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ABSTRACT: We live in a complex chemical environment where there are an estimated 350 000 chemical compounds or mixtures commercially produced. A strong body of literature shows that there are time points during early development when an organism's epigenome is particularly sensitive to chemicals in its environment. What is less understood is how gene-environment and epigenetic-environment interactions change with age. This question is bidirectional: (1) how do chemicals in the environment affect the aging process and (2) how does aging affect an organism's response to its chemical environment? The study of gene-environment interactions with age is especially important because, in many parts of the world, older individuals are a large and rapidly growing proportion of the population and because aging is a process universal to most of the animal kingdom. Epigenetics has emerged as a crucial framework for studying aging as epigenetic pathways, often triggered by environmental stimuli, have been shown to be essential regulators of the aging process. In this perspective article, we delineate the connection between aging, epigenetics, and environmental exposures. We discuss why it is essential to consider age when researching how an organism interacts with its environment. We describe recent advances in understanding how the chemical environment affects aging and the gap in research on how age affects an organism's response to the environment. Finally, we highlight how model organisms and network approaches can help fill this crucial gap. Taken together, systemic changes that occur in the epigenome with age indicate that adult organisms cannot be treated as a homogeneous population and that there are discrete mechanisms modulating the aging epigenome that we do not yet understand.

KEYWORDS: Biology of aging, environmental toxicology, epigenetics

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Introduction

How does aging affect gene-environment interactions? How can we use epigenetics as a framework for understanding how gene-environment interactions change with age? Gene-environment interactions as a concept emerged largely in the past few decades, and studying aging is also relatively new when compared with the long history of the field of Medicine. It was not until the mid-18th century that lifespan in Europe increased to the extent that people were living to old age and dying of chronic conditions—not solely communicable diseases.[1] Physician Ignatz Nascher coined the term geriatrics in 1909 because he believed there was a need for studying "senility [senescence] and its diseases apart from maturity [adulthood]."[1] Although the term was coined in 1909, historian Pat Thane observed that "for most of recorded time neither philosophical nor medical comment on old age (a small proportion of the full range of medical discourse) touched the actual lives of most older people."[1]

One way to conceptualize aging is that it describes an imbalance between stress and stress-buffering capacity.[2] Stress-buffering capacity is controlled by specific molecular pathways—for example, in mammals, protein misfolding is mitigated by the activity of chaperone proteins, and oxidative stress elicits a potent antioxidant response via the transcription factor NRF2.[3,4] While environmental stressors can take many shapes, from physical to psychological, this commentary—focused on the intersection of toxicoepigenetics and aging—will narrowly define the term "environment" as the collection of chemicals in an organism's surroundings and "stress" as the physiological and molecular responses these chemicals elicit in that organism's body. Epigenetics is the collection of mechanisms by which environmental stressors affect gene expression but not the underlying genetic sequence. These mechanisms primarily involve DNA methylation, histone modifications, and gene regulation by noncoding RNA.[5-7] Epigenetics, like aging, is a multifaceted concept whose definition is debated and in flux.[8-11] The recent development of epigenetics as a field has provided a mechanistic framework with which to study gene-environment interactions to identify critical windows of exposure during development[12] and also potentially with age.

The impetus to study gene-environment interactions over the life course is highlighted by the exponential increase in the manufacturing and use of chemicals. Based on a global inventory, it is estimated that currently there are more than 350 000 chemicals and mixtures of chemicals registered for commercial production.[13] That number, however, does not consider the interaction between chemicals that an individual might be exposed to daily, and therefore the potential for additive and synergistic effects those multiple exposures might have. Although it is a daunting task to unravel gene-environment

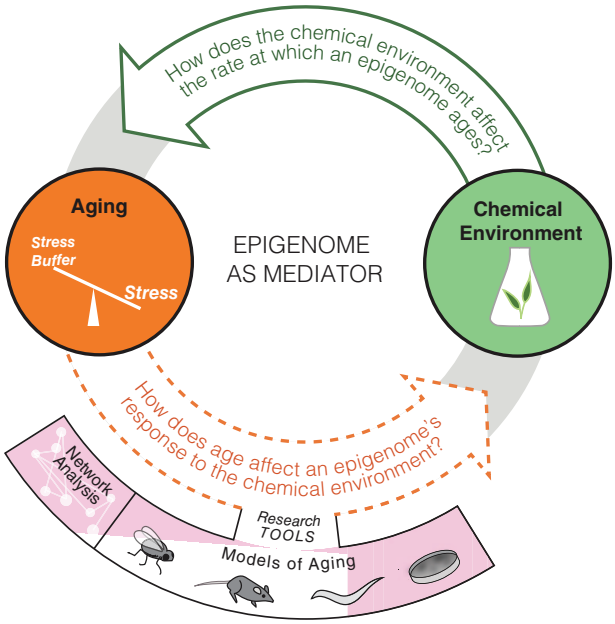

Figure 1. The relationship between epigenetics and aging. Aging can be defined as an imbalance between stress and stress-buffering capacity. The chemical environment influences aging principally by increasing or decreasing the rate of epigenetic aging and/or by increasing the variation in epigenetic marks. What is largely unknown, however, is how aging affects an organism's response to its chemical environment and what role the epigenome plays in that response. To understand how aging impacts epigenetic-environment interactions at the mechanistic level, network analysis and model organisms, such as *Caenorhabditis elegans*, should be leveraged.

interactions, especially across the lifespan, it has never been a more important task.

In this commentary, we will discuss why it is essential to consider age when studying epigenetics, one specific mechanism of gene-environment interactions—both regarding how the environment affects aging and how aging changes an organism's response to the environment (Figure 1). We will also highlight progress and gaps in the field of gene-environment interactions as they relate to age. We will conclude by describing model organisms and methods of analysis that would be beneficial in advancing the intersectional study of aging, epigenetics, and the environment.

Why Is It Important to Study Aging?

Aging involves a myriad of changes at the organ, tissue, and cellular levels.[11] Hallmarks of aging are factors which when upregulated accelerate aging and when downregulated slow aging. These factors include increases in protein misfolding and aggregation, increases in inflammation, increases in DNA damage, a decrease in mitochondrial efficiency, telomere shortening, and dysregulation of nutrient sensing.[11] As it will be explored in more detail below, epigenetic marks are heavily modulated over the lifespan. DNA methylation, specifically 5-methylcytosine (5mC) at CpG dinucleotides, shows a well-characterized trend toward global hypomethylation and focal hypermethylation with age.[5,11] Histone modifications show varied changes with age depending on the mark: for example, an increase in activating histone marks such as histone H3 lysine 4 trimethylation (H3K4me3), a decrease in repressive histone marks such as histone H3 lysine 9 trimethylation (H3K9me3), and a decrease in total histone protein, although these changes have tissue- and species-specific variations.[5,11] Additional recognized age-related changes include nuclear lamina breakdown, leading to alteration of heterochromatin that is normally anchored to the nuclear lamina and changes in chromosome structure and gene regulation which lead to increases in transcriptional noise with age.[5] However, before understanding why it is important to study aging at the micro/epigenetic level, we must first examine why aging is important to study also at the macro/physiological level.

From a broader perspective, aging is fundamental to understand because it is universal to all mammals and to most multicellular species.[1] Furthermore, older adults are becoming an increasingly large proportion of the human world population. The National Institutes of Health (NIH) projects that the percentage of individuals worldwide aged 65 and over will jump from 8.5% in 2015 to 17% in 2050.[14] These statistics stand in sharp contrast to the lack of clinical trials that include elderly participants, as low as 32%.[15,16] The lack of focus on aging is also demonstrated by the fact that the NIH does not articulate separate research guidelines for working with elderly population but does include specific instructions for working with pregnant women, fetuses, neonates, and children under age 18.[17] This disparity between the proportion of the population who is elderly and the proportion of research on aging becomes even more paradoxical when considering that elderly patients are the most likely to have chronic conditions such as cancer and cardiovascular disease. For example, in the United States, elderly individuals account for 61% of all new cancer cases and 70% of all cancer deaths; however, between 1993 and 1996, the elderly comprised only 32% of participants in cancer clinical trials.[15] As another example, a distinct subtype of heart disease known as heart failure with preserved ejection fraction (HFpEF) shows a highly skewed incidence in elderly patients. Yet HFpEF is a subtype that is poorly understood, likely because of a lack of enrollment of elderly patients in heart failure studies.[18] Thus, the aging population is underrepresented in research despite being particularly sensitive to the presentation of many diseases. The necessity for more aging research extends beyond the clinic and to the laboratory. There is still a need for a better understanding of the fundamental processes underlying aging, as demonstrated by the latest 2020-2025 strategic plan and accompanying goals from the NIH's National Institute on Aging.[19] Some of these basic research goals include identifying the genetic, molecular, and cellular factors that determine the rate of aging and/or identifying reliable biomarkers of aging.[19] The strategic plan also mentions the need to identify the environmental exposures that may modify aging, although the definition of exposure in that context is vague and no mention of environmental chemicals is made.[19]

Yet, from a toxicological/pharmacological perspective, elderly patients have distinct physiological responses, especially regarding drug metabolism (reviewed in detail in Jafri[20]). Differences in drug metabolism are predominantly due to changes in hepatic and renal function with age, decreased absorption in the gut, decreased gastric acid secretion, decreased peristalsis, and decreased albumin.[16,21] This is highlighted by the conserved decrease in phase I metabolism (especially CYP450 levels) with age in rodents and humans.[22-24] Examples of age-dependent changes in metabolism include the decreased clearance rate of toxic metabolites of dichloroacetate in older humans and rats and an increased susceptibility of older women to the side effects of heparin.[25,26] Given that elderly patients have unique pharmacological profiles and require many drugs to treat chronic and age-related conditions, one would expect elderly patients to be widely studied in clinical research. However, when Konrat and colleagues studied published reports on 4 medications—pioglitazone, rosuvastatin, risedronate, and valsartan—commonly used by elderly patients, they found that elderly patients were grossly underrepresented.[27] In 2006 and 2007, of the 155 randomized control trials done, only 3 studies were exclusively of elderly patients, and the majority of trials had a proportion of elderly patients that was less than half the proportion actually treated in the clinic.[27]

In this context emerges a moral and ethical imperative to push for further research on aging organisms and populations. Clinical research on aging is also economically beneficial: in 2017-2018, $737 billion (22% of personal health care) in the United States was spent on Medicare, the insurance program primarily serving individuals aged above 65.[28] Research on aging may help improve and direct how this $737 billion is spent. However, this research needs to extend beyond clinical trials to study molecular, genetic, and epigenetic mechanisms of aging, mechanisms which will serve as a strong foundation for the development of age-appropriate therapies and risk assessments.

Why Do We Need to Study Aging through an Epigenetic Lens?

Not only are elderly patients not adequately represented in clinical trials, the fundamental molecular mechanisms underlying the aging process, not just correlating with it, are in need of more research. An increasingly recognized component of aging research involves understanding the epigenetic changes that occur with age. This intensifying focus on epigenetics over the life course has been sparked by the recognition that changes in epigenetic marks are one of the most accurate ways to calculate someone's biological age.[29] For example, algorithms such as the Horvath clock, which uses DNA methylation to evaluate the rate at which someone is aging, are extremely accurate across tissues and across species (from wolves to mice), with an age correlation of $r = 0.96$ in humans[29-31] The Horvath[31] clock, based on the methylation levels of 353 CpGs, indicates that DNA CpG methylation predictably changes with age, with these CpGs localized near genes related to cell death, cellular proliferation, and tissue development. However, studies, primarily in yeast and worms (*Caenorhabditis elegans*), show that histone modifications also change predictably with age.[5,32] Although the epigenetic regulation of *C. elegans* does not include DNA methylation, it does include a wide array of histone modifications (including methylation, acetylation, and phosphorylation) and small RNA which regulate chromatin compaction, RNA transcription, and protein translation.[33] Many of the histone-modifying complexes are highly conserved between *C. elegans* and mammals, for example, NuRD, CoREST, Sin3, DRM, and SET1/COMPASS.[33] Histone posttranslational modifications in *C. elegans*, like DNA methylation in mammals, change in a predictable way with age. For example, the repressive marks H3K9me3 and H3 lysine 27 trimethylation both decrease with age in *C. elegans* somatic tissue in a *set-26*-dependent manner as detected by Western blotting.[34] Furthermore, acetylation of histone H3 on lysine 56 decreases with age in yeast, and trimethylation of histone H3 lysine 36 decreases with age in yeast, mice, and *C. elegans*.[32,35] Given that epigenetic marks are some of the most accurate ways to measure aging, it is likely that many of the mechanisms closely tied to aging are epigenetic in nature. Yet, we have not fully elucidated the mechanisms by which these epigenetic changes are controlled. Epigenetic clocks are independent of mitotic divisions and senescence and change most rapidly during development, suggesting that the clock's rate of change represents the work done to maintain epigenetic stability.[31,36] The hypothesis that epigenetic stability decreases with age is consistent with the fact that epigenetic marks increase in variation, not just level, with age. This phenomenon has been observed for both DNA methylation and histone H3 and H4 acetylation in many different human tissues and cell types, including whole blood, lymphocytes, leukocytes, epithelial mouth cells, intra-abdominal fat, skeletal muscle biopsies, and brain.[37-43]

Interestingly, in some context, the connection between the epigenome, environment, healthspan (the length of time an organism lives free of chronic disease), and lifespan (the length of time an organism lives) is remarkably well understood. Caloric restriction is the prime example here. In mice, caloric restriction can reduce the rate of age-related changes in DNA methylation by modulating the methylation of DNA regulatory enzymes and thus their transcription, particularly reducing the messenger RNA expression of the methylcytosine dioxygenase TET3.[44] Rhesus monkeys exposed to caloric restriction for more than 10 years have DNA methylation signatures that make them appear 7 years younger.[45] Caloric restriction also increases the activity of sirtuin Sir2 and its mammalian homologues SIRT1-SIRT7. Sir2 is a histone deacetylase (HDAC) that acts as a positive regulator of several key pathways that promote healthy aging.[46] As HDACs, sirtuins' activities lead to a global decrease in acetylation and a local redistribution of histone H3 lysine 9 acetylation.[47-51] Mechanistic studies in *C. elegans* show that caloric restriction activates Sir2 by shifting the metabolism toward oxidative phosphorylation and thus

Table 1. Examples of environmental perturbations that either accelerate or decelerate aging.

ENVIRONMENTAL PERTURBATIONS	ORGANISM	EFFECT ON AGING	EPIGENETIC PATHWAY INVOLVED	REFERENCES
Calorie restriction	*C. elegans*, mice, monkeys, yeast	Decelerate	DNA methylation, histone acetylation	30, 45–49, 51, 52
Arsenic	*C. elegans*	Accelerate/decelerate[a]	Unknown	60, 63
Sodium butyrate	*C. elegans*, mice	Decelerate	Histone acetylation	54, 55
Valproic acid	*C. elegans*	Decelerate	Unknown	58
Exercise	*C. elegans*	Decelerate	Unknown	62
Monounsaturated fatty acids	*C. elegans*	Decelerate	Histone H3K4 methylation	57
Oxidative stress	*C. elegans*, humans	Accelerate	DNA methylation	61, 64, 65
Stress, high glucocorticoid levels, posttraumatic stress disorder	Humans	Accelerate	DNA methylation	66, 67
Obesity	Humans	Accelerate	DNA methylation	68
Alcohol	Humans	Accelerate	DNA methylation	69

[a]Acceleration versus deceleration depends on the dose.

producing an excess of nicotinamide adenine dinucleotide, a metabolite that Sir2 recycles.[52]

Other studies in *C. elegans* have demonstrated that different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways.[53] In both *C. elegans* and mammals, butyrate extends lifespan by inhibiting specific HDACs.[54,55] Inhibiting HDACs increases histone H3 lysine 9 acetylation and the expression of FOXO3, a transcription factor that coordinates antioxidant responses.[55,56] In addition, experiments in *C. elegans* have also shown that decreases in histone H3K4me3 extend lifespan by increasing the levels of monounsaturated fatty acids.[57]

Caloric restriction is an example of how an environmental stressor affects aging by activating specific epigenetic pathways. Because epigenetic writers and readers modulate aging, aging is not a stochastic process but rather one that can be dissected through scientific investigation. If we understand how the epigenome governs aging, we can better harness the environment to induce epigenetic changes that may mitigate age-related decline in biological functions. For example, screens in *C. elegans* found a previously approved US Food and Drug Administration drug, the HDAC inhibitor valproic acid, that increases lifespan and delays age-related declines in body movement.[58]

What Do We Know About the Intersection of Toxicoepigenetics and Aging, and What Gaps Remain?

Environmental factors such as caloric restriction (see above), arsenic, oxidative stress, and exercise accelerate or decelerate aging.[59-62] Table 1 provides information about the environmental cues linked to aging acceleration/deceleration, the species, and the epigenetic pathways involved. Although many correlations have been made between environmental factors and healthspan/lifespan, few causal relationships have been elucidated. Arsenic stands as a rare example of a chemical whose aging mechanisms have begun to be understood. Yet arsenic's dose-response relationship with longevity is neither simple nor monotonic. For example, experiments exposing the nematode *C. elegans* to arsenic show that a low dose (100 nm) of sodium arsenite extends lifespan, whereas higher doses (10 µM and above) shorten lifespan.[63]

Arsenic has a clear and profound impact on the epigenome.[70] Arsenic metabolism requires methylation using the cofactor S-adenosylmethionine (SAM).[70] S-adenosylmethionine is also a cofactor used by DNA methyltransferases DNMT1 and DNMT3A, meaning arsenic likely competes with DNA methyltransferases for available SAM cofactors.[70] Consistent with this, exposure of cell lines to 25 µM arsenic for 24 hours decreased SAM concentrations, increased global DNA hypomethylation, and repressed *Dnmt1* and *Dnmt3a* expression.[59,70] In addition, mice exposed to 100 µg/L arsenic concentrations in utero demonstrated global hypoacetylation at histone H3 lysine 9. The concentrations of arsenic required for epigenetic perturbations are physiologically relevant to human health; 17% of wells in the Western United States have arsenic concentrations above 100 µg/L.[71] Importantly, it is shown that arsenic may affect DNA methylation patterns in older populations (men with a mean age of 72 years); specifically, arsenic was correlated with a decrease in LINE-1 DNA methylation and an increase in Alu DNA methylation (LINE-1, $\beta = -0.03$, 95% confidence interval [CI] = −0.11 to 0.03; Alu, $\beta = 0.04$; 95% CI = −0.004 to 0.083] per 1 interquartile range [0.06 µg/g] increase in arsenic). These results suggest that the impact of arsenic on epigenetic pathways may interact with changes in DNA methylation that occur with age.[72]

Although arsenic represents an interesting case study at the intersection of epigenetics, toxicology, and aging, our understanding of these relationships with arsenic is still limited, and we know very little about the modulation of mechanisms of other chemicals with age. However, a framework has been proposed for studying the aging epigenome's interactions with chemical exposures that include the distinction between epigenetic drift and age-related epigenetic changes.[73] Age-related epigenetic changes are predictable, age-specific changes in epigenetic marks during physiological aging, whereas epigenetic drift describes stochastic changes in the variability of epigenetic marks with age.[73] These two concepts help distinguish two different mechanisms by which chemicals in the environment can affect aging. As discussed earlier, environmental stressors and chemical exposures can cause perturbations in age-related epigenetic changes. Lifetime psychological stress, high body mass index, and alcohol use all accelerate age-related DNA methylation changes that occur in human whole blood or liver.[66-69] Epigenetic drift, on the contrary, is superimposed onto the aforementioned age-related epigenetic changes and usually increases the variation in epigenetic marks with age—as demonstrated by comparing twin pairs of different ages.[74,75] Thus, in addition to age-related epigenetic changes, environmental chemicals can also deflect (perturb) epigenetic drift. For example, exposure to trichloroethylene increased DNA methylation variance at several gene regions in mouse CD4+ T cells.[76] Epigenetic drift increases mostly due to a gradual decrease in the efficiency of epigenetic writers and readers with age.[73] Toxicants likely increase epigenetic drift directly by inhibiting epigenetic writers or indirectly by altering gene expression and corresponding cell signaling.[73] For example, exposing monkeys and mice early in life to lead resulted in altered levels of epigenetic writers in brain tissue; in mice, expression of DNA methyl transferase 1 and MeCP2 decreased, levels of histone H3 lysine 9 acetylation and H3 lysine 4 dimethyl decreased, and levels of H3 lysine 27 trimethyl increased.[77,78]

It is evident that chemicals in the environment can directly or indirectly interfere with epigenetic writers' ability to maintain stability with age. Because toxicants can alter the ability of proteins to maintain epigenetic stability across the life course, it is reasonable to assume that age affects the epigenome's response to chemical exposures. Yet, this very question is dramatically underexplored. Tammen et al. compared global hepatic hydroxymethylcytosine levels when younger mice (4 months) or older mice (18 months) were exposed to alcohol. Younger mice, when compared with age-matched controls, had reduced global DNA hydroxymethylation, whereas older mice did not.[79] Age influences how an epigenome responds to a chemical, in this case alcohol exposure. Although there is a scarcity of information on different DNA methylation responses due to different ages of exposure, to our knowledge there are no published studies examining similar differential responses with histone modifications or small RNA. To adequately analyze the safety of chemicals we introduce into our environment, we need to know how organisms of different ages will respond. How sensitive an organism is to chemical exposures intimately depends on the organism's age and epigenetic regulatory machinery.

Given the importance of studying gene-environment interactions in the context of aging, it may be puzzling why this field is not more thoroughly explored. At the practical level, studying subtle changes in molecular trajectories over long periods of time inherently requires extensive amounts of resources. Furthermore, aging is a complex interconnected process that occurs systematically through all organ systems, yet not necessarily synchronously, and at multiple omics levels (genetic, epigenetic, proteomic, metabolomic).[5,11] This complexity requires that aging research be a collaboration between scientists from different backgrounds. This web of interconnectedness expands even further when considering the myriad of environmental chemicals that interact with the aging epigenome.

Recommendations: What Model Organisms and Technologies Can Lead to New Mechanistic Discovery in the Field of Gene-Environmental Interactions With Age?

Studying gene-environment interactions in the context of aging is important because we live in a complex chemical environment, humans (and most species) experience aging, and a growing proportion of humans are elderly. Although unraveling these interconnected and bidirectional networks is challenging, we have an array of biological and technological tools to help us achieve these goals.

Using the model organisms yeast, worms, flies, killifish, zebrafish, and mice can accelerate research on the connection between environment, epigenetics, and aging[5,80] (Figure 1). *Caenorhabditis elegans*, a species of roundworm, in particular offers important advantages over other model organisms when studying aging because it has a short lifespan, approximately 12 to 18 days at 20°C; is easily amenable to genetic manipulation; and is transparent, which facilitates imaging. Furthermore, epigenetic pathways regulating aging (except for 5mC DNA methylation) and metabolic pathways controlling toxicant metabolism are highly conserved between *C. elegans* and mammals.[33,81-83] Finally, *C. elegans' small size, short lifespan*, and inexpensive culture requirements make it amenable to high-throughput screenings necessary for us to understand the health consequences of the more than 350 000 chemicals and chemical mixtures that we interact with daily.[13] Because of the many advantages of using *C. elegans*, its has been at the forefront of aging research, especially research that considers chemical stressors. For example, our knowledge of how oxidative stress and diet affect aging and how the transcription factor FOXO coordinates aging largely originated from seminal *C. elegans* studies.[64,83-85] As *C. elegans* is highly tractable, it offers the possibility for mechanistic experiments that go beyond correlations to actual causal inferences. For example, *C. elegans* was the model organisms used to dissect

mechanisms by which metformin, a drug commonly used to treat diabetes, improves metabolism and lifespan.[86] Metformin also provides an example of how studies in *C. elegans* can be both mechanistic and directly relevant to the clinic.

Technology also has an important role to play, and researchers have developed a wide array of computational modeling techniques that will help us analyze the complex web of gene-environment interactions that we live in. Computationally modeling many of these interacting networks is valuable because it can help us visualize, identify, and interpret multiple dimensions in a single graph—allowing for us to make inferences that we would otherwise not be able to draw.[87-89] One example of a computational tool that could be applied to the question of aging is the Mergeomics pipeline. Mergeomics is a publicly available R package that can integrate epigenetic, transcriptomic, proteomic, and genetic information to infer causal relationships.[90] The Mergeomics pipeline integrates these data by, first, calculating the association between particular omics data sets and a disease, for example, genes correlated with a disease that then form a coexpression network, and, second, by overlaying these disease-associated pathways on molecular interaction networks (Bayesian networks) to identify hubs in the network that are key upstream regulators of disease.[90] Thus, the Mergeomics pipeline is advantageous because it can integrate many different types of data (genetic, transcriptomic, proteomic) as well as data from different species. In this context, adding age as a study variable for the generation of multi-omics data to be fed into Mergeomics would help identify regulatory networks that explain age-dependent responses to environmental stressors.

It is perhaps human nature to show apprehension toward aging. As Simone de Beauvoir[91] writes in *The Coming of Age*:

> When we look at the image of our own future provided by the old we do not believe it: an absurd inner voice whispers that that will never happen to us—when that happens it will no longer be ourselves that it happens to.

Yet, with both the aforementioned model organisms and technological advances in genetics, epigenetics, and toxicology, we have the tools, and perhaps the moral imperative, to understand how aging pathways are regulated by chemicals and how age sensitizes an individual to chemical exposures.

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Author Contributions

RBC and PA wrote the manuscript.

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