

10-10-2025 1:00 PM

# Antibiotic Pollution and the Making of a Global Environmental Crisis (1943-2002)

James C. Caldwell

Supervisor: Turkel, William J., *The University of Western Ontario*

A thesis submitted in partial fulfillment of the requirements for the Master of Arts degree in History

© James C. Caldwell 2025

## Abstract

This thesis examines how antibiotic pollution in aquatic environments shifted from an inferred concern to a measurable environmental problem between 1943 and 2002. A mixed-methods approach combining bibliometric analysis and historical investigation reveals three distinct phases. First, decades of chemical imperceptibility (1943-1989), when scientists could only infer pollution through proxy measures of resistance while lacking the means to detect antibiotics directly. Second, an analytical revolution (1989-1998), as solid-phase extraction, atmospheric-pressure ionization interfaces, and commercial liquid chromatography-mass spectrometry enabled routine detection at trace concentrations. Third, a phase of field formation (1998-2002), when these technical advances converged with growing public health concern over antibiotic resistance, producing seminal reviews, standardized methods, and the U.S. Geological Survey's national reconnaissance confirming widespread contamination. This convergence of instrument capability and political mandate transformed scattered observations into a coherent environmental science, demonstrating how analytical technologies shape what becomes visible as an environmental crisis.

*Keywords:* antibiotic pollution, aquatic environments, antibiotic resistance, environmental history, computational history, science and technology studies, bibliometrics, liquid chromatography-mass spectrometry, atmospheric-pressure ionization, solid-phase extraction

## Summary

Antibiotics are among the greatest medical breakthroughs of the twentieth century. Since the 1940s, they have saved countless lives by treating infections that once killed millions. Yet as their use spread across medicine, agriculture, and industry, traces of these drugs began to move beyond human bodies. Because the body does not fully absorb antibiotics, some of the drug passes through into urine and feces. In addition, large quantities are used on farms where they can wash into local waterways. In both cases, the result is the same: antibiotics end up in rivers, lakes, and drinking water. Over time, these invisible residues posed an unexpected danger: they pressured bacteria in the environment to develop resistance, threatening the very effectiveness of antibiotics.

This research investigates how scientists came to recognize antibiotic pollution in water as a global environmental problem. For decades, researchers could only see the biological consequences of bacteria becoming resistant without being able to measure the chemicals themselves. The tools available at the time, such as gas chromatography, were designed for different pollutants and could not detect fragile antibiotic molecules.

That changed in the late 1980s and 1990s. Advances in sample-preparation techniques, new ionization methods, and powerful instruments capable of separating and identifying molecules at trace levels finally made antibiotics measurable in environmental samples. By the end of the 1990s, these tools were widely adopted in laboratories. At the same time, rising alarm about antibiotic resistance reframed the issue as a pressing public health crisis. Together, the new technology and the new urgency converged, producing reviews, guidelines, and landmark studies that confirmed the widespread presence of antibiotics in water.

The U.S. Geological Survey's 1999-2000 national survey of rivers and streams marked a turning point. For the first time, antibiotic pollution was measured on a broad scale, demonstrating that the problem was real, widespread, and required policy attention.

This study combines large-scale analysis of thousands of scientific papers with close reading of the most influential publications. It shows that the recognition of antibiotic pollution was not simply a matter of discovering contamination but of developing the tools and frameworks to make it measurable. More broadly, it highlights how new technologies shape what becomes visible as an environmental crisis, and why visibility is essential for action.

## Table of Contents

<b>Abstract</b> .....	<b>ii</b>
<b>Summary</b> .....	<b>iii</b>
<b>Table of Contents</b> .....	<b>v</b>
<b>List of Tables</b> .....	<b>vi</b>
<b>List of Figures</b> .....	<b>vii</b>
<b>List of Appendices</b> .....	<b>viii</b>
<b>List of Abbreviations, Symbols, Nomenclature</b> .....	<b>ix</b>
<b>Antibiotic Pollution and the Making of a Global Environmental Crisis (1943-2002)</b> .....	<b>1</b>
Introduction.....	1
The Paradox of a Miracle.....	1
Historiography.....	10
Aquatic Antibiotic Pollution.....	10
Historiographical Contribution.....	15
Methodology.....	18
Data Collection and Database Construction.....	18
Machine Learning Classification and Relevance Filtering.....	20
Data Cleaning and Standardization.....	20
Geographic Scope.....	21
Bibliometric Analysis.....	22
Qualitative Analysis and Synthesis.....	23
Limitations.....	24
Methodological Contribution.....	26
Chapter 1: The Golden Age and its Shadows: Biological Visibility and Chemical Imperceptibility (1943-1989).....	28
A Lengthening Shadow.....	28
Up Against the Analytical Wall.....	35
Chapter 2: The Analytical Revolution (1989-1998).....	48
Chapter 3: Convergence and Formation: A New Field Emerges (1998-2002).....	69
A New Public Health Mandate.....	69
The New Intellectual Framework.....	75
Political and Regulatory Mandates.....	81
Formation and Crisis.....	83
Conclusion.....	86
<b>Bibliography</b> .....	<b>96</b>
<b>Appendix A: Web of Science Search Term</b> .....	<b>107</b>
<b>Appendix B: Python and R Scripts</b> .....	<b>108</b>

## **List of Tables**

Table 1: Chronology of selected LC-MS interfaces.....	56
Table 2: Relative applicability of major ionization techniques to antibiotics in aquatic environmental samples.....	62

## List of Figures

Figure 1: Reference Publication Year Spectroscopy.....	3
Figure 2: Growth in the number of antibiotic resistance publications contained in the Web of Science database.....	49
Figure 3: Annual scientific production between 1933 and 1989.....	51
Figure 4: Keyword burst detection post-1998 to 2020.....	87
Figure 5: Topic evolution based on author keywords.....	88
Figure 6: Productivity bursts of the highest locally cited researchers.....	90
Figure 7: Historiograph of the most impactful papers and their influence on subsequent publications.....	92
Figure 8: Collaboration network of the most productive researchers as of 2009.....	92
Figure 9: Scientific production by country.....	94

## **List of Appendices**

Appendix A: Web of Science Search Term

Appendix B: Python and R Scripts

## **List of Abbreviations, Symbols, Nomenclature**

AGP - Antibiotic Growth Promoters

APCI - Atmospheric Pressure Chemical Ionization

API - Atmospheric Pressure Ionization

ASM - American Society for Microbiology

$\beta$ -lactam - Ring-structured antibiotic class

$\beta$ -lactamase - Enzyme conferring resistance to  $\beta$ -lactams

CDC - Centers for Disease Control and Prevention

CF-FAB - Continuous-Flow Fast-Atom Bombardment

Da - Daltons (unit of molecular mass)

DESI - Drug Efficacy Study Implementation

Derivatization - Chemical modification to aid detection (commonly for GC-MS)

DLI - Direct Liquid Introduction

EARSS - European Antimicrobial Resistance Surveillance System

US EPA - United States Environmental Protection Agency

ESI - Electrospray Ionization

EU - European Union

US FDA - United States Food and Drug Administration

GC-MS - Gas Chromatography-Mass Spectrometry

HLB - Hydrophilic-Lipophilic Balanced

HPLC - High Performance Liquid Chromatography

IoM - Institute of Medicine

LoD - Limit of Detection

LoQ - Limit of Quantification

LC-MS - Liquid Chromatography-Mass Spectrometry

LC-MS/MS - Liquid Chromatography-Tandem Mass Spectrometry

Local citations - Citations within the bibliographic corpus

MAR - Multiple Antibiotic Resistance

MBI - Moving-Belt Interface

MS/MS or MS<sup>2</sup> - Tandem Mass Spectrometry

m/z - Mass-to-charge ratio measured in MS

NARMS - National Antibiotic Resistance Monitoring System

NAS-NRC - National Academy of Sciences - National Research Council

ng/L - nanograms per liter

NEPA - National Environmental Policy Act (1969)

NIH - National Institutes of Health

PBI - Particle-Beam Interface

PCBs - Polychlorinated Biphenyls

PhACs - Pharmaceutically Active Compounds

ppb - parts per billion

PPCPs - Pharmaceuticals and Personal Care Products

Q1, Q2, Q3 - Quadrupole 1, 2, and 3 (mass spectrometer components)

Plasmid R-Factor - Extrachromosomal DNA conferring transferable drug resistance

SPE - Solid-Phase Extraction

SRM - Selected Reaction Monitoring

STW - Sewage Treatment Works

Torr - unit of pressure (vacuum measurement)

TSP - Thermospray

µg/L - micrograms per liter

µl - microliters

USDA - United States Department of Agriculture

USGS - United States Geological Survey

WHO - World Health Organization

WWTP - Wastewater Treatment Plant

## **Antibiotic Pollution and the Making of a Global Environmental Crisis (1943-2002)**

### **Introduction**

#### ***The Paradox of a Miracle***

For fifty years, the twentieth century's greatest medical miracle was simultaneously creating one of its most pervasive and analytically invisible environmental crises. The commercialization of penicillin in 1943 inaugurated a golden age of antibiotic discovery, transforming previously fatal infections into treatable conditions and fundamentally reshaping human health. Yet, this "miracle cure" carried a hidden consequence. As millions of tons of these life-saving compounds were produced, consumed, and excreted,<sup>1</sup> they began to accumulate in the environment, creating a subtle, pervasive form of pollution that threatened to undermine their own efficacy by creating evolutionary pressures against antibiotic mechanisms in bacteria exposed to this pollution. This thesis investigates how aquatic antibiotic pollution was transformed from an invisible environmental concern, inferred only through its biological consequences, into a visible, measurable, and manageable global research priority between 1943 and 2002.

The scientific field dedicated to this problem did not emerge from a linear process of discovery. Rather, it was the result of a critical convergence in the 1990s between two distinct and powerful forces: first, an internal scientific revolution in analytical chemistry that provided the technical ability to detect and measure antibiotic compounds at environmentally relevant concentrations; and second, an external public health crisis defined by a growing, global fear of untreatable antibiotic resistance.

---

<sup>1</sup> Landecker, "Antibiotic Resistance and the Biology of History," 24.

For decades, the scientists studying this problem were akin to prisoners in Plato's allegorical cave. They could clearly observe the distorted biological shadows of pollution – the alarming rise of antibiotic resistance genes and multi-drug resistant bacteria in the environment – but they were instrumentally unable to see the chemical reality casting them. Lacking the tools to measure the antibiotic compounds themselves, they could only study the consequences, inferring the cause but never directly observing it. This thesis explains how, in the 1990s, they were finally able to leave the cave. It traces the technological and political convergence that allowed them to turn away from the shadows on the wall and face the chemical source, transforming the chemical presence of antibiotics from an inferred cause of biological shadows into a directly measurable and manageable environmental crisis.

This historical explanation is supported by a mixed-methods approach that integrates a large-scale bibliometric analysis of the scientific literature with a close reading of foundational texts and an examination of the external socio-political context. The quantitative data, such as the reference publication year spectroscopy below (see Figure 1), reveals a clear inflection point in the history of this field. Prior to the 1990s research was sparse and dominated by microbiological studies of resistance. After a period of technological and political convergence in the 1990s, the number of publications exploded, with a distinct "burst" of activity occurring between 1998 and 2002. This thesis provides the historical narrative that explains this quantitative pattern, tracing the intellectual, technological, and political developments that made the field's emergence possible. It is important to note, however, that this analysis operates within certain constraints. The bibliographic dataset was originally constructed for the broader topic of aquatic pharmaceutical pollution and later adapted for this more focused study of antibiotics. Consequently, quantitative insights prior to 1995 are limited by a low document count,

necessitating a greater reliance on qualitative evidence for the early period. This is explained in more detail in the methodology section below.

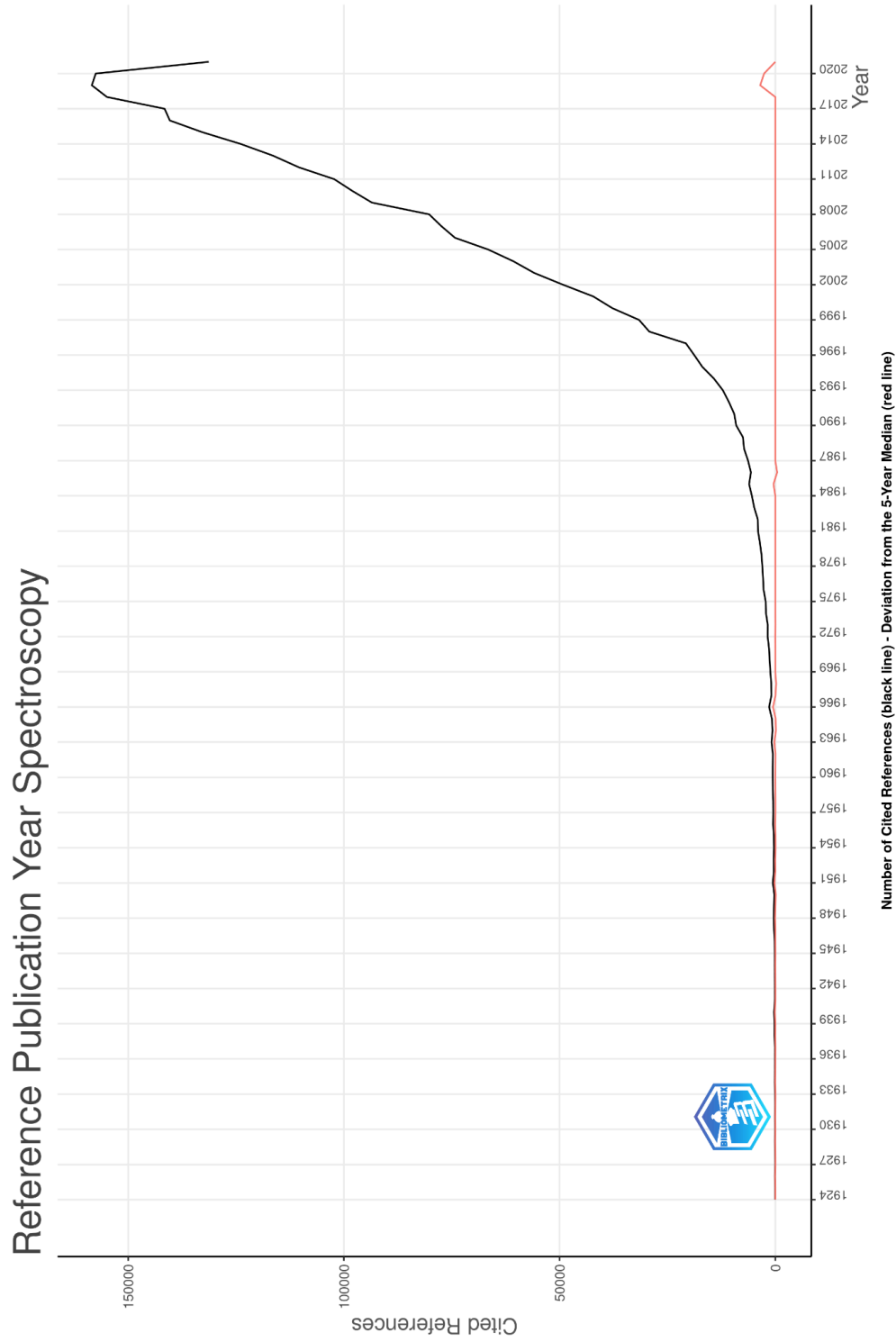


Figure 1. Reference Publication Year Spectroscopy. Note the sudden increase in cited references from 1997 to 1998.

The narrative unfolds chronologically across three distinct phases. The first phase (1940s-1980s) was an era of *chemical imperceptibility*. Scientists could perceive the biological shadows of antibiotic pollution through the study of resistance genes and the development of proxy measures like the Multiple Antibiotic Resistance (MAR) index. However, they lacked the analytical means to detect the compounds themselves. Standard instruments of the period, above all gas chromatography-mass spectrometry (GC-MS), demanded volatility and thermal stability, properties most antibiotics did not possess. The environmental presence of these drugs thus remained inferred rather than directly observed.

The second phase (1989-1998) marked the *analytical revolution* that broke down this barrier. Three technologies matured and diffused into laboratories, enabling for the first time the routine detection of antibiotics in environmental samples: (1) advanced solid-phase extraction (SPE) methods, such as the Oasis HLB cartridge, that provided reliable sample preparation; (2) atmospheric-pressure ionization (API) interfaces that could gently ionize delicate antibiotic molecules; and (3) the commercial SCIEX API III System, which integrated high performance liquid chromatography with tandem mass spectrometry to provide sensitivity and selectivity at trace levels. By the late 1990s, these advances had established the technical capacity for systematic investigation. The broader political and public-health mandate that converged with this capability is taken up in Chapter 3.

The third phase (1998-2002) details the formation of the field as the new analytical paradigm was consolidated and defined. Robust analytical methods now coincided with

mounting concern over antimicrobial resistance, reframed by international bodies as a global health emergency. Seminal reviews by researchers such as Christian Daughton and Thomas Ternes provided a conceptual framework, defining pharmaceuticals as "agents of subtle change" and creating a shared vocabulary for a generation of scientists.<sup>2</sup> In parallel, pioneering investigative studies such as those by Hirsch et al. (1998) operationalized the technological convergence by demonstrating the first reliable, multi-class methods for quantifying antibiotics at trace concentrations in environmental waters.<sup>3</sup> Together with the policy attention of the late 1990s, these methodological and synthetic works transformed scattered findings into a coherent domain of inquiry, culminating in the U.S. Geological Survey's 2002 national reconnaissance – an event that marked antibiotic pollution as a measurable, recognized environmental crisis.

To understand the historical challenge of making this pollution visible, it is first necessary to understand its scientific nature and the pathways by which it enters the environment. The unique chemical properties of pharmaceuticals dictated the types of tools scientists would need to find them, while their biological effects – especially the selection for antibiotic resistance – shaped the political urgency to look for them in the first place. The following section will therefore briefly outline the nature of pharmaceutically active compounds (PhACs), their persistence in aquatic systems, and the specific evolutionary pressure that antibiotics exert, providing essential scientific context for the historical narrative that follows.

PhACs in the environment are part of a class of pollution called emerging contaminants, labeled as such because our ability to routinely detect them in environmental matrices (such as water or soil) only recently developed alongside advancements in analytical chemistry during the

---

<sup>2</sup> Daughton and Ternes, "Pharmaceuticals and Personal Care Products in the Environment."

<sup>3</sup> Hirsch et al., "Determination of Antibiotics in Different Water Compartments via Liquid Chromatography-Electrospray Tandem Mass Spectrometry."

1990s.<sup>4</sup> Antibiotics are a particularly noteworthy group of pharmaceuticals as their chemical structure made them more difficult to detect in the environment compared to other pharmaceuticals. Some scientists such as Addison (1984) developed mathematical models and simulations of antibiotic pollution but specifically noted knowledge and technology gaps that prevented further environmental studies from being conducted.<sup>5</sup> Scientists did not begin to directly measure antibiotics in environmental samples until Watts et al. (1984) investigated non-volatile organics in English river water and drinking water.<sup>6</sup> Although this heralded a few more detection studies of antibiotics in water throughout the 1980s, such as the works of Richardson and Bowron (1985),<sup>7</sup> and Jacobsen and Berglind (1988),<sup>8</sup> it was not until the 1990s that concern regarding pharmaceuticals (including antibiotics) in the environment significantly increased with advancements in analytical techniques which enabled the routine detection of these compounds at extremely low concentrations (ng/L to µg/L).

A PhAC is a chemical compound or a metabolized derivative of a compound which accomplishes some alteration in an organism's anatomy or physiology to treat an illness or disease. This is an important distinction from other emerging contaminants which may still impact health but were not originally intended for a medical purpose. For example, microplastics are also classified as emerging contaminants, are known to harm endocrine system functioning, and are pervasive throughout the aqueous environment. However, they were not designed to be

---

<sup>4</sup> Fernandes et al., "Pharmaceutical Compounds in Aquatic Environments—Occurrence, Fate and Bioremediation Prospective," 2; Patel et al., "Pharmaceuticals of Emerging Concern in Aquatic Systems," 3525; Santos et al., "Ecotoxicological Aspects Related to the Presence of Pharmaceuticals in the Aquatic Environment," 46.

<sup>5</sup> Addison, "Antibiotics in Sediments and Run-off Waters from Feedlots."

<sup>6</sup> Watts et al., "Identification of Non-Volatile Organics in Water Using Field Desorption Mass Spectrometry and High Performance Liquid Chromatography."

<sup>7</sup> Richardson and Bowron, "The Fate of Pharmaceutical Chemicals in the Aquatic Environment."

<sup>8</sup> Jacobsen and Berglind, "Persistence of Oxytetracycline in Sediments from Fish Farms."

ingested for the purposes of altering health.<sup>9</sup> On the other hand, a chemical compound like oxytetracycline (a tetracycline class antibiotic) is regularly used to treat illnesses or increase animal growth, making it pharmaceutical in nature.<sup>10</sup>

PhACs enter the environment after being administered to an organism due to the pharmacokinetics of drug absorption. They are not usually completely bioavailable unless administered intravenously, meaning the organism does not absorb and make use of the entire compound if not administered directly through the bloodstream.<sup>11</sup> The biomechanics of metabolization vary depending on the compound, but typically a small portion is unused by the body while the rest is metabolized to alter anatomy and physiology, transforming the used portion of the compound into PhAC metabolites which may still be pharmaceutically active. Both these PhAC metabolites and unused PhACs are then excreted from the organism via feces and urine. PhACs may bypass an organism and enter the environment in other ways as well – for example through use in animal feedlots and aquaculture for improving livestock growth and health, industrial discharge, or improper disposal of medication. The key issue here is that these compounds are highly persistent in aqueous environments and still have the potential to impact the health of an organism it encounters.<sup>12</sup> PhAC persistence depends on the chemical structure of that PhAC, but essentially a small portion of known PhACs can be filtered out or changed into an innocuous compound while a much larger proportion of PhACs are either persistent (cannot be

---

<sup>9</sup> Ullah et al., “A Review of the Endocrine Disrupting Effects of Micro and Nano Plastic and Their Associated Chemicals in Mammals.”

<sup>10</sup> Boxall et al., “Veterinary Medicines in the Environment.”

<sup>11</sup> Kümmerer, *Pharmaceuticals in the Environment*, 144; Pokluda, “A Comprehensive Review of the Environmental Impacts and Human Health Risks Associated with the Occurrence of Waste Pharmaceuticals in Water Sources of the United States, and Policy Implications”; Flynn, “Pharmacokinetic Parameters”; Monteiro and Boxall, “Occurrence and Fate of Human Pharmaceuticals in the Environment.”

<sup>12</sup> Patel et al., “Pharmaceuticals of Emerging Concern in Aquatic Systems,” 3575.

removed from the environment) or pseudo-persistent (enter the environment faster than we can remove them).<sup>13</sup> Most antibiotics are known to be pseudo-persistent in water due to their pervasive usage, although some classes (such as sulfonamides, fluoroquinolones, and macrolides) tend to persist due to their resistance to hydrolysis in typical aquatic conditions.<sup>14</sup> And although we can filter out or modify some of these compounds via physical, thermal, biological, chemical, and other methods, the technologies required to do so are high-cost, unconventional, and still maturing. As a result, they are typically not installed in most wastewater treatment plant (WWTP) infrastructure across the globe.<sup>15</sup> PhACs which are not filtered out at the WWTP stage (or those that bypass human-serving infrastructure completely) then flow through the aqueous environment, entering surface water, groundwater, drinking water, sediment, soil, and sludge.<sup>16</sup>

Once in water, PhACs have the potential to directly compromise both human and aquatic life through several pathways. One such pathway is biomagnification, which occurs when a compound increases in concentration as it moves up the food chain.<sup>17</sup> For example, a population of phytoplankton may absorb a low-level concentration of fluoxetine (a selective serotonin reuptake inhibitor) present in its ecosystem. A crab then consumes a portion of the phytoplankton population which results in a larger concentration of fluoxetine in the crab. As

---

<sup>13</sup> Khetan and Collins, “Human Pharmaceuticals in the Aquatic Environment,” 2325; Patel et al., “Pharmaceuticals of Emerging Concern in Aquatic Systems,” 3573; Calisto and Esteves, “Psychiatric Pharmaceuticals in the Environment,” 1266.

<sup>14</sup> Khetan and Collins, “Human Pharmaceuticals in the Aquatic Environment,” 2342.

<sup>15</sup> Patel et al., “Pharmaceuticals of Emerging Concern in Aquatic Systems,” 3567; Fernandes et al., “Pharmaceutical Compounds in Aquatic Environments—Occurrence, Fate and Bioremediation Prospective,” 17.

<sup>16</sup> Fernandes et al., “Pharmaceutical Compounds in Aquatic Environments—Occurrence, Fate and Bioremediation Prospective,” 18; Boxall et al., “Pharmaceuticals and Personal Care Products in the Environment,” 1224; Patel et al., “Pharmaceuticals of Emerging Concern in Aquatic Systems.”

<sup>17</sup> Singh et al., “Occurrence and Fate of Antidepressants in the Aquatic Environment,” 2.

organisms continue to consume other organisms lower on the food chain there is a summative effect on the concentration of fluoxetine at each level resulting in a higher level of toxicity as the trophic level of the food chain increases. A second pathway is bioaccumulation, where a PhAC gradually increases in concentration within any given organism over time as a function of its length of exposure to that PhAC in the ecosystem.<sup>18</sup> The longer an organism is exposed to the PhAC in its ecosystem, the larger the concentration of that PhAC in the organism. A third and critically understudied pathway is through the “cocktail effect,” where the presence of multiple different PhACs in an environment interact, both forming new compounds and producing emergent effects in exposed organisms.<sup>19</sup>

All organisms must consume water to survive, so regardless of pathway every organism on earth is being exposed to PhACs in water. PhACs in the environment have already harmed non-human life (for example, by altering zebra mussel reproduction rate, or sex differentiation in fish, or by killing algae), but as concentrations increase due to an increased emphasis on pharmaceutical interventions in medicine, human life will be affected too.<sup>20</sup>

There is a particular concern surrounding antibiotics that does not apply to other classes of pharmaceuticals: the selection and spread of antibiotic resistance genes in bacteria exposed to low levels of antibiotics in their environment. Antibiotic resistance genes arise through mutation or horizontal gene transfer, and persistent low-level antibiotic exposure selects for bacteria

---

<sup>18</sup> Silori et al., “Global Groundwater Vulnerability for Pharmaceutical and Personal Care Products (PPCPs),” 3.

<sup>19</sup> Ortúzar et al., “Pharmaceutical Pollution in Aquatic Environments,” 15.

<sup>20</sup> Fong, “Zebra Mussel Spawning Is Induced in Low Concentrations of Putative Serotonin Reuptake Inhibitors”; Daughton and Ternes, “Pharmaceuticals and Personal Care Products in the Environment,” 928; Pfluger and Dietrich, “Effects on Pharmaceuticals in the Environment — an Overview and Principle Considerations”; Brooks et al., “Aquatic Ecotoxicology of Fluoxetine”; Crane et al., “Chronic Aquatic Environmental Risks from Exposure to Human Pharmaceuticals.”

carrying these genes. Imagine a hypothetical population of *E. coli* that exists in a wastewater treatment plant lagoon. As this population is continuously exposed to low levels of an antibiotic (say, penicillin), the bacteria unable to produce the  $\beta$ -lactamase required to neutralize the  $\beta$ -lactam in the penicillin are killed or inhibited, while those carrying the  $\beta$ -lactamase genes survive. Because *E. coli* reproduce asexually through binary fission,<sup>21</sup> the resistant fraction increases with each generation. At the same time, plasmid-mediated  $\beta$ -lactamase genes can spread between cells through horizontal gene transfer, accelerating the rise of resistance. Over time, this proportion of resistant bacteria grows as non-resistant cells are progressively eliminated by the selective pressure of antibiotics in the environment.

Furthermore, bacteria in these environments are rarely exposed to a single antibiotic in isolation. Because wastewater often contains a complex mixture of pharmaceutical residues from human and agricultural use, bacteria are frequently exposed to multiple classes of antibiotics at once. This co-exposure creates a powerful and continuous selective pressure for the evolution of multiple antibiotic resistance (MAR). The development of any new class of antibiotics, therefore, does not represent a final victory, but rather the start of a new evolutionary arms race against resistance.

## ***Historiography***

### ***Aquatic Antibiotic Pollution***

This thesis represents the first comprehensive historical examination of aquatic antibiotic pollution as a scientific and environmental phenomenon. While scientists have produced chronological accounts of analytical developments, particularly in the evolution of liquid chromatography-mass spectrometry interfaces, these accounts function as technical genealogies

---

<sup>21</sup> Stewart et al., "Aging and Death in *E. Coli*."

rather than historical analyses. They catalog innovations—the progression from thermospray to atmospheric pressure ionization, the refinement of solid-phase extraction techniques—without examining the broader scientific, political, and social contexts that shaped these technological trajectories. Similarly, the proliferation of scientific review papers after 1998, particularly those by Halling-Sørensen et al. (1998) and Daughton and Ternes (1999), synthesized detection studies and analytical methods to guide future research rather than to understand how antibiotic pollution emerged as a recognized environmental problem. These reviews, while invaluable for establishing the field's scientific foundations, were written by practitioners looking forward rather than historians looking back.

The existing historical scholarship on antibiotics, such as Podolsky's *The Antibiotic Era* and Kirchhelle's *Pyrrhic Progress*, approaches the history of antibiotics in a way that leaves the environmental dimension largely unexplored. Podolsky chronicles the struggles of antibiotic reformers from the 1940s onward, examining their efforts to promote "rational therapeutics" at the contested intersection of pharmaceutical marketing, physician autonomy, regulatory oversight, and public health concerns. His focus remains on the human medical sphere: the politics of prescribing, the battles over drug efficacy standards, and the tensions between professional independence and public health imperatives. Kirchhelle, meanwhile, traces the agricultural side of the antibiotic revolution, analyzing how these drugs transformed Anglo-American food production after 1945 through their use in disease prevention, growth promotion, and the creation of what he terms "antibiotic infrastructures" in farming. While Kirchhelle addresses the environmental pathways through which agricultural antibiotics enter ecosystems, his primary concern is with risk regulation, consumer politics, and the divergent policy responses in the US and UK. Both works treat the actual environmental presence of

antibiotics – their movement through water systems, their persistence in aquatic environments, their detection and measurement – as contextual background rather than as a central historical problem. This thesis, by contrast, examines antibiotics' unintended second life as environmental contaminants and the decades-long struggle to make that contamination scientifically legible. Where Podolsky and Kirchhelle focus on the politics of use and regulation in human medicine and agriculture respectively, this work traces the technological and epistemological transformation that allowed antibiotics to shift from inferred shadows to measurable environmental pollutants. This work draws together two previously disconnected scientific traditions: the microbiologists who studied antibiotic resistance as a biological phenomenon from the 1960s through the 1980s, and the analytical chemists who developed the tools to detect antibiotic molecules directly in the 1990s. By connecting these traditions under a single narrative arc – from inference through biological proxies to direct chemical measurement – this thesis reveals how the "shadows" of resistance genes ultimately guided scientists toward the chemical sources casting them.

The historiography can be segmented into three distinct periods. From 1940 until the 1980s scientific investigations were primarily conducted by microbiologists and focused on antibiotic resistance, the shadow of antibiotic pollution. These publications were sparse and usually low-impact with the exception of a few classic studies, such as the initial publication by Abraham and Chain (1940) of antibiotic-resistant bacteria;<sup>22</sup> Watanabe's (1963) finding of plasmid "R factors," which brought horizontal gene transfer of antibiotic resistance into scientific discourse;<sup>23</sup> and Levy, FitzGerald, and Macone's (1973) creation of an experimental farm and subsequent finding of antibiotic-resistant bacteria in the intestines of the farm workers

---

<sup>22</sup> Abraham and Chain, "An Enzyme from Bacteria Able to Destroy Penicillin."

<sup>23</sup> Watanabe, "Infective Heredity of Multiple Drug Resistance in Bacteria."

(linking antibiotics in animal feed to antibiotic resistance).<sup>24</sup> These high-impact studies were often impactful because of their novelty, or because they introduced a new concept to the scientific discourse.

The second historiographical period began in the 1980s and was characterized by publications which actually measured antibiotics in environmental samples. There were not many such publications in the 1980s as methodologies for measuring concentrations in environmental samples were in their infancy. As a result, these few studies (such as those by Watts et al. (1984)<sup>25</sup> and Richardson and Bowron (1985)<sup>26</sup>) frequently entered scientific discourse as judged by their high citation counts. Traditional “proxy” publications still dominate this period, but the introduction of the *ability* to measure antibiotics in water changed how proxy studies conceptualized antibiotics and environmental pollution. By 1996 analytical methodologies matured to the point they had been established as standard approaches, which resulted in a significant increase in studies which measured antibiotics directly, though the focus of this research remained on human medicine and animal husbandry. This period continued until 1998, at which point new analytical tools and growing concern about antibiotic resistance converged.

The third historiographical period begins in 1998 when our analytical technologies matured to the point they were widely commercially available and had been adopted by the majority of laboratories. This period begins with review publications like those from

---

<sup>24</sup> Levy et al., “Changes in Intestinal Flora of Farm Personnel after Introduction of a Tetracycline-Supplemented Feed on a Farm.”

<sup>25</sup> Watts et al., “Identification of Non-Volatile Organics in Water Using Field Desorption Mass Spectrometry and High Performance Liquid Chromatography.”

<sup>26</sup> Richardson and Bowron, “The Fate of Pharmaceutical Chemicals in the Aquatic Environment.”

Halling-Sørensen et al. (1998)<sup>27</sup> and Daughton and Ternes (1999).<sup>28</sup> It is also marked by an increase in political discourse and initiatives regarding antibiotic resistance, such as increased funding for research,<sup>29</sup> the banning of some antibiotics in animal feed,<sup>30</sup> and the United States Geological Survey's national reconnaissance program.<sup>31</sup> This brings about three important shifts in scholarship: first, it became routine for scientists to measure antibiotics in environmental samples directly rather than infer routes of antibiotic pollution through antibiotic resistance and proximity to antibiotic usage hotspots. Second, the ability to directly measure antibiotic pollution allowed for greater geographic expansion of these studies. During the proxy years and convergence years, studies would examine only a small geographic region such as a small segment of a river, a single wastewater treatment plant, or an individual animal feedlot, as this provided a causal link to pollution. For example, scientists knew from their studies of general pharmaceutical pollution (e.g., the Garrison et al. (1976)<sup>32</sup> and Heberer and Stan (1996)<sup>33</sup> investigations of clofibric acid) that pharmaceutically active compounds were in wastewater treatment plant influent by virtue of their consumption and passing (see bioavailability discussion above). Studying the antibiotic resistance of bacteria in a wastewater treatment plant effluent lagoon could therefore provide a clue toward whether these WWTPs were completely filtering out antibiotics during the treatment stage or if they were passing through into effluent.

But, with the widespread acceptance that antibiotic pollution was real and that it was known to

---

<sup>27</sup> Halling-Sørensen et al., "Occurrence, Fate and Effects of Pharmaceutical Substances in the Environment- A Review."

<sup>28</sup> Daughton and Ternes, "Pharmaceuticals and Personal Care Products in the Environment."

<sup>29</sup> Podolsky, *The Antibiotic Era*, 173.

<sup>30</sup> Kümmerer, *Pharmaceuticals in the Environment*; Podolsky, *The Antibiotic Era*, 172; Martinez, "Environmental Pollution by Antibiotics and by Antibiotic Resistance Determinants," 2896.

<sup>31</sup> Kolpin et al., "Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999–2000."

<sup>32</sup> Garrison et al., *GC/MS Analysis of Organic Compounds in Domestic Wastewaters*.

<sup>33</sup> Heberer and Stan, "Determination of Clofibric Acid and N-(Phenylsulfonyl)-Sarcosine in Sewage, River and Drinking Water."

come from animal feedlots, hospitals, pharmaceutical factories, WWTPs, and other known hotspots, scientists could begin to survey for pollution more broadly.<sup>34</sup>

### ***Historiographical Contribution***

This thesis makes its primary contribution to the history of environmental science, yet it is written in direct and critical conversation with the field of environmental history. Many works of environmental history have focused on visible phenomena: the transformation of landscapes, the politics of wilderness, the social conflicts over tangible resources, and so on. My work continues this tradition, but directs it toward a history of the invisible. I argue that to write this history, one must build on the foundations of environmental history by extending its methods with those of Science and Technology Studies. This broadens environmental history's longstanding focus on legibility, pushing it down to the molecular and microbial scales. The central historical problem of antibiotic pollution was not one of conservation or land use, but one of detection.

The foundational text for this history of the invisible is Rachel Carson's *Silent Spring*.<sup>35</sup> Carson was the first to successfully make a systemic, unseen chemical threat visible to the public, documenting the pervasive contamination of the planet by insecticides, herbicides, and other chemicals, what she powerfully re-termed "biocides" due to their ability to kill life

---

<sup>34</sup> Halling-Sørensen et al., "Occurrence, Fate and Effects of Pharmaceutical Substances in the Environment- A Review"; Daughton and Ternes, "Pharmaceuticals and Personal Care Products in the Environment"; Heberer, "Occurrence, Fate, and Removal of Pharmaceutical Residues in the Aquatic Environment"; Fent et al., "Ecotoxicology of Human Pharmaceuticals"; Sarmah et al., "A Global Perspective on the Use, Sales, Exposure Pathways, Occurrence, Fate and Effects of Veterinary Antibiotics (VAs) in the Environment"; Khetan and Collins, "Human Pharmaceuticals in the Aquatic Environment"; Calisto and Esteves, "Psychiatric Pharmaceuticals in the Environment"; Santos et al., "Ecotoxicological Aspects Related to the Presence of Pharmaceuticals in the Aquatic Environment."

<sup>35</sup> Carson, *Silent Spring*.

indiscriminately. Carson herself grappled with making invisible threats visible: she wrote of chemicals that accumulate in tissues where they cannot be seen, detected only through their delayed effects that may not manifest for years or generations. Carson's project was fundamentally about rendering visible what industry and government wished to keep hidden – the presence and persistence of synthetic chemicals in the environment.

My work builds on Carson's project but confronts a crisis of deeper invisibility. While the ecological harm Carson studied was chemical in nature, it had powerful, visible manifestations: the dead robins on the lawn, the eagle's fragile egg, a pervasive 'shadow of death.' Carson could point to dying birds, fishless streams, and poisoned wildlife. My thesis, however, examines a threat that operates at a second order of invisibility. The primary environmental danger of antibiotic pollution is not a die-off of charming fauna but a silent, molecular process: the horizontal transfer of resistance genes in microbial communities and the mutation of genes in individual bacteria. There are no dead birds and no absence of birdsong, only altered genomes invisible to the unaided eye. This profound imperceptibility demanded a different kind of 'seeing,' one that could not be achieved through ecological observation alone but required sophisticated analytical instrumentation

This deeper level of imperceptibility is where my work engages with, and ultimately departs from, many conversations in environmental history. Rob Nixon, for instance, has provided a framework for understanding the "representational challenge" of what he terms "slow violence" – a form of attritional, long-term harm that unfolds gradually and remains largely unobserved.<sup>36</sup> Likewise, historian Finis Dunaway has demonstrated how public environmental consciousness has been overwhelmingly shaped by spectacular, emotionally charged, and visible

---

<sup>36</sup> Nixon, *Slow Violence and the Environmentalism of the Poor*.

crises.<sup>37</sup> Antibiotic pollution is an example of the type of problem these scholars identify: a systemic, accretive threat with no iconic imagery to galvanize public feeling. However, where Nixon champions the translative power of the "writer-activist" and Dunaway analyzes visual media, my thesis argues that for this specific type of threat, the most historically significant actor was the analytical chemist. I provide a history not of public representation, but of scientific visibility. I trace how a community of scientists, faced with a crisis that was invisible to the camera and the naked eye, built a new sensory apparatus out of laboratory instruments and chemical techniques. This is a history of data-driven, instrument-dependent environmental science – a necessary alternative path to visibility when the traditional avenues of analysis fail.

By adopting this history of science approach, this project offers a novel perspective on the dynamics of state environmental governance, a central theme in James C. Scott's *Seeing Like a State*. Scott argues that high-modernist states, in their quest to make complex social and natural systems "legible" and administratively manageable, simplify complex problems.<sup>38</sup> My research reveals the creation of the EPA and its list of "priority pollutants" in the 1970s as a strong example of this process. Faced with a complex chemical world that had been mostly discussed in terms of the classical pollutants, the state created a schematic map that made a specific slice of pollution visible to regulators. This new regulatory focus created a powerful institutional demand for a specific kind of chemical data, a demand that overwhelmingly favored the use of the mature and widely available detection technology best suited to the task: GC-MS. However, as Scott's work predicts, this simplification had unintended consequences. While the primary barrier to understanding antibiotic pollution was the lack of adequate analytical technology, the state's priorities on classical pollutants actively reinforced this technological blind spot. By defining a

---

<sup>37</sup> Dunaway, *Seeing Green*.

<sup>38</sup> Scott, *Seeing like a State*, 2.

narrow set of priorities, the state created a powerful incentive structure that encouraged scientific and financial investment toward the study of these classical pollutants, which in turn prolonged the technological ignorance surrounding antibiotics. My work thus moves beyond a simple policy history to uncover this interplay between state power and technology, demonstrating how a high-modernist framework exacerbated and prolonged a state of technological ignorance. This history reveals how the uncontained waste products of antibiotics created a vast, invisible system connecting pharmaceutical manufacturing, human bodies, and microbial communities in a planetary-scale cycle of chemical circulation and biological evolution. It is precisely by focusing on the instruments and practices of scientific detection that these intertwined histories of technology, state power, and environmental change are made visible.

### ***Methodology***

This study employs a mixed-methods approach combining bibliometric analysis with qualitative historical investigation to examine the development of scientific research on antibiotic pollution and resistance from 1943 to 2002. The methodology integrates quantitative data analysis of scientific publications with close reading of key texts and examination of external socio-political factors influencing the field's development.

The methodology has two components: the quantitative approach used to map the broad landscape of the research field and the qualitative approach used to build the detailed chronological narrative.

### ***Data Collection and Database Construction***

The primary dataset consists of English-language journal articles retrieved from the Web of Science Core Collection, selected for its superior metadata quality and temporal coverage

extending back to the early twentieth century. Best practices of bibliometrics involve merging multiple databases to reduce any possible collection bias in each database.<sup>39</sup> Initial data collection plans included integrating records from Scopus, PubMed, and Dimensions databases. However, extended maintenance of the Scopus platform during the data collection period and the absence of citation metadata in PubMed records necessitated reliance on Web of Science as the sole bibliographic source. This decision was further justified by Web of Science's established reputation for maintaining higher quality standards and more comprehensive historical coverage compared to alternative databases.<sup>40</sup>

The search strategy evolved through multiple iterations to balance comprehensiveness with precision. The final search query (see Appendix A) employed a complex Boolean structure combining pharmaceutical terms with aquatic environment descriptors and pollution indicators. The search explicitly excluded clinical trials and animal studies unrelated to environmental contamination. Document types were restricted to articles, review articles, retracted publications, corrections, and publications with expressions of concern to capture the full scholarly discourse while excluding gray literature that would complicate bibliometric analysis.

Following initial exploration and supervisory guidance, a decision was made to narrow the narrative scope of the thesis to the history of antibiotic pollution and antibiotic resistance from the commercialization of penicillin to the research burst of 1998-2002. This decision was based on two key observations from the data: 1) the literature naturally bifurcates into a broad, multi-pollutant focus (pesticides, heavy metals, etc. in addition to pharmaceuticals) *or* a highly specific focus on antibiotics, and 2) the post-1998 explosion in publications represents a distinct

---

<sup>39</sup> Echchakoui, "Why and How to Merge Scopus and Web of Science during Bibliometric Analysis."

<sup>40</sup> Stahlschmidt and Stephen, *Comparison of Web of Science, Scopus and Dimensions Databases*.

historical era, making the pre-1998 period a coherent and manageable unit of analysis and also an interesting story that explains the origins of the 1998 boom. This strategic pivot allowed for a deeper, more focused historical account appropriate for a master's thesis.

### ***Machine Learning Classification and Relevance Filtering***

Given the broad nature of the search terms and the interdisciplinary character of pharmaceutical pollution research, a machine learning approach was developed to identify relevant records within the retrieved dataset. The classification model was trained using a hybrid approach combining established relevant papers from Daughton's core pharmaceuticals in the environment bibliography<sup>41</sup> with manual annotation of randomly selected abstracts.

The training dataset comprised approximately 1,500 relevant and 2,500 irrelevant examples, manually verified through active learning iterations. The classification model analyzed merged textual features from abstracts, titles, and keywords to determine relevance probability. A confidence threshold of 86 percent was established through systematic spot-checking of classification outcomes against manual assessment. Records were considered relevant only if they demonstrated a clear causal link to aquatic pharmaceutical pollution, excluding studies that merely investigated drug properties or clinical applications without environmental context.

### ***Data Cleaning and Standardization***

The data cleaning process involved multiple stages of standardization and disambiguation conducted primarily using OpenRefine with custom Python and R scripts (see Appendix B) for complex transformations. Author names presented challenges due to inconsistent formatting

---

<sup>41</sup> Daughton, "Pharmaceuticals and the Environment (PiE)."

across records, with variations including different ordering conventions, use of initials versus full names, and transliteration inconsistencies particularly prevalent among Chinese authors.

Author name disambiguation was performed on the top five percent of papers by local citation count, representing 2,276 records. This subset was selected as these highly cited works exerted disproportionate influence on the field's development. The disambiguation process employed iterative clustering algorithms including fingerprint matching, n-gram analysis, Levenshtein distance calculations, and phonetic matching. Co-authorship patterns and institutional affiliations served as additional validation criteria for identifying unique authors.

Keyword standardization involved consolidating variant spellings, singular and plural forms, and synonym groups identified through collaborative analysis with large language models and verified by hand. A custom synonym list was generated for keywords appearing ten or more times in the dataset, resulting in approximately 100,000 cell modifications that significantly improved the reliability of keyword-based analyses.

Finally, a rigorous manual artifact removal process was applied to pre-1999 publications. This entailed writing an R script to identify records with high disparities between global and local citation counts, which successfully flagged influential papers from other fields that were not central to the aquatic pollution discourse. Close reading of pre-1999 article abstracts was conducted to manually remove false positives missed by the model, such as studies on naturally occurring algal antibiotics or research where "water" referred to bodily fluids.

### ***Geographic Scope***

The historical narrative developed in this thesis is weighted toward developments in the United States and Western Europe, a geographic concentration that reflects the origins of the

analytical technologies central to the story and the accessibility of archival materials. The key analytical breakthroughs that enabled direct antibiotic detection – atmospheric pressure ionization, commercial LC-MS/MS systems, and advanced solid-phase extraction – were developed, commercialized, and initially deployed primarily in North American and European laboratories between 1989 and 2002. The SCIEX API III system, instrumental in making routine antibiotic detection possible, was a Canadian-American development that first saw widespread adoption in US EPA laboratories, academic institutions in Europe, and government research programs like the US Geological Survey's national reconnaissance.

This geographic focus also reflects pragmatic constraints. The bibliometric dataset relies primarily on English-language scientific publications indexed in Web of Science, which underrepresents early contributions from non-Anglophone regions, particularly research conducted in Japan, China, India, and Eastern Europe during the 1970s and 1980s. While Japanese scientists were pioneers in studying antibiotic resistance genes (notably Watanabe's work on R factors), subsequent Japanese-language environmental studies may not appear in this dataset.

### ***Bibliometric Analysis***

Quantitative analysis was conducted using the R-based Bibliometrix package,<sup>42</sup> supplemented by custom R scripts for specialized analyses. The analytical framework encompassed performance analysis to identify key contributors and institutions, science mapping to reveal intellectual and social structures, and temporal analysis to identify research bursts and evolutionary patterns. These quantitative insights provided the essential scaffolding for the

---

<sup>42</sup> Aria and Cuccurullo, “*Bibliometrix*.”

historical narrative, identifying key landmarks, influential papers, and temporal shifts that required deeper qualitative investigation.

Network analyses employed the Walktrap and Louvain clustering algorithms for collaboration network construction. The analysis examined networks at five-year intervals initially, with subsequent refinement based on natural breakpoints identified through burst detection algorithms.

### ***Qualitative Analysis and Synthesis***

The core of the qualitative analysis was a close reading of the top 200 most locally cited articles within the dataset. Local citation count was chosen as the primary metric for influence, as it best reflects which papers were most impactful to the community of researchers being studied. This core set of scientific articles was supplemented with a targeted selection of external sources (including historical books on the antibiotic era, government policy documents like the Swann Report, and media reports) to provide the necessary social, political, and cultural context.

A novel aspect of this methodology was the extensive use of Google's NotebookLM to structure the qualitative analysis. The top 200 articles were uploaded into a series of thematic notebooks. For example, a dedicated notebook was created for all papers related to a specific technology, such as "gas chromatography-mass spectrometry (GC-MS)." This allowed for targeted queries to find information on specific scientific developments for close reading.

This AI-assisted process was used to systematically extract key internal scientific events from the literature—such as the introduction of a new concept (e.g., the "R Factor"), the development of a new method (e.g., the MAR index), or a significant finding (e.g., the detection

of clofibric acid in tap water). These events were organized into a master chronological timeline which aided in narrative construction.

This internal timeline was then enriched with external historical context. Large language models were used to brainstorm potential external events (for example, the passage of the Clean Water Act, the publication of *Silent Spring*), which were then manually verified for relevance and accuracy to correct for any AI errors or hallucinations. Additionally, systematic examination of regulatory documents, government reports, and contemporary news coverage provided additional information for the timeline.

The final step was the synthesis of the quantitative and qualitative data streams. The quantitative analysis identified what happened and when, while the qualitative analysis explained how and why. For example, the burst detection analysis quantitatively identified 1998 as a landmark year of explosive growth. The qualitative timeline then provided the explanation for this burst, revealing a self-reinforcing cycle. The maturation of quadrupole high performance liquid chromatography tandem mass spectrometry (LC-MS/MS) technology (an internal factor) converged with an external one: the framing of antibiotic resistance as a public health emergency by scientists, governments, and non-governmental organizations (an external factor). This growing sense of crisis, itself an effect of earlier resistance studies, created an urgent mandate for the new technology, which in turn generated more data that amplified the emergency.

### ***Limitations***

Several limitations constrain the scope and generalizability of this analysis. The restriction to English-language publications potentially underrepresents contributions from non-Anglophone countries, particularly significant given the documented rise of Chinese and

Indian research productivity post-2010. The machine learning classification model, while achieving acceptable accuracy, was trained on pharmaceutical pollution research instead of antibiotic pollution and antibiotic resistance research. Improvements in accuracy could be made by recompiling the bibliographic dataset using purely antibiotic-oriented keywords instead of the general pharmaceutical keywords contained in Appendix A.

The use of citation counts as a measure of impact presents particular challenges when dealing with review articles. Highly cited review papers may achieve elevated citation metrics not due to original contributions but because they synthesize existing literature, making them convenient references for researchers seeking comprehensive overviews of a field. This can artificially inflate the apparent importance of certain publications in bibliometric analyses. In this study, several of the most highly cited papers – particularly Halling-Sørensen et al. (1998) and Daughton and Ternes (1999) – are review articles. While these works were indeed influential in consolidating and disseminating knowledge about pharmaceutical pollution, their high citation counts reflect both their genuine intellectual contribution and their utility as comprehensive reference sources. This dynamic is acknowledged throughout the analysis, with review articles discussed primarily for their role in field formation and knowledge synthesis rather than for original empirical contributions.

The dataset's original construction for general aquatic pharmaceutical pollution rather than specifically antibiotic pollution and resistance created gaps in coverage for the pre-1995 period. With only four documents in the dataset before 1970 and 94 documents from 1970 to 1990, quantitative analysis for these early periods relies primarily on qualitative investigation supplemented by secondary sources.

The author name disambiguation process, while rigorous for the top five percent of papers, leaves most of the dataset with potential attribution errors. This limitation is partially mitigated by the focus on highly cited works that shaped the field's trajectory, but it constrains network analyses of collaboration patterns to this influential subset.

Finally, the reliance on published articles cannot fully capture the informal networks, failed experiments, and informal collaborations that shape scientific progress. This methodology, therefore, tells the history of the published record of the field, a necessary and acknowledged boundary for this project.

Despite these limitations, the methodology provides a robust framework for examining the historical development of antibiotic pollution research, combining the explanatory power of large-scale bibliometric analysis with the interpretive depth of qualitative historical investigation.

### ***Methodological Contribution***

This thesis makes a methodological contribution by demonstrating how computational tools can expand the evidentiary base available to historians of science and the environment. The integration of large-scale bibliometrics with machine-learning relevance filtering enabled the construction of a comprehensive corpus of research on aquatic pharmaceutical pollution. This dataset revealed chronological inflection points and clusters of scientific attention that would be extremely difficult to discern through traditional close reading or archival work alone, especially within the compressed timeline of a master's thesis.

However, computational methods by themselves cannot explain why such inflections occurred. To address this, I combined the corpus analysis with a qualitative close reading of the two hundred most locally cited articles, supplemented by especially influential papers outside the

dataset. This triangulated approach provided a data-driven periodization that forms the central pillar of the thesis's argument. For example, reference publication year spectroscopy quantitatively demonstrated that 1998 marked the beginning of a dramatic burst of activity. This empirical finding dictated my approach: rather than offering a general chronological overview up to the present, the challenge became explaining the causes of this 1998 event. The quantitative map thus guided the qualitative close reading, leading directly to the investigation of the methodological convergence of LC-MS interfaces, solid-phase extraction, and commercial tandem mass spectrometers.

The combination of bibliometrics, machine learning, and close reading ultimately allowed for a different kind of environmental history to be told. It made possible a chronological narrative of antibiotic pollution that emphasizes the construction of legibility itself – the shift from biological shadows to direct chemical detection. The methodological innovation here lies less in any one technique than in their integration. By aligning large-scale pattern analysis with an interpretive history of science, this project reveals developments that remain hidden when either approach is used in isolation, and demonstrates how computational tools can be fruitfully integrated into environmental history.

## Chapter 1: The Golden Age and its Shadows: Biological Visibility and Chemical Imperceptibility (1943-1989)

### *A Lengthening Shadow*

The story of antibiotic pollution began not with environmental concern and the pioneering detection studies of the 1980s, but with an earlier medical triumph. The modern pharmaceutical era began with the discovery of penicillin in 1928 by Alexander Fleming. By 1943, mass production had been achieved and dramatically scaled to supply Allied forces during World War II, marking the beginning of penicillin's commercial availability. Following the war, civilian access expanded, and by 1946 penicillin had become widely available on the open market.<sup>43</sup>

The widespread availability of penicillin after 1943 coincided with a broader cultural and scientific optimism that defined the post-war era. The technological mobilization of the Second World War and subsequent translation of these technological advances into civilian life (especially in Western Europe and North America) created a powerful public belief in scientific progress.<sup>44</sup> This context is crucial for understanding the period. Antibiotics were not just a routine advancement in medical science. They were also a symbol of a new era where science could solve humanity's most intractable problems.

The rapid, successive discovery of new antibiotic classes during the 1940s to the 1960s reflected the “cumulative, collaborative, [...] [and] contemporary” zeitgeist of post-war science.<sup>45</sup>

---

<sup>43</sup> Carvalho and Santos, “Antibiotics in the Aquatic Environments,” 373; Neushul, “Science, Government, and the Mass Production of Penicillin.”

<sup>44</sup> Doel, “Internationalism in Science After 1940,” 60–61; American Chemical Society National Historic Chemical Landmarks, “Legacy of Rachel Carsons Silent Spring.”

<sup>45</sup> De Bellis, *Bibliometrics and Citation Analysis*, 10.

Within this paradigm of inevitable progress, early signs of antibiotic resistance were often viewed not as a fundamental threat but as a temporary challenge that the next discovery would soon render obsolete. This created a cat-and-mouse dynamic in drug discovery: if one antibiotic failed, another was always on the horizon. It was this pervasive sense of scientific mastery and forward momentum during the golden age of antibiotics that helps explain why the subtle, environmental "shadows" of pollution and resistance remained largely unexamined. The focus was on the next miracle, not the accumulating consequences of the last one.

The first signs of antibiotic resistance appeared almost immediately. In 1940, Abraham and Chain reported that an *E. coli* strain could inactivate penicillin by producing penicillinase, representing the first recorded case of antibiotic-resistant bacteria.<sup>46</sup> By 1942 the first clinical case of penicillin-resistant *Staphylococcus aureus* was documented in a hospital setting, establishing the threat outside the laboratory.<sup>47</sup>

Despite early warnings that bacteria exposed to any given antibiotic could develop resistance to that antibiotic, environmental consideration of antibiotic pollution and its impact on health or the contribution to the development of antibiotic resistance genes emerged sporadically and without systematic attention. The first noted discussion of antibiotics causing harm to aquatic life appears to have been published by Marshall and Orr (1958), specifically in regards to the inhibition of "the feeding of copepods" when antibiotics were added to sea water.<sup>48</sup> By 1960, Carlucci and Pramer found that "some antibiotics added to sea water persisted for long enough periods to exert a biological effect" on *E. coli*.<sup>49</sup>

---

<sup>46</sup> Abraham and Chain, "An Enzyme from Bacteria Able to Destroy Penicillin."

<sup>47</sup> Rammelkamp and Maxon, "Resistance of *Staphylococcus Aureus* to the Action of Penicillin."

<sup>48</sup> Marshall and Orr, "Some Uses of Antibiotics in Physiological Experiments in Sea Water."

<sup>49</sup> Carlucci and Pramer, "An Evaluation of Factors Affecting the Survival of *Escherichia Coli* in Sea Water," 253.

Antibiotic resistance discourse would be “permanently transformed” by Tsutomu Watanabe’s 1963 *Bacteriological Reviews* article, *Infective Heredity of Multiple Drug Resistance in Bacteria*.<sup>50</sup> In this work, Watanabe described the "R Factor" – an extrachromosomal genetic element that could confer multiple drug resistance and be transferred between bacteria by conjugation. This introduced into mainstream scientific discourse the concept of "infective heredity."<sup>51</sup> Resistance was not only vertically inherited but could spread horizontally across species, such as from *Shigella* to *E. coli*. Put another way, resistance was recognized as transmissible in a population, not simply the product of isolated mutation. Watanabe’s findings built on Joshua Lederberg’s earlier demonstration of bacterial conjugation (1946) and his introduction of the term “plasmid” in 1952, but shifted the focus to resistance specifically.<sup>52</sup> By the mid-1960s, this work had redefined antibiotic resistance as both hereditary and “infectious,” a framing that made plasmid-mediated resistance central to research and policy debates throughout the 1960s and 1970s.<sup>53</sup>

A critical pathway for environmental contamination was established in 1951 when the United States Food and Drug Administration (FDA) approved the use of antibiotics as growth promoters in animal feedlots.<sup>54</sup> This practice would continue globally for decades with minimal oversight or environmental consideration until it became a concern in the late 1990s and early 2000s, initially in Europe.<sup>55</sup>

---

<sup>50</sup> Podolsky, *The Antibiotic Era*.

<sup>51</sup> Watanabe, “Infective Heredity of Multiple Drug Resistance in Bacteria.”

<sup>52</sup> Lederberg, “Cell Genetics and Hereditary Symbiosis”; Lederberg, “Plasmid (1952-1997).”

<sup>53</sup> Podolsky, *The Antibiotic Era*.

<sup>54</sup> Addison, “Antibiotics in Sediments and Run-off Waters from Feedlots”; Sarmah et al., “A Global Perspective on the Use, Sales, Exposure Pathways, Occurrence, Fate and Effects of Veterinary Antibiotics (VAs) in the Environment.”

<sup>55</sup> Addison, “Antibiotics in Sediments and Run-off Waters from Feedlots.”

The end of this pre-environmental era roughly coincided with the end of the golden age of antibiotics. Throughout this period scientists continuously discovered new classes of antibiotics. Antibiotic resistance is best thought of as an evolutionary arms race, where targeted organisms evolve resistance to classes of antibiotics as a function of their exposure. With the rapid development of new classes during this time, antibiotic resistance was much slower to take hold since antibiotic attack vectors were constantly changing. However, progress in developing new classes of antibiotics has significantly slowed since the end of this era. As a result, antibiotic resistance grew along with concern in our ability to outpace the evolution of antibiotic resistance genes in targeted organisms.

This golden age of discovery, stretching from the 1940s into the late 1960s, was defined by the rapid and successive introduction of over twenty new classes of antibiotic drugs.<sup>56</sup> This constant innovation created what was essentially a therapeutic arms race: as bacteria inevitably evolved resistance to one antibiotic, scientists could reliably counter with a novel compound that targeted a new vulnerability. This sustained success fostered a paradigm of scientific optimism where resistance was often viewed as a temporary hurdle in a cycle of innovation and obsolescence, rather than a fundamental threat. However, by the late 1960s, the pipeline of new antibiotic classes had slowed dramatically. With fewer novel drugs on the horizon, the steady rise of resistance was no longer a problem with a foreseeable solution but an escalating crisis. The prospect of losing this arms race became increasingly real, creating an impetus to thoroughly understand the mechanisms of antibiotic resistance, including the environmental pathways that might be contributing to it, such as the use of antibiotics in animal husbandry.

---

<sup>56</sup> Davies, “Where Have All the Antibiotics Gone?”; Coates et al., “Novel Classes of Antibiotics or More of the Same?”

Early attempts to regulate antibiotic use based on the growing evidence of resistance were met with organized and effective opposition from both industry and professional groups. The 1969 Swann Report in the United Kingdom, which called for a ban on the nontherapeutic use of antibiotics in animal feed, was one of the first such examples of this resistance.<sup>57</sup> While the report was a landmark in acknowledging the ecological links of resistance, its recommendations were largely ignored. According to a 2010 perspective, the Swann recommendations proved "impossible to enforce in many countries to this day," partly due to the strong resistance to regulation within the animal husbandry industry.<sup>58</sup> Scientific concerns regarding antibiotic resistance were often systematically undermined by powerful economic interests in this way.

This opposition was not limited to Europe or the agricultural sector. In the United States, attempts to restrict clinical antibiotic use to reduce the rate of antibiotic resistance gene development faced pushback from medical practitioners protective of their professional autonomy. In 1973 the FDA convened a National Task Force on the Clinical Use of Antibiotics to address overuse, but quickly found that a core premise among its participants was that "physicians do not want the major ruling on antibiotic usage to come from Washington."<sup>59</sup> This opposition to top-down regulation was commonplace and complicated efforts to control antibiotic prescribing or use in feedlots at the time.

The most illustrative example of this coordinated opposition occurred in the mid-1980s with the derailing of the National Institute of Health's (NIH) Fogarty International Task Force on

---

<sup>57</sup> Swann et al., *Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine*.

<sup>58</sup> Davies and Davies, "Origins and Evolution of Antibiotic Resistance."

<sup>59</sup> Podolsky, *The Antibiotic Era*, 132.

Antibiotic Use. This ambitious global effort led by Stuart Levy, a prominent antibiotics researcher, was designed to consolidate data and recommend policy on antibiotic resistance.<sup>60</sup> However, it was perceived as a direct threat by pharmaceutical manufacturers. Industry executives accused the organizers of spreading “propaganda” and harboring a “general anti-industry bias.” In letters to government officials, one vice-president wrote that the planned conference was an “outright attack on free enterprise” and explicitly asked the Assistant Secretary for Health to “use the influence of [his] office to limit this activity.”<sup>61</sup>

This pressure was effective and the planned major international conference was dramatically scaled back by the NIH, leading reformers to conclude that the effort was “disregarded and even downplayed under the guise that the problem [of antibiotic resistance] was being overstated.”<sup>62</sup> While the task force ultimately generated valuable data, the pushback they faced limited their ability to catalyze significant policy changes. This episode was emblematic of the period. By the end of the 1980s, despite growing scientific consensus and the formation of dedicated organizations, a broad, unified, and consistently funded crusade against antibiotic resistance had not fully taken off, reflecting these ongoing political and economic resistances.

Government and non-governmental organizations were still often sidelined in their efforts to address antibiotic overuse. Discourse at the time frequently revolved around promoting “rational therapeutics,” the idea that physicians should prescribe antibiotics less, and only when

---

<sup>60</sup> Podolsky, *The Antibiotic Era*, 165–68.

<sup>61</sup> Beardsley, “NIH Retreat from Controversy.”

<sup>62</sup> Knobler et al., *The Resistance Phenomenon in Microbes and Infectious Disease Vectors*, 37.

absolutely necessary. However, attempts to enforce this through centralized regulation were often perceived as an attack on "free enterprise" and the professional autonomy of physicians.<sup>63</sup>

An example of this conflict was the Drug Efficacy Study Implementation (DESI) process, initiated by the US Food and Drug Administration (FDA) in 1966. Mandated by the 1962 Kefauver-Harris Amendments to the Food, Drug, and Cosmetic Act in response to the Thalidomide tragedy, DESI's goal was to re-evaluate all drugs approved between 1938 and 1962 for efficacy, as previously studies had only considered safety. The FDA contracted with the National Academy of Sciences – National Research Council (NAS-NRC), which convened nearly 200 experts across thirty panels to undertake the review.<sup>64</sup>

The process quickly zeroed in on fixed-dose combination antibiotics as the most problematic category. These drugs, which combined multiple antibiotics into a single pill, were overwhelmingly viewed by the expert panels as "irrational" therapy. By 1968, the panels recommended that all such combinations be removed from the market, arguing that the therapeutic benefit was no greater than that of one of the individual components.<sup>65</sup>

This recommendation sparked a significant backlash. When the FDA moved to decertify prominent combination drugs like Upjohn's *Panalba*, the company challenged the agency's authority, arguing the decision ignored the "realities of medical practice."<sup>66</sup> The ensuing legal battle, which Upjohn ultimately lost in 1970, roused fierce opposition from some physicians who saw the DESI process as a direct assault on their prescribing autonomy. While DESI was a "high-water mark" for government regulation – eventually leading to the removal of nearly three

---

<sup>63</sup> Beardsley, "NIH Retreat from Controversy."

<sup>64</sup> Podolsky, *The Antibiotic Era*.

<sup>65</sup> Podolsky, *The Antibiotic Era*.

<sup>66</sup> Podolsky, *The Antibiotic Era*, 106.

hundred drugs, including all fixed-dose combination antibiotics – it also fostered antagonism to centralized oversight in the professional lives of physicians. This conflict over clinical prescribing, driven by industry's defense of its products and physicians' defense of their autonomy, established a pattern of organized opposition that would be replayed decades later when the environmental consequences of antibiotic use came under scrutiny.<sup>67</sup>

By the end of the 1960s, the paradox of the antibiotic era was clear. The scientific community had identified the lengthening shadows of resistance and traced their origins to both clinical and agricultural use, but political and economic opposition had stalled any meaningful regulatory action. As this medical arms race continued, a powerful new social force was emerging outside the clinic and the feedlot: the modern environmental movement. One might have expected this movement to shine a light on antibiotic pollution. However, the specific way it came to define the environment and its priority pollutants would have a paradoxical effect, inadvertently deepening the shadows around pharmaceuticals for another two decades.

### ***Up Against the Analytical Wall***

The new environmental consciousness of the 1970s paradoxically lengthened the biological shadows of antibiotic pollution. The groundwork was laid in the previous decade, most notably by Rachel Carson's landmark 1962 book *Silent Spring*,<sup>68</sup> which exposed the dangers of pesticides and galvanized a nascent environmental movement. This new public consciousness resulted in the first Earth Day in 1970,<sup>69</sup> creating a powerful mandate to address chemical pollution. However, this energy was channeled into tackling a specific set of "priority pollutants"

---

<sup>67</sup> Podolsky, *The Antibiotic Era*, 111.

<sup>68</sup> Carson, *Silent Spring*.

<sup>69</sup> Lewis, "The Spirit of the First Earth Day."

identified by the newly formed U.S. Environmental Protection Agency (EPA). This stated policy of exclusion is evident in a landmark 1970 EPA report on organic chemical pollution in freshwater:

Americans have become increasingly involved in the area of drug abuse and the probability of drugs reaching water supplies has increased. It is recognized that disposal of drugs in the waste water system is one means of destruction by both police departments and hospitals. This specific area of concern was excluded from consideration in this report.<sup>70</sup>

This deliberate exclusion of pharmaceuticals from the environmental agenda was reinforced by the 1976 Toxics Consent Decree, which mandated the regulation of 129 priority industrial and agricultural chemicals, none of which were pharmaceuticals.<sup>71</sup>

This narrow regulatory framework dictated the direction of environmental science by creating an institutional demand for the analytical tool best suited to find the priority pollutants: gas chromatography-mass spectrometry (GC-MS). The widespread adoption of GC-MS was therefore not a purely scientific choice, but a direct response to a policy already blind to the chemical nature of most antibiotics which are generally non-volatile and thermally labile, and thus degraded by the technique. This created a self-reinforcing cycle: policy defined the problem in a way that privileged a specific technology, and that technology, in turn, made it nearly impossible to see any problem that lay outside those definitions. In this way, the very success of the 1970s environmental movement in targeting classical pollutants inadvertently deepened the analytical and regulatory blind spot that kept antibiotic pollution invisible.

---

<sup>70</sup> US Environmental Protection Agency, Water Quality Criteria Data Book Volume I: Organic Chemical Pollution of Freshwater, 8.

<sup>71</sup> US EPA, "Toxic and Priority Pollutants Under the Clean Water Act"; Toxic Pollutants.

In terms of regulatory initiatives, this period saw some discussion of regulation to combat the development of antibiotic resistance, but not any implementation of such regulation. The late 1960s and early 1970s also saw the introduction of many environmental initiatives under President Nixon, such as the creation of the US EPA and the National Environmental Policy Act of 1969, but these were focused on the classical pollutants of the time (such as pesticides, herbicides, PCBs, and so on). This regulatory focus encouraged scientists to prioritize these pollutants at the expense of other emerging pollutants like pharmaceuticals. This does not mean that scientists focused exclusively on classical pollutants, as regulation does not go hand-in-hand with scientific advancements. There were isolated studies on pharmaceuticals in the environment even if US policy considerations did not take them into account or take them seriously.

Even as the official regulatory focus remained on classical pollutants, the 1970s also saw the first pioneering studies on the chemical detection of pharmaceuticals in aquatic environments. New approaches examined water, sediment, and sludge for pharmaceutically active compounds instead of organisms and their antibiotic resistance genes. For example, Tabak & Bunch (1970) discovered synthetic hormones in lake sediment in Michigan and Wisconsin.<sup>72</sup> A few years later, researchers began applying new analytical techniques to the problem, with Garrison et al. (1976) and Hignite & Azarnoff (1977) conducting some of the earliest studies using gas chromatography/mass spectrometry (GC-MS).<sup>73</sup> Garrison's team used GC-MS to measure clofibric acid in treated wastewater, while Hignite & Azarnoff discovered chlorophenoxyisobutyrate and salicylic acid in sewage water. These compounds stood in contrast

---

<sup>72</sup> Tabak and Bunch, "Steroid Hormones as Water Pollutants. I. Metabolism of Natural and Synthetic Ovulation-Inhibiting Hormones by Microorganisms of Activated Sludge and Primary Settled Sewage."

<sup>73</sup> Garrison et al., *GC/MS Analysis of Organic Compounds in Domestic Wastewaters*; Hignite and Azarnoff, "Drugs and Drug Metabolites as Environmental Contaminants."

to antibiotics because of their polarity, and the technology required to detect their molecular signature. Studies at the time used GC-MS, a tool well-suited for their targets but, as will be discussed further in chapter 2, was fundamentally incapable of detecting most antibiotics.

The establishment of the U.S. Environmental Protection Agency in 1970 and the passage of the Clean Water Act in 1972 created an institutional framework for addressing water pollution, though pharmaceuticals were still not on the regulatory radar of any nation. Although the aforementioned studies demonstrated that pharmaceutical contamination extended beyond wastewater to potable water sources, regulation would not seriously be considered until the 1990s.

And despite these early detections, there was a recognized lack of comprehensive information regarding the fate of the sheer number of drugs in use, and limited further literature activity on the topic occurred until the late 1990s.<sup>74</sup>

The 1980s witnessed broadening geographical detection and the identification of biological impacts, although the total number of studies remained relatively low. In 1981 drug residues were detected in UK rivers at concentrations reaching up to roughly 1 µg/L.<sup>75</sup> In 1984, Watts et al. published what would seem to be the first classically cited paper, one that detected antibiotics in English surface water due to antibiotic use in fish farming, establishing aquaculture as a source of contamination.

---

<sup>74</sup> Daughton and Ternes, “Pharmaceuticals and Personal Care Products in the Environment”; Ternes, “Occurrence of Drugs in German Sewage Treatment Plants and Rivers.”

<sup>75</sup> Richardson and Bowron, “The Fate of Pharmaceutical Chemicals in the Aquatic Environment,” 4.

The Watts et al. study was remarkable in that it is an example of measuring antibiotics in water prior to the ubiquity of analytical chemistry techniques for routine detection. It was also remarkable in that the researchers set out to measure multiple residues in water. A targeted approach was designed to include compounds that were “non-volatile organics, in widespread use, produced in large amounts, of potential toxicological concern, [and were] likely to enter the water cycle.”<sup>76</sup> In practice, this meant pesticides and pharmaceuticals were targeted for analysis. These compounds posed a significant analytical challenge at this time. Standard methods like GC-MS required compounds to be volatile enough to be turned into a gas, but most antibiotics were non-volatile and thermally labile, meaning they would decompose under the intense heat required for analysis. This analytical challenge meant that successful detections, like those conducted by Watts et al., required researchers to look beyond the standard methods of the day. Watts and colleagues explicitly conclude that “[t]he use of [high performance liquid chromatography and field desorption mass spectrometry] affords a solution to the long-standing problem of analyzing non-volatile organics in river and drinking waters.” They also highlight that “[m]any of these compounds have been identified, some for the first time, in such samples.”<sup>77</sup>

The Watts et al. (1984) study was not alone in its success, though such achievements were rare and methodologically distinct from the research that would follow in the 1990s. These early detections succeeded because they used clever workarounds to avoid the fatal flaw of the standard GC-MS approach: its destructive heat. The main challenge was getting large, fragile antibiotic molecules into the mass spectrometer without boiling them to the point of

---

<sup>76</sup> Watts et al., “Identification of Non-Volatile Organics in Water Using Field Desorption Mass Spectrometry and High Performance Liquid Chromatography.”

<sup>77</sup> Watts et al., “Identification of Non-Volatile Organics in Water Using Field Desorption Mass Spectrometry and High Performance Liquid Chromatography.”

decomposition. Watts and his colleagues solved this by using a highly specialized technique called field desorption mass spectrometry. This method involved placing the sample on a tiny wire emitter and using a powerful electric field to gently lift the intact molecules into the machine without heat, as if using a magnet to lift sprinkles off an ice cream cone without melting it.<sup>78</sup> Jacobsen and Berglind used a different tactic. After separating chemicals with HPLC, they used a simple ultraviolet detector as a checkpoint.<sup>79</sup> This detector could not identify a molecule's specific structure, but it could spot the known wavelength signature of oxytetracycline as it passed by – similar to a guard who cannot read IDs but can spot everyone wearing a specific color of shirt.<sup>80</sup> What set these studies apart was their reliance on such highly customized solutions. These methods were technically demanding, like field desorption, or less specific and only suitable for high-concentration samples, like the UV detector. The revolution of the 1990s lay in the arrival of robust, versatile, and commercially available liquid chromatography-tandem mass spectrometry (LC-MS/MS) systems that transformed the detection of antibiotics from an exceptional discovery into a routine analytical capability.

The scarcity of antibiotic detection data from this period was not due to a lack of scientific concern, but to these well-documented analytical barriers. Scientists at the time plainly stated the challenge. In 1985, Richardson and Bowron concluded that routine analysis for most pharmaceuticals was simply "not practical," noting that standard methods like GC-MS could only detect "a maximum of some 20-25% of chemicals considered to be present."<sup>81</sup> A year

---

<sup>78</sup> Beckey and Schulten, "Field Desorption Mass Spectrometry"; Watts et al., "Identification of Non-Volatile Organics in Water Using Field Desorption Mass Spectrometry and High Performance Liquid Chromatography."

<sup>79</sup> Jacobsen and Berglind, "Persistence of Oxytetracycline in Sediments from Fish Farms," 366.

<sup>80</sup> Ardrey, *Liquid Chromatography-Mass Spectrometry*; Niessen, *Liquid Chromatography-Mass Spectrometry*; Jacobsen and Berglind, "Persistence of Oxytetracycline in Sediments from Fish Farms."

<sup>81</sup> Richardson and Bowron, "The Fate of Pharmaceutical Chemicals in the Aquatic Environment."

earlier, Watts et al. had quantified this analytical gap, explaining that the non-volatile compounds inaccessible to GC-MS constituted up to 80% of the total organic material in water samples. As they stated, "[n]o similar combined separation-identification method or mass spectral data bank is available for non-volatile organics and this has proved a major obstacle to more widespread research."<sup>82</sup> These accounts from the biological shadows period serve as evidence that researchers were aware of the potential problem but were prevented from observing its chemical reality by the instrumental limitations of their time.

By and large, these decades were dominated by the use of antibiotic resistance as a proxy for measuring the presence and impact of antibiotic pollution. This approach represented a consistent scientific trend throughout the 1970s and early 1980s, as researchers developed methods to trace the biological consequences of chemical contamination they could not yet directly measure.

This method of inquiry is clearly visible as early as 1973 with the work of Grabow and Prozesky,<sup>83</sup> who compared drug resistance in coliform bacteria from hospital sewage versus general city sewage. Their approach was to compare a known antibiotic hotspot with a more diffuse environment. The results were stark, showing that 26% of coliforms in hospital wastewater had transferable resistance, compared to just 4% in city sewage. The study concluded by discussing the "environmental pollution with [resistant] bacteria" and the dissemination of these organisms, establishing a clear conceptual link between concentrated antibiotic use and a measurable biological footprint in the environment.<sup>84</sup>

---

<sup>82</sup> Watts et al., "Identification of Non-Volatile Organics in Water Using Field Desorption Mass Spectrometry and High Performance Liquid Chromatography."

<sup>83</sup> Grabow and Prozesky, "Drug Resistance of Coliform Bacteria in Hospital and City Sewage."

<sup>84</sup> Grabow and Prozesky, "Drug Resistance of Coliform Bacteria in Hospital and City Sewage," 178.

Three years later, Cooke (1976) conceptually reinforced this link by explicitly framing antibiotic resistance itself as a form of environmental pollution. After comparing resistance patterns in bacteria from natural waters and effluents, Cooke argued that the evidence "strongly suggests that the increased incidence of [resistant] bacteria in the natural environment is due to selection by antibiotics."<sup>85</sup> This moved the idea beyond simple correlation to a causal argument, where the presence of resistant bacteria was an indicator of antibiotic selection pressure in the environment. The application of these proxy methods continued to expand, with studies like Goyal et al. (1979) investigating transferable drug resistance in the bacteria of coastal canal water and sediment, further demonstrating the broadening geographic and environmental scope of these investigations.<sup>86</sup> This proxy-based approach, which used bacterial resistance to infer the presence of antibiotics, became the dominant mode of inquiry until the analytical revolution of the 1990s. Unable to measure the chemical compounds directly, microbiologists instead became adept at interpreting their effects.

This trend culminated in the highly influential work of Krumperman (1983), which formalized the proxy approach into a standardized, quantitative tool: the multiple antibiotic resistance (MAR) index. Krumperman's key contribution was creating a numerical system that could differentiate between sources of contamination. He defined "high-risk" sources, such as commercial poultry and humans, as those with MAR indices of 0.200 or greater, while "low-risk" sources from wild animals had indices below 0.200.<sup>87</sup> The MAR index was not a direct measure of chemicals, but a tool for measuring their biological consequence, acting as a

---

<sup>85</sup> Cooke, "Antibiotic Resistance in Coliform and Faecal Coliform Bacteria from Natural Waters and Effluents."

<sup>86</sup> Goyal et al., "Transferable Drug Resistance in Bacteria of Coastal Canal Water and Sediment."

<sup>87</sup> Krumperman, "Multiple Antibiotic Resistance Indexing of Escherichia Coli to Identify High-Risk Sources of Fecal Contamination of Foods."

"signature" that pointed back to human-centric reservoirs of resistance. Krumperman's work built upon the established logic of earlier proxy studies, refining it into a robust, reproducible methodology that became a classic example of how scientists inferred the presence of antibiotic pollution.

The basic principle of the index is that environments with heavy, routine multiple antibiotic use (like commercial animal husbandry and human populations) create a powerful selective pressure that fosters the emergence of bacteria resistant to many drugs at once. The MAR index captures this phenomenon numerically. For a single bacterial isolate, the index is calculated by dividing the number of antibiotics it is resistant to by the total number of antibiotics it was exposed to. So, for example, "if the isolate were exposed to 12 antibiotics and were resistant to 6, the index for the isolate would be  $6/12$ , or 0.50."<sup>88</sup>

This numerical threshold made the index a powerful diagnostic tool for environmental research. The MAR index was not a direct measurement of antibiotic chemicals in the water. Rather, it was a quantitative measure of the biological consequence of that pollution. It acted as a signature or tracer, allowing scientists to infer the origin of contamination through correlation of MAR index with known pollution hotspots, like commercial aquaculture sites. A high MAR index in a water sample pointed directly back to the human-centric reservoirs of resistance created by modern agriculture and medicine. This provided a clever and essential method for making the invisible problem of antibiotic pollution quantifiable, long before the chemical reality could be routinely measured.

---

<sup>88</sup> Krumperman, "Multiple Antibiotic Resistance Indexing of *Escherichia Coli* to Identify High-Risk Sources of Fecal Contamination of Foods," 167.

By examining the resistance profile of *E. coli*, the MAR index allows scientists to infer whether the contamination it represents comes from a source where antibiotics are heavily used. Krumperman showed that a high index signals a higher potential risk of encountering dangerous pathogens, thus providing crucial information beyond just knowing that contamination is present.

Put another way, the MAR index is a tool for measuring how human practices create a specific, measurable signature of biological pollution, providing a tool to see the unseen risk in using antibiotics as feed additives.

Krumperman (1983) took *E. coli* samples from water, sewage, animals, and humans in Oregon and measured their resistance against a panel of 12 antibiotics.<sup>89</sup> The degree of MAR exhibited by the *E. coli* population allowed for environmental pathways of antibiotic pollution to be evaluated by how much danger they posed in the development of antibiotic resistance.

The study found that high-risk sources ( $\geq 0.200$  MAR index) included humans (from raw sewage and rectal swabs), food animals (poultry, swine, dairy cows), and "metropolitan vector animals" (like rats, mice, insects) found near metropolitan areas or poultry/swine farms. Conversely, low-risk sources included grazing sheep and cattle, wild animals (deer, fox, raccoon, rat, mouse, skunk), and rural vector animals. Essentially, the MAR index could be used to evaluate pathways of environmental contamination for antibiotics. Pathways that did not involve the use of antibiotics for improving health or fattening animals produced the least environmental risk. Despite the inability to directly measure antibiotic pollution, they also suggested it was present to a lesser extent. Meanwhile, *E. coli* samples taken from places with a high amount of antibiotic usage, like livestock feedlots, or caged chicken farms, had a high MAR index. The

---

<sup>89</sup> Krumperman, "Multiple Antibiotic Resistance Indexing of Escherichia Coli to Identify High-Risk Sources of Fecal Contamination of Foods."

proxy of antibiotic resistance genes served to make antibiotic pollution increasingly visible to scientists.

Krumperman's work became a foundational methodology for researchers tracing sources of fecal pollution and antibiotic resistance. By providing a standardized and quantitative metric, the MAR index was adopted as a key tool for differentiating contamination from high-risk agricultural and human sources versus low-risk wildlife sources.<sup>90</sup> Its influence is demonstrated not only by its conceptual elegance but also by its status as one of the most highly cited papers of the era on the topic of environmental antibiotic resistance. It served as a methodological touchstone for subsequent studies, providing a robust way to measure the biological shadow of antibiotic pollution and assess its public health implications long before the chemical sources could be routinely and directly measured.

An early indication that reached beyond the laboratory emerged in the mid-1980s, when British anglers reported hermaphrodite roach fish in the lagoons of sewage treatment works (STWs).<sup>91</sup> Follow-up studies by scientists confirmed these observations and "stimulated a [further] number of studies using caged rainbow trout that identified effluent from STWs as being estrogenic."<sup>92</sup> This established a direct link between wastewater and biological effects in aquatic organisms. This was not an effect of antibiotic pollution, but it reinforced the logic of using biological endpoints as proxies when direct chemical measurement lagged.

---

<sup>90</sup> Stoeckel and Harwood, "Performance, Design, and Analysis in Microbial Source Tracking Studies"; Scott et al., "Microbial Source Tracking."

<sup>91</sup> Purdom et al., "Estrogenic Effects of Effluents from Sewage Treatment Works."

<sup>92</sup> Routledge et al., "Identification of Estrogenic Chemicals in STW Effluent. 2. In Vivo Responses in Trout and Roach," 1559.

Meanwhile, evidence of pharmaceutical contamination expanded geographically. In 1986, ibuprofen and naproxen were identified in Canadian wastewaters,<sup>93</sup> and by 1988, antibiotics from fish farming were documented in sediments beneath fish farms, confirming persistent environmental contamination.<sup>94</sup> However, significant analytical limitations continued to persist. As the reviews of 1998-2002 made clear,<sup>95</sup> "[u]ntil the early nineties the analytical techniques needed to accurately detect these compounds in complex environmental samples such as sewage or river waters did not exist".<sup>96</sup>

Additionally, future growth could be explained by the extreme focus during this period on classic aquatic pollutants such as PCBs, heavy metals, pesticides, and herbicides. My study of the post-2000 quantitative data suggests that researchers almost never focused exclusively on pharmaceutically active compounds as water pollutants. Instead, researchers tended to fall into two camps: either they focused on pharmaceuticals *and* pesticides *and* herbicides *and* heavy metals *and so on* in water, *or* they focused solely on antibiotics in all environmental matrices. Given that analytical technologies for detecting volatile, thermally labile compounds in environmental samples were not yet ubiquitous, that classical water pollutants were still being emphasized (e.g., US EPA priority list), and that the field of aquatic pharmaceutical pollution had not been formalized yet, the attention of many future antibiotic pollutant researchers was elsewhere at this time.

---

<sup>93</sup> Rogers et al., "Organic Extractables in Municipal Wastewater Vancouver, British Columbia."

<sup>94</sup> Jacobsen and Berglind, "Persistence of Oxytetracycline in Sediments from Fish Farms."

<sup>95</sup> Halling-Sørensen et al., "Occurrence, Fate and Effects of Pharmaceutical Substances in the Environment- A Review"; Daughton and Ternes, "Pharmaceuticals and Personal Care Products in the Environment."

<sup>96</sup> Jones et al., "Aquatic Environmental Assessment of the Top 25 English Prescription Pharmaceuticals," 5013.

By the close of the 1980s, the paradox of antibiotic pollution was fully entrenched. On one hand, the biological shadows of the problem were sharper than ever, traced through the sophisticated proxy methods of microbiologists and reinforced by alarming observations of pharmaceutical impacts on aquatic life. On the other, the chemical reality remained stubbornly obscured behind a self-reinforcing wall of regulatory priorities and technological limitations. Scientists were now fully aware of the biological consequences, but they remained instrumentally paralyzed, unable to directly measure the chemical source of the problem. Making that source visible would require an analytical revolution and a political crisis powerful enough to demand that the world finally look.

## Chapter 2: The Analytical Revolution (1989-1998)

By the late 1980s, researchers faced an untenable position: decades of microbiological studies had established that environmental antibiotic resistance was spreading through environmental pathways, yet the antibiotic compounds responsible remained analytically inaccessible. This disconnect undermined scientists' credibility with policymakers who required measurable chemical evidence to justify regulatory action. As the broader antimicrobial resistance crisis—encompassing drug-resistant tuberculosis, MRSA, and resistant hospital-acquired infections—moved from a specialized concern to a broader public health anxiety in the early 1990s, pressure mounted from both sides: internally from environmental researchers frustrated at studying biological shadows rather than chemical substances, and externally from regulators who demanded direct measurements of antibiotics in water before acting on biological warnings. The analytical revolution that followed was therefore not simply the next stage of instrument development, but a focused response to these converging pressures. Its enabling technologies – atmospheric pressure ionization, commercial liquid chromatography-tandem mass spectrometry systems, and refined solid-phase extraction – were pulled into prominence by the urgent need to measure the chemical source of environmental resistance. The surge in scientific attention to antibiotic resistance across clinical, agricultural, and environmental contexts, visible in the publication record after 1990 (see Figure 2), signaled that the era of chemical imperceptibility was ending.

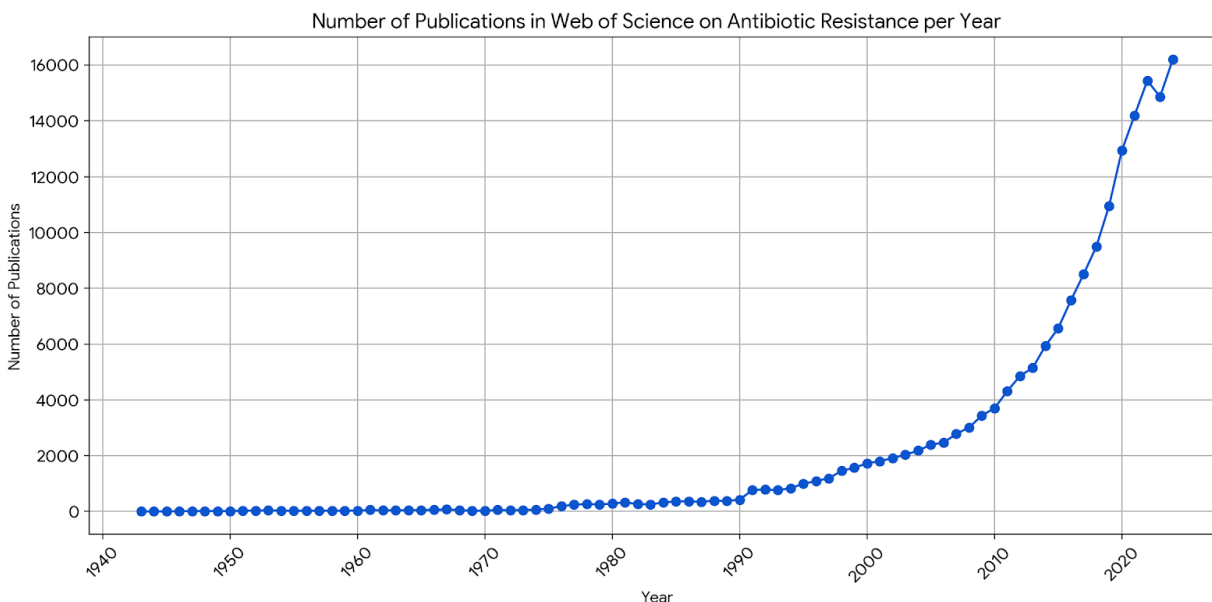


Figure 2. Growth in the number of antibiotic resistance publications contained in the Web of Science database. Note the growth after 1990.

It is important to clarify the scope of concern during this period. The broader antimicrobial resistance crisis of the 1990s encompassed the full spectrum of resistance in clinical, agricultural, and environmental settings. Drug-resistant tuberculosis, methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant enterococci dominated headlines and policy discussions. However, within this broader crisis, environmental scientists were specifically concerned with a distinct but related phenomenon: the presence of antibiotics in aquatic environments and the selection for resistant bacteria in wastewater, rivers, and soil. While the clinical resistance crisis provided the political urgency and public attention, the technical challenge addressed in this chapter was narrower – developing methods to detect the chemical source (antibiotics in water) that environmental microbiologists had been inferring through proxy measures of resistance. The two phenomena were interconnected: environmental

reservoirs of resistance genes could potentially transfer to human pathogens, making the environmental dimension a critical piece of the larger resistance puzzle.

The successful detection of non-antibiotic pharmaceuticals like clofibrac acid and synthetic hormones in the 1970s and 1980s relied on a mature analytical paradigm: gas chromatography-mass spectrometry (GC-MS) (see Figure 3 below). This established tool was the standard for measuring the classical pollutants of the era, such as pesticides and industrial chemicals, but its success hinged on a critical two-part thermal challenge that made the analysis of many antibiotics impossible. First, a compound had to be volatile enough to vaporize completely to be analyzed by the system; second, it had to be thermally stable enough to withstand those high temperatures required for vaporization without decomposing.

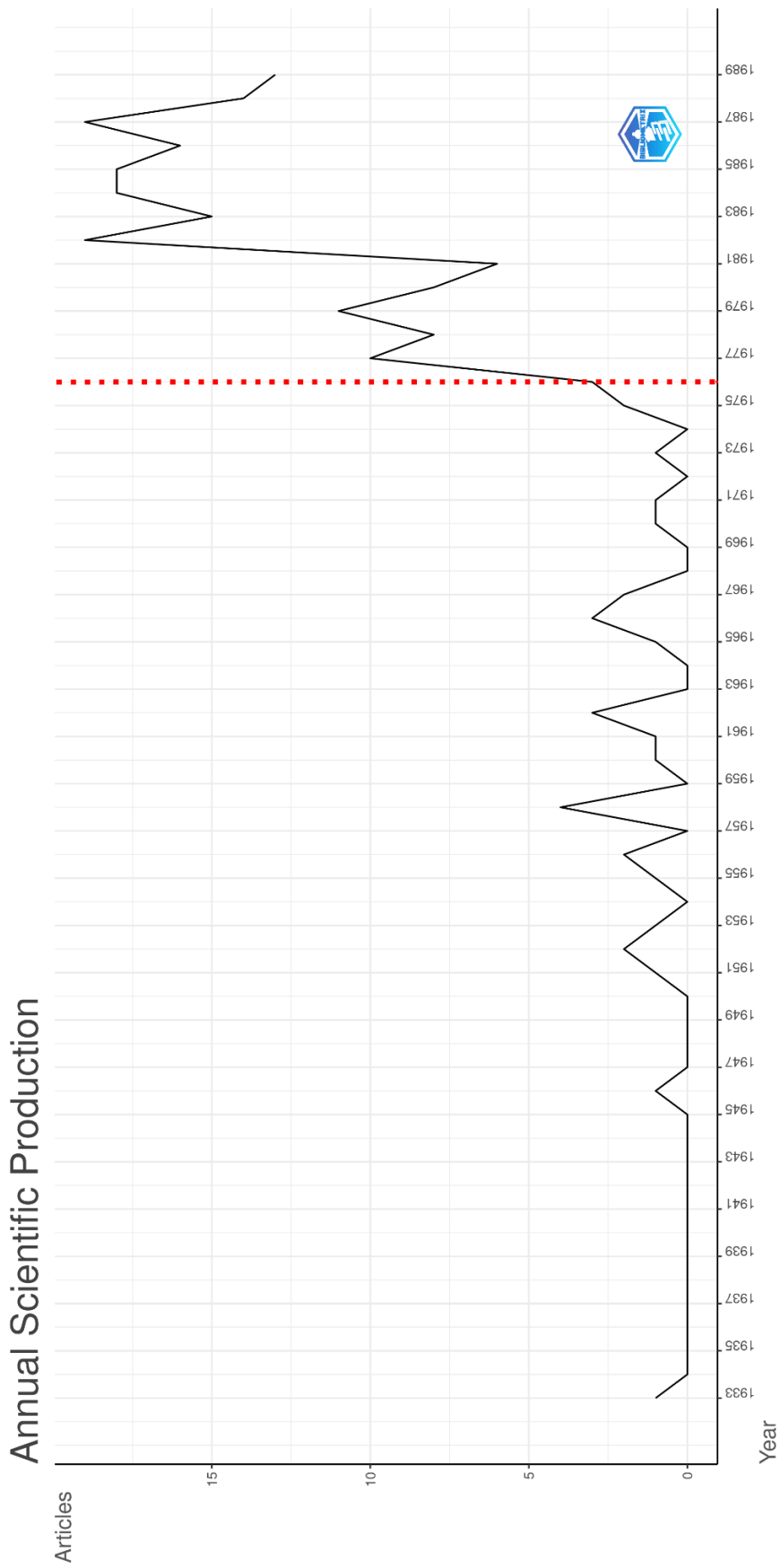


Figure 3. Annual scientific production between 1933 and 1989. Note the growth of aquatic pharmaceutical pollution publications to the right of the dotted red line prior to the standardization of LC-API-MS and SPE.

Most antibiotic classes (such as macrolides, tetracyclines, and the especially delicate  $\beta$ -lactams) fail this test. Antibiotics typically have a large molecular mass, but compounds with a molecular weight above 1000 daltons are rarely volatile.<sup>97</sup> Since they are typically non-volatile they resist the transition into a gaseous state which is required by GC-MS (hence ‘gas’ spectrometry). Forcing them to vaporize into a gas requires extreme heat, but due to their thermal lability that very heat destroys their delicate structures before they can be turned into a vapor.<sup>98</sup> It is like trying to analyze a raw egg by boiling it. You do not get a "gaseous egg," you just get a hard-boiled egg. Furthermore, the aqueous output of essential sample preparation techniques like solid-phase extraction could not be easily coupled to the dry conditions of a GC inlet. This fundamental gas/liquid mismatch is the technical reason why antibiotics remained chemically invisible for so long considering the tools that were available during the 1970s and much of the 1980s. A new approach was needed, one that could analyze compounds directly from a liquid state without destructive heat. This fundamental incompatibility created an analytical wall that forced scientists to seek a new approach which could analyze non-volatile compounds without destructive heat. Even the thermospray, the most commercially successful interface of the era, while successful for many polar compounds, still relied on high heat that could degrade the most fragile antibiotics. What finally broke this impasse was the maturation and commercialization of

---

<sup>97</sup> Chew et al., “Choices of Chromatographic Methods as Stability Indicating Assays for Pharmaceutical Products”; Richardson, “Environmental Mass Spectrometry.”

<sup>98</sup> Niessen, *Liquid Chromatography-Mass Spectrometry*.

a suite of technologies that, when integrated, solved the core challenges of sample preparation, ionization, and detection.

Before a sample could even reach a mass spectrometer, the target analytes had to be isolated and concentrated from the complex environmental matrix. The process began by taking an environmental sample out in the field. This could be water, soil, sludge, or some other material. For water samples, an SPE format (typically a cartridge or disk packed with sorbent material) can be used while out in the field. SPE was generally the approach of choice as it “eliminate[d] several disadvantages, including many organic solvents, a lengthy operation time and numerous steps, potential sources of error, and high costs” of preparing an environmental sample.<sup>99</sup> Cartridges and disks accomplish the same task but differ in how they process water. Cartridges are typically more compact than disks but have a more restricted flow rate which reduces the speed of processing and increases the likelihood of clogging when particulates are present in the water sample.<sup>100</sup>

An important contribution to SPE came in 1996 with the commercial introduction of hydrophilic-lipophilic balanced (HLB) polymeric sorbents, most notably the Waters Corporation Oasis HLB cartridge. At introduction these sorbents offered clear advantages. They could directly accept samples without preconditioning and were designed to capture a broad spectrum of analytes (“acidic, basic and neutral compounds whether polar or non-polar”).<sup>101</sup> But the method was not perfected from the start. Early users had to refine conditioning, washing, and

---

<sup>99</sup> Badawy et al., “A Review of the Modern Principles and Applications of Solid-Phase Extraction Techniques in Chromatographic Analysis.”

<sup>100</sup> Hennion, “Solid-Phase Extraction.”

<sup>101</sup> Richardson, “Environmental Mass Spectrometry”; Badawy et al., “A Review of the Modern Principles and Applications of Solid-Phase Extraction Techniques in Chromatographic Analysis.”

elution steps before the cartridges delivered consistent recoveries across diverse antibiotics.

Through iterative refinements over the late 1990s, HLB developed into a robust, reliable, and forgiving technology for routine use. By the late 1990s, the mature HLB method had become the standard clean-up step: it removed matrix interferences, enabled multi-residue extraction with a single cartridge, and unlocked the full analytical sensitivity of LC-API-MS systems, allowing signals to be resolved against noisy backgrounds.

The water sample is passed through the disk or cartridge, either through the application of light pressure from the top of the housing via a piston or a vacuum applying downward pressure through the housing.<sup>102</sup> Ideally this water is filtered for particulate matter before being introduced to the cartridge or disk, but some specialized disk formats have been designed to filter particulates while also adsorbing (not absorbing) compounds of interest.<sup>103</sup>

If the sample of interest is not originally liquid (such as soil, sludge, or sediment), an extra step (called solid-liquid extraction) needs to be applied to turn the solid matrix sample into a liquid phase. The sample is collected by core sample or by scooping, then transported to the laboratory. A solvent (like hot water, acetonitrile, or acetone, selected depending on what compound the researcher wishes to extract) is applied to the solid sample. This solvent diffuses through the solid matrix and dissolves the target compounds. The liquid solvent is then extracted from the sample, at which point it can be fed into a SPE cartridge or disk to separate the analytes of interest from other contaminants contained in the solvent.<sup>104</sup>

---

<sup>102</sup> Badawy et al., “A Review of the Modern Principles and Applications of Solid-Phase Extraction Techniques in Chromatographic Analysis.”

<sup>103</sup> Hennion, “Solid-Phase Extraction.”

<sup>104</sup> Berk, “Chapter 11 - Extraction”; Vas and Vékey, “Solid-phase Microextraction”; Badawy et al., “A Review of the Modern Principles and Applications of Solid-Phase Extraction Techniques in Chromatographic Analysis”; Niessen, *Liquid Chromatography-Mass Spectrometry*.

Once the analytes have bonded to the sorbent, the sorbents are then washed to remove any remaining contaminants, then eluted with an appropriate solvent to release the analytes from the sorbent material into the liquid phase solvent, which is then fed into the liquid chromatography system.<sup>105</sup>

A chromatographic system generally consists of four main parts: a pump, an injector, a stationary phase, and a detector.<sup>106</sup> First, a pump pressurizes the liquid mobile phase (the sample purified using SPE) so it flows through the system at a constant rate (which reduces the likelihood of imprecise or incorrect measurements). Next, a loop injector introduces a small, precise volume of the purified sample from the SPE step into this flowing mobile phase. The mobile phase then carries the sample into the stationary phase within the analytical column. For antibiotics, this is almost always a reversed-phase column with a non-polar surface.<sup>107</sup>

The separation occurs based on the differing affinities of the antibiotic molecules for the stationary and mobile phases. The most polar antibiotics have a low affinity for the non-polar column and prefer to stay in the polar mobile phase. They are swept through the column quickly, resulting in a short elution time (or the amount of time it takes them to move through the column). Conversely, less-polar antibiotics have a higher affinity for the non-polar stationary phase, causing them to be retained longer and resulting in a long elution time. This process separates the initial SPE purified mixture into a sequence of individual compounds, which then enter the detector (almost always a mass spectrometer) for identification.<sup>108</sup>

---

<sup>105</sup> Badawy et al., "A Review of the Modern Principles and Applications of Solid-Phase Extraction Techniques in Chromatographic Analysis."

<sup>106</sup> Ardrey, *Liquid Chromatography-Mass Spectrometry*.

<sup>107</sup> Niessen, *Liquid Chromatography-Mass Spectrometry*; Ardrey, *Liquid Chromatography-Mass Spectrometry*.

<sup>108</sup> Majors, *Historical Developments in HPLC and UHPLC Column Technology*; Niessen, *Liquid Chromatography-Mass Spectrometry*; Badawy et al., "A Review of the Modern Principles and

The final stage of analyzing a sample occurs in the mass spectrometer, but actually getting the individual compounds into the detector was also a problem that needed solving. Coupling chromatography with mass spectrometry was one of the primary challenges in this history, and also explains why non-antibiotics were detected much more frequently than antibiotics in environmental samples until the introduction of two atmospheric pressure ionization (API) techniques: electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI).

<b>LC-MS Interface Technology</b>	<b>Year of Commercial Availability</b>	<b>Operating Principle</b>
Moving-Belt Interface (MBI)	1977	Deposits LC effluent onto conveyor belt into MS
Direct Liquid Introduction (DLI)	1980	Sprays LC effluent directly into MS
Thermospray (TSP)	1983	Heated tube vaporizes LC effluent before spraying into MS
Electrospray Ionization (ESI)	1989	Uses high voltage to nebulize LC effluent into spray. Residual charge is transferred to analytes, which then are transferred to MS
Atmospheric Pressure Chemical Ionization (APCI)	1989	Uses heated tube to evaporate LC effluent, then ionizes gas-vapor mixture with corona discharge needle and transfers analytes into MS

Table 1. Chronology of selected LC-MS interfaces.

The interface between the liquid chromatography system and the mass spectrometer is crucial because it bridges two fundamentally different environments. Liquid chromatography requires high pressure liquid to separate analytes into groups, but mass spectrometry requires a strong vacuum (about  $10^{-6}$  Torr) and a gaseous mixture of ions.<sup>109</sup> The interface between each system resolves this incompatibility by turning the liquid phase into a fine aerosol or vapor from which ions can be generated and sampled into the vacuum.

The earliest attempts during the 1970s and 1980s at bridging the LC and MS gap were innovative, but each solution introduced its own set of frustrating trade-offs that made the detection of antibiotics unfeasible (see Table 1 above). These technologies formed a graveyard of promising but ultimately flawed interfaces that proved unsuitable for the delicate task of routinely identifying trace-level, thermally labile (that is, easily broken down by heat) antibiotic compounds in complex samples.

One of the earliest approaches, Direct Liquid Introduction (DLI), attempted the most straightforward solution: spraying a fine jet of LC effluent directly into the mass spectrometer. While mechanically simple and avoiding the destructive heat of other methods, its design was its downfall. To maintain the MS vacuum, the DLI could only accept an input flow rate of about 50  $\mu\text{l}/\text{minute}$  or less, while the LC output about 500-1000  $\mu\text{l}/\text{minute}$ . As a result, the DLI needed to discard about 96-99% of the sample.<sup>110</sup> This crippling loss of sensitivity made it impractical for the trace-level analysis required in environmental monitoring. Additionally, the tiny pinhole

---

<sup>109</sup> Ardrey, *Liquid Chromatography-Mass Spectrometry*.

<sup>110</sup> de Koster and Schoenmakers, "Chapter 3.1 - History of Liquid Chromatography—Mass Spectrometry Couplings"; Niessen, *Liquid Chromatography-Mass Spectrometry*; Ardrey, *Liquid Chromatography-Mass Spectrometry*.

required for the spray was notoriously prone to clogging, making the entire system unreliable for routine use.<sup>111</sup>

A more mechanically ambitious solution was the Moving-Belt Interface (MBI), which used a tiny conveyor belt to process the sample then physically carry it into the mass spectrometer. The liquid mobile phase effluent was first sprayed onto the belt, then the solvent was evaporated off with heaters and vacuums until just the analytes remained. Once the solvent had evaporated, the dried analyte was then flash desorbed or vaporized into the ion source.<sup>112</sup> This design offered the major advantage of allowing chemists to use traditional electron ionization methods they were familiar with.<sup>113</sup> However, the MBI was a mechanically complex and finicky device. It suffered from "memory effects," where traces of a previous sample would cling to the belt and contaminate the next analysis, reducing reliability and increasing busywork for the scientist. More importantly, its reliance on heat to evaporate the solvent could still degrade thermally labile antibiotics, and its inability to handle some solvents favored by scientists for separating polar compounds made the detection of some antibiotics impossible.<sup>114</sup>

The most commercially successful interface of the 1980s was thermospray (TSP). It represented a breakthrough as it was the "first [widely available] spray interface that could be

---

<sup>111</sup> de Koster and Schoenmakers, "Chapter 3.1 - History of Liquid Chromatography—Mass Spectrometry Couplings."

<sup>112</sup> Pullen, "The Fascinating History of the Development of LC-MS; a Personal Perspective"; de Koster and Schoenmakers, "Chapter 3.1 - History of Liquid Chromatography—Mass Spectrometry Couplings"; Ardrey, *Liquid Chromatography-Mass Spectrometry*.

<sup>113</sup> Niessen, *Liquid Chromatography-Mass Spectrometry*; Ardrey, *Liquid Chromatography-Mass Spectrometry*.

<sup>114</sup> Niessen, *Liquid Chromatography-Mass Spectrometry*; de Koster and Schoenmakers, "Chapter 3.1 - History of Liquid Chromatography—Mass Spectrometry Couplings"; Ardrey, *Liquid Chromatography-Mass Spectrometry*; Pullen, "The Fascinating History of the Development of LC-MS; a Personal Perspective."

used to couple mainstream LC systems” to mass spectrometers.<sup>115</sup> TSP uses a heated probe to rapidly vaporize the liquid mobile phase, making it the first interface capable of handling the high flow rates of reversed-phase chromatography. Key to its operation was a heated probe that rapidly vaporized the LC effluent and an ion skimmer that sampled only the core of the resulting spray into the mass spectrometer. This skimmer design was one of the primary reasons TSP could manage high flow rates. Ionization happened directly within the spray through a process of solvent-induced chemical ionization, using ions already present in the liquid. This synergy with LC systems "heralded the beginning of biological MS" by enabling, for the first time, the routine analysis of highly polar analytes.<sup>116</sup>

The problem with TSP was that its reliance on heat – while effective for creating a stable spray – remained a threat to the most delicate molecules. For certain classes of antibiotics like  $\beta$ -lactams, the high temperatures of the TSP source were observed to cause degradation, breaking the molecule's characteristic ring structure.<sup>117</sup> Once again the thermal lability of antibiotics was a limiting factor in their ability to be passed between the liquid chromatography system and the mass spectrometer.

Other specialized interfaces also revealed the breadth of the challenge. The particle-beam interface (PBI) (also known as the monodisperse-aerosol-generator interface or MAGIC) was another attempt to achieve high quality analysis, but it too struggled with thermal lability and

---

<sup>115</sup> de Koster and Schoenmakers, “Chapter 3.1 - History of Liquid Chromatography—Mass Spectrometry Couplings.”

<sup>116</sup> Hennion, “Solid-Phase Extraction”; Niessen, *Liquid Chromatography-Mass Spectrometry*; de Koster and Schoenmakers, “Chapter 3.1 - History of Liquid Chromatography—Mass Spectrometry Couplings”; Ardrey, *Liquid Chromatography-Mass Spectrometry*.

<sup>117</sup> de Koster and Schoenmakers, “Chapter 3.1 - History of Liquid Chromatography—Mass Spectrometry Couplings.”

some solvents used for antibiotics.<sup>118</sup> Continuous-Flow Fast-Atom Bombardment (CF-FAB), a forerunner to modern techniques, could handle highly polar and ionic compounds but was hampered by its own set of limitations: it required impractically low flow rates resulting in extremely low sensitivity (even lower than DLI), and the ionization technique used provided very little structural information needed to identify complex compounds.<sup>119</sup>

Each of these technologies solved one part of the puzzle while failing at another, leaving the scientific community searching for a single, robust interface that could gently, reliably, and sensitively analyze the full, diverse spectrum of antibiotic compounds. These limitations meant that LC-MS, despite its potential, was not widely adopted as a routine analytical technique for pharmaceuticals in water.

The breakthrough that finally solved the interface puzzle arrived with the maturation and commercial availability of API interfaces around the early 1990s. Unlike previous interfaces that tried to force samples into the harsh vacuum before ionizing them, API's revolutionary insight was to flip the process: the ions were generated first, directly from the liquid effluent at atmospheric pressure, and *then* guided into the mass spectrometer.<sup>120</sup> This approach offered incredible sensitivity while its "soft" ionization processes preserved the molecular structure of thermally labile antibiotics (like  $\beta$ -lactam class antibiotics), solving the two key problems that had plagued earlier technologies.

---

<sup>118</sup> Niessen, *Liquid Chromatography-Mass Spectrometry*; de Koster and Schoenmakers, "Chapter 3.1 - History of Liquid Chromatography—Mass Spectrometry Couplings"; Ardrey, *Liquid Chromatography-Mass Spectrometry*.

<sup>119</sup> Niessen, *Liquid Chromatography-Mass Spectrometry*; de Koster and Schoenmakers, "Chapter 3.1 - History of Liquid Chromatography—Mass Spectrometry Couplings"; Ardrey, *Liquid Chromatography-Mass Spectrometry*.

<sup>120</sup> Ardrey, *Liquid Chromatography-Mass Spectrometry*; Niessen, *Liquid Chromatography-Mass Spectrometry*.

While the concept of API was explored as early as 1975 by Horning, Dziric, and Carrol, the paradigm shift was driven by the parallel, pioneering work of two research groups in the 1980s. At Cornell University, Jack Henion and colleagues demonstrated the power of an LC-APCI-MS approach for drug determination in biological fluids in 1982.<sup>121</sup> Around the same time at Yale University, John Fenn and colleagues developed the first atmospheric pressure electrospray source, a breakthrough that would later contribute to Fenn being awarded a Nobel Prize.<sup>122</sup> Both Henion and Fenn’s work rapidly demonstrated the extraordinary sensitivity of these new ionization techniques for compounds such as antibiotics, fundamentally reshaping the practice of liquid chromatography.<sup>123</sup> Contemporary scientists described these interfaces with phrases like “a marriage made in heaven,”<sup>124</sup> saying that our capabilities to measure antibiotics without it “would not exist.”<sup>125</sup> Although these foundational inventions occurred in the 1980s, their widespread adoption had to wait for commercial availability in the early 1990s, and the first dedicated environmental applications in the mid-to-late 1990s.<sup>126</sup> This new toolkit was defined by two complementary technologies: Atmospheric Pressure Chemical Ionization (APCI) and Electrospray Ionization (ESI).

<b>Technique</b>	<b>Typical Mass Range (Da)</b>	<b>Polarity Range</b>	<b>Works Well For</b>	<b>Limitations For Antibiotics</b>
------------------	--------------------------------	-----------------------	-----------------------	------------------------------------

<sup>121</sup> Henion et al., “Determination of Sulfa Drugs in Biological Fluids by Liquid Chromatography/Mass Spectrometry/Mass Spectrometry.”

<sup>122</sup> Pitt, “Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry”; de Koster and Schoenmakers, “Chapter 3.1 - History of Liquid Chromatography—Mass Spectrometry Couplings.”

<sup>123</sup> Pullen, “The Fascinating History of the Development of LC-MS; a Personal Perspective.”

<sup>124</sup> Pullen, “The Fascinating History of the Development of LC-MS; a Personal Perspective.”

<sup>125</sup> Jones et al., “Aquatic Environmental Assessment of the Top 25 English Prescription Pharmaceuticals.”

<sup>126</sup> Niessen, *Liquid Chromatography-Mass Spectrometry*; Pullen, “The Fascinating History of the Development of LC-MS; a Personal Perspective.”

GC-MS (EI)	Up to ~800 Da	Non-polar to moderately polar	Volatile, thermally stable pollutants (e.g., pesticides)	Most antibiotics are too polar and thermally labile; derivatization rarely practical
APCI	~100-1000 Da	Low to moderate polarity (overlaps GC-MS and ESI)	Moderately polar antibiotics (e.g., some sulfonamides, quinolones)	Less effective for highly polar antibiotics
ESI	~200-10,000+ Da	Moderate to high polarity	Polar antibiotics (e.g., tetracyclines, macrolides, $\beta$ -lactams, vancomycin)	Less suitable for non-polar antibiotics

Table 2. Relative applicability of major ionization techniques to antibiotics in aquatic environmental samples.

APCI proved to be a valuable tool for the few less- to moderately-polar antibiotics that could withstand being heated into a vapor. It works by using a high-voltage corona discharge needle to create a cascade of primary electrons, which ionize the vaporized solvent from the mobile phase. These solvent ions then act as reagents in a series of gentle, gas-phase chemical reactions that transfer a charge to the analyte molecules, allowing them to be detected without fragmentation. This "soft," low-energy event preserved the molecular structure of the antibiotic, providing a clear signal for its molecular weight.<sup>127</sup> One of the earliest published applications demonstrating its potential was a 1995 study by Doerge and Bajic, who used an APCI interface

---

<sup>127</sup> Ardrey, *Liquid Chromatography-Mass Spectrometry*; Niessen, *Liquid Chromatography-Mass Spectrometry*; de Koster and Schoenmakers, "Chapter 3.1 - History of Liquid Chromatography—Mass Spectrometry Couplings."

for the multi-residue determination of quinolone antibiotics in catfish tissue.<sup>128</sup> While a biological matrix, this study showcased the method's utility for complex samples relevant to environmental pathways and was soon followed by applications to explicitly environmental matrices, such as the analysis of sulfonamides in manure in 2002.<sup>129</sup>

The true revolution, however, came with Electrospray Ionization (ESI), a remarkably gentle technique that finally allowed for analyzing a wide range of highly polar and thermally fragile antibiotics. ESI completely sidestepped the problem of heat by using a strong electric field to spray the liquid sample into a fine, charged mist. As the tiny droplets evaporated, the electrical charge became concentrated on the antibiotic molecules, turning them into gas-phase ions without ever needing to boil or bake them. This ability to create ions directly from a liquid solution was a "great impact," particularly for compounds like aminoglycosides and  $\beta$ -lactams that were notoriously prone to thermal degradation.<sup>130</sup>

Following its commercialization in the early 1990s,<sup>131</sup> a landmark study that marked the solidification of ESI as the go-to technique for environmental antibiotic monitoring was published in 1998 by Hirsch et al.<sup>132</sup> They successfully used LC-ESI-MS/MS to quantify 18 different antibiotics across six major classes in various water compartments, achieving the low

---

<sup>128</sup> Doerge and Bajic, "Multiresidue Determination of Quinolone Antibiotics Using Liquid Chromatography Coupled to Atmospheric-Pressure Chemical Ionization Mass Spectrometry and Tandem Mass Spectrometry."

<sup>129</sup> Pfeifer et al., "Determination of Selected Sulfonamide Antibiotics and Trimethoprim in Manure by Electrospray and Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry"; Niessen, *Liquid Chromatography-Mass Spectrometry*.

<sup>130</sup> Pitt, "Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry."

<sup>131</sup> Pullen, "The Fascinating History of the Development of LC-MS; a Personal Perspective"; Niessen, *Liquid Chromatography-Mass Spectrometry*.

<sup>132</sup> Hirsch et al., "Determination of Antibiotics in Different Water Compartments via Liquid Chromatography-Electrospray Tandem Mass Spectrometry."

nanogram-per-liter detection limits necessary for environmental relevance. This breakthrough was immediately followed by a wave of validating studies, such as Hartig et al. (1999),<sup>133</sup> who used LC-ESI-MS/MS to detect sulfonamides in municipal wastewater, cementing ESI's role as a comprehensive tool for making antibiotic pollution visible in aquatic systems.

Together, the complementary nature of APCI and ESI created a powerful new toolkit. For the first time, scientists had reliable, sensitive, and "soft" ionization methods that could handle virtually any antibiotic thrown at them. This technological convergence provided the "incredible sensitivity" needed to find antibiotics at the low nanogram-per-liter concentrations relevant to environmental systems.<sup>134</sup> The arrival of API was an essential piece of the technological puzzle that contributed to the transformation of antibiotic pollution from a series of isolated, difficult detections into a coherent and measurable field of inquiry.

Now that these compounds could be purified with SPE, separated with liquid chromatography, and softly ionized, they could be fed into the mass spectrometer for identification. However, the interface only solved the problem of getting the molecules into the instrument; the analyzer itself had to be able to find and definitively identify them in a complex environmental sample.

This presented another analytical challenge. Unlike GC-MS, which benefited from extensive mass spectral libraries and robust electron ionization (EI) that provided rich structural information, early LC-MS systems with their "soft" ionization techniques were different. While

---

<sup>133</sup> Hartig et al., "Detection and Identification of Sulphonamide Drugs in Municipal Waste Water by Liquid Chromatography Coupled with Electrospray Ionisation Tandem Mass Spectrometry."

<sup>134</sup> Pullen, "The Fascinating History of the Development of LC-MS; a Personal Perspective"; Hirsch et al., "Determination of Antibiotics in Different Water Compartments via Liquid Chromatography-Electrospray Tandem Mass Spectrometry."

excellent at determining a molecule's weight, they provided very little of the fragmentation data needed to confirm its precise structure. This lack of comprehensive spectral libraries for LC-MS made identifying unknown compounds difficult and created a risk of false positives based on molecular weight alone.<sup>135</sup>

The solution was tandem mass spectrometry (MS/MS), but to understand its power, one must first understand the core component on which it relies. At the heart of the mass spectrometer is a mass filter, a component that uses electric fields to separate ions based on their mass-to-charge ratio.<sup>136</sup> The most common type, and the one crucial to this history, is the quadrupole – a precise arrangement of four parallel metal rods. By applying specific, oscillating voltages to these rods, a stable flight path is created down the center, but only for ions of a single, targeted mass-to-charge ratio. All other ions have unstable trajectories, causing them to collide with the rods and never reach the detector.<sup>137</sup> Quadrupole-based mass spectrometers at this time were typically built with a single quadrupole (just MS) or three quadrupoles (called tandem MS, MS/MS, or MS<sup>2</sup>).<sup>138</sup>

A basic mass spectrometer with a single quadrupole is like a librarian searching for a book based only on its total number of pages. It can pull every 400-page book from the shelves, but in a large library, many different books will match that single criterion. This lack of

---

<sup>135</sup> Chew et al., “Choices of Chromatographic Methods as Stability Indicating Assays for Pharmaceutical Products”; Richardson, “Environmental Mass Spectrometry”; Niessen, *Liquid Chromatography-Mass Spectrometry*.

<sup>136</sup> Pitt, “Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry”; Ardrey, *Liquid Chromatography-Mass Spectrometry*; Niessen, *Liquid Chromatography-Mass Spectrometry*.

<sup>137</sup> Niessen, *Liquid Chromatography-Mass Spectrometry*; Ardrey, *Liquid Chromatography-Mass Spectrometry*.

<sup>138</sup> Pitt, “Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry”; Niessen, *Liquid Chromatography-Mass Spectrometry*.

specificity makes it difficult to be certain you have found the exact item you are looking for, creating the risk of false positives.

Tandem mass spectrometry (MS/MS), performed with a triple quadrupole system, solves this problem of non-specificity. This technique is like giving the librarian three successive, highly specific instructions to find one unique edition of a single book. First, the initial quadrupole (Q1) acts as the first filter, instructed to pull only the 400-page books from the shelf (the *parent ion*). Next, in the collision cell (Q2), the librarian performs a confirmation step: they open each selected book to a specific page where a unique, identifying phrase is known to exist, analogous to fragmenting the parent ion into its predictable smaller pieces (*daughter ions*). Finally, the third quadrupole (Q3) performs the ultimate verification, scanning for that exact identifying phrase (a specific *daughter ion*) and discarding any books that do not match.<sup>139</sup> This multi-step verification, known as Selected Reaction Monitoring (SRM),<sup>140</sup> allowed scientists to find a specific molecule of interest with near-perfect certainty, even at trace concentrations in a chemically noisy sample. It was this ability to definitively find the molecular "book" in an environmental "library" that made the triple quadrupole mass spectrometer an essential tool for measuring aquatic antibiotic pollution.

In 1989 SCIEX released the API III, the first widely adopted system that integrated high performance liquid chromatography; an atmospheric-pressure ionization interface, most often configured as electrospray with APCI options beginning to appear at the same time; and

---

<sup>139</sup> Pitt, "Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry"; Niessen, *Liquid Chromatography-Mass Spectrometry*.

<sup>140</sup> Richardson, "Environmental Mass Spectrometry"; Pitt, "Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry"; Badawy et al., "A Review of the Modern Principles and Applications of Solid-Phase Extraction Techniques in Chromatographic Analysis."

triple-quadrupole mass spectrometry in one package.<sup>141</sup> This was identified by scientists as what “unarguably [led] the way for the acceptance of API LC/MS techniques” required for the measurement of antibiotics in environmental samples.<sup>142</sup> Indeed, a survey of investigations after the introduction of the API III in 1989 reveals strong uptake by the mid-1990s by both governmental organizations (for instance, the US EPA, albeit with the non-volatile and low molecular weight pesticide Clothianidin and its degradates)<sup>143</sup> and academic research labs.<sup>144</sup>

While the SCIEX API III was commercially introduced in 1989, its transformation of the field was not immediate. A period of maturation was necessary before it saw widespread adoption. By the mid-1990s, improvements in the price and performance of LC-MS instruments made them accessible beyond specialized research labs and into clinical and environmental monitoring settings.<sup>145</sup> The system's performance was a monumental leap over previous technologies. Its atmospheric pressure-to-vacuum interface was vastly more sensitive than earlier designs and solved critical practical problems like clogging and spectral noise, making it far more robust and reliable for routine use.<sup>146</sup> This reliability, combined with the high specificity of

---

<sup>141</sup> Niessen, *Liquid Chromatography-Mass Spectrometry*.

<sup>142</sup> Thomson, “Atmospheric Pressure Ionization and Liquid Chromatography/Mass Spectrometry—Together at Last.”

<sup>143</sup> Cassidy et al., *Analytical Method for the Determination of TI-435 and the Degradates, TZNG, TZMU, MNG, and TMG in Soil by Liquid Chromatography with APCI MS/MS-Detection*.

<sup>144</sup> Pullen, “The Fascinating History of the Development of LC-MS; a Personal Perspective”; Niessen, *Liquid Chromatography-Mass Spectrometry*; Ardrey, *Liquid Chromatography-Mass Spectrometry*; Pitt, “Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry”; Thomson, “Atmospheric Pressure Ionization and Liquid Chromatography/Mass Spectrometry—Together at Last”; Homem and Santos, “Degradation and Removal Methods of Antibiotics from Aqueous Matrices--a Review.”

<sup>145</sup> Pitt, “Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry,” 19.

<sup>146</sup> Thomson, “Atmospheric Pressure Ionization and Liquid Chromatography/Mass Spectrometry—Together at Last,” 191; SCIEX, “The History of SCIEX”; Pullen, “The Fascinating History of the Development of LC-MS; a Personal Perspective”; Niessen, *Liquid Chromatography-Mass Spectrometry*.

its integrated triple-quadrupole analyzer, gave scientists the confidence to definitively identify compounds at trace levels. The new API LC-MS/MS approach also enabled high-throughput analysis with early demonstrations showing sample processing times as fast as one per minute, a speed essential for the large-scale screening that environmental reconnaissance demanded. It was this combination of increasing accessibility, revolutionary sensitivity, and high-speed capability that established the technology as the essential tool that would finally make widespread antibiotic pollution visible.

By integrating robust sample preparation from SPE, "soft" ionization from API interfaces, and the specificity of tandem mass spectrometry, the analytical revolution was complete. For the first time, scientists possessed a reliable and sensitive toolkit capable of moving beyond the biological shadows to measure the chemical source of pollution directly. The following chapter details how this new technical capability converged with a political and public health crisis powerful enough to transform what was *possible* to measure into what was *essential* to know.

### **Chapter 3: Convergence and Formation: A New Field Emerges (1998-2002)**

The previous chapter detailed the analytical revolution that, by 1998, provided the scientific community with the tools to make antibiotic pollution visible. However, technology alone does not create a new field of science. This chapter argues that the formation of aquatic antibiotic pollution as a coherent area of inquiry required the convergence of this new technical capability with a powerful external force: a growing political and public health crisis over antibiotic resistance. It was the fusion of the means to measure with the mandate to look that, between 1998 and 2002, produced the intellectual frameworks, methodological blueprints, and regulatory will to transform a series of isolated findings into a recognized environmental issue.

Although the roots of this public health mandate lie in the early 1990s, its formation into concrete scientific and political action occurred after 1998. This chapter will first trace these crucial antecedents before focusing on the 1998-2002 period.

#### ***A New Public Health Mandate***

As the new analytical tools began to emerge from specialized labs in the 1990s, researchers started applying them to known contamination hotspots. In 1990 and 1991, studies by Björklund et al. continued to confirm the persistence of antibiotics in sediments beneath fish farms, reinforcing aquaculture as a significant and now measurable source of pollution.<sup>147</sup> A more critical turning point came in 1994, when research by Stan et al. (1994) explicitly linked the presence of the lipid-regulating drug clofibric acid in Berlin tap water to its use in human

---

<sup>147</sup> Björklund et al., “Residues of Oxytetracycline in Wild Fish and Sediments from Fish Farms”; Björklund et al., “Residues of Oxolinic Acid and Oxytetracycline in Fish and Sediments from Fish Farms.”

medicine.<sup>148</sup> Although clofibric acid is not an antibiotic, it nonetheless provided proof that pharmaceuticals consumed by the public could survive wastewater treatment and persist directly into aquatic environments, including drinking water. Regulators were now armed with evidence that the possibility of receiving an active dose of drugs through seemingly potable water was a problem to be seriously considered. These early signals, made visible by the nascent technology, helped build the factual foundation for the wave of political and social anxiety that was about to mount. The rapid uptake of these findings by policymakers reflected the unusually charged context of the 1990s: antibiotic resistance was already framed as a looming medical crisis, regulators were primed by prior controversies over pesticides and hormones in food, and environmental agencies were under pressure to demonstrate responsiveness to emerging risks. As a result, even modest detections of pharmaceuticals in tap water or sediments resonated far beyond the lab. Scientists could present their results not as abstract traces but as evidence of a direct pathway from human consumption to human exposure, a framing that lent itself immediately to public health mandates. This convergence of heightened scientific credibility and political sensitivity explains how actors like the House of Lords could move from first reports to formal debate within only a few years.

A pivotal moment came in 1992 with the release of the U.S. Institute of Medicine's (IoM) report, *Emerging Infections: Microbial Threats to Health in the United States*.<sup>149</sup> The report's unique power to galvanize scientific and political opinion, where previous warnings had failed, stemmed from its framing, historical timing, and strategic dissemination. Previous efforts had

---

<sup>148</sup> Stan et al., "Occurrence of Clofibric Acid in the Aquatic System-- Is the Use in Human Medical Care the Source of the Contamination of Surface, Ground and Drinking Water?"

<sup>149</sup> Institute of Medicine (US) Committee on Emerging Microbial Threats to Health, *Emerging Infections*, ed. Lederberg et al. (National Academies Press (US), 1992).

often focused narrowly on specific issues like antibiotic residues in food,<sup>150</sup> but the IoM report, pushed by Nobel laureate Joshua Lederberg, instead defined "emerging infectious diseases" as a broad-scale microbial threat and explicitly included antibiotic resistance as a core component. Critically, it linked this threat to "systemic complacency and the collapse of public health infrastructure," a particularly resonant message in an era still grappling with the AIDS epidemic.<sup>151</sup>

This alarming frame was amplified by a powerful "scale politics" that connected local actions to global consequences.<sup>152</sup> As Lederberg famously articulated, "[t]he microbe that felled one child in a distant continent yesterday can reach yours today and seed a global pandemic tomorrow."<sup>153</sup> Like microbes, individual prescriptions could have global repercussions. This narrative was backed by concrete, actionable recommendations for government agencies, including calls for enhanced surveillance, user education, and new incentives for drug development. This combination of a broad, alarming frame and a clear policy roadmap was then skillfully translated into an eye-catching story for the public by influential science writers and major media outlets.

Two Pulitzer prize winning works – Laurie Garrett’s 1994 book *The Coming Plague*,<sup>154</sup> and Mike Toner’s *When Bugs Fight Back*, a 10-part investigative journalism piece published in the *Atlanta Journal-Constitution*<sup>155</sup> – translated the issue to non-scientists, while provocative

---

<sup>150</sup> Podolsky, *The Antibiotic Era*; Kirchhelle, *Pyrrhic Progress*.

<sup>151</sup> Podolsky, *The Antibiotic Era*.

<sup>152</sup> Podolsky, *The Antibiotic Era*; Kirchhelle, *Pyrrhic Progress*.

<sup>153</sup> Lederberg, "Medical Science, Infectious Disease, and the Unity of Humankind."

<sup>154</sup> Garrett, *The Coming Plague*.

<sup>155</sup> *The New York Times*, "Winners of the 1993 Pulitzer Prizes for Journalism, Literature and the Arts"; Podolsky, *The Antibiotic Era*.

headlines like “Revenge of the Killer Microbes” in *Time*,<sup>156</sup> and “Drug resistance: The next AIDS crisis” in New York’s *The Village Voice*<sup>157</sup> allowed members of the public to imagine a potentially disastrous future resulting from antibiotic pollution and resistance. This media attention elevated antibiotic resistance from a clinical concern to a national crisis, and the IoM’s report explicitly called for U.S. government agencies like the CDC, FDA, and USDA to establish measures for educating healthcare and agricultural users on rational antibiotic use and, crucially, to conduct surveillance of newly resistant organisms.<sup>158</sup>

While experts and policymakers were beginning to frame antibiotic resistance as a national crisis, public perception lagged significantly behind, still focused on the classical pollutants of a previous era. This disconnect was captured in a 1994 proceeding on public perceptions of freshwater issues, where O’Connor and colleagues observed that while “most people view water quality problems as serious and getting worse,” they also “tend to think that serious water problems are somewhere else.”<sup>159</sup>

This perception that the problem was distant and abstract stemmed from the fact that the threat of antibiotic pollution was still chemically invisible to the public. Decades of focus on industrial chemicals and pesticides had shaped public concern, leaving little room for a niche, unseen contaminant like antibiotics. This public blind spot highlights a challenge facing regulatory efforts to address antibiotic pollution: the looming threat was scientifically real but not yet socially resonant, a reality that the US Geological Survey national reconnaissance study at

---

<sup>156</sup> Time Magazine, “Revenge of the Killer Microbes.”

<sup>157</sup> Schoofs, “Drug Resistance.”

<sup>158</sup> Institute of Medicine (US) Committee on Emerging Microbial Threats to Health, *Emerging Infections*, ed. Lederberg et al. (National Academies Press (US), 1992); Podolsky, *The Antibiotic Era*.

<sup>159</sup> O’Connor et al., “Public Perceptions of Fresh Water Issues.”

the end of the decade overturned, by demonstrating that antibiotic residues were already widespread and environmentally significant.

This call to action created a domino effect throughout the scientific and public health communities. The American Society for Microbiology (ASM) followed with its own task force report in 1995, calling for coordinated surveillance, decreased antibiotic usage in animal husbandry, and increased research funding.<sup>160</sup> The CDC, leveraging the IoM report's momentum, produced its own landmark strategy in 1994 and saw its funding for emerging infections skyrocket from \$1 million in 1994 to \$59.1 million by 1998.<sup>161</sup> Most importantly, this push resulted in the creation of concrete systems to gather data on antibiotic resistance and pollution. In 1996, the CDC partnered with the FDA and USDA to establish the National Antibiotic Resistance Monitoring System (NARMS), a program designed to test for resistance in bacteria like *Salmonella* and *Campylobacter*.<sup>162</sup> This institutionalization of surveillance created a direct, government-backed demand for the very analytical capabilities that were just becoming available. Crucially, these new surveillance systems were still tracking the biological shadow of resistance; the alarming data they produced, however, created an intense demand to find and quantify the chemical cause (the antibiotics themselves) in the environment.

This sense of urgency was global. European nations, facilitated by the EU, became highly attentive to the issue. After reports linked the growth promoter antibiotic avoparcin to cross-resistance with a clinically important antibiotic vancomycin, Denmark issued a ban on

---

<sup>160</sup> Jones, “The Emergent Needs for Basic Research, Education, and Surveillance of Antimicrobial Resistance. Problems Facing the Report from the American Society for Microbiology Task Force on Antibiotic Resistance”; Podolsky, *The Antibiotic Era*.

<sup>161</sup> US Centers for Disease Control and Prevention, *Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States*; Podolsky, *The Antibiotic Era*.

<sup>162</sup> Kirchhelle, *Pyrrhic Progress*; Podolsky, *The Antibiotic Era*.

avoparcin's use in animal feed in 1995, which was soon followed by other EU members.<sup>163</sup> The WHO convened meetings throughout the decade, with a 1997 session reiterating calls to exclude key antibiotics from animal feed and a 1998 meeting warning against the use of quinolones for "performance enhancement" in animals.<sup>164</sup>

The year 1998 marked the apex of this political and social crescendo. In a flurry of activity, the British House of Lords issued a report citing the "continuing threat to human health from imprudent use of antibiotics in animals,"<sup>165</sup> while the British House of Commons Select Committee on Agriculture recommended an outright ban on AGPs.<sup>166</sup> On December 17, 1998, the EU Commission acted decisively, banning four popular AGPs.<sup>167</sup> That same year, the EU established the European Antimicrobial Resistance Surveillance System (EARSS) to coordinate monitoring across the continent.<sup>168</sup>

By the close of the decade, a powerful, multi-faceted, and international consensus had been forged. Antibiotic resistance and pollution were no longer just a scientific concern – they were a political and public health priority backed by new surveillance networks, regulatory bans, and significant funding. A clear and urgent mandate for measurement was in place.

---

<sup>163</sup> European Commission, "Ban on the Antibiotic 'Avoparcin' in Animal Feed"; Kirchhelle, *Pyrrhic Progress*.

<sup>164</sup> Podolsky, *The Antibiotic Era*; Kirchhelle, *Pyrrhic Progress*.

<sup>165</sup> House of Lords, Select Committee Appointed to Consider Science and Technology, *Resistance to Antibiotics: Select Committee Report (Hansard, 16 November 1998)*.

<sup>166</sup> Kirchhelle, *Pyrrhic Progress*.

<sup>167</sup> Kirchhelle, *Pyrrhic Progress*.

<sup>168</sup> Kirchhelle, *Pyrrhic Progress*.

### *The New Intellectual Framework*

The explosion of analytical data in the mid-1990s created an urgent need for intellectual synthesis. The rapid accumulation of data from individual detection studies created a critical gap: the scientific community had a growing collection of facts but lacked a unified language or big picture synthesis piece to draw collective meaning from these individual studies. This challenge was met between 1998 and 1999 by a pair of reviews that transformed decades of scattered findings into a coherent field of inquiry. These works moved beyond simple data consolidation to provide the emerging scientific community with a shared vocabulary, a new theoretical paradigm, and a sense of its own history.

The first of these foundational texts, “Occurrence, Fate and Effects of Pharmaceutical Substances in the Environment,” was published in 1998 by Halling-Sørensen et al.<sup>169</sup> This review serves as the first comprehensive effort to consolidate fragmented global knowledge on the topic. It systematically mapped the known world of pharmaceutical pollution by outlining anticipated exposure routes from both human and veterinary sources; summarizing the patchwork of existing environmental legislation in Europe and the United States; and compiling occurrence data from every environmental compartment, including groundwater, rivers, sediments, and soil. The review moved beyond documenting the presence of these substances to examining their environmental behavior, summarizing the limited data on fate processes like biodegradation, soil binding, and hydrolysis. Crucially, however, the review's most significant contribution was its stark illustration of how much remained unknown. After methodically assembling the available evidence, the authors concluded that for many areas, “the knowledge is practically zero” and that

---

<sup>169</sup> Halling-Sørensen et al., “Occurrence, Fate and Effects of Pharmaceutical Substances in the Environment- A Review.”

further research in this "hitherto little explored field would be necessary."<sup>170</sup> By providing this exhaustive baseline and explicitly identifying the vast knowledge gaps, the Halling-Sørensen review was a call to action, defining the boundaries of the field and highlighting the urgent need for a more unified conceptual approach.

While the Halling-Sørensen review consolidated the existing data, it was the 1999 publication by Christian Daughton and Thomas Ternes that delivered the conceptual framing required to catalyze a focused, international scientific discussion. Recognizing that the existing literature was "highly fragmented, disjointed, uneven, and difficult to assess," Daughton and Ternes aimed to provide context for what they defined as a major, overlooked environmental problem.<sup>171</sup> Their paper "Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?" provided the first standardized language to discuss pharmaceutical pollution (including antibiotic pollution) for a generation of scientists. Its influence stems from several key contributions. First, it introduced the unifying term "Pharmaceuticals and Personal Care Products (PPCPs)," framing these substances as a major class of pollutants distinct from the conventional "priority" pollutants that had long dominated regulatory attention.

Second, Daughton and Ternes proposed a new paradigm for understanding the environmental threat. By defining PPCPs as "agents of subtle change," they shifted the toxicological focus away from acute, high-dose effects and toward the unique risks posed by the continuous, low-level introduction of drugs into aquatic ecosystems. They emphasized the potential for "subtle, cumulative, and insidious effects" on non-target organisms resulting from "continual life-cycle, multigenerational exposure," a risk profile that existing toxicity screening

---

<sup>170</sup> Halling-Sørensen et al., "Occurrence, Fate and Effects of Pharmaceutical Substances in the Environment- A Review," 386.

<sup>171</sup> Daughton and Ternes, "Pharmaceuticals and Personal Care Products in the Environment," 908.

methods were not designed to assess.<sup>172</sup> Finally, the paper served as an explicit call to action. By identifying research gaps and providing recommendations, the authors aimed to "catalyze a concerted effort" among environmental scientists to investigate the issue further.<sup>173</sup> They also validated the "inferred-to-measured" narrative by noting the 15-year gap in research that followed the initial 1970s detections of pharmaceuticals in WWTP effluent, giving context and significance to the recent analytical revolution that made their own work possible. It was this combination of synthesis, conceptual reframing, and a clear research agenda that provided the definitive intellectual architecture for the emerging field.

While these foundational reviews addressed the entire spectrum of pharmaceuticals and personal care products, it was nonetheless critical for formalizing the specific problem of antibiotic pollution. As a class of drugs, antibiotics fit within the new paradigms being proposed. They were the most prominent and concerning subclass of PPCPs at the time due to the direct and growing threat of antibiotic resistance. The concept of "agents of subtle change" was also applicable: the subtle, cumulative effect of antibiotics in the environment was not only ecological but also evolutionary, exerting the exact selection pressure that fosters the proliferation of resistance genes. By situating antibiotics within the broader category of PPCPs, these reviews moved the issue of antibiotic pollution beyond the confines of microbiology and public health and placed it squarely within the domain of environmental science. They provided the essential intellectual justification for treating antibiotics as critical environmental contaminants, making them a central pillar of the emerging field of environmental pharmaceutical research.

---

<sup>172</sup> Daughton and Ternes, "Pharmaceuticals and Personal Care Products in the Environment," 907–8.

<sup>173</sup> Daughton and Ternes, "Pharmaceuticals and Personal Care Products in the Environment," 908.

The intellectual frameworks established in 1998 and 1999 provided the "why" for the emerging field, but it was the concurrent development of a robust analytical blueprint for operationalizing these techniques at scale that provided the essential "how." The mature analytical tools were now applied to answer the urgent questions posed by the new political mandate, with one landmark study in particular serving as the definitive proof-of-concept for the new paradigm.

That methodological capstone was the 1998 study by Hirsch et al., "Determination of Antibiotics in Different Water Compartments via Liquid Chromatography-Electrospray Tandem Mass Spectrometry."<sup>174</sup> The study's authors explicitly identified a critical gap in the existing science. While the presence of drugs in aquatic systems was known, most analytical methods for antibiotics were designed for biological matrices like meat or milk, were often specific to a single class of compounds, and achieved relatively high detection limits of hundreds of nanograms per liter.<sup>175</sup> Motivated to close the critical analytical gaps that prevented the routine monitoring of antibiotics, a task made urgent by the growing concern over antibiotic resistance, their achievement was a comprehensive analytical multi-method capable of determining 18 different antibiotics across six major classes in water samples. Using the now-mature technique of LC-ESI-MS/MS, they reached the "lower ng/l range" detection limits necessary for environmental relevance, a feat that would have been impossible just a few years prior.<sup>176</sup> By successfully applying their method to detect five different antibiotics in a German river, they provided both a theoretical model and a field-validated tool. This study contributed to the

---

<sup>174</sup> Hirsch et al., "Determination of Antibiotics in Different Water Compartments via Liquid Chromatography-Electrospray Tandem Mass Spectrometry."

<sup>175</sup> Hirsch et al., "Determination of Antibiotics in Different Water Compartments via Liquid Chromatography-Electrospray Tandem Mass Spectrometry," 216.

<sup>176</sup> Hirsch et al., "Determination of Antibiotics in Different Water Compartments via Liquid Chromatography-Electrospray Tandem Mass Spectrometry," 215–16.

analytical blueprint for a new generation of research, demonstrating a reproducible method that could make a wide swath of antibiotic pollution visible at once.

The impact of this new blueprint was immediate. In a follow-up study the next year, Hirsch and colleagues expanded on the 1998 blueprint, publishing one of the first comprehensive reports on the occurrence of antibiotics in German rivers and sewage treatment plants.<sup>177</sup> This 1999 study was crucial as it moved from demonstrating a method to providing concrete environmental data, confirming that a variety of antibiotics were indeed present in the aquatic environment and setting the stage for larger-scale investigations.

This definitive demonstration of a new standard was not an anomaly. The scientific community quickly moved to validate and apply the new blueprint as evidenced by the 1999 study from Hartig, Storm, and Jekel on sulfonamide drugs in municipal wastewater.<sup>178</sup> This work demonstrated a sophisticated awareness of the emerging field. It explicitly referenced the recent comprehensive review by Halling-Sørensen et al. and situated its own research within the known environmental pathways for sulfonamides from both human and veterinary use. The authors provided a concise history of analytical techniques, noting that while older, specialized methods existed, the new LC-MS/MS approach necessary for high-quality measurements had "became favoured only in the last few years."<sup>179</sup> By improving the methodology for detecting 13 different sulfonamides in the low ng/L range, the Hartig et al. study refined and extended these methods

---

<sup>177</sup> Hirsch et al., "Occurrence of Antibiotics in the Aquatic Environment."

<sup>178</sup> Hartig et al., "Detection and Identification of Sulphonamide Drugs in Municipal Waste Water by Liquid Chromatography Coupled with Electrospray Ionisation Tandem Mass Spectrometry."

<sup>179</sup> Hartig et al., "Detection and Identification of Sulphonamide Drugs in Municipal Waste Water by Liquid Chromatography Coupled with Electrospray Ionisation Tandem Mass Spectrometry," 164.

for a specific and highly relevant class of antibiotics, confirming the robustness of the new analytical paradigm and solidifying its role as the way forward.

The final piece of the analytical puzzle was cemented that same year with a comprehensive 1999 review of Solid-Phase Extraction (SPE) by Marie-Claire Hennion.<sup>180</sup> The power of the new LC-MS/MS systems could only be realized if the sample introduced was sufficiently clean and concentrated, a challenge that older methods struggled to meet for the polar compounds typical of antibiotics. Hennion's review detailed the maturation of the technology that solved this problem, highlighting the development of a new generation of sorbents, such as polymeric and carbon-based materials, that were specifically capable of trapping the polar and water-soluble analytes that previous materials missed. A particularly transformative development was the complete automation of SPE through its on-line coupling with liquid chromatography. Described as a "fast, modern and reliable approach to trace analysis," this technique integrated the sample enrichment and analytical separation steps into a single, automated workflow.<sup>181</sup> Using small, pressure-resistant precolumns and switching valves, the system could automatically process a sample and elute the concentrated analytes directly into the LC-MS system. This eliminated manual handling, which dramatically reduced the risk of contamination and analyte loss while improving productivity and reproducibility. This automated, high-throughput capability was essential for the large-scale environmental monitoring that the field now demanded. Hennion's review thus documented the maturation of the entire analytical chain, from sample collection to final detection. The complete, validated blueprint (from automated sample preparation with on-line SPE-LC to highly specific analysis

---

<sup>180</sup> Hennion, "Solid-Phase Extraction."

<sup>181</sup> Hennion, "Solid-Phase Extraction," 5.

with tandem mass spectrometry) had been established, providing the methodological justification for the national-scale reconnaissance that would define the next era of research.

### ***Political and Regulatory Mandates***

While the previous section described the framing of the crisis, this section details the formal policy and regulatory actions that became established around 1998, creating a direct, institutional demand for the new analytical science.

The new intellectual frameworks and analytical blueprints that emerged in 1998 and 1999 did not develop in a vacuum. They were both a cause and a consequence of a concurrent political and regulatory surge that transformed the growing scientific visibility of antibiotic pollution into an urgent mandate for action. This period saw high-level governmental bodies, spurred by the escalating crisis of antibiotic resistance, create the policy frameworks and institutional demand for the exact kind of chemical data that the new analytical methods could now supply.

The year 1998 marked a critical turning point as the threat of antibiotic resistance achieved global political urgency, particularly in Europe. In the United Kingdom a landmark report from the House of Lords Select Committee on Science and Technology framed the issue in stark terms, calling their inquiry an "alarming experience" and concluding that resistance constituted a "major threat to public health."<sup>182</sup> The debate was underscored by warnings of a potential "return to the pre-antibiotic era" and sharp critiques of perceived government "complacency" in the face of the crisis. While acknowledging the controversy surrounding antibiotics used as growth promoters in animal husbandry, the committee deemed it "imprudent

---

<sup>182</sup> House of Lords, Select Committee Appointed to Consider Science and Technology, *Resistance to Antibiotics: Select Committee Report (Hansard, 16 November 1998)*, sec. 1053.

to dismiss" the growing circumstantial evidence linking the practice to resistance in human pathogens.<sup>183</sup>

The report's impact was immediate and substantial, catalyzing a series of decisive policy actions. The British House of Commons Select Committee on Agriculture recommended an outright ban on antibiotic growth promoters, a move soon adopted by the European Union, which acted decisively to ban four popular agents on December 17, 1998.<sup>184</sup> Further, the House of Lords report directly led to the establishment of the European Antimicrobial Resistance Surveillance System (EARSS, later EARS-Net) and a 100 million-euro infusion of research funding.<sup>185</sup> These coordinated actions at the highest levels of government signaled a definitive policy shift: the biological shadow of resistance was now a political priority, and the new mandate required the direct measurement of its chemical cause.

In the United States, the response materialized not as an outright ban but as the creation of a new regulatory framework that linked a drug's market approval directly to its potential environmental fate. In 1998, the U.S. Food and Drug Administration (FDA) finalized its Environmental Assessment guidance for human drugs. This policy, for the first time, established a concrete regulatory trigger: an environmental assessment would be required for any new drug whose estimated aquatic introduction concentration was at or above 1 part per billion (ppb).<sup>186</sup> This created an explicit, quantitative link between a drug's usage, its predicted environmental

---

<sup>183</sup> House of Lords, Select Committee Appointed to Consider Science and Technology, *Resistance to Antibiotics: Select Committee Report (Hansard, 16 November 1998)*, secs. 1054–1067.

<sup>184</sup> Kirchhelle, *Pyrrhic Progress*.

<sup>185</sup> Podolsky, *The Antibiotic Era*; Kirchhelle, *Pyrrhic Progress*.

<sup>186</sup> US Food and Drug Administration, *Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications*; Patel et al., "Pharmaceuticals of Emerging Concern in Aquatic Systems."

concentration, and the need for ecotoxicity data, thereby embedding environmental risk assessment directly into the pharmaceutical approval process.

This alignment of political will, regulatory structure, and analytical capability resulted in the initiation of the U.S. Geological Survey (USGS) National Reconnaissance study in 1999.<sup>187</sup> This ambitious undertaking represented the synthesis of the decade's developments. The political urgency driven by the antibiotic resistance crisis provided the impetus, the new FDA guidance provided a clear regulatory context, and the analytical blueprint established by researchers like Hirsch et al. (1998) provided the necessary technical capability. For the first time, it was possible to ask and answer the question of pharmaceutical (including antibiotic) pollution on a national scale, setting the stage for the landmark findings that would transform the issue into an undeniable environmental crisis in the new millennium.

### ***Formation and Crisis***

By the turn of the millennium, the intellectual, methodological, and political foundations of aquatic antibiotic pollution research had solidified. The issue was now firmly embedded in scientific and regulatory consciousness, a fact underscored by the official adoption of the European Water Framework Directive in 2000.<sup>188</sup> This legislation provided a comprehensive framework for water quality management that would later incorporate substances of emerging concern, signaling that the era of institutional blindness was ending. With the field's new analytical blueprint established and a clear political mandate in place, the stage was set for the

---

<sup>187</sup> Kolpin et al., "Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999–2000."

<sup>188</sup> Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy.

narrative's climax: a national-scale investigation that would definitively reveal the extent of the problem.

That revelation arrived in 2002 with the publication of the U.S. Geological Survey's national reconnaissance study, led by Dana Kolpin.<sup>189</sup> Prior to this, knowledge of the environmental occurrence of pharmaceuticals in the United States was fragmented and geographically limited, constrained by prior analytical method issues. The study was the direct application of the newly operationalized analytical paradigm at an unprecedented scale, using the newly developed analytical methods to measure 95 contaminants in water samples from 139 streams across 30 states. Contaminants were detected in 80% of the streams sampled, with 82 of the 95 target compounds detected at least once. The study also revealed the prevalence of complex mixtures, finding a median of seven different contaminants per sample and as many as 38 in a single stream. Kolpin and colleagues' findings were a landmark in the history of environmental science as they demonstrated the ability to conduct these investigations both on a large geographic scale and with many target compounds.

The publication of the Kolpin et al. study marked the moment the source of the shadows on the wall were irrevocably revealed. Made possible by the technological and political convergence of the late 1990s, it provided the first comprehensive, nationwide chemical data confirming that the presence of these compounds was not an isolated or regional anomaly, but a pervasive condition in American waterways. The widespread detection implied that many compounds, including antibiotics, were surviving wastewater treatment processes and persisting in the environment. It transformed antibiotic and pharmaceutical pollution from a specialized

---

<sup>189</sup> Kolpin et al., "Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999–2000."

scientific concern into an undeniable and widespread environmental crisis, setting a new baseline for public understanding and establishing the research priorities and monitoring strategies for the decades of work that would follow.

## Conclusion

This thesis has traced the transformation of aquatic antibiotic pollution from a threat understood only through its distorted biological shadows into a directly measurable chemical reality between 1943 and 2002. I have argued that this shift from biological shadow to chemical reality was not the result of a single discovery, but rather a critical convergence during the 1990s of two powerful, reinforcing forces: a revolution in analytical chemistry that provided the technical means to see the problem, and a concurrent political and public health crisis over antibiotic resistance that created the urgent mandate to look. The period between 1998 and 2002 represents the culmination of this convergence, a moment when the intellectual, methodological, and political foundations were laid for a new and enduring field of environmental science.

The historical narrative unfolded across three distinct phases. For decades, during an era of chemical imperceptibility, microbiologists studied the biological shadows of pollution. Lacking the tools to measure antibiotics directly, they traced these effects by studying resistance patterns and developing proxy measures like the Multiple Antibiotic Resistance (MAR) index. The 1990s marked a turning point. The maturation of a new analytical toolkit – combining Solid-Phase Extraction (SPE), Atmospheric-Pressure Ionization (API) interfaces, and commercially available Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) systems – provided the means to move beyond the shadows and measure the chemical source directly. This technological leap occurred in parallel with the framing of antibiotic resistance as a global public health emergency, crystallized between 1998 and 2002 as reviews by Daughton and Ternes provide a conceptual framework, pioneering studies by Hirsch et al. (1998) established an analytical blueprint, and the landmark U.S. Geological Survey national reconnaissance study led by Kolpin et al. provided undeniable, large-scale evidence of the problem's pervasiveness.

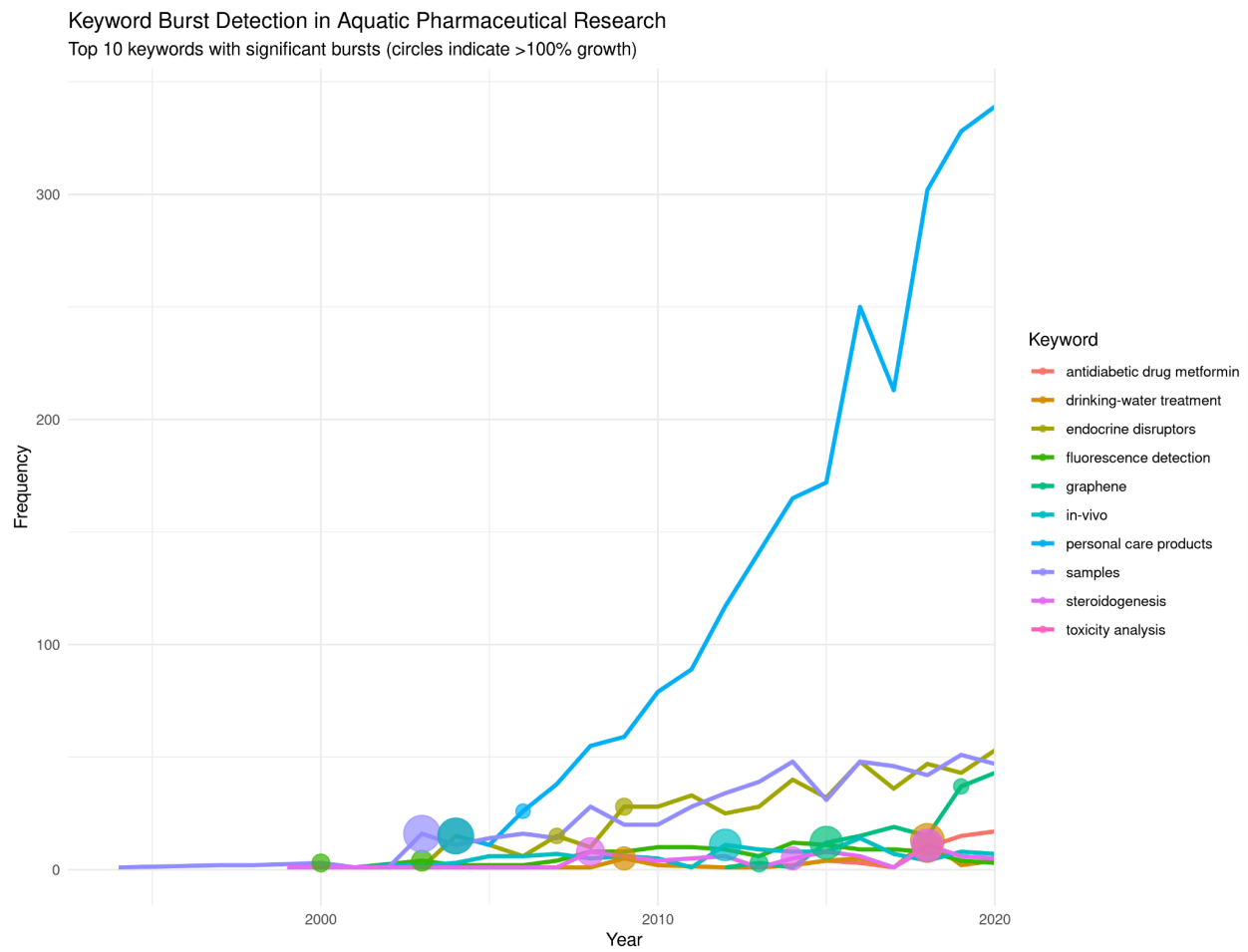


Figure 4. Keyword burst detection post-1998 to 2020.

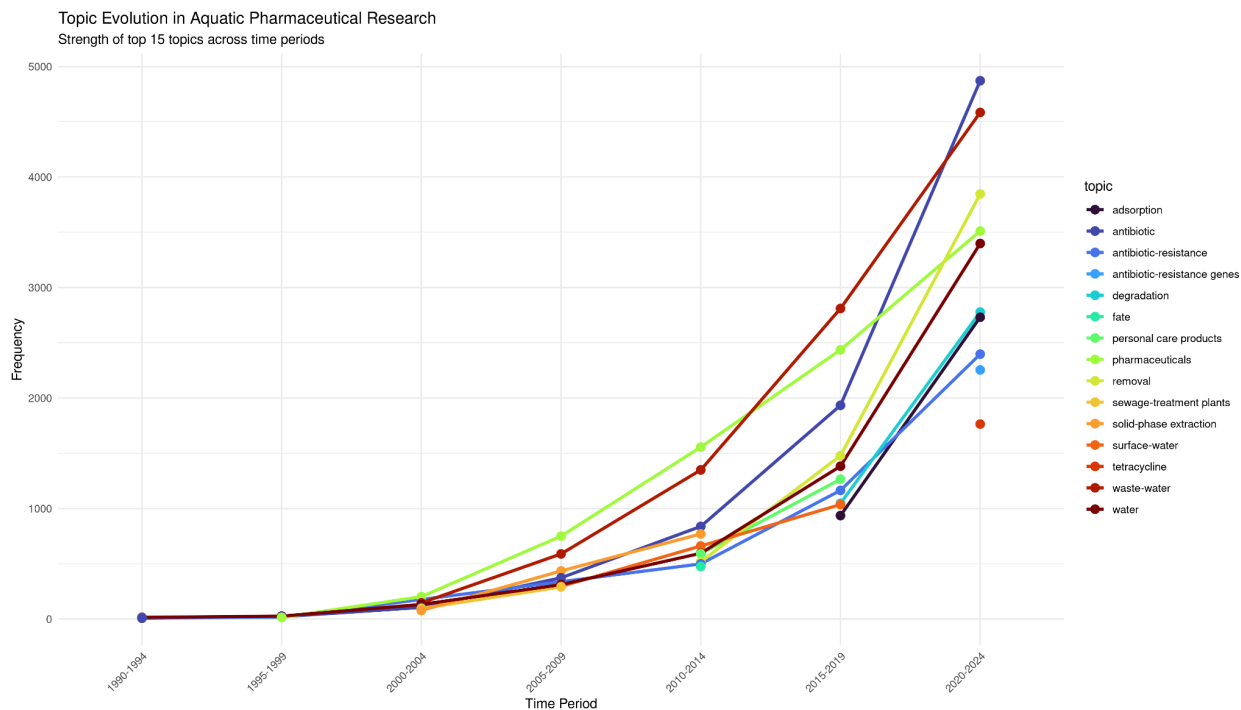


Figure 5. Topic evolution based on author keywords.

The formation of the field between 1998 and 2002 marks both an end for this thesis and a new beginning for the study of pharmaceutical pollution within environmental science. The intellectual and methodological architecture constructed during this period became the foundation for an explosive and global expansion of research, as bibliometric data reveals (see Figure 9). This post-2002 era was characterized by a distinct evolution in the field's thematic focus. As the initial challenge of detection was overcome, research priorities shifted toward understanding the full complexity of contaminants and developing effective remediation technologies. Scientists increasingly recognized that not only active pharmaceutical ingredients but also their metabolites and transformation products were crucial environmental contaminants. Consequently, intense efforts were directed at improving the performance of wastewater treatment plants through advanced techniques like ozonation and membrane filtration. This

thematic shift is quantitatively visible in the literature (see Figure 5); whereas pre-1998 keywords were general, the post-2002 lexicon became more specialized, with terms like "removal," "adsorption," and "waste-water" gaining prominence. Keyword bursts (see Figure 4) in topics such as "steroidogenesis" and "toxicity analysis" signaled a deepening concern with biological consequences, while later bursts in terms like "graphene" pointed to a growing focus on novel removal technologies.

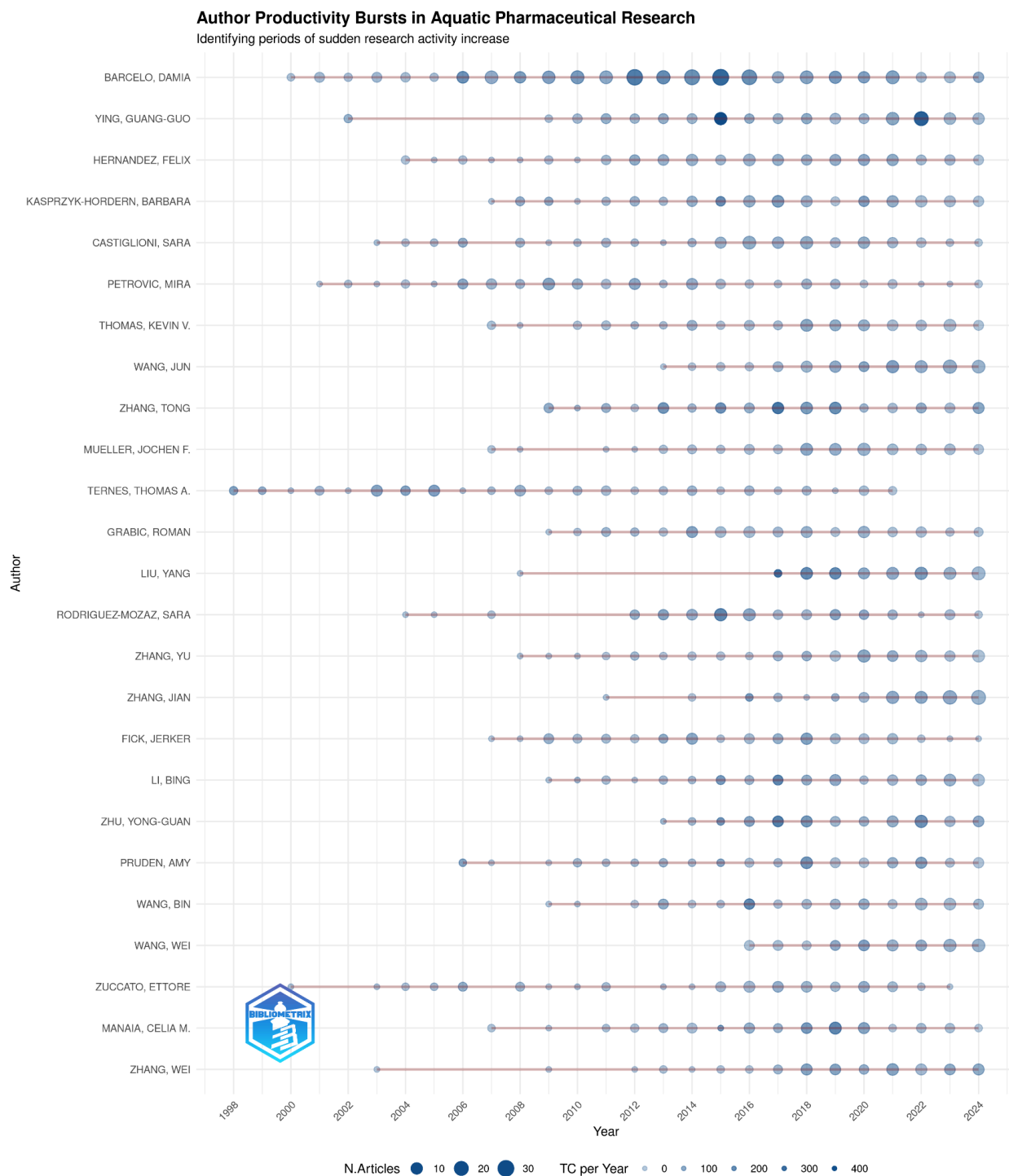


Figure 6. Productivity bursts of the highest locally cited researchers. “TC” means total citations.

All of the most productive researchers began publishing in 1998 or later.

Additionally, the social and geopolitical structure of the field underwent significant development after 1998. A historiographic analysis of the most impactful publications (see Figure 7) demonstrates that the key works of the 1998-2002 period became the central, highly cited nodes upon which subsequent research was built. The most productive authors who dominate the field today almost all began publishing during or immediately after this foundational era, cementing the influence of a core group of researchers (see Figure 6). This early momentum appears to have contributed to a surprising trend in the field's social structure. While network theory predicts that scientific fields undergo continuous splitting and merging as researchers come and go and new sub-specialties form, the core collaboration network for aquatic pharmaceutical pollution stabilized around 2009 (see Figure 8). This suggests the formation of a durable, foundational group and a potential Matthew effect, where established laboratories disproportionately shape the field's trajectory. While this social structure was forming, the geography of knowledge production also underwent a transformation. Early research was concentrated in North America and Europe, but after 2010, research productivity from China began to increase exponentially, overtaking the United States by the mid-2010s (see Figure 9). This surge reflected both the rapid expansion of China's scientific infrastructure and the country's central role as a global consumer and producer of antibiotics, often spurred by national initiatives like the "Beautiful China" program.



Figure 8. Collaboration network of the most productive researchers as of 2009.

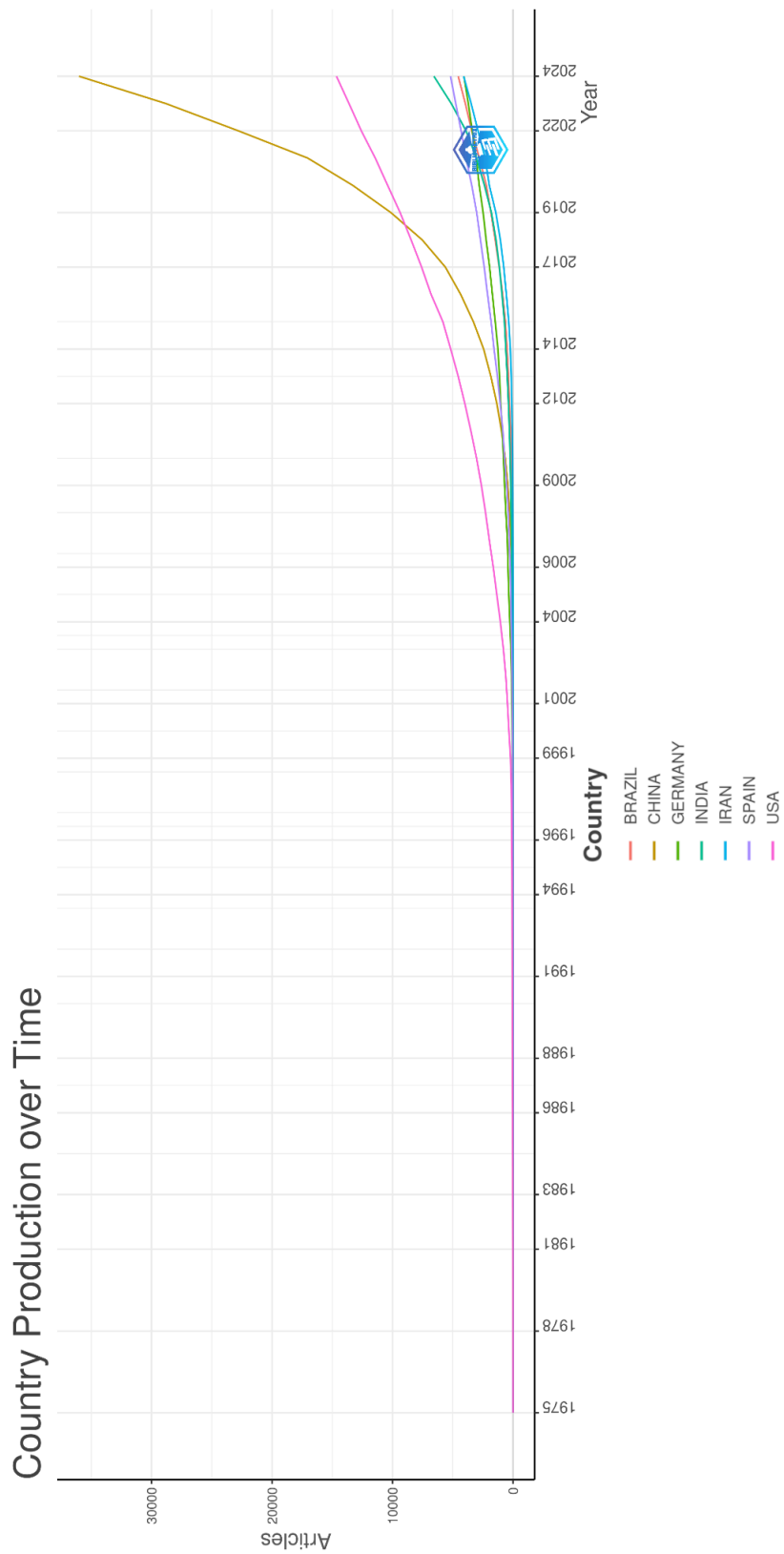


Figure 9. Scientific production by country.

By making antibiotic pollution chemically visible, the convergence of the late 1990s transformed a series of scattered findings into a coherent field of inquiry. It established the analytical tools, conceptual language, and institutional justification for the decades of research that followed. Yet, this visibility alone has not solved the challenge of antibiotic pollution. The pharmaceutical industry has often resisted substantive regulatory measures, and new environmental threats continue to emerge.

By 2002, antibiotics in aquatic environments had shifted from a threat perceived only through its distorted biological shadows to a directly measurable chemical reality, and from isolated findings to a recognized environmental issue. This transformation was enabled by a convergence of analytical tools and catalyzed by public health anxieties over resistance. Despite the immense growth in scientific knowledge, the full extent of antibiotics' impact on ecosystems, the precise mechanisms driving environmental resistance, and the most effective methods for mitigating these risks remain subjects of intense and ongoing debate. The journey from shadow to reality was a monumental scientific achievement, but it marks only the first step in addressing the enduring consequences of pollution.

## Bibliography

- Abraham, E. P., and E. Chain. "An Enzyme from Bacteria Able to Destroy Penicillin." *Nature* 146, no. 3713 (1940): 837–837. <https://doi.org/10.1038/146837a0>.
- Addison, J. B. "Antibiotics in Sediments and Run-off Waters from Feedlots." In *Residue Reviews*, edited by Francis A. Gunther and Jane Davies Gunther. Springer New York, 1984. [https://doi.org/10.1007/978-1-4612-5266-5\\_1](https://doi.org/10.1007/978-1-4612-5266-5_1).
- American Chemical Society National Historic Chemical Landmarks. "Legacy of Rachel Carsons Silent Spring." American Chemical Society. Accessed December 19, 2023. <https://www.acs.org/education/whatischemistry/landmarks/rachel-carson-silent-spring.html>.
- Ardrey, R. E. *Liquid Chromatography-Mass Spectrometry: An Introduction*. Analytical Techniques in the Sciences. J. Wiley, 2003.
- Aria, Massimo, and Corrado Cuccurullo. "Bibliometrix: An R-Tool for Comprehensive Science Mapping Analysis." *Journal of Informetrics* 11, no. 4 (2017): 959–75. <https://doi.org/10.1016/j.joi.2017.08.007>.
- Badawy, Mohamed E. I., Mahmoud A. M. El-Nouby, Paul K. Kimani, Lee W. Lim, and Entsar I. Rabea. "A Review of the Modern Principles and Applications of Solid-Phase Extraction Techniques in Chromatographic Analysis." *Analytical Sciences* 38, no. 12 (2022): 1457–87. <https://doi.org/10.1007/s44211-022-00190-8>.
- Beardsley, Tim. "NIH Retreat from Controversy." *Nature* 319, no. 6055 (1986): 611–611. <https://doi.org/10.1038/319611a0>.
- Beckey, H. D., and H. -R. Schulten. "Field Desorption Mass Spectrometry." *Angewandte Chemie International Edition in English* 14, no. 6 (1975): 403–15. <https://doi.org/10.1002/anie.197504031>.
- Berk, Zeki. "Chapter 11 - Extraction." In *Food Process Engineering and Technology*, edited by Zeki Berk. Food Science and Technology. Academic Press, 2009. <https://doi.org/10.1016/B978-0-12-373660-4.00011-9>.
- Björklund, H. V., C. M. I. Råbergh, and G. Bylund. "Residues of Oxolinic Acid and Oxytetracycline in Fish and Sediments from Fish Farms." *Aquaculture* 97, no. 1 (1991): 85–96. [https://doi.org/10.1016/0044-8486\(91\)90281-B](https://doi.org/10.1016/0044-8486(91)90281-B).
- Björklund, Harry, Johan Bondestam, and Göran Bylund. "Residues of Oxytetracycline in Wild Fish and Sediments from Fish Farms." *Aquaculture* 86, no. 4 (1990): 359–67. [https://doi.org/10.1016/0044-8486\(90\)90324-G](https://doi.org/10.1016/0044-8486(90)90324-G).
- Boxall, A. B. A., L. A. Fogg, P. A. Blackwell, et al. "Veterinary Medicines in the Environment." In *Reviews of Environmental Contamination and Toxicology*, edited by George W. Ware,

- Lilia A. Albert, D. G. Crosby, et al., vol. 180. *Reviews of Environmental Contamination and Toxicology*. Springer New York, 2004. [https://doi.org/10.1007/0-387-21729-0\\_1](https://doi.org/10.1007/0-387-21729-0_1).
- Boxall, Alistair B.A., Murray A. Rudd, Bryan W. Brooks, et al. "Pharmaceuticals and Personal Care Products in the Environment: What Are the Big Questions?" *Environmental Health Perspectives* 120, no. 9 (2012): 1221–29. <https://doi.org/10.1289/ehp.1104477>.
- Brooks, Bryan W., Christy M. Foran, Sean M. Richards, et al. "Aquatic Ecotoxicology of Fluoxetine." *Toxicology Letters*, Hot Spot Pollutants: Pharmaceuticals in the Environment, vol. 142, no. 3 (2003): 169–83. [https://doi.org/10.1016/S0378-4274\(03\)00066-3](https://doi.org/10.1016/S0378-4274(03)00066-3).
- Calisto, Vânia, and Valdemar I. Esteves. "Psychiatric Pharmaceuticals in the Environment." *Chemosphere* 77, no. 10 (2009): 1257–74. <https://doi.org/10.1016/j.chemosphere.2009.09.021>.
- Carlucci, A. F., and David Pramer. "An Evaluation of Factors Affecting the Survival of Escherichia Coli in Sea Water: III. Antibiotics." *Applied Microbiology* 8, no. 4 (1960): 251. <https://doi.org/10.1128/am.8.4.251-254.1960>.
- Carson, Rachel. *Silent Spring*. Houghton Mifflin Company, 1962.
- Carvalho, Isabel T., and Lúcia Santos. "Antibiotics in the Aquatic Environments: A Review of the European Scenario." *Environment International* 94 (September 2016): 736–57. <https://doi.org/10.1016/j.envint.2016.06.025>.
- Cassidy, P. S., Y. X. Li, and D. G. Dyer. *Analytical Method for the Determination of TI-435 and the Degradates, TZNG, TZMU, MNG, and TMG in Soil by Liquid Chromatography with APCI MS/MS-Detection*. Environmental Chemistry Method No. 110263. US EPA, 2001. <https://19january2021snapshot.epa.gov/sites/static/files/2014-12/documents/454229-02-s.pdf>.
- Chew, Yik-Ling, Mei-Ann Khor, and Yau-Yan Lim. "Choices of Chromatographic Methods as Stability Indicating Assays for Pharmaceutical Products: A Review." *Heliyon* 7, no. 3 (2021): e06553. <https://doi.org/10.1016/j.heliyon.2021.e06553>.
- Coates, Anthony RM, Gerry Halls, and Yanmin Hu. "Novel Classes of Antibiotics or More of the Same?" *British Journal of Pharmacology* 163, no. 1 (2011): 184–94. <https://doi.org/10.1111/j.1476-5381.2011.01250.x>.
- Code of Federal Regulations, 44 FR 44502 § 401.15 (1979). <https://www.govinfo.gov/content/pkg/CFR-2014-title40-vol29/xml/CFR-2014-title40-vol29-sec401-15.xml>.
- Cooke, Marylyn D. "Antibiotic Resistance in Coliform and Faecal Coliform Bacteria from Natural Waters and Effluents." *New Zealand Journal of Marine and Freshwater Research* 10, no. 3 (1976): 391–97. <https://doi.org/10.1080/00288330.1976.9515625>.

- Crane, Mark, Chris Watts, and Tatiana Boucard. "Chronic Aquatic Environmental Risks from Exposure to Human Pharmaceuticals." *Science of The Total Environment* 367, no. 1 (2006): 23–41. <https://doi.org/10.1016/j.scitotenv.2006.04.010>.
- Daughton, C G, and T A Ternes. "Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?" *Environmental Health Perspectives* 107, no. Suppl 6 (1999): 907–38.
- Daughton, Christian G. "Pharmaceuticals and the Environment (PiE): Evolution and Impact of the Published Literature Revealed by Bibliometric Analysis." *Science of The Total Environment* 562 (August 2016): 391–426. <https://doi.org/10.1016/j.scitotenv.2016.03.109>.
- Davies, Julian. "Where Have All the Antibiotics Gone?" *Canadian Journal of Infectious Diseases and Medical Microbiology* 17, no. 5 (2006): 707296. <https://doi.org/10.1155/2006/707296>.
- Davies, Julian, and Dorothy Davies. "Origins and Evolution of Antibiotic Resistance." *Microbiology and Molecular Biology Reviews : MMBR* 74, no. 3 (2010): 417–33. <https://doi.org/10.1128/MMBR.00016-10>.
- De Bellis, Nicola. *Bibliometrics and Citation Analysis: From the Science Citation Index to Cybermetrics*. Scarecrow Press, 2009.
- Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy, EP, CONSIL, 327 OJ L (2000). <http://data.europa.eu/eli/dir/2000/60/oj/eng>.
- Doel, Ronald E. "Internationalism in Science After 1940." In *The Cambridge History of Science*, 1st ed., edited by Hugh Richard Slotten, Ronald L. Numbers, and David N. Livingstone. Cambridge University Press, 2020. <https://doi.org/10.1017/9781139044301.006>.
- Doerge, D. R., and S. Bajic. "Multiresidue Determination of Quinolone Antibiotics Using Liquid Chromatography Coupled to Atmospheric-Pressure Chemical Ionization Mass Spectrometry and Tandem Mass Spectrometry." *Rapid Communications in Mass Spectrometry: RCM* 9, no. 11 (1995): 1012–16. <https://doi.org/10.1002/rcm.1290091108>.
- Dunaway, Finis. *Seeing Green: The Use and Abuse of American Environmental Images*. Paperback edition. The University of Chicago Press, 2018.
- Echchakoui, Saïd. "Why and How to Merge Scopus and Web of Science during Bibliometric Analysis: The Case of Sales Force Literature from 1912 to 2019." *Journal of Marketing Analytics* 8, no. 3 (2020): 165–84. <https://doi.org/10.1057/s41270-020-00081-9>.
- European Commission. "Ban on the Antibiotic 'Avoparcin' in Animal Feed." Text. Press Corner, January 29, 1997. [https://ec.europa.eu/commission/presscorner/detail/en/ip\\_97\\_71](https://ec.europa.eu/commission/presscorner/detail/en/ip_97_71).

- Fent, K, A Weston, and D Caminada. "Ecotoxicology of Human Pharmaceuticals." *Aquatic Toxicology* 76, no. 2 (2006): 122–59. <https://doi.org/10.1016/j.aquatox.2005.09.009>.
- Fernandes, Joana P., C. Marisa R. Almeida, Maria A. Salgado, Maria F. Carvalho, and Ana P. Mucha. "Pharmaceutical Compounds in Aquatic Environments—Occurrence, Fate and Bioremediation Prospective." *Toxics* 9, no. 10 (2021): 257. <https://doi.org/10.3390/toxics9100257>.
- Flynn, Edward. "Pharmacokinetic Parameters." In *xPharm: The Comprehensive Pharmacology Reference*, edited by S. J. Enna and David B. Bylund. Elsevier, 2007. <https://doi.org/10.1016/B978-008055232-3.60034-0>.
- Fong, P. P. "Zebra Mussel Spawning Is Induced in Low Concentrations of Putative Serotonin Reuptake Inhibitors." *The Biological Bulletin* 194, no. 2 (1998): 143–49. <https://doi.org/10.2307/1543044>.
- Garrett, Laurie. *The Coming Plague: Newly Emerging Diseases in a World out of Balance*. Farrar, Straus and Giroux, 1994.
- Garrison, A. W., J. D. Pope, and F. R. Allen. *GC/MS Analysis of Organic Compounds in Domestic Wastewaters*. Ann Arbor Science Publishers, 1976.
- Goyal, Sagar M., Charles P. Gerba, and Joseph L. Melnick. "Transferable Drug Resistance in Bacteria of Coastal Canal Water and Sediment." *Water Research* 13, no. 4 (1979): 349–56. [https://doi.org/10.1016/0043-1354\(79\)90022-8](https://doi.org/10.1016/0043-1354(79)90022-8).
- Grabow, W. O. K., and O. W. Prozesky. "Drug Resistance of Coliform Bacteria in Hospital and City Sewage." *Antimicrobial Agents and Chemotherapy* 3, no. 2 (1973): 175–80. <https://doi.org/10.1128/aac.3.2.175>.
- Halling-Sørensen, B., S. Nors Nielsen, P. F. Lanzky, F. Ingerslev, H. C. Holten Lützhøft, and S. E. Jørgensen. "Occurrence, Fate and Effects of Pharmaceutical Substances in the Environment- A Review." *Chemosphere* 36, no. 2 (1998): 357–93. [https://doi.org/10.1016/S0045-6535\(97\)00354-8](https://doi.org/10.1016/S0045-6535(97)00354-8).
- Hartig, C, T Storm, and M Jekel. "Detection and Identification of Sulphonamide Drugs in Municipal Waste Water by Liquid Chromatography Coupled with Electrospray Ionisation Tandem Mass Spectrometry." *Journal of Chromatography. A* 854, nos. 1–2 (1999). [https://doi.org/10.1016/s0021-9673\(99\)00378-7](https://doi.org/10.1016/s0021-9673(99)00378-7).
- Heberer, Th., and H.-J. Stan. "Determination of Clofibric Acid and N-(Phenylsulfonyl)-Sarcosine in Sewage, River and Drinking Water." *International Journal of Environmental Analytical Chemistry* 67, nos. 1–4 (1997): 113–24. <https://doi.org/10.1080/03067319708031398>.
- Heberer, Thomas. "Occurrence, Fate, and Removal of Pharmaceutical Residues in the Aquatic Environment: A Review of Recent Research Data." *Toxicology Letters* 131, nos. 1–2 (2002): 5–17. [https://doi.org/10.1016/S0378-4274\(02\)00041-3](https://doi.org/10.1016/S0378-4274(02)00041-3).

- Henion, Jack, Bruce Thomson, and Peter Dawson. "Determination of Sulfa Drugs in Biological Fluids by Liquid Chromatography/Mass Spectrometry/Mass Spectrometry." *Analytical Chemistry* 54 (April 1982): 451–56. <https://doi.org/10.1021/ac00240a023>.
- Hennion, M. C. "Solid-Phase Extraction: Method Development, Sorbents, and Coupling with Liquid Chromatography." *Journal of Chromatography. A* 856, nos. 1–2 (1999): 3–54. [https://doi.org/10.1016/s0021-9673\(99\)00832-8](https://doi.org/10.1016/s0021-9673(99)00832-8).
- Hignite, C., and D. L. Azarnoff. "Drugs and Drug Metabolites as Environmental Contaminants: Chlorophenoxyisobutyrate and Salicylic Acid in Sewage Water Effluent." *Life Sciences* 20, no. 2 (1977): 337–41. [https://doi.org/10.1016/0024-3205\(77\)90329-0](https://doi.org/10.1016/0024-3205(77)90329-0).
- Hirsch, R., T. A. Ternes, K. Haberer, A. Mehlich, F. Ballwanz, and K. L. Kratz. "Determination of Antibiotics in Different Water Compartments via Liquid Chromatography-Electrospray Tandem Mass Spectrometry." *Journal of Chromatography. A* 815, no. 2 (1998): 213–23. [https://doi.org/10.1016/s0021-9673\(98\)00335-5](https://doi.org/10.1016/s0021-9673(98)00335-5).
- Hirsch, Roman, Thomas Ternes, Klaus Haberer, and Karl-Ludwig Kratz. "Occurrence of Antibiotics in the Aquatic Environment." *Science of The Total Environment* 225, no. 1 (1999): 109–18. [https://doi.org/10.1016/S0048-9697\(98\)00337-4](https://doi.org/10.1016/S0048-9697(98)00337-4).
- Homem, Vera, and Lucia Santos. "Degradation and Removal Methods of Antibiotics from Aqueous Matrices--a Review." *Journal of Environmental Management* 92, no. 10 (2011). <https://doi.org/10.1016/j.jenvman.2011.05.023>.
- House of Lords, Select Committee Appointed to Consider Science and Technology. *Resistance to Antibiotics: Select Committee Report (Hansard, 16 November 1998)*. Nos. Cc1043-95. 1998. <https://api.parliament.uk/historic-hansard/lords/1998/nov/16/resistance-to-antibiotics-select>.
- Institute of Medicine (US) Committee on Emerging Microbial Threats to Health. *Emerging Infections: Microbial Threats to Health in the United States*. Edited by Joshua Lederberg, Robert E. Shope, and Stanley C. Oaks. National Academies Press (US), 1992. <http://www.ncbi.nlm.nih.gov/books/NBK234855/>.
- Jacobsen, Pål, and Lasse Berglund. "Persistence of Oxytetracycline in Sediments from Fish Farms." *Aquaculture* 70, no. 4 (1988): 365–70. [https://doi.org/10.1016/0044-8486\(88\)90120-2](https://doi.org/10.1016/0044-8486(88)90120-2).
- Jones, O. A. H, N Voulvoulis, and J. N Lester. "Aquatic Environmental Assessment of the Top 25 English Prescription Pharmaceuticals." *Water Research* 36, no. 20 (2002): 5013–22. [https://doi.org/10.1016/S0043-1354\(02\)00227-0](https://doi.org/10.1016/S0043-1354(02)00227-0).
- Jones, R. N. "The Emergent Needs for Basic Research, Education, and Surveillance of Antimicrobial Resistance. Problems Facing the Report from the American Society for Microbiology Task Force on Antibiotic Resistance." *Diagnostic Microbiology and*

- Infectious Disease* 25, no. 4 (1996): 153–61.  
[https://doi.org/10.1016/s0732-8893\(96\)00099-5](https://doi.org/10.1016/s0732-8893(96)00099-5).
- Khetan, Sushil K., and Terrence J. Collins. “Human Pharmaceuticals in the Aquatic Environment: A Challenge to Green Chemistry.” *Chemical Reviews* 107, no. 6 (2007): 2319–64. <https://doi.org/10.1021/cr020441w>.
- Kirchhelle, Claas. *Pyrrhic Progress: The History of Antibiotics in Anglo-American Food Production*. Wellcome Trust–Funded Monographs and Book Chapters. Rutgers University Press, 2020. <http://www.ncbi.nlm.nih.gov/books/NBK554200/>.
- Knobler, Stacey L., Stanley M. Lemon, Marian Najafi, and Tom Burroughs, eds. *The Resistance Phenomenon in Microbes and Infectious Disease Vectors: Implications for Human Health and Strategies for Containment -- Workshop Summary*. National Academies Press, 2003. <https://doi.org/10.17226/10651>.
- Kolpin, Dana W., Edward T. Furlong, Michael T. Meyer, et al. “Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999–2000: A National Reconnaissance.” *Environmental Science & Technology* 36, no. 6 (2002): 1202–11. <https://doi.org/10.1021/es011055j>.
- Koster, Chris G. de, and Peter J. Schoenmakers. “Chapter 3.1 - History of Liquid Chromatography—Mass Spectrometry Couplings.” In *Hyphenations of Capillary Chromatography with Mass Spectrometry*, edited by Peter Q. Tranchida and Luigi Mondello. Elsevier, 2020. <https://doi.org/10.1016/B978-0-12-809638-3.00007-7>.
- Krumperman, P. H. “Multiple Antibiotic Resistance Indexing of Escherichia Coli to Identify High-Risk Sources of Fecal Contamination of Foods.” *Applied and Environmental Microbiology* 46, no. 1 (1983): 165–70. <https://doi.org/10.1128/aem.46.1.165-170.1983>.
- Kümmerer, Klaus, ed. *Pharmaceuticals in the Environment*. Springer Berlin Heidelberg, 2004. <https://doi.org/10.1007/978-3-662-09259-0>.
- Landecker, Hannah. “Antibiotic Resistance and the Biology of History.” *Body & Society* 22, no. 4 (2016): 19–52. <https://doi.org/10.1177/1357034X14561341>.
- Lederberg, J. “Cell Genetics and Hereditary Symbiosis.” *Physiological Reviews* 32, no. 4 (1952): 403–30. <https://doi.org/10.1152/physrev.1952.32.4.403>.
- Lederberg, J. “Plasmid (1952-1997).” *Plasmid* 39, no. 1 (1998). <https://doi.org/10.1006/plas.1997.1320>.
- Lederberg, Joshua. “Medical Science, Infectious Disease, and the Unity of Humankind.” *JAMA: The Journal of the American Medical Association* 260, no. 5 (1988): 684. <https://doi.org/10.1001/jama.1988.03410050104039>.
- Levy, Stuart B., George B. FitzGerald, and Ann B. Macone. “Changes in Intestinal Flora of Farm Personnel after Introduction of a Tetracycline-Supplemented Feed on a Farm.” *New*

- England Journal of Medicine* 295, no. 11 (1976): 583–88.  
<https://doi.org/10.1056/NEJM197609092951103>.
- Lewis, Jack. “The Spirit of the First Earth Day.” *EPA Journal* 16, no. 1 (1990): 8–12.
- Majors, Ronald. *Historical Developments in HPLC and UHPLC Column Technology: The Past 25 Years*. LCGC North America-11-01-2015, vol. 33 (November 2015): 818–40.
- Marshall, S., and A. Orr. “Some Uses of Antibiotics in Physiological Experiments in Sea Water.” *Journal of Marine Research* 17, no. 1 (1958).  
[https://elischolar.library.yale.edu/journal\\_of\\_marine\\_research/925](https://elischolar.library.yale.edu/journal_of_marine_research/925).
- Martinez, Jose Luis. “Environmental Pollution by Antibiotics and by Antibiotic Resistance Determinants.” *Environmental Pollution (Barking, Essex: 1987)* 157, no. 11 (2009): 2893–902. <https://doi.org/10.1016/j.envpol.2009.05.051>.
- Monteiro, Sara C., and Alistair B.A. Boxall. “Occurrence and Fate of Human Pharmaceuticals in the Environment.” In *Reviews of Environmental Contamination and Toxicology*, vol. 202. *Reviews of Environmental Contamination and Toxicology*. Springer New York, 2010.  
[https://doi.org/10.1007/978-1-4419-1157-5\\_2](https://doi.org/10.1007/978-1-4419-1157-5_2).
- Neushul, Peter. “Science, government and the mass production of penicillin.” *Journal of the history of medicine and allied sciences* 48, no. 4 (1993): 371-395.
- Niessen, Wilfried M. A. *Liquid Chromatography-Mass Spectrometry*. 3rd ed. *Chromatographic Science Series*, volume 97. CRC, 2006.
- Nixon, Rob. *Slow Violence and the Environmentalism of the Poor*. Harvard University Press, 2011.
- O’Connor, Robert E., Richard J. Bord, and Ann Fisher. “Public Perceptions of Fresh Water Issues.” In *Risk Based Decision Making in Water Resources. 6: Proceedings of the Sixth Conference [on Risk-Based Decision Making] ; Santa Barbara, California, October 31 - November 5, 1993*, by Yacov Y. Haimes, David A. Moser, and Eugene L. Stakhiv. Conference on Risk Based Decision Making, New York, NY. American Soc. of Civil Engineers, 1994.
- Ortúzar, Maite, Maranda Esterhuizen, Darío Rafael Olicón-Hernández, Jesús González-López, and Elisabet Aranda. “Pharmaceutical Pollution in Aquatic Environments: A Concise Review of Environmental Impacts and Bioremediation Systems.” *Frontiers in Microbiology* 13 (2022). <https://www.frontiersin.org/articles/10.3389/fmicb.2022.869332>.
- Patel, Manvendra, Rahul Kumar, Kamal Kishor, Todd Mlsna, Charles U. Jr. Pittman, and Dinesh Mohan. “Pharmaceuticals of Emerging Concern in Aquatic Systems: Chemistry, Occurrence, Effects, and Removal Methods.” *Chemical Reviews* 119, no. 6 (2019): 3510–673. <https://doi.org/10.1021/acs.chemrev.8b00299>.

- Pfeifer, Thomas, Jochen Tuerk, Kai Bester, and Michael Spiteller. "Determination of Selected Sulfonamide Antibiotics and Trimethoprim in Manure by Electrospray and Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry." *Rapid Communications in Mass Spectrometry* 16, no. 7 (2002): 663–69. <https://doi.org/10.1002/rcm.624>.
- Pfluger, P., and D. R. Dietrich. "Effects on Pharmaceuticals in the Environment — an Overview and Principle Considerations." In *Pharmaceuticals in the Environment*, edited by Klaus Kümmerer. Springer Berlin Heidelberg, 2001. [https://doi.org/10.1007/978-3-662-04634-0\\_2](https://doi.org/10.1007/978-3-662-04634-0_2).
- Pitt, James J. "Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry." *The Clinical Biochemist Reviews* 30, no. 1 (2009): 19–34.
- Podolsky, Scott H., ed. *The Antibiotic Era: Reform, Resistance, and the Pursuit of a Rational Therapeutics*. Johns Hopkins University Press, 2015.
- Pokluda, Michael. "A Comprehensive Review of the Environmental Impacts and Human Health Risks Associated with the Occurrence of Waste Pharmaceuticals in Water Sources of the United States, and Policy Implications." M.P.H., The University of Texas School of Public Health, 2010. <https://www.proquest.com/docview/815250476/abstract/C327F58841FE456EPQ/1>.
- Pullen, Frank. "The Fascinating History of the Development of LC-MS; a Personal Perspective." *Chromatography Today*, March 2010. <http://www.chromatographytoday.com/article/hplc-uhplc/31/unassigned-independent-article/the-fascinating-history-of-the-development-of-lc-ms-a-personal-perspective/601>.
- Purdom, C. E., P. A. Hardiman, V. V. J. Bye, N. C. Eno, C. R. Tyler, and J. P. Sumpter. "Estrogenic Effects of Effluents from Sewage Treatment Works." *Chemistry and Ecology* 8, no. 4 (1994): 275–85. <https://doi.org/10.1080/02757549408038554>.
- Rammelkamp, C. H., and T. Maxon. "Resistance of Staphylococcus Aureus to the Action of Penicillin." *Experimental Biology and Medicine* 51, no. 3 (1942): 386–89. <https://doi.org/10.3181/00379727-51-13986>.
- Richardson, Mervyn L, and Judith M Bowron. "The Fate of Pharmaceutical Chemicals in the Aquatic Environment." *Journal of Pharmacy and Pharmacology* 37, no. 1 (1985): 1–12. <https://doi.org/10.1111/j.2042-7158.1985.tb04922.x>.
- Richardson, Susan D. "Environmental Mass Spectrometry: Emerging Contaminants and Current Issues." *Analytical Chemistry* 84, no. 2 (2012): 747–78. <https://doi.org/10.1021/ac202903d>.
- Rogers, Ian H., Ian K. Birtwell, and George M. Kruzynski. "Organic Extractables in Municipal Wastewater Vancouver, British Columbia." *Water Quality Research Journal* 21, no. 2 (1986): 187–204. <https://doi.org/10.2166/wqrj.1986.014>.

- Routledge, E. J., D. Sheahan, C. Desbrow, G. C. Brighty, M. Waldock, and J. P. Sumpter. "Identification of Estrogenic Chemicals in STW Effluent. 2. In Vivo Responses in Trout and Roach." *Environmental Science & Technology* 32, no. 11 (1998): 1559–65. <https://doi.org/10.1021/es970796a>.
- Santos, Lúcia H.M.L.M., A.N. Araújo, Adriano Fachini, A. Pena, C. Delerue-Matos, and M.C.B.S.M. Montenegro. "Ecotoxicological Aspects Related to the Presence of Pharmaceuticals in the Aquatic Environment." *Journal of Hazardous Materials* 175, nos. 1–3 (2010): 45–95. <https://doi.org/10.1016/j.jhazmat.2009.10.100>.
- Sarmah, Ajit K., Michael T. Meyer, and Alistair B.A. Boxall. "A Global Perspective on the Use, Sales, Exposure Pathways, Occurrence, Fate and Effects of Veterinary Antibiotics (VAs) in the Environment." *Chemosphere* 65, no. 5 (2006): 725–59. <https://doi.org/10.1016/j.chemosphere.2006.03.026>.
- Schoofs, Mark. "Drug Resistance: The next AIDS Crisis." *The Village Voice* (New York, United States), July 9, 1996.
- SCIEX. "The History of SCIEX." [https://www.asms.org/docs/default-source/history-posters/vendor\\_sciex\\_history\\_poster\\_d8\\_print\\_final-\(1\).pdf](https://www.asms.org/docs/default-source/history-posters/vendor_sciex_history_poster_d8_print_final-(1).pdf).
- Scott, Troy M., Joan B. Rose, Tracie M. Jenkins, Samuel R. Farrah, and Jerzy Lukasik. "Microbial Source Tracking: Current Methodology and Future Directions." *Applied and Environmental Microbiology* 68, no. 12 (2002): 5796–803. <https://doi.org/10.1128/AEM.68.12.5796-5803.2002>.
- Scott, James C. *Seeing Like a State: How Certain Schemes to Improve the Human Condition Have Failed*. Yale University Press, 1998.
- Silori, Rahul, Vikalp Shrivastava, Ashwin Singh, et al. "Global Groundwater Vulnerability for Pharmaceutical and Personal Care Products (PPCPs): The Scenario of Second Decade of 21st Century." *Journal of Environmental Management* 320 (October 2022): 115703. <https://doi.org/10.1016/j.jenvman.2022.115703>.
- Singh, Adarsh, Duduku Saidulu, Ashok Kumar Gupta, and Vijay Kubsad. "Occurrence and Fate of Antidepressants in the Aquatic Environment: Insights into Toxicological Effects on the Aquatic Life, Analytical Methods, and Removal Techniques." *Journal of Environmental Chemical Engineering* 10, no. 6 (2022): 109012. <https://doi.org/10.1016/j.jece.2022.109012>.
- Stahlschmidt, Stephan, and Dimity Stephen. *Comparison of Web of Science, Scopus and Dimensions Databases*. n.d.
- Stan, H.-J., Thomas Heberer, and Manfred Linkerhägner. "Occurrence of Clofibric Acid in the Aquatic System-- Is the Use in Human Medical Care the Source of the Contamination of Surface, Ground and Drinking Water?" *Vom Wasser. Weinheim* 83 (1994): 57–68.

- Stewart, EJ, R Madden, G Paul, and F Taddei. "Aging and Death in E. Coli." *PLoS Biology* 3, no. 2 (2005): e58. <https://doi.org/10.1371/journal.pbio.0030058>.
- Stoeckel, Donald M., and Valerie J. Harwood. "Performance, Design, and Analysis in Microbial Source Tracking Studies." *Applied and Environmental Microbiology* 73, no. 8 (2007): 2405–15. <https://doi.org/10.1128/AEM.02473-06>.
- Swann, M. M., K. L. Blaxter, H. I. Field, et al. *Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine*. No. 4190. London, n.d. Wellcome Collection. Accessed August 22, 2025. <https://wellcomecollection.org/works/cqvewh54>.
- Tabak, H. H., and R Bunch. "Steroid Hormones as Water Pollutants. I. Metabolism of Natural and Synthetic Ovulation-Inhibiting Hormones by Microorganisms of Activated Sludge and Primary Settled Sewage." *Developments in Industrial Microbiology* 11 (1970): 367–76.
- Ternes, Thomas A. "Occurrence of Drugs in German Sewage Treatment Plants and Rivers." *Water Research* 32, no. 11 (1998): 3245–60. [https://doi.org/10.1016/S0043-1354\(98\)00099-2](https://doi.org/10.1016/S0043-1354(98)00099-2).
- The New York Times*. "Winners of the 1993 Pulitzer Prizes for Journalism, Literature and the Arts." U.S. April 14, 1993. <https://www.nytimes.com/1993/04/14/us/winners-of-the-1993-pulitzer-prizes-for-journalism-literature-and-the-arts.html>.
- Thomson, Bruce A. "Atmospheric Pressure Ionization and Liquid Chromatography/Mass Spectrometry—Together at Last." *Journal of the American Society for Mass Spectrometry* 9, no. 3 (1998): 187–93. [https://doi.org/10.1016/S1044-0305\(97\)00285-7](https://doi.org/10.1016/S1044-0305(97)00285-7).
- Time Magazine. "Revenge of the Killer Microbes - Sep. 12, 1994." *Time*, n.d. Accessed August 20, 2025. <https://content.time.com/time/covers/0,16641,19940912,00.html>.
- Ullah, Sana, Shahid Ahmad, Xinle Guo, et al. "A Review of the Endocrine Disrupting Effects of Micro and Nano Plastic and Their Associated Chemicals in Mammals." *Frontiers in Endocrinology* 13 (January 2023): 1084236. <https://doi.org/10.3389/fendo.2022.1084236>.
- US Centers for Disease Control and Prevention. *Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States*. U.S. Department of Health and Human Services, Public Health Service, 1994. <https://stacks.cdc.gov/view/cdc/13231>.
- US Environmental Protection Agency. *Water Quality Criteria Data Book Volume I: Organic Chemical Pollution of Freshwater*. No. 18010DPV1270. 1970. <https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=9101SCYK.txt>.
- US EPA, OW. "Toxic and Priority Pollutants Under the Clean Water Act." Overviews and Factsheets. Accessed August 28, 2025. <https://19january2017snapshot.epa.gov/eg/toxic-and-priority-pollutants-under-clean-water-act>.

- US Food and Drug Administration. *Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications*. FDA, 1998.  
<https://www.fda.gov/regulatory-information/search-fda-guidance-documents/environmental-assessment-human-drug-and-biologics-applications>.
- Vas, György, and Károly Vékey. “Solid-phase Microextraction: A Powerful Sample Preparation Tool Prior to Mass Spectrometric Analysis.” *Journal of Mass Spectrometry* 39, no. 3 (2004): 233–54. <https://doi.org/10.1002/jms.606>.
- Watanabe, Tsutomu. “Infective Heredity of Multiple Drug Resistance in Bacteria.” *Bacteriological Reviews* 27, no. 1 (1963): 87–115.  
<https://doi.org/10.1128/br.27.1.87-115.1963>.
- Watts, C. D., B. Crathorne, M. Fielding, and C. P. Steel. “Identification of Non-Volatile Organics in Water Using Field Desorption Mass Spectrometry and High Performance Liquid Chromatography.” In *Analysis of Organic Micropollutants in Water*, edited by G. Angeletti and A. Bjørseth. Springer Netherlands, 1984.  
[https://doi.org/10.1007/978-94-009-6345-0\\_13](https://doi.org/10.1007/978-94-009-6345-0_13).

### Appendix A: Web of Science Search Term

TS=(( ( ("pharmaceutical pollution" OR "pharmaceutical contaminant\*" OR "pharmaceutical residue\*" OR "drug pollution" OR "drug contaminant\*" OR "antibiotic contamination" OR "antibiotic residue\*" OR PhAC\* OR "active pharmaceutical ingredient\*" OR antibiotic\* OR analgesic\* OR hormone\* OR steroid\* OR "contraceptive hormone\*" OR medication\* OR "birth control" OR drug\*) ) AND ( water OR wastewater OR "waste water" OR sewage OR effluent\* OR aquat\* OR "aquatic environment" OR "aquatic system" OR marine OR ocean\* OR sea OR estuar\* OR lake\* OR river\* OR stream\* OR wetland\* OR reservoir\* OR "drinking water" OR groundwat\* OR "ground water" OR brackish OR "coastal environment" OR "coastal water" ) AND ( "environmental pollutant\*" OR "environmental contaminant\*" OR "environmental contamination" OR pollutan\* OR contaminant\* OR presence OR occur\* OR distribut\* OR detect\* OR measur\* OR sampling OR monitor\* OR expos\* OR toxic\* OR ecotoxic\* OR hazard\* OR fate OR removal OR treat\* OR degrad\* OR "risk assessment" ) AND ( theor\* OR conceptual\* OR "literature review" OR "systematic review" OR "meta analysis" OR "meta-analysis" OR modeling OR modelling OR "global perspective" OR "international perspective" OR detect\* OR measur\* OR sampling OR monitor\* OR analy\* OR quantif\* OR "field stud\*" OR "field measurement\*" OR "field sampling" OR "field-based" OR "site-based" OR "environmental data" ) ) NOT ( "clinical trial" OR "drug trial" OR "randomized trial" OR RCT OR "animal trial" OR "phase I" OR "phase II" OR "phase III" ) ) and English (Languages) and Article or Review Article or Retracted Publication or Publication With Expression Of Concern or Correction or Retraction (Document Types)

## **Appendix B: Python and R Scripts**

See: <https://github.com/James-C-000/MA-Thesis-Python-and-R-Scripts>