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Next generation toolbox for greener pharmaceuticals design and manufacturing towards reduced environmental impact

D6.2 – The ENVIROMED registry of pharmaceuticals and LCA

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February 2026

Funded by the European Union

Deliverable	D6.2 – The ENVIROMED registry of pharmaceuticals and LCA
Work Package(s)	WP6 – Better understanding of pharmaceuticals' environmental impact
Task(s)	T6.4 – registry of pharmaceutical micropollutants in wastewater & the environment T6.5 – Green pharmaceuticals Life Cycle Analysis
Dissemination Level	Public
Due Date	30-11-2025
Actual Submission Date	05-02-2026
WP Leader	IRES
Task Leaders	RISA / IRES
Deliverable Leader	RISA
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Document History				
Version	**Date**	**Description**	**Comments**	**Editor(s)**
v0.1	14-11-2025	First version	Table of Contents	S. Camarinopoulos (RISA)
v0.2	14-01-2026	Second version	Addition of Introduction and Chapters 3 and 4	K.R. Chatzipanagiotou (IRES) A. Pappas (IRES) G. Antonaropoulos (IRES)
v0.3	30-01-2026	Third version	Addition of Chapter 2	M. Dimitriadou (RISA)
v0.4	05-02-2026	Version ready for peer review	Addition of Conclusion, updates and completion of the deliverable	S. Camarinopoulos (RISA)
v0.5	24-02-2026	Reviewed document draft	Peer review	C. McSweeney (PFIZER)
v0.6	20-03-2026	Revised version	Updated version based on reviewers' comments	K.R. Chatzipanagiotou (IRES) A. Bon (IRES) G. Antonaropoulos (IRES)
v0.7	22-04-2026	Version ready for peer review	Updated version based on reviewers' comments and Quality Management review	M. Dimitriadou (RISA) U. Hussels (RISA) A. Giakoumaki (RISA)
v0.8	06-05-2026	Reviewed document draft	Peer review	C. McSweeney (PFIZER)
v0.9	07-05-2026	Reviewed version	Quality Management Review	A. Giakoumaki (RISA)

v1.0	08-05-2026	Final version	Ready for submission	S. Camarinopoulos (RISA)

Executive Summary

Pharmaceutical products comprise a heterogeneous group of substances, encompassing diverse chemical classes, each exhibiting unique environmental behaviours due to differences in physicochemical properties, metabolism, and fate processes. In this context, some Active Pharmaceutical Ingredients (APIs) and their transformation products may be of environmental concern depending on their persistence, biological activity, and sources of exposure. In response to growing regulatory scrutiny and sustainability expectations, the ENVIROMED project aims to improve the understanding, monitoring, and mitigation of environmental impacts associated with pharmaceutical manufacturing. Deliverable D6.2 contributes to this objective by integrating two complementary approaches: the development of a registry for pharmaceutical micropollutants and the application of Life Cycle Assessment (LCA) to selected pharmaceutical production systems.

The first part of this deliverable presents the ENVIROMED registry of Pharmaceutical Micropollutants, developed under Task 6.4. The registry aims to provide a structured, auditable, and scalable digital framework for documenting wastewater volumes, API and other chemical concentrations and loads, and energy consumption associated with pharmaceutical manufacturing activities. Designed as a database-driven web application, the registry captures data at process level and aggregates them hierarchically across products, manufacturing units, buildings, and sites. It incorporates data management and control mechanisms such as role-based access control, versioning, data historisation, and audit trails, ensuring data quality, and traceability. By supporting structured data imports, validation procedures, and standardized reporting outputs, the registry bridges operational environmental monitoring with enhanced environmental assessment capabilities.

The second part of the deliverable reports the outcomes of LCA activities conducted under Task 6.5. LCA was applied to selected biopharmaceutical production systems at different scales, using a combination of primary data from project partners and secondary data from established LCA databases. Insulin was used as a high-volume biopharmaceutical to investigate environmental hotspots across upstream production, energy use, and selected downstream stages. In addition, laboratory-scale production of a recombinant antibody fragment was assessed to compare alternative fermentation strategies. Across the assessed systems, the LCA results identified carbon and nutrient sources, electricity and heat demand, and sterilization processes as dominant contributors to environmental impacts within the defined system boundaries, particularly for climate change and water use indicators. Scenario analyses demonstrated the potential for significant impact reductions through yield improvement, process intensification, and decarbonization of energy supply.

A key contribution of Deliverable D6.2 lies in the conceptual integration of the registry and LCA approaches. While conventional LCA studies often rely on generic wastewater datasets and assumptions, the ENVIROMED registry enables the generation of high-resolution, process-specific Life Cycle Inventory (LCI) data for wastewater and energy flows. This provides a foundation for improving the representation of pharmaceutical-specific emissions, supporting more accurate allocation of environmental burdens, and enabling future incorporation of ecotoxicity impacts related to manufacturing effluents. The registry therefore acts as a critical interface between operational data collection and life cycle-based environmental assessment.

Overall, Deliverable D6.2 outcomes contribute to ENVIROMED's broader objectives of reducing the environmental footprint of pharmaceuticals, supporting operational efficiency, data management, and enabling future development of pharmaceutical-specific LCA methodologies and impact pathways.

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List of Acronyms

Table 1: Acronyms and abbreviations

Term	Definition
API	Active Pharmaceutical Ingredient / Application Programming Interface
CF	Characterisation Factor
EF	Environmental Footprint
GUI	Graphical User Interface
LCA	Life Cycle Assessment
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
LIMS	Laboratory Information and Management System
PCR	Product Category Rules
PEC	Predicted Environmental Concentration
PNEC	Predicted No-Effect Concentration
RFB	Repetitive Fed Batch

1 Introduction

The release of pharmaceutical micropollutants into the environment has become a growing concern for policy makers, industries, and the scientific community. Human and animal pharmaceuticals may enter the environment throughout their life cycle, including manufacturing, use, and disposal. While the use of pharmaceutical products is the main contributor to environmental loading, emissions from pharmaceutical manufacturing remain relevant from an environmental stewardship perspective. During the manufacturing of Active Pharmaceutical Ingredients (APIs), various waste streams, particularly wastewater, can carry trace amounts of unreacted substrates, reaction by-products, intermediates, solvents, and final pharmaceutical products. These constituents, often collectively referred to as pharmaceutical micropollutants, may pose environmental and regulatory challenges. As European regulators continue to strengthen industrial wastewater requirements to reduce the presence of emerging substances of concern, pharmaceutical manufacturers may face increasing pressure to understand, quantify, and manage these pollutants within their production processes.

Tracking the origin, composition, and volume of wastewater generated throughout the pharmaceutical production chain is therefore essential for to help support compliance and environmental stewardship. Wastewater can arise not only from reaction effluents but also from cleaning processes between batches, solvent exchanges, and auxiliary operations, each contributing to a unique pollutant profile. A systematic approach to collecting and organizing this information, can enhance companies' ability to demonstrate compliance with existing regulations or to anticipate the impacts of potential future API-specific discharge limits. A robust data-management system supports process efficiency, transparency, reproducibility of reporting, and can support proactive responses to policy development.

To support this approach, the development of a dedicated registry for pharmaceutical micropollutants offers a practical and strategic solution. Such a registry can enable companies to more efficiently record key information, including wastewater volumes, pollutant types, and process-related waste characteristics, for each stage of pharmaceutical manufacturing. Beyond serving as a compliance support tool, the registry can provide a structured framework for environmental performance assessment, internal decision-making, and long-term sustainability planning. It also supports industry-wide efforts to better understand emissions pathways and to adopt cleaner and more efficient production practices.

In this project, different stages, inputs and outputs, were investigated along the lifecycle of APIs from a sustainability perspective, using Life Cycle Assessment (LCA) approach. LCA was performed at different scales of production (i.e., lab-scale and industrial scale), focusing on processes and products that represent the activities and expertise of different consortium members. Specifically, primary data were received by project partners (TUW, NOVO) for API production, and literature-based data were also utilized, to complement the information generated within the consortium.

Insulin, produced through recombinant fermentation using engineered microorganisms under tightly controlled biocatalytic and cultivation conditions, represents a globally high-volume biopharmaceutical whose extensive market demand make it an especially relevant model API for investigating environmental impacts within life-cycle assessment frameworks. Previous reports on biopharmaceutical production have identified different inputs and processes contributing most to the environmental impacts, including the cultivation medium components and electricity use as well as the reliance on resource-intensive upstream processes. [1], [2]

In the context of the ENVIROMED project, we applied LCA approach to the upstream production of different biopharmaceuticals (i.e., medicinal products derived from

biotechnological processes involving living organisms or biological systems, as opposed to chemically synthesized APIs). For insulin, we used literature data to map hot-spots associated with growth media compositions, at different manufacturing scales (i.e., from lab scale to pilot scale) and reactor operation modes (e.g., batch, fed-batch, continuous). [3]

Besides insulin production at different scales with literature-based data, the lab-scale production of *E. coli*-based antibody fragment was also evaluated, using primary data from experiments performed at the facilities of TUW. The analysis compared different reactor operation modes, media compositions, and feeding regimes, incorporating real-time measurements of electricity consumption. The evaluation focused specifically on global warming potential and water usage as the selected impact indicators. In addition, several sensitivity scenarios were explored, including alternative electricity mixes (e.g., country-specific, European average, and renewable), different reactor sterilization approaches between batches, and variations in API productivity. Finally, using industrially relevant primary data from the project partner NOVO, an impact assessment was performed on the large-scale production of insulin.

While the present work focuses on upstream manufacturing processes, several other life cycle phases of pharmaceuticals contribute significantly to their overall environmental footprint [4], [5]. The use phase is critical, as APIs are excreted by human and animal patients and enter wastewater systems, where conventional treatment often fails to remove them completely, leading to their potential persistence in surface and groundwater and potential ecotoxicological effects on aquatic organisms. Packaging also represents a notable impact hotspot, given the reliance on plastics and multilayer materials that are difficult to recycle; recent trends emphasize biodegradable polymers and smart packaging to reduce waste and improve sustainability. Additionally, consumables used in healthcare settings, such as syringes, tubing, and diagnostic kits, generate substantial plastic waste and require energy-intensive sterilization processes, further increasing greenhouse gas emissions and resource use throughout the product's life cycle. Addressing these stages through improved wastewater treatment, eco-design of packaging, and circular strategies for medical consumables is essential for reducing the cumulative environmental burden of pharmaceuticals.

Pharmaceuticals enter the environment through multiple pathways across their lifecycle, including manufacturing and use. During production, effluents from synthesis and formulation processes often contain high concentrations of APIs and intermediates, which, if inadequately treated, can contaminate surface waters and soils, creating hotspots for antimicrobial resistance and ecotoxicity. In the use phase, APIs are excreted by patients and discharged via municipal wastewater systems, where conventional treatment technologies frequently fail to remove them completely, leading to their potential persistence in aquatic environments and uptake by biota. Additional sources include hospital effluents, improper disposal of unused medicines, and agricultural applications of sewage sludge, which can introduce pharmaceuticals and their metabolites into soils and plants. [6], [7], [8], [9] In the context of the ENVIROMED project, a wide screening of different APIs and metabolites in wastewaters and natural waters in Greece was performed as a case study, to estimate the risk of different APIs. Our review adopted a risk-based prioritization approach, integrating consumption data, environmental occurrence, and ecotoxicity thresholds to calculate Risk Quotients (RQs). This method revealed significant mismatches between pharmaceutical use and monitoring practices, highlighting high-risk compounds such as NSAIDs, macrolide antibiotics, synthetic hormones, and contrast agents that may warrant routine surveillance in wastewater and natural waters. Such correlation-driven strategies are essential for identifying substances with the greatest ecological impact and guiding targeted monitoring and mitigation efforts. The outcomes of this review are reported in Deliverable D6.1.

The lifecycle of pharmaceutical products, from cradle to grave, is shown schematically in Figure 1. Different data sources were used within the context of the ENVIROMED project, to build the Life Cycle Inventory (LCI) for the LCA of pharmaceuticals, including primary sources (i.e., generated from project partners NOVO and TUW) and secondary sources (LCA dataset library Ecoinvent, literature).

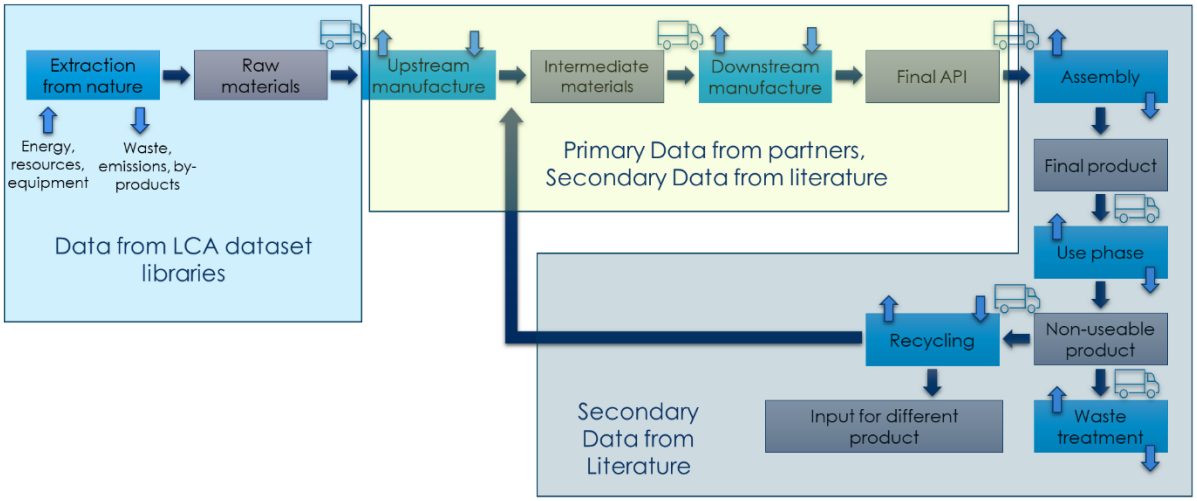

Figure 1: Life Cycle of pharmaceutical products. Along the graph, the primary and secondary different data sources are indicated, that were used for the Life Cycle Inventory of the LCA in the ENVIROMED project.

Although the LCA work performed in the context of ENVIROMED did not address the emissions or contaminants discharged in manufacturing waste, it provided crucial insight into the environmental burdens associated with the resource use and upstream inputs for complex biopharmaceuticals. The results build towards the broader objectives of the project, which aims to assess environmental occurrence, persistence, fate, and toxicity of pharmaceuticals, and to promote greener, more efficient manufacturing. Considering the entire lifecycle of APIs, the registry complements the LCA-based findings, by capturing data on wastewater volumes and pollutant types from real manufacturing operations. By doing so, it enables a cradle-to-grave environmental assessment that aligns with the project's objectives to reduce the environmental footprint of pharmaceuticals throughout their lifecycle: from growth media and resource use, through production and waste streams, to eventual environmental release and fate.

In this report, different outcomes of the work performed under Tasks 6.4 and 6.5 of the ENVIROMED project will be presented, which link to different life cycle stages and processes along the lifecycle of selected pharmaceuticals (Figure 1).

Chapter 2 presents the ENVIROMED registry of pharmaceutical micropollutants, a structured digital system designed to document, quantify, and manage wastewater volumes, pollutant concentrations and loads, and associated energy use arising from pharmaceutical manufacturing processes. The registry addresses a gap in current environmental assessment practice for pharmaceuticals, namely the lack of systematic, process-resolved data on manufacturing-related emissions to wastewater. Pharmaceutical production is characterised by batch-wise operation, variable process configurations, and low but potentially environmentally relevant pollutant concentrations, which are not adequately represented by generic wastewater datasets typically used in life cycle assessment. By capturing wastewater generation, pollutant loads, and energy consumption at process and product level and aggregating them hierarchically across manufacturing units and sites, the registry provides a traceable and

auditable data foundation for operational environmental management and the generation of life cycle inventory data. In this way, the registry complements life cycle assessment activities by providing high-resolution, process-specific inventory data and supports an integrated perspective on pharmaceutical environmental impacts across manufacturing and assessment domains.

In Chapter 3, the LCA results are shown, related to the upstream and downstream manufacture of different biopharmaceuticals at various scales. Commercial LCA dataset libraries (Ecoinvent 3.10) are used for the upstream production of input materials and resources, while emissions are not considered, since specific data on emissions from manufacture could not be retrieved. Data regarding the packaging, downstream (post-production) transport, and use-phase related consumables (and resulting waste), were retrieved from literature and LCA dataset libraries.

Insulin is a peptide hormone that undergoes complete enzymatic degradation in the human body to its constituent amino acids, which are subsequently metabolized to carbon dioxide (CO_2), water, and ammonia (NH_3) through standard protein catabolic pathways. [10] It does not persist as an API (or a specific metabolite) in wastewater after patient use and is not suitable for assessing API fate in the aquatic environment. Therefore, emissions during the use phase were excluded from the results reported here. Nevertheless, different APIs of interest within the ENVIROMED project (such as diclofenac, ibuprofen, carbamazepine, metformin, metoprolol) were included in our approach in the form of a literature-based review on the presence of APIs and their metabolites in the environment, correlated data on pharmaceutical consumption, monitoring in wastewater and the environment, and ecotoxicity. The detailed outcomes of this analysis are included in Deliverable 6.1, while their relevance to the overall LCA insights is discussed in Chapter 3 (i.e., comparison of waste treatment process for aqueous based solvents or mixed solvent waste). Finally, the possibility to combine emission estimations during the use phase and End of Life phase of pharmaceuticals, which can be further linked to specific emission impacts modelled in the LCA of the complete API lifecycle, are also discussed in this chapter.

The final chapter of the deliverable, Chapter 4, reflects on the potential link between an integrated pharmaceutical registry, which contains specific information on the volume and type of contaminants from different pharmaceutical production processes, and the LCA assessment, which can incorporate the ecotoxicity impacts for specific manufacturing waste to the overall lifecycle impact assessment.

2 ENVIROMED registry of pharmaceutical micropollutants

2.1 Purpose and Scope

Pharmaceutical manufacturing is characterised by batch-oriented production, complex process chains, and the use of a wide range of active pharmaceutical ingredients and auxiliary substances. These characteristics pose specific challenges for wastewater management and environmental assessment, particularly when addressing pharmaceutical micropollutants that may occur at very low concentrations but are potentially environmentally relevant. In contrast to continuous production industries, wastewater streams in pharmaceutical production must be attributed to discrete process steps, batches, and products, often under conditions of limited analytical coverage and variable production patterns.

To address these challenges, the ENVIROMED registry of pharmaceutical micropollutants has been developed as a comprehensive digital platform for the systematic documentation, quantification, and evaluation of wastewater streams and associated pollutant loads generated by pharmaceutical manufacturing activities. The registry is designed to support operational wastewater management and the generation of high-quality, traceable environmental data for life cycle assessment (LCA).

A central design objective is to enable a generic yet highly adaptable approach that allows fast and efficient configuration of the registry to the specific characteristics of different production sites. At the same time, the platform prioritises usability and findability, ensuring that relevant information can be efficiently accessed by industrial operators, environmental agencies, and public authorities. Particular emphasis is placed on the reliable mapping of batch-based manufacturing processes in order to link ingredients, production activities, and wastewater streams in a consistent and retrievable manner.

The registry builds on extensive prior experience in the development and operation of wastewater cadastres for pharmaceutical production sites, including systems used both for regulatory reporting and for internal substance and process queries. Within ENVIROMED, this expertise is consolidated and extended to create a stakeholder-oriented registry that bridges operational wastewater data, environmental monitoring, and environmental assessment needs.

2.2 Conceptual Design

The ENVIROMED registry is designed as an integrated environmental data management system that links operational production data, analytical measurements, and wastewater information within a consistent conceptual framework. Its overall architecture supports the structured ingestion, storage, processing, and export of environmental data while remaining adaptable to site-specific conditions and data availability. The logical and digital architecture of the registry, including its main functional layers and interfaces, is illustrated in Figure 2.

The registry is structured around a layered architecture that separates data ingestion, storage, processing, and reporting functions. Incoming data from heterogeneous sources, including laboratory analytical results, historical environmental datasets, and automated monitoring systems, are collected through structured interfaces and application programming interfaces (APIs). These data are stored in relational and non-relational databases and processed through dedicated calculation and analytics modules, which perform pollutant load calculations, aggregation and allocation of wastewater flows, energy consumption assessments, and data

validation procedures. Reporting and interoperability layers enable standardized outputs for internal environmental management and life cycle assessment (LCA) applications.

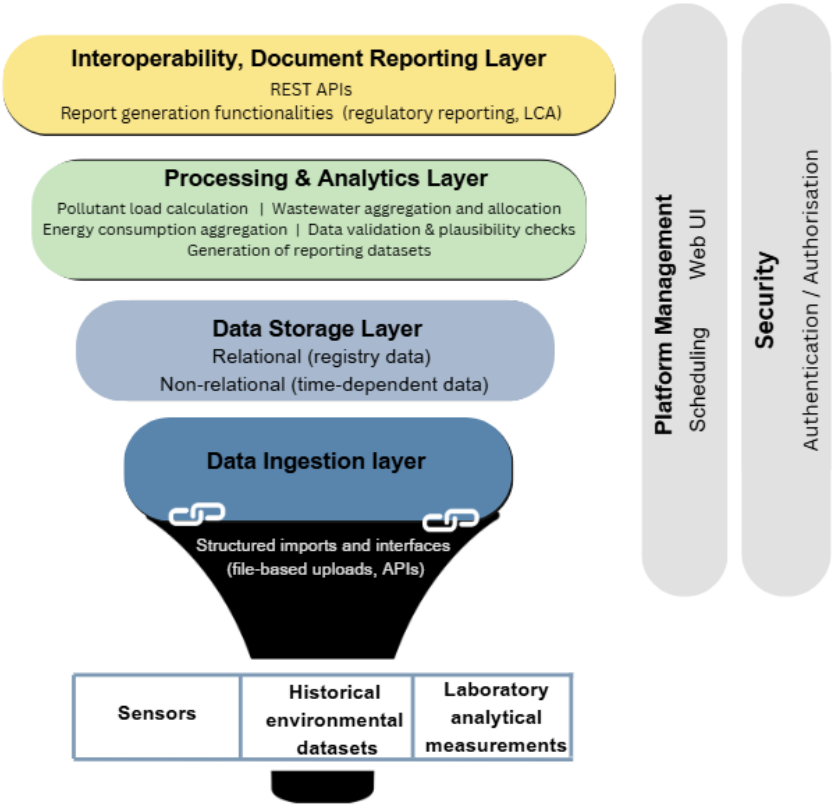

Figure 2: Conceptual architecture of the ENVIROMED registry

In parallel to the logical system architecture, the registry explicitly defines and captures the physical system boundaries of pharmaceutical production sites. Figure 3 illustrates the physical water, wastewater, and waste flow structure that underpins the registry's data model. Municipal drinking water enters the site and is subject to on-site water treatment to produce drinking water and demineralized water, which are distributed to buildings hosting production, laboratories, workshop, and office activities. Within these buildings, water use results in distinct wastewater streams, including sanitary wastewater, process wastewater, and high-load wastewater, which are routed to the wastewater treatment process. Liquid waste streams that cannot be managed via the wastewater system are directed to external disposal, while solid waste and products leave the system via defined output pathways.

Figure 3: Physical system boundaries and water, wastewater, and waste flow structure within a pharmaceutical production site as represented in the ENVIROMED registry.

The registry covers all wastewater streams generated at pharmaceutical production sites that are routed to central wastewater treatment systems, as well as the associated water treatment steps and the energy consumption relevant to wastewater generation and treatment. Data are captured at multiple organisational and technical levels, including individual process steps, products, manufacturing units, buildings, and the overall site. Relevant non-production-related contributions, such as laboratory wastewater, sanitary wastewater, and building-related energy use, are included where necessary to ensure completeness and consistency of the environmental inventory.

System boundaries and temporal resolution are defined to support longitudinal analyses and compatibility with life cycle inventory (LCI) modelling. The registry accommodates both high-resolution, process-level data where available and aggregated annual data where finer temporal resolution is not feasible. This flexible but clearly defined approach ensures methodological consistency across reporting and assessment contexts, while maintaining traceability between physical material flows, operational activities, and calculated environmental indicators.

Together, Figures 2 and 3 illustrate the dual perspective of the ENVIROMED registry: a logical, digital architecture for managing environmental data and a physical representation of water, wastewater, and waste flows at pharmaceutical production sites. This combined perspective provides the foundation for consistent data aggregation, robust environmental management, and integration of registry outputs into life cycle assessment frameworks.

2.3 Data model and calculation logic

The registry's data model is explicitly structured into two complementary components: a production-related model and a structure-related model. The production-related model captures pharmaceutical products, batch groups, production volumes, process steps, analytical samples, and pollutant parameters. The structure-related model represents organisational units, buildings, wastewater inflows and outflows, treatment infrastructure, and relevant media connections. This separation enables transparent attribution and aggregation of wastewater volumes, pollutant loads, and energy consumption from individual process steps to site level.

Hierarchical relationships are applied throughout the data model to reflect real-world dependencies. Each process step is defined such that generated wastewater is assigned to a

single disposal or treatment route, enabling unambiguous aggregation. To limit data maintenance effort while preserving environmental relevance, multiple production phases or process steps may be consolidated where justified. Such simplifications are applied according to documented rules defined within the specific implementation context and maintain full traceability across aggregation levels.

Environmental calculations follow a bottom-up logic. Pollutant loads are derived by combining analytical concentration data with corresponding wastewater volumes at the most detailed level available. These results are then aggregated hierarchically to higher organisational levels using explicitly defined relationships and scaling factors. Batch-oriented production is represented through production volumes and batch multiplicators; where manufacturing conditions are identical, batches may be grouped to reduce data entry effort while maintaining environmental relevance.

Energy consumption associated with wastewater generation and treatment is captured and allocated using the same structural and hierarchical logic as wastewater data. This integrated approach enables consistent assessment of wastewater-related pollutant releases and energy-related environmental impacts and supports the identification of efficiency improvement potentials. In this context, the water treatment process is also considered, taking into account the different purity levels of the drinking water used and its distribution to the buildings.

Finally, liquid waste that cannot be disposed of via the wastewater system is also considered. This part of the inventory can be linked to waste management or, if necessary, can also take over other aspects of waste management.

2.4 Data Sources and Quality Assurance

The registry integrates data from multiple organisational sources, including production units, wastewater laboratories, building-level metering systems, on-site monitoring systems (where available), and central wastewater treatment facilities. These data sources differ in structure, granularity, and temporal resolution and are harmonised within the registry's data model.

Initial population of the registry may involve structured migration of historical datasets previously maintained in non-systematic formats. Migration is typically performed in stages, including test imports, plausibility checks, and validation against independently measured reference values, such as wastewater inflows to treatment plants.

Ongoing data quality assurance combines formal relational constraints with plausibility checks and consistency checks across independent aggregation pathways. For example, a balance sheet of water quantities across a building and the entire site. Analytical data quality is supported through structured sample management, including defined approval states, role-based editing rights, and controlled use of calculated or virtual samples where direct measurements are unavailable.

The registry supports versioning and historisation to ensure reproducibility and auditability. Versioning enables the preservation of frozen data states, while historisation captures temporal changes in selected data elements using defined validity periods or reporting years. Comprehensive change logging and controlled data protection functions ensure compliance with data protection requirements while maintaining transparency.

2.5 Outputs, Use Cases, and LCA Interface

The ENVIROMED registry supports a wide range of outputs for operational, analytical, and policy development purposes. Its design ensures that environmental data collected at process level can be reliably aggregated, documented, and reused across different reporting and assessment contexts, including internal environmental management and life cycle assessment (LCA).

Outputs and reporting

Environmental datasets generated by the registry can be exported in structured formats to enable further analysis, documentation, and exchange with external tools. These outputs include wastewater volumes, pollutant concentrations and loads, and energy consumption data aggregated at different organisational levels (e.g. process step, product, manufacturing unit, building, and site). Standardised export formats support reproducible data exchange and long-term archiving.

In addition to raw data exports, the registry generates standardised reports based on predefined layouts and frozen data states. These reports are suitable for process efficiency enhancement, audits, internal reviews, and can help to ensure consistency between operational data management and externally communicated results. Versioning mechanisms ensure that reported datasets remain traceable and reproducible even as underlying operational data evolve over time.

Operational use and decision support

The registry supports day-to-day operational use within pharmaceutical production sites. Structured data access and guided workflows enable users to explore wastewater generation, pollutant loads, and energy consumption across different processes and time periods. This functionality supports internal monitoring, plausibility checks, and identification of potential optimisation opportunities, such as high-load wastewater streams or energy-intensive operations.

By providing a consolidated and transparent view of wastewater-relevant data, the registry reduces dependency manual data management processes and supports consistent environmental data management across organisational units and reporting periods.

Interface to life cycle assessment

A key design objective of the ENVIROMED registry is to provide structured, traceable, and LCA-compatible life cycle inventory (LCI) data, particularly for wastewater-related emissions and associated energy use. By capturing wastewater volumes, pollutant concentrations and loads, and energy consumption at the level of individual processes and products, the registry establishes a foreground data foundation for life cycle assessment modelling.

Structured data exports allow registry outputs to be directly integrated into LCA software tools and workflows, supporting the assessment of wastewater-related impact categories, including freshwater ecotoxicity, as well as energy-related environmental impacts. The explicit documentation of aggregation logic, allocation rules, and data provenance enhances the scientific interpretability of integrated environmental assessments.

While the registry itself does not perform impact assessment or characterisation, it provides the necessary inventory-level data and metadata required for advanced LCA applications. In this way, the registry acts as a reliable interface between operational environmental monitoring and life cycle-based environmental assessment, complementing the LCA analyses presented in Chapter 3 and supporting the integrative perspective discussed in Chapter 4.

2.5.1 Graphical user interface and usability

The ENVIROMED registry is implemented as a web-based application with a Graphical User Interface (GUI) designed to support efficient data entry, review, and retrieval by users with different roles and levels of technical expertise (Figure 4). Usability and findability were key design objectives, reflecting the need to support both routine operational tasks and more advanced analytical and reporting activities.

Figure 4: ENVIROMED registry of pharmaceutical micropollutants.

The GUI follows a role-based access concept, ensuring that users are presented only with the functions and data relevant to their responsibilities. Typical user roles include production and environmental data providers, laboratory staff, environmental managers, and reporting or assessment specialists. Role-based permissions support data integrity and compliance by restricting editing, approval, and export functions as appropriate.

Navigation and information structure

The registry interface is organised around a hierarchical navigation structure that mirrors the underlying data model. Core navigation elements allow users to access data at different aggregation levels, including process steps, products, manufacturing units, buildings, and site level. This structure enables intuitive movement between detailed operational data and aggregated environmental results.

Key entities such as products, processes, wastewater streams, and analytical samples are accessible through dedicated overview pages that provide both summary information and direct links to related objects. Contextual navigation elements allow users to trace relationships between production activities, wastewater generation, and analytical results, supporting transparency and interpretability.

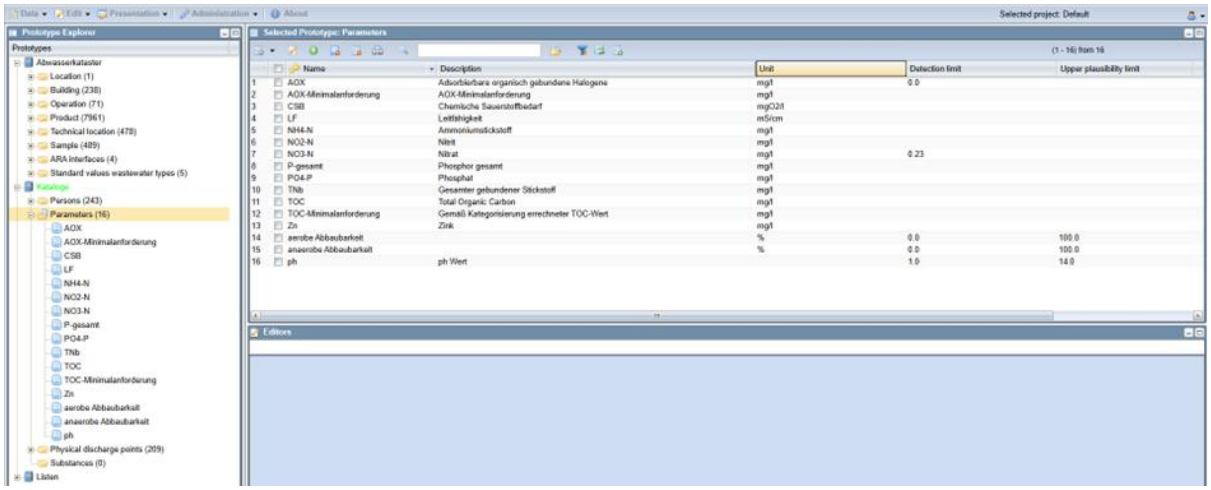

Figure 5: Overview screen showing hierarchical navigation and key registry entities.

Data entry and maintenance

Data entry and maintenance workflows are designed to minimise manual effort while ensuring data quality and consistency (Figure 6). Structured input forms guide users through required data fields and apply validation rules to prevent incomplete or inconsistent entries. Where appropriate, default values, reference data, and reusable master-data elements (e.g. parameter catalogues and standard wastewater definitions) reduce redundancy and support harmonised data management.

For recurring data imports, such as analytical laboratory results or production volume data, the GUI supports structured file uploads and interface-based data ingestion. Import routines provide immediate feedback on data completeness and plausibility, allowing users to identify and resolve issues before data are committed to the registry. The wastewater register performs some of the tasks of a Laboratory Information and Management System (LIMS). It can also communicate with LIMS to avoid duplicate data entry.

Approval states and visual status indicators are used to distinguish between draft, reviewed, and validated data, supporting coordinated data management across organisational units.

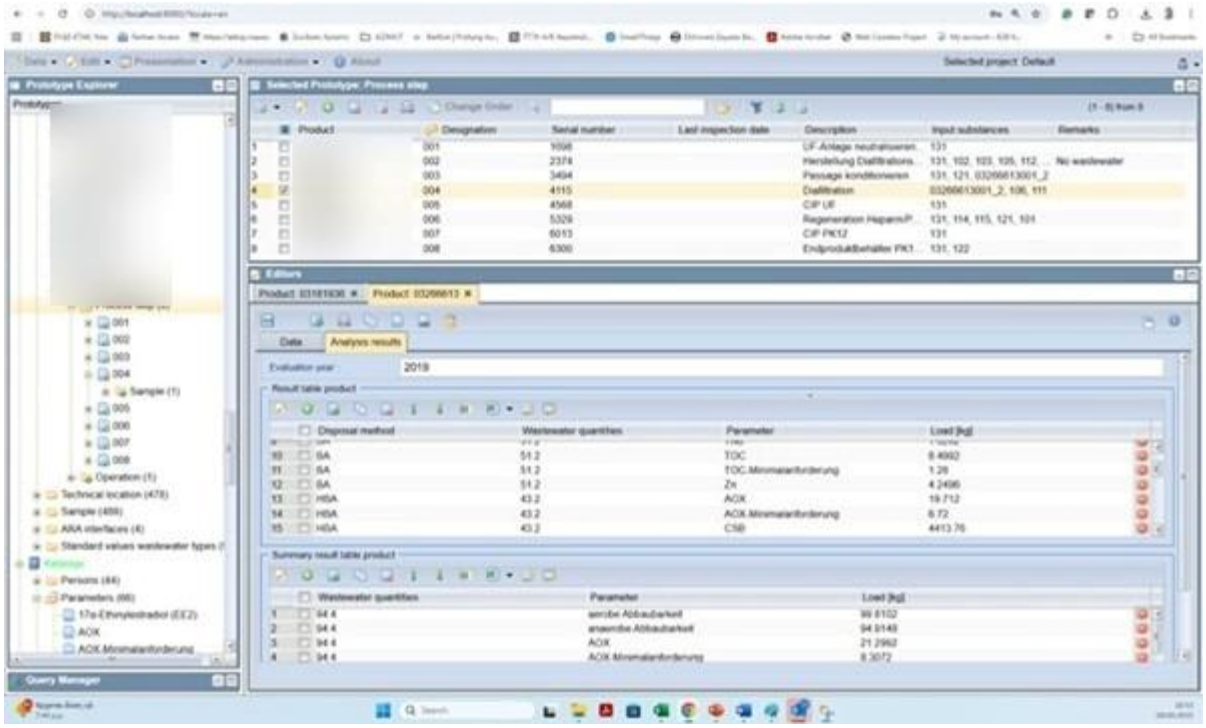

Figure 6: Data entry and validation screen with status indicators.

Query, visualisation, and reporting support

The GUI provides flexible query and filtering functions that allow users to retrieve information based on products, processes, time periods, wastewater streams, or specific pollutant parameters. Search and filter tools are designed to support rapid access to relevant entries, reflecting the registry's focus on optimised findability. Full-text searches in associated PDF documents are also possible.

Results can be displayed in tabular form or as aggregated summaries at different organisational levels. Where applicable, the interface provides basic visualisations (e.g. time series or aggregated pollutant loads) to support exploratory analysis and plausibility checks prior to reporting or export.

Reporting functions are integrated into the GUI and allow authorised users to generate standardised reports and data exports based on defined reporting periods and frozen data states. This ensures consistency between operational data management and reporting outputs.

Support for long-term operation

To ensure long-term usability, the GUI combines generic access to registry data with guided workflows for key tasks such as data import, validation, and reporting. Inline help texts, tooltips, and structured layouts support users who are not domain experts, reducing dependency on individual key users and facilitating onboarding of new personnel.

By aligning the GUI structure closely with the underlying data model and calculation logic, the registry ensures that complex environmental information remains accessible, transparent, and usable across different stakeholder groups and over extended reporting periods.

The GUI design explicitly supports the dual role of the registry as both an operational wastewater management tool and a structured provider of LCA-ready environmental data.

2.6 Illustrative data flow example: from production to reporting

To illustrate the practical functioning of the ENVIROMED registry and the interaction between production data, analytical measurements, and environmental calculations, this section provides an exemplary data flow narrative for a representative pharmaceutical production case. The example is intended to clarify how heterogeneous operational data are transformed into consistent, traceable environmental outputs within the registry.

In a typical scenario, a pharmaceutical product is manufactured in discrete batches within a defined production unit. Each batch follows a sequence of process steps, such as synthesis, purification, formulation, and cleaning operations. Within the registry, these process steps are represented as production-related entities and linked to corresponding batch groups and annual or campaign-based production volumes.

During production, wastewater is generated at several process steps, for example during equipment cleaning or aqueous purification stages. Each wastewater stream is assigned to the originating process step and mapped to a defined disposal or treatment route, such as discharge to an on-site wastewater treatment plant. Where multiple process steps generate wastewater with comparable characteristics, they may be grouped in accordance with predefined simplification rules.

Analytical sampling of wastewater is performed at defined points, either at the level of individual process streams or at aggregated inflows to treatment facilities. Analytical results, including concentrations of relevant pharmaceutical micropollutants and conventional wastewater parameters, are imported into the registry via structured interfaces. Each analytical sample is linked to metadata describing sampling location, time period, and associated wastewater streams.

Within the calculation logic of the registry, pollutant loads are derived by combining analytical concentration data with corresponding wastewater volumes. Calculations are first performed at the most detailed level available (e.g. process step or batch group) and then aggregated hierarchically to higher levels, such as product, production unit, building, and site. Where analytical data are available only at aggregated levels, the registry applies documented allocation and scaling rules to distribute pollutant loads consistently across contributing processes.

In parallel, energy consumption data associated with wastewater generation and treatment are captured and allocated using the same hierarchical structure. This ensures that both pollutant releases and energy-related environmental burdens can be evaluated consistently for the same functional units.

The resulting aggregated datasets can then be used for multiple purposes, including internal environmental performance evaluation and export to life cycle assessment tools. Throughout this process, the registry preserves the linkage between production activities, wastewater generation, analytical measurements, and calculated results, enabling full traceability and reproducibility of outputs.

2.7 Data considerations, simplifications, and methodological limitations

The design and operation of the ENVIROMED registry involve practical considerations and simplifications that reflect practical constraints in data availability, monitoring feasibility, and long-term maintainability. These aspects are explicitly documented to ensure transparency and consistency in data reporting.

One key consideration concerns the level of process resolution. While the registry supports detailed representation of individual process steps, it is not always feasible or necessary to capture all theoretical production phases separately. In such cases, multiple steps or batches may be grouped where production conditions and wastewater characteristics are comparable. These simplifications are applied conservatively and are designed to preserve the overall representativeness of pollutant loads and energy use.

Analytical data availability represents another limitation. For many pharmaceutical micropollutants, concentrations in manufacturing wastewater streams may be low and sampling frequencies may be limited. Where direct measurements are unavailable, the registry allows the inclusion of estimated values, which may be defined by the data provider or derived using user-specified approaches. Such values are clearly documented within the system and distinguished from measured data to ensure transparency. The registry does not prescribe calculation methods but ensures that all values are traceable and appropriately recorded.

Temporal alignment between production activities, wastewater generation, and analytical sampling is addressed through defined reporting periods and aggregation rules. However, short-term fluctuations within production campaigns may not always be fully captured when only aggregated annual data are available. The registry mitigates this limitation by maintaining consistent temporal boundaries and by enabling longitudinal analyses across reporting periods.

From a system boundary perspective, the registry focuses on manufacturing-related wastewater streams, including those associated with in-house treatment systems (e.g. influent and effluent), as well as associated energy consumption. Emissions occurring outside these boundaries, such as diffuse releases during product use or disposal, are not covered and must be addressed through complementary assessment approaches.

Finally, while the registry is designed to support LCA-compatible life cycle inventory data, it does not itself perform impact assessment or characterisation. The quality of downstream LCA results therefore also depends on the suitability of selected impact assessment methods and characterisation factors, particularly for pharmaceutical micropollutants.

By explicitly acknowledging these considerations and limitations, the registry establishes a transparent basis for data collection and reporting. This clarity supports appropriate interpretation of results and facilitates informed use of registry outputs by industry, policy makers, and environmental assessment practitioners.

3 Life Cycle Assessment (LCA)

The Life Cycle Assessment (LCA) methodology is briefly described in Section 3.1, wherein the Goal and Scope of the LCA employed in the ENVIROMED project is detailed. In Section 3.2, the method used to collect the Life Cycle Inventory (LCI) for selected products is explained. In Section 3.3, the final outcomes and interpretation of the Life Cycle Impact Assessment are presented. The results presented in this chapter are specific to the case studies analysed within the ENVIROMED project and should not be interpreted as representative of all pharmaceutical or biopharmaceutical production systems.

3.1 LCA Method Description, Goal and Scope Definition for ENVIROMED

LCA is a scientific method used to evaluate the environmental impacts of a product or process across its entire life cycle—from raw material extraction to manufacturing, use, and disposal. LCA is standardized under ISO 14040 [11] and ISO 14044, [12] which define the principles, framework, and detailed requirements for conducting LCA studies, including goal and scope definition, inventory analysis, impact assessment, and interpretation, to ensure consistency and transparency across applications. The LCA process begins with **Goal and Scope definition**, which includes the following aspects about the LCA:

- Goal of the Study

Defines the purpose of the LCA, why it is being conducted, and how the results will be used (e.g., for product improvement, policy-making, or communication).

- Functional Unit

The unit of measurement of the product or process, which is defined based on its (desired) function, and to which all inputs and outputs are related to, ensuring comparability among different products / processes (e.g., “1 dose of medication” or “1 liter of solution”).

- System Boundaries

Specifies which life cycle stages are included (e.g., raw material extraction, manufacturing, use, disposal) and what is excluded.

- Impact Assessment, Database and Software Selection

Determines which environmental impact categories will be assessed (e.g., climate change, water pollution, human toxicity) and which scientific model will be used (e.g., ReCiPe, CML, etc). The software and additional background LCA dataset libraries should also be defined.

- Assumptions and Limitations

Lists any simplifications or data gaps that could influence results, ensuring transparency.

- Stakeholders and Audience

Identifies who will use the results (e.g., researchers, industry, policy makers) and how detailed or technical the report should be.

- Allocation Rules (if applicable)

Explains how environmental burdens are divided among co-products or processes when multiple outputs are produced. In the context of the ENVIROMED project, the Goal and Scope Definition are described in Table 2.

Table 2: Goal and Scope Definition for the LCA performed in the ENVIROMED project

Goal and Scope definition	In the ENVIROMED project
Goal of the Study	1. To assess the **environmental impact of specific pharmaceuticals along their entire lifecycle** (i.e., production, use, end-of-life and final disposal), including **toxicity damage** (as calculated by the Environmental Footprint method). 2. To identify **potential key impacts** along the manufacturing of materials, transportation, and energy used in the facility or connected with the disposal of waste. To investigate ingredients (organic and non-organic) and electricity as **potential hotspots** of impacts for pharmaceuticals and investigate (if relevant) **mitigation strategies** such as solvent replacement, elimination of solvent waste, energy adjustment, and synergistic analysis. 3. To discuss methods of **decreasing the leak of natural gas** to the environment (and its subsequent toxicity to the aquatic environment) during the manufacturing of the investigated pharmaceuticals, and (if relevant) consider strategies such as adjusting the source of energy in pharmaceutical production. 4. To propose **effective strategies to mitigate** environmental damage of the pharmaceuticals under study. To evaluate **different process alternatives** regarding their environmental impact, to **identify bottlenecks** and **improvement potentials** for further process development activities. 5. To contribute towards a **decision-making framework**, promoting green pharmaceutical processes. 6. To propose an approach for incorporating **pharmaceutical-specific Product Category Rules** (PCRs) for LCA, and to incorporate **pharmaceutical-specific impact pathways** in Life Cycle Impact Assessment, i.e., via the development of **new characterisation factors** for pharmaceuticals using existing ecotoxicity models.
Functional Unit	Different Functional Units were selected for different sections of the LCA: - For the production of insulin at different scales, based on Inventory Data from literature, [3] one Liter of reactor

Goal and Scope definition	In the ENVIROMED project
	medium was used as a basis for analysis, to allow comparison among different reaction scales. - For the production of biopharmaceuticals at the facilities of project partners (TUW, NOVO), 1 kg of produced API was used as the functional unit.
System Boundaries	The entire lifecycle of the pharmaceutical was considered in the LCA, i.e., cradle-to-grave system boundaries.
Impact Assessment, Software and LCA dataset library	The Impact Assessment method Environmental Footprint 3.1 [13]was used in this study. The software employed for LCA is SimaPro (version 9.6.0.1), with LCA dataset Ecoinvent (version 3.10, cut-off, U)
Assumptions and Limitations	LCI data from NOVO is confidential, and cannot be reported in the present deliverable LCI datasets regarding the waste solvent at NOVO, after manufacturing of the API, did not include the specific impacts of dissolved contaminants, as this data was not known Limitations and assumptions related to insulin production, as described in the corresponding paper [3] LCI data for transport, packaging and use phase of investigated pharmaceuticals is based on secondary (literature) sources
Stakeholders & Audience	The detailed outcomes of the LCA are intended to be shared within the consortium. The present report excludes confidential information related to manufacturing activities within the facilities of involved partners and provides an overall perspective on the sustainability of pharmaceuticals along their lifecycle, to a wide audience, as a public deliverable of the project.

The next step in the LCA process is the **Life Cycle Inventory** (LCI). This involves collecting data on all inputs (like energy, water, and raw materials) and outputs (such as emissions and waste) associated with each stage of the life cycle. This inventory provides a detailed picture of resource use and pollutant release for each stage. Primary data collection was performed in the project using dedicated, excel-based questionnaires to corresponding partners. Secondary data was collected from peer-reviewed, scientific publications.

To translate inventory data into meaningful environmental impacts, LCA uses a step called **Life Cycle Impact Assessment** (LCIA). Here, emissions and resource uses are grouped into categories such as climate change, water pollution, or human toxicity. Each substance in the inventory is multiplied by a characterisation factor, which expresses its relative contribution to a specific impact. For example, carbon dioxide emitted to the air has a characterisation factor for global warming potential, while heavy metals emitted to the environment have factors for (eco)toxicity. These factors are based on scientific models of how substances behave in the environment and affect ecosystems or human health, while a more detailed description of the methodology behind them can be found in literature describing the selected impact assessment method (e.g., [13]). By applying these factors, LCA converts raw data (i.e., flows of resources

and emissions between the environment and the system boundaries, collected in the LCI) into indicators like "kilograms of CO_2-equivalent" or "toxicity potential," making it easier to compare options and identify hotspots for improvement.

Finally, the **Interpretation step** in LCA is where the results are analysed and translated into actionable insights. It involves checking the consistency and reliability of the study, identifying the most significant contributors to environmental impacts (known as "hotspots"), and drawing conclusions that support decision-making. Through interpretation, organizations can determine whether one product or process is more sustainable than another, identify opportunities for improvement, and understand trade-offs between different impact categories and life cycle stages. This step ensures that the findings are meaningful and aligned with the original goal of the study, helping stakeholders use LCA results for strategies such as eco-design, policy development, or supply chain optimization.

3.2 Life Cycle Inventory Data collection for ENVIROMED

LCA within the ENVIROMED project was performed as three separate assessments, namely:

- LCA based on literature data for different process alternatives in insulin production, as described in an open-access publication that resulted from this project [3]. The LCA focused on different formulations of growth media and the electricity use during their preparation for downstream insulin production, while excluded the generated waste, as well as the production process itself.
- Quantification of global warming potential and water usage for the upstream production of a recombinant antibody fragment (Ranibizumab Fab) at TUW, comparing four operational strategies (fed-batch, repetitive fed-batch, continuous, and cascade). The analysis considers upstream processing steps only, including sterilization, preculture, main fermentation, and cleaning operations, while excluding downstream processing.
- LCA on the production of insulin at the facilities of NOVO. Due to the confidential nature of LCI data, this will not be disclosed here, and only insights from the analysis will be reported in the rest of Chapter 3.

3.2.1 LCI of upstream fermentation strategies from TUW

Four operational strategies were investigated from TUW to produce the antibody fragment Ranibizumab Fab.

- Fed-batch: A single cultivation cycle comprising batch and fed-batch phases, followed by a full harvest at the end of the run.
- Repetitive fed-batch (RFB): Successive auto-induced fed-batch phases with multiple partial harvests, increasing cumulative product output per campaign.
- Continuous (chemostat): Steady-state operation established after an initial batch phase, enabling continuous harvesting of product-containing biomass.
- Cascade: Two reactors operated in series to decouple growth and production phases, allowing continuous harvest under intensified process conditions.

The detailed material and energy balances of the four upstream processing configurations are summarized in this section. For clarity, the inventory is organized into four categories: nutrients, consumables, utilities, and wastes which are described below.

Nutrients:

Table 3: Nutrient consumption per gram of API in the four fermentation modes

Nutrients	Fedbatch	RFB	Continuous	Cascade	Units
C6H12O6 * H2O	498.57	817.04	496.01	858.83	g/g API
KH2PO4	38.58	23.99	23.14	23.27	g/g API
(NH4)2HPO4	11.60	22.33	22.83	35.11	g/g API
Citric acid	4.93	4.84	15.42	12.89	g/g API
MgSO4 * 7 H2O	23.96	39.51	13.02	11.41	g/g API
Fe(III) citrate	0.04	0.22	0.78	0.70	g/g API

Only the six dominant medium components were included in this analysis, as they collectively account for more than 99.5 % of the total mass input among all medium constituents (> 17 in total). These components glucose, phosphate and ammonium salts, citric acid, magnesium sulfate, and ferric citrate represent the main sources of carbon, nitrogen, and essential ions in the culture medium. Nutrient consumption showed notable variation among the four fermentation modes. Glucose was the dominant input in all cases, ranging from roughly 500 g g^{-1} API in the continuous setup to more than 800 g g^{-1} API in the repetitive fed-batch process (RFB), reflecting differences in productivity and yield. Phosphate and ammonium salts contributed smaller but non-negligible fractions to the total mass input, while trace components such as magnesium sulfate and ferric citrate remained nearly constant. Overall, the RFB required the highest nutrient load per gram of product, whereas the chemostat process achieved the most efficient substrate utilization.

Consumables:

Table 4: Consumable use (process water, antifoam, cleaning agents) per gram of API

Consumables	Fed-batch	RFB	Chemostat	Cascade	Units
Process water	6.83	3.25	1.52	1.74	L/g API
Antifoam	3.41	2.28	8.54	9.25	g/g API
Ammonia	134	64	30	34	g/g API
NaOH (cleaning)	137	65	30	35	g/g API
H3PO4 (cleaning)	68	33	15	17	g/g API

Consumable use, mainly process water, antifoam, ammonia for pH control, and cleaning chemicals, was strongly affected by the operating regime. Continuous cultivation minimized process-water demand (< 2 L g^{-1} API), whereas cascade and fed-batch operations required larger volumes due to their lower production capacity and the relatively higher weight of one-off consumable inputs per batch.

Utilities:

Table 5: Specific electricity consumption of reactor and autoclave operations per gram of API.

Consumables	Fed-batch	RFB	Chemostat	Cascade	Units
Electricity (Reactor)	1.10	7.81	5.24	5.27	kWh/g API
Electricity (Autoclave)	13.41	6.39	3.00	3.42	kWh/g API
Electricity (Total)	14.51	14.20	8.23	8.69	kWh/g API

The autoclave accounted for the largest share in batch and RFB operations (up to 13 kWh g^{-1} API) because the fixed sterilization energy per run was distributed over a smaller amount of product. In contrast, the continuous mode required less than 3 kWh g^{-1} API, as the same one-off sterilization effort was spread over a longer production period and higher API yield. Bioreactor power consumption increased with process duration and aeration intensity, being highest in the RFB. When aggregated, total specific electricity use ranged from 8 to 14 kWh g^{-1} API, confirming the dominance of sterilization and agitation as energy hotspots.

Fermentation output streams

Table 6: Output streams (biomass and product stream) per gram of API

Fermentation output	Fedbatch	Rep fedbatch	Continuous	Cascade	Units
Biomass (dry sludge)	182	204	88	187	g/g API
Broth supernatant	3.06	3.80	4.37	9.32	g/g API
Cleaning wastewater	10.24	4.88	2.29	2.61	L/g API

The biomass (dry sludge) corresponds to the dry cell weight (DCW) measured at the end of fermentation and represents the product-containing stream, as the target product was assumed to be intracellular and therefore associated with the harvested biomass. The broth supernatant represents the clarified liquid phase remaining after cell removal and was treated as a waste stream in the downstream processing, as it does not contain the product. Finally, the cleaning wastewater was estimated as the total amount of cleaning and rinsing solutions (NaOH, H_3PO_4, and deionized water) used during reactor cleaning, normalized to the amount of API produced.

Interpretation of mass and energy balances

Among the evaluated configurations, the RFB mode exhibited the highest overall material and energy demand per unit of investigated product, mainly due to its elevated glucose and phosphate requirements. The continuous process showed the lowest electricity consumption and nutrient use per g API, reflecting its higher productivity and reduced downtime between runs. The cascade configuration presented intermediate values, while the fed-batch process displayed moderate energy intensity but higher specific water and base consumption. These data form the quantitative basis for calculating the Process Mass Intensity (PMI) and Energetic Footprint, enabling subsequent evaluation of environmental hotspots through the life-cycle assessment.

3.2.2 LCI of insulin production at the facilities of NOVO

For the production of insulin at the facilities of NOVO, different scenarios were investigated regarding the source of electricity and heat. Furthermore, different datasets from Ecoinvent were used to model the End-of-Life treatment of the waste solvent used during the process.

While the composition and contained contaminants in the waste solvent are not known, investigating different alternative waste treatment scenarios allows to predict a range of potential impacts from the manufacturing process. The different alternative Ecoinvent datasets used for these scenarios are listed in Table 7.

An Ecoinvent dataset for road transport (Transport, freight, lorry 7.5-16 metric ton, EURO6, market, RER) over a distance of 2787 km (i.e., from the manufacturing site of NOVO in Kalundborg, Denmark to Athens, Greece), was used to estimate the impacts of the transport of the packaged products to its final consumer. Regarding packaging and other consumables related to the use phase of the insulin product, previously reported findings for the carbon footprint of NOVO devices and their corresponding packaging were used as a proxy. [14], [15] Therefore, complete life cycle assessment of insulin is only performed for the Climate Change potential.

Table 7: Different Ecoinvent datasets used to investigate scenarios for electricity and heat upstream production, as well as alternative waste solvent treatment at the facilities of NOVO. For waste solvent treatment, the density was assumed to be equal to water (1 kg/Liter), when comparing processes expressing data in different units.

Resource	Ecoinvent process alternatives
Electricity	Electricity, high voltage {DK}| market for electricity, high voltage
	Electricity, high voltage {DK}| electricity, high voltage, production mix
	Electricity, high voltage, renewable energy products, production mix {CH}
	Electricity, high voltage, renewable energy products, market for {CH}
	Electricity, high voltage, renewable energy products {CH}| electricity, high voltage, wind power, import from Germany, renewable energy products
	Electricity, high voltage, renewable energy products {CH}| electricity production, wind, <1MW turbine, onshore, renewable energy products
	Electricity, high voltage, renewable energy products {CH}| electricity production, wind, >3MW turbine, onshore, renewable energy products
	Electricity, high voltage, renewable energy products {CH}| electricity production, wind, 1-3MW turbine, onshore, renewable energy products
	Electricity, high voltage {RER}| market group for electricity, high voltage
	Electricity, high voltage {Europe without Switzerland}| market group for electricity, high voltage
Heat	Heat, district or industrial, natural gas {DK}| heat and power co-generation, natural gas, combined cycle power plant, 400MW electrical
	Heat, district or industrial, natural gas {DK}| heat and power co-generation, natural gas, conventional power plant, 100MW electrical
	Heat, district or industrial, other than natural gas {DK}| heat and power co-generation, hard coal
	Heat, district or industrial, other than natural gas {DK}| heat and power co-generation, oil

Resource	Ecoinvent process alternatives
	Heat, district or industrial, other than natural gas {DK}| heat and power co-generation, wood chips, 6667 kW, state-of-the-art 2014
	Heat, from steam, in chemical industry {RER}| market for heat, from steam, in chemical industry
Waste Solvent	Wastewater from maize starch production {CH}| treatment of wastewater from maize starch production, wastewater treatment
	Wastewater from maize starch production {RoW}| treatment of wastewater from maize starch production, wastewater treatment
	Wastewater from potato starch production {CH}| treatment of wastewater from potato starch production, wastewater treatment
	Wastewater from potato starch production {RoW}| treatment of wastewater from potato starch production, wastewater treatment
	Wastewater, average {Europe without Switzerland}| treatment of wastewater, average, wastewater treatment
	Spent solvent mixture {CH}| treatment of spent solvent mixture, hazardous waste incineration
	Spent solvent mixture {CH}| treatment of spent solvent mixture, hazardous waste incineration, with energy recovery
	Spent solvent mixture {Europe without Switzerland}| treatment of spent solvent mixture, hazardous waste incineration
	Spent solvent mixture {Europe without Switzerland}| treatment of spent solvent mixture, hazardous waste incineration, with energy recovery
	Spent solvent mixture {RoW}| treatment of spent solvent mixture, hazardous waste incineration
	Spent solvent mixture {RoW}| treatment of spent solvent mixture, hazardous waste incineration, with energy recovery

3.3 Life Cycle Impact Assessment (LCIA) and Interpretation

The outcomes of the individual LCAs performed in the context of the ENVIROMED project are discussed in Sections 3.3.1 – 3.3.3. The complete life cycle impacts, per kg of insulin, are presented in Section 3.3.4. Concluding remarks from the LCA are presented in Section 3.3.5.

3.3.1 Comparative LCA of growth media compositions for insulin production

The objective of this comparative LCA, as reported in our manuscript [3] was to analyze the environmental impacts of some growth medium components commonly employed in the production of recombinant human insulin, using *E. coli* and yeast. This assessment links directly to Objectives 2 and 4 (Table 2) of the LCA in the ENVIROMED project.

Several publications describing laboratory- and pilot-scale microbial cultivation strategies for insulin production were reviewed. A subset of papers with detailed reporting of materials,

resources, and process steps was selected as the basis for the LCI. Because the analysis relied on secondary data and assumptions, the aim was not to conduct a full sustainability comparison among alternative insulin production processes. Instead, the focus was on comparing different reaction media to support bioprocess developers in selecting material options. The study provides a detailed mapping of upstream operations and key inputs and outputs, highlighting the components that drive environmental impacts within the investigated insulin production systems. These insights help clarify how specific elementary flows influence the final product's footprint, enabling more informed decisions in biopharmaceutical manufacturing. Identifying environmental hotspots can further support targeted process optimizations that reduce the overall impact of insulin production. To allow comparison of different media independent of the scale, the functional unit of 1 Liter of medium was selected.

The contribution of different components to the impacts of pre-cultivation growth media for *E. coli* was analyzed based on the work of Cho and co-workers. [16] **Glucose carbon source** is a hotspot primarily for Resource Use (Minerals & Metals) and Water Use. Tellurium use is primarily linked to the Resource Depletion impacts of glucose, as well as fodder yeast (proxy for **yeast extract**), as part of the upstream production of copper, which plays a role in agriculture. Water Use impacts are strongly linked to the upstream production of glucose and yeast via irrigation, while the use of decarbonized water in the upstream production of electricity also contributes to measurable contribution for the **electricity-consuming autoclaving** process. The **ultrapure water** needed to produce 1 Liter of medium also had a significant contribution to Water Use, Freshwater Eutrophication (mainly from phosphate ions released during ultrapure water production) and Ozone Depletion (via the upstream production of membranes used in reverse osmosis). Finally, upstream production of **fodder yeast and glucose** is also associated with measurable Land Use impacts per Liter of medium, linked to the land requirements for the upstream production of cow milk and maize grain, respectively.

The **autoclaving process**, and specifically its **electricity use**, is also identified as a hotspot for Acidification (mainly due to nitrogen and sulfur oxide emissions from coal-based electricity), Climate Change (mainly due to fossil CO_2 emissions from coal-based electricity), Particulate Matter (mainly due to particulate emissions from coal mining), Eutrophication (mainly due to nitrogen oxide and phosphate emissions along the value chain of electricity production), Human Toxicity (primarily due to anthracene emissions from coke production, and mercury emissions from electricity generation with hard coal), Ionizing Radiation (mainly due to the upstream production of nuclear energy and its associated emissions), Photochemical Ozone Formation (due to nitrogen oxide emissions), and Fossil Resource Use (particularly from the use of hard coal, natural gas and uranium in the electricity mix).

Antibiotics can have a significant contribution to impacts, depending on their concentration, as was shown for media utilized by Shin and co-workers, [17] particularly for Freshwater Ecotoxicity (due to the upstream use of pesticides), Minerals and Metals Resource Use (mainly due to copper use for the production of glucose, used for antibiotic manufacture), Human Toxicity (mainly due to the upstream production of heat and electricity, glucose, and other organic substrates for the production of antibiotics) and Water Use (mainly due to the upstream production of glucose).

Besides *E. coli*, yeast such as *P. pastoris* and *S. cerevisiae* have also been extensively used for insulin production, and an assessment of different compounds used for microbial growth media was performed, based on the compositions described by Nurdiani and co-workers [18] and Kazemi Seresht and co-workers, [19] respectively. For *P. pastoris*, The upstream production

of soybean, rape seed, alfalfa grass and cow milk were primarily associated with the Marine Eutrophication and Land Use impact categories, and more specifically the production of **glycerol, peptone and yeast extract** used for the growth medium. **Glycerol** production is further associated with Ozone Depletion (mainly associated with the upstream production of chlorinated compounds used for pesticides and herbicides), Terrestrial Eutrophication (mainly due to ammonia emissions associated with upstream agricultural activities), Human Toxicity and Ecotoxicity impacts (mainly due to upstream pesticide and mercury emissions from agricultural activities). **Potassium phosphate**, serving as a macronutrient and buffer component, was associated as a hotspot compound with several impact categories for yeast growth media, including Water Use (primarily due to the water intensive upstream process of phosphoric acid production), and Freshwater Eutrophication (primarily due to phosphate-containing residues generated from the production of phosphoric acid), while the **ultrapure water** used to produce the medium also contributes significantly to Water Use, Ozone depletion (related to the upstream production of membranes for reverse osmosis) and Freshwater Eutrophication (due to upstream phosphate emissions during reverse osmosis). Human Toxicity from the upstream production of **potassium phosphate** is primarily linked to lead, arsenic and cadmium emissions, as part of the upstream production of sulfuric acid and copper, the latter also being a strong predictor of Minerals and Metals resource use per Liter of buffer, particularly due to Tellurium depletion in the production of copper. Finally, Particulate Matter impacts for potassium phosphate are linked to upstream sulfur dioxide and particulates emissions to air, with the former also significantly contributing to the Acidification potential per Liter of buffer solution.

For *S. cerevisiae*, several hotspots were identified, specifically **glucose** (for Water Use and Land Use impacts), **electricity-driven autoclaving** (for Ionizing Radiation), **macronutrients** (mainly **ammonium sulfate**, particularly for Minerals and Metals Resource Use, as ammonium sulfate is partially produced as a co-product of zinc production, and is therefore being allocated part of the impacts of zinc, lead, silver, copper and gold co-extraction). Finally, **vitamins** also constitute a hotspot for multiple impact categories. Regarding vitamins, a significant variation of up to 97% was calculated, when alternative upstream manufacturing protocols were modelled for the same proxy vitamin, and therefore it is advisable to consider more specific and accurate proxies to draw reliable conclusions about the specific hotspots.

In order to trigger the expression of the target gene for insulin production for both *E. coli* and yeast, different inducers were compared from literature, including **chemical inducers** (IPTG (Isopropyl β-D-1-thiogalactopyranoside); [20] and methanol), [18] or temperature-induced expression. [21] **Methanol** was shown to be a hotspot particularly for Fossil Resource Use at high concentrations, whereas **IPTG** had a much higher contribution in several impact categories, of up to 151 times higher (for Ozone Depletion potential). It should be noted however that these insights are of limited relevance at the commercial scale, since alternative inducers such as lactose or methanol are typically preferred.

Different media are used at different stages during microbial production of insulin in fed-batch growth. The **starting growth medium** is the initial, nutrient-rich medium added to the bioreactor before inoculation with microorganisms, containing the essential components for early-stage growth and biomass accumulation. The **feed growth medium** is the medium supplied incrementally or continuously over time to the bioreactor, in order to replenish nutrients and sustain microbial growth without excessively diluting the culture volume. Typically, the starting growth medium has more complex composition, while the feed medium contains higher concentrations of carbon source (e.g., glucose) and yeast extract (i.e., the source

of vitamins, proteins, and minerals to sustain growth). Increased concentration of **glucose and yeast extract** systematically leads to an increase of impacts in every impact category in the investigated literature, with over 100% increase for Water Use and Land Use in some cases. **Trace elements**, while carrying significant impacts per gram of component (when compared to macronutrients), did not exceed 5% contribution to the overall impacts, due to their low concentration. On the contrary, **macronutrients**, and particularly **potassium phosphate**, were a hotspot for several impact categories, as was extensively discussed in previous paragraphs regarding the buffer impacts, [18] namely Human Toxicity, Particulate Matter, Land Use (associated primarily with upstream phosphate mining and landfilling of resulting waste), Water Use, Acidification, and Mineral and Metal Resource Use.

Overall, the outcomes of this literature-based assessment of impacts from the upstream production of insulin reveal different hotspots, including the **carbon source** (i.e., glucose, glycerol) and commonly employed **low-cost sources of nutrients** (i.e., yeast extract), in agreement with previous reports on the impacts of glucose on microbial fermentation processes. The **ultrapure water** was shown to be a hotspot, in agreement with previous LCA reports from bioprocesses and other industrial activities with requirements for high water purity. **Macronutrients** (i.e., ammonium sulfate) and/or **Buffer components** (potassium phosphate) were additional hotspots, in agreement with previously reported results on bio-catalytic microbial processes, while **vitamins and antibiotics** were also identified as hotspots, as previously shown for growth medium components. For the latter two, it is particularly important to select more specific and accurate proxy components for LCA modelling in future studies. Finally, **electricity consumption for media sterilization** via autoclaving was revealed as a hotspot in several impact categories, in agreement with previous LCA studies on (bio)processes.

These insights were used to propose potential strategies for impact mitigation, namely: identifying and selecting sustainable and locally-sourced upstream **carbon sources and yeast extract** and production processes; investigating closed-loop or open-loop strategies for **water purification and re-use** and/or **buffer recycling**; investigating **alternative upstream production routes for ultrapure water**; re-evaluating the upstream impacts of **macronutrients** based on specific resources used, to **avoid uncertainty related to impact allocation** with metal extraction processes; selecting **more specific and accurate proxies for vitamins and antibiotics**; investigating strategies to **decrease the impacts of electricity use in sterilization** (e.g., renewable electricity, heat recovery, improving sterilization energy use efficiency, opting for steam-based sterilization).

3.3.2 Comparative LCA of different reactor operation scenarios at the lab scale in TUW

3.3.2.1 Global warming potential

Using the upstream life cycle inventories established in Section 3.2.1, the carbon footprint results for the four reactor operation scenarios under assessment are presented in Table 8 and Figure 7.

Overall, the total cradle-to-upstream emissions ranged from 3.85 kg CO_2-eq g^{-1} API for the continuous process to 6.46 kg CO_2-eq g^{-1} API for the RFB. The autoclave electricity demand was identified as the dominant hotspot across all modes, contributing up to 69 % of the total emissions in the fed-batch configuration. The bioreactor electricity use and glucose supply

were the next most relevant contributors, reflecting the influence of both energy intensity and carbon-based feedstock requirements.

Continuous operation achieved the lowest specific impact mainly because the single sterilization event was distributed over a longer fermentation duration and higher volumetric productivity, thereby reducing the relative contribution of start-up energy to the overall footprint.

The cascade mode displayed intermediate performance, combining moderate electricity use with lower specific media demand. Consumables and cleaning agents contributed marginally (< 5 %), indicating that process-energy optimization offers the greatest potential for emission reduction. These findings highlight sterilization and stirrer dissipation energy as key environmental hotspots in lab-scale antibody fragment production and point toward significant benefits from renewable electricity integration or waste-heat-based sterilization strategies.

Table 8: Carbon footprint of upstream Fab production under different fermentation modes (per g API). Breakdown of CO_2-equivalent emissions by source category for the four process configurations.

kg CO_2/g API	Fedbatch	Rep fedbatch	Continuous	Cascade
Electricity autoclave	4.38	2.09	0.97	1.12
Electricity bioreactor	0.36	2.55	1.70	1.72
Glucose	0.85	1.40	0.85	1.47
Nutrients	0.51	0.30	0.23	0.28
Consumables	0.02	0.01	0.04	0.04
Cleaning agents	0.23	0.11	0.05	0.06
Rest (wastes, other)	0.01	0.01	0.00	0.01
SUM	6.35	6.46	3.85	4.70

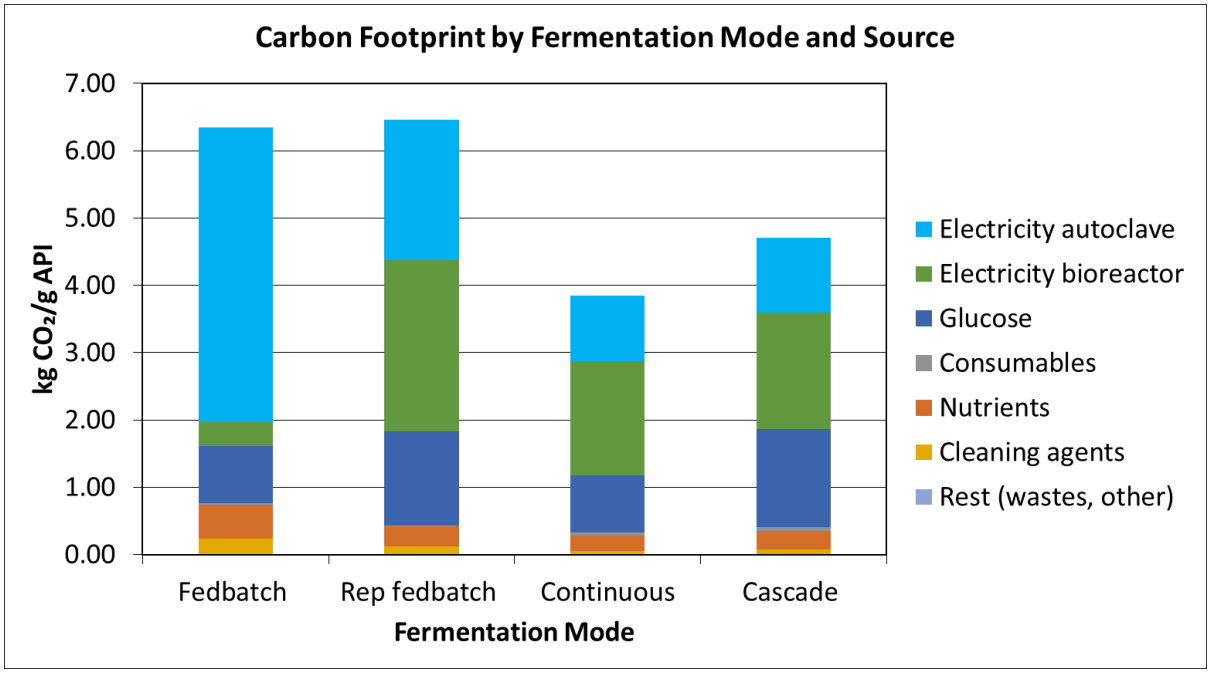

Figure 7: CO_2 footprint by fermentation mode and emission source. Stacked bar chart showing the relative contribution of electricity, substrate, and auxiliary material inputs to the total carbon footprint (kg CO_2 g^{-1} API) for fed-batch, repeated fed-batch, continuous, and cascade modes.

3.3.2.2 Water footprint assessment

The water footprint results for the four fermentation modes assessed are shown in Figure 8. The figure presents the total water consumption per gram of API and the relative contribution of each upstream input, including deionized water, medium components, cleaning operations, electricity generation, and auxiliary chemicals.

Across the investigated modes, the overall pattern closely mirrors the trends observed for the carbon footprint. The continuous mode shows the lowest water use per gram of API, followed by fed-batch, cascade, and with repeated fed-batch exhibiting the highest specific water demand. This behaviour results from the fact that several major water-consuming activities such as sterilization, aeration, and cleaning are fixed per run and therefore exert a proportionally smaller influence when the process operates for longer durations and produces more product, as in continuous mode.

A major contributor to the water footprint across all investigated modes is the water embodied in electricity generation. This arises not from the fermentation itself but from the upstream production of electricity within the EU average grid mix, which relies heavily on thermal power plants (coal, gas, and nuclear) which require very large volumes of cooling water. Consequently, electricity-related water consumption decreases substantially from fed-batch to continuous mode because the same fixed energy demand is distributed over a larger amount of product.

In contrast, glucose production remains a consistently significant contributor to water consumption. This reflects the intrinsically water-intensive nature of agricultural cultivation and glucose processing, combined with the large mass of glucose required per gram of API. Unlike electricity and cleaning water, which scale with operational duration, glucose consumption is driven primarily by metabolic demand and therefore remains a major hotspot in all modes.

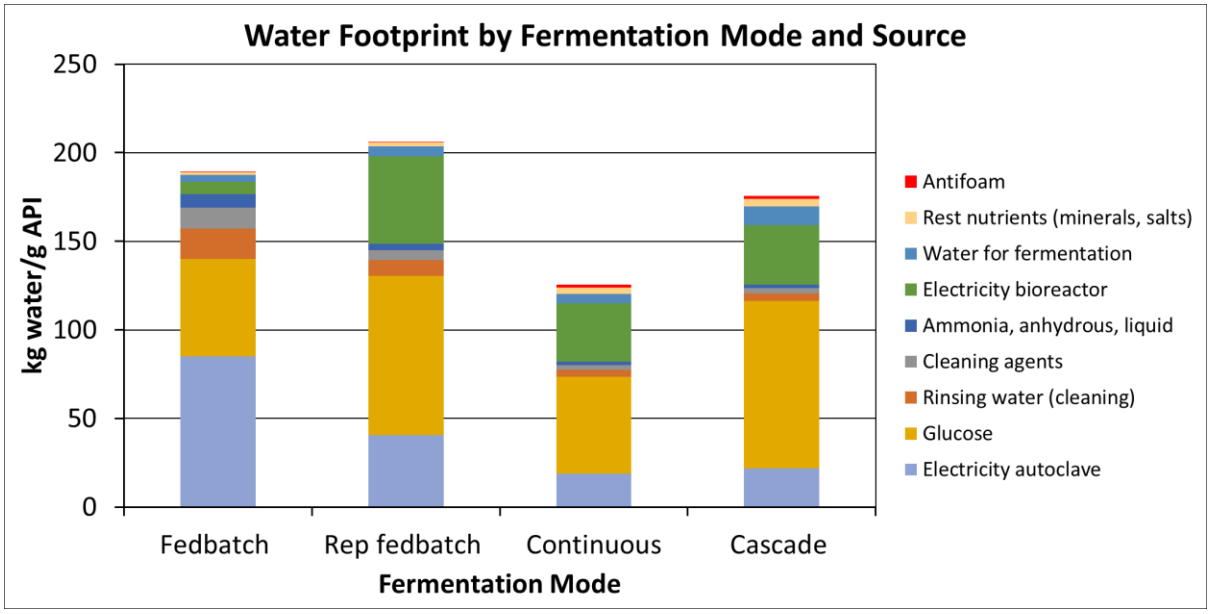

Figure 8: Water footprint of upstream Fab production for the four fermentation modes, broken down by contributing processes.

Overall, these results confirm that for the investigated cases:

- Electricity dominates due to upstream cooling-water demands in the EU grid mix.
- Glucose remains a substantial contributor regardless of mode.
- Specific water use decreases from fed-batch toward continuous mode for the same reason as the CO_2 footprint: fixed upstream burdens are spread over higher product yields.

This analysis highlights that both energy decarbonization and medium optimization represent key levers for reducing the water footprint of upstream Fab production.

3.3.2.3 Sensitivity and scenarios

Effect of renewable electricity vs EU mix:

To evaluate the effect of energy source on the total carbon footprint, two electricity supply scenarios were considered: the current EU average grid mix and a 100 % renewable (wind-based) mix. Figure 9 illustrates the impact of these scenarios across the four fermentation modes.

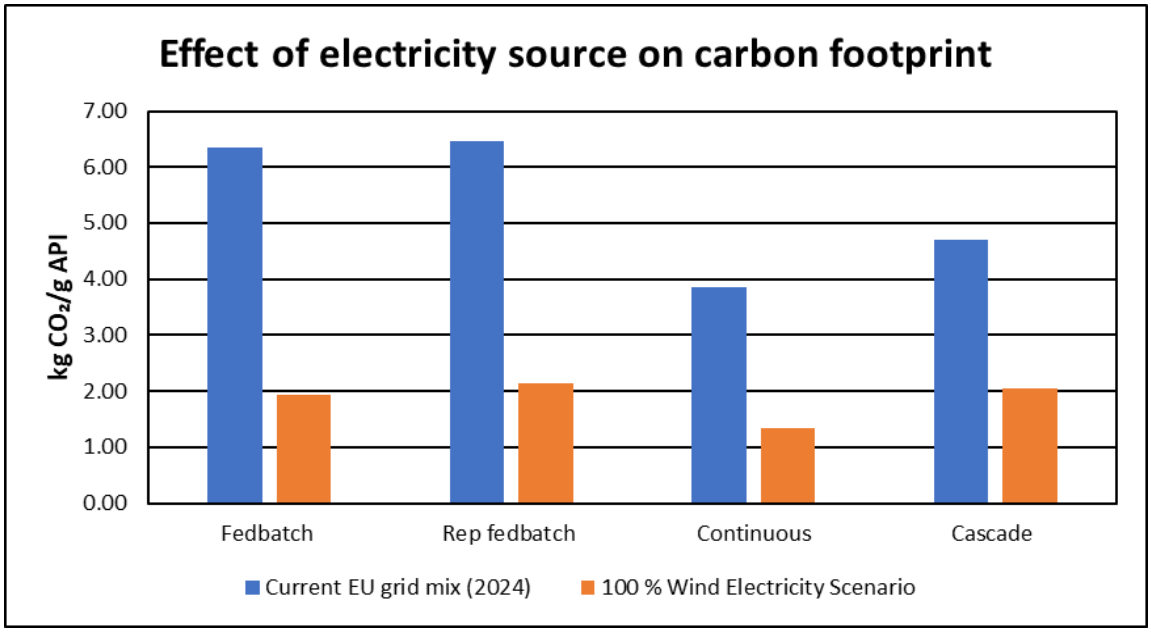

Figure 9: Effect of electricity source on the carbon footprint of Fab upstream production. Comparison between the current EU grid and a 100 % wind-based electricity scenario for all process configurations (values expressed as kg CO_2 g^{-1} API).

Replacing the current grid electricity with 100 % wind power led to a substantial decrease in total emissions for all process modes. The most pronounced reductions occurred for the fed-batch and repeated fed-batch operations, where electricity use mainly from autoclaving and stirring constitutes the dominant contribution to the overall footprint. Under the renewable scenario, their specific carbon intensity decreased by roughly 65–70 %, while the continuous and cascade processes, already less electricity-intensive, showed more moderate improvements. These results confirm that decarbonizing the electricity supply provides the greatest opportunity for reducing the upstream environmental burden of Fab production.

Effect of glucose to product yield:

A sensitivity analysis was performed assuming a fivefold increase in glucose-to-product yield (Figure 10), based on consultation with TU Wien to reflect the upper bound of a realistically achievable improvement through strain and process optimization.

As shown in Figure 10, this assumption results in a pronounced reduction in the climate change impact across all four operational modes, confirming glucose consumption as a dominant contributor to the overall footprint. Despite the significant decrease in absolute values, the relative ranking of the configurations remains unchanged, with the continuous process showing the lowest impact, followed by the cascade system, and higher impacts for the fed-batch and repetitive fed-batch modes. This analysis highlights yield improvement as a critical lever for reducing upstream environmental burdens within realistic performance limits.

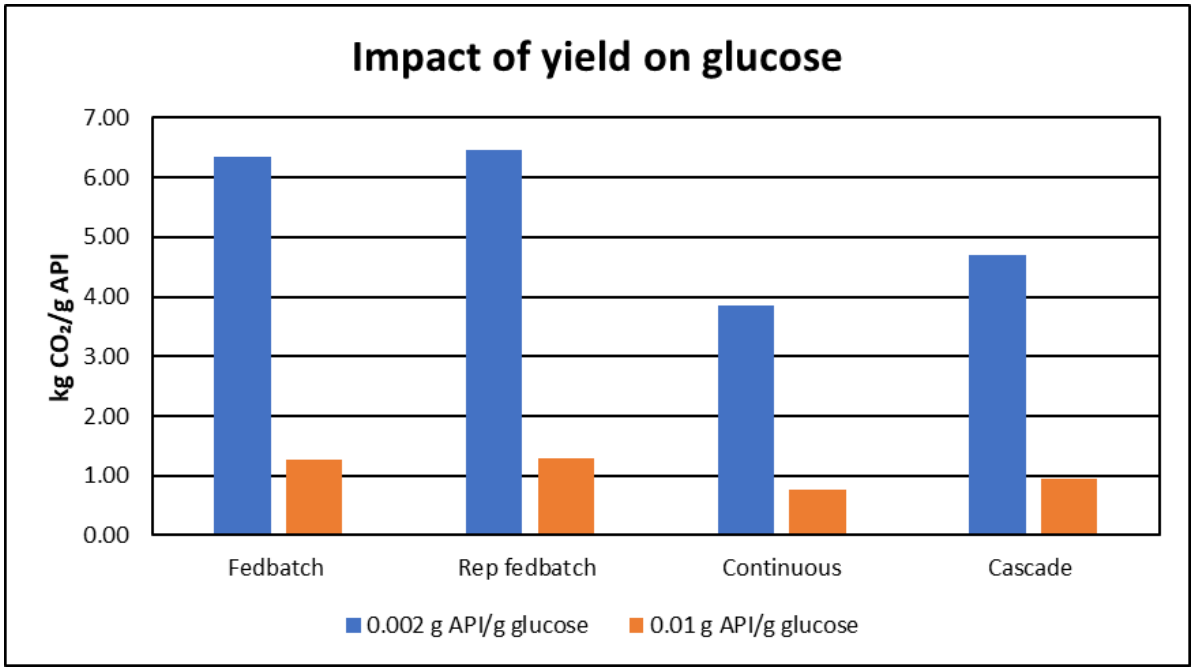

Figure 10: Sensitivity analysis of the climate change impact (kg CO_2-eq per g API) for the four operational modes assuming a baseline glucose yield (blue bar) and a fivefold increase in glucose-to-product yield (orange bar)

3.3.3 LCA and scenario analysis for insulin production at the industrial scale (NOVO)

The manufacturing process per mass of product output (i.e., kg of insulin) was analyzed using data for the production of insulin at the facilities of NOVO. The contribution of different resources, materials and waste flows are shown in Figure 11. As can be observed, the majority of impacts (over 90% for Land Use) in most impact categories originate from the carbon and nutrient source (red section in Figure 11), as also found in the literature-based assessment of insulin production (Section 3.3.1). Another significant contributor is the energy use for the manufacture (orange section in the figure, nearly 40% contribution to Ionizing Radiation potential), including electricity and heat. The organic chemicals (green section in the figure) reached a contribution of up to 30% (for Fossil Resource Use potential), followed by the inorganic chemicals (purple section, up to 20% for Freshwater Ecotoxicity and Minerals and Metals Resource Use potential) and the waste treatment (black section in Figure 11, up to 14% contribution, for Marine Eutrophication potential).

As per the Objectives of this LCA study (Objective 2, Table 2), a sensitivity analysis was performed on different process alternatives for process steps that constitute LCA hotspots, to investigate how different upstream scenarios can improve the overall sustainability of API production. Considering that the carbon and nutrient source, as well as the organic and inorganic chemicals used for industrial insulin production are optimized for the process, no alternatives were investigated for these inputs. Instead, different sources for energy (i.e., electricity and heat) were analysed, per output of product (i.e., per MJ of output energy), and the results are shown in Figure 12 and Figure 13.

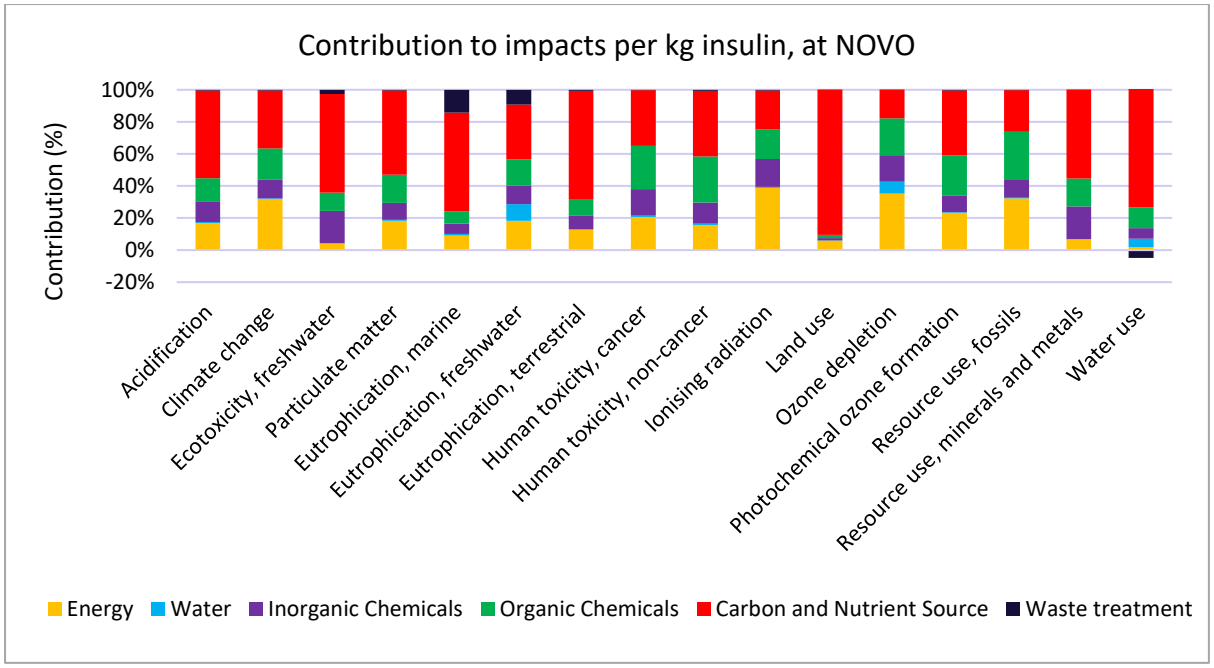

Figure 11: Contribution to environmental impacts of different inputs and outputs along the production of insulin at NOVO facilities

For the different upstream sources of heat (Figure 12), it can be concluded that heat produced from steam in the chemical industry (grey line) has the highest impact in most impact categories, followed by heat produced from oil (orange line). Besides oil, other sources to produce heat (excluding natural gas), i.e., hard coal and wood chips, result in intermediate impacts, while exhibiting some peaks in selected impact categories, namely Non-Cancer Human Toxicity (for both, up to 89% comparative impacts), Land Use (for wood chips) and Terrestrial Eutrophication (for hard coal). Finally, the two **heat and power co-generation processes based on natural gas result in the overall lowest environmental impacts**, considering all impact categories. Such insights can serve as a basis for recommendations to the pharmaceutical industry, to select upstream processes for the production of heat that minimize the environmental impact, as natural gas is shown to be here.

While natural gas is shown to be a sustainable source for heat in biopharmaceutical processes, its upstream environmental impacts should be discussed, as also described in Objective 3 of this assessment (Table 2). Natural gas extraction and transportation often result in methane leakage, a serious environmental concern. Methane is a potent greenhouse gas, with a global warming potential 84 × that of CO_2 over 20 years, and 30 × over 100 years. In the United States alone, pipeline leaks release approximately 1.2 to 2.6 million tons of methane annually, exacerbating near-term global warming and contributing significantly to CO_2-equivalent emission. [22] On a global scale, the liquefied natural gas supply chain emits an estimated 350 Mt CO_2-equivalent per year, of which ~30% (≈105 Mt CO_2-eq) is due to methane leakage. [23] This leakage not only undermines the climate benefits gained by displacing coal but also affects aquatic ecosystems. Underwater methane releases, such as from pipeline or well blowouts, can trigger anoxic conditions and oxygen depletion, harming aquatic organisms and disrupting marine ecosystems. [24]

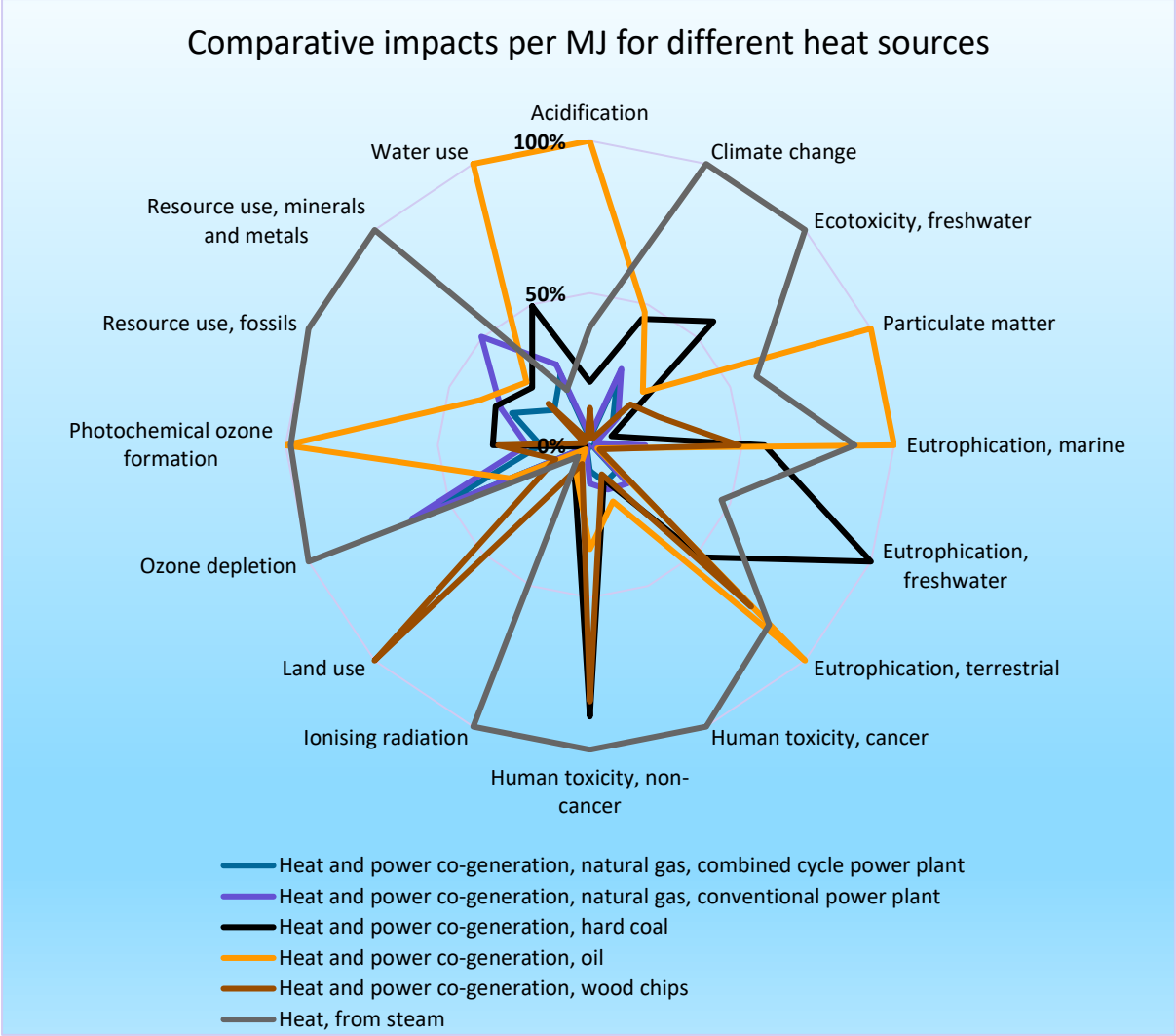

Figure 12: Comparative LCA assessment of different Heat sources from the Ecoinvent library.

Mitigation strategies for methane leaks have advanced significantly in recent years. For example, according to a report from the International Energy Agency, [23] applying best-available methane abatement technologies across the liquefied natural gas supply chain can reduce methane emissions by ~90 Mt CO_2-equivalent annually, cutting total emissions by ~25% at little or no net cost. Detection methods have improved with real-time leak monitoring, infrared cameras, and AI-powered sensors, enabling rapid identification and repair of leaks before they escalate. [25] Beyond formal regulation, organizations such as the Environmental Defense Fund in the U.S. have recommended mitigation measures including automatic shut-off valves, remote-control valves, and enhanced supervisory controls to isolate leaks and prevent large releases. Research and deployment funded by the DOE's Methane Mitigation Technologies Program advance cost-effective tools across upstream infrastructure, helping operators routinely detect, quantify, and eliminate methane leaks. [25], [26] Collectively, these measures present a robust pathway toward minimizing natural gas's environmental footprint. The European Union has also taken strong action: through the EU Methane Regulation (Regulation (EU) 2024/1787), which came into effect in 2024 as part of the EU Methane Strategy and Green Deal, operators across the natural gas lifecycle (including production, transmission, and imports) are now required to monitor, report, verify methane emissions,

implement mandatory leak detection and repair , ban routine venting and flaring, and ensure transparency on methane emissions from imported fossil fuels. [27]

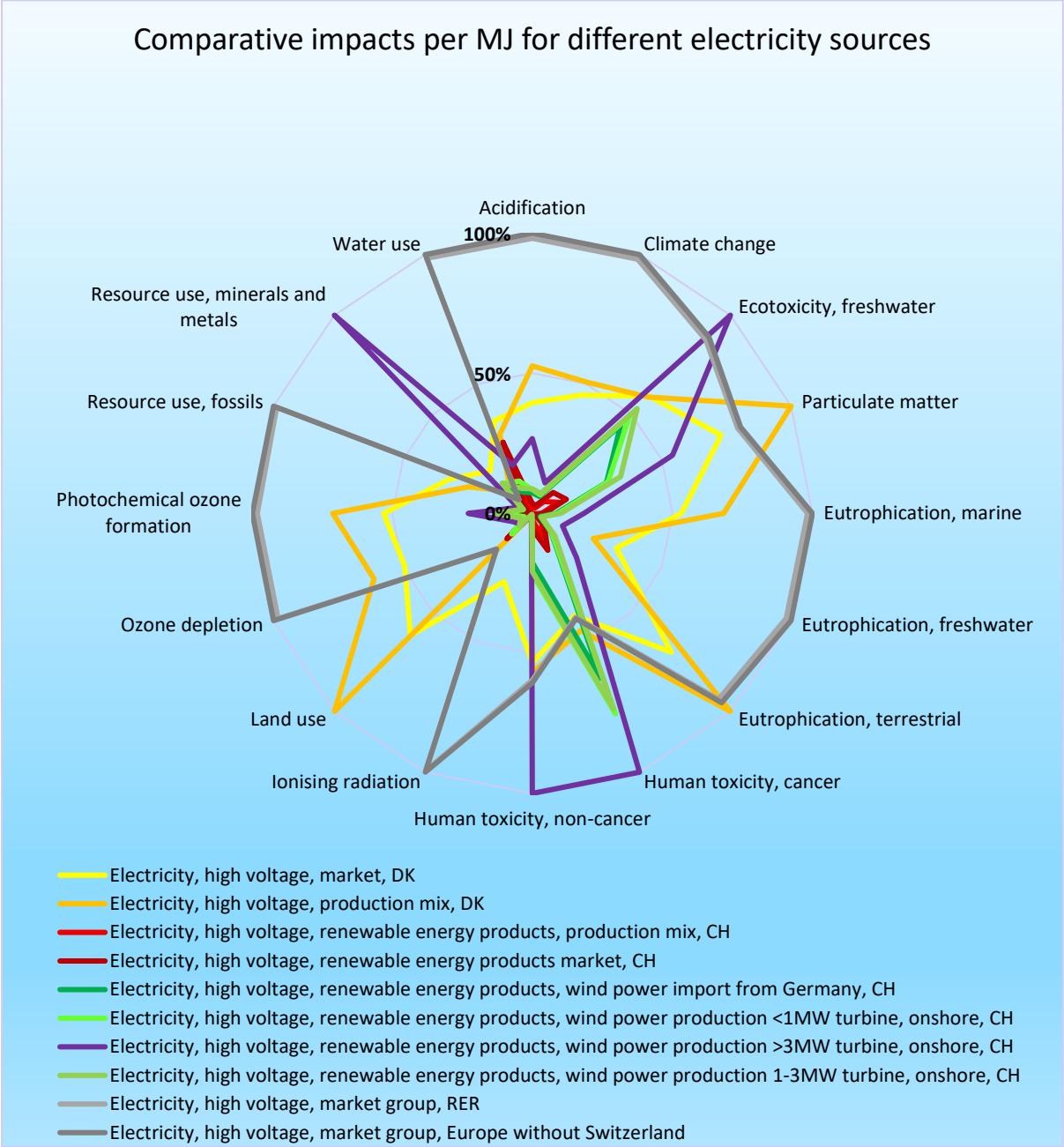

Figure 13: Comparative LCA assessment of different Electricity sources from the Ecoinvent library.

A comparative assessment was also performed over different electricity sources, and the results are shown in Figure 13. The assessed datasets can be grouped, based on their resulting environmental impacts. For example, electricity modelled in Europe (i.e., RER and Europe without Switzerland datasets, light and dark grey lines in the figure) exhibit almost the same impacts in all categories, which are generally the highest among all examined datasets. In terms of overall impacts for all impact categories, the second highest impacts were calculated for the two market-average datasets representing the electricity mix of Denmark (orange and yellow lines in the figure), where the largest insulin production line of NOVO is located. [28] These

market-average electricity datasets representing European and country-specific electricity mixes were modelled as part of the sensitivity assessment, to estimate whether the location of the manufacturing facility within Europe has a significant contribution to the overall impacts. Based on these results, as well as the high contribution of energy (Figure 12, heat and electricity) to the overall process, **country-specific upstream processes for energy production** appear to be important, to accurately predict the overall environmental impacts of biopharmaceutical manufacture.

Besides the country-specific energy mix for Denmark, some renewable energy scenarios were investigated, particularly focusing on wind energy. Wind energy plays an important role on the renewable energy mix of Denmark, based on current data and future scenarios. Wind power is used to generate nearly 60 % of the country's electricity in 2023, making it the highest share of wind in any national electricity mix. [29], [30] Future energy roadmap scenarios anticipate wind energy continuing to play a central role, with capacity expansions and offshore projects like Bornholm Energy Island further increasing wind's contribution toward Denmark's goal of 100 % renewable electricity by 2030. [30]

In Figure 13, a third cluster of datasets (with different shades of green) represents renewable electricity, and more specifically wind energy, produced at <1MW or 1-3MW onshore wind turbines, or imported from Germany to Switzerland (CH in Figure captions), while wind energy produced with onshore wind turbines >3M resulted in significantly higher impacts, especially in the categories Freshwater Ecotoxicity, Human Toxicity, and Minerals and Metals Resource Use. The sub-process-specific impacts of different Ecoinvent datasets for wind energy are outside the scope of this work, and therefore differences for the >3MW wind turbines will not be further discussed. Data for Switzerland were used for renewable electricity scenarios, as they were the only ones available in Ecoinvent. Finally, the group with the smallest comparative environmental impacts over all impact categories are a market / production mix of renewable energy products (red and dark red lines in the figure), modelled in Switzerland. While the specific impacts of different upstream production processes for renewable electricity are outside the scope of this work, the present results indicate the **significant impact decrease potential of employing renewable energy sources in the biopharmaceutical cases investigated**.

One final scenario was investigated regarding the process at NOVO, focusing on the waste solvent treatment (i.e., the reaction medium for insulin production), and the comparative impacts per Liter of waste solvent are shown in Figure 14. Specifically, two types of waste solvent (i.e., water or a mix of waste solvents), undergoing different types of treatment (i.e., wastewater treatment, or incineration as a hazardous waste, with and without energy recovery). For wastewater, one additional scenario was investigated, modelling as either average wastewater composition, or as wastewater from starch production (to better reflect the Chemical Oxygen Demand content of a biopharmaceutical waste solvent, considering the input organic materials, e.g., as discussed in Section 3.3.1). As can be observed, when modelled as a waste solvent undergoing incineration, the reaction effluent has a significantly higher contribution in every impact category, compared to wastewater. The highest comparative impacts for the wastewater treatment processes are calculated for Marine Eutrophication (up to 76%, for wastewater originating from maize starch production), while a negative environmental impact (i.e., environmental benefit) is calculated for some of the wastewater treatment processes, as clean water is modelled as an output of the process in Ecoinvent.

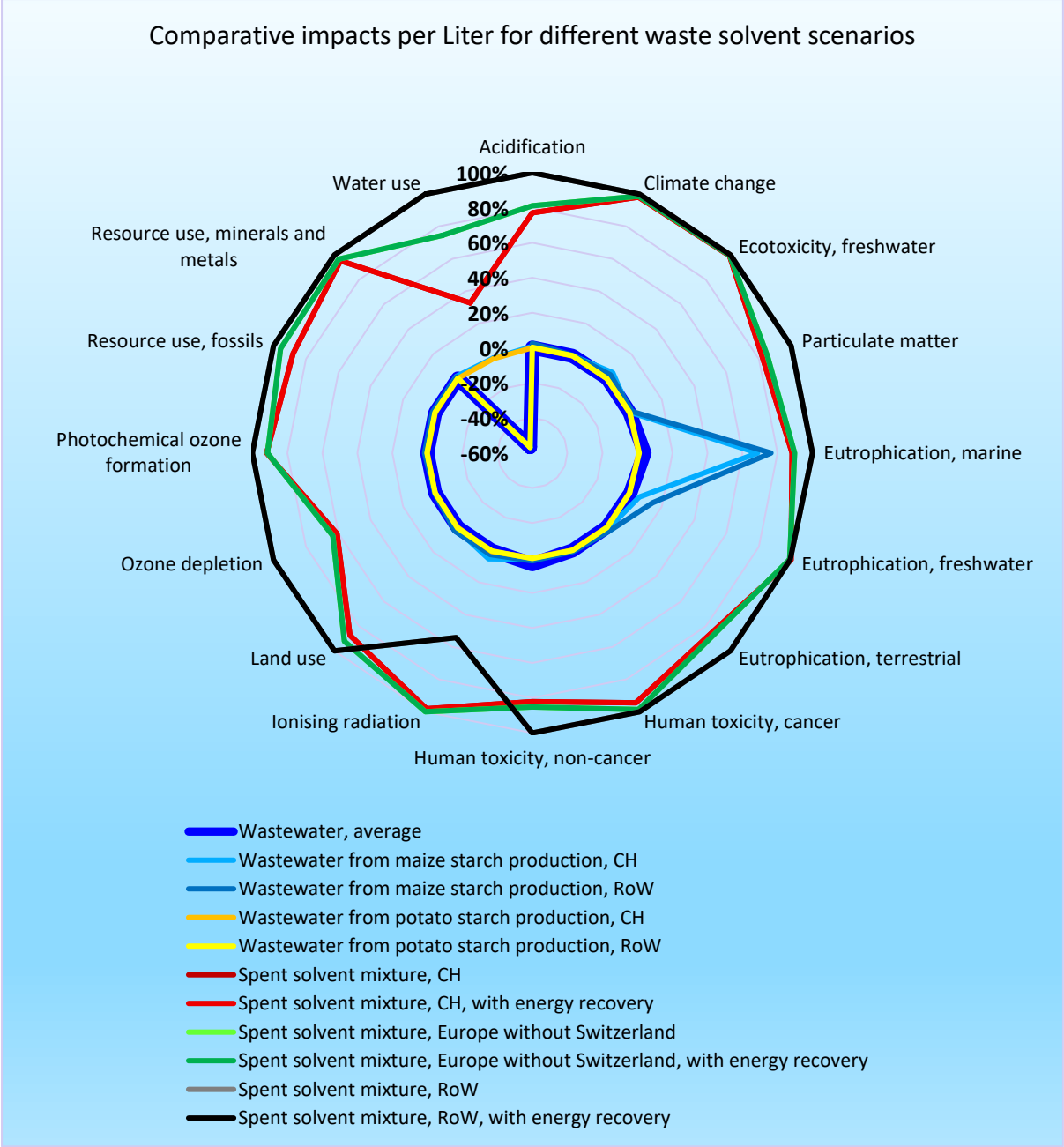

Figure 14: Comparative LCA assessment of different waste solvent treatment datasets from the Ecoinvent library.

Based on the minor overall environmental impact of the waste solvent treatment process to the total impacts per kg of produced insulin (Figure 11), a more detailed assessment of the waste treatment scenarios, and corresponding recommendation for selection of more sustainable solvents (i.e., Objective 2 in Table 2), is not considered relevant. Nevertheless, it should be noted that API-specific impacts (e.g., intermediates, final product, specific reaction media components and other potential contaminants in the waste solvent) are not included in the LCA modelling of the waste and could potentially affect the results if modelled as emissions, particularly for ecotoxicity impacts. Finally, while the solvent composition (and resulting waste treatment impacts) is not a relevant factor in the LCA results for NOVO, such insights in different waste treatment impacts may be of relevance for other pharmaceutical manufacturing

processes, particularly employing chemical instead of biological catalysts, which often use solvents of higher chemical complexity and toxicity. [31], [32], [33], [34]

3.3.4 Carbon Footprint over the entire lifecycle for insulin

The Carbon Footprint was calculated over the entire lifecycle of insulin, assumed to be produced via the process modelled at NOVO, packaged in a DuraTouch® pen, and transported to Greece to be used by one patient for one year, and the results are shown in Figure 15. The packaging weight (for the transport impacts) was estimated based on an observational literature study for daily use injections, with an average monthly weight per patient of approximately 3.1 lb (1.4 kg). [35] As can be observed, transport has negligible impacts, while consumables (i.e., needle, cartridge, and delivery system) have o total contribution of 76% to the Carbon Footrpint, followed by the insulin itself (20%) and the packaging (4%). It should be noted that API-specific impacts in the form of emissions during the manufacture, use phase and End of Life (EoL) please are not included. Nevertheless, overall, such findings highlight the importance of considering the entire lifecycle when proposing impact mitigation measures for biopharmaceuticals.

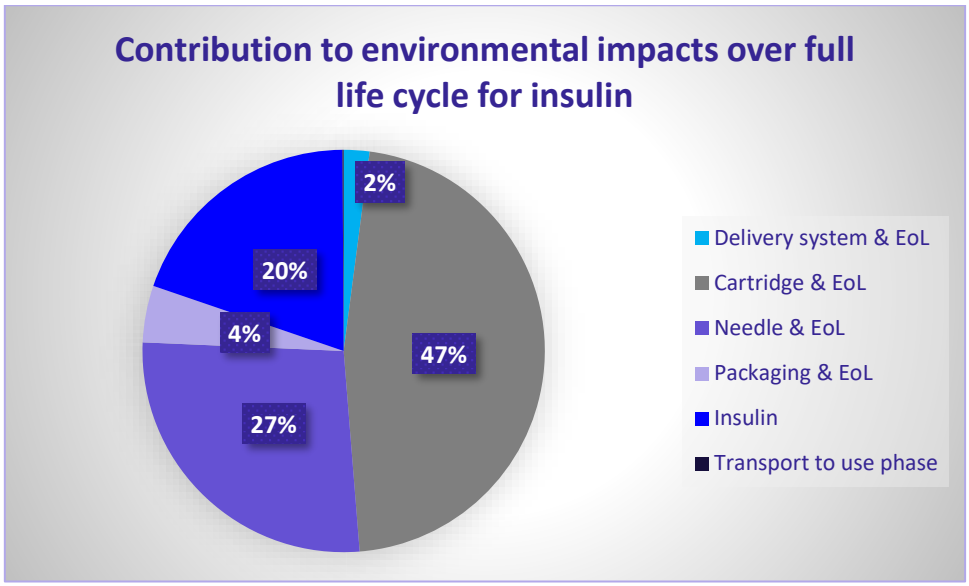

Figure 15: Contribution to Carbon Footprint of different inputs and sub-processes along the entire lifecycle of insulin.

3.3.5 Concluding remarks from LCA

In this project, three different LCA assessments / sustainability metrics were applied at different scales and on different APIs and manufacturing processes, focusing on biopharmaceuticals of the ENVIROMED project. The overall assessment revealed several hotspots of impacts along the lifecycle of the investigated biopharmaceuticals, and mitigation measures were suggested to end-users (i.e., in Section 3.3). The identified hotspots from the literature-based assessment of insulin production were the carbon source (i.e., glucose, glycerol), ultrapure water, commonly employed low-cost sources of nutrients (i.e., yeast extract), inorganic components (i.e., potassium phosphate and ammonium sulfate), vitamins, antibiotics, and electricity for growth media sterilization as the major contributors across several impact categories, including climate change and water use.

At laboratory scale, the comparative sustainability metrics calculated for the four upstream operational strategies for antibody fragment production performed at TU Wien showed that the climate change potential and water footprint were primarily driven by glucose consumption and electricity demand, with sterilization and agitation representing the dominant energy-related contributors. In line with ENVIROMED Objective 2 (Table 2), these results pinpoint the upstream stages and process operations where material and energy use concentrate the highest environmental burdens, providing a clear basis for targeted mitigation strategies

Continuous operation consistently exhibited the lowest impacts due to higher productivity and reduced downtime, while fed-batch and repetitive fed-batch modes showed higher specific burdens associated with lower yields and increased material and utility inputs per functional unit. The cascade configuration achieved intermediate performance by partially decoupling growth and production phases. These findings are aligned with the LCA research studies on insulin production and the industrial-scale assessment at NOVO, where carbon source supply and electricity use were likewise identified as main environmental hotspots. The convergence of results across lab-scale modelling, literature data, and industrial-scale analysis highlights yield improvement and energy decarbonization as the most effective levers for impact reduction across scales. This work directly contributes to Objective 1 (Table 2) by quantitatively assessing the environmental impacts of pharmaceutical production, focusing on climate change potential and water footprint (Figure 7, Figure 8). The comparative evaluation of alternative reactor operation strategies and mitigation scenarios supports Objective 4 (Table 2), by demonstrating how process configuration, productivity improvements, and energy decarbonization can effectively reduce environmental burdens in the assessed biopharmaceutical manufacturing (Figure 9, Figure 10).

The assessment on manufacturing at industrial scale, identified as main hotspots were the carbon and nutrient sources, energy (electricity and heat), followed by organic and inorganic input chemicals. Scenarios related to alternative upstream sourcing of electricity and heat were investigated, with the potential to significantly improve the environmental impact, per MJ of resource. Finally, along the entire lifecycle of insulin, impacts related to the auxiliary equipment for its use (e.g., needles, pens, etc.) have the most significant contribution to the carbon footprint, followed by the insulin product itself and the packaging. It should be noted that API-specific emissions (such as the API itself, its intermediates, specific contaminants in the reaction medium, or metabolites after the use phase) were not included in the assessment. Nevertheless, several important conclusions can be drawn regarding the different hotspots, which can inform possible mitigation actions for the biopharmaceuticals studied, as per the ambition of Objective 1 (Table 2).

The outputs from this work, encompassing both primary and secondary data, demonstrate how LCA can serve as a decision-support tool for identifying environmental hotspots and guiding process optimization in pharmaceutical manufacturing (objective 5, Table 2). By applying LCA to growth media, lab-scale biomanufacturing regimes, and industrial insulin production, we provide an overview of resource use and emissions across different scales. This approach supports more informed decision-making by identifying main contributors to environmental impacts and potential areas for improvement and mitigation measures, when moving from laboratory toward scaled-up pharmaceutical research activities.

The results of our assessments highlight critical hotspots, which dominate the environmental footprint of the investigated biopharmaceutical production research activities. By modelling scenarios for alternative energy sources, such as renewable electricity and low-impact heat generation, we quantify the potential reductions in impact categories such as greenhouse gas emissions and resource depletion. These insights allow decision-makers to prioritize

interventions with the highest impact, such as switching to renewable energy or optimizing feedstock use, thereby embedding sustainability into process design and operational planning.

Beyond technical optimization, our findings contribute to a broader framework for green pharmaceutical processes, by illustrating how LCA can inform both industry practices and policy development. For example, the different waste solvent scenarios compared between aqueous-based (suitable for biobased products) or a mixed chemical solvent (more suitable for chemically-catalyzed API synthesis) underscores the potential environmental advantages of water-based biomanufacturing over solvent-intensive chemical synthesis, providing insight into how process design choices may influence environmental impacts. It is of course critical to add that a comparison between biologically- and chemically-catalyzed API production is outside the scope of the LCA performed here, and it should be performed on a functional unit basis (e.g., per kg of produce API), to account for differences in scales and employed volumes of reaction media per unit API output. Ultimately, our work provides insights that help sustainability-oriented decision-making in pharmaceutical process development.

Finally, the approach followed in this work towards the LCA assessment along the lifecycle of insulin followed previously proposed methodologies and definitions, as required for assessment based on Product Category Rules (PCRs), in agreement with Objective 6 (Table 2). PCRs codify what to measure, how to model, and how to report, when performing LCA for a product belonging to a product class, so that different LCA studies become methodologically comparable rather than bespoke and non-comparable. PCRs they are developed under ISO 14025 Type III declarations with dedicated guidance in ISO/TS 14027 for PCR development, review and updating, all anchored to ISO 14040/44 LCA principles. [36] In the EU, the Product Environmental Footprint (PEF) initiative goes further, by recommending harmonized LCA rules and PEF Category Rules, to prioritize “comparability over flexibility” across studies. [37] The European Commission’s Green Forum and JRC materials explain how PEF and PCRs are meant to provide a common rule set for modeling flows, emissions, and impact methods, using EF-compliant datasets and guidance documents, thereby setting the stage for sector-specific PCRs (including pharmaceuticals) in certification schemes and declarations (e.g., Environmental Product Declarations, EPDs). [38], [39]

PCRs define functional units, system boundaries, data quality rules, and impact methods, so that results are transparent and compatible with third party verification in EPD programmes. The International EPD System describes PCRs as the cornerstone for consistent EPDs and provides a formal, open, transparent development process in line with ISO/TS 14027, including consultation, review, and publication. [40], [41], [42] Following the proposed PCR guidelines, the present study selected a functional unit based on the daily patient dose for insulin, allowing standardized comparisons across products, formulations, or delivery modes. This can be done in a more objective manner when the declared unit reflects real world use.

For pharmaceuticals, specific limitations are identified towards implementing complete LCA assessments, which also emphasize the importance of a PCR-based approach in LCA. For example, inconsistencies and missing pharma specific impact pathways have been previously discussed for pharmaceutical products, indicating that pharma PCRs and tailored LCIA models are needed, for comparisons to be fair and reproducible. Moreover, confidential and incomplete datasets in pharma supply chains can limit transparency. To that end, EU guidance stresses company specific data quality requirements and EF compliant datasets to ensure sufficient data availability, without compromising confidentiality of protected processes. In that sense, PCRs mandate minimum data quality scores and verification protocols, while recognizing that some proprietary data will remain a barrier to full comparability. [37] In this context, the British Standards Institution (BSI) has released PAS 2090:2025, marking the first global standard that

sets out a formal methodology for conducting product-level LCA of pharmaceutical products [43]. To fulfil the second part of Objective 6, research towards the development and implementation of pharmaceutical specific impact pathways, with new characterisation factors, is necessary. The EU JRC explicitly supports updating characterisation models and normalization/weighting factors in EF methods, serving as an entry point for pharma specific CFs integrated into EF / PCR frameworks. Building such CFs can leverage in silico ecotoxicity tools, such as the ones developed within the ENVIROMED project, [44] [45] or existing ones from literature. [46], [47], [48], [49], [50] This approach of incorporating pharma-specific ecotoxicity metrics from in silico tools in LCA was not implemented as part of the ENVIROMED LCA, as models were developed in parallel with the LCA work, and therefore sufficient time for integration was not available within the context of the project. Nevertheless, research initiatives that follow a multidisciplinary approach, such as the one represented by the ENVIROMED project consortium, have the potential to develop strong digital solutions for data exchange and dissemination across the value chain.

4 Towards an integrated pharmaceutical registry and LCA assessment

This study underscores that robust, **company-level data** on wastewater volumes and contaminant profiles (APIs, intermediates, solvents, and by-products) is the missing link to integrate current, credible, concentration-based environmental risk assessments, with LCA results for pharmaceutical manufacturing. The LCA performed in the context of this deliverable focused on resource use and upstream inputs for complex biopharmaceuticals. The proposed **pharmaceutical micropollutant registry** provides detailed data on **wastewater flows and pollutant types** from manufacturing processes. This information can support the improved representation of manufacturing-related emissions in LCA models, including the estimation of potential ecotoxicity impacts based on established methodologies. This is particularly relevant in the context of increasing regulatory attention on pharmaceuticals in the environment and the persistent evidence that **conventional wastewater treatment** may only partially remove certain APIs, which can persist or transform in receiving waters. [51], [52], [53]

Critically, the registry addresses a well-documented gap: **generic inventory datasets** (e.g., standard municipal/industrial wastewater treatment from LCI datasets) rarely capture **pharma-specific emissions, fate, and removal efficiencies** (the effectiveness of treatment processes in reducing the concentration or mass of a substance) for distinct molecules. As a result, LCAs often model downstream releases with **average removal assumptions** that can under- or over-estimate ecotoxicity, especially for compounds resistant to biodegradation or prone to forming **transformation products** (i.e., compounds formed through chemical modifications of pharmaceutical substances during biological, environmental, or treatment processes) during treatment. Evidence shows that activated sludge removal is highly variable; some APIs (e.g., fluoxetine, diclofenac, carbamazepine) can survive or even **increase in effluent concentrations** post-treatment due to partitioning and transformation dynamics. [53] A **company-fed registry** with real concentrations and in-house wastewater **treatment data** (e.g., influent/effluent concentrations and applied treatment technologies such as ozonation or activated carbon) [54], [55] would allow **chemically resolved inventory flows** and better mapping to support the development of fate and transport models, improving potential downstream impact estimates for both biopharmaceuticals and chemically synthesized APIs. By providing site- and process-specific information, the registry can support the identification of relevant emission pathways and improve scenario analyses.

Within LCA, toxicity impact categories differ conceptually from regulatory PEC/PNEC-based environmental risk assessment. While PEC/PNEC approaches are designed to derive compound-specific risk quotients, LCA ecotoxicity assesses several substances simultaneously across the entire life cycle, capturing potential impacts across multiple environmental compartments, enabling comparison of alternatives. From a **methodological** standpoint, the EU's Environmental Footprint impact assessment method and existing models to develop characterisation factors, such as **USEtox**, provide an **entry point** for integrating **pharma-specific characterisation factors**. Such an integration is in line with recent versions of the Environmental Footprint method, which provide updated toxicity-related characterisation factors and calculation principles, and explicitly encourage ongoing updates to characterisation, normalization, and weighting factors. [56], [57], [58] USEtox already supports **chemical-specific fate, exposure, and effect parameters** across scales; [59] thus, coupling registry data (emissions, compartments, removal efficiency) with **API properties** (degradation, partitioning, bioaccumulation) would allow derivation or refinement of **characterisation factors, tailored to pharmaceutical releases from manufacturing** rather than relying on generic wastewater processes. A pragmatic route for **rapid characterisation**

factor development, capitalizing on the extensive body of knowledge and available tools for in-silico predictions, is to leverage **in silico ecotoxicity tools** to fill **data gaps** for APIs and transformation products, especially where analytical standards or long-term tests are unavailable. Recent work shows how **(Q)SAR** and read-across can screen **TPs formed by oxidative/tertiary treatments** and provide preliminary **effect endpoints** (e.g., carcinogenicity, mutagenicity, PNEC), which are then compatible with LCIA workflows. [60], [61] Such metrics can be combined with **USEtox parameterization** to generate **provisional characterisation factors**, later validated against in vivo / in vitro / experimentally acquired data as the registry matures, and particularly prioritizing high-risk compounds from an initial screening based on existing tools.

Finally, while companies select among different **advanced treatment** options (e.g., ozonation, activated carbon), life-cycle trade-offs must be quantified; not only **removal efficiency** (i.e., the ability of a process to reduce or eliminate the concentration of a specific contaminant or component from a stream) and **residual toxicity**, but also the environmental impacts of alternative treatment processes ought to be considered, such as impacts of **energy, chemicals, and by-product formation**. While advanced treatments can achieve high removal for most compounds, their **life-cycle burdens** may offset some benefits, unless the wastewater treatment systems are optimized and targeted to the **actual contaminant mix**. [62], [63], [64] The proposed registry's **high-resolution, site-specific data** would enable **scenario LCAs** that compare treatment options against real loadings, and would support **risk-based management** of manufacturing effluents; moving the sector from generic assumptions to **evidence-based, pharma-specific decision-making**. In other words, the registry improves the quality and granularity of data entering LCA models.

In sum, coupling LCA with an **industry–wide registry** of wastewater volumes, API / intermediate concentrations, and treatment performance, provides the **data backbone** for credible **pharma-specific characterisation factors**, which can drastically improve the capabilities of LCA to capture specific (eco)toxicity impacts along the lifecycle, and identify potential trade-offs. With such an integration, future pharmaceutical LCAs, both for biopharmaceuticals and chemically synthesized APIs, can reflect **real emissions and removal**, rather than **generic proxies**, advancing ENVIROMED's objectives to **reduce environmental footprints across the value chain** and enabling more meaningful **policy and transition towards** greener manufacturing.

5 Conclusions

Deliverable D6.2 presents an integrated approach for improving the understanding and management of the environmental impacts associated with pharmaceutical manufacturing and use. By combining the development of a pharmaceutical micropollutant registry with the application of Life Cycle Assessment (LCA) methodologies, this work addresses a critical gap between operational environmental monitoring and life cycle-based environmental assessment within the pharmaceutical sector.

The ENVIROMED registry of Pharmaceutical Micropollutants provides a structured, auditable, and scalable framework for the systematic capture of wastewater volumes, pollutant concentrations and loads, and energy consumption at pharmaceutical manufacturing sites. Designed with hierarchical aggregation, role-based access control, data validation, and versioning mechanisms, the registry ensures high data quality, traceability, and reproducibility. Its modular architecture enables consistent environmental reporting while remaining flexible and extensible to accommodate evolving policy frameworks and site-specific data availability. As such, the registry establishes a robust digital backbone for managing pharmaceutical manufacturing-related environmental data and for supporting informed decision-making on wastewater management and emission reduction measures.

The LCA activities conducted within the scope of this deliverable demonstrate the value of life cycle thinking for identifying environmental hotspots and guiding sustainability-oriented decision-making in pharmaceutical production. Assessments performed at laboratory, pilot, and industrial scales consistently identified key contributors to environmental impacts of the investigated studies, including carbon and nutrient sources, electricity and heat demand, and energy-intensive sterilization processes. Scenario analyses highlighted the effectiveness of mitigation strategies such as process intensification, yield improvement, and the transition towards low-carbon energy sources in reducing greenhouse gas emissions and resource use.

A central outcome of this deliverable is the establishment of a practical and methodologically consistent linkage between the registry and LCA. By generating process-level, traceable, and LCI-compatible data, the registry enables a more accurate representation of wastewater and energy flows in life cycle models than is achievable using generic datasets. This integration improves the transparency and robustness of environmental assessments and lays the groundwork for future incorporation of pharmaceutical-specific emissions and ecotoxicity impacts into LCA frameworks.

While the LCA studies presented here did not explicitly model API-specific emissions from manufacturing effluents or the use phase, the combined registry–LCA approach establishes the necessary infrastructure to support such analyses in future work. The availability of high-resolution wastewater and pollutant data creates opportunities for developing pharmaceutical-specific characterisation factors, refining impact pathways, and improving environmental risk assessments related to pharmaceuticals in the environment.

In conclusion, Deliverable D6.2 demonstrates how digital environmental data management and life cycle assessment can be effectively combined to support greener pharmaceutical manufacturing. The results contribute to ENVIROMED's objectives by enabling evidence-based identification of environmental hotspots and providing a foundation for future methodological advances in pharmaceutical sustainability assessment.

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