Cynthia V. Rider · Jane Ellen Simmons
Editors

Chemical Mixtures and Combined Chemical and Nonchemical Stressors

Exposure, Toxicity, Analysis, and Risk

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Foreword by Linda S. Birnbaum

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To all who came before, paving the way, to those currently engaged in the active practice of mixtures research – from unraveling complex exposures to performing toxicological evaluations of mixtures and assessing cumulative risk. In particular, we recognize George Alexeeff, who dedicated himself to advancing public health and understanding cumulative health impacts, with particular attention to vulnerable populations overburdened by multiple sources of pollution.

Foreword

Mixtures are our everyday reality. We are exposed to numerous chemicals throughout our lifetimes from various sources in our environment – personal care products, food and water contaminants, occupational exposures, traffic pollution, molds and allergens, pesticides, pharmaceuticals, and too many others to list. These external exposures are influenced by our internal milieu, which reflects background genetics and acquired epigenetic changes, as well as a host of nonchemical environmental factors (e.g., microbiome, psychosocial stressors, disease states, nutritional status). Considering this complex and dynamic exposure scenario, it has long been recognized that evaluating exposures and their effects on a chemical-by-chemical basis is not adequate for protecting public health. However, there has not been a clear path forward for changing the paradigm, and the complexities involved in mixtures research and risk assessment have often been used as justification for perpetuating the standard approach of assessing one chemical or one exposure at a time. Despite the challenges, many researchers across diverse fields of science have been actively engaged in the study of mixtures. Through these efforts, we have gained a significant understanding of the key issues in mixtures science and developed many approaches for addressing these issues. In this book, insights from leading-edge researchers and analysts have been pulled together to present a comprehensive picture of the current state of mixtures science and provide tools for practitioners engaged in assessment of risk from exposure to mixtures.

My interest in mixtures has spanned the breadth of my career. Through my own research program and as director of the National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program (NTP), I have had the opportunity to be a part of the mixtures research story. One of my early interests was in mixtures of dioxin-like chemicals. Work from my lab and others contributed to some of the first efforts to account for the cumulative risk associated with exposure to mixtures. Dioxins, typified by the reference contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), represent persistent organic pollutants that are highly toxic and exist in complex mixtures. Dioxins accumulate in the food chain, and people are exposed today mainly through food consumption. The toxic

equivalency factor approach, developed to sum the total burden of dioxin-like chemicals, was a pioneering effort to move beyond single chemical analyses of risk and account for the cumulative effects of dioxin-containing mixtures. Lessons learned through that effort have been applied to many other classes of environmental contaminants. I have since been involved in mixtures research touching upon endocrine disruptors, flame retardants, organophosphates, and perfluorinated compounds, among others.

During my tenure as director of NIEHS, I have had the pleasure of seeing mixtures research elevated through numerous workshops and NIEHS-wide research efforts. In 2011, NIEHS hosted a meeting titled "Advancing Research on Mixtures: New Perspectives and Approaches for Predicting Adverse Human Health Effects." This workshop brought together mixtures experts from exposure science, toxicology, epidemiology, statistics, and risk assessment to outline challenges in mixtures research and discuss approaches to address those challenges. More recently, NIEHS organized a workshop on "Statistical Approaches for Assessing Health Effects of Environmental Chemical Mixtures in Epidemiology Studies." Development of statistical methods for analysis of mixtures in epidemiological studies was an area specifically identified in the 2011 workshop as requiring research attention. During this innovative workshop, participants were given multiple epidemiological datasets and asked to apply their analysis methods, which were then compared. In addition to workshops, the NIEHS has also demonstrated a commitment to mixtures research by including "Understand how combined environmental exposures affect disease pathogenesis" as Goal #4 in the 2012–2017 NIEHS Strategic Plan, which identifies priority areas of research. This goal includes assessing the joint action of multiple environmental factors, including both chemical and nonchemical stressors. Finally, numerous projects led by NIEHS scientists and grantees are dedicated to better understanding the potential health effects of exposure to mixtures. Projects supported by NIEHS range widely from defining the totality of human exposure through research into the exposome to targeted projects that address the toxicity of specific complex mixtures, such as toxicity testing of botanical dietary supplements at the NTP.

The future of mixtures research is bright. Mixtures research has moved beyond simply combining chemicals to look for greater than additive interactions. Instead, we are using the latest understanding of biological systems to predict how combinations of chemicals and nonchemical factors might interact by targeting an adverse outcome pathway. We are developing hypotheses of combined effects and using every tool available to test these hypotheses. Combinations of in silico, in vitro, alternative animal and traditional toxicity studies are being employed to prioritize mixtures for study and to routinely assess both defined and complex mixtures. We are developing more sophisticated methods to analyze "big data" resulting from high-content assays and refining methods to predict mixture effects. All of these efforts inform risk assessment efforts that are increasingly expanding beyond single chemicals to address cumulative and community-specific risks. As we move forward with mixtures research, we are critically evaluating findings in the context of our historical knowledge of mixtures.

This book is an excellent example of the type of thoughtful collaboration that is required to understand the human health consequences of a life lived, beginning before conception, in a soup of chemical and nonchemical stressors. The editors and the authors collaborated on producing a book that stands in sharp contrast to most multiauthored books. The authors agreed to use a common set of definitions and terminology, greatly enhancing the ability of the reader to move between chapters and sections. Conference calls were held at the request of various writing teams with other writing teams. Further, the authors shared draft chapters within and between sections to ensure continuity and lack of duplication. The book and the reader directly benefit from the intense effort required to accomplish this level of integration. The book loosely follows the risk assessment paradigm (exposure, hazard identification, risk characterization), providing the reader with essential information and tools. It also highlights some recent advances in predicting co-occurrence, using the new adverse outcome pathway concept to group chemicals and to identify the appropriate risk assessment strategy, and environment-wide association studies (EWAS) for identification of the effect drivers within complex exposures. The book concludes with suggested approaches for incorporating nonchemical stressors into cumulative risk assessment. This book provides a sound foundation for anyone engaging in some aspect of consideration of chemical mixtures and chemical and nonchemical stressors and their impact on human health.

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Contents

Chapter 1
Introduction

Cynthia V. Rider and Jane Ellen Simmons

Abstract All people are exposed to complex and dynamic mixtures of chemicals and nonchemical stressors throughout their lives. Understanding how these combined exposures impact human health is an active area of research spanning exposure science, toxicology, epidemiology, statistics, and risk analysis. Mixtures under study range from simple combinations of chemicals to the complete exposure profile known as the exposome. Research efforts to explore mixtures have used individual chemical data to estimate mixture effects in a bottom-up approach and have evaluated the effects of whole mixtures in a top-down approach. Considering the numerous perspectives and approaches, mixture terminology has been particularly challenging. In this introductory chapter, we lay the groundwork for the book by providing a rationale for the study of mixtures, defining important terms, and describing the flow of the book.

Keywords Mixtures · Nonchemical stressors · Combined exposures · Mixture terminology

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1.1 To Study Mixtures or Not to Study Mixtures, That Is the Question

The study of mixtures is often paradoxically referred to as essential and as prohibitively complicated. Essential, because it is widely accepted that humans are not exposed to a single chemical throughout their lifetimes, but to a complex milieu of chemicals that differ circumstantially and across time (Pohl and Abadin 2008). Therefore, efforts to better understand how public health and ecological systems are affected by their ambient conditions must include consideration of mixtures (Sexton 2012). Prohibitively complicated, because as researchers move toward addressing more environmentally relevant mixtures, uncertainty increases in terms of study design, interpretation of results, and translation into risk estimates (Fig. 1.1). This sentiment is captured in a 2004 paper titled "Chemical Mixtures: An Unsolvable Riddle?" which details the increased uncertainty in assessing the risk of mixtures as compared to single chemicals (Borgert 2004). These two opposing viewpoints (i.e., essentiality versus prohibitive complexity) have provided the backdrop for decisions on whether to continue on a path of assessing chemicals individually or to move toward more standard consideration of mixtures. Fortunately, many investigators have braved the various obstacles and contributed to the significant body of mixtures research that now exists. It is important to note that the study of mixtures has included effort directed at building on knowledge from the study of individual chemicals and simple mixtures and developing new ways to interpret information from the study of complex mixtures. In other words, mixture studies have included bottom-up approaches to understand how components of a mixture contribute to mixture effects and top-down approaches involving assessment of complex mixtures and developing methods to interpret and apply information gained.

1.1.1 Mixtures Are Reality

According to the Environmental Protection Agency's Toxic Substances Control Act (TSCA) Inventory, there are approximately 85,000 chemical substances manufactured or processed in the United States (U.S. EPA 2017a). People are constantly exposed to dynamic mixtures of chemicals, through the air we breathe; our diets; use of personal care and household products; pharmaceutical intake;

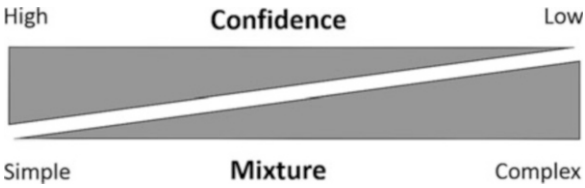

Fig. 1.1 As the complexity of the study mixture increases, confidence in associated data and interpretation decreases

occupational, recreational, accidental, and intentional exposures; etc. It is clear from biomonitoring efforts such as the National Health and Nutrition Examination Survey (NHANES), which measures over 200 chemicals in human samples, that chemicals are making their way from the environment into our bodies (CDC 2015). Although chemicals have been the focus of the majority of mixtures research, many of the concepts also apply to nonchemical factors. There are many pathways for nonchemical factors to influence toxicity outcomes.

Consideration of the many nonchemical factors that can potentially affect human health or modify our response to chemical exposures moves us closer to the real-world scenario. People are exposed to various nonchemical factors that can include physical stressors (e.g., heat, radiation, allergens, and noise) as well as psychosocial stressors (e.g., circumstances or events that elicit an acute or chronic stress response). Furthermore, there are some factors that do not fit neatly into categories. For example, over-consumption of an essential trace mineral (e.g., manganese) could be considered as a chemical exposure with the potential to elicit toxicity, while a trace mineral deficiency could be considered as a nonchemical factor that could also negatively affect health but with a different constellation of effects.

People are exposed to different mixtures throughout their lifetime, and exposure profiles can change drastically based on behavior as well as age. For example, crawling and hand-to-mouth behaviors in an infant may result in relatively high exposure to house dust and chemicals attached to dust, as compared to adults (Pohl and Abadin 2008). Product use and diet can change over time, as can surroundings. Whereas young children may spend a significant portion of their time at home, outside, or in a daycare setting, adults may be exposed to very different mixtures depending on their occupation. In addition to changing exposures over time, there are also differences in the responses of individuals to exposure that can depend on genetic background, life stage, or disease state.

1.1.2 What Mixtures Are Being Studied?

Accepting that there are an infinite number of potential combined exposures for study, it is important to define the term "mixture" before discussing the associated science. Although the majority of this book addresses mixtures of chemicals, combined exposures to any factors (chemical, physical, or psychosocial) to which a human, animal, or cell are exposed, either concurrently or separated in time, may also be relevant determinants of outcome. Mixtures span a wide range of complexity. The following is a presentation of common terms and definitions used to describe mixtures. It is not meant to be exhaustive, but to provide illustrative examples of the types of mixtures that are the focus of research attention.

Mixture Types The term *binary mixtures* refers to combinations of two factors and represent the simplest possible mixtures. Good examples of binary mixtures can be found in the pharmacology field, where binary combinations of drugs are either recommended for increased efficacy/selectivity (e.g., combination

chemotherapy) or discouraged based on the potential for increased toxicity (e.g., many drugs are counter-indicated for people taking the anticoagulant warfarin). A specific example of a well-studied binary mixture can be found in combined use of alcohol and acetaminophen – a combination that can lead to increased hepatotoxicity as compared to use of one or the other alone. Typically, binary mixtures are the subject of focused studies to assess interactions among select factors. Ternary and quaternary mixtures can be considered as incrementally more complex versions of this simple binary mixture category.

Following binary mixtures, the next level of complexity can be captured by the term *defined mixtures* (which subsumes binary, ternary, and quaternary combinations). In defined mixtures, all of the constituents and their concentrations are known. Many studies with defined mixtures have been conducted to assess the performance of predictive models of mixture toxicity based on single chemical data (i.e., component-based approaches) (Kortenkamp 2007; Howdeshell et al. 2017). Defined mixtures are also studied under the assumption that complex mixture toxicity can be estimated by evaluating a subset of known active constituents. For example, research efforts can focus on a subset of dioxin-like chemicals that have demonstrated binding to the aryl hydrocarbon receptor, while the actual mixture to which people are exposed could contain a more complex suite of structurally diverse chemicals. Alternatively, exposure data can be used to identify a subset of chemicals from which to build defined mixtures for study. Although defined mixtures do not recapitulate the complexity of real-world exposures, they offer an intermediate step in understanding the behavior of chemicals acting jointly.

Moving beyond defined mixtures are different types of *whole mixtures* or *complex mixtures*. Recently, the more descriptive term to categorize these mixtures, "chemical substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials," abbreviated as UVCB, has been used by the EPA in the Toxic Substances Control Act (TSCA) Chemical Substance Inventory (U.S. EPA 2017b) and by the European Chemicals Agency (ECHA) (ECHA 2017). Complex mixtures can be further subcategorized into *formulations* and *environmental mixtures*. Formulations are whole mixtures that contain one or more purported active chemical(s) and a number of potentially inert ingredients. Although it is possible for formulations to be defined mixtures, they are categorized as whole mixtures here, because many of their ingredients can be proprietary or can be variable in terms of their concentration. Consider personal care products, which may list "fragrance" as an ingredient, or commercial formulations of flame retardants, such as Firemaster 550, which include a proprietary mixture of different active classes of chemicals (e.g., brominated and non-halogen flame retardants). Although the active constituent(s) are often identified in formulations, they are often unknown in environmental mixtures. These mixtures include samples taken from hazardous waste sites (e.g., Superfund sites), spills or catastrophic events (e.g., Elk River chemical spill, Gulf oil spill, Fukushima disaster), or those created by intentional processes (e.g., drinking water disinfection, gas extraction via hydraulic fracturing).

Finally, the most complex and challenging type of mixture to study is that encompassed by the *exposome* concept – all exposures over a lifetime. This concept includes all factors (chemical, physical, biological, psychosocial) that an individual is exposed to from conception to death (Wild 2005). Essentially, the exposome represents the real-world scenario. The difficulties in assessing the exposome are in measuring the totality of exposures and deconvoluting how these exposures collectively affect health. It is important to note that to develop effective interventions, the drivers of harm must be identified.

In addition to defining the types of mixtures that are typically the focus of research, there is also a need to define the relevant terms that have been used in mixture science. As discussed below, terminology has been particularly confusing in the field of mixtures, with multiple meanings associated with a single term and meanings differing between disciplines. The next section does not offer an official consensus on terminology, but instead attempts to clarify meanings for the terms as they are used in this volume.

1.1.3 Additional Mixture Terminology

The topic of mixtures has been of interest to many diverse fields, including ecology, epidemiology, exposure science, pharmacology, risk analysis science, statistics, and toxicology. Scientists in these fields approach mixtures from perspectives informed by their expertise and apply specialized terminology to address mixture questions most relevant to their discipline. While this multidisciplinary treatment is critical to understanding environmental and human health effects resulting from exposure to mixtures, it has also led to confusion over terminology.

A list of common terms and definitions is provided below to clearly articulate the interpretation of each term applied within this text. Throughout the book, the terms will be used as defined below with exceptions explicitly noted by chapter authors.

1.1.3.1 Terms to Identify the Combined Exposure or Mixture of Interest

Combined Exposure This represents a broad term that encompasses any combination of two or more exposures or factors of interest. Exposures can include chemical and/or nonchemical stressors and can be concurrent or separated in time. Often, this term is used interchangeably with the term "mixture." However, while "combined exposure" can refer to chemical and/or nonchemical exposures, the term "mixture" is limited to chemical exposures in this text.

Mixture A mixture is any combination of two or more chemicals of interest present concurrently or separated in time. See additional definitions of mixture types above. A *simple mixture* is one where the effects of the mixture can be

reasonably estimated based on knowledge of its components. Although the upper limit of a simple mixture has not yet been defined and may depend on the nature of the component chemicals, it is estimated that mixtures between 2 and 25 components would qualify as simple mixtures. A *complex mixture* contains too many components to allow for a reasonable estimate of mixture effects based on component chemicals and usually contains some *unidentified fraction* (portion of a mixture that has not been chemically characterized). In contrast, a *defined mixture* contains chemicals that are identified and present in known quantities (see above for more detailed description).

Chemical and Nonchemical Factors The term "chemical" refers to any natural or anthropogenic substance to which a person or animal can be exposed. Unless noted, this does not include endogenous chemicals (e.g., natural hormones produced in the body). Chemicals are differentiated from nonchemical factors, which can be subdivided into *physical* and *psychosocial stressors*. Physical stressors are defined here as biological agents (e.g., viruses) or external forces (e.g., radiation, noise) that can modify exposure or elicit a physiological response from the exposed organism. Psychosocial stressors are defined as factors in the external environment that are perceived to be harmful (e.g., fear of violence), which can result in physiological changes (e.g., increased production of cortisol).

1.1.3.2 Terms to Describe the Joint Action of Exposures

Dose Addition Dose addition and *concentration addition* are essentially the same concept, with the term "dose" applying where appropriate (e.g., oral gavage to rodents, ingestion of pharmaceuticals) and the term "concentration" applying where appropriate (e.g., aquatic exposures, cell-based experiments). Under dose addition, the effect (response) of the mixture is predicted by summing the exposure doses of the component chemicals. A key concept is that the doses of the component chemicals are weighted by their toxic potency. In the idealized situation, the component chemicals behave as concentrations or dilutions of each other. Dose addition is thought to be best applied to those chemicals that share a common or similar mode of action or similarity of target organ. Thus, the behavior of a chemical mixture is considered dose additive if the effects of the combined components (i.e., the effect of the mixture) can be estimated from the sum of the scaled doses of the individual components.

Independent Action Under independent action (also called *independent joint action* or *response addition*), the effect (response) of the mixture is predicted by summing the effects (responses) of the component chemicals. A key concept is that the mixture response is predictable by the sum of the responses of the components using the formula for the sum of the probabilities of independent events. Independent action is thought to be best applied to mixtures of chemicals that have dissimilar modes of action; these chemicals are toxicologically independent (i.e., the biological response to each chemical is the same whether or not the other

chemical(s) is present). Thus, the behavior of a chemical mixture is considered to be consistent with independent action if the effects of the combined components (the effect of the mixture) can be estimated from combining the responses of the individual chemicals using an equation to describe the probability of independent events co-occurring. *Effect summation*, which represents a simple summation of component effects, can be viewed as a special case of independent action. Although effect summation is commonly used in the mixture literature, its application should be limited (see Chap. 9).

Interaction The term "interaction" is common in both toxicology and epidemiology and has notably different implications depending on the context (see Chap. 10). Unless otherwise stated, "interaction" will be used to describe a joint action among combined exposures that differs significantly from the clearly stated expectation of additivity (e.g., predicted effects based on dose addition or independent action).

Greater-than-Additive and Less-than-Additive It is recommended that conclusions regarding interactions be drawn as to whether the response of the mixture in question is consistent with a specific definition of additivity as in "no detectable deviation from additivity" or inconsistent with the specific definition, showing "greater-than-additive" or "less-than-additive" responses. The definition of additivity should be specific as to dose addition or independent action with appropriate reference to the underlying literature. It is highly recommended that the use of the terms "synergy" and "antagonism" be avoided due to the vast confusion that has plagued chemical mixture toxicology and risk assessment. In effect, they are problematic because of the many differing definitions of these terms and their widespread use without articulation of the meaning ascribed by the user. To avoid confusion "synergy" is replaced with "greater-than-additive," and "antagonism" is replaced with "less-than-additive." When the term "synergy" cannot be avoided, it should be defined within the context of the definition of additivity being used.

1.1.3.3 Terms to Describe Exposure or Risk

Aggregate Exposure, Aggregate Risk The term "aggregate" is used here to indicate the summing of exposure for an individual chemical across all relevant routes, so that the total dose to the person/animal model can be used to estimate the aggregate risk. For example, in the case of bromodichloromethane, multiple routes of exposure (i.e., oral, inhalation, and dermal) make significant contributions to internal dose and contribute to the aggregate exposure and aggregate risk (Haddad et al. 2006).

Cumulative Exposure, Cumulative Risk The term "cumulative" is used here to indicate consideration of more than one stressor (chemical or nonchemical) in an exposure or risk assessment. This is a general term that can be applied to any exposure characterization or risk assessment that includes multiple factors (e.g.,

cumulative assessment of organophosphate pesticides, community-based assessment involving chemical and nonchemical exposures in a select population) and can be contrasted against single chemical exposure or risk evaluations. *Cumulative is notably distinct from aggregate and should not be used interchangeably. However, an exposure characterization or risk assessment can be both aggregate and cumulative*. It is an umbrella term that does not dictate the specific model used to assess cumulative risk, and concepts of either dose addition or independent action can be used as a basis for the calculation of cumulative risk. It is important to note the distinction between the concepts used to describe joint action (dose addition and independent action) and the methods available for calculation of risk (e.g., hazard index, relative potency factors) that are built upon those concepts.

Exposure/Response Modifier Due to inconsistent definitions of the terms susceptibility, vulnerability, and sensitivity, as well as interchangeable use in the literature, the single term "exposure/response modifier" has been suggested as an umbrella term to capture any condition or state that could alter exposure or response to a chemical or nonchemical stressor or buffer. Although the use of this more generic term is recommended, the terms *susceptibility, vulnerability, and sensitivity* have a long history of use, particularly in epidemiology, and do appear in this volume. Throughout the book, these three terms are used interchangeably and defined as any factor or set of factors that increases the likelihood of harm from an exposure. Examples include low socioeconomic status and other psychosocial stressors (e.g., exposure to violence, lack of access to healthcare) within a population that contribute to decreasing the resiliency reservoir required to maintain health. For more information, see Gee and Payne-Sturges (2004). The traditional use of "genetic susceptibility" refers specifically to genetic variations that increase the likelihood of harm from an exposure. For example, inherited mutations in the tumor suppressor genes *BRCA1* and *BRCA2* predispose women to development of breast cancer (Valencia et al. 2017).

1.2 Challenges in Mixtures Research

As discussed previously, mixtures can be approached from a reductionist (i.e., bottom-up) or holistic (i.e., top-down) perspective. The challenges associated with mixtures research differ depending on which approach is used. In the reductionist approach, the challenges can lie in relating findings from carefully controlled experiments with defined mixtures to the real-world scenario. On the other hand, the holistic approach presents a different set of challenges in understanding how to interpret data and develop targeted interventions based on findings from complex mixtures.

Specific challenges associated with a reductionist approach include deciding on chemicals to incorporate in the defined mixture for study and understanding how the defined mixture fits into the bigger exposure picture. Prioritizing chemicals for

inclusion in mixtures research or cumulative risk assessment can be driven by exposure information or biological considerations (e.g., common toxicity target). The NHANES database has been an excellent source of information for better understanding of co-exposures. In terms of using biological information to prioritize mixtures for study, there has been movement from focusing on isolated targets (e.g., estrogen receptor, liver) toward a systems biology view that considers the complex network of signaling pathways involved in disease manifestation. This evolution has expanded our view on which chemicals and/or factors to include in defined mixture studies.

Challenges in the study of whole mixtures include identifying which chemicals are present in the mixture of concern and which chemicals are responsible for eliciting the observed effects. Identifying the active constituents within a complex mixture can require significant investment in chemical analysis without guaranteed success. Furthermore, efforts to confirm the biological activity of possible active constituents by isolating, identifying, and testing individual chemicals are complicated by the possibility of interactions among constituents. In effect, it is often very difficult to disentangle the problem when the mixture, the biological target, and the interaction of the mixture with the biological target are all complex.

1.3 Progress to Date and Future Directions

Despite the many challenges associated with the study of mixtures, there have been significant advancements over the past century. The first major step was the development of modeling approaches that used single chemical dose-response data to predict mixture effects in the mid-1900s (detailed in Chap. 9). Almost half a century later, these models were further advanced and applied to different types of mixtures. There followed a period defined by the search for unexpected interactions, thereby beginning the controversy over use of the term "synergy." Perhaps a lack of discovery of the "holy grail" (i.e., chemicals that produced thousandfold greater effects when tested in combination versus alone) shifted focus away from the search for such remarkable deviations from additivity toward more sophisticated modeling of predicted mixture responses.

This advancement in modeling of predicted mixture toxicity continues today. Work in this area represents a joint effort between statisticians and toxicologists to design more efficient and appropriate studies to assess mixtures, to develop methods to better fit individual chemical data, and to utilize appropriate statistical methods for comparing predicted responses to observed responses. Increasingly, there has been a recognition that methods to assess mixtures should be fit for purpose, not one size fits all.

Another more recent evolution is from mixtures research focusing exclusively on simple mixtures to an expansion into complex mixtures. Although there is a long history of assessing the toxicity and risk associated with complex mixtures (e.g., diesel exhaust, tobacco smoke) as a single entity, attempts to better understand

complex mixtures and compare across them represent a relatively recent development. There are still only a handful of studies that attempt to assess whether or not complex mixtures are *sufficiently similar*, meaning that data from a well-characterized reference mixture can be applied to a related, untested mixture of interest. This represents an area where more research is needed.

Finally, incorporation of nonchemical stressors, including both physical and psychosocial, into mixtures research and cumulative risk assessments remains an area that requires significant attention. The default has been to apply methods from chemical mixtures to these nonchemical stressors, but more work is needed to validate this application. The development of case studies that incorporate both chemical and nonchemical stressors will offer opportunities to identify data needs and areas that require refinement.

1.4 Flow of Book Sections

In this volume, the current state of the science on mixtures is presented. Beginning with exposure, the measurement of chemicals is addressed, including both internal measures of chemicals in human samples (biomonitoring) (Chap. 2) and external measures of chemicals in the environment (Chap. 3). These chapters on exposure measurement are complemented with a chapter covering modeling approaches to describe exposure (Chap. 4). The set of three chapters provides insight into the characterization of mixtures in the environment, people, and populations.

Following identification of chemicals, a prioritization step is needed to focus attention on mixtures that require research attention. Prioritization can be based on the presence and concentration of chemicals (e.g., high levels measured in an NHANES population), or it can be driven by a particular goal (e.g., occupational risk assessment). The section on prioritization of chemicals focuses on newer approaches that have been developed to intelligently design mixtures for study. These include using statistical tools for determining association of exposure to effect markers in Environment-Wide Association Studies (EWAS; Chap. 5), methods adapted from the field of biogeography to examine chemical co-occurrence (Chap. 6), and building adverse outcome networks using mechanistic data to inform mixture decisions (Chap. 7).

Having identified the mixture(s) of interest, it is then critical to understand how to go about evaluating the mixture. In the section on Mixture Toxicology, there is a thorough coverage of evaluating defined mixtures using component-based approaches. A chapter on dose-response evaluation addresses single chemical data that are used to understand mixtures (Chap. 8). Predicting mixture effects is addressed in multiple chapters, which cover models of additivity conceptually (Chap. 9), contrasting toxicological and epidemiological approaches (Chap. 10), and statistical comparison of observed and predicted mixture responses (Chap. 11). The challenging topic of physiologically based pharmacokinetic modeling of

mixture toxicity is also addressed (Chap. 12). Finally, elements of experimental design in the study of mixtures are examined (Chap. 13).

The risk assessment section covers both component-based (Chap. 14) and whole mixture (Chap. 15) approaches. There are many well-established component-based methods for estimating cumulative risk. Whole mixtures are often handled like single chemicals; however, there is a need for methods to determine sufficient similarity of whole mixtures. Throughout these chapters, areas of uncertainty are highlighted.

In the last section of the book, nonchemical stressors are brought into the picture. Physical stressors (e.g., heat, radiation) are discussed in terms of how to begin to quantify the effects of these stressors and bring them into cumulative risk assessments (Chap. 16). Psychosocial stressors (e.g., fear of violence) represent a challenging area of combined exposure research and cumulative risk evaluation that include the unique aspect of perception (Chap. 17). Finally, many of the concepts presented throughout the book are brought together by providing an example of community-based cumulative impact assessment (Chap. 18).

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References

Borgert, C.J. 2004. Chemical mixtures: An unsolvable riddle? *Human and Ecological Risk Assessment* 10: 619–629.

CDC. 2015. Fourth National Report on Human Exposure to Environmental Chemicals. In*:* CDC (ed.).

ECHA. 2017. *What is a substance?* [Online]. Available: https://echa.europa.eu/support/substance-identification/what-is-a-substance. Accessed 15 Dec 2017.

Gee, G.C., and D.C. Payne-Sturges. 2004. Environmental health disparities: A framework integrating psychosocial and environmental concepts. *Environmental Health Perspectives* 112: 1645–1653.

Haddad, S., G.C. Tardif, and R. Tardif. 2006. Development of physiologically based toxicokinetic models for improving the human indoor exposure assessment to water contaminants: Trichloroethylene and trihalomethanes. *Journal of Toxicology and Environmental Health-Part A-Current Issues* 69: 2095–2136.

Howdeshell, K.L., A.K. Hotchkiss, and L.E. Gray. 2017. Cumulative effects of antiandrogenic chemical mixtures and their relevance to human health risk assessment. *International Journal of Hygiene and Environmental Health* 220: 179–188.

Kortenkamp, A. 2007. Ten years of mixing cocktails: A review of combination effects of endocrine-disrupting chemicals. *Environmental Health Perspectives* 115: 98–105.

Pohl, H.R., and H.G. Abadin. 2008. Chemical mixtures: Evaluation of risk for child-specific exposures in a mufti-stressor environment. *Toxicology and Applied Pharmacology* 233: 116–125.

Sexton, K. 2012. Cumulative risk assessment: An overview of methodological approaches for evaluating combined health effects from exposure to multiple environmental stressors. *International Journal of Environmental Research and Public Health* 9: 370–390.

U.S. EPA. 2017a. *About the TSCA chemical substance inventory* [Online]. U.S. EPA. Accessed 15 Dec 2017.

———. 2017b. *Chemical substances of unknown or variable composition, complex reaction products and biological materials (UVCB Substance) on the TSCA Inventory* [Online]. U.S. EPA. Available: https://www.epa.gov/tsca-inventory/chemical-substances-unknown-or-variable-composition-complex-reaction-products-and. Accessed 15 Dec 2017.

Valencia, O.M., S.E. Samuel, R.K. Viscusi, T.S. Riall, L.A. Neumayer, and H. Aziz. 2017. The role of genetic testing in patients with breast cancer a review. *JAMA Surgery* 152 (6): 589–594.

Wild, C.P. 2005. Complementing the genome with an exposome: The outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiology, Biomarkers & Prevention* 14: 1847–1850.

Part I
Combined Exposures

Chapter 2
Biomonitoring to Assess Exposures to Mixtures of Environmental Chemicals

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Abstract In modern societies, humans may be exposed to a wide spectrum of environmental stressors, including mixtures of anthropogenic chemicals. Furthermore, because human exposure does not occur under controlled conditions of dose-response evaluations in animal studies, exposure assessment is complex. Three main tools have been used to assess human exposures: history/questionnaire information, environmental monitoring, and biomonitoring (i.e., measuring concentrations of the chemicals or their metabolites or adducts in human specimens). In this chapter, we will discuss the suitability of biomonitoring data for evaluating exposures to mixtures of environmental chemicals.

Keywords Biomonitoring · Exposure · NHANES · Endocrine disruptors · Environmental chemicals

2.1 Introduction

In the course of their daily routines, humans are exposed to a large number and variety of physical, biological, psychosocial, and chemical stressors. All of these stressors, their timing, and duration along with each person's genetic makeup, diet, and lifestyle can affect human health (Needham et al. 2005a; Birnbaum 2010). Because of the complexity of such exposures and their interactions, understanding the potential effects of the exposures on health requires a multidisciplinary approach—a topic of interest to several scientific fields including, among others, chemistry, ecology, epidemiology, exposure science, pharmacology, risk analysis, statistics, and toxicology (Carlin et al. 2013).

Disclaimer The findings and conclusions in this report are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC).

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Three main tools have been used to assess chemical exposures: history/questionnaire information, environmental monitoring, and biomonitoring (i.e., measuring concentrations of the chemicals or their metabolites or adducts in human specimens) (Calafat et al. 2006; Sexton et al. 2004; Needham et al. 2005b). Exposure models, covered in the last chapter of this section, usually incorporate information from the three approaches. The use of history/questionnaire data to assess human exposure to environmental chemicals falls within the purview of environmental epidemiology. Indirect measures of exposure (e.g., environmental monitoring) are the subject of the second chapter of this section. In this chapter, we will cover the assessment of internal exposures using biomonitoring.

2.2 Biomonitoring Overview

Biomonitoring is the assessment of internal dose (i.e., body burden) by measuring the parent chemical (or its metabolite or reaction product) in human samples. Biomonitoring, a "gold standard for assessing exposure to chemicals" (Sexton et al. 2004), has many potential uses in the public health context of preventing disease related to people's exposure to chemicals. Biomonitoring can be used to detect and monitor chemical exposures, to assess people's health risk as a result of such exposures, to develop and implement interventions to reduce exposures, and to evaluate the effectiveness of those interventions (CDC 2009; National Research Council 2012).

In some cases, evidence of chemical exposures and their human health effects (e.g., lead poisoning) have been known since antiquity (Waldron 1973; U.S. EPA 1985), although the use of biomonitoring to track lead poisoning did not start until the late 1890s with the screening of factory workers' blood and urine (Sexton et al. 2004). Since then and thanks, in part, to access to and availability of sophisticated analytical chemistry techniques, trace levels of lead and many other chemicals in a person's body can be routinely measured with high precision and accuracy (Angerer et al. 2007; Pirkle et al. 1995).

These scientific and technologic advances along with the increase in global production of chemicals and their use in a myriad of industrial and consumer products starting in the twentieth century (UNEP 2013) have contributed to the remarkable growth of human biomonitoring research in the last few decades (Angerer et al. 2006; National Research Council 2006; Needham et al. 2007). For example, biomonitoring concentrations are increasingly used to categorize exposures (e.g., low, medium, high) within populations to assess internal exposure to environmental chemicals (National Research Council 2012). However, the scenario of chemical human exposures is complex (Table 2.1). First, controlled conditions, as in traditional animal studies based on the administration of a single chemical and identification of potential target organs (Carlin et al. 2013), do not generally apply. Second, intensity, duration, and frequency of the exposures are normally unknown and often changing. Third, the timing of the exposure is seldom known. Fourth,

Table 2.1 Typical scenarios of human vs animal exposures to environmental chemicals

Chemical-dependent Variable	Human	Animal
Dose	Low? (known?)	High (controlled/ known)
Intensity	Unknown	Known
Timing	Variable (known?)	Fixed
Frequency	Unknown, likely episodic yet chronic	Known
Pathway	Multiple (known?)	Single and identifiable
Chemicals	Many (known?) Metabolites?	Single (mixtures)
Target organ	Accessible? (known?)	Accessible

exposure routes and sources are numerous and, at times, even unknown. Finally, in a world where more than 80,000 chemicals are used in commerce (Bell and Edwards 2015), people are exposed to "cocktails" (multiple/mixtures) of chemicals. Therefore, mixtures encompass the large majority of environmental or background chemical exposures, even in situations when other exposures to mainly single chemicals or chemical classes may occur (e.g., accidental exposures). The fact that all of the above considerations would apply to each of the individual components of the mixtures may further complicate the interpretation of human biomonitoring data. Nevertheless, because biomonitoring per its nature provides an aggregate measure of exposure, biomonitoring has the potential to provide invaluable information for the exposure assessment of chemical mixtures.

2.3 Analytical Aspects of Biomonitoring

Biomonitoring relies on a targeted analysis to provide a quantitative measure of the amount of a chemical or chemicals present in the human body. These chemical biomarkers can be markers of exposure, effect, or susceptibility (National Research Council 2006). As defined by the World Health Organization (WHO), a biomarker of exposure is a "chemical or its metabolite or the product of an interaction between a chemical and some target molecule or cell that is measured in a compartment in an organism," a biomarker of effect is "a measurable biochemical, physiologic, behavioral, or other alteration in an organism that, depending on the magnitude can be recognized as associated with an established or possible health impairment or disease" (e.g., DNA adduct), and a biomarker of susceptibility is "an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific chemical substance" (e.g., glucose-6-phosphate dehydrogenase deficiency) (National Research Council 2006). In this chapter, we will focus on biomarkers of exposure.

The success of a biomonitoring approach greatly depends on the adequate selection of the exposure biomarkers, the matrix, and the analytical method (Needham et al. 2007). Knowledge of the physicochemical properties of the target chemicals is important in the choice of exposure biomarkers (e.g., parent compound vs metabolite) and biomonitoring matrix. In general, persistent compounds are commonly measured in blood or blood products, while metabolites of nonpersistent compounds (chemicals with half-lives of the order of hours) are measured in urine (Needham et al. 2007). Measuring blood concentrations of nonpersistent chemicals may be possible when the blood is collected soon after the exposure and if the analytical method is sensitive enough to detect the much lower blood concentrations of the chemical than of its metabolites in urine (Needham and Sexton 2000).

Because exogenous chemicals are present in the biological matrix at much lower concentrations than other endogenous compounds, biomonitoring methods generally include steps to (a) preconcentrate the target analytes and eliminate unwanted matrix components and (b) separate the target analytes from each other and from residual matrix constituents before quantification. The chemical nature of the biomarker and availability of instrumentation can impact the choice of preconcentration (e.g., liquid-liquid extraction, solid-phase extraction) and separation (e.g., chromatography) techniques. For example, separation of volatile organic chemicals is generally achieved by gas chromatography, while liquid chromatography separates nonvolatile organic compounds; nonvolatile organic compounds may also be amenable to gas chromatography after suitable chemical derivatization (Needham et al. 2005c). For human biomonitoring of organic chemicals, isotope-dilution mass spectrometry is generally considered the gold standard quantification technique (Needham et al. 2005c; WHO 2011). Other techniques (e.g., enzyme-linked immunosorbent assay [ELISA], fluorescence) may have the required sensitivity but generally lack adequate analytical selectivity and specificity (WHO 2011).

Biomonitoring methods, rooted in their analytical chemistry foundation, must be sensitive (i.e., capable of accurately measuring small amounts of a given substance in a sample (Saah and Hoover 1997)), selective and specific (i.e., able to measure one particular substance, rather than others in a sample (Saah and Hoover 1997)), and accurate and precise at trace levels (Needham et al. 2005c; Calafat and Needham 2009). Thanks to the scientific and technical advances in robotics and analytical instrumentation in the past few decades, such methods have become increasingly common in many laboratories (Angerer et al. 2007). In addition, biomonitoring methods should preferably use minimal matrix volume, be high throughput, show sustained reproducibility, and concurrently quantify multiple biomarkers. To achieve such characteristics, biomonitoring requires highly trained staff, top quality analytical standards (often custom-synthesized), and the use and maintenance of state-of-the-art instrumentation and facilities. Because of the uniqueness of these resources, biomonitoring is relatively expensive. Nevertheless, despite cost and other challenges associated with the interpretation of biomonitoring data (e.g., study design, communication of results), the use of biomonitoring in environmental public health is on the rise (National Research

Council 2012, 2006; Albertini et al. 2006; Morello-Frosch et al. 2015). Specifically, the possibility of measuring multiple chemicals simultaneously in a small amount of biospecimen makes biomonitoring uniquely suited to study human exposures to chemical mixtures and the potential effects of such exposures on health.

2.4 Interpretation of Biomonitoring Data

Biomonitoring provides information on the concentrations (i.e., amount) of select chemicals that were absorbed into the body after contact between the chemicals—or their precursors—and the body (Needham et al. 2005b). Noteworthy, such concentrations are in the range of trace levels (compared to the generally higher concentrations of the chemicals in the environment) and integrate all environmental pathways and routes (e.g., food, water, air, dust, product use). Furthermore, using biomonitoring concentrations to estimate exposure can pose study design challenges related both to the nature of the biomarker (e.g., specificity, temporality) as well as to the adequacy of the sampling process.

Specificity of the Biomarker Interpreting biomonitoring data requires a good understanding of the toxicokinetics of the target biomarkers. In general, relying on the concentrations of the most abundant biomarker for a given chemical will likely minimize exposure misclassification. For example, di-isononyl phthalate (DINP) metabolizes into mono-isononyl phthalate (MINP) before forming several oxidative metabolites which are the major DINP metabolites in urine (Koch and Angerer 2007). In general population settings, using urinary concentrations of MINP may underestimate exposure to DINP because MINP represents only a minor fraction (~2%) of the DINP excreted in urine compared to the oxidative metabolites (~44%). In fact, exposure to DINP in approximately 82% of Americans would have been misclassified based on the concentrations of the insensitive biomarker MINP, highlighting the importance of selecting a priori the best biomarkers for the intended purposes of the study (Calafat et al. 2011). An additional benefit of measuring phthalate oxidative metabolites is that they cannot be formed as a result of external contamination with the parent phthalate (Koch and Calafat 2009), thus also increasing the specificity of the measurement. In general, when a compound is converted to multiple metabolites, the quantification of all metabolites provides the best biomonitoring approach for exposure assessment. Depending on the aims of the study, exposure categorization can then be based on the concentrations of the individual biomarkers (e.g., four di (2-ethylhexyl) phthalate [DEHP] metabolites) and/or their sum (e.g., sum of all measured DEHP metabolites) (Dales et al. 2018; Kasper-Sonnenberg et al. 2017; Sathyanarayana et al. 2016; Huang et al. 2016; Axelsson et al. 2015; Ferguson et al. 2014; Guo et al. 2014; Kim and Hong 2014; Larsson et al. 2014; Mervish et al. 2014; Watkins et al. 2014; Zhang et al. 2014; Kim et al. 2013; Park et al. 2013; Tellez-Rojo et al. 2013; Upson et al.

2013; Braun et al. 2012; James-Todd et al. 2012; Kasper-Sonnenberg et al. 2012; Teitelbaum et al. 2012; Frederiksen et al. 2011; Romero-Franco et al. 2011).

The fact that several chemicals can metabolize into the same end product may also complicate the interpretation of biomonitoring data. For example, a number of synthetic pyrethroid insecticides are converted to 3-phenoxybenzoic acid (Leng et al. 2003). Therefore, the presence of 3-phenoxybenzoic acid in urine suggests exposure to pyrethroids, but 3-phenoxybenzoic acid concentrations per se cannot pinpoint the specific pyrethroid(s) to which exposure occurred. Similarly, certain chemicals (e.g., organophosphate insecticides) may degrade in the environment, and exposure could be to both the parent compound and the preformed degradate (e.g., dialkylphosphates) (Needham et al. 2005a; Barr et al. 2004). Yet, biomonitoring concentrations of degradates will reflect exposure to both the parent chemical and the preformed metabolites. In the above scenarios, interpreting the concentrations of the biomarkers may be challenging, particularly when the bioactivity of the precursors/parent compounds and their corresponding degradates or non-specific metabolites differ (Duggan et al. 2003). Nonetheless, use of these "non-specific" urinary biomarkers can still provide useful information about cumulative exposure to the parent class of compounds (e.g., pyrethroids, organophosphates).

Temporality of the Biomarker Exposure biomarkers should reflect a person's exposure to the target chemicals or their precursors within a specific time period (e.g., pregnancy) (Calafat et al. 2015). However, with a few exceptions of defined patterns of exposure, such as scheduled tasks in occupational settings (Arnold et al. 2013), the timing, duration, and intensity of chemical exposures are generally unknown and likely different for each chemical in a mixture. As a result, even though biomarker concentrations can accurately rank a person's exposure at a single time point, to evaluate exposure over weeks, months, or years may require different approaches.

In general, for persistent compounds, the timing of the exposure relative to sample collection is not critical. Regardless of the nature of the exposure (i.e., constant vs episodic), its duration, intensity, or timing, a single biomarker concentration at a given time point adequately represents exposure over an extended time (e.g., years) because persistent compounds have relative long elimination half-lives (Needham et al. 2005a; Meeker et al. 2009; Makey et al. 2014). Nonetheless, in certain situations, more than one sample may be needed. For example, chemical-specific toxicokinetics, including transplacental transfer or distribution into breast milk, can result in biomarker concentration changes which, in turn, could impact long-term exposure estimates obtained using a single sample collected during pregnancy or lactation (Adetona et al. 2013; Hooper et al. 2007; Glynn et al. 2012; Kato et al. 2014).

Variability in concentrations is much more pronounced for nonpersistent than for persistent chemicals because concentrations of the target biomarkers for non-persistent chemicals increase and decay rapidly in blood and urine after exposure