until analyses. See AESGP (2022) for more details on the equipment. Site, sampling date(s), sampling hours(s) and signature of responsible sampler should be indicated on the sample containers.

Sample analyses

As monitoring APIs in wastewater may not be common practice, it is often necessary to partner with an external laboratory to gain access to or develop analytical methods enabling the measurement of an API concentration required to meet the relevant PNECs. The target concentration limit should be based on the corresponding PNECs and this would determine the required method's LOQ. If the *Stringent* level is desired, the LOQ (as applied to undiluted effluent) needs to be lower than the PNECres and not more than 10 times higher than the PNECeco. If only the *Good* level is desired (and still applying chemical analyses, as it is an option), the LOQ needs to be no more than 10 times higher than the PNECres and the PNECeco. For quality control of the laboratory results, duplicate analyses might be necessary with the prerequisite that the sample volume provided is sufficient (most often, one litre is enough).

Analytical method selection and quantitation limit

The determination of the type of analytical method should be agreed beforehand with the laboratory, but would normally use solid-phase extraction (SPE) for sample preparation, and liquid chromatography coupled with tandem mass spectrometry for identification and quantification. QA/QC specifications, limit of detection and LOQ with a comparable sample matrix must be investigated and made available to auditors.

QA/QC

Establish the data quality objectives with the laboratory to enable it to integrate QA/QC into the analysis. Multiple QA/QC measures can serve to interpret the quality of the laboratory data.

Analysis of spikes

A spike is a known quantity of a target analyte added to a part of a sample before analysis. The accuracy of the analysis is measured through the recovery of a spike from a sample (percentages of the spike concentration). Accuracy refers to the closeness of the measurement to the true concentration of the target analyte in a sample. The laboratory is responsible for determining the acceptable range of recoveries. Parallel analyses with spiked aliquots of the same samples (before SPE) should ideally be conducted to evaluate possible matrix effects. Stable isotope-labelled standards of the same API should be added to the samples prior to SPE and analysed in parallel to the target API.

Analysis of duplicates

Duplicate analyses (technical replicates) serve to evaluate precision in the chemical analyses. The laboratory should establish an acceptable precision range in a specific sample matrix. The variability in the measurements of API may vary based on the sample matrix and should be determined for each type of sample.

Analysis of blanks

A blank refers to a sample that should be entirely free of the target API. The blank aims to detect contamination and interference problems, or report their absence. Blanks can be incorporated at various points in the sampling and analytical process. Different types of blanks exist: trip; field; equipment; method; and instrument. If selected, field or equipment blanks are required to be programmed into the sampling plan. Lastly, field blanks should be deemed for analysis.

Analysis of standards

Standards assess instrument calibration and method performance. Most instrumental test methods necessitate analysis of calibration standards every day the instrument is utilized and additional standards checks are carried out with each batch of samples analysed. The laboratory commonly prepares both the standards and acceptance criteria.

Matrix interferences

Matrix interferences (physical and chemical), often occurring in complex samples/untreated/partially treated process wastewaters, can trigger poor precision, poor recovery, and/or signal suppression or enhancements in a sample. Severe interference may hinder the achievement of the method performance requirements. Dilution of the sample can potentially alleviate matrix interferences, but may also render the analyte undetectable because the concentration might become lower than the LOQ. Identify samples with high risk of matrix interferences so that the analytical laboratory can implement procedures to try to diminish matrix interferences. Please also see the section on the use of spikes (including isotope-labelled spikes) to control for possible matrix interference.

Data evaluation

The average API concentration of technical replicates shall be used in your risk analysis. If several samples are analysed representing, for example, different time points or days within a production cycle, the one with the highest concentration should be used. When the risk assessment indicates that ECs are lower than PNECs, generally no further action is needed. However, the opposite requires actions to identify the sources of contaminations and reduction of discharges, followed by new sampling and analyses. If an internal audit has identified unacceptable emissions followed by documented actions to reduce discharges, and the next sampling and analyses shows acceptable emissions, the latter should be the data used to qualify for certification.

Records of sampling and results of analysis of wastewater samples should be systematized and updated regularly as soon as laboratory results are received. Each sample needs to be traceable to the specific manufacturing batch and to all personnel involved in the process, starting with the sample collection, storage, shipment/transportation up to the laboratory analysis of the sample. If analyses cannot be conducted immediately in-house, samples need to be cooled down and frozen directly and remain so until analyses. For record-keeping purposes, consider using a checklist such as the one available in AESGP (2022).

Annex 6
Audits

This annex focuses on the essential aspect of environmental compliance maintained in the antibiotics manufacturing facilities in order to minimize the risk that emissions of antibiotics either lead to selection of resistant bacteria and/or ecological effects in the environment.

Frequency of audits

There needs to be a balance between controlling actions to prevent risks of discharging high levels of antibiotics from a manufacturing site and the resources needed and available to conduct these audits.

Internal verification monitoring that also provides data for the external (third-party) audit should be done on a yearly basis, but also if there is a large change in manufacturing volume (on a per batch basis, not per year) or if a new equipment or process that might impact the nature of effluent has been recently introduced to the process. This internal audit should be carried out by personnel who understand not only the manufacturing processes, but also the waste management operations, and preferably have experience in auditing environmental management systems.

External audits that form the basis for certification should be periodically conducted every three years after the initial external audit, as long as there has been no change in manufactured product, no major change in batch production volume (i.e. > 100% increase compared with the largest batches assessed during verification monitoring forming the basis for the current certification), or change of equipment or processes with plausible implications for risks of emissions, as any of these changes would trigger a need for new assessment.

External auditors should have experience in technical auditing and should also have some experience working within the pharmaceutical industry to ensure an understanding of the essential processes that might contribute to the release of APIs to the environment. A good understanding of the details of this guidance as well as good familiarity with local regulations is an absolute prerequisite. Having an engineering, scientific or operational background is valuable.

Timing of audits

Certification audits are usually initiated by the auditee. Given the logistics and preparations needed for an efficient audit, it is suggested that external third-party audits be scheduled in good time so that relevant documentation is ready and personnel in charge are available to answer questions from the auditors. There should be live production of the API/antibiotic during the assessment. If this is not possible, but there is sufficient in-house audit data including emission/exposure estimates collected at earlier campaigns of that specific antibiotic, it may still be possible to achieve certification under certain circumstances (with annotation in the certification document). This includes that a similar product using the same equipment and operated by personnel of the same production line is ongoing at the time of the external audit, that the assessment of exposure against PNECs are based on the previously collected data for the API in question, and that an additional site visit by external auditors is scheduled for the next production campaign of the API in question.

The questions in Table A6.1 serve as a preliminary framework outlining key considerations for conducting an environmental performance audit within an antibiotics manufacturing plant. They are not intended as detailed audit inquiries or specific criteria questions, but can be used as a starting point to integrate environmental aspects in existing audit mechanisms.

Table A6.1: Example audit checklist

Example audit checklist		
Does the site have proof of regulatory compliance? (i.e. manufacturing permit, GMP certification, etc.)	Provide documentation	
Do the permits correspond to the actual manufacturing operations?	Review documentation and compare with operation records	
Does the site have the required environmental permits from local/national authorities?	Provide valid documentation	
Environmental management system		
Does the site have environmental policies and procedures in place specific to the management of wastes containing APIs?	Provide documentation	
Does the site have third-party certification (i.e. British Standards Institution, ISO)?	Provide valid documentation	
Implementation and operation		
Staff competence and employee awareness	Proof of training	
Hazard assessment	Provide documentation	
Operational control	Provide documentation	
Internal verification for achieving exposure lower than PNECs and technology targets	Provide documentation	
Corrective and preventive measures taken	Provide documentation	
Waste management procedures		
Does the site have on-site WWTP? If yes, describe pretreatment method of processed water and final treatment method before discharge.	Provide documentation	
Does the site discharge treated/partially/untreated wastewater to a water body (i.e. lake, river, etc.)?		
Does the site collect and analyse samples of treated wastewater and sludge to quantify residues of API? Describe the procedure for management and disposal of sludge/solid wastes (i.e. landfill, incineration, etc.).		
Describe other pollution control measures (i.e. air pollution) at the site, if applicable.		
Does the site have a procedure to select third-party waste management facilities and monitor their operations?	Provide documentation on the monitoring of the third-party waste disposal records	

Documentation		
Is there a periodic report on waste stream monitoring (quantity and nature of wastes and analytical data of API levels)?	Provide documentation	
Is there a periodic report on collection and disposal of solid, sludge and liquid wastes?	Provide documentation	
Does the site have verified documentation on the compliance of waste disposal management of the off-site third-party suppliers (both solid and liquid wastes if applicable)?	Provide documentation	
Storage of products and/or wastes		
Does the site have storage tanks and storage rooms for finished API products?		
Are the storage spaces and containers regularly cleaned and inspected for accidental release of APIs?		
Does the site store solid/liquid waste in the facility or outside the facility? For how long?	Describe storage and handling procedure	
Are procedures and systems in place to ensure that waste stored does not end up in the environment?	Provide documentation	
Is there a record of chemical analyses and risk assessment for liquid run-off from a storage site?	Provide documentation	
Handling of spills (spill control)		
Are there containment systems specific for handling accidental spill of APIs?		
Is there a procedure for routine monitoring to detect occurrences of spills or leakage of APIs?	Provide documentation	
Transparency		
Does the site (or the parent company) publish a voluntary report to disclose environmental performance?	Provide documentation	
Is the manufacturing site registered in the Eco-Management and Audit Scheme?	Provide documentation	
Does the site regularly provide data of their environmental performance for a third-party report? (i.e. Access to Medicines Foundation's AMR Benchmark)		
Would the site provide information on the API suppliers or the contract manufacturers (contract development and manufacturing organizations) of each of their API products or finished formulations?		

Control of pollution from antibiotic manufacturing is a key part of safeguarding the longevity of antibiotics for all. Pollution contributes to antibiotic resistance and potentially undermines the effectiveness of medicines. High levels of antibiotics in water bodies downstream of manufacturing sites have been widely documented. Currently, antibiotic pollution from manufacturing is largely unregulated and quality assurance criteria typically do not address environmental emissions.

This guidance has been called for by a myriad of international bodies, strategies and reports. Its purpose is to provide an independent scientific basis for inclusion of targets in binding instruments to prevent the emergence and spread of antibiotic resistance.

The target audiences are: regulatory bodies (national or regional) responsible for the regulation of pharmaceutical product manufacturing or wastewater and solid waste (in countries or regions that manufacture); procurers of antibiotics for human, animal and plant use; entities responsible for generic substitution schemes and reimbursement decisions; third-party audit and inspection bodies; industrial actors in all stages of the antibiotic production chain and their collective organizations and initiatives; investors in the sector; and waste and wastewater management services that handle antibiotic wastes.

This guidance also includes best practices for risk management plans, including internal and external audit and public transparency. Crucially, this guidance includes considerations for progressive implementation, and stepwise improvement, when needed recognizing the need to protect and strengthen the global supply, and to ensure appropriate, affordable and equitable access to quality-assured antibiotics.

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