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1 Introduction

A key aspect of evaluating the environmental impact and sustainability of pharmaceuticals is assessing the risk that they may pose to ecosystems. Regulatory bodies around the world enforce this by requiring or recommending environmental risk assessments be performed. The choice of exactly how to perform these risk assessments is highly complex, and regulators must take into account a broad range of scientific knowledge on environmental exposures and potential effects on organisms. They then must distil this knowledge into pragmatic risk assessment requirements that are both protective of the environment but not unduly onerous to complete. These requirements need continuously updating as the science evolves.

Within the European Union (EU), the European Medicines Agency (EMA) oversees pharmaceutical regulation. The current guidance on the environmental risk assessment of pharmaceuticals was published in 2006 and is widely regarded as needing a significant overhaul. Accordingly, under the European Commission (EC) Pharmaceutical Strategy for Europe, a new draft Directive and Regulation was published in May 2023, which significantly enhances the protection offered to the environment. In the first part of this deliverable, we review this regulation, commenting on significant changes in the new proposed legislation. In the context of the new legislation requiring broader life cycle assessment (LCA), we also review the current status of pharmaceutical life cycle assessment.

Whilst the new legislation offers enhanced protection in several areas, it is prudent to consider areas where it could be strengthened. In the remainder of this deliverable, we discuss gaps and potential areas for improvement, and lay out steps that could be taken to address these. These recommendations cover the full range of assessing environmental risk, from advancing our assessment of environmental exposure, to ensuring effects testing is broad enough to consider sub-lethal effects that might have significant long-term impacts on ecosystems.

2 Review of current environmental risk assessment and life-cycle assessment requirements

2.1 Environmental risk assessment

2.1.1 Current EMA Guideline on pharmaceutical products for human use

Medicinal products are authorised and monitored in the EU by the European medicines regulatory network. This network is a partnership between the EC, the medicines regulatory authorities in EU Member States and the European Economic Area and the EMA (EMA, 2023). The current regulatory guideline (EMA, 2006b) on the environmental risk assessment of medicinal products for human use states, that as part of the authorisation process, an Environmental Risk Assessment (ERA) is usually required when a marketing application is made for a new product. If an ERA is not included in the authorisation application a justification for its absence should be provided (e.g. due to product nature significant risk to the environment is unlikely to occur). The risks to the environment that require assessment relate to those arising from the use, storage and disposal of medicinal products and not those from the synthesis or manufacture of medicinal products (European Commission, 2001). An ERA is not required for renewal applications or type IA variations (minor change to a marketing authorisation that has a minimal or no impact on the quality, safety or efficacy of the product) or type IB variations (minor change to a marketing authorisation that requires marketing-authorisation holder to notify the regulatory authority before implementation, but which does not require formal approval). For type II variations (major change to a marketing authorisation that may have a significant impact on the quality, safety or efficacy of a product, but does not involve a change to the active substance, its strength or the route of administration, requires formal approval), an ERA is required if there is a potential increase in environmental exposure (European Commission, 2008). Vaccines, herbal medicines and products containing e.g. vitamins, proteins and lipids etc. as active ingredients also require an ERA (or justification for its absence). The environmental impact of medicinal products should be assessed on a case-by-case basis and arrangements should be put in place to limit impacts; however environmental impact is not a criteria for refusal of a marketing authorisation. Separate guidelines/directives apply for marketing authorisations for medicinal products consisting of genetically modified organisms (EMA, 2006a) and radio-pharmaceuticals (European Commission, 2013).

The guideline on pharmaceutical products for human use currently in force describes the environment risk assessment process to be followed to gain a marketing authorisation. The risk assessment process is a phased procedure and a summary is provided here and in Table 1. Phase 1 estimates exposure and Phase 2, which is divided in two parts (Tier A and B), is where information about the fate and effects in the environment is obtained and assessed. Phase 1 is a pre-screening risk assessment with the objective of estimating exposure. Products with a $\log K_{ow}$ >4.5 (log n-octanol/water partition coefficient, OSPAR, 1992) should be screened for persistence, bioaccumulation and toxicity in a step-wise procedure according to procedures described in ECB, 2003. The Predicted Environmental Concentration (PEC) should also be calculated; this calculation is restricted to the aquatic compartment ($PEC_{SURFACEWATER}$). If the value is below 0.01 µg L^{-1} (value mainly based on acute toxicity data) and no other environmental concerns are apparent (see below re reproduction effects), it is assumed that the medicinal product being assessed is unlikely to be a risk to the environment and the assessment may be terminated. If however, the $PEC_{SURFACEWATER}$ value is equal to or greater than 0.01 µg L^{-1} then a Phase 2 assessment should be conducted. A Phase 2 risk assessment is always required for products that may affect the reproduction of vertebrates (or lower animals) at concentrations lower than 0.01 µg L^{-1}; the risk assessment should address all modes of action and justified

actions taken/recommended. Highly lipophilic compounds and potential endocrine disruptors (for example) may need to be assessed irrespective of the quantity released into the environment.

Table 1. The phased approach to environmental risk assessment in the EMA regulatory guideline on medicinal products for human use. Adapted from CHMP and EMA, 2006.

Stage in regulatory evaluation	Stage in risk assessment	Objective	Method	Test / data requirement
Phase I	Pre-screening	Estimation of exposure	Action limit	Consumption data, logK_{ow}
Phase II Tier A	Screening	Initial prediction of risk	Risk assessment	Base set aquatic toxicology and fate
Phase II Tier B	Extended	Substance and compartment-specific refinement and risk assessment	Risk assessment	Extended data set on emission, fate and effects

Phase 2 (Tier A) is also a screening assessment with the objective of providing a prediction of environmental risk; a default dataset of aquatic toxicology and fate data is used for initial screening. As part of this phase a biodegradability test should be conducted to determine the K_{ow} and K_{oc} (adsorption coefficient) values of the product. A long term toxicity test (on fish, daphnia or algae) should also be conducted to determine $PNEC_{WATER}$ (Predicted No Effect Concentration); the $PNEC_{WATER}$ is calculated by applying an assessment factor (AF) to the no-observed-effect-concentration(s) (NOEC) from relevant effects studies. An exposure assessment for groundwater is also required. If the ratio $PEC_{SURFACEWATER}$: $PNEC_{WATER}$ for the product is less than 1, then further testing in the aquatic compartment is not required as the product (or its metabolites) are unlikely to represent a risk; if the ratio is above 1 (and also in other circumstances, see below) then further assessment in Phase 2 (Tier B) is required. All experimental studies should follow test protocols issued by the European Commission, Economic Co-operation and Development (OECD) or the International Organisation for Standardisation (ISO); if other protocols are used their use should be justified in the Environmental Risk Assessment Report.

Phase 2 (Tier B) is an extension of Phase 2 (Tier A). If a potential risk/environmental impact is identified in Tier A then a Tier B assessment should be performed. In Tier B, refined PEC and PNEC values should be used based on worst case data relating to physical-chemical properties, pharmacodynamics, toxicology, metabolism, excretion, degradability and persistence from Phase 2 Tier A. As for Tier A, a number of recommend protocols are recommended for use. The $PEC_{SURFACEWATER}$ may be refined using the SimpleTreat model (Struijs, 2015) in Tier B. If the product is not readily biodegradable then the effects on sediment organisms should be investigated in Tier B. If the ratio $PEC_{SURFACEWATER}$: $PNEC_{MICROORGANISM}$ is above 0.1, further evaluation is required in Tier B. If the K_{ow} indicates transfer into aquatic organisms and a potential to bio-accumulate (K_{ow} >1000) then further evaluation of the bio-concentration factor should be considered in Tier B. If the adsorption/desorption data indicates binding to sewage sludge (K_{OC} >10,000 L kg^{-1}) an assessment in the terrestrial compartment should be conducted in Tier B, unless the substance is readily biodegradable. The terrestrial assessment does not replace the aquatic assessment.

All the data/information collated during the risk assessment process should be collated into an Environmental Risk Assessment Report to be submitted for evaluation by the European medicines regulatory network. The report should include (or justify the absence of):

- An estimate of potential environmental exposure
- An assessment of possible risks to the environment (and data to support this)

- An evaluation of precautionary and safety measures to be taken regarding the environmental release from use and from disposal of unused products/related waste
- Proposals for labelling (when the possibility of environmental risks cannot be excluded labelling describing the risks and storage and disposal guidelines should be stated on the product packaging)
- A *curriculum vitae* for the report author.

2.1.2 Draft European Commission Regulation and Directive

The European Commission just completed conducting a wide-ranging revision of the EU general pharmaceuticals legislation. A public consultation and feedback opportunity has been ongoing since March 2021 and closed on 8th November 2023. The revision of the regulation aims to achieve the following objectives:

- Make sure all patients across the EU have timely and equitable access to safe, effective, and affordable medicines.
- Enhance the security of supply and ensure medicines are available to patients, regardless of where they live in the EU.
- Continue to offer an attractive and innovation-friendly environment for research, development, and production of medicines in Europe.
- Make medicines more environmentally sustainable.
- Address antimicrobial resistance (AMR) and the presence of pharmaceuticals in the environment through a One Health approach.

More information on the revision of the regulation is available from European Commission, 2023.

The ETERNAL project is particularly focused on the environmental aspects of the regulation, i.e. the area highlighted in Figure 1.

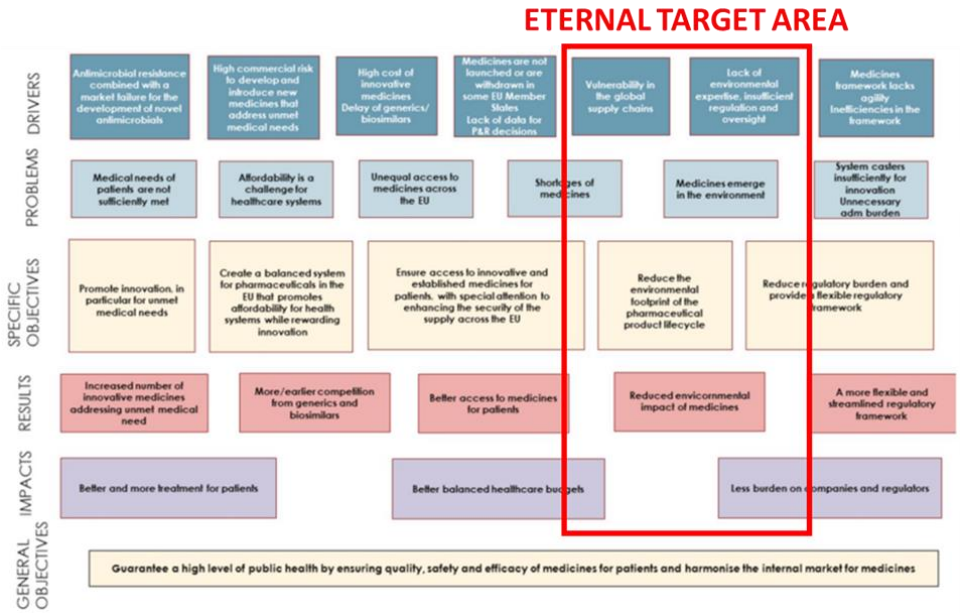

Figure 1. Overview of the proposed revisions to the EU general pharmaceuticals regulation; ETERNAL project focus area highlighted (figure adapted from European Commission, 2023c).

A summary of the information related to the Environmental Risk Assessment (ERA) process within the proposed regulation (European Commission, 2023a) is provided here. The reader should be aware that the information summarised may not include all relevant information and is based on the consultation documents (European Commission, 2023b). Amendments to the regulation are likely therefore it is advised the final documentation is consulted for more up-to-date information once the entry into force of the regulation.

The proposed regulation has, for the first time, a requirement to assess the environmental impact of entire manufacturing process (i.e. all stages of a product life-cycle, Figure 2). This may lead to longer timelines for product development and investment may be required to identify less harmful ingredients. Failure to comply with the requirements could result in penalties and reputational damage.

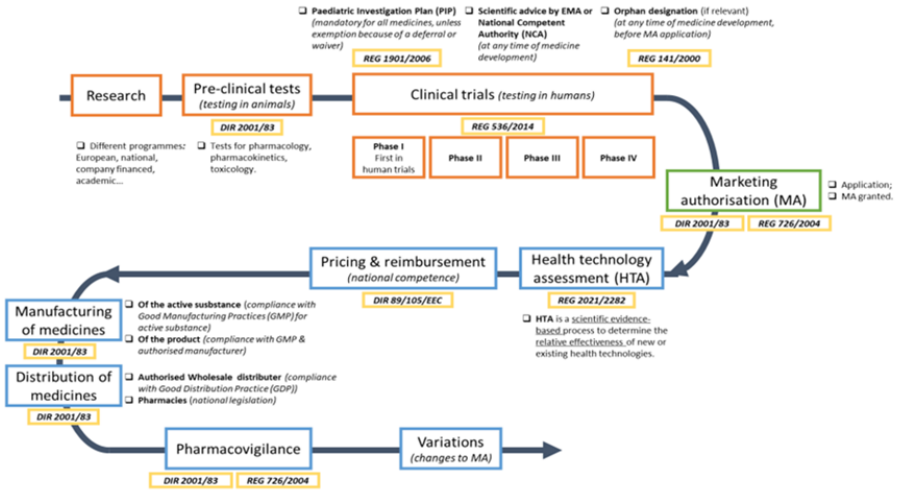

Figure 2. Visual overview of the life cycle of a medical product, including links to the legal framework (from European Commission, 2023d).

There are pre-authorisation requirements suggested within the proposed regulation. It reinforces the current mandatory ERA requirement for introducing products into the EU market however, for the first time, if an ERA is 'not adequate' or the public benefit-environmental risk profile is not favourable then the EU authorities could refuse, suspend, or vary an authorisation based on environmental harm. A time limit will be set for the submission of missing information and if the information is not received within that time limit, then the application will be considered withdrawn.

Post-authorisation ERAs could also be imposed. For products authorised prior to 30th October 2005, there is a proposed requirement for them to be conducted within 30 months of the regulation coming into effect. The European Medicines Agency (EMA) will likely request priority be given to those products/substances posing the highest potential risk.

It is also proposed that ERAs should be continuously updated with any new information that could change their conclusions; new information should be added 'without undue delay'. The proposed regulation also proposes wider consideration and evaluation of published literature within ERAs. ERA updates could be requested at any time up to 18 months after the entry into force of the proposed regulation. Any products (or their ingredients/constituents) containing PBT (Persistent, Bio-accumulative and Toxic), vPvB (very Persistent and very Bio-accumulative), PMT (Persistent, Mobile and Toxic), vPvM (very persistent and very mobile) or Endocrine-disrupting chemicals (EDC) should be identified in ERAs and risk mitigation measures included which avoid or limit emissions to air, water and soil (and human health for PBT substances). ERAs for Genetically Modified Organisms (GMO's) must identify risks to the environment, animals and human health; information on containment measures may also be required.

There is also a significant scope extension to assess antimicrobial resistance (AMR). The risk is to be evaluated for both human health and the environment covering the use and disposal of the product for the entire manufacturing supply chain both inside and outside of the EU. New antibiotics are required to submit a "stewardship plan" that includes a plan to monitor the development of AMR. The proposed regulation also includes the introduction of measures to encourage the innovation of novel antimicrobials to avoid the development of antibiotic-resistant bacteria.

Also proposed is the setting up of an active substance based review system of ERA data for authorised medicinal products ('ERA monographs'). It is proposed that an ERA monograph should include a comprehensive set of physicochemical data, fate data and effect data and that the system would be based on a risk-based prioritisation of active substances. Information, studies and data may be requested from competent authorities of the Member States and from marketing authorisation holders during the preparation of the monographs and, in cooperation with the competent authorities of the Member States, a proof-of-concept pilot study is proposed that would be completed within three years of the directive entering into force. Also, in collaboration with the competent authorities of the Member States, it is proposed a register of ERA studies is set up and maintained (unless such information is made public in the EC by different means (e.g. potentially within the European Medicines Agency's European Public Assessment Report (EPAR)). Information within the register would be publicly available unless restrictions were necessary to protect commercially confidential information. For the purpose of setting up the register, marketing authorisation holders and competent authorities may be requested to submit results from studies already completed for products authorised within 24 months of the directive entering into force.

It is also suggested that a joint inspectorate is established between EU authorities and the European Medicines Agency to reinforce current capacity; it will likely focus on conducting inspections in both EU and third countries to build efficiency in surveillance and support marketing authorisation procedures (Finan *et al.*, 2023; Pharmavibes, 2023).

More detailed information can be found in the proposed regulation text (European Commission, 2023b) and within other documents and blogs summarising the proposed changes; some of which were consulted during the preparation of this summary (EFPIA, AESGP and Medicines for Europe, 2023; Finan *et al.*, 2023; Martuscelli and Cater, 2023; McKenna, K, 2023; Pharmavibes, 2023; Schofield, 2023).

2.2 Life cycle assessment

Life cycle assessment (LCA) is a well-established paradigm by which to evaluate all potential impacts of a product throughout its manufacture, use and disposal. Traditional methods used to assess the sustainability of a product, such as Process Mass Intensity, are often constrained to considering only the manufacturing process. On the other hand, LCA takes a broad picture of the entire value chain, from raw material extraction to end-of-life, with consideration of environmental emissions along the way. This makes it an invaluable tool for quantifying the full environmental impact of a pharmaceutical product.

Despite this, the use of LCA in the pharmaceutical sector is still not widespread, highlighting difficulties in its application, largely due to lack of methodological harmonisation and data unavailability. Furthermore, when a LCA is performed, it often does not take into account ecotoxicological hazards in its impact categories (Emara *et al.*, 2019), and commonly covers only cradle-to-gate (raw material extraction to pre-distribution) rather than cradle-to-grave

(also covering use and disposal) (Siegert *et al.*, 2019). Figure 3 presents a conceptualisation of the generic life cycle of a pharmaceutical product and its relation to cradle-to-gate and -grave.

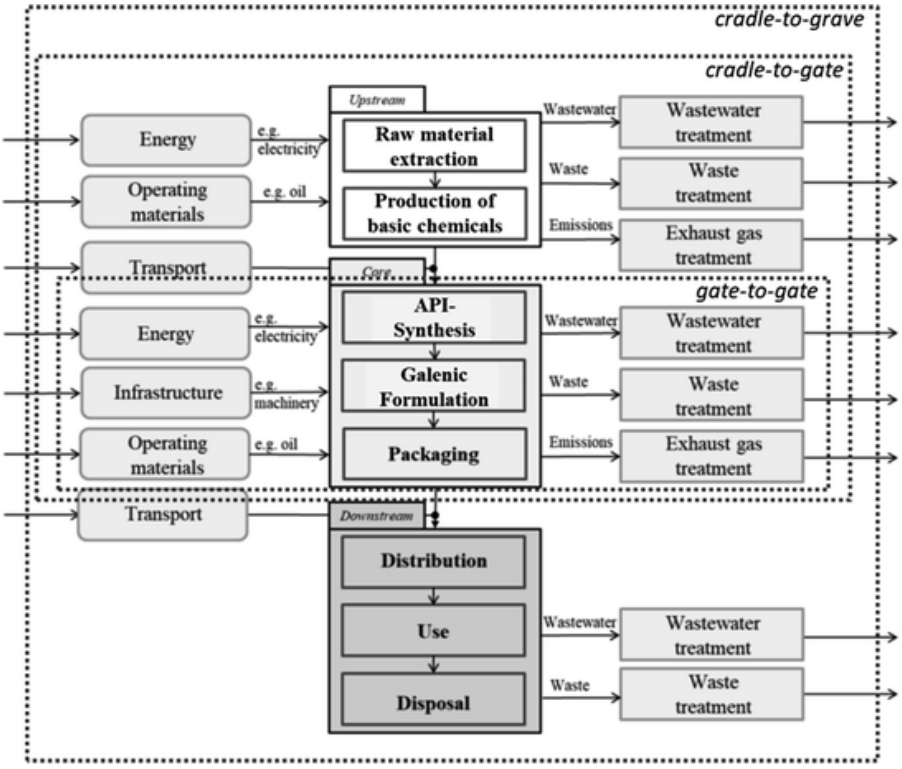

Figure 3. Life cycle of a pharmaceutical product from cradle (raw material extraction) to gate and grave. Taken from Emara *et al.*, 2018.

2.2.1 Life cycle assessment in regulation

It is worth noting, in the context of the previous section on the draft regulation and directive proposed by the EC, where LCA fits within the regulatory landscape. Though a full LCA is not mandatory when submitting a product for market authorisation, manufacturers must provide evidence around many of the points covered in LCA. In particular, the proposed regulation strengthens the life cycle aspect by requiring that environmental risk be assessed at all stages all the product life cycle. In the sense that environmental risk post-distribution are accounted for in ERA, this goes beyond many LCA that only assess cradle-to-gate.

2.2.2 Lack of harmonisation

Amongst the purported difficulties in performing LCA on pharmaceutical products, lack of harmonisation is often cited as the largest (Emara *et al.*, 2018; Siegert *et al.*, 2019), leading to different companies developing their own LCA approaches and databases that are usually confidential. Specific elements of LCA that are not well harmonised include:

- Definition of an appropriate **functional unit**. Generally, functional units employed are either effect-based (e.g. treatment of an individual for a specific period) or mass-based (e.g. production of X kg of API), though the units used within these are inconsistent. For example, mass-based functional units can use either an absolute mass (kg) or dose-dependent mass (number of defined daily doses).

- **System boundaries**, which define what the extent of the life cycle that is covered, for example, whether raw material extraction and consumer use is included. As highlighted above, LCA studies already performed are mostly cradle-to-gate, which covers only raw material extraction to pre-distribution. It is likely that the main risk to the environment comes from the use and disposal phase, and so excluding these phases severely limits the use of LCA for environmental risk assessment.
- **Impact categories** and the **method used to assess the impact**. Notably, ecotoxicity impacts are often excluded. For example, the American Chemical Society Green Chemistry Institute (ACS-GCI) Pharmaceutical Roundtable proposed nine impact categories as part of a streamlined LCA tool (Jiménez-González *et al.*, 2013) in 2013, none of which were related to the ecotoxicity of pharmaceutical products. Later efforts around harmonisation sought to rectify this issue, for example, Siegert *et al.*, 2019 proposes separate impact categories for ecotoxicity, as detailed in Table 2. Furthermore, they recommend refinement and expansion of the impact categories where appropriate for a given API, to, for example, cover sub-lethal effects such as endocrine disruption and antimicrobial resistance potential. They also make recommendations on models used to perform the impact assessment, harmonising methods as well as categories.

Table 2. Impact categories and assessment models suggested by Siegert et al 2019.

Impact category (indicator)	Impact assessment model
Climate change (global warming potential GWP)	▪ IPCC model for Global Warming Potential (GWP) over a 100 year time horizon (IPCC 2013)
Human toxicity (human toxicity potential, cancerogenic/non-cancerogenic)	USEtox model (Rosenbaum et al. 2008, 2011)
Ecotoxicity (freshwater ecotoxicity)	▪ USEtox model (Rosenbaum et al. 2008; Henderson et al. 2011)
Ecotoxicity (marine ecotoxicity, terrestrial ecotoxicity)	▪ USES-LCA 2.0 (Van Zelm et al. 2009)
Abiotic resource consumption (abiotic depletion potential (ADP) fossil and minerals)	▪ Minerals and metals: ADP model (Guinée 1995; Van Oers et al. 2002) (ADP-ultimate reserves) ▪ Energy carriers: ADP model (Guinée 1995; Van Oers et al. 2002) (ADP-fossil)
New pharma-specific impact categories	▪ New characterization models

It has been proposed that a potential step towards solving this harmonisation problem is the use of Product Category Rules (PCRs). PCRs comprise a set of harmonised rules to conduct LCA studies, and were originally developed to provide category-specific guidance for different industries. Siegert *et al.*, 2019 propose a set of rules covering system boundaries, functional unit, use- and end of life phases, impact assessment and provision of additional information (e.g. side effects, pharma-specific impacts).

More recently, there has been a renewed focus on harmonisation of LCA. The Innovative Health Initiative (IHI) have recently published a call (https://www.ihi.europa.eu/apply-funding/ihi-call-4) that includes LCA harmonisation within its scope, on a topic of "Sustainable circular development and manufacturing of healthcare products and their quantitative environmental impact assessment". The expected impact of this call states that "The harmonisation of environmental sustainability assessment methodologies across the whole healthcare sector will influence European environmental regulations to make life cycle assessments (LCA) comparable

between different pharmaceutical manufacturing processes and will contribute to establishing a novel European LCA guideline…".

The Sustainable Markets Initiative (https://www.sustainable-markets.org/) has also recently convened a joint action of seven large pharmaceutical manufacturers to work towards harmonised LCAs, through the definition of PCRs for the pharmaceutical sector.

2.2.2.1 FATE AND EMISSIONS MODELLING WITHIN LCA

A full cradle-to-grave LCA of any API requires the modelling of emissions, fate and exposure in the environment, and it is important that these models are applied along realistic, potentially API-specific exposure pathways. The models and tools often used to perform these assessments, such as USEtox, are not well suited to this task and there is a need for pharma-specific modelling to provide realistic predictions of emissions, fate and degradation. Limitations in existing models includes the unsuitability of conventional partitioning models (such as USEtox) due to the specific chemical behaviour of pharmaceuticals in the environment, inability to model degradation products, and inability to account for environmental heterogeneity and its effects on fate and ecotoxicity. These limitations are discussed more broadly in the context of ERA in Section 3.3.

2.2.3 Lack of data

Lack of (access to) data to drive LCA models is often cited as a difficultly in their application to pharmaceutical products (Jiménez-González and Overcash, 2014; Kralisch, Ott and Gericke, 2015; Emara *et al.*, 2018; Siegert *et al.*, 2019). This lack of data can be attributed to different factors:

- Limited studies providing data: For example, there may be limited data on ecotoxicological endpoints to inform impact assessments for particular pharmaceuticals, especially those new to the market or for non-standard endpoints.
- Complex life cycles and supply chains: Data is often confused by complex, opaque supply chains on which there are little data.
- Confidential data: Where data are available, they are often for proprietary products with confidential synthesis routes, and so only available to the original manufacturer or via commercial life cycle impact databases (Emara *et al.*, 2019). This limits the sharing of data, such as inventories, amongst pharmaceutical manufacturers for the purpose of LCA.

The proposed new regulation and directive from the EC (Section 2.1.2) explicitly requires the whole pharmaceutical product life cycle to be considered. Also, as detailed above, the EC is proposing the creation of an ERA monograph system, comprising a comprehensive set of physicochemical, fate and effect data. It is also proposing the creation of a register of ERA studies. Both of these are likely to increase the availability of data for performing LCA, particularly for impact categories focussing on ecotoxicity and environmental risk.

3 Gap analysis of existing approaches

The prospect of strengthened environmental protection offered by the proposed EC regulation and directive is a welcome step forward, and already begins to address gaps that have been highlighted over the past decade (Boxall *et al.*, 2012; Helwig *et al.*, 2023). In this section, we comment on these gaps and discuss further areas in which regulation could be strengthened.

3.1 More holistic effects testing

Historically, regulatory ecotoxicity assessments have been weighted heavily towards standard endpoints such as mortality, growth inhibition and reproduction including maturation timing and offspring production. This approach risks missing important sub-lethal effects, long-term impacts and modes of actions that could have significant effects on populations, communities and ecosystem services. A growing body of scientific literature is providing evidence that chronic exposure to (multiple) chemicals over extended times-scales may result in effects on physiological traits that are not measured in classical ecotoxicity studies. When such effects on "non classical" endpoints occur, this can plausibly lead to changes in individual performance and vital rates, these may propagate on to population and community impacts.

Moving beyond acute testing on classical endpoints. Recent work on biologically active chemicals (pesticides, biocides, pharmaceuticals) has identified a range of such modes of action that may not themselves directly affect conventional apical (survival, growth, reproduction) endpoints, but that are likely to affect the physiology of species in the wild in ways that will lead to effects relevant to the population level. Example of such mechanisms are neurotoxic (when affecting behavioural endpoints), immune modulation (when affecting disease/parasitism vulnerability) and genotoxicity (when affecting offspring quality or other fitness parameters). These types of effects are identified as being more likely to occur during chronic, low level exposures and also under multigenerational exposure scenarios. Such effects under such low-level, chronic exposures can be subtle and are often challenging to identify, characterise and quantify. Integrative approaches are needed to mechanistically link complex chronic exposures to the subtle non-lethal effects at different levels of biological complexity (from molecule to individual) to establish the causality of dose-response relationships and assess risks of chronic low-level exposures to chemicals. For this linkage, AOP (Adverse Outcome Pathway) approaches may be a promising approach to make linkages between mode of action and effect. Any such impact models, however, need to be operationalised quantitatively for application by linking components of the life-cycle to individual physiological and behavioural changes that feed through to apical endpoint effects understood in regulatory settings.

Endocrine disruption. A key example of a potential effect that is population relevant is through is endocrine disruption. It is welcome to see the proposed regulation includes a requirement to label whether substances are endocrine disruptors. However despite this recognition, there are some limitations with the current approach. One of the major concerns is that the focus on endocrine disruption generally considers effects through three major axes, the oestrogen, androgen and thyroid hormone systems. These endocrine axes are clearly important for their effects on maturation and reproduction for oestrogen and androgen and for development (e.g. metamorphosis in amphibians) and some aspects of metabolism. These three pathways are, however, not the only endocrine system that chemicals can affect. Other hormone pathways, for example, the corticosteroid, retinoic acid and PPAR receptor pathways also act as critical controls of physiological responses including development and metabolism; chemicals have been shown to interact with these pathways leading to biological effects. Hence, as evidence develops there may be a need for consideration for a wider range of endocrine effects within assessments. The use of a biosensor method that allows an assessment of whether a substance can act with each of these hormone receptors (usually using the human variant), would be a first step to understanding how frequent endocrine activity through these additional pathways may be.

Other specific pathways, e.g. Neurotoxicity and behaviour. A second example of a potential impact currently not assessed in classical ecotoxicology testing that is relevant to consider for populations is through neuronal system interactions that lead to changes in behaviour. Such effects were found to be critical in the impact of neonicotinoid insecticides on pollinators. Ultimately these impacts have underpinned declines in pollinator species at a national scale. Pesticides are not the only chemicals that can interact with nervous systems. Many pharmaceuticals have been specifically designed to have such effects (e.g. treatment for many mental health conditions). There is already some evidence on the effects of antidepressants on wildlife at realistic environmental concentrations. Such effects would not be picked up in classic tests. Studies to specifically measure behavioural effects would be needed. A range of such methods have been proposed by Peterson *et al.*, 2017, but to date none have yet been proposed for standardisation.

Including the use of toxicokinetic (TK) toxicodynamic (TD) models. To cause endocrine and neurotoxic linked behavioural effects, chemicals need to be able to be both taken up and to interact with potential receptors involved in, e.g. endocrine and nervous system pathways. Such uptake and receptor interactions would be expected to differ between species indicating the potential for differences in such effects (Klerks, Xie and Levinton, 2011). As part of this, coupling toxicokinetic trait information to physiological effect models to develop TK/TD models is an important step towards integrating exposure and effect assessment. Especially in those cases where it is possible to link these TK/TD models to dynamic energy budget (DEB) approaches, it can be possible to generate data from exposure that predicts effects for key population relevant life-cycle traits (Cropp, Nash and Hawker, 2014). Thus, these models can provide insights into internal chemical fate, exposure and vulnerability to potential effects on survival, growth and reproduction. As more molecular data becomes available, it is also possible to now characterise the number, diversity and potential activity of the genes physiological pathways responsible for xenobiotic metabolism, including the phase I, II, and III pathways, cellular defence mechanisms and the targets of pesticide effects (Spurgeon *et al.*, 2020). Using such data, which is rapidly being generated by wildlife DNA sequencing projects, such as the Darwin Tree of Life project (https://www.darwintreeoflife.org/), it is possible to study how species differ in the range and expression of the pathways that both detoxify a chemical, and also lead to its effect. Such approaches have clear potential value for species vulnerability assessment.

Characterising the Adverse Outcome Pathway for pharmaceuticals. Development of the AOP concept as an approach that combines current understanding of xenobiotic interactions with a particular biomolecule as the molecular initiating event leading to specific endpoints across all levels of biological organisation (Ankley *et al.*, 2010). The AOP approach provides a tractable and potentially quantitative approach to linking exposure and the ongoing accumulation of a chemical, to the mechanistic triggering of biological effect and its later apical toxicity consequences. Using ecotoxicological data accessible via searchable databases it is possible to use key identifiable steps in the AOP to assess sensitivity relationships between species to develop evolutionary and correlative models. Through this, the AOP concept can be applied to understand the degree of divergence or conservation of toxicologically relevant biochemical pathways or other traits that affect whether a pharmaceutical will cause an effect through a specific pathway and how the severity of that effect will vary between species (Rivetti *et al.*, 2020).

Mechanistic modelling. Mechanistic models like those developed for deriving extrapolation factors for pesticides (e.g. EFSA *et al.*, 2023) may play an important future role in developing improved approach to understand the potential ecotoxicology of pharmaceuticals, as they may reveal patterns related to toxicokinetic and toxicodynamic processes that can increase confidence in extrapolation. Such methods can make greater use of relatively new measurement technologies (e.g., genomics, proteomics), computational tools (e.g., quantitative structure activity relationships), and have the potential to contribute to an understanding of how the

underlying molecular pathways evolve. However, development of these methods will likely need to consider how they link into measured endpoints used during the regulatory assessment (e.g. survival, growth, reproduction). Clear links with the attribute defined to assess potential effects for vulnerable species will also be needed. Such methods will also need to undergo extensive validation to translate the effect assessment to an effect on colony/population size. The development of mechanistic models would be underpinned by the initial step of developing a Mode of Action (MoA) catalogue for pharmaceutical and tools (e.g. EcoDRUG, https://ecodrug.org/) that are able to screen species data (e.g. genomic sequence data) to identify the conservation of key receptor linked to the specific mode of action across species offer a promising starting point.

Assessing potential to cause antimicrobial resistance in the environment. Some pharmaceuticals, most notably antibiotic and antifungals, but also some active ingredients with other uses when released into the environment can cause an increase in the frequency of antibiotic resistance genes (AMR) in the resident microbial populations. It has been suggested that the presence of AMR genes in natural populations may act as a reservoir for these genes that can then be transferred from the environment to medical settings. It is again welcome to see the proposed regulation tackle this issue with the requirement for manufacturers to track the development of AMR post-authorisation, and develop a stewardship plan to reduce its likelihood. However, the potential to increase AMR is not yet a routine part of any authorisation assessment. Methods to measure the potential to cause AMR can use established pipelines for the amplification of DNA samples extracted from environmental samples and assessment for AMR presence by polymerase chain reaction (PCR), and real-time quantification of genes using fluorescence measurements. For high throughput, the use of a microchips with microreactors, allowing for the simultaneous quantification of hundreds to thousands of genes. Theoretically any sequence can be run including taxonomically informative genes (e.g. 16S rDNA, ITS) or functional genes linked to biochemical pathways. Further, because they can use DNA collected from many types of "dirty" environmental sample (e.g. river water, sludge wastes, sediment, soil) it is possible to screen production site and municipal and industrial effluent discharge point to see whether releases are causing any increase in antibiotic resistance in the environment.

3.2 Development of methodologies and risk assessment schemes for mixtures

Pharmaceuticals do not exist in the environment as individual compounds but as complex mixtures co-occurring alongside other potentially harmful chemicals. For example, in monitoring schemes in a range of European countries (e.g. France, UK, Spain) the presence of multiple pharmaceuticals has been identified in environmental media such as surface water and groundwater, including some identified as potentially being of greatest potential to cause effects in aquatic ecosystems.

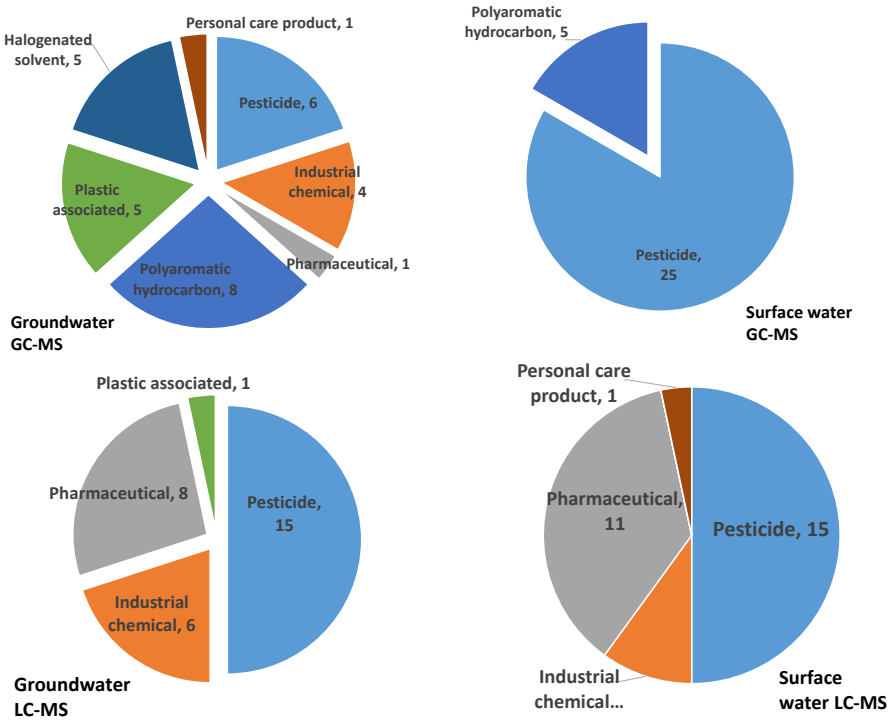

Figure 4. Main use categories for the top 30 chemicals ranked according to the potential to cause risks for aquatic habitats that were detected by GC-MS (top) and LC-MS (bottom) in the UK groundwater (left) and surface water (right) monitoring datasets, collected from samples analysed in the period 2010-2023; note most pharmaceuticals are measured in the LC-MS dataset and comprise approximately one third of the 30 chemicals of highest concern; in all case the pharmaceutical detected occur as part of a mixture of up to 70 detected substances (average in LC-MS data-set = 14).

With pharmaceuticals known to be widely present in the environment and known to nearly always be found alongside each other and also other types of chemical, there is a recognised need to consider how such mixtures of compounds may affect ecosystems. The basis of an assessment of the effects of mixtures can leverage the principles of mixture toxicology, that the joint effects of chemicals are additive in a manner governed by the similarity or dissimilarity of their mechanism of action. Within mixture assessment, perhaps one of the greatest challenges is to predict those cases (perhaps 10% of mixture combinations) where synergism or antagonism occurs (with synergism being the effect of greatest concern for risk assessment, Cedergreen, 2014), or where the prevalence of synergism and antagonism has a complicated dependence on the concentration, ratios and number of other chemicals present (Silva *et al.*, 2022) – see Figure 5). Studies of the mechanisms of synergism have begun to link some types of chemical effects, notably interactions with toxicokinetic mechanism, as one of the more major causes of such effects. In cases where such interactions are known or suspected, the potential for synergistic effects can be taken into account within the mixture modelling framework to include such non-additive effects in the risk assessment.

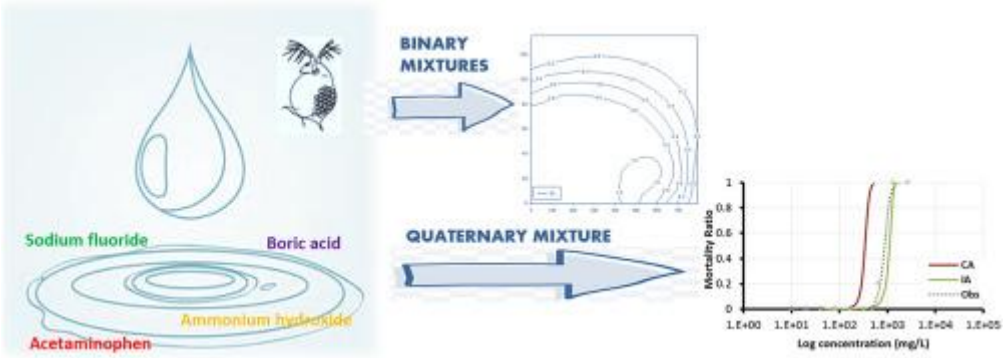

Figure 5. Whether a mixture is synergistic or antagonistic can have a complicated dependence on the concentration and ratio of chemicals present. Taken from Silva et al 2022.

Mixture assessment presents a significant challenge to the regulatory system, in the sense that assessment for the potential for environmental effects are almost always performed for individual products. Mixture assessment would present the need for manufacturers to assess their products in the context of likely co-exposures when emitted to the environment. One proposed approach is the use Mixture Assessment Factors (MAFs), which are adjustment factors applied to risk assessments to account for the risk from mixtures. The development of such MAFs is a difficult subject, and research has shown that the use of generic MAFs applied to all chemicals does not adequately capture mixture effects (Viaene, Nys and Verdonck, 2021). Therefore, the development of targeted MAFs specific to pharmaceuticals, perhaps tailored to different intended MoAs, would therefore be prudent.

The development of such MAFs also raises the important point that we need data on chemical mixtures present in the environment. Monitoring data gives useful snapshots (e.g. Wilkinson *et al.*, 2022) but exposure modelling is needed to fill gaps and generate predictions for prospective assessments. Hence, there is a need to make sure that the modelling methods that are being developed will both be flexible enough to be used for a range of different activities and also that the outcomes lead to a better understanding of the types of mixtures that will commonly be present. Few exposure models currently exist that take mixtures into account, and to the authors' best knowledge, none have been applied to pharmaceuticals. Within the ETERNAL project, we aim to develop such a model to enable robust mixtures assessment.

3.3 Better accounting for the effects of environmental heterogeneity and pharmaceutical-specific chemistry

Current ERA approaches and exposure models that underpin them treat environmental compartments as homogenous, spatially-averaged boxes, but the environment is a complex and dynamic system. Whilst spatial averages give a reasonable indication of average exposure, hazards and risk, they risk missing local, seasonal or other dynamic issues caused by the influence of environmental heterogeneity. For example, elevated concentrations of pharmaceuticals downstream of wastewater treatment plants might have locally significant ecosystem effects, but will not be picked up by spatially-averaged exposure assessments. Similarly, periods of drought that cause low water levels and thus potentially elevated concentrations will not be accounted for by steady-state predictions. These impacts may become more significant under a changing climate and different socioeconomic pathways.

To tackle these issues, exposure models used in regulation tend to make conservative assumptions in order to represent the worst-case scenario. However, this approach is not fool proof, and it is important that periodic comparisons are made between these low tier models and more realistic spatiotemporal exposure models or observation data, in order to ensure this

conservatism. Recent work on nanomaterials in EU Horizon Europe project ASINA (deliverable submitted) showed that, for certain release scenarios and geographical regions, the low tier exposure model SimpleBox(4nano) failed to detect peaks in nanomaterial predicted environmental concentrations as modelled by a spatiotemporal exposure model. Whilst this is for a different class of chemicals, the conclusions are likely to hold for pharmaceuticals.

The motivation for the use of low tier exposure assessment models is their pragmatic data requirements and ease of use. We contend that careful development, pre-processing of data and provision of map-based GUIs for more complex spatiotemporal exposure models would make them just as easy to use and therefore facilitate their use to provide more realistic regulatory exposure assessments.

Another aspect of current exposure assessment approaches that requires improvement is that the exposure assessment models used are often not specifically developed for pharmaceuticals. For example, SimpleBox is a steady-state equilibrium partitioning model that relies on partition coefficients such as the octanol-water partition coefficient (Kow), which may not fully account for the behaviour of polar pharmaceuticals. Furthermore, metabolites (degradation products) are often not considered in exposure modelling or risk assessment in general, but these may present their own risk to the environment. The development of pharmaceutical-specific models such as ePIE (Oldenkamp *et al.*, 2018) is a welcome step to address these issues.

4 Recommendations for integrating scientific knowledge into risk assessment

The previous section has highlighted the current status of regulation in the EU, and gaps that exist in current and proposed legislation that would, if filled, strengthen environmental protection. From these assessments, we can make several recommendations for integrating the latest scientific knowledge into pharmaceutical risk assessment to help fill these gaps:

- The European Commission should adopt their draft Regulation and Directive without significant alterations that would reduce the environmental protection it offers. For example, there is concern from industry on the requirement to retrospectively risk assess legacy products. Whilst modifications to this requirement can be considered to ensure this process is as streamlined as possible for industry, the overall requirement is critical in ensuring the risk from *all* pharmaceutical products, not just new ones, is considered in regulation.
- Mixtures should be accounted for in regulation. This will likely require the development of a tailored mixtures assessment framework for pharmaceuticals. This should be built in close consultation with academia and industry, and leverage the latest scientific knowledge and advancement in this area over recent years. The possibility of effects from mixtures of pharmaceuticals with other chemicals and substances should be considered.
- There is a need for the development or enhancement of methods, models and standards for better effects testing. Amongst others, this could include new methodologies and parameters for assessing the potential for endocrine disruption (such as YES assays using modified yeast cells containing the gene for human oestrogen) and TK-TD modelling and other complimentary mechanistic effects modelling.
- Regulation should consider environmental heterogeneity, at least such that screening-level assessments remain conservative for extremes encountered in the environment (with potentially increasing frequency with a changing climate). Locally elevated concentrations that could have significant local effects on ecosystems should be considered, as should extremes in temporal dynamics (e.g. river flows) that might become more prevalent under a changing climate. The impact of heterogeneity on hazard (e.g. the effect of pH and DOC on bioavailability) should also be considered.
- Exposure modelling plays a key role in enabling the aforementioned updates, for example through mixture modelling and accounting for the importance of environmental heterogeneity. Spatiotemporal exposure modelling, which provides the most realistic picture of pharmaceuticals in the environment, can be made accessible to non-experts through user-friendly interfaces and automated data parsing, but significant investment is required to enable these updates.

Within the ETERNAL project, our work package is working on several of these elements, such as improved testing for endocrine disruption, and enhanced spatiotemporal exposure modelling.

Going beyond environmental risk considerations, there is also a need to properly define how industry can measure the environmental sustainability – the *greenness* – of a pharmaceutical product. This sustainability should take environmental risk into consideration alongside broader impacts such as energy and water usage and potential environmental degradation caused through supply chains. Difficulties arise in weighting different considerations against each other. For example, are risks to ecosystems a price worth paying for a life-saving medicine? Or is a higher carbon footprint acceptable for a product that causes less environmental risk?

5 Conclusions

In this deliverable, we have presented an overview of the current EMA regulation covering the environmental risk assessment of pharmaceuticals, followed by a comparison against the new proposed Regulation and Directive published earlier in 2023. Generally, we found that the proposed legislation offers better environmental protection on several fronts. For example, the requirement to consider the whole lifecycle of products, the ability for authorities to refuse market authorisation based on risk of environmental harm, the requirement for retrospective risk assessment of products already on the market, and the requirement for continually updated risk assessments. In addition, manufacturers must flag when substances are PBT (persistent, bio-accumulative, toxic), vPvB (very persistent, very bio-accumulative), PMT (persistent, mobile, toxic), vPvM (very persistent, very mobile) or endocrine disrupting.

Despite this enhanced protection, there are important aspects that are not addressed. For example, the risk posed by chemical mixtures, which could be included through the development of a robust mixtures assessment framework. Whilst endocrine disruption is explicitly mentioned, there is room for improvement in testing for it, alongside testing for other sub-lethal effects, such as neurotoxicity (affecting behaviour), immune modulation and genotoxicity leading to inter- and trans-generational effects. In general, environmental heterogeneity (in space and time) is not accounted for, requiring the better use of data on the effects of, for example, pH and DOC on bioavailability and climatic scenarios on temporal dynamics. Integration of these aspects would lead to better understanding, supporting strengthened environmental protection for pharmaceutical products.

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