CESPU
INSTITUTO UNIVERSITÁRIO
DE CIÊNCIAS DA SAÚDE
UNIVERSITY INSTITUTE OF HEALTH SCIENCES

ENVIRONMENTAL IMPACT OF ORAL ANTICANCER MEDICATIONS (OAMs) CONTAINED IN THE NIOSH LIST OF HAZARDOUS DRUGS USED IN THE TREATMENT OF BREAST CANCER – EUROPEAN AND ASIAN REALITIES

Pierre Norreddine Tarik Mahammed

Dissertação conducente ao **Grau de Mestre** em **Ciências Farmacêuticas (Ciclo Integrado)**

Gandra, **outubro de 2025**

CESPU
INSTITUTO UNIVERSITÁRIO
DE CIÊNCIAS DA SAÚDE
UNIVERSITY INSTITUTE OF HEALTH SCIENCES

Pierre Norreddine Tarik Mahammed

Dissertação conducente ao Grau de Mestre em Ciências Farmacêuticas (Ciclo Integrado)

ENVIRONMENTAL IMPACT OF ORAL ANTICANCER MEDICATIONS (OAMs) CONTAINED IN THE NIOSH LIST OF HAZARDOUS DRUGS USED IN THE TREATMENT OF BREAST CANCER – EUROPEAN AND ASIAN REALITIES

Clique ou toque aqui para introduzir texto.

Trabalho realizado sob a Orientação de
Alexandra Sofia Moreira Amendolia da Costa Maia

CESPU
INSTITUTO UNIVERSITÁRIO
DE CIÊNCIAS DA SAÚDE
UNIVERSITY INSTITUTE OF HEALTH SCIENCES

DECLARATION OF INTEGRITY

I, Pierre Norreddine Tarik Mahammed, duly identified above, declare, for all due purposes, that this work is original, was prepared by me, and is the result of my own personal effort, having been developed with absolute integrity.

I further declare that this work has not been previously submitted, in whole or in part, for any other academic purpose and that, throughout its preparation, I did not resort to any form of result falsification or plagiarism; all sources consulted and third-party contributions are duly identified and referenced throughout the text, in accordance with the applicable academic and scientific standards.

I also declare that any similarity with other works, whether published or not, is purely coincidental or the result of properly indicated citation.

Gandra, 11/10/2025

Pierre Norreddine Tarik Mahammed

CONTRIBUTION OF THE DISSERTATION TO THE UNITED NATIONS SUSTAINABLE DEVELOPMENT GOALS (SDGs)

This dissertation contributes to the achievement of the United Nations Sustainable Development Goals (SDGs), particularly SDG 3 (Good Health and Well-being), SDG 6 (Clean Water and Sanitation), and SDG 12 (Responsible Consumption and Production). By evaluating the environmental impact of oral anticancer drugs classified as hazardous by the NIOSH list, this dissertation enhances the understanding of the potential risks associated with their use, excretion, and disposal. The comparative analysis between European and Asian contexts highlights regulatory and technological differences, fostering the dissemination of sustainable practices and the development of more effective public policies for pharmaceutical waste management. Ultimately, this work aims to support the implementation of global strategies that reduce the contamination of natural resources and promote a safer and more environmentally responsible healthcare system.

ACKNOWLEDGEMENTS

As I reach the end of these five years of study in Pharmaceutical Sciences, a journey marked by profound personal and professional growth, I look back with immense gratitude to all the people who have been part of this chapter of my life. Throughout this path, I have been surrounded by incredible individuals who have supported, guided, and motivated me, leaving an indelible mark on my academic and personal development. It is with deep appreciation that I would like to express my sincere thanks:

To Prof. Alexandra Sofia Moreira Amendolia da Costa Maia, my supervisor, I owe my deepest gratitude. Her guidance, encouragement, and invaluable insights were essential throughout the development of this dissertation. Her patience, constructive feedback, and dedication not only helped me overcome challenges but also inspired me to grow as a researcher and to pursue excellence in my work. I am truly thankful for her continuous support and for believing in my potential.

To all the professors of the Faculty, thank you for your dedication, for the knowledge you shared, and for your words of encouragement throughout the course. Your commitment has played a crucial role in shaping my education and preparing me to embrace the profession I have chosen with passion and responsibility.

To my classmates, who have been there since the very first day of university, I am deeply grateful for the friendship, camaraderie, and shared experiences. Together, we have faced challenges, celebrated achievements, and created unforgettable memories. I wish you all the very best as you embark on your own paths.

A special word of thanks goes to my dearest friends Pauline, Linda, Nine, Léa, and Chloé, who have been by my side through every joy and hardship. Your unwavering friendship, support, and the countless unforgettable moments we've shared mean the world to me. The memories we've created together will always hold a special place in my heart.

I would also like to express my gratitude to the friends I made within the university community, including those from different courses and areas of study. Whether through casual conversations, shared breaks, or fun moments around CESPU and Gandra, your presence brought joy, laughter, and balance to my life. You made this journey even more enriching by creating memories beyond the classroom, and for that, I am deeply thankful.

Finally, I wish to express my deepest gratitude to my family. Even from a distance, you have always believed in me, offering your unconditional love, encouragement, and support throughout this entire journey. None of this would have been possible without your trust and constant presence in my life.

GENERAL INDEX

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

5-FU – 5-Fluorouracil

AI – Artificial Intelligence

AOP – Advanced Oxidation Process

API – Active Pharmaceutical Ingredient

ASIR – Age-Standardized Incidence Rate

ASMR – Age-Standardized Mortality Rate

BCF – Bioconcentration Factor

CAP – Capecitabine

CMR – Carcinogenic, Mutagenic, or Reprotoxic

CoPMed (CoPMED) – Committee on Oversight of Pharmaceuticals and Medical Devices Safety Regulation (MHLW, Japan)

CSCL – Chemical Substances Control Law (Japan)

DNA – Deoxyribonucleic Acid

EC – European Commission

EC50 – Median Effective Concentration

ECHA – European Chemicals Agency

ECIS – European Cancer Information System

EFPIA – European Federation of Pharmaceutical Industries and Associations

EMA – European Medicines Agency

ENCR – European Network of Cancer Registries

EPA – Environmental Protection Agency (US)

EPR – Extended Producer Responsibility

EPS – Eco-Pharmaco-Stewardship

ERA – Environmental Risk Assessment

EU – European Union

FAIR – Findable, Accessible, Interoperable, and Reusable

HDI – Human Development Index

HQ – Hazard Quotient

HQmix – Mixture Hazard Quotient

HRT – Hormone Replacement Therapy

IARC – International Agency for Research on Cancer

IV – Intravenous

JRC – Joint Research Centre (European Commission)

MEC – Measured Environmental Concentration

MHLW – Ministry of Health, Labour and Welfare (Japan)

MSHI – Manufacturer's Special Handling Information

NIOSH – National Institute for Occupational Safety and Health

NOEC – No Observed Effect Concentration

NTP – National Toxicology Program (US)

OAMs – Oral Anticancer Medications

OECD – Organisation for Economic Co-operation and Development

PBT – Persistent, Bioaccumulative, and Toxic

PEC – Predicted Environmental Concentration

PNEC – Predicted No-Effect Concentration

QSAR – Quantitative Structure–Activity Relationship

REACH – Registration, Evaluation, Authorisation and Restriction of Chemicals (EU Regulation (EC) No 1907/2006)

SERM – Selective Estrogen Receptor Modulator

SVHC – Substance of Very High Concern (under REACH)

TP – Transformation Product

UWWTD – Urban Wastewater Treatment Directive

UV – Ultraviolet

vB – Very Bioaccumulative

vP – Very Persistent

vPvB – Very Persistent and Very Bioaccumulative

WFD – Water Framework Directive

WHO – World Health Organization

WWTPs – Wastewater Treatment Plants

ABSTRACT

Oral anticancer medications (OAMs) commonly used in breast cancer therapy such as Capecitabine, Cyclophosphamide, Methotrexate, and Tamoxifen are emerging as contaminants of environmental concern due to their persistence, incomplete metabolism, and limited removal in conventional wastewater treatment systems. This study assesses their environmental fate, degradation behavior, and ecotoxicological risks, with a comparative focus on European and Asian regulatory contexts.

The results reveal that these compounds exhibit low biodegradability and variable toxicity toward aquatic species. Cyclophosphamide and Tamoxifen display high persistence and endocrine-disrupting effects, while Capecitabine and Methotrexate contribute to acute and chronic toxicity through the generation of transformation products with equal or greater environmental hazard. Their continuous release, even at trace concentrations, may lead to cumulative and long-term ecological impacts, particularly in regions lacking advanced wastewater treatment technologies.

From a regulatory standpoint, the European Union has recently reinforced its environmental risk assessment (ERA) framework through legislative revisions and updated water protection policies. Nonetheless, ERA implementation remains inconsistent, and post-marketing environmental monitoring is still limited. In contrast, Asian countries face greater challenges due to fragmented regulatory systems and insufficient waste management infrastructure.

This research highlights the urgent need to modernize ERA approaches by incorporating predictive modeling, effect-based monitoring, and periodic reassessment of high-risk substances. Promoting green pharmaceutical design, improving data transparency, and fostering international cooperation within a One Health and ecopharmacovigilance framework are essential to mitigate pharmaceutical pollution and ensure the environmental sustainability of anticancer therapies.

Keywords: oral anticancer medications; environmental contamination; pharmaceutical pollution;wastewater treatment; ecotoxicology; biodegradability; Environmental Risk Assessment ; ecopharmacovigilance.

RESUMO

Os medicamentos anticancerígenos orais (OAMs) comumente utilizados na terapia do cancro de mama, como Capecitabina, Ciclofosfamida, Metotrexato e Tamoxifeno, estão emergindo como contaminantes de preocupação ambiental devido à sua persistência, metabolismo incompleto e remoção limitada em sistemas convencionais de tratamento de águas residuais. Este estudo avalia o destino ambiental, o comportamento de degradação e os riscos ecotoxicológicos desses compostos, com foco comparativo nos contextos regulatórios europeu e asiático.

Os resultados revelam que esses compostos apresentam baixa biodegradabilidade e toxicidade variável para espécies aquáticas. A Ciclofosfamida e o Tamoxifeno exibem alta persistência e efeitos de disrupção endócrina, enquanto a Capecitabina e o Metotrexato contribuem para a toxicidade aguda e crônica por meio da geração de produtos de transformação com perigo ambiental igual ou superior. Sua liberação contínua, mesmo em concentrações traço, pode levar a impactos ecológicos cumulativos e de longo prazo, especialmente em regiões que carecem de tecnologias avançadas de tratamento de águas residuais.

Do ponto de vista regulatório, a União Europeia reforçou recentemente o seu quadro de Avaliação de Risco Ambiental (ERA) por meio de revisões legislativas e políticas atualizadas de proteção da água. No entanto, a implementação da ERA continua inconsistente, e o monitoramento ambiental pós-comercialização ainda é limitado. Em contraste, os países asiáticos enfrentam maiores desafios devido a sistemas regulatórios fragmentados e infraestrutura insuficiente de gestão de resíduos.

Esta pesquisa destaca a necessidade urgente de modernizar as abordagens da ERA, incorporando modelagem preditiva, monitoramento baseado em efeitos e reavaliação periódica de substâncias de alto risco. Promover o design farmacêutico verde, melhorar a transparência dos dados e fomentar a cooperação internacional dentro de um enquadramento One Health e de ecofarmacovigilância são medidas essenciais para mitigar a poluição farmacêutica e garantir a sustentabilidade ambiental das terapias anticancerígenas.

Palavras-chave: medicamentos anticancerígenos orais; contaminação ambiental; poluição farmacêutica; tratamento de águas residuais; ecotoxicologia; biodegradabilidade; Avaliação de Risco Ambiental; ecofarmacovigilância.

I. INTRODUCTION

The global incidence of breast cancer has continued to rise, making it the most commonly diagnosed cancer among women and a leading cause of mortality worldwide (1). This trend has been accompanied by an increasing reliance on OAMs, which have gained prominence due to their convenience, improved patient autonomy, and better quality of life compared to conventional intravenous chemotherapy. Among these, drugs such as Capecitabine, Cyclophosphamide, Tamoxifen, and Methotrexate are extensively used in breast cancer therapy and are included in the NIOSH list of hazardous drugs because of their carcinogenic, mutagenic, and teratogenic properties (2,3).

While these substances are therapeutically effective, their mechanism of action disrupting essential cellular pathways such as DNA replication and hormone signaling renders them potentially harmful contaminants once released into the environment (4) . Because OAMs are often incompletely metabolized, large fractions of the parent compounds and active metabolites are excreted and subsequently discharged into wastewater (5). Conventional wastewater treatment plants (WWTPs) are generally unable to fully remove such persistent micropollutants, leading to their detection in surface waters at concentrations ranging from ng/L to µg/L (6) . Evidence has shown that cytotoxic drugs at environmentally relevant levels can induce genotoxicity, endocrine disruption, and developmental toxicity in aquatic organisms (3).

The environmental concern over OAMs is increasingly reflected in regulatory discussions. In the European Union, pharmaceutical residues are recognized as "contaminants of emerging concern" under the Water Framework Directive and the Zero Pollution Action Plan, with a revised European Medicines Agency (EMA) guideline on Environmental Risk Assessment (ERA) (2024) providing a more comprehensive, tiered framework to evaluate persistence, bioaccumulation, and toxicity of active pharmaceutical ingredients (7). In Japan, the Ministry of Health, Labour and Welfare (MHLW) issued guidance for ERA in 2016, but the framework remains less detailed, relying heavily on EU and US standards for methodological steps (8). This highlights the persistent gap in regulatory harmonization across regions (8).

Despite these advances, ERA implementation is uneven: while high-income regions like the EU and Japan are moving towards more stringent monitoring and precautionary approaches, many Asian countries with rapidly expanding pharmaceutical markets still lack adequate wastewater infrastructure and enforceable legislation(8). This discrepancy raises concerns about disproportionate ecological risks, especially in areas undergoing rapid urbanization and industrial growth.

Within this context, the four OAMs central to this dissertation: Capecitabine (and its metabolite 5-FU), Methotrexate, Tamoxifen, and Cyclophosphamide illustrate the broader challenge of aligning therapeutic innovation with environmental sustainability. Recent ERA data suggest varying degrees of persistence and ecotoxicological profiles: for instance, Capecitabine/5-FU shows partial biodegradability but ecotoxic effects on algae and cyanobacteria (9) ; Methotrexate resists degradation and generates toxic transformation products during advanced oxidation treatments; Tamoxifen has endocrine-disrupting potential in fish despite relatively low predicted environmental risks (10); and Cyclophosphamide metabolites can yield toxic, non-biodegradable products in aquatic environments(11).

Taken together, these findings point to critical knowledge and governance gaps at the intersection of pharmacology, ecotoxicology, and environmental policy. This dissertation therefore investigates the environmental impact of selected OAMs used in breast cancer therapy across European and Asian contexts. It examines the persistence, toxicity, and bioaccumulation potential of these drugs, evaluates differences in wastewater treatment and ERA implementation, and highlights the urgent need for global ecopharmacovigilance strategies and sustained investment in advanced treatment technologies(7,8).

- Aims and Research Questions

Anchored in the foregoing context, this dissertation pursues four interrelated aims centered on the environmental fate and risks of four key OAMs used in breast cancer therapy: Capecitabine (and its metabolite 5-fluorouracil), Cyclophosphamide, Tamoxifen, and Methotrexate. These drugs are highly prescribed, persistent, and frequently detected in aquatic environments(3,4,5). Therefore, the objectives of this work are:

- Synthesize current knowledge on the environmental occurrence, persistence, and ecotoxicological effects of the selected OAMs, with emphasis on their bioaccumulation potential and endocrine-disrupting properties(12,13).
- Evaluate wastewater treatment performance, comparing conventional and advanced treatment methods. This includes assessing removal efficiencies across secondary, tertiary, and quaternary treatments, and considering the unintended formation of toxic transformation products (TPs) during advanced oxidation processes (3,4).
- Compare regulatory frameworks in the European Union and Japan (as a representative Asian jurisdiction), with a focus on ERA procedures. The EU's revised guideline (7) and Japan's MHLW scheme (2016) offer contrasting levels of detail and enforcement, illustrating gaps in harmonization (8) .
- Identify critical research and policy gaps, particularly in monitoring, data availability, and cross-sectoral communication. This includes examining the potential role of extended producer responsibility (EPR) as a financing tool for advanced micropollutant removal (8).

- Significance of the Work

This dissertation makes three main contributions at the interface of pharmacology, ecotoxicology, and regulatory science.

- Evidence consolidation: It brings together fragmented ecotoxicological and ERA data on the four selected OAMs, strengthening the scientific basis for their prioritization in environmental monitoring (5,9).
- Treatment byproduct dimension: It highlights how wastewater treatment processes, while intended to reduce pharmaceutical loads, can generate toxic or poorly biodegradable transformation products, thereby introducing new risks (3,4).
- Policy relevance: It aligns environmental pharmacology with broader sustainability agendas such as One Health and the EU's zero-pollution objectives, emphasizing how European advances in regulatory science and funding mechanisms like Extended Producer Responsibility (EPR) could be adapted to Asian contexts where infrastructure is rapidly evolving (7,8).

By framing OAMs as a critical test case for global ecopharmacovigilance, this work underscores the need for coordinated international responses to pharmaceutical pollution. Bridging the scientific and regulatory gaps between Europe and Asia offers not only ecological benefits but also a unique opportunity to advance sustainable cancer care practices.

METHODS

A. Search Strategy

This research was conducted using PubMed, ScienceDirect, and ResearchGate databases between March and September 2025. Medical Subject Headings (MeSH) descriptors and keywords were combined to develop the following search strategy:

("Breast Neoplasms") AND ("Antineoplastic Agents" OR "Drug Therapy, Oral") AND ("Environmental Exposure" OR "Water Pollution" OR "Drug Residues" OR "Toxicity") AND ("Occupational Exposure") AND ("Europe" OR "Asia") AND ("Biotransformation" OR "Biological Availability") AND ("Health Policy" OR "Government Regulation")

This search aimed to identify studies focused on the environmental impact of oral antineoplastic drugs used in breast cancer treatment within European and Asian contexts.

B. Inclusion and Exclusion Criteria

- Studies were included if they met the following criteria:
- Scientific articles published in peer-reviewed journals.
- Official documents, technical reports, or public policies issued by regulatory agencies.
- Publications dated between 2010 and 2025.
- Studies addressing the use of oral antineoplastic drugs in the treatment of breast cancer.
- Research focused on European or Asian countries.
- Studies involving at least one drug from the NIOSH hazardous drug list.
- Original articles presenting data on the effects of selected compounds in environmental compartments or on organisms.

- Studies were excluded if they met any of the following criteria:
- Literature reviews, opinion pieces, or letters to the editor.
- Thesis, dissertations, or works not published in peer-reviewed journals.
- Research involving intravenous or non-antineoplastic drugs.
- Studies with a purely clinical or pharmacological focus, without environmental relevance.
- Studies conducted outside Europe or Asia.

II. BACKGROUND AND ENVIRONMENTAL CHALLENGES OF OAMs

A. Oral Anticancer Drugs in Breast Cancer Treatment

1. Epidemiology of Breast Cancer

a) Breast Cancer in the European Union

Breast cancer is the most frequently diagnosed malignancy and remains the leading cause of cancer-related mortality among women worldwide(14). In 2022, an estimated 2.3 million new cases and 666,000 deaths were reported globally, accounting for 11.6% of all cancer cases and 6.9% of cancer deaths(15). Despite significant advances in early detection and therapy, the global burden of breast cancer continues to increase, particularly in middle-income and developing countries where healthcare resources remain limited. Together, Europe and Asia account for over two-thirds of the world's total breast cancer incidence and mortality (14).

Within the European Union (EU), breast cancer remains a major public health challenge. According to the Joint Research Centre (16), approximately 374,800 women were newly diagnosed with breast cancer in 2022, and around 95,800 women died from the disease, representing a lifetime risk of one in eleven women developing breast cancer before the age of 75 (16).

Although breast cancer primarily affects women, the disease also occurs in men. In 2022, about 4,400 men were diagnosed and 1,200 deaths were reported across the EU (16). Among women, half of the new cases occurred in the 45–69 age group, which corresponds to the primary target of organized mammography screening programs.

Conversely, about two-thirds of all breast cancer–related deaths occurred in individuals aged 70 or older, underscoring the increased vulnerability of elderly populations (16).

Substantial geographical variations exist across the EU. As illustrated in Figure 1, age-standardized incidence rates in 2022 ranged up to 190 cases per 100,000 women, while mortality rates reached 45 deaths per 100,000 (14,16). These differences are strongly associated with disparities in access to and participation in screening programs, as well as heterogeneity in risk factors, including older maternal age at first childbirth, reduced parity, and lower breastfeeding prevalence (16). Socioeconomic inequalities, variations in healthcare infrastructures, and differences in public health literacy further amplify these regional disparities (16).

Despite these challenges, breast cancer mortality in Europe has shown a steady decline over the past two decades, particularly in Northern and Western Europe. This improvement is largely attributed to the expansion of early detection programs, enhanced awareness campaigns, advances in targeted and hormone therapies, and the reduced use of hormone replacement therapy (HRT) after 2003, which contributed to a decline in incidence and risk (14,16).

The European Cancer Information System (ECIS), maintained collaboratively by the JRC, International Agency for Research on Cancer (IARC), and European Network of Cancer Registries (ENCR), plays a crucial role in harmonizing cancer data collection and supporting evidence-based decision-making across Member States (16). Through ECIS, Member States can monitor temporal trends, evaluate screening efficiency, and guide resource allocation for national cancer control strategies.

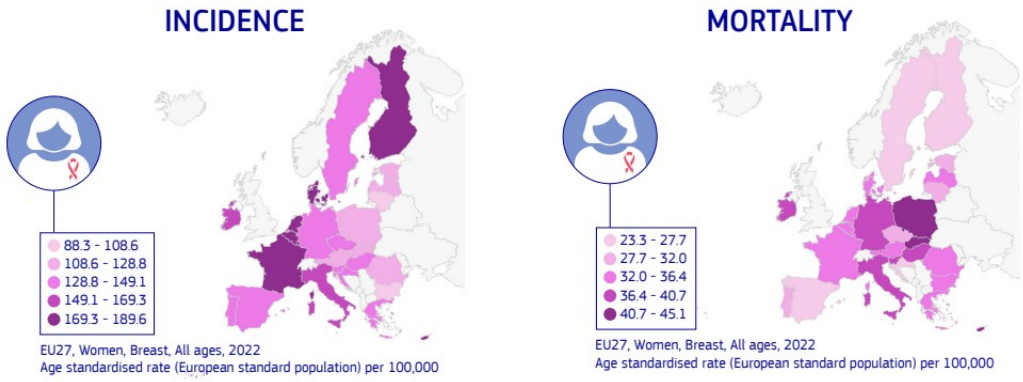

Figure 1: Estimated incidence and mortality rates of breast cancer among women across EU27 countries (Data extracted from the European Comission Joint Research Centre, 2023)

b) Breast Cancer in Asia

In Asia, breast cancer has become the most prevalent cancer among women and a major cause of cancer-related death(14,15). In 2022, the continent accounted for approximately 43% of global breast cancer cases and 47% of all related deaths, equivalent to about 985,000 new cases and 315,000 deaths (15). The age-standardized incidence rate (ASIR) was 34.3 per 100,000, while the age-standardized mortality rate (ASMR) reached 10.5 per 100,000 (14).

Marked differences in incidence and mortality exist between countries with differing levels of Human Development Index (HDI). High-HDI countries such as Japan, the Republic of Korea, Israel, and Singapore report higher incidence rates due to changing reproductive behaviors (delayed childbearing, lower parity) and lifestyle risk factors, but lower mortality thanks to better screening and treatment infrastructure (15). In contrast, low-HDI countries such as Bangladesh, Nepal, and Yemen exhibit much higher fatality rates, primarily because of limited access to early diagnosis and effective treatment (14,15).

China and India dominate the regional burden, representing 36% and 31% of all new cases and deaths, respectively (15). In South Korea, incidence among women aged 20–49 has risen sharply, driven by lifestyle and reproductive changes, while countries like Bhutan, Mongolia, and Nepal maintain the lowest recorded rates but remain vulnerable due to fragile healthcare systems and limited screening coverage (15).

Future projections are alarming. By 2050, breast cancer incidence in Asia is expected to exceed 1.4 million new cases and 500,000 deaths annually, with the sharpest increases in low-HDI countries (14,15). This anticipated rise reflects both demographic expansion and a growing prevalence of risk factors linked to urbanization and westernized lifestyles.

2. Oral Chemotherapy and Key OAMs used in Treatment

Over the last decade, oral chemotherapy has gained a crucial role in the treatment of metastatic breast cancer (MBC), mirroring the broader evolution of oncology toward patient-centered, decentralized models of care(17). The development and widespread availability of oral agents such as Capecitabine, oral Vinorelbine, and Tamoxifen have provided effective alternatives to traditional intravenous (IV) administration, offering comparable efficacy in overall and progression-free survival while improving treatment convenience (17).

A longitudinal analysis conducted across national cancer centers demonstrated that the use of oral anticancer therapies more than doubled between 2008 and 2020, with a parallel increase in pharmaceutical expenditure largely associated with the introduction of targeted oral agents (17). This reflects a structural transformation in oncology practice, enabling greater treatment continuity outside hospitals and reducing healthcare system burden (17).

From the patient's viewpoint, the preference for oral therapy over IV regimens is well documented. In a multicentric study of metastatic breast and lung cancer patients, 77.7% indicated a clear preference for oral chemotherapy, identifying reduced hospital visits, preserved autonomy, and lower psychological stress as decisive benefits(18). Similar findings were observed in a systematic literature review encompassing multiple cancer types, where 84.6% of patients favored oral therapy, particularly because of its convenience and perceived quality-of-life improvements(19). These studies underline that patient experience and autonomy have become essential determinants in modern treatment planning (18,19).

Despite these advantages, adherence remains a major clinical concern. According to Eek et al. (2016), adherence rates to oral chemotherapy range widely from 50% to 80%, influenced by complex dosing schedules, treatment duration, and adverse event profiles (19). Ciruelos et al. (2019) reported that non-adherence often arises from gastrointestinal toxicity, forgetfulness, or the mistaken perception that oral drugs are less potent than IV formulations(18). Such inconsistencies can reduce therapeutic efficacy and, in some cases, contribute to disease progression(18,19) .

To improve compliance, healthcare teams have increasingly adopted multidisciplinary interventions that include structured patient education, digital reminder systems, and pharmacist-led follow-up programs(17). These approaches have been shown to improve treatment continuity and patient satisfaction, highlighting the importance of regular communication and supportive monitoring (17,18,19). According to Eek et al. (2016), adherence enhancement strategies are most effective when they combine educational reinforcement with psychological and logistical support tailored to each patient's needs (19).

Overall, the growing reliance on oral chemotherapy marks a fundamental transition in breast cancer management, balancing efficacy with patient quality of life (17,18,19) . As emphasized by Moreira et al. (2022), this therapeutic model demands not only pharmacological innovation but also robust organizational and educational infrastructures to ensure treatment safety, adherence, and equitable access (17). Integrating these elements will be essential for maximizing the benefits of oral therapies while minimizing the risks associated with self-administered regimens.

3. NIOSH Classification Criteria

The National Institute for Occupational Safety and Health (NIOSH) is a U.S. federal agency responsible for researching and recommending ways to prevent work-related injuries and illnesses (2). In healthcare and pharmaceutical environments, NIOSH plays a critical role in identifying drugs that require special handling due to health risks (2).

A drug is considered hazardous by NIOSH if it meets one or more of the following six criteria (2):

- **Carcinogenicity**: Known or suspected of causing cancer, based on human or animal studies.
- **Genotoxicity**: Can damage DNA or cause mutations.
- **Reproductive Toxicity**: May impair fertility or harm fetal development.
- **Teratogenicity**: Can cause birth defects or developmental issues in a fetus.
- **Organ Toxicity at Low Doses**: Causes serious organ damage even at low exposures.
- **Structure/Toxicity Profile of New Drugs**: Similar in structure or toxicity to known hazardous drugs.

NIOSH classifies hazardous drugs into three groups (2):

- Group 1: Antineoplastic and Strongly Carcinogenic Drugs

This group includes drugs with a Manufacturer's Special Handling Information (MSHI) or those classified by the National Toxicology Program (NTP) as known human carcinogens, or by the International Agency for Research on Cancer (IARC) as Group 1 (Carcinogenic) or Group 2A (Probably Carcinogenic). Many are cytotoxic and pose risks to workers who are pregnant, breastfeeding, or trying to conceive, as these substances may be excreted in breast milk. Not all are antineoplastic.

- Group 2: Non-Antineoplastic Drugs with Other Hazards

Drugs in this group meet NIOSH's hazard criteria but do not have MSHI and are not classified by NTP or IARC as carcinogens. Despite this, they may still cause genotoxicity, reproductive or developmental toxicity, or organ damage at low doses. Like Group 1, they may present reproductive risks to exposed healthcare workers.

- Group 3: Drugs with Reproductive Risks

Group 3 includes drugs that primarily pose reproductive risks, such as effects on fertility, pregnancy, or fetal development. They may not meet other NIOSH hazard criteria but are included due to their potential impact on reproductive health, especially for pregnant or breastfeeding workers.

B. Environmental Fate and Pathways of OAMs

1. Sources of contamination: Excretion, Hospital Discharge, Improper Disposal

Pharmaceutical residues contaminate the environment at every stage of their life cycle from production and consumption to disposal (Figure 2)(21). In the European Union, nearly 600 active pharmaceutical ingredients (APIs) have been detected in environmental samples above analytical detection limits, occurring as either parent compounds, metabolites, or transformation products (21).

Among these stages, human consumption and excretion represent the principal pathway through which pharmaceuticals reach aquatic ecosystems in industrialized countries. After ingestion, between 30% and 90% of orally administered drugs are excreted as pharmacologically active substances in urine and feces, entering wastewater networks and eventually WWTPs (21). However, conventional WWTPs are designed primarily for nutrient and organic matter removal rather than complex micropollutants, resulting in incomplete elimination of pharmaceutical residues (11). Consequently, active compounds and their metabolites are discharged into rivers, lakes, and even drinking water at trace concentrations(Figure 2) (21).

While hospital effluents can contain high concentrations of specialized pharmaceuticals, their overall contribution to municipal wastewater contamination remains relatively limited. Most pharmaceutical residues originate from community and household use, since most medicines are consumed outside hospitals (21). Estimates suggest that only about 20% of the total API load in municipal wastewater comes from healthcare facilities, and in the Netherlands this share can be as low as 5%, with few hospitals exceeding 10% (21). Nevertheless, hospital discharges are considered critical point sources because they release highly potent substances such as cytostatics, antibiotics, and contrast media often in much higher concentrations than those found in domestic wastewater (21).

The contamination profile of hospital effluents varies depending on factors such as local disease prevalence, hospital size, and pharmaceutical formulary. For instance, Ferrando-Climent et al. (2014) identified multiple anticancer drugs including Cyclophosphamide, Methotrexate, Tamoxifen, and Capecitabine in hospital effluents, WWTP influents, and even in surface waters downstream of treatment plants (21). Although partial removal was observed, persistent cytostatics such as Cyclophosphamide and Tamoxifen were still detected at concentrations up to several tens of nanograms per liter in receiving rivers, confirming their incomplete degradation during wastewater treatment.

Cytostatic drugs are of environmental concern due to their carcinogenic, mutagenic, and teratogenic properties. Human metabolism of these compounds is known to be variable (11), but their removal efficiency in WWTPs ranges from 0 to 96%, depending on the compound. For many, removal rates below 2% have been reported due to low biodegradability(22). Excretion through urine, feces, sweat, and vomit constitutes the main emission pathway for these compounds (21). These pathways and sources are illustrated in Figure 2.

In recent years, the increase in oral cancer therapies and the growing trend toward home-based treatments have shifted the contamination burden toward domestic wastewater, since patients excrete active substances after hospital discharge. This shift complicates mitigation efforts, as decentralized emissions are more difficult to monitor and control than point-source discharges. Furthermore, rural hospitals pose a particular risk, as they often serve large catchment areas but rely on small or outdated wastewater treatment systems, creating potential local pollution hotspots (21).

Altogether, the combination of widespread drug use, inefficient wastewater treatment, and emerging outpatient cancer care practices underscores the need for integrated pharmaceutical stewardship that addresses both hospital and community sources of contamination.

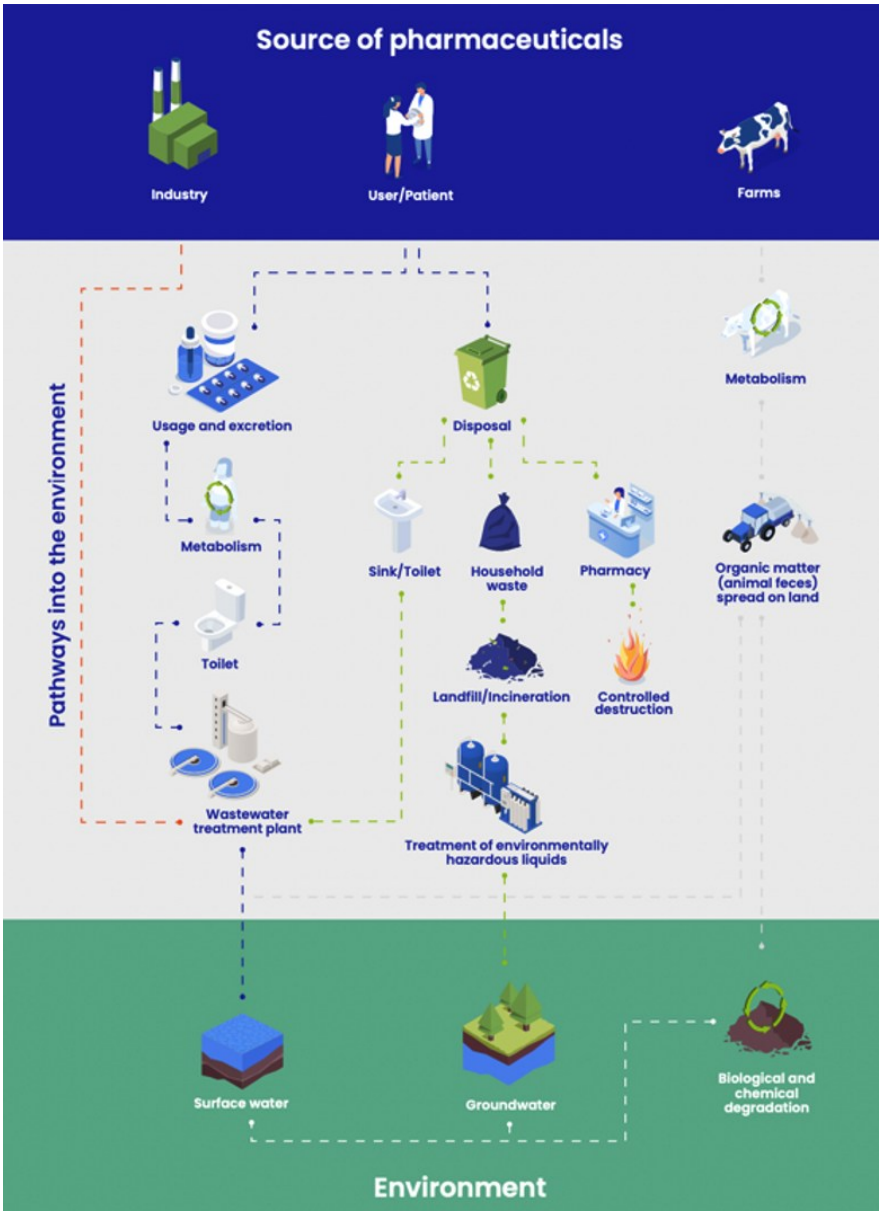

Figure 2: Main sources and pathways of Pharmaceuticals in the environment (Extracted from the European Federation of Pharmaceutical Industries and Associations (EFPIA, 2023))

2. Persistence, Bioaccumulation and Toxicity: Concepts and European Regulatory Criteria

Persistence refers to the ability of a substance to resist degradation in the environment. According to Commission Delegated Regulation (EU) 2023/707 , a substance is defined as *persistent (P)* when it exhibits resistance to degradation in the environment beyond established thresholds. Specifically, a chemical is considered persistent if its half-life exceeds 60 days in marine water, 40 days in freshwater or estuarine water, 180 days in marine sediment, 120 days in freshwater or estuarine sediment, or 120 days in soil. A substance is deemed *very persistent (vP)* when its degradation half-life surpasses 60 days in any water compartment and 180 days in sediment or soil. Persistence implies that once released into the environment, a compound may remain for extended periods, potentially causing long-term exposure risks to aquatic and terrestrial ecosystems, even after emissions have been reduced or discontinued (23).

Under the Regulation (EC) No 1907/2006 (REACH), bioaccumulation refers to the ability of a substance to build up in living organisms over time, often through the food web (24). The key indicator is the bioconcentration factor (BCF) measured in aquatic species. A chemical is classified as *bioaccumulative (B)* if its BCF exceeds 2,000, and *very bioaccumulative (vB)* if it is greater than 5,000. These thresholds are outlined in Annex XIII of REACH, which defines the criteria for identifying persistent, bioaccumulative, and toxic (PBT) or *very persistent and very bioaccumulative* (*vPvB*) substances. Bioaccumulation may lead to increasing concentrations in organisms at higher trophic levels and potential human exposure through biomagnification across the food chain (24).

Toxicity refers to the capacity of a substance to induce harmful or disruptive effects on organisms, ecosystems, or human health. According to the Commission Delegated Regulation (EU) 2023/707, a compound is classified as *toxic (T)* when it causes chronic effects at low concentrations, typically when its No Observed Effect Concentration (NOEC) for aquatic organisms is below 0.01 mg/L (23).

This classification also applies to substances that meet the hazard criteria for carcinogenicity, mutagenicity, reproductive toxicity, or specific target organ toxicity after repeated exposure, as well as to those identified as Category 1 endocrine disruptors for humans or the environment. Such compounds can interfere with physiological and hormonal systems, leading to long-term biological impacts. Toxicity may manifest as acute

or chronic, affecting not only individual organisms but also populations and ecological balance.

Within the EU's PBT/vPvB assessment framework, substances that combine persistence, bioaccumulation, and toxicity are considered particularly critical, as their continued presence in the environment can generate cumulative and potentially irreversible effects even long after emissions have ceased (23).

3. Examples of OAMs: Environmental Fate, Concentrations and PBT

a) CAPECITABINE

Capecitabine is an oral chemotherapeutic agent belonging to the class of fluoropyrimidine antimetabolites(25). It is widely used in the treatment of various solid tumors, particularly breast and colorectal cancers, either as monotherapy or in combination with other anticancer drugs. Its oral administration offers a more convenient alternative to traditional intravenous chemotherapy, improving patient comfort and adherence to treatment. Because of its extensive use in cancer therapy, Capecitabine is recognized as a hazardous drug, and its presence in the environment has raised concerns about potential risks to ecosystems and human health(26).

(1) Environmental fate and wastewater treatment of 5-FU and Capecitabine

Once released into municipal sewage systems, Capecitabine undergoes only partial removal in conventional WWTPs, with reported removal efficiencies around 50%(27). As a result, substantial quantities may pass through treatment barriers and enter receiving water bodies. This persistence is concerning, since Capecitabine and its active metabolite 5-fluorouracil (5-FU) are cytostatic agents capable of exerting toxic effects on non-target aquatic organisms, even at trace concentrations(28). Moreover, the chronic and mixture effects of Capecitabine with other pharmaceuticals remain insufficiently characterized, suggesting potential long-term ecological impacts(22,28). Capecitabine is frequently detected at higher concentrations than other anticancer drugs in both wastewaters and surface waters(29), qualifying it as a contaminant of emerging concern (28). This issue is

particularly critical in regions where treated wastewater is reused for potable water production, increasing the potential for human exposure (27).

Overall, the occurrence of Capecitabine and 5-FU in aquatic environments underscores the need for improved pharmaceutical stewardship, advanced wastewater treatment technologies, and comprehensive research into their environmental fate, transformation products, and long-term toxicity(27,30).

- Biodegradation

At low environmental concentrations, 5-FU can be biodegraded in biological treatment systems, but at higher concentrations (e.g., in hospital effluents), it shows pronounced resistance and may persist even after prolonged treatment (28). This indicates that conventional biological WWTP processes alone are often insufficient for its complete removal. Capecitabine, by contrast, exhibits even greater resistance to biodegradation due to its chemical stability and limited hydrolysis under aerobic conditions (22). Laboratory-scale studies have demonstrated that Capecitabine remains largely unaltered during activated sludge treatment, emphasizing the need for advanced degradation processes to ensure its elimination(22,27).

- Photolysis (Natural and assisted UV treatment)

Both 5-FU and Capecitabine are resistant to direct natural sunlight photolysis, showing minimal degradation under environmental irradiation(22,31). However, assisted photochemical processes can greatly enhance their breakdown. The degradation of 5-FU increases substantially when exposed to UV irradiation combined with hydrogen peroxide (UV/H_2O_2) or photocatalysts such as titanium dioxide (TiO_2) and zinc oxide (ZnO). The efficiency of these systems depends strongly on operational parameters, with optimal results occurring at acidic to neutral pH, while bicarbonate and chloride ions versus nitrate ions inhibit and promote photodegradation (32,33,34).

Capecitabine is particularly responsive to UV-assisted oxidation. Under optimized UV/H_2O_2 conditions, complete degradation can occur within minutes of exposure (27,35). Similar findings were reported by Franquet-Griell et al. (2017), who demonstrated that UV/H_2O_2 treatments efficiently eliminated Capecitabine and other cytostatics within four minutes, whereas direct photolysis alone was largely ineffective(22). This highlights the strong

potential of UV-based advanced oxidation processes (AOPs) for mitigating the environmental presence of these drugs.

- Ferrate Oxidation

Ferrate (VI) oxidation has recently emerged as a highly promising method for the removal of Capecitabine in water. The process operates through direct electron transfer, hydroxyl substitution, and cleavage of C–O and C–N bonds, enabling efficient degradation at neutral pH (30). Although natural organic matter, particularly humic acids, can partially inhibit this process by scavenging oxidants, Capecitabine is still rapidly degraded, and its oxidation by-products are generally less toxic than the parent compound. To date, only limited studies have examined the application of ferrate oxidation to 5-FU, leaving its efficacy less established (30).

In summary, both 5-FU and Capecitabine display persistence in aquatic systems, though their degradation behaviors differ. Capecitabine is more resistant to biodegradation and natural photolysis than 5-FU, requiring advanced oxidation processes such as UV/H_2O_2, photocatalysis, or ferrate(VI) oxidation for efficient removal(22,27,30). Meanwhile, 5-FU exhibits partial biodegradability at low concentrations but remains stable at higher levels, particularly in hospital wastewater, where photochemical assistance is required for significant removal(28,31).

Although advanced oxidation processes can drastically reduce concentrations of both drugs, further investigation is necessary to identify and assess the toxicity of intermediate transformation products. These by-products may persist or even exhibit distinct toxicological profiles. Understanding their formation, stability, and ecological effects remains critical for accurately evaluating the environmental safety and long-term impacts of these anticancer agents (22,28,30).

(2) Environmental Concentrations of Capecitabine

Capecitabine is one of the cytostatic drugs with high consumption levels and a significant predicted environmental concentration (PEC) in the studied coastal region of the Ría de Vigo, Northwest Spain. The study identified this compound as one of the main

antineoplastic agents of concern due to its potential to impact both wastewater systems and marine ecosystems(36).

The EMA requires an ERA for any pharmaceutical whose PEC reaches or exceeds 0.01 µg/L (7). Capecitabine surpassed this threshold, indicating a notable environmental risk. Even when a standard marine dilution factor was applied, its concentration remained above the regulatory limit. It was calculated that a much higher dilution would be needed for the drug to no longer pose a potential hazard, which highlights its persistence and the challenge it represents for environmental management.

(3) Bioaccumulation and Toxicity of Capecitabine

Capecitabine has a low tendency to bioaccumulate in aquatic organisms, mainly due to its physicochemical properties such as high water solubility and low lipophilicity, which hinder its partition into biological tissues. According to environmental risk assessment criteria, compounds with a BCF below 1000 are considered non-bioaccumulative. Capecitabine falls within this range, suggesting that although it is continuously discharged into aquatic systems, it is unlikely to biomagnify through the food chain or pose long-term bioaccumulation risks (36).

Despite its low bioaccumulation potential, Capecitabine exhibits notable toxicity toward aquatic organisms. Laboratory studies assessing its effects on algae, crustaceans, and fish have demonstrated that crustaceans are the most sensitive species, with a NOEC of 0.6 µg L^{-1} (37,38) . Similarly, another study reported that Capecitabine showed chronic toxicity toward *Daphnia magna* and *Ceriodaphnia dubia*, with reproduction inhibition observed at concentrations in the low microgram per liter range, confirming its potential ecological risk even at environmentally relevant levels(38) . According to the U.S. Environmental Protection Agency (EPA) classification, any compound with a chronic toxicity value below 10 mg L^{-1} is considered hazardous, implying that even trace levels of Capecitabine can interfere with vital biological processes in aquatic species.

To evaluate the environmental risk of Capecitabine, a study applied the Hazard Quotient (HQ) method (Figure 3), which compares the measured environmental concentration (MEC) with the predicted no-effect concentration (PNEC), the concentration below which no adverse effects are expected for aquatic organisms (37).

$$HQ = \frac{MEC}{PNEC}$$

Figure 3: Formula of "Hazard Quotient"

In Olalla et al. study, the PNEC for Capecitabine was established at 0.006 mg/L (6 µg/L). The analysis of hospital effluents revealed maximum concentrations of 1.139 µg/L in effluent 1 and 1.749 µg/L in effluent 2. These values correspond to HQs of 0.2 and 0.3, respectively (37).

According to European environmental guidelines:

- HQ < 0.1 indicates a negligible risk,
- 0.1 ≤ HQ < 1 represents a low but relevant risk, where long-term effects cannot be excluded,
- HQ ≥ 1 suggests a moderate to high risk, requiring urgent attention.

These results place Capecitabine in the low but relevant environmental risk category. While acute or immediate ecological impacts are unlikely, chronic exposure especially in the presence of other cytostatic drugs co-occurring in wastewater may progressively impact aquatic organisms and ecosystem health (36,37).

(4) Case study

The study by Venâncio et al. (2023) assessed the environmental concentrations and ecotoxicological effects of Capecitabine and 5-FU on different aquatic organisms, including algae, invertebrates, and fish(39). The research provides key insights into their environmental pathways, toxicity profiles, and ecological risks in both European and Asian contexts, highlighting significant differences related to consumption patterns and wastewater treatment capacities.

The main findings for Capecitabine and 5-FU, including environmental concentrations and hazard quotients, are summarized in Table 5.

- **Capecitabine**

Capecitabine is an oral prodrug of 5-fluorouracil (5-FU) commonly used to treat breast and colorectal cancers (39). After administration, a large fraction is excreted unchanged, entering WWTPs where removal efficiency is low. As a result, Capecitabine residues are frequently detected in aquatic systems, with reported concentrations up to 30 µg/L in Japan and 1.139 µg/L in Spain (39).

In ecotoxicity tests, algae were the most sensitive species, showing growth and yield inhibition with EC_{50} values of 0.63 mg/L and 0.077 mg/L, respectively. *Hydra viridissima* exhibited feeding inhibition at 22 mg/L, while *Danio rerio* showed no adverse effects up to 800 mg/L (39). These results indicate low acute toxicity, with effects mainly limited to primary producers. The HQ = 0.26 suggests a low environmental risk, though the conversion of Capecitabine into 5-FU after excretion significantly increases its overall ecological impact (39).

- **5-Fluorouracil (5-FU)**

5-FU, the active metabolite of Capecitabine, is also used directly as an antineoplastic drug and is known for its persistence and poor removal in WWTPs (39). Detected concentrations range from 0.16 µg/L in Taiwan and 0.578 µg/L in Thailand to 6.7 µg/L in French hospital effluents, reflecting global differences in consumption and treatment capacity (39).

Toxicity assays revealed stronger effects for 5-FU than for Capecitabine. In *Hydra viridissima*, mortality occurred at LC_{50} = 55.4 mg/L and feeding inhibition at EC_{50} = 67.9

mg/L, while Danio rerio embryos showed LC_{50} = 4546 mg/L and developmental alterations at EC_{50} = 2459 mg/L (39). Algal responses were variable but confirmed sensitivity at lower trophic levels. The hazard quotient (HQ = 7.71) indicates a high environmental risk, which increases when co-occurring with Capecitabine (HQmix = 7.97), confirming a cumulative toxic effect (39).

Parameter	Capecitabine (CAP)	(5-FU)
Detected Concentrations	Up to 30 µg/L (Japan); 1.139 µg/L (Spain)	0.16 µg/L (Taiwan); 0.578 µg/L (Thailand); 6.7 µg/L (France)
Most sensitive organism	*Raphidocelis subcapitata* (alga)	*Raphidocelis subcapitata* (alga)
Key Toxicity Values	- Algae: EC_{50} (growth) = 0.63 mg/L; EC_{50} (yield) = 0.077 mg/L (*R. subcapitata*) - Invertebrates: EC_{50} (feeding) = 22 mg/L (*H. viridissima*) - Fish: LC_{50} > 800 mg/L (*D. rerio*)	- Algae: variable sensitivity (*R. subcapitata*) - Invertebrates: LC_{50} = 55.4 mg/L; EC_{50} (feeding) = 67.9 mg/L (*H. viridissima*) - Fish: LC_{50} = 4546 mg/L; EC_{50} (malformations) = 2459 mg/L (*D. rerio*)
Ecotoxicity	Low acute toxicity; algae most affected	Broader toxicity profile; invertebrates most sensitive
HQ	0.26 (low risk)	7.71 (high risk) 7.97 (CAP + 5-FU, significant combined effect)
Overall Conclusion	Low direct risk; contributes via 5-FU formation	High ecological risk, especially near hospital effluents

Table 1: Summary of environmental occurrence and ecotoxicological effects of Capecitabine and 5-Fluorouracil, according to Venâncio et al. (2023).

In Europe, contamination is more localized, with peaks mainly observed in hospital effluents. The risk is mitigated by the presence of more advanced WWTPs, which include tertiary or even quaternary treatment steps, leading to higher removal efficiency.

In Asia, concentrations detected in surface waters are much higher, reflecting both greater drug consumption and lower overall treatment efficiency due to less advanced infrastructure and regional disparities.

5-FU remains the main environmental risk factor, especially in regions with high rates of oral chemotherapy combined with insufficient wastewater management systems.

b) CYCLOPHOSPHAMIDE

Cyclophosphamide is an alkylating agent derived from nitrogen mustards, administered orally or intravenously, and primarily used in the management and treatment of various cancers, including multiple myeloma, sarcomas, and breast cancer (26). In the body, it acts as a prodrug requiring hepatic activation. This activation leads to the formation of 4-hydroxycyclophosphamide, which is in equilibrium with aldophosphamide. The latter is then converted into phosphoramide mustard, responsible for DNA alkylation, as well as acrolein, a toxic metabolite (26). The main action of cyclophosphamide is to form stable bonds with guanine residues in DNA, thereby blocking DNA replication and inducing apoptosis in cancer cells (26).

(1) Environmental fate and wastewater treatment of Cyclophosphamide

Cyclophosphamide plays a critical role in cancer chemotherapy but is also a substance of environmental concern due to its high persistence and toxicity. After administration, a significant proportion of Cyclophosphamide and its metabolites are excreted unchanged in urine and feces. These substances are then released directly into hospital wastewater and eventually enter municipal sewage systems (40).

Hospitals act as major point sources of pharmaceutical contamination because their effluents contain high concentrations of cytotoxic drugs. Since hospital wastewater is rarely treated before mixing with municipal wastewater, and because conventional WWTPs are not designed to remove complex cytostatic compounds, Cyclophosphamide largely passes through treatment systems unchanged(37,40). Once released, it easily reaches rivers, lakes, and other surface waters, leading to continuous environmental exposure (37,41).

Cyclophosphamide exhibits extremely low biodegradability. At environmentally relevant concentrations, it shows virtually no biodegradation over 24 hours, making it highly persistent in both wastewater treatment plants and natural aquatic systems (42). Even over longer periods, only minimal degradation occurs, indicating that natural microbial communities are unable to effectively break down the compound.

In addition to biological stability, Cyclophosphamide is also resistant to abiotic processes.

- Photodegradation

It absorbs very little solar light, which makes direct photodegradation under natural sunlight negligible. Laboratory tests using simulated sunlight or UV lamps demonstrated only partial degradation, and such conditions are difficult to reproduce in real aquatic environments (43).

- Hydrolysis

Under neutral pH, typical of natural waters, hydrolysis is negligible. Even under alkaline conditions, Cyclophosphamide remains highly stable (42).

- Advanced oxidation and membrane treatment

Treatments such as ozonation or nanofiltration show variable results. A pilot-scale nanofiltration system removed an average of 68 ± 23 % of several anticancer drugs, yet Cyclophosphamide persisted in the permeate and still exhibited a high ecological risk (44). Biological treatment achieved only ~59 % removal, whereas advanced oxidation combining UV/O_3/H_2O_2 (5 g L^{-1}, 120 min) achieved ≈ 99 % degradation (40). However, even advanced processes can produce transformation by-products that may be equally or more toxic and persistent (42).

Due to this unique combination of poor biodegradability, low susceptibility to photolysis and hydrolysis, and resistance to treatment technologies, Cyclophosphamide is considered a highly persistent contaminant. Its continuous release and stability explain why it is frequently detected in both hospital effluents and municipal wastewater and why it can persist throughout the aquatic environment (40,41) .

(2) Environmental Concentrations of Cyclophosphamide

Cyclophosphamide has been widely detected in different aquatic environments, highlighting its persistence and limited removal during wastewater treatment. Concentrations measured in hospital effluents range between 14 and 22,000 ng L^{-1}, while municipal WWTP influents contain about 19 – 27 ng L^{-1}, and effluents still hold approximately 17 ng L^{-1} (40) . In surface waters, the compound has been reported at trace levels, mostly within the ng L^{-1} range in Portuguese rivers (41). Globally, concentrations generally vary from 2.4 to 656 ng L^{-1}, with exceptional cases reaching 1.9 µg L^{-1} in the Chao Phraya River, Thailand (41) .

Although modern technologies such as nanofiltration and AOPs improve removal efficiency, complete elimination remains difficult. Nanofiltration achieves around 68 ± 23 % removal, while optimized UV/O_3/H_2O_2 systems can reach up to 99 % degradation (40,44). Nonetheless, residual concentrations and transformation products persist, indicating that Cyclophosphamide is a recalcitrant and ubiquitous contaminant in aquatic systems (37).

(3) Bioaccumulation and Toxicity of Cyclophosphamide

Due to its persistence and hazardous mode of action, Cyclophosphamide is frequently listed among priority pollutants in environmental risk assessments. Within the PBT framework, it ranks high in persistence and toxicity but low in bioaccumulation potential, as its chemical properties limit accumulation in aquatic organisms (26,37).

Toxicological evaluations have demonstrated that Cyclophosphamide exerts cytotoxic, mutagenic, and genotoxic effects, potentially affecting non-target species. Experimental assays revealed growth inhibition in the microalga *Raphidocelis subcapitata*, as well as developmental abnormalities in zebrafish embryos (Danio rerio) and other aquatic invertebrates, though most effects occurred at concentrations in the milligram per litre range (43,45).

When environmental concentrations are compared to these toxicity thresholds, the resulting HQ is consistently below 1, suggesting low acute ecological risk under current exposure levels (45). However, Cyclophosphamide's carcinogenic classification (IARC Group 1) and its genotoxic properties mean that even minimal chronic exposure could have long-term consequences for both wildlife and human health (41).

Additionally, co-occurrence with other cytostatic agents in wastewater may lead to additive or synergistic effects, increasing the overall ecotoxicological burden of contaminated aquatic systems (41).

(4) Case Studies

- **Case study 1 (Europe):** Cytotoxic effects of Cyclophosphamide residues on the marine polychaete *Nereis diversicolor* (46)

This study investigated, for the first time, the effects of Cyclophosphamide at environmentally relevant concentrations on *Nereis diversicolor*, a key benthic species in European estuarine ecosystems. The aim was to evaluate how this cytotoxic drug, commonly detected in aquatic environments, impacts marine organisms at multiple biological levels.

To achieve this, specimens of *Nereis diversicolor* were exposed for seven days to three concentrations of cyclophosphamide, 0.01 µg/L, 0.1 µg/L, and 1 µg/L , covering the range typically reported in natural water bodies. Researchers examined several biomarkers, including antioxidant enzyme activities (catalase and glutathione S-transferase), indicators of oxidative stress such as lipid peroxidation, DNA damage using the Comet assay, and behavioral changes, particularly burrowing activity.

The results revealed that even at the lowest concentration tested, Cyclophosphamide induced oxidative stress, demonstrated by increased activities of antioxidant enzymes and higher levels of lipid peroxidation. Moreover, DNA damage was detected across all exposure levels, confirming the genotoxic potential of Cyclophosphamide on this marine polychaete. In addition to these cellular and molecular effects, behavioral alterations were observed, with a notable reduction in burrowing activity. Such behavioral disruptions are particularly concerning, as they can affect sediment turnover and nutrient cycling, ultimately impacting the ecological functioning of estuarine ecosystems.

This study demonstrates that environmentally relevant concentrations of Cyclophosphamide are sufficient to induce oxidative stress, DNA damage, and behavioral changes in *Nereis diversicolor*, a key benthic species in European estuarine ecosystems. The observed reduction in burrowing activity highlights potential disruptions to sediment turnover and nutrient cycling, which are essential for ecosystem balance. These findings emphasize the ecological risks posed by anticancer drug residues, underscoring the urgent

need for environmental monitoring and improved wastewater management strategies to mitigate the presence of cytotoxic pharmaceuticals in aquatic environments.

A concise overview of Cyclophosphamide's effects on Nereis diversicolor and related biomarker responses is provided in Table 5.

Criteria	Case Study – Europe (46)
Study Focus	Cytotoxic effects of Cyclophosphamide on Nereis diversicolor, a key benthic species in European estuarine ecosystems
Ecosystem Type	Estuarine and marine ecosystems (Europe)
Concentrations Tested	0.01, 0.1, and 1 µg/L (levels commonly reported in natural water bodies)
Biomarkers measured	- Antioxidant enzyme activities (CAT, GST) - Lipid peroxidation (oxidative stress) - DNA damage (Comet assay) - Behavioral changes (burrowing activity)
Main Findings	- Oxidative stress observed even at the lowest concentration - Significant DNA damage at all concentrations - Reduced burrowing activity, impacting sediment turnover and nutrient cycling
Ecological Implications	Demonstrates that environmentally relevant concentrations of cyclophosphamide can harm benthic organisms and disrupt estuarine ecosystem functions

Table 2: Summary table of the European study on Cyclophosphamide (Fonseca et al., 2018)

- **Case study 2 (Asia):** Effects of Cyclophosphamide on the immune system and disease resistance of Asian catfish *Clarias batrachus* (47)

A study published in 2005 investigated the effects of Cyclophosphamide on the immune health of Asian catfish (*Clarias batrachus*), a species of significant aquaculture importance. The results showed a clear dose-dependent immunosuppressive effect, with reduced leukocyte counts, phagocytic activity, and respiratory burst activity, as well as lower serum protein levels (47). Fish exposed to Cyclophosphamide were also more susceptible to *Aeromonas hydrophila* infections, displaying higher mortality rates and more severe symptoms (47).

Although these findings highlight the ecological risks of Cyclophosphamide in freshwater environments, this study falls outside the 2010–2025 publication window used for this thesis. Furthermore, there is a notable lack of recent research on the environmental impact of Cyclophosphamide, indicating that this remains a largely unexplored area.

c) TAMOXIFEN

Tamoxifen is a non-steroidal compound classified among antiestrogens, and more specifically as a selective estrogen receptor modulator (SERM) (26) . Its primary mechanism of action involves competitive antagonism with endogenous estrogens at their receptor sites, which are expressed on the surface of target cells such as mammary and uterine tissues. By binding to these receptors, Tamoxifen inhibits the estrogen-induced cellular stimulation and thereby limits the proliferation of hormone-dependent cancer cells (26). It remains the gold standard in the management of hormone receptor-positive breast cancer, both for therapeutic purposes and in the prevention of disease among high-risk populations. Additionally, due to its selective nature, Tamoxifen can also display partial agonist activity on estrogen receptors in other tissues, which accounts for certain side effects and its unique pharmacological profile (26).

(1) Environmental fate and wastewater treatment of Tamoxifen and its metabolites

Following therapeutic use, Tamoxifen and its active metabolites, mainly 4-hydroxytamoxifen and endoxifen, are excreted largely unchanged and enter both hospital and municipal wastewater systems. Because conventional WWTPs are not designed to remove complex and persistent pharmaceuticals, Tamoxifen is only partially eliminated and can persist through treatment processes, eventually reaching rivers, estuaries, and marine environments (48,49).

- Chlorination Processes

The behavior of Tamoxifen during chlorination has been widely investigated to assess the potential formation of toxic transformation products (48). A study demonstrated that when exposed to free chlorine for 90 minutes at neutral pH, Tamoxifen remains highly stable, while its metabolites 4-hydroxytamoxifen and endoxifen degrade rapidly. Thirteen chlorinated TPs were identified, some of which were predicted by QSAR modeling to be up to 110 times more toxic than the parent compound. In real wastewater samples, degradation was slower due to the competition of organic matter for chlorine, indicating that chlorination may generate more hazardous by-products even as it degrades certain metabolites (48).

- Ozonation

Ozonation is one of the most effective advanced oxidation processes for removing Tamoxifen from wastewater. According to Knoop et al. (2018), ozonation at neutral pH nearly eliminates Tamoxifen's endocrine-disrupting activity (50). The process leads to the formation of three N-oxides and two aromatic derivatives (TP270 and TP388), whose distribution depends strongly on the pH. Under acidic conditions, TP270 exhibits strong anti-estrogenic activity, highlighting the importance of optimizing pH to minimize ecotoxic transformation products (50). Ecotoxicity assays confirmed that although overall toxicity to Daphnia magna decreases after ozonation, residual algal growth inhibition remains, largely due to TP270 (50). Therefore, process control is crucial to achieve effective removal while preventing the formation of harmful intermediates (50).

- Biodegradation by Microorgansisms and Fungi

Tamoxifen exhibits very low biodegradability under standard treatment conditions . An early microbial screening by El-Sharkawy (1991) found that among 48 bacterial species tested, only a few were able to partially metabolize the compound, producing minor derivatives such as Tamoxifen N-oxide and desmethyltamoxifen (51) .

Although this study dates to the early 1990s, outside the 2010–2015 timeframe considered in this thesis, it remains one of the few investigations addressing microbial degradation of Tamoxifen and therefore underscores the scarcity of more recent research on this topic. In contrast, Ferrando-Climent et al. (2015) explored a non-conventional biological treatment using the white-rot fungus *Trametes versicolor* (52). This process achieved up to 99% removal of Tamoxifen after nine days, mainly through adsorption to fungal biomass. Two hydroxylated metabolites were detected exclusively in active fungal cultures, indicating limited enzymatic transformation. These results show that fungal systems may complement conventional WWTPs in removing cytostatic drugs (52).

- Photodegradation

Photodegradation represents another possible, though slow, removal pathway. DellaGreca et al. (2007) observed that Tamoxifen is photochemically stable in the dark but undergoes partial transformation under sunlight exposure, with approximately 50% degradation after 30 days of irradiation(53). The main products included the *cis*-isomer of Tamoxifen, phenanthrene derivatives, a ketone, and benzoic acid, resulting from photoisomerization and photocyclization reactions (53). Although this work precedes the 2010–2015 period considered in this thesis, it remains an important reference as it highlights the lack of more recent experimental studies focused on the photochemical behavior of Tamoxifen in aquatic systems.

Overall, Tamoxifen displays low biodegradability, limited photoreactivity, and only partial oxidation, explaining its persistence through treatment processes and its widespread detection in aquatic environments (48,50,52,53).

(2) Environmental Concentrations of Tamoxifen

Tamoxifen has been detected in a variety of aquatic environments, from hospital effluents to surface waters, demonstrating its wide distribution and environmental persistence. In Europe, concentrations range between 0.133 and 0.201 µg L^{-1} in hospital effluents and 14–133 ng L^{-1} in municipal influents and treated effluents (48,49,54). These findings confirm that both hospital and domestic discharges contribute to its environmental release.

In surface waters, Tamoxifen concentrations typically lie within 10–200 ng L^{-1} across European rivers (49,55) , while in Asia, notably in the Yangtze River Estuary, levels reach up to 212 ng L^{-1} in water and 212–431 ng g^{-1} in sediments (56). Its strong affinity for organic matter and suspended particles promotes long-range transport and accumulation in sediments and colloids, which act as reservoirs for persistent contamination (56,57).

Overall, Tamoxifen levels in the Yangtze Estuary were comparable to or even higher than those reported in European locations. The findings confirm that Tamoxifen contamination is not only widespread but also persistent, with its presence in sediments and suspended matter posing risks to benthic organisms and contributing to long-term ecological impacts. These measured concentrations, although often in the low-ng L^{-1} range, remain ecologically relevant given the compound's persistence and biological activity, necessitating global-scale risk evaluations.

(3) Bioaccumulation and Toxicity of Tamoxifen

Due to its lipophilic nature, Tamoxifen has a high tendency to bioaccumulate in aquatic organisms and sediments. Predictive modeling indicates a BCF > 5000, exceeding the regulatory threshold for bioaccumulative substances (55,57). This property facilitates its retention in biota and its potential transfer along trophic chains.

Experimental studies show that Tamoxifen exerts multi-level toxicity on aquatic organisms. In algae (*Raphidocelis subcapitata*), it inhibits growth and photosynthesis(54); in crustaceans (*Daphnia magna*), it causes reduced reproduction and altered feeding behavior (50,54); and in fish embryos (*Danio rerio*), it induces developmental abnormalities and increased mortality. The PNEC for aquatic systems has been estimated at 81 ng L^{-1}, and measured concentrations in natural waters frequently yield HQ ≥ 1, indicating a tangible ecological risk (54,57).

A recent global risk assessment, encompassing 41 endocrine-disrupting substances, confirmed Tamoxifen's detection in over 60% of monitoring sites worldwide. Reported median concentrations ranged from 1 to 50 ng L^{-1}, with maxima exceeding 500 ng L^{-1} (57). The study calculated HQs up to 1974, often surpassing the PNEC, thereby identifying Tamoxifen as one of the highest ecological risk factors among endocrine-active pharmaceuticals (57).

As a SERM, Tamoxifen interferes with vitellogenin synthesis in fish and induces endocrine alterations such as skewed sex ratios and reduced fertility (50,54,57). The persistence of these effects, even at sublethal concentrations, indicates long-term risks for population dynamics and biodiversity.

Collectively, the compound's persistence, bioaccumulation capacity, cytotoxicity, and endocrine-disrupting activity justify its classification as an emerging contaminant of high concern, both regionally and globally (50,54,55,57).

(4) Case Studies

- **Case study (Europe):** Multigenerational effects of Tamoxifen and 4-hydroxy-Tamoxifen on *Daphnia pulex* (58)

This study was conducted at the University of Lausanne (Switzerland) to assess the multigenerational toxicity of Tamoxifen and its active metabolite 4-hydroxy-tamoxifen (4-OH-Tam) on the freshwater crustacean *Daphnia pulex*. Four successive generations (F0–F3) were exposed to environmentally relevant concentrations of both compounds (0.12 – 5.26 µg/L for Tamoxifen and 0.16–72.99 µg/L for 4-OH-Tam) (58).

Both substances caused significant reductions in reproduction and body size, with Tamoxifen showing greater toxicity. At 5.26 µg/L, reproduction dropped by over 90%, and growth decreased by 30%. No complete recovery occurred after exposure ceased, indicating persistent physiological effects. In combined exposures, even low, individually non-toxic concentrations produced a 21% decline in reproduction, suggesting synergistic toxicity (58). These results demonstrate that Tamoxifen and 4-OH-Tam can induce endocrine-disrupting effects at concentrations close to those found in hospital and municipal effluents. The study highlights the ecological relevance of chronic and multigenerational exposure to anticancer drugs and emphasizes the need for long-term and mixture-based risk assessments in aquatic environments (58). The chronic and multigenerational responses of Daphnia pulex to Tamoxifen and 4-OH-Tam are summarized in Table 5, alongside comparable marine data.

- **Case study (Asia):** Toxicity of Tamoxifen on the Swimming Crab *Portunus trituberculatus* in China (59)

Recent research has revealed the toxic effects of Tamoxifen, a widely used anti-estrogenic pharmaceutical, on aquatic invertebrates (59). This study examined its impact on the swimming crab *Portunus trituberculatus*, a species of major ecological and economic value in the western Pacific, especially along the coasts of China, Japan, and Korea, where it supports important fisheries and aquaculture activities (59).

In this experiment, female crabs were exposed to different doses of Tamoxifen for five weeks. Analyses focused on the hepatopancreas, a vital organ responsible for metabolism, energy storage, and detoxification. Tamoxifen exposure caused significant disruption of lipid metabolism, with genes involved in lipid synthesis and catabolism being

downregulated, while genes linked to fatty acid oxidation were upregulated (59). These molecular changes led to a marked decrease in total lipids, phospholipids, and free fatty acids, as well as alterations in the fatty acid composition, with reduced monounsaturated fatty acids and increased levels of certain polyunsaturated fatty acids (59).

Histological observations revealed clear, dose-dependent tissue damage. The hepatopancreatic tubules became thinner and more fragile, with an increase in blister-like cells and a decrease in resorptive cells, indicating impaired structural integrity and reduced metabolic capacity (59).

These findings show that Tamoxifen can significantly affect the health, growth, and reproductive capacity of *Portunus trituberculatus*. Given the wide distribution and economic importance of this species, the study highlights the ecological risks of Tamoxifen contamination in marine environments and underscores the need for stricter monitoring and assessment of pharmaceutical pollutants in aquatic ecosystems (59). Comparative effects of Tamoxifen in marine and freshwater systems are included in Table 5, illustrating its cross-ecosystem toxicity.

Criteria	Case Study – Europe (58)	Case Study – Asia (59)
Study Focus	Chronic and multigenerational toxicity of Tamoxifen and 4-hydroxy-tamoxifen; reproductive and growth effects	Toxic effects of Tamoxifen, an anti-estrogenic drug, on the swimming crab *Portunus trituberculatus*, a species of ecological and economic importance in East Asia
Species/ Ecosystem Type	*Daphnia pulex* (freshwater invertebrate)	Portunus trituberculatus (marine crustacean)
Exposure conditions	Waterborne exposure for four generations (F0 – F3), 14 days per generation; recovery and mixture tests included	Intramuscular injections every 7 days for 35 days (four treatment groups including control)
Concentrations Tested	- Tamoxifen: 0.12 – 5.26 µg/L - 4-OH-Tam: 0.16 – 72.99 µg/L	0 (control), 6.7, 13.4, 20 µg/g body weight
Main Findings	– Reproduction ↓ up to 93% – Growth ↓ 31% – Persistent effects after exposure removal – Synergistic toxicity in Tamoxifen + 4-OH-Tam mixtures	– Downregulation of lipogenesis genes – Upregulation of CPT-I (β-oxidation) – Decrease in total lipids, phospholipids, and cholesterol – Histological damage to hepatopancreas
Ecological Relevance	Demonstrates endocrine and reproductive disruption at environmentally relevant concentrations; potential population-level effects in freshwater ecosystems	Tamoxifen exposure compromises health, growth, and reproductive capacity of *P. trituberculatus*, threatening fisheries and aquaculture.

Table 3: Summary table of the European and Asian ecotoxicological studies on Tamoxifen (Borgatta et al., 2016 and Liu et al., 2019)

Both studies consistently demonstrate that Tamoxifen induces adverse effects across multiple trophic levels and aquatic environments. In freshwater ecosystems, it primarily acts as an endocrine and reproductive toxicant, disrupting hormonal balance and impairing population dynamics. In marine systems, Tamoxifen functions as a metabolic disruptor, damaging hepatopancreatic tissues and altering lipid homeostasis.

Overall, these findings confirm Tamoxifen's classification as an emerging contaminant of global concern, capable of producing chronic, multi-level, and persistent toxicity in aquatic organisms.

d) METHOTREXATE

Methotrexate is a cornerstone anticancer drug belonging to the class of antifolates, which are themselves a subgroup of antimetabolites (26). Antifolates exert their cytostatic effect by inhibiting dihydrofolate reductase, a key enzyme involved in folic acid metabolism. Since folic acid is essential for the synthesis of purines and thymidine, this inhibition ultimately blocks DNA synthesis and prevents the proliferation of tumor cells (26).

Methotrexate is the principal agent within the antifolate group and is widely used in oncology. It plays a major role in the treatment of acute lymphoblastic leukemia, B-cell non-Hodgkin's lymphomas, and is also administered prophylactically in cases of brain tumors(26). In addition to its established indications, Methotrexate can be administered orally, which represents a convenient route, particularly in the management of breast cancer, either as part of combination chemotherapy protocols or, in selected cases, as monotherapy. Its versatility in terms of administration and its proven clinical efficacy make Methotrexate a fundamental component in the therapeutic arsenal against various cancers (26).

(1) Environmental fate and wastewater treatment of Methotrexate and its metabolites

Methotrexate's physicochemical properties allow it to move easily through soil and water once released into the environment. With a pKa of 4.70, it mainly exists in an anionic form, which is non-volatile and highly mobile. Its low Koc indicates minimal adsorption to sediments or particles, meaning it stays mostly dissolved in water (26).

These characteristics favor its widespread distribution, increasing exposure risks for aquatic organisms (26). Understanding degradation processes such as photolysis, advanced oxidation, and biodegradation is therefore crucial to assess its persistence and environmental impact (26).

- Biodegradation

Biodegradation represents an important natural mechanism for the removal of Methotrexate from the environment when conditions are favorable. Under appropriate circumstances, biological processes can break down a large proportion of the compound, with up to 95% degradation observed within five days in tests conducted following the Organisation for Economic Co-operation and Development (OECD) guidelines (26). Despite this high removal rate, one of the main transformation products, 7-hydroxymethotrexate, is known to be both toxic and persistent (26). As a result, even though Methotrexate itself can be efficiently degraded, its by-products may persist and continue to pose environmental and ecological risks (26).

- Photodegradation

Methotrexate absorbs UV radiation above 290 nm, allowing it to undergo photochemical reactions under sunlight (60,61). Under laboratory UV-C exposure, up to 60% of Methotrexate degraded within one hour via decarboxylation, demethylation, and molecular cleavage (60). The addition of titanium dioxide (TiO_2) as a photocatalyst markedly enhanced degradation, achieving complete removal within 30 minutes due to hydroxyl radical generation (60).

Photolysis yields various TPs, mainly hydroxylated and oxidized derivatives (61). Yet, under environmentally relevant conditions, efficiency drops significantly natural organic matter and turbidity hinder UV penetration, while nitrate ions can enhance degradation by producing secondary radicals (34,61). Thus, although Methotrexate is photoreactive, its natural degradation in surface waters remains limited.

- Advanced Oxidation Processes

Combining UV irradiation with oxidants notably improves Methotrexate degradation. Studies reported removal efficiencies above 90% using systems such as UV/H_2O_2, UV/Fe^{2+}/H_2O_2 (photo-Fenton), and UV/TiO_2. The photo-Fenton process showed the highest performance (62).

Main reactions include hydroxylation, oxidative cleavage, and deamination of aromatic bonds (61,62). Although complete mineralization was not achieved, most TPs exhibited lower toxicity than the parent compound (62). These results highlight AOPs as promising technologies for Methotrexate removal, despite the persistence of some oxidation intermediates.

- Hydrolysis

Hydrolysis is not considered a major pathway for the degradation of Methotrexate in the environment (26). This compound does not contain functional groups that are prone to hydrolytic reactions under typical environmental conditions, which means it remains chemically stable in natural waters (26). As a result, in the absence of other degradation mechanisms such as photolysis or oxidation, Methotrexate can persist for extended periods without significant structural changes. This stability highlights the need for alternative treatment processes, such as advanced oxidation or biological degradation, to effectively remove the compound from wastewater and reduce its potential ecological impact.

(2) Environmental concentrations of Methotrexate

Methotrexate has been consistently detected in hospital and municipal wastewaters across Europe, confirming its incomplete removal during treatment. In Valencia (Spain), Methotrexate concentrations ranged from ≤ 0.1 ng/L to 4,756 ng/L, while its metabolite 7-hydroxymethotrexate was found between ≤ 0.2 ng/L and 846 ng/L (37) . Similar findings were reported in Barcelona and Slovenia, where concentrations varied from a few to several tens of ng/L, depending on sampling conditions and treatment efficiency (63). These data highlight that hospital effluents are major sources of Methotrexate and that conventional biological processes in WWTPs achieve only partial elimination (63).

The persistence of Methotrexate and its metabolites in treated effluents allows their transfer to receiving surface waters, where chronic exposure of aquatic organisms may occur. Continuous emissions from hospitals maintain low but constant environmental levels, classifying Methotrexate as a pseudo-persistent contaminant (37,63).

No quantitative data have been reported for Asian waters, revealing a major regional knowledge gap that calls for additional monitoring to assess the global distribution of this compound.

(3) Bioaccumulation and Toxicity of Methotrexate

Methotrexate exhibits low bioaccumulation potential due to its strong hydrophilicity (log K_ow ≈ –1.85), which prevents partitioning into fatty tissues (26). Nevertheless, its continuous discharge leads to chronic exposure that may trigger long-term biological effects even at trace concentrations (63). The drug's mechanism, inhibition of dihydrofolate reductase (DHFR), interferes with DNA synthesis and cell replication, producing cytotoxic and genotoxic effects in non-target organisms (37).

Experimental studies have identified crustaceans such as *Daphnia magna* as particularly sensitive, showing moderate acute toxicity and sublethal responses including oxidative stress, DNA damage, and reduced reproduction (64)Transformation products like 7-hydroxymethotrexate can also display comparable toxicity, further amplifying ecological risks (37).

ERA conducted near hospital discharge zones in Spain and Slovenia reported HQ values above 1, confirming a potential hazard to aquatic ecosystems (37,63). Despite its low

tendency to bioaccumulate, the persistence, cytotoxicity, and constant input of Methotrexate justify its classification as a priority contaminant for environmental monitoring and improved wastewater management.

(4) Cases Studies

- **Case Study**: Acute aquatic toxicity assessment of six anti-cancer drugs and one metabolite using biotest battery e Biological effects and stability under test conditions (64).

The acute aquatic toxicity of Methotrexate and its main metabolite 7-hydroxymethotrexate (7-OH-MTX) was comprehensively evaluated through a multi-species biotest battery covering different trophic levels. The study assessed the effects of Methotrexate on the bacterium *Vibrio fischeri*, the green alga *Raphidocelis subcapitata*, the crustacean Daphnia magna, and the aquatic macrophyte *Lemna minor* under standardized OECD testing conditions (64).

Methotrexate exhibited a pronounced variability in species sensitivity. The compound was classified as very toxic to aquatic plants, with *Lemna minor* showing an EC_{50} between 0.08 and 0.16 mg L^{-1}, and toxic to algae, with *Raphidocelis subcapitata* showing an EC_{50} of 9.51 mg L^{-1}. In contrast, *Daphnia magna* and *Vibrio fischeri* were only weakly affected, with EC_{50} values exceeding 100 mg L^{-1}, suggesting low acute toxicity toward crustaceans and bacteria. The metabolite 7-OH-MTX was consistently less toxic, indicating that biotransformation may reduce but not eliminate environmental hazard (64).

Stability analysis showed that Methotrexate is photolabile, undergoing partial degradation when exposed to light, whereas it remains stable in dark conditions. This behavior identifies photolysis as the dominant degradation pathway in surface waters, while hydrolysis and adsorption processes appear negligible (64).

Overall, the study concluded that Methotrexate presents selective but significant toxicity toward photosynthetic organisms, consistent with its pharmacological mode of action as a dihydrofolate reductase inhibitor, which disrupts DNA synthesis and cell division in actively growing cells. Although environmental concentrations are typically far below acute EC_{50} values, its continuous discharge from hospital effluents and its high toxicity to primary producers raise concerns about chronic ecological impacts on aquatic food webs (64) . The

acute toxicity endpoints and photostability results for Methotrexate and 7-OH-MTX are detailed in Table 5.

Criteria	Case Study – Acute aquatic toxicity assessment of six anti-cancer drugs and one metabolite using biotest battery. Biological effects and stability under test conditions.
Study Focus	Acute aquatic toxicity and stability of Methotrexate and its metabolite 7-hydroxymethotrexate (7-OH-MTX) using a multi-species biotest battery.
Species / Ecosystem Type	- *Vibrio fischeri* (bacterium) - *Raphidocelis subcapitata* (alga) - *Daphnia magna* (crustacean) - *Lemna minor* (macrophyte)
Exposure Conditions	Standardized OECD bioassays under controlled laboratory conditions; tests performed in light and dark environments to assess chemical stability.
Concentrations Tested	Range: 0.01–100 mg/L depending on species; metabolite 7-OH-MTX tested at comparable levels.
Main Findings	- *Lemna minor*: EC_{50} = 0.08–0.16 mg/L (very toxic) - *Raphidocelis subcapitata*: EC_{50} = 9.51 mg/L → (toxic) - *Daphnia magna* and *Vibrio fischeri*: EC_{50} > 100 mg/L (non-toxic) - 7-OH-MTX less toxic than parent compound - Photodegradation under light; stability in dark conditions
Ecological Relevance	Demonstrates high phytotoxicity and selective sensitivity of primary producers, linked to Methotrexate's inhibition of DNA synthesis. Indicates risk of chronic impacts on photosynthetic organisms at the base of aquatic food webs despite low environmental concentrations.

Table 4: Summary table of the ecotoxicological study on Methotrexate (Białk-Bielińska et al., 2017)

Compound	Ecotoxicity	Ecotoxicological Risk (HQ)	Bioaccumulation (BCF)	Persistence
Capecitabine	- CAP: algae (*Raphidocelis subcapitata*) very sensitive (EC50 = 0.077 mg/L) - 5-FU: *Hydra viridissima* feeding inhibition (EC50 ~67 mg/L), zebrafish embryo malformations (EC50 = 2459 mg/L) - Mixture CAP+5-FU: combined toxicity significant	- CAP: HQ = 0.26 (low) - 5-FU: HQ = 7.71 (high) - CAP+5-FU: HQmix = 7.97 (high risk)	Low (not bioaccumulative)	- Persistent in water; poorly removed by WWTPs; - 5-FU highly resistant
Cyclophosphamide	- *Nereis diversicolor*: oxidative stress, DNA damage, reduced burrowing activity at 0.01–1 µg/L - Fish: genotoxicity (DNA strand breaks) - Algae: moderate growth inhibition	HQ < 1 (moderate risk), but high concern due to genotoxicity	Low (not bioaccumulative)	Very persistent; not readily biodegradable; resistant to photolysis & hydrolysis; passes WWTPs
Tamoxifen	- *Daphnia magna*: reproductive effects across generations (NOEC ~0.1 µg/L) - Fish: reproductive and developmental disruption - Acts as a potent endocrine disruptor	HQ > 1 (high risk), metabolites increase risk	High (BCF > 5000, lipophilic, accumulates in tissues and food webs)	Persistent, low biodegradability, stable in water and sediments
Methotrexate	- *Lemna minor*: very sensitive (EC50 = 0.08–0.16 mg L^{-1}) - Algae (*R. subcapitata*): toxic (EC50 = 9.51 mg L^{-1}) - *Daphnia magna*, *V. fischeri*: low acute sensitivity (EC50 > 100 mg L^{-1}) - Zebrafish liver cells: proliferation inhibition; mono-chloro-MTX bioactive	HQ ~0.1–0.5 (moderate risk) but higher with metabolites	Low (hydrophilic, log Kow < 0)	Pseudo-persistent (continuous discharge); stable in dark, photolabile under UV; hydrolysis negligible; 7-OH-MTX more stable/toxic

Table 5: Recapitulative table of the ecotoxicological and environmental profiles of the 4 selected oral anticancer drugs

III. REGULATORY FRAMEWORK IN EUROPEAN UNION

A. Overview of EU Directives

The European Union has progressively reinforced its environmental legislation to address the growing issue of pharmaceutical contamination, including cytotoxic and cytostatic compounds such as OAMs.

The cornerstone of EU water policy is the Water Framework Directive (WFD, 2000/60/EC), which establishes a common framework for protecting and managing surface and groundwater across Member States (65). Although the WFD does not explicitly list oral anticancer agents among its priority substances, it introduces the principle of progressive inclusion of emerging micropollutants within its regulatory scope. Through Annex X and the "Watch List" mechanism, the directive enables the identification and monitoring of contaminants that pose potential environmental and health risks (65).

In recent years, the WFD has undergone important updates to address the emergence of new pollutants. In 2024, the European Commission and the Council of the EU initiated a revision of the Directive, aiming to strengthen environmental quality standards and to update the lists of priority and hazardous substances (65). The proposed amendments expected to enter into force between 2025 and 2026, include new pharmaceutical and pesticide compounds, in line with the EU's commitment to reduce chemical pollution in water bodies and achieve "good ecological status" by 2030 (65). These revisions reflect growing concern about micropollutants, particularly pharmaceutical residues such as cytostatics, hormones, and antibiotics, which are now recognized as contaminants of emerging concern (65).

The Commission Implementing Decision (EU) 2022/1307, followed by the 2025 update of the EU Surface Water Watch List, reinforced this approach by including additional categories of emerging substances. These include pharmaceuticals that are increasingly detected in aquatic environments, notably Cyclophosphamide, Capecitabine, Methotrexate, and Tamoxifen, which are frequently used in breast cancer treatment and excreted through patient metabolism (European Commission, 2025).

Furthermore, while pharmaceutical products are generally exempt from registration under the REACH Regulation (EC No. 1907/2006) (66), the regulation indirectly contributes to the

environmental management of hazardous compounds. It establishes obligations related to hazard assessment, traceability, and control of PBT substances, which may apply to some intermediates and excipients used in the production of anticancer drugs (66).

Finally, the European Pharmaceutical Strategy explicitly recognizes the environmental dimension of pharmaceutical development (67). It advocates for the creation of greener pharmaceuticals, the strengthening of ERA during the marketing authorization process, and the improvement of collection systems for unused or expired medicines, a particularly relevant issue for oral anticancer agents administered at home (67).

B. Hazardous pharmaceutical waste management policies

The Waste Framework Directive (2008/98/EC) classifies cytotoxic and cytostatic pharmaceutical waste, including oral anticancer drugs prescribed for breast cancer treatment, as "hazardous waste" because of their toxic, mutagenic, and carcinogenic properties (68). It explicitly prohibits their disposal through household waste or domestic sewage and mandates specialized collection, transportation, and treatment systems to prevent environmental contamination (68).

Since 1978, the European pharmaceutical sector, represented by the European Federation of Pharmaceutical Industries and Associations (EFPIA), has undertaken initiatives to minimize pharmaceutical emissions to water systems (69). One key initiative is the Eco-Pharmaco-Stewardship (EPS) program, which seeks to (69):

- Identify and prioritize active pharmaceutical ingredients (APIs) that present significant environmental risks;
- Enhance analytical methods for detecting pharmaceutical residues in the environment;
- Promote cooperation between healthcare and environmental authorities to reduce discharges and improve wastewater management.

The most significant recent reform in this area is the recast of the Urban Wastewater Treatment Directive , adopted in April 2024 and entering into force in January 2025 (70). The new directive introduces quaternary treatment requirements in wastewater treatment plants to effectively remove micropollutants, including cytotoxic and cytostatic drug residues (70).

A key innovation is the implementation of an Extended Producer Responsibility (EPR) system, which obliges the pharmaceutical and cosmetic industries to finance at least 80% of the additional costs associated with advanced treatment technologies. The directive provides a gradual implementation timeline extending to 2045, depending on the size and environmental impact of wastewater treatment facilities.

This financial mechanism, however, has generated significant debate. The EFPIA has publicly criticized the cost-sharing model, claiming that It (69):

- Places disproportionate responsibility on the pharmaceutical sector while neglecting other contributors to micropollutant pollution;
- Contradicts the Polluter Pays Principle, which calls for proportional burden-sharing;
- Fails to create incentives for all sectors to reduce emissions collectively.

In March 2025, EFPIA announced its intention to challenge the directive before the EU General Court, demanding a fairer distribution of costs based on each sector's real contribution to pollution and improved transparency in the decision-making process (69). This legal dispute underscores the persistent tension between environmental protection objectives and industrial equity within EU regulatory frameworks (69).

Although pharmaceutical manufacturing emissions have declined significantly in recent decades, the primary source of cytotoxic residues in the environment now stems from patients' excretion following oral treatments administered at home (69). This shift calls for integrated strategies combining (69):

1. Investment in advanced wastewater treatment technologies;
2. Public awareness campaigns to promote safe disposal of unused medicines;
3. Balanced policy approaches that distribute environmental and financial responsibility fairly across sectors.

C. Role of the European Medicines Agency (EMA) and National Agencies

The EMA is the central regulatory authority responsible for the scientific evaluation, supervision, and safety monitoring of medicines within the European Union. One of its roles is to ensure that all medicinal products, including oral anticancer agents, undergo a thorough assessment not only for their safety and efficacy in patients but also for their potential environmental impact (7).

As illustrated in Figure 4, the EMA Guideline on Environmental Risk Assessment of Medicinal Products outlines a stepwise framework to evaluate the risk posed by pharmaceuticals to the environment. This structured approach ensures regulatory consistency and transparency throughout the evaluation process (7).

The process begins with Phase I, an initial screening stage based on predictive calculations and environmental exposure scenarios (see Figure 4). The primary aim of Phase I is to determine whether a medicinal product considering its prescribed dose, frequency of use, and excretion patterns might reach concentrations of concern in natural waters (7).

During this stage, companies must calculate the Predicted Environmental Concentration (PEC) using drug usage and excretion data. If the PEC is below a defined threshold (usually 0.01 μg L^{-1} for most medicines), the product is considered to pose a negligible environmental risk, and the risk assessment can be concluded at Phase I, as shown in Figure 4 (7).

However, if the predicted concentration exceeds this threshold, or if the active substance displays properties such as persistence, bioaccumulation, or toxicity, the process advances to Phase II. In this phase, a comprehensive experimental assessment of the drug's environmental fate, behavior, and potential ecotoxicological hazards is required, following the methodological guidance provided in Section 4.2 of the EMA document (7).

This tiered decision-making process (Figure 4) enables regulators to focus detailed testing on compounds that present the greatest environmental concern while streamlining approval for those with minimal risk (7).

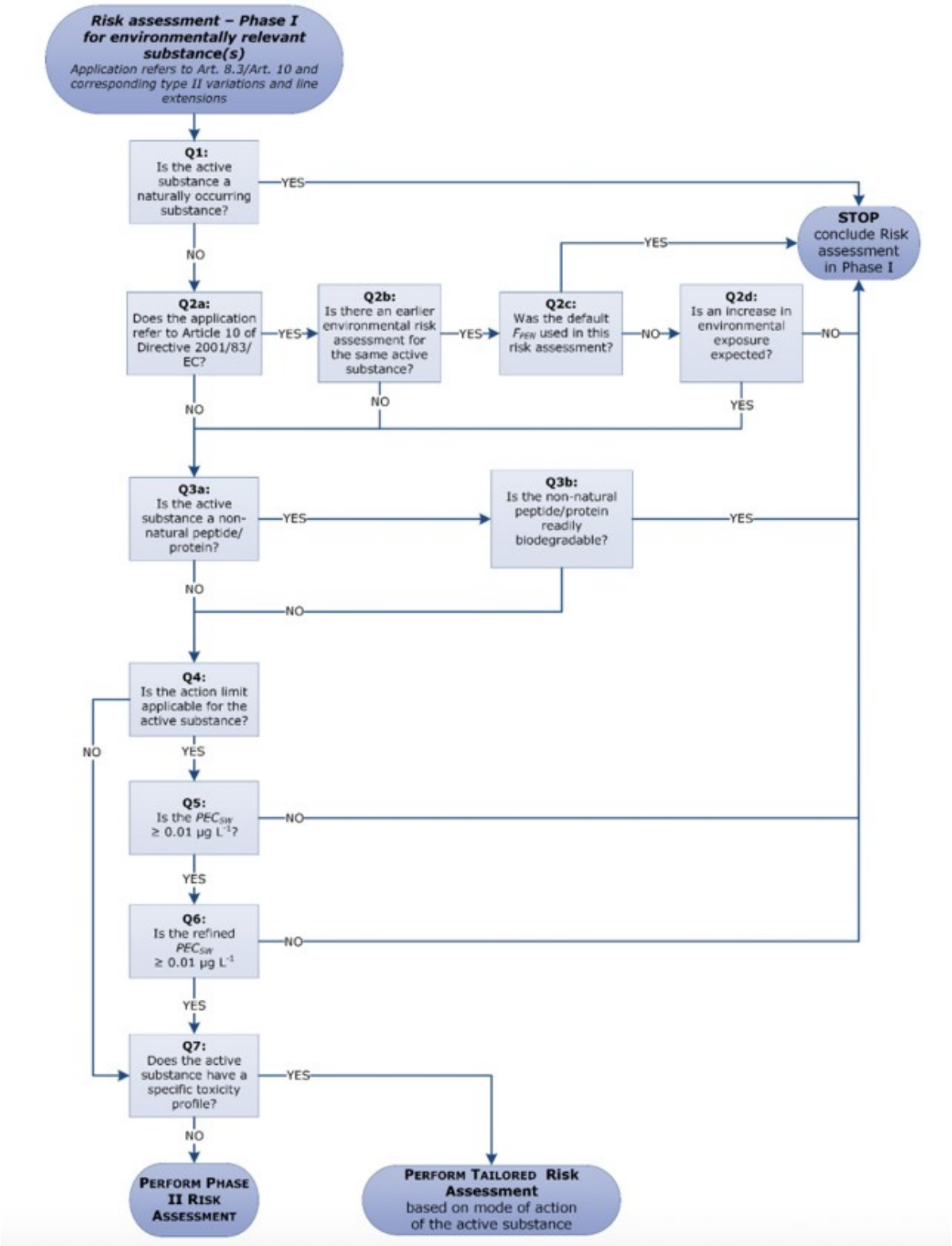

Figure 4: Decision tree of Phase 1 for environmentally relevant substances (extracted from the EMA Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use, 2024)

Phase II of the ERA, as defined by the EMA, is carried out when Phase I indicates that a pharmaceutical, such as an oral anticancer drug, may pose an environmental risk (7). It uses standardized OECD guidelines to generate reliable and internationally comparable data on a drug's degradation, bioaccumulation, and ecotoxicity (7).

IV. REGULATORY FRAMEWORK IN ASIA

In this thesis, the focus is placed exclusively on the Japanese regulatory landscape, as clear and reliable information for other countries, such as China and India, was not readily available.

A. Regulatory landscape in Japan

Japan has developed a robust system for evaluating the environmental risks of chemicals through the Chemical Substances Control Law (CSCL) (71). The main objective of this law is to prevent environmental pollution that could harm human health or disrupt ecosystems by affecting the growth and survival of flora and fauna (71). It primarily focuses on industrial chemicals synthesized through chemical processes. Substances already governed by other specific laws, such as pharmaceuticals, cosmetics, or pesticides, are excluded from its direct scope (72). However, pharmaceuticals like oral anticancer medications (OAMs) can still fall under CSCL regulations if their production volumes or environmental release levels exceed certain thresholds (72).

The Ministry of Health, Labour and Welfare (MHLW) plays a central role in ensuring pharmaceutical safety in Japan. In 2020, the Committee on Oversight of Pharmaceuticals and Medical Devices Safety Regulation (CoPMed) was established within the MHLW (73). This committee is responsible for monitoring and assessing the implementation of administrative safety regulations for pharmaceuticals and medical devices. Its main objectives are to ensure the safe use of pharmaceuticals and to prevent the occurrence or spread of health and hygiene-related hazards. CoPMed also provides recommendations to the MHLW, which can then implement additional safety measures if needed. This mechanism strengthens oversight of pharmaceuticals, including their potential environmental implications, by ensuring compliance and accountability (73).

1. Japanese Chemical Substances Control Law (CSCL)

The Japanese Chemical Substances Control Law (CSCL) establishes a comprehensive framework for evaluating and managing chemicals based on their PBT characteristics. As illustrated in Figure 5, the CSCL employs a three-tiered risk assessment process designed to classify and regulate chemicals according to their potential environmental and human health hazards (72).

- First Tier: Screening Assessment (74)

The first tier identifies substances that may pose environmental or human health risks (see Figure 5). During this stage, total emissions and environmental exposure levels are estimated, and hazards are assessed to prioritize substances for further study. Compounds that are persistent and bioaccumulative receive the highest priority, while low-risk substances may proceed to market approval with minimal restrictions.

- Second Tier: Primary Risk Assessment (74)

The second tier, also outlined in Figure 5, focuses on high-priority substances for which long-term toxicity data are not yet available. This stage is divided into three sequential steps.

- Risk Assessment I evaluates existing toxicity data and predicted environmental concentrations.
- Risk Assessment II integrates environmental monitoring data and pollutant release inventories.
- Risk Assessment III addresses remaining data gaps, including potential carcinogenic or mutagenic effects. Substances that are still considered hazardous after this stage are designated as Priority Assessment Chemicals and are subjected to long-term toxicity testing.

- Third Tier: Secondary Risk Assessment (74)

The third tier uses comprehensive long-term toxicity data to confirm the classification of a substance (Figure 5). Under the CSCL framework, chemicals are classified into three main regulatory categories based on their persistence, bioaccumulation, and toxicity.

- Class I Specified Chemicals are those identified as PBT. These substances pose the highest level of risk and are therefore subject to the strictest regulatory measures,

including complete bans or severe restrictions on their manufacture, use, or import.

- Class II Specified Chemicals include substances that have been confirmed, through comprehensive long-term toxicity studies, to present unacceptable risks to the environment or human health. These chemicals are managed through strict controls, such as limits on production, use, and importation. Substances in this category that pose more manageable risks may continue to be used, but they are subject to rigorous monitoring and mandatory reporting requirements to ensure safe management and minimize potential harm.
- Monitoring Chemicals are substances that are also persistent and bioaccumulative but lack sufficient toxicity data to fully assess their environmental and health risks. Because of this uncertainty, they are not immediately restricted but are placed under continuous observation and monitoring to identify potential emerging hazards over time.

The CSCL also includes pre- and post-marketing evaluation mechanisms (72). Before a new chemical can be placed on the market, manufacturers must submit environmental safety data. Substances produced in quantities exceeding one ton per year must undergo extensive studies on environmental behavior and toxicity. Once a chemical enters the market, it is subject to ongoing surveillance through annual reporting of production and import volumes. If new evidence suggests an increased environmental risk, the government can require additional testing or impose stricter regulatory measures (72).

Although pharmaceuticals like oral anticancer medications are not explicitly regulated under the CSCL, they may be indirectly managed when production or environmental release exceeds certain thresholds. In practice, systematic environmental monitoring of pharmaceutical residues in Japan remains limited and is primarily driven by academic research rather than mandatory regulation. However, the integration of CoPMed into this framework represents an important step forward, strengthening the monitoring and evaluation of pharmaceuticals, including their environmental impacts, and providing a platform for future regulatory action if needed (73).

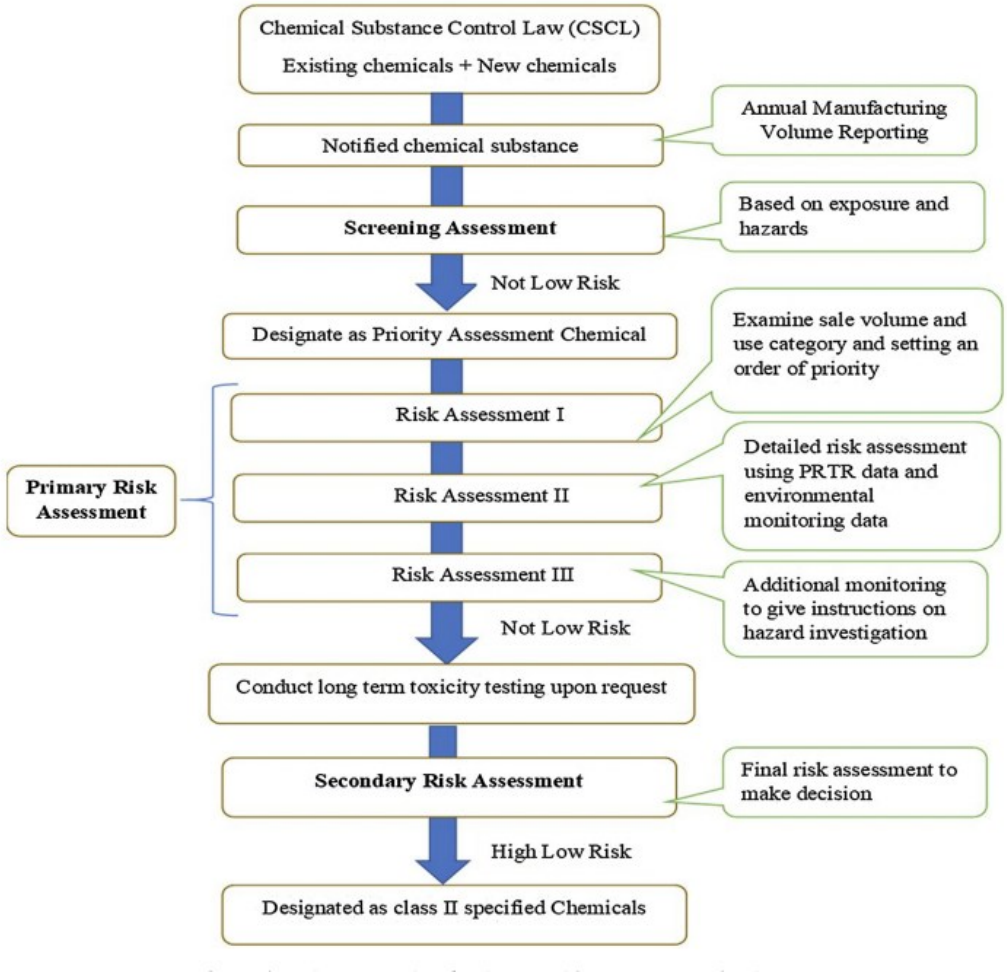

Figure 5: Schematic representation of environment risk assessment procedure in Japan (extracted from "Comparison of the regulatory outline of ecopharmacovigilance of pharmaceuticals in Europe, USA, Japan and Australia" (Zhou et al., 2021)).

V. EUROPE VS ASIA: A COMPARATIVE ASSESSMENT

A. CSCL VS REACH

The Japanese CSCL and the European Union's REACH Regulation (Registration, Evaluation, Authorisation and Restriction of Chemicals) represent two distinct but complementary frameworks for chemical management, each reflecting the regulatory priorities and socio-environmental contexts of their respective regions. (75)

The CSCL, first enacted in 1973 and most recently updated in 2011, was developed with the primary objective of protecting human health and the environment through long-term pollution prevention and exposure control. In contrast, REACH, which came into force in 2007, aims to protect workers, consumers, and the environment by ensuring the safe use of chemicals throughout their entire lifecycle (75).

In terms of scope, the CSCL mainly targets newly developed industrial chemicals produced or imported in quantities exceeding one ton per year, with a particular emphasis on those synthesized through chemical processes. REACH, on the other hand, applies to both new and existing chemicals, including imported substances, as long as they are manufactured or brought into the EU in quantities above one ton per year (75).

A fundamental difference between the two systems lies in their market timing and registration approaches. The CSCL operates as a pre-market system, requiring a full environmental and human health risk assessment before a substance can be marketed. REACH, however, is a post-market system, allowing substances to be placed on the market after registration. Under REACH, registrants (typically companies) are responsible for submitting data, which are then reviewed by regulatory agencies such as the European Chemicals Agency (ECHA) (75).

Regarding the evaluation process, the CSCL is managed entirely by government authorities following a tiered structure that includes a screening phase, a primary risk assessment, and a secondary risk assessment. REACH adopts a more decentralized approach, where evaluations are primarily carried out by industry registrants and subsequently reviewed by ECHA and national competent authorities (75).

The responsibility for risk assessment also differs significantly. Under the CSCL, risk assessment and decision-making are government-led with centralized oversight. In contrast, REACH places the burden of proof on industry, requiring companies to generate, submit, and update safety data for all registered substances (75).

In terms of long-term monitoring, the CSCL mandates annual reporting of production and import volumes. The Japanese government retains the authority to request additional studies whenever new environmental risks are identified. REACH also emphasizes continuous data updates and periodic reporting by registrants, allowing regulatory bodies to impose additional restrictions or require substitution plans if necessary (75).

When it comes to PBT evaluation, both frameworks share a similar conceptual foundation but differ in implementation. Under the CSCL, substances are categorized into Class I Specified Chemicals (persistent, bioaccumulative, and toxic, subject to strict bans or severe restrictions), Monitoring Chemicals (persistent and bioaccumulative but with incomplete toxicity data, requiring continuous observation), and Class II Specified Chemicals (toxic but of lower risk, allowed under controlled conditions with mandatory reporting). REACH integrates PBT assessment into its broader risk evaluation for all substances. Compounds meeting the criteria for Substances of Very High Concern (SVHCs) are added to the Candidate List, potentially leading to restrictions or authorization requirements (75).

Finally, the regulatory focus of each framework highlights their differing philosophies. The CSCL places strong emphasis on controlling environmental emissions and ecosystem protection, using multi-step monitoring of air, water, and soil to prevent long-term contamination. REACH, in contrast, adopts a more balanced approach, integrating environmental protection with human health and occupational safety objectives. It ensures that both worker and consumer exposure are adequately managed through comprehensive lifecycle oversight of chemical substances (75).

In summary, while both the CSCL and REACH aim to safeguard human and environmental health, the Japanese system prioritizes preventive, government-led environmental management, whereas the European model emphasizes shared responsibility, transparency, and continuous risk reassessment throughout a chemical's lifecycle (75). Table 6 shows a comparative overview of Japan's and EU regulations.

	CSCL (Japan)	REACH (European Union)
Start Date	1973, last major update in 2011	Came into force in 2007
Main Objectives	Protect human health and the environment with a focus on long-term environmental pollution control through environmental exposure management.	Protect workers, consumers, and the environment by ensuring safe use of chemicals throughout their lifecycle.
Scope	Primarily targets new industrial chemicals produced through chemical synthesis.	Applies to both new and existing chemicals, including imported substances.
Registration Target	Mainly new chemicals produced or imported in quantities >1 ton/year.	All substances produced or imported in quantities >1 ton/year, regardless of whether they are new or existing.
Pre-Market VS Post-Market	Pre-market system: Risk assessment must be completed before the substance can enter the market.	Post-market system: Substances can be placed on the market after registration, with random checks and automatic IT screening during pre-market steps.
Evaluation Process	Managed entirely by government authorities through a tiered approach: Screening → Primary Risk Assessment → Secondary Risk Assessment.	Conducted primarily by registrants (companies), reviewed by regulatory bodies such as ECHA (European Chemicals Agency).
Responsibility for Risk Assessment	Government-led, with centralized oversight.	Industry-led, with companies responsible for generating and submitting data.
Long-Term Monitoring	Annual reporting of production/import volumes. Government can demand additional studies if new environmental risks are identified.	Continuous update and periodic reporting by registrants. Authorities can impose additional restrictions or require substitution plans.
PBT Evaluation	Substances are classified into: - Class I Specified Chemicals: Persistent, bioaccumulative, toxic → strict bans or severe restrictions. - Monitoring Chemicals: Persistent and bioaccumulative, incomplete toxicity data → continuous observation. - Class II Specified Chemicals: Toxic but lower risk → controlled use with reporting.	PBT assessment integrated into risk evaluation for all substances. Substances with very high concern (SVHC) are placed on the Candidate List for potential restrictions or authorizations.
Focus on Environmental Exposure	Strong emphasis on environmental emissions and ecosystem protection, with multi-step monitoring (air, water, soil).	Balanced focus on human health and environmental exposure, with worker and consumer protection integrated.

Table 6: Comparative overview of the CSCL (Japan) and REACH Regulation (EU)

B. Comparison of Environmental Risk Assessment Scheme on Pharmaceuticals between EU and Japan

The European Union and Japan have both developed regulatory frameworks to evaluate the environmental risks of pharmaceuticals, but these systems differ significantly in terms of maturity, structure, and methodological scope (76).

The European Union possesses a well-established, comprehensive, and highly structured ERA system, developed and refined through decades of environmental legislation under the EMA. In contrast, Japan's ERA framework, formally introduced in 2016, is still evolving and less detailed, reflecting its relatively recent implementation and ongoing refinement within the national regulatory structure (76).

In terms of overall structure, the European ERA follows a stepwise, multi-tiered approach, consisting of Phase I (initial screening) and Phase II, which is subdivided into Tier A (basic testing) and Tier B (advanced long-term studies). Japan employs a similar two-phase structure, but the higher-tier testing corresponding to the EU's Tier B is not yet fully developed or standardized (76). Table 7 provides an overall comparison of ERA systems for pharmaceuticals between the EU and Japan.

The screening criteria for Phase I are largely aligned in both regions. In the EU, a PEC threshold of 0.01 µg/L and a $\log K_o w > 4.5$ trigger bioaccumulation studies. Japan applies the same PEC threshold but uses a slightly lower $\log K_o w$ trigger value of 3.5, which reflects a more precautionary approach in identifying potentially bioaccumulative compounds.

When calculating PEC values, the two systems diverge in their methodological basis. The EU relies on estimated maximum dosage and population exposure data, which can sometimes overestimate real-world environmental concentrations. In contrast, Japan's method uses empirical prescription and distribution data derived from the national health insurance database, providing more realistic and accurate exposure estimates for pharmaceuticals in use (76).

The scope of Phase I testing in the European system is broader, encompassing biodegradability, initial ecotoxicity tests on algae, daphnia, and fish, as well as assessments of wastewater treatment microorganisms. Japan's equivalent phase remains more limited, focusing mainly on biodegradability and basic acute toxicity testing on similar aquatic organisms (76).

During Phase II (Tier A), the EU framework expands to include environmental fate studies, covering persistence, partitioning, and extended ecotoxicity tests used to calculate the PNEC. Japan's corresponding stage is present but less comprehensive, with fewer defined tests and less emphasis on modeling and chronic effects (77).

The Phase II (Tier B) component in the EU represents the most advanced stage of ERA, involving long-term and chronic toxicity testing across multiple trophic levels, including sediment organisms. Japan's ERA system has not yet fully established this phase, leaving notable gaps in chronic and higher-tier testing, which limits its ability to assess cumulative and long-term ecological risks (77).

Regarding PBT assessment, the EU's system is fully integrated into the ERA process, with well-defined criteria to identify and prioritize high-risk substances for further evaluation or mitigation. In contrast, Japan's PBT assessment framework remains under development and has not yet been systematically applied to pharmaceutical substances (77).

Each system also presents distinct strengths and weaknesses. The EU's ERA benefits from a clear regulatory framework, defined thresholds, and a predictable decision-making process, ensuring transparency and consistency across Member States. However, its reliance on theoretical PEC estimates can sometimes overstate environmental exposure compared to observed field data. Japan's system, by contrast, offers the advantage of using real-world prescription data for more accurate PEC calculations, but it suffers from limited scope, a lack of advanced testing, and incomplete higher-tier evaluations, which constrain its overall effectiveness (77).

In summary, while both the European and Japanese ERA frameworks share the same goal of minimizing pharmaceutical pollution, the EU's system currently demonstrates greater regulatory maturity and technical depth, whereas Japan's model remains in a developmental stage, gradually integrating scientific advancements and real-world exposure data to strengthen its environmental protection capacity (77).

	European Union	Japan
Regulatory Maturity	Well-established, comprehensive, and highly structured ERA system.	Recently introduced (2016), still evolving and less detailed.
Overall Structure	Stepwise, multi-tiered approach with Phase I (initial screening) and Phase II divided into Tier A (basic testing) and Tier B (advanced long-term studies).	Similar two-phase structure, but higher-tier testing (equivalent to Tier B) not yet fully developed.
Phase I Screening Criteria	PEC (Predicted Environmental Concentration) threshold of 0.01 µg/L and LogPow > 4.5 trigger bioaccumulation studies.	PEC threshold also 0.01 µg/L, but lower LogPow trigger value of 3.5.
PEC Calculations Method	Based on maximum dosage and population exposure estimates.	Based on real-world prescription and distribution data from the national health insurance database, giving more realistic exposure estimates.
Scope of Phase I Tests	Includes biodegradability, initial ecotoxicity tests (algae, daphnia, fish), and evaluation of wastewater treatment microorganisms.	Limited mainly to biodegradability and basic acute toxicity tests (algae, daphnia, fish).
Phase II (Tier A)	Environmental fate studies (persistence, partitioning) and initial ecotoxicity testing to calculate PNEC (Predicted No Effect Concentration).	Present but less comprehensive, with fewer defined tests.
Phase II (Tier B)	Advanced and long-term studies, including sediment toxicity and chronic toxicity tests across multiple trophic levels.	Not yet established, leaving gaps in chronic and higher-tier evaluations.
PBT assessment	Fully integrated into ERA with clear criteria for identifying high-risk substances.	Still under development; not yet fully implemented in pharmaceutical evaluations.
Strengths	Clear regulatory framework, well-defined thresholds, and predictable decision-making process.	Use of real-world health insurance data provides highly accurate PEC estimates.
Weaknesses	PEC estimates are theoretical and may overestimate actual environmental exposure.	Limited scope of testing and lack of higher-tier studies for comprehensive risk assessment.

Table 7: Comparative overview of ERA systems for pharmaceuticals between the EU and Japan

VI. DISCUSSION AND FUTURE PERSPECTIVES

The findings of this study confirm that OAMs used in breast cancer therapy, such as Cyclophosphamide, Capecitabine, Methotrexate, and Tamoxifen, represent a persistent and complex class of contaminants of emerging concern. Their incomplete metabolism and resistance to conventional wastewater treatment processes contribute to their continuous release and accumulation in aquatic ecosystems. Among them, Cyclophosphamide and Tamoxifen stand out due to their persistence, endocrine-disrupting activity, and potential for bioaccumulation, while Capecitabine, although less bioaccumulative, exhibits acute toxicity to aquatic invertebrates. Methotrexate forms transformation products that can be as toxic or even more toxic than the parent compound. The combined effects of these drugs may lead to chronic ecological stress, especially in systems already burdened by multiple pharmaceutical contaminants.

From a regulatory standpoint, this research highlights significant disparities between Europe and Asia. The EU has developed a relatively advanced and structured ERA framework, incorporating defined thresholds, standardized ecotoxicological testing, and gradual integration of emerging pollutants through instruments such as the Water Framework Directive (2000/60/EC) and its 2024 revision. In contrast, Asian regulatory systems remain fragmented. Japan has made progress under the CSCL, but its evaluation process lacks harmonized criteria and higher tier ecotoxicological assessments. In many developing Asian countries, weak waste management systems and limited regulatory oversight amplify the accumulation of pharmaceutical residues in local "pollution hotspots". These findings are consistent with broader observations by Oldenkamp et al. (2024), who demonstrated that current ERA frameworks even in well-regulated countries such as the Netherlands often fail to prevent ecotoxicological effects from pharmaceuticals (8). This shortcoming results from incomplete toxicity data, neglect of mixture effects, and the absence of systematic post-market environmental monitoring. The authors stress that ERA must evolve from a purely predictive model toward a data-driven, iterative process, integrating both "prospective" and "retrospective" evaluations to reflect real environmental exposure conditions (8).

- **Policy Recommendations**

To mitigate these risks, environmental regulations should require ERA for all newly authorized drugs, regardless of administration route or therapeutic class (76). Emphasis should be placed on the monitoring of hospital effluents, where high concentrations of cytotoxic agents are likely, and on the safe disposal of unused oral anticancer medications prescribed in outpatient care.

Furthermore, applying an EPR principle would encourage pharmaceutical companies to invest in green drug design, prioritizing molecules with higher biodegradability, reduced persistence, and lower aquatic toxicity (76) . This aligns with the EU's call for "greener pharmaceuticals" under the European Pharmaceutical Strategy (2020).

At a strategic level, the adoption of a "solution-focused risk assessment" model integrating emission reduction scenarios, realistic exposure modeling, and wastewater treatment optimization directly into ERA processes is advocated (8) .This approach would bridge the current gap between risk assessment and management, ensuring that ERA outcomes lead to concrete, applicable mitigation measures (8).

- **Research Priorities**

Future studies should address chronic and sublethal effects of OAMs on multiple trophic levels, as well as their combined mixture toxicity. Transformation products and metabolites require particular attention, as they often exhibit comparable or higher toxicity and greater environmental mobility than the parent compounds (8).

A major challenge remains the scarcity of ecotoxicological data for older pharmaceuticals, those authorized before the implementation of the current ERA framework in 2006. Revisiting these "legacy" substances and integrating new data according to the FAIR principles (Findable, Accessible, Interoperable, Reusable) is essential to ensure data transparency and scientific reproducibility (76).

In addition, effect-based monitoring and ecological modeling should be incorporated into ERA practice to capture real-world mixture effects (8,77). Developing integrated monitoring programs that combine chemical analytics with bioassays to detect cumulative toxic impacts at low concentrations would improve ERA's predictive value and align it more closely with ecosystem-based management principles.

- **Future Directions for Improving ERA**

Recent reviews propose several directions to make ERA more efficient, transparent, and protective of the environment:

1. *Legislative strengthening and periodic reassessment*

The revised EU pharmaceutical legislation should explicitly allow marketing authorization refusal when unacceptable environmental risks are identified. Moreover, periodic re-evaluation of ERA data should be mandatory, reflecting evolving environmental monitoring results and new scientific evidence (76).

2. *Integration of prospective and retrospective ERA*

ERA should no longer end at market authorization. Instead, retrospective ERA based on MECs and field observations should complement predictive models, helping regulators adjust environmental thresholds and refine risk management in real time (8).

3. *Enhanced data transparency and harmonization*

A centralized, open-access ERA database at the EU level should be established to share ecotoxicological data among agencies, researchers, and manufacturers. Harmonizing ERA methodologies through the principle of "One Substance – One Assessment" would eliminate redundancy and strengthen international comparability (76).

4. *Incorporation of advanced computational tools*

The integration of artificial intelligence (AI), quantitative structure–activity relationship (QSAR) modeling, and machine-learning algorithms could accelerate hazard prediction and identify high-risk compounds before clinical development, making ERA more proactive and cost-effective (8).

5. Promotion of "green chemistry" and sustainable design

True sustainability must begin at the design phase. Incorporating environmental degradability and low toxicity into early drug development known as benign-by-design chemistry would prevent the release of hazardous APIs rather than treating them post-emission (76).

6. Interdisciplinary and global collaboration

International data sharing and capacity building, especially in low- and middle-income countries where monitoring infrastructure is limited, are crucial. Establishing a global ecopharmacovigilance network shared between Europe and Asia would promote harmonization of ERA standards, encourage collaborative monitoring programs, and facilitate technology transfer for sustainable wastewater management (8).

BIBLIOGRAFICAL REFERENCES

1. Kim J, Harper A, McCormack V, Sung H, Houssami N, Morgan E, et al. Global patterns and trends in breast cancer incidence and mortality across 185 countries. Nat Med. 2025 Apr;31(4):1154-62.

2. NIOSH List of Hazardous Drugs in Healthcare Settings, 2024. [Internet]. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health; 2024 Dec [cited 2025 Oct 14]. Available from: https://www.cdc.gov/niosh/docs/2025-103/

3. Zhang S, Ye C, Li J, Yu X, Feng M. Treatment-driven removal efficiency, product formation, and toxicity evolution of antineoplastic agents: Current status and implications for water safety assessment. Water Research [Internet]. 2021 Nov [cited 2025 Oct 14];206:117729. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0043135421009234

4. González-Burciaga LA, García-Prieto JC, Núñez-Núñez CM, Proal-Nájera JB. Statistical Analysis of Methotrexate Degradation by UV-C Photolysis and UV-C/TiO2 Photocatalysis. International Journal of Molecular Sciences [Internet]. 2023 Jan [cited 2025 Oct 14];24(11):9595. Available from: https://www.mdpi.com/1422-0067/24/11/9595

5. Li D, Chen H, Liu H, Schlenk D, Mu J, Lacorte S, et al. Anticancer drugs in the aquatic ecosystem: Environmental occurrence, ecotoxicological effect and risk assessment. Environment International [Internet]. 2021 Aug [cited 2025 Oct 14];153:106543. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0160412021001689

6. aus der Beek T, Weber F, Bergmann A, Hickmann S, Ebert I, Hein A, et al. Pharmaceuticals in the environment–Global occurrences and perspectives. Environmental Toxicology and Chemistry [Internet]. 2016 Apr 1 [cited 2025 Oct 14];35(4):823-35. Available from: https://doi.org/10.1002/etc.3339

7. Environmental riskassessment of medicinal products for human use - Scientific guideline |European Medicines Agency (EMA) [Internet]. 2006 [cited 2025 Oct 11]. Availablefrom:https://www.ema.europa.eu/en/environmental-risk-assessment-medicinal-products-human-use-scientific-guideline

8. Oldenkamp R, Hamers T, Wilkinson J, Slootweg J, Posthuma L. Regulatory Risk Assessment of Pharmaceuticals in the Environment: Current Practice and Future Priorities. Environmental Toxicology and Chemistry [Internet]. 2024 Mar 1 [cited 2025 Oct 14];43(3):611-22. Available from: https://academic.oup.com/etc/article/43/3/611/7728760

9. F. Hoffmann-La Roche Ltd. Environmental Risk Assessment Summary: Capecitabine / 5-Fluorouracil. Basel: F. Hoffmann-La Roche Ltd, Group SHE; 2024. 6 p. Available from: https://www.roche.com

10. Tamoxifen-citrate.pdf [Internet]. [cited 2025 Oct 14]. Available from: https://www.astrazeneca.com/content/dam/az/PDF/Sustainability/era/Tamoxifen-citrate.pdf

11. Jureczko M, Kalka J. Cytostatic pharmaceuticals as water contaminants. European Journal of Pharmacology [Internet]. 2020 Jan [cited 2025 Oct 14];866:172816. Available from: https://linkinghub.elsevier.com/retrieve/pii/S001429991930768X

12. Castellano-Hinojosa A, Gallardo-Altamirano MJ, González-López J, González-Martínez A. Anticancer drugs in wastewater and natural environments: A review on their occurrence, environmental persistence, treatment, and ecological risks. Journal of Hazardous Materials [Internet]. 2023 Apr [cited 2025 Oct 14];447:130818. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0304389423001000

13. Ortúzar M, Esterhuizen M, Olicón-Hernández DR, González-López J, Aranda E. Pharmaceutical Pollution in Aquatic Environments: A Concise Review of Environmental Impacts and Bioremediation Systems. Front Microbiol [Internet]. 2022 Apr 26 [cited 2025 Oct 14];13. Available from: https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2022.869332/full

14. 20-breast-fact-sheet.pdf [Internet]. [cited 2025 Oct 14]. Available from: https://gco.iarc.who.int/media/globocan/factsheets/cancers/20-breast-fact-sheet.pdf

15. Fu M, Peng Z, Wu M, Lv D, Li Y, Lyu S. Current and future burden of breast cancer in Asia: A GLOBOCAN data analysis for 2022 and 2050. The Breast [Internet]. 2025 Feb [cited 2025 Oct 14];79:103835. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0960977624001668

16. jrc_Breast_cancer_2022_Oct_2023.pdf [Internet]. [cited 2025 Oct 14]. Available from: https://ecis.jrc.ec.europa.eu/sites/default/files/2024-01/jrc_Breast_cancer_2022_Oct_2023.pdf

17. Moreira A, Bernardo C, Ramos C, Aguiar P, Alves da Costa F. National trends in the use of oral chemotherapy over 13 years. Front Pharmacol [Internet]. 2022 Aug 10 [cited 2025 Oct 14];13. Available from: https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2022.909948/full

18. Ciruelos EM, Díaz MN, Isla MD, López R, Bernabé R, González E, et al. Patient preference for oral chemotherapy in the treatment of metastatic breast and lung cancer. Eur J Cancer Care (Engl). 2019 Nov;28(6):e13164.

19. Krohe M, Eek D, Mazar I, Horsfield A, Pompilus F, Friebe R, et al. Patient-reported preferences for oral versus intravenous administration for the treatment of cancer: a review of the literature. PPA [Internet]. 2016 Aug [cited 2025 Oct 14];Volume 10:1609-21. Available from: https://www.dovepress.com/patient-reported-preferences-for-oral-versus-intravenous-administratio-peer-reviewed-article-PPA

20. Krohe M, Eek D, Mazar I, Horsfield A, Pompilus F, Friebe R, et al. Patient-reported preferences for oral versus intravenous administration for the treatment of cancer: a review of the literature. PPA [Internet]. 2016 Aug [cited 2025 Oct 14];Volume 10:1609–21. Available from: https://www.dovepress.com/patient-reported-preferences-for-oral-versus-intravenous-administratio-peer-reviewed-article-PPA

21.14-07-2021_Pharmaceutical-residues-in-hospital-wastewater-FINAL.pdf [Internet]. [cited 2025 Oct 14]. Available from: https://europe.noharm.org/sites/default/files/documents-files/6831/14-07-2021_Pharmaceutical-residues-in-hospital-wastewater-FINAL.pdf

21.Ferrando-Climent L, Rodriguez-Mozaz S, Barceló D. Incidence of anticancer drugs in an aquatic urban system: From hospital effluents through urban wastewater to natural environment. Environmental Pollution [Internet]. 2014 Oct [cited 2025 Oct 14];193:216–23. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0269749114002887

22. Franquet-Griell H, Gómez-Canela C, Ventura F, Lacorte S. Anticancer drugs: Consumption trends in Spain, prediction of environmental concentrations and potential risks. Environmental Pollution [Internet]. 2017 Oct [cited 2025 Oct 14];229:505–15. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0269749117312046

23. Delegated regulation - 2023/707 - EN - EUR-Lex [Internet]. [cited 2025 Oct 14]. Available from: https://eur-lex.europa.eu/eli/reg_del/2023/707/oj/eng

24. Regulation - 1907/2006 - EN - REACH - EUR-Lex [Internet]. [cited 2025 Oct 14]. Available from: https://eur-lex.europa.eu/eli/reg/2006/1907/oj/eng

25. Capecitabine [Internet]. [cited 2025 Oct 14]. Available from: https://go.drugbank.com/drugs/DB01101

26. Hazardous Substances Data Bank (HSDB) - PubChem Data Source [Internet]. [cited 2025 Oct 14]. Available from: https://pubchem.ncbi.nlm.nih.gov/source/11933

27. Tang S, Xu L, Yu X, Chen S, Li H, Huang Y, et al. Degradation of anticancer drug capecitabine in aquatic media by three advanced oxidation processes: Mechanisms, toxicity changes and energy cost evaluation. Chemical Engineering Journal [Internet]. 2021 June [cited 2025 Oct 14];413:127489. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1385894720336135

28. Kosjek T, Perko S, Žigon D, Heath E. Fluorouracil in the environment: Analysis, occurrence, degradation and transformation. Journal of Chromatography A [Internet]. 2013 May [cited 2025 Oct 14];1290:62–72. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021967313005116

29. Cristóvão MB, Bento-Silva A, Bronze MR, Crespo JG, Pereira VJ. Detection of anticancer drugs in wastewater effluents: Grab versus passive sampling. Science of The Total Environment [Internet]. 2021 Sept [cited 2025 Oct 14];786:147477. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0048969721025481

30. Yao B, Lu Y, Li A, Ma L, Wang W, Wu K, et al. Ferrate(VI) oxidative degradation of capecitabine in aquatic matrices: Degradation performance, mechanisms, and toxicity assessment. Journal of Environmental Chemical Engineering [Internet]. 2024 Dec [cited 2025 Oct 14];12(6):114535. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2213343724026666

31. Koltsakidou A, Antonopoulou M, Sykiotou M, Evgenidou E, Konstantinou I, Lambropoulou DA. Photo-Fenton and Fenton-like processes for the treatment of the antineoplastic drug 5-fluorouracil under simulated solar radiation. Environ Sci Pollut Res [Internet]. 2017 Feb 1 [cited 2025 Oct 14];24(5):4791–800. Available from: https://doi.org/10.1007/s11356-016-8138-3

32. Lin AYC, Wang XH, Lee WN. Phototransformation Determines the Fate of 5-Fluorouracil and Cyclophosphamide in Natural Surface Waters. Environ Sci Technol [Internet]. 2013 May 7 [cited 2025 Oct 14];47(9):4104–12. Available from: https://doi.org/10.1021/es304976q

33. Lai WWP, Chuang YC, Lin AYC. The effects and the toxicity increases caused by bicarbonate, chloride, and other water components during the UV/TiO2 degradation of oxazaphosphorine drugs. Environ Sci Pollut Res [Internet]. 2017 June 1 [cited 2025 Oct 14];24(17):14595–604. Available from: https://doi.org/10.1007/s11356-017-9005-6

34. Zhang Y, Zhang J, Xiao Y, Chang VWC, Lim TT. Direct and indirect photodegradation pathways of cytostatic drugs under UV germicidal irradiation: Process kinetics and influences of water matrix species and oxidant dosing. Journal of Hazardous Materials [Internet]. 2017 Feb [cited 2025 Oct 14];324:481–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0304389416310202

35. Zimmermann S, Revel M, Borowska E, Horn H. Degradation and mineralization of anti-cancer drugs Capecitabine, Bicalutamide and Irinotecan by UV-irradiation and ozone. Chemosphere [Internet]. 2024 May [cited 2025 Oct 14];356:141780. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0045653524006738

36. Couñago-Fernández M, Otero P, Samartín-Ucha M, Paradela-Carreiro A, Muniategui-Lorenzo S, Martínez-López De Castro N. Predicted concentrations of antineoplastic drugs in the aquatic environment: The case of Ría de Vigo (NW, Spain). Marine Pollution Bulletin [Internet]. 2024 June [cited 2025 Oct 14];203:116399. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0025326X2400376X

37. Olalla A, Negreira N, López De Alda M, Barceló D, Valcárcel Y. A case study to identify priority cytostatic contaminants in hospital effluents. Chemosphere [Internet]. 2018 Jan [cited 2025 Oct 14];190:417–30. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0045653517315564

38. Parrella A, Lavorgna M, Criscuolo E, Russo C, Fiumano V, Isidori M. Acute and chronic toxicity of six anticancer drugs on rotifers and crustaceans. Chemosphere [Internet]. 2014 Nov [cited 2025 Oct 14];115:59–66. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0045653514000794

39. Venâncio C, Monteiro B, Lopes I, Sousa ACA. Assessing the risks of capecitabine and its active metabolite 5-fluorouracil to freshwater biota. Environ Sci Pollut Res [Internet]. 2023 Apr 1 [cited 2025 Oct 14];30(20):58841–54. Available from: https://doi.org/10.1007/s11356-023-26505-4

40. Česen M, Kosjek T, Laimou-Geraniou M, Kompare B, Širok B, Lambropolou D, et al. Occurrence of cyclophosphamide and ifosfamide in aqueous environment and their removal by biological and abiotic wastewater treatment processes. Science of The Total Environment [Internet]. 2015 Sept [cited 2025 Oct 14];527–528:465–73. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0048969715300437

41. Gouveia TIA, Mota IH, Silva AMT, Alves A, Santos MSF. Are cytostatic drugs in surface waters a potential threat? Science of The Total Environment [Internet]. 2022 Dec [cited 2025 Oct 14];853:158559. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0048969722056583

42. Suk M, Kümmerer K. Environmental degradation of human metabolites of cyclophosphamide leads to toxic and non-biodegradable transformation products. Science of The Total Environment [Internet]. 2023 Jan [cited 2025 Oct 14];857:159454. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0048969722065536

43. Lutterbeck CA, Wilde ML, Baginska E, Leder C, Machado ÊL, Kümmerer K. Degradation of cyclophosphamide and 5-fluorouracil by UV and simulated sunlight treatments: Assessment of the enhancement of the biodegradability and toxicity. Environmental Pollution [Internet]. 2016 Jan [cited 2025 Oct 14];208:467–76. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0269749115301196

44. Gouveia TIA, Cristóvão MB, Pereira VJ, Crespo JG, Alves A, Ribeiro AR, et al. Antineoplastic drugs in urban wastewater: Occurrence, nanofiltration treatment and toxicity screening. Environmental Pollution [Internet]. 2023 Sept [cited 2025 Oct 14];332:121944. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0269749123009466

45. Monteiro B, Venâncio C, Francisco R, Sousa ACA, Lopes I. Contributions towards the hazard evaluation of two widely used cytostatic drugs. Environ Sci Pollut Res [Internet]. 2023 Feb 1 [cited 2025 Oct 14];30(6):15641–54. Available from: https://doi.org/10.1007/s11356-022-23120-7

46. Fonseca TG, Auguste M, Ribeiro F, Cardoso C, Mestre NC, Abessa DMS, et al. Environmental relevant levels of the cytotoxic drug cyclophosphamide produce harmful effects in the polychaete Nereis diversicolor. Science of The Total Environment [Internet]. 2018 Sept [cited 2025 Oct 14];636:798–809. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0048969718314979

47. Kumari J, Sahoo P. Effects of cyclophosphamide on the immune system and disease resistance of Asian catfish. Fish & Shellfish Immunology [Internet]. 2005 Oct [cited 2025 Oct 14];19(4):307–16. Available from: https://linkinghub.elsevier.com/retrieve/pii/S105046480500029X

48. Negreira N, Regueiro J, López De Alda M, Barceló D. Transformation of tamoxifen and its major metabolites during water chlorination: Identification and in silico toxicity assessment of their disinfection byproducts. Water Research [Internet]. 2015 Nov [cited 2025 Oct 14];85:199–207. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0043135415301871

49. Ferrando-Climent L, Rodriguez-Mozaz S, Barceló D. Development of a UPLC-MS/MS method for the determination of ten anticancer drugs in hospital and urban wastewaters, and its application for the screening of human metabolites assisted by information-dependent acquisition tool (IDA) in sewage samples. Anal Bioanal Chem [Internet]. 2013 July 1 [cited 2025 Oct 14];405(18):5937–52. Available from: https://doi.org/10.1007/s00216-013-6794-4

50. Knoop O, Woermann M, Lutze HV, Sures B, Schmidt TC. Ecotoxicological effects prior to and after the ozonation of tamoxifen. Journal of Hazardous Materials [Internet]. 2018 Sept 15 [cited 2025 Oct 14];358:286–93. Available from: https://www.sciencedirect.com/science/article/pii/S0304389418305144

51. El-Sharkawy SH. Microbial conversion of tamoxifen. Appl Microbiol Biotechnol [Internet]. 1991 July 1 [cited 2025 Oct 14];35(4):436–9. Available from: https://doi.org/10.1007/BF00169745

52. Ferrando-Climent L, Cruz-Morató C, Marco-Urrea E, Vicent T, Sarrà M, Rodriguez-Mozaz S, et al. Non conventional biological treatment based on Trametes versicolor for the elimination of recalcitrant anticancer drugs in hospital wastewater. Chemosphere [Internet]. 2015 Oct [cited 2025 Oct 14];136:9–19. Available from: https://linkinghub.elsevier.com/retrieve/pii/S004565351500274X

53. DellaGreca M, Iesce MR, Isidori M, Nardelli A, Previtera L, Rubino M. Phototransformation products of tamoxifen by sunlight in water. Toxicity of the drug and its derivatives on aquatic organisms. Chemosphere [Internet]. 2007 May [cited 2025 Oct 14];67(10):1933–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0045653506017346

54. Orias F, Bony S, Devaux A, Durrieu C, Aubrat M, Hombert T, et al. Tamoxifen ecotoxicity and resulting risks for aquatic ecosystems. Chemosphere [Internet]. 2015 June [cited 2025 Oct 14];128:79–84. Available from: https://linkinghub.elsevier.com/retrieve/pii/S004565351500017X

55. Jean J, Perrodin Y, Pivot C, Trepo D, Perraud M, Droguet J, et al. Identification and prioritization of bioaccumulable pharmaceutical substances discharged in hospital effluents. Journal of Environmental Management [Internet]. 2012 July [cited 2025 Oct 14];103:113–21. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0301479712001235

56. Yang Y, Fu J, Peng H, Hou L, Liu M, Zhou JL. Occurrence and phase distribution of selected pharmaceuticals in the Yangtze Estuary and its coastal zone. Journal of Hazardous Materials [Internet]. 2011 June [cited 2025 Oct 14];190(1–3):588–96. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0304389411003955

57. Grobin A, Roškar R, Trontelj J. Multi-parameter risk assessment of forty-one selected substances with endocrine disruptive properties in surface waters worldwide. Chemosphere [Internet]. 2022 Jan [cited 2025 Oct 14];287:132195. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0045653521026679

58. Borgatta M, Waridel P, Decosterd LA, Buclin T, Chèvre N. Multigenerational effects of the anticancer drug tamoxifen and its metabolite 4-hydroxy-tamoxifen on Daphnia pulex. Science of The Total Environment [Internet]. 2016 Mar [cited 2025 Oct 14];545–546:21–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0048969715311360

59. Liu M, Feng Q, Francis DS, Turchini GM, Zeng C, Wu X. Tamoxifen affects the histology and hepatopancreatic lipid metabolism of swimming crab Portunus trituberculatus. Aquatic Toxicology [Internet]. 2019 Aug [cited 2025 Oct 14];213:105220. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0166445X19302760

60. Calza P, Medana C, Sarro M, Rosato V, Aigotti R, Baiocchi C, et al. Photocatalytic degradation of selected anticancer drugs and identification of their transformation products in water by liquid chromatography–high resolution mass spectrometry. Journal of Chromatography A [Internet]. 2014 Oct [cited 2025 Oct 14];1362:135–44. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021967314012837

61. Espinosa A, Nélieu S, Lieben P, Skarbek C, Labruère R, Benoit P. Photodegradation of methotrexate in aqueous solution: degradation kinetics and identification of transformation products. Environ Sci Pollut Res [Internet]. 2022 Jan 1 [cited 2025 Oct 14];29(4):6060–71. Available from: https://doi.org/10.1007/s11356-021-15820-3

62. Lutterbeck CA, Baginska E, Machado ÊL, Kümmerer K. Removal of the anti-cancer drug methotrexate from water by advanced oxidation processes: Aerobic biodegradation and toxicity studies after treatment. Chemosphere [Internet]. 2015 Dec [cited 2025 Oct 14];141:290–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0045653515007900

63. Isidori M, Lavorgna M, Russo C, Kundi M, Žegura B, Novak M, et al. Chemical and toxicological characterisation of anticancer drugs in hospital and municipal wastewaters from Slovenia and Spain. Environmental Pollution [Internet]. 2016 Dec [cited 2025 Oct 14];219:275–87. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0269749116317432

64. Białk-Bielińska A, Mulkiewicz E, Stokowski M, Stolte S, Stepnowski P. Acute aquatic toxicity assessment of six anti-cancer drugs and one metabolite using biotest battery – Biological effects and stability under test conditions. Chemosphere [Internet]. 2017 Dec [cited 2025 Oct 14];189:689–98. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0045653517313942

65. EUR-Lex - 02000L0060-20141120 - EN - EUR-Lex [Internet]. [cited 2025 Oct 14]. Available from: https://eur-lex.europa.eu/eli/dir/2000/60/2014-11-20/eng

66. COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS Pharmaceutical Strategy for Europe [Internet]. 2020. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52020DC0761

67. EUR-Lex - 02008L0098-20240218 - EN - EUR-Lex [Internet]. [cited 2025 Oct 14]. Available from: https://eur-lex.europa.eu/eli/dir/2008/98/2024-02-18/eng

68. Water - Pharmaceuticals in the environment [Internet]. [cited 2025 Oct 14]. Available from: https://efpia.eu/about-medicines/development-of-medicines/regulations-safety-supply/environment-health-safety-sustainability/water-pharmaceuticals-in-the-environment/

70. EUR-Lex - 02024L3019-20241212 - EN - EUR-Lex [Internet]. [cited 2025 Oct 14]. Available from: https://eur-lex.europa.eu/eli/dir/2024/3019/2024-12-12/eng

71. Team C. ChemLinked. [cited 2025 Oct 14]. Japan CSCL - Chemical Substance Control Law. Available from: http://chemical.chemlinked.com/chempedia/japan-cscl-chemical-substance-control-law

72. CSCL(Chemical Substances Control Law)（METI） [Internet]. [cited 2025 Oct 14]. Available from: https://www.meti.go.jp/policy/chemical_management/english/cscl/

73. Ministry of Health, Labour and Welfare: CoPMed [Internet]. [cited 2025 Oct 14]. Available from: https://www.mhlw.go.jp/english/policy/other/CoPMed/index.html

74. Inohana S, Kaminishi M, de Roode DF. Comparison of Environmental Risk Assessment Scheme on Pharmaceuticals between EU and Japan.

75. Jose J, Sandra Pinto J, Kotian B, Mathew Thomas A, Narayana Charyulu R. Comparison of the regulatory outline of ecopharmacovigilance of pharmaceuticals in Europe, USA, Japan and Australia. Science of The Total Environment [Internet]. 2020 Mar [cited 2025 Oct 14];709:134815. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0048969719348065

76. Gildemeister D, Moermond CTA, Berg C, Bergstrom U, Bielská L, Evandri MG, et al. Improving the regulatory environmental risk assessment of human pharmaceuticals: Required changes in the new legislation. Regulatory Toxicology and Pharmacology [Internet]. 2023 Aug [cited 2025 Oct 14];142:105437. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0273230023001058

77. Ågerstrand M, Berg C, Björlenius B, Breitholtz M, Brunström B, Fick J, et al. Improving Environmental Risk Assessment of Human Pharmaceuticals. Environ Sci Technol [Internet]. 2015 May 5 [cited 2025 Oct 14];49(9):5336–45. Available from: https://doi.org/10.1021/acs.est.5b00302