1
Introduction to Mixtures Toxicology and Risk Assessment

M. Moiz Mumtaz, William A. Suk, and Raymond S.H. Yang

1.1
Chemical Mixtures Exposure

When humans are exposed to chemicals, they are not exposed to just one chemical at a time. A vast number of chemicals pervade our environment. Exposures, whether simultaneous or sequential, are to chemical mixtures. The standard definition of a chemical mixture is any set of multiple chemicals regardless of source that may or may not be identifiable that may contribute to joint toxicity in a target population [1, 2].

By some estimates, up to 6 billion tons of waste is produced annually in the United States. Several years ago, the US Office of Technology Assessment estimated 275 million of those tons were hazardous. Most waste finds its way to more than 30 000 toxic waste disposal sites across the United States, a majority of which the US EPA has categorized as uncontrolled hazardous waste sites [3]. Thus far, traditional risk assessment, even with its inherent shortcomings, has helped to control chemical exposures to that waste reasonably well, as evidenced by statistics on longevity, health status, and world population growth. Yet, new health and environment indicators have raised disquieting questions, and a consequent growing concern is that this success might be short-lived. One reason is an alarming, logarithmic increase in the synthesis, manufacture, and use of chemicals worldwide as "developed" and "developing" countries compete to provide their populations an improved quality of life. To help meet these concerns, the World Health Organization (WHO), as part of its harmonization of approaches project, recently published a report on methods and approaches for risk assessment of chemical mixtures [4, 5].

Former US Secretary of Defense Donald Rumsfeld once said with regard to intelligence reports

> There are known knowns. There are things we know we know. We also know there are known unknowns. That is to say, we know there are some things we do not know. But there are also unknown unknowns, the ones we don't know we don't know [6].

Principles and Practice of Mixtures Toxicology. Edited by Moiz Mumtaz

ISBN: 978-3-527-31992-3

Rumsfeld's wisdom also applies to the state of chemical toxicology, particularly to toxicology of chemical mixtures. Among the three categories, the *unknown unknowns* are, in the science of chemical mixture toxicology, the ones that cause the most worry.

Mixtures are one of the toxicology's huge unknowns. The concerns for chemical mixture toxicology's potential but unknown problems are illustrated by an examination of the presence of many chemicals, albeit at low levels, in our bodies. On December 10, 2009, the Centers for Disease Control and Prevention (CDC) released its Fourth National Report on Human Exposure to Environmental Chemicals [7]. This is the most comprehensive assessment to date of the exposure of the US population to chemicals in the environment. CDC has measured 212 chemicals in people's blood or urine, 75 of which have never before been measured in the US population. Similar to its three predecessors, but with expanded effort, this report contains exposure data for the US population for environmental chemicals monitored during 1999–2000, 2001–2002, and 2003–2004. The number 212 is not magic; it is merely the number of chemicals that could be identified and quantified per the established analytical laboratory protocol. The actual numbers of environmental pollutants in our body could be much higher than 212. The sample size ranged from hundreds to a few thousands, for example, a low of 1854 samples for 2,2′,3,3′,4,4′,5,5′,6,6′-decachlorobiphenyl (PCB 209) and a high of 8945 for cadmium or lead analyses. With such large sample sizes and the CDC staff's meticulous work, this report's results are widely viewed as a fair representation of those environmental chemicals and their respective concentrations that inhabit the general US population [7]. Recognizing, however, that associations are not causations, the CDC emphasizes in these reports that ". . . the measurement of an environmental chemical in a person's blood or urine does not by itself mean that the chemical causes disease."

Such a cautionary statement is understandable from a government agency responsible for public health. But toxicologically speaking, the 212 chemicals analyzed in the serum or urine samples were from a fairly large population sample. This then raises an important issue regarding the toxicological importance of a "mixture cocktail" in our bodies, albeit at very low concentrations. In many ways, this is the kind of *unknown unknowns* in chemical mixtures toxicology that should draw toxicologists to this real-life challenge: how do we assess the impact of these chemicals on current or future human health?

No one yet knows the answer for certain, but the question can be viewed from two different perspectives. A liberal perspective would hold that the presence of these chemicals in our bodies is merely a nuisance; they are the price paid for living in a modern, industrialized society that generates thousands of chemicals. These chemicals in our bodies are a necessary evil without any toxicological importance, particularly given such low levels. The average life span of our society is increasing, and the benefits derived from these xenobiotics outweigh their potential risks [8]. In fact, some scientists even believe that a small amount of any chemical might have certain beneficial effects [9–12].

But a second, more conservative and more cautionary perspective is that the presence of these chemicals in our bodies represents the toxicological *unknown unknowns*. These chemicals could potentially harm us. Thus, if we are to err, we

should err on the safe side. Exposure to persistent chemicals such as metals, dioxins, and polychlorinated biphenyls (PCBs) could lead to their accumulation in our bodies and lead further to increasingly high tissue concentrations. Several lipophilic, persistent organic pollutants (POPs) can, for example, concentrate in breast milk and during pregnancy and through lactation expose the growing fetus and babies. Such chemical body burdens and their possible variations from person to person, together with the unlimited combinations of chemical mixtures that may be inherent in human populations, are beyond the capacity of traditional toxicity testing. The precautionary principle could be a solution, founded as it is on the use of comprehensive, coordinated research to protect human and environmental health. When the Collegium Ramazzini opened in 1983, Professor Irving Selikoff wrote, "Science is no stranger to uncertainty and incomplete data. The Collegium will utilize science to help unlock the rigidities of those fixed in legalistic and regulatory combats that prevent progress in environmental and occupational health" [13].

As environmental health hazards become increasingly complex and international in scope, this principle might play an increasingly important role. Its spirit is embodied in the 1992 Rio de Janeiro declaration: "Where there are threats of serious or irreversible damage, scientific uncertainty shall not be used to postpone cost-effective measures to prevent environmental degradation." That chemical mixtures or complex exposures are part of our lives is a subject of increased realization and awareness; to play a central role in environmental protection and public health, traditional risk assessment must accommodate such challenges [14]. The US Congress enacted, for example, clean air, clean water, and environmental laws because of concerns that contaminants in air and water and hazardous waste might cause adverse health effects before development of any antidotal, comprehensive body of biomedical science [15]. But such science is possible only by continued coordination and collaboration of efforts to develop alternative methods and transparent strategies; science that allows dynamic participation of data generators, data users, researchers, stakeholders, and decision makers.

Almost all applied and basic science underpinning current regulations tested one chemical at a time. Several US environmental laws including Superfund,[1)] the Safe Drinking Water Act Amendments (SDWAs), and the Clean Air Act (CAA) have acknowledged the significance of potential exposure to, and the health effects of, chemical mixtures. The Food Quality Protection Act (FQPA) even states that mixtures are the rule rather than the exception. Thus began the groundwork for a new approach to the study of chemical mixtures. Now, when calculating whether exposures exceed tolerance levels, compounds with similar mechanisms of action must receive joint consideration. FQPA has therefore initiated the cognitive transition – and the logical progression – from single to multiple chemical risk evaluation.

During the past two decades, quantitative risk assessment research has devised formulas, written documents, held workshops, and developed guidelines to address chemical mixtures issues [16, 17]. Recently, guidelines and guidance have incorpo-

1) The Comprehensive Environmental Response, Compensation and Liability Act of 1980, as amended by the Superfund Amendments Reauthorization Act (SARA) of 1986.

rated chemical interaction concepts and have suggested methods to evaluate the possible influence of such interactions on the overall joint toxicity of chemical mixtures [1, 2, 18].

The methods these guidelines propose have been, and could be applied to contrived mixtures, simple mixtures, complex mixtures, particulate matter, food additives, intended and unintended exposures of short-term and long-term episodic durations, and to environmental stressors. The evolution of the methods to evaluate the joint toxicity of mixtures and their success have provided the confidence to apply them to real-world exposures.

The past two decades have undoubtedly witnessed the gradual maturing of the toxicology of chemical mixtures. Symposia and major conferences dedicated to chemical mixtures have appeared with increasing frequency at annual meetings of major scientific societies, specifically the Society of Toxicology [19], the Society of Risk Analysis, the European Conference on Combination Toxicology, the ATSDR International Conference on Chemical Mixtures 2002 [20–24]. Concerns about chemical mixtures prompted the Society of Toxicology to establish a mixtures specialty in 2007, and mixtures were the theme of the 2009 annual meeting of Society of Toxicology, Canada. Recently, the ILSI Health and Environmental Sciences Institute (HESI) risk assessment methodologies technical committee convened a working group of academic, government, and industry representatives to explore and to improve methodologies available for assessing mixtures risk. The team elected to explore screening-level risk assessment methodologies that could address risks from low-dose exposure to mixtures and completed a critical analysis of chemical interactions and their magnitude [25]. As is true with any developing area, issues remain that are unique to chemical mixtures. Researchers must deal with an array of concerns about chemical mixtures, and many factors affect research and methods' development, including scientific advances, expert opinions, regulatory needs, administrative priorities, public interest, and legislative actions [26].

1.2 Superfund Research Program

The National Institute of Environmental Health Sciences (NIEHS) Superfund Research Program (SRP) was created as a network of multi- and interdisciplinary teams of researchers. Its purpose is to address broad, complex health and environmental issues that arise from the multimedia nature of hazardous waste sites – particularly, from both long-known and emerging environmental contaminants (http://www.niehs.nih.gov/sbrp) [27]. By creating multiproject and multidisciplinary programs, SRP encourages and fosters partnerships among diverse scientific disciplines. Recent technological advances have the capacity to stimulate interdisciplinary research in such disciplines as follows:

- Genomics, proteomics, and metabolomics technologies;
- Molecular, cellular, and whole-animal imaging methodologies;

- Miniaturized tools/technologies (i.e., at the micro- and nanolevel); and
- Improved cyber infrastructure and bioinformatic tools to gather, assimilate, and interrogate large diverse data sets.

Establishing multidisciplinary research programs provides a more comprehensive understanding of complex environmental issues. The knowledge gained through these research efforts has proven useful in supporting decisions made by state, local, and federal agencies, private organizations, and in industries involving the management of hazardous substances.

The mandates under which the SRP operates provide a framework that has, for example, allowed the NIEHS the latitude to address a wide array of scientific uncertainties facing the national Superfund Program (http://www.niehs.nih.gov/research/supported/srp/about/index.cfm). These mandates include the development of

1) Methods and technologies to detect hazardous substances in the environment;
2) Advanced techniques for the detection, assessment, and evaluation of the effect of hazardous substances on human health;
3) Methods to assess the risks to human health presented by hazardous substances; and
4) Basic biological, chemical, and physical methods to reduce the amount and toxicity of hazardous substances.

The methods grew out of Congress's recognition that the strategies for the cleanup of Superfund sites and the technologies available to implement these cleanups were inadequate to address the magnitude and complexity of the problems. Congress accordingly enacted the Superfund's 1986 SARA amendments.

To address the complex, interdependent, yet fundamental issues that arise in relation to hazardous waste integration, cooperation is needed from many disciplines. A holistic approach that borrows theories and methodologies from many diverse scientific disciplines is the future for integrated environmental health as it relates to the Superfund [28]. Ultimately, this approach will enable basic research findings to transition into epidemiological, clinical, ecological, and remediation studies, all of which are important for the public health decision-making process.

A central SRP premise recognizes the link between chemical exposure and health effects leading to disease outcome will assist in understanding, identifying, and establishing new or improved prevention and intervention modalities (Figure 1.1) [29]. Contributing factors that modulate the exposure–disease paradigm include

- Temporal factors (age and developmental stage);
- Spatial factors (geographic locations);
- Genetic factors (single-nucleotide polymorphisms (snps), methylation patterns); and
- Unique circumstances (e.g., comorbid conditions, nutritional status, etc.).

The considerable interplay between exposure and response results in "real-world" mixture exposures with widely varied biological effects. Ultimately, developing

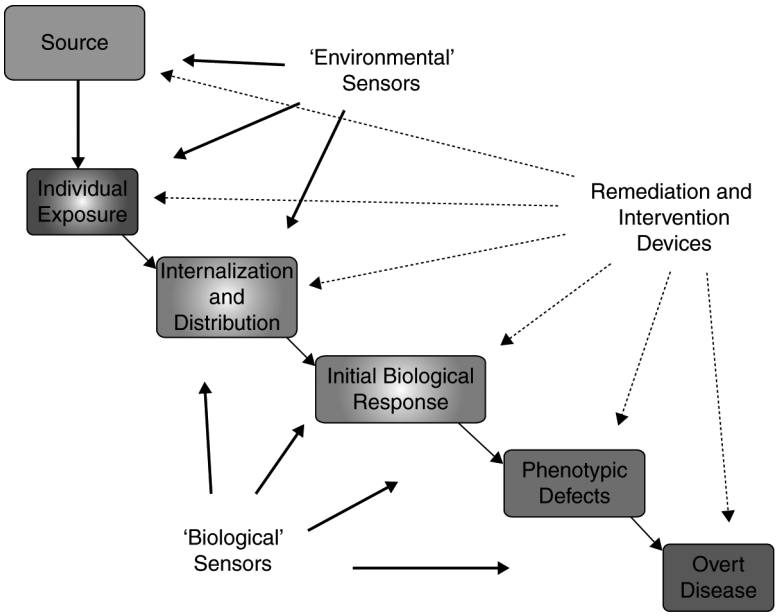

Figure 1.1 The environmental health sciences working paradigm of environmental exposures leading to disease through a cascade of events.

strategies must consider this foundational fact, not only to minimize the effect of exposure on disease risk but also to develop risk assessment models that incorporate these diverse parameters. Only in this way will science provide the biologically relevant information needed to make informed, human-health protective choices.

Thus, SRP-supported research is a continuum from basic to applied research. Its state-of-the-art techniques improve the sensitivity and specificity for detecting adverse effects on humans or on ecosystems exposed to hazardous substances. This research also promotes a better understanding of the underlying biology responsible for such adverse effects.

1.3 SRP and Mixtures Research

From a public health perspective, the inability to predict whether chemical agents act in an additive, synergistic, or antagonistic manner at concentrations encountered in the environment creates real problems for human health risk assessment. Many examples confirm that interactions of chemicals with each other or with other physical or biological agents affect health to a greater extent than would have been predicted given the toxicity of individual components. A critical issue related to hazardous waste sites for remediation or health effects research is that the concentrations at which chemicals occur in the environment are extremely low, and

exposures are long-term and continual with simultaneous exposure to multiple chemicals. Thus, whether the subject is remediation strategies, exposure to humans or ecosystems, site characterization, bioavailability, or the development of risk assessment models, chemical mixtures are an issue of concern. Furthermore, real-life scenarios rarely reflect biomedical research, exposure assessments, or remediation strategies based on exposures to single substances in isolation. Indeed, such oversimplification fails to consider

- Prior exposure history and vulnerability (i.e., susceptibility);
- Interactions with other stressors of similar/dissimilar mechanisms of action;
- Potentiation or sensitization by chemicals not toxic in themselves; and
- Interaction of chemicals that could lead to synergistic or antagonistic effects [30].

In fact, the majority of diseases are the consequence of both environmental exposures and genetic factors [31]. Individual susceptibility to environmentally induced disease is a source of uncertainty. A better understanding of genetic influences on environmental response could lead to more accurate estimates of disease risks and could provide a basis for disease prevention. Researchers, environmental policy makers, and public health officials are challenged to design and implement strategies to reduce human disease and dysfunction resulting from exposure to chemical mixtures.

Interactions among mixture components and gene–environment interactions should be seen not as a limit to scientific progress but as a challenge to develop more complex and sensitive methods. To address the complexities and uncertainties surrounding human exposures to mixtures of chemical contaminants, researchers must fully utilize and integrate cellular and molecular biology methodologies, mechanistically based short-term toxicology studies, computational technology, and mathematical and statistical modeling [32, 33].

With continued development of and refinement in the available repertoire of advanced tools and approaches, the ability to better assess the effect of mixtures on human health is reachable. The types of research related to mixtures important to addressing issues within the SRP include the following:

- Development of computational toxicology approaches to understand dose/effect relationship in the context of chemical interactions;
- Application of high throughput functional assays to define critical mechanistic end points associated with potential adverse biological consequences of exposure to chemical mixtures;
- Integration of diverse data sets to develop biologically based predictive models for chemical mixtures;
- Application of metagenomics to understand the impact of chemical mixtures on the structure and function of microbial communities;
- Development of nanotechnologies to detect and measure individual components within complex mixtures in real time;
- Development of innovative approaches to remediate chemical mixtures in environmental media; and

- Adaptation and application of fate and transport models to predict and assess the influence of chemical mixtures on the efficiency and effectiveness of applied remediation approaches.

Multi-, inter-, and transdisciplinary research strategies are not easy to implement; many government, industry, and academic programs tend to foster and reward narrow approaches to problem solving. The NIEHS SRP, however, serves as a model of a successful program where biomedical researchers cooperate and collaborate with, for example, ecologists, engineers, and mathematicians. This results in creative synergisms and novel approaches to address complex problems, especially the problems of chemical mixtures at Superfund sites.

1.4 Drug–drug Interactions and Nanomaterials

For those interested in the toxicology of chemical mixtures, two areas of toxicological sciences – drug–drug interactions and nanomaterials – are particularly challenging. The former, though a long-time issue in the pharmaceutical industry, remains a toxicological *unknown unknown*. With regard to serious toxicological interactions, it has not received attention it deserves.

And nanomaterials are a completely new area. The challenges to toxicologists are particularly relevant from the perspective that many nanomaterials are "chemical mixtures" and their unique physicochemical properties would raise some highly unusual physiological, biochemical, and toxicological issues (see Chapter 21). These two areas warrant some special discussion.

Before prescribing multiple drugs, some physicians consider potential drug interactions. Physicians try to minimize these interactions by taking into consideration the time needed for each drug to reach maximum blood concentration, its half-life ($t_{1/2}$), its bioavailability, and its mode of action. Until recently, however, institutions would not allocate resources to study toxicological interactions from multiple drug intakes. In addition to combination therapy (i.e., polytherapy or polypharmacy), multiple drug intake could easily be realized when different doctors treat patients for multiple illnesses, particularly aging patients. As the exposure dose levels from drug intake are usually much higher than are doses of environmental chemicals, drug–drug toxicological interactions can become a serious problem. A number of case studies quoted below provide a glimpse of the seriousness of this issue.

Using a meta-analysis, in 1994 over 2.2 million cases of serious adverse drug reactions (ADRs) occurred among US hospital patients [34]. During hospital stays, the patients were given an average of eight drugs. Some 106 000 serious drug interaction cases were fatal, making ADRs the fourth to sixth leading cause of death for that year in the United States. In 1998, the US Food and Drug Administration (FDA) established the "Adverse Event Reporting System." Data analysis collected under this system revealed that from 1998 through 2005, serious adverse drug reactions increased 2.6 fold, from 34 966 to 89 842, and fatal adverse drug incidence

increased 2.7 fold, from 5519 to 15 107 [35]. These results highlight the importance of ADRs as a public health problem.

An exposure situation and its related complications may also influence toxicological interactions. The anesthetic agent Fluroxene was safely used in clinical medicine for almost 20 years before the first fatal incident [36, 37]. In 1972, an epileptic surgical patient who was on a regimen of phenobarbital and diphenylhydantoin died within 36 h of operation due to massive hepatic necrosis. That fatal lesion was quickly confirmed with experimental animal toxicology studies; the cause of death was attributed to potentiation of fluroxene hepatotoxicity by phenobarbital and diphenylhydantoin through enzyme induction [36, 37].

In an experimental toxicology study, 7-day old infant rats were administered a combination of drugs commonly used in pediatric anesthesia (i.e., midazolam, nitrous oxide, and isoflurane) in doses sufficient to maintain a surgical plane of anesthesia for 6 h [38]. Researchers observed that such a common therapeutic practice combination in the infant rats caused widespread apoptotic neurodegeneration in the developing brain, deficits in hippocampal synaptic function, and persistent memory/learning impairments.

The intrinsic functions of the subject exposed to chemicals may modulate toxicological interactions. Renal dysfunction may change drug disposition such that the likelihood of drug–drug interactions would increase. A clinical example is the interaction between aminoglycoside antibiotics and penicillins in patients with impaired renal function [39]. In solution, these antibiotics bind to inactivate each other, but the reaction is slow. Because penicillin(s) is usually given in great molar excess to the aminoglycosides, the major consequence of such drug–drug interaction is inactivation of aminoglycoside to subtherapeutic concentration. This interaction, however, seems to occur only in patients with renal dysfunction. The reason has been attributed to the retention of both antibiotics in patients with impaired renal function, thereby allowing sufficient time for this interaction to take place.

Manufactured nanomaterials is the second area of science that toxicologists believe will pose a challenge to the study of chemical mixtures in the foreseeable future. The advancement of nanotechnology in the twenty-first century is probably so important that it represents yet another phase of the Industrial Revolution. Some estimates are that in few years, worldwide commerce involving nanomaterials will reach $1 trillion [40]. At present, more than 600 commercial products are known to contain nanomaterials [41]. Because these nanoparticles are invisible – usually under 100 nm in diameter – and because nothing much is known about their toxicities, concerns have been raised about their health effects on humans [42, 43]. Many of these nanomaterials have a core that consists of a number of metals [40, 44], hence arises the chemical mixtures issue. Moreover, nanomaterials have some unique physicochemical properties – some have rather persistent tissue pharmacokinetics [43, 45, 46]. In one of the first published physiologically based pharmacokinetic (PBPK) modeling papers on a nanoparticle, Quantum Dot 705 (QD705) in mice (Figure 1.2), the authors pointed out that such unique and worrisome pharmacokinetic nanoparticle properties might have a silver lining [43, 46]. That is, while the persistence of QD705 was of health concern specifically in the spleen, kidney, and

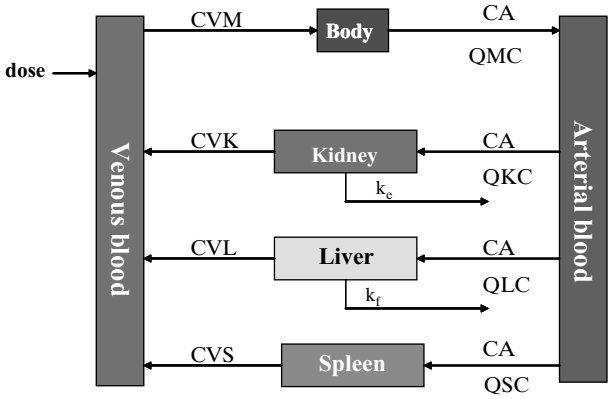

Figure 1.2 A conceptual PBPK model for QD705 in mice. CVM, CVK, CVL, and CVS represent QD 705 concentrations in venous blood, kidneys, liver, and spleen, respectively. CA is QD 705 concentration in arterial blood. QMC, QKC, QLC, and QSC represent blood flow to body, kidneys, liver, and spleen. (Reproduced with permission from Yang *et al.* (2008) *Environ. Sci. Technology*)

liver for up to an experimental duration of 6 months, the nanoparticles' affinity toward these tissues might be exploited to design drug delivery systems for potential targets in these same tissues. Thus, nanomaterials' unique properties will undoubtedly present an important future challenge for scientists in the environmental and occupational toxicology and risk assessment areas.

1.5 Waste Sites and Mixtures Risk Assessment

Communities near waste sites – particularly Superfund sites – can potentially be exposed to low levels of a wide range of chemicals originating from the site. Communities can also be exposed to various other environmental chemicals from nearby manufacturing, transportation, and other sources. At very low-level exposures, human populations do not show any observable health effects. Chemical(s) remain as body burdens showing no discernible effect on a person's overall health. Physiologically, the body adjusts to the presence of chemicals at this level through adaptive mechanisms. As the pollutant exposure levels increase, some effects may be observed.

But effects such as enzyme induction and certain biochemical and subcellular changes may be of uncertain importance. At this level of pollutant exposure, the body may have compensatory mechanisms [47]. Yet, as pollutant levels continue to increase, significant, readily observable adverse effects may ensue. At these higher pollutant levels, the body has exhausted its adaptive and compensatory mechanisms,

and its functioning could be compromised. Such adverse effects could lead to organ function impairment through compromise of physiological processes, leading to pathophysiological changes such as fatty changes and necrosis resulting in significant organ function impairment. Exposure to higher levels of pollutants could lead to morbidity and ultimately to death. In this continuum of effects, exposures from multiple sources may cause some persons to cross the threshold for adverse health effects. Considering that the human population is heterogeneous and therefore lacks biochemical characteristic homogeneity, some persons within the population will be more susceptible than others to adverse effects. At the either end of the bell curve then, a small fraction of the population may be hypersensitive to pollutant burdens and exhibit adverse effects to levels of exposure that may otherwise be considered low. Moreover, as emphasized in a recent National Research Council report [48], both endogenous and exogenous background exposure and underlying disease processes contribute to population background risk by affecting the dose–response relationships of environmental chemicals.

The goal of waste-site risk assessment is to ensure "healthy people in healthy environment" through protecting the public from unintentional exposures to toxic substances. Determining the health risks of complex mixtures is daunting both to toxicologists using experimental approaches and to epidemiologists using observational approaches. Risk assessment is a four-step process that includes hazard identification, dose–response assessment, exposure assessment, and risk characterization [49]. Just as researchers often confront large data gaps, chemical mixture risk assessment of waste sites is often limited, incomplete, or inconclusive. Hazard assessment is the fundamental basis of the overall risk assessment process. If data were available on the whole mixture of concern, a toxicity index analogous to MRLs/RfDs would be calculated for the mixture [50]. Often, however, whole mixture data are not available; they often are available for some but not all mixture components. In such cases, the hazard index (HI) approach uses the doses of the individual mixture components after they have been scaled for toxic potency relative to each other. In practice, a screening level analysis is performed, summing across all target organs. If the HI value exceeds 1, this initial analysis is repeated by developing effect-specific HIs. Conceptually, this approach helps to construct the plausible toxicity index of a mixture that would have been calculated had the mixture itself been tested. Using this HI approach, if exposure or toxicity screening data are unavailable for all the components of the mixture, the risk could possibly be underestimated [51].

When using the HI approach, its limitations should be understood and special attention should be given to multiple target toxicities, the role of chemical interactions [52], and novel or new toxicity end points. Rarely does a chemical have single end point toxicity. Most chemicals cause multiple toxicities and cause them in multiple target organs in multiple cell types as a function of dose (Figure 1.3). Single chemicals can affect multiple organs/end points as a function of dose, and multiple chemicals can affect a single organ or system. For example, lead (Pb) can affect nervous, reproductive, and hematopoietic systems. On the other hand, arsenic, cadmium, chromium, and Pb can affect the nervous system, thus increasing the

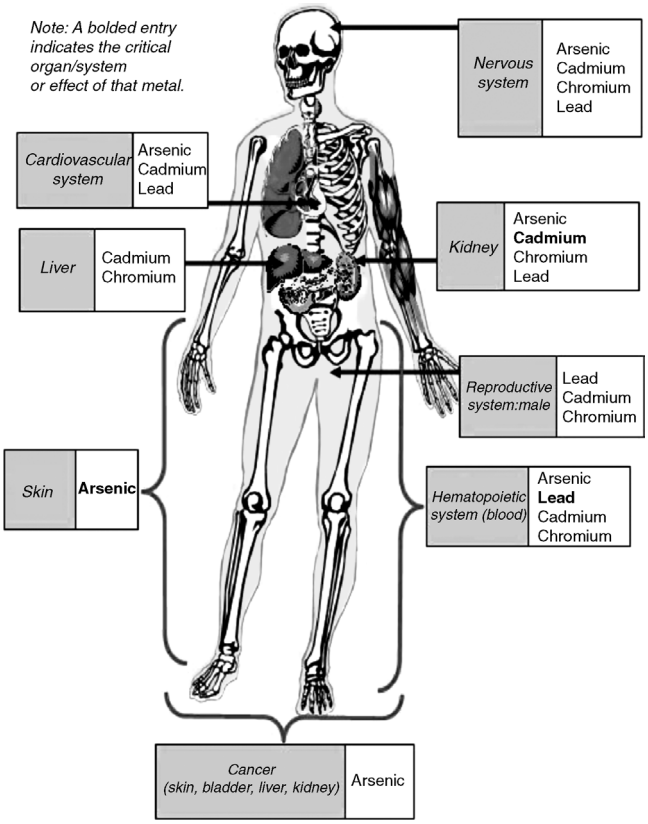

Figure 1.3 Single chemicals can affect multiple organs/end points and multiple chemicals can affect a single organ or system as a function of dose.

chances of chemical interactions and increasing overall joint toxicity. The point is that effects caused in the nervous system could be quite different from those caused in the reproductive system or in the liver or kidney, resulting in different disease outcomes. Often hazard assessment of chemical(s) is limited to critical effect or most sensitive effect. A full understanding of chemical mixtures' potential hazards is essential and a thorough evaluation of multiple end points is achievable. In this regard, a physiologically based, pharmacokinetic/pharmacodynamic model may serve as an integrator for all the relevant physiological and toxicological processes in the body – the essence of systems biology ([53–55], see Chapter 22). A full range of multiple toxicity values should be derived for all the secondary effects of a chemical component, analogous to its critical effect. Thus, if minimal risk levels (MRLs), reference doses (RfDs), threshold level values (TLVs), and other allowable levels are derived for hepatotoxicity as critical effect, then analogous values the target organ toxicity doses (TTDs) should be derived for all secondary effects such as nephrotoxicity, hematotoxicity, and immunotoxicity [56]. At times, in the absence of experimental toxicity

data, computational tools such as structure–activity relationships (SAR) models can be used to derive such values.

The second aspect for consideration while using the HI approach is the role of potential of chemical interactions in the overall expression of chemical mixture joint toxicity. Ample studies demonstrate that chemicals can interact with one another and at times, by influencing the toxicity of other components of the mixture, can increase or decrease a mixture's overall toxicity. People are exposed to complex and highly variable mixtures of chemicals of naturally occurring and synthetic origin. The body in general disposes of all natural or synthetic chemicals by the same limited pathways. Thus, the probability arises of simple or complex interactions occurring at multiple levels in an organism. These interactions could be toxicokinetic (see Chapter 9) and toxicodynamic in nature (see Chapter 6); for realistic and accurate risk assessments, the interactions' consideration and, if needed, their integration into the overall toxicity assessment of a mixture, is important [57]. Often, this type of interaction assessment might lead to the conclusion that the interactions are insignificant, but it will serve the purpose by alleviating the concerns of communities cognizant of exposure to chemical mixtures.

Another more sophisticated approach – PBPK modeling – has also been used to study, validate, or verify interactions ([51, 53–55, 58, 59], see Chapter 20). Many early PBPK modeling efforts were based on the SimuSolv software. But at present, support for that is not forthcoming. More recently, the Advanced Continuous Simulation Language (ACSL; AEgis Technologies, Huntsville, AL) and Berkeley Madonna (the University of California, Berkley, CA) are being widely used. In addition to these dedicated computer software packages [60], the application of a spreadsheet program to support a PBPK model has also been demonstrated [61], and the Trent University (Peterborough, Ontario, Canada, updated 2003) has made available a spreadsheet program to run PBPK models.

PBPK models are mathematical representations of the animal or human body that group tissues or organs into compartments. Physiological and anatomical considerations of the sizes and blood flow of the organs they represent dictate the characteristics and links between these compartments. Thus, the model simulation is the resolving of a set of equations. These models were originally developed to understand the relationship between dose delivered to a target organ/tissue and its toxic response (s). Because of their increased biological relevance and reliability (fidelity), these models are now applied to study various aspects of toxicity of chemicals and interactions. During the past two decades, several mixtures, their mechanisms of interactions, and in some instances the threshold of such interactions, have been studied using PBPK models (see Chapter 7). Through these mixtures modeling exercises, great insights were acquired into the most commonly occurring mechanisms of interactions in biological systems such as competitive, noncompetitive, and noncompetitive enzyme inhibition or enzyme induction. With experience, increasing sophistication has been incorporated into new models for evaluating defined mixtures consisting of -2, -3, -4, -5 components and complex mixtures.

The third and last issue to consider for the HI approach is the possibility of new or novel toxicities the chemical mixtures might cause that individual components might

not cause. This can happen when a shift occurs from chemical-specific, toxic responses to mixture-specific responses. If interactions occur (in the toxicokinetic or toxicodynamic phase), mixtures are likely to induce effects not seen in the individual chemicals. In both similar or dissimilar mechanisms of action, novel effects of mixtures are not likely to occur at low dose or no observed adverse effect levels of individual components. At high dose or adverse effect levels of the individual compounds, however, novel adverse effects of the mixture may occur and indeed have been observed [62, 63]. Such studies also show that some of the adverse effects seen with the individual chemicals are not found after exposure to the mixture at comparable dose levels.

In this challenging era of toxicology, application of transcriptomics,[2)] proteomics, and metabolomics are proving to be powerful tools. Transcriptomics using expression microarrays has provided increased insight into toxic actions and has led to the findings of gene expression signatures associated with types of action such as genotoxic or nongenotoxic carcinogenicity, peroxisome proliferators, oxidative stressors, and others [64–71]. These gene expression signatures are valuable in predicting the potential toxic actions of new compounds. Importantly, transcriptomics also provides information on the pathways and molecular processes affected by chemicals. While the number of publications on transcriptomics of individual chemicals is increasing, very few studies have applied transcriptomics to the effects of chemicals within mixtures. Transcriptomics can, however, address such important issues as

- Are profiles of the mixtures a simple sum of the profiles of the individual compounds or do one or two compounds dominate the effect of other toxins?
- Which processes and pathways are affected by the compounds?
- Do the mixtures affect genes or processes not affected by individual compounds?
- Which of the affected genes and processes can be linked to the pathology and clinical data?
- Can transcriptomics detect the initiation of potential harmful processes not detected by the classical toxicology methods?

Toward this end, recent transcriptomic studies with mixtures have shown several genes affected by the ternary mixture but not by single compounds or binary mixtures' synergistic action [72]. In the liver, the high-dose ternary mixture upregulated 57 genes, not significantly upregulated by any individual compound (Figure 1.4). Only 8 of these 57 genes were upregulated by at least one of the binary mixtures, leaving 49 genes uniquely upregulated by the ternary mixture. These 49 genes included those that influence cellular proliferation, apoptosis, and tissue-specific functions – a majority of these are stress genes not induced by individual chemical components [72]. The highly sensitive results from such new techniques need to be integrated into the hazard assessment step; the results allow detection and evaluation of end points undetected by classical toxicological testing. Thus, by integration of such molecular biomarkers into

2) The study of the transcriptome, that is, the complete set of RNA transcripts produced by the genome at any one time.

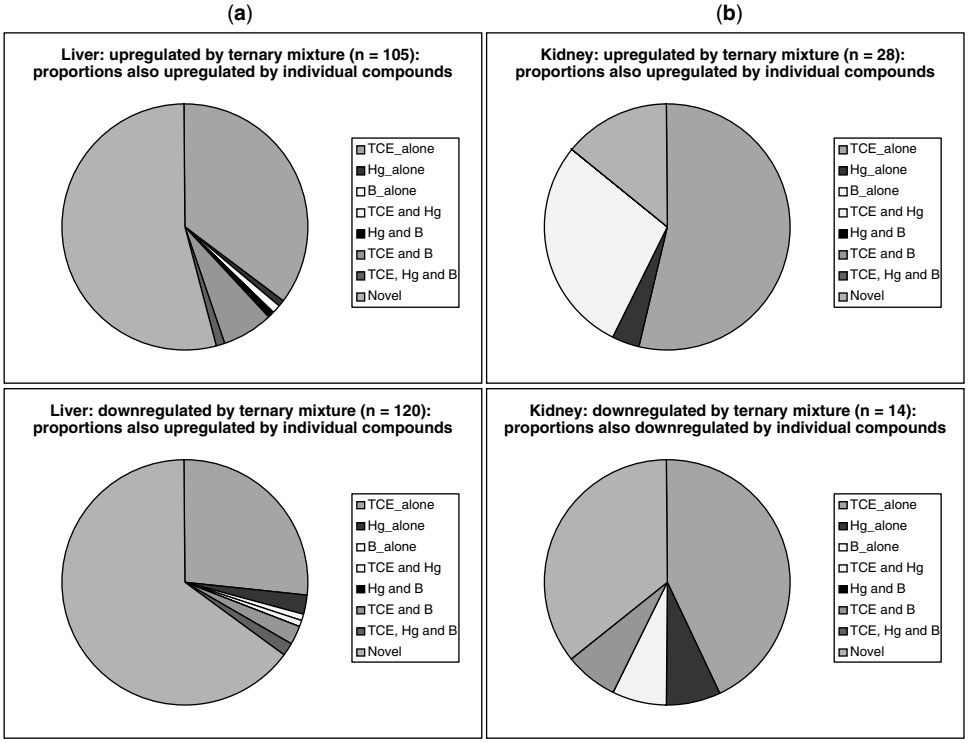

Figure 1.4 A majority of genes induced by a ternary mixture are stress genes not induced by individual chemical components.

the overall assessment process, unexpected outcomes following exposure to chemical mixtures can be avoided.

The first key step of the risk assessment process is accurate exposure assessment. For an accurate and realistic hazard assessment, the identification of all chemicals and stressors and their exposure assessment should be as complete as possible. This includes a thorough documentation of chemical mixtures and their compositions and concentrations, including bioavailability information (see Chapter 2). More recently, cumulative risk assessment – that is, the combined risk from aggregate exposures via multiple routes to multiple stressors, namely, chemical, biological, physical, and others – is gaining recognition as a pragmatic approach to characterize real-world risk [73] (see Chapter 10). Only with such advances can risk characterization of hazardous waste sites that integrates all the available information on toxicity of chemicals and their mixtures project, with some certainty, the frequency, as well as the severity of adverse health effects in potentially exposed populations. The fuller the risk characterization, the easier the comparison of the results of toxicological assessment with those of epidemiological studies to establish cause and effect relationships. Thus, the weight of evidence regarding human health effects of chemical mixtures should be derived from emerging evidence in the broad areas

of toxicology and epidemiology. Once such a relationship(s) is established, steps can be taken for remediation of hazardous waste site and protection of public health – the fundamental goal of risk assessment.

1.6 Alternative Testing Methods

In August 2005, Hurricane Katrina brought unprecedented destruction to the Gulf Coast. Huge storm surges, widespread wind damage, and flooding of New Orleans displaced hundreds of thousands of people, damaged thousands of homes beyond repair, and disrupted thousands of lives and businesses. The City of New Orleans was particularly hard hit; its levees broke, flooding large parts of the city. Apart from the hundreds of lives lost, several chemicals were released from storage into the environment, chemicals that industries used as intermediates, reagents, and catalysts, mostly with unknown toxicity. Since Hurricanes Katrina and Rita, personnel from the multiagency unified command in Metairie, Louisiana, made important advances in the assessment, investigation, and oversight of the environmental cleanup efforts in southeast Louisiana. The unified command agencies, along with their local, state, and federal partners, have recovered millions of pounds of hazardous material/oil and disposed of large amounts of debris.

Such emergency situations and the shortcomings of the risk assessment community in providing solutions to real-life challenges brought a new awareness in the public both affected and unaffected by the happenings. This awareness has turned into demands for issue resolution pertaining to unknown chemical toxicities. In response, alternative testing protocols are being developed that could save time and could husband resources. The Interagency Coordination Committee for Validation of Alternative Methods (ICCVAMs) Authorization Act passed by the US Congress has authorized tests that will also achieve the goal of humane treatment of animals by reducing, refining, and replacing animal toxicity testing [74]. The ICCVAM has undertaken a mixture toxicity testing study of new products submitted for US EPA registration, 89% of which are chemical mixtures. They belong to a variety of chemical classes, including anti-inflammatory/analgesic agents, respiratory stimulants, barbiturates, pesticides (insecticides, herbicides, nematicides, algicides, and fungicides), and surfactants. This study was undertaken for the evaluation of the *in vitro* neutral red uptake (NRU) basal cytotoxicity test method. Researchers wanted to determine its usefulness for predicting the *in vivo* acute oral toxicity of chemical mixtures. One of the study's goals is to assess the relevance, including the accuracy, of NRU in an *in vitro* cytotoxicity assay for estimating rat oral LD_{50} values of mixtures representing the five Global Harmonization System (GHS) categories of acute oral toxicity [75].

Through these activities, toxicology adopts a more aggressive approach to overcome the data paucity – toxicology now accesses a broad panel of *in vitro* assays that the drug discovery industry has been using for years [76]. Several newly developed assays are now available that allow chemical bioactivity evaluation using an array of

protein family pathways, critical cell signaling pathways, and cell health parameters. Building these assays into a series of screens to decipher the mechanisms of toxicity remains a formidable challenge in itself, but it can make the resulting information usable in risk assessment.

So much optimism surrounds this approach that several US government agencies and other organizations around the world are integrating these methods for high-throughput screening of chemicals. Noteworthy government-funded programs include ToxCast and Tox21. Registration, Evaluation, Authorization, and Restriction of Chemical substances (REACH) is a European initiative that will generate experimental data at a pace never duplicated in the history of toxicology. Tox21, a collaborative project of NIH, US EPA, and NIEHS combines advance automation and a growing assortment of *in vitro* assays and computational methods to reveal the interaction of chemicals with biological targets. The *in vitro* methods used an array of biochemical (e.g., metabolic kinase, multiple protein pathways, and protease) and cell-based assays (e.g., nuclear receptor, phenotypic, protease, signaling, and spicing). These assays can assess cell viability, nuclear receptors, pathways, and DNA damage.

ToxCast, a US EPA project, evaluates chemical properties and bioactivity profiles using a broad spectrum of gene assays, proteins, and metabolites that comprise the cellular "interactome" [77]. This data can help develop methods of prioritizing chemicals for further screening and testing to assist US EPA programs in the management and regulation of environmental contaminants and their mixtures. These pathways could serve as a good middle ground between biochemical or other target-focused assays and more phenomenological, phenotypic, or high-content assays. An important complement to ToxCast data will be that they are obtained from assays for detecting biotransformation and complex toxicities that use complex formats of human, nonhuman primate, or rodent cells. The ToxCast data can help identify overall patterns across many assays and data types that could be toxicity predictors. This type of testing takes advantage of HTS and toxicogenomic technologies for bioactivity profiling of environmental chemicals related in structure or in mechanism of action. Although the primary purpose is not to identify mechanisms of action of environmental toxicants per se, this might be a future benefit of the ToxCast program.

A scientific-method development norm is that whenever a new method is developed, it is compared with existing methods to show its advantages and define its limitations. The National Toxicology Program (NTP) has conducted methodical toxicological testing for the better part of this century. The results obtained using these new alternative methods should be compared and correlated with historical data, specifically those generated by the NTP. Similar correlative research would use data generated through those previous US EPA and FDA programs that guided toxicity testing for specific registration and regulation purposes.

Paralleling this newfound evolution of immense data generation openness and transparency is the emergence of data sharing. For example, ToxCast is making all of its data publicly available [78]. Establishment of databases such as ACToR, eChemPortal, REACH, and Comparative Toxicogenomics Database (CTD) [79] will enhance

data mining and interpretation. ACToR is a central database of toxicity information for thousands of chemicals that can be accessed to study chemical toxicity. Bioinformatics, the science of turning data into information, will play a critical role in experimental design and conduct of chemical toxicity studies.

Independent of these developments, in two separate reports, the National Research Council recently emphasized the use of toxicogenomics to link biological response indicators (biomarkers) to toxicity mechanisms, an approach susceptible of ready application to chemical mixtures [80]. These recommendations could enhance efforts to evaluate and remediate Superfund sites, and to reduce their effects on human health from exposure to chemical mixtures. The National Academy of Sciences (NAS) [81] has also recommended yet another alternative approach to traditional toxicity testing: the use of a complex array of animal/human studies and bioassays for the identification of toxicity pathways. This approach uses a systems understanding of the interconnected pathways composed of complex biochemical interactions operative in normal human and animal functioning. Following identification of these pathways, their qualitative and quantitative perturbations should be studied as a function of exposure to chemicals or their mixtures. This recommendation is predicated on the hypothesis that when sufficiently large biological perturbations of these pathways occur following exposures to chemicals or their mixtures' toxicity, adverse health effects or diseases result. For this to happen, the perturbations must be large enough that they exceed the adapting capacity of the host organism, namely, animals or humans. Thus, the degree of toxicity is host-specific and therefore dependant on a person's underlying health and disease status and on his or her individual ability to adapt [81].

Knowledge about the dose–response relationship (including its shape and slope) is a major factor in describing the toxicological characteristics of chemical(s) and their mixtures. The dose–response curve plays a key role in the assessment of health risks. Although toxicology deals with adverse effects and not with physiological changes, this latest NAS recommendation brings into focus the transitional research between physiological and harmful effects, assuming that nonadverse physiological changes (often controlled by homeostatic processes) seen at lower doses precede adverse effects observed at higher doses. Detailed knowledge of the relationship between biochemical changes at lower dose levels and adverse effects seen at higher doses will improve our understanding of mixture toxicology and will significantly contribute to a more knowledge-based risk assessment. Ultimately, this NAS recommendation will require standardization of magnitude of toxicity pathway perturbations and deviations from normal functioning of biological systems and will relate to toxicity and disease outcomes.

1.7 Translational Research

The scientific community increasingly recognizes that an understanding of risk requires consideration of the characteristics of the host population, the environ-

mental chemical or chemical mixture, and the exposure milieu. If these factors are not adequately addressed, the shape and low-dose characteristics of dose–response relationships for environmental toxicants may be substantially misrepresented. Also, though recognized as scientifically important, site assessments give little if any consideration to nonchemical stressors, to population vulnerability, or to various background exposure and other risk factors. Affected stakeholder communities often question risk assessments as inadequate and point to their narrow focus and lack of comprehensive scope. Long-term basic research needs to generate the underlying scientific understanding to support assessments that would more realistically characterize low-dose risk. Any strategic planning exercise should consider promising lines of research to advance the ability to better predict risk from interactions of sensitive population groups and life stages and from chemical and nonchemical stressors.

In the near term, some advances are possible in the study of complex mixtures with high throughput, toxicogenomic studies. Complex mixtures of toxicants are a significant problem in Superfund sites as well as in other areas of toxicology and environmental health. These toxicant mixtures are known to interact in unexpected or poorly understood ways. Unfortunately, most toxicological studies use purified compounds, reconstructed mixtures, or both. Data from these studies are the basis for regulation of individual compounds and mixtures. As the number of components in the mixture increases, however, the study of reconstructed mixtures becomes more and more difficult and less and less valuable. This type of needed basic research might gain knowledge but will be of little value unless it can be applied in risk assessment to protect public health. And even if it is applied, it has to effectively bring about change in the decision-making process. Translational research is needed to transform basic and applied research into a risk assessment tool. Computational toxicology – a rapidly advancing discipline of toxicology that combines the modern-day computational power with the wisdom gained from conventional toxicity testing – is breathing optimism in this area of translational research and risk assessment tool development.

Once they are developed, computational tools could be made easily accessible, could decrease the cost of toxicity testing, and could meet the present demand for filling the fundamental knowledge gaps in chemical mixture studies. Modern computational chemistry and molecular and cellular biology tools allow researchers to characterize a broad spectrum of physical and biological properties for large numbers of chemicals [82].

Genomics, transcriptomics, proteomics, and metabolomics technologies are becoming integral components of the modern biology toolkit. Linking these molecular biology changes to adverse outcomes represents a significant research challenge that must be addressed before such data can provide information essential to support risk assessment. However, establishing a quantitative relationship between such changes and adverse responses will provide key information. Such information can be very relevant and, at times, critical to risk assessment by providing mechanistically oriented insight into the hazard identification, dose–response, and exposure portions of risk assessments [83]. Together with computational toxicology methods, researchers are using as biomarkers complimentary, alternative, *in vitro* methods in com-

bination with -omics responses. As experience is gained through an increased use of such crosscutting science methods and technology, a more efficient approach to fill critical gaps in our knowledge base to support risk assessment will evolve. Public health and environmental medicine will then emerge together to solve chronic health problems such as obesity, diabetes, and other metabolic diseases linked to environmental factors. These changing perspectives have led to the evolution of the concepts of green chemistry, which has the potential to drastically reduce the synthesis, use, and production of hazardous chemicals, and largely limit the introduction of superfluous chemicals in our environment [84].

Exposure to environmental contaminants or toxicants is one of the many conditions or factors that compromise human quality of life. Toxic chemicals have been linked to deaths and to mortality increases from cancer, respiratory, and cardiovascular diseases [85]. The characteristics and patterns of exposures from waste sites, unplanned releases, and other sources of pollution need to be understood clearly to prevent potential adverse human health effects and diminished quality of life. Ideally, data from epidemiological findings supported by animal studies to verify mechanisms leading to the toxicity of chemical mixtures would be the most appropriate information needed for risk assessment [86–88]. Yet human and animal studies are costly and time-consuming and sometimes lead to inconclusive results. Available epidemiological studies that have examined the health effects of mixtures are usually based on retrospective epidemiological data, where exposure duration and concentrations can only be approximated. Apart from this, those epidemiological studies suffer from confounding factors such as genetic susceptibility, nutritional status, and lifestyle factors.

Looking at the science of toxicology holistically, a realization emerges as to how little is known about the millions of chemicals generally, or the over 80 thousand chemicals in commerce, let alone their mixtures [89]. The effects of chemical mixtures are extremely complex and vary as a function of the chemical composition of each mixture. This complexity is a major reason why mixtures have not been well studied. Thus, writings on chemical mixtures are more often a presentation of what we do not know than of what we do know. As with the expert, the more we understand, the more we realize how little we know. To quote,

> Learning is but an adjunct to ourself,
>
> And where we are, our learning likewise is.
>
> Shakespeare (*Love's Labor's Lost* 4,3)

Acknowledgment

We acknowledge the editorial review and suggestions of Wallace Sagendorph, Division of Creative Services, National Center for Health Marketing, Centers for Disease Control and Prevention (CDC).

References

1 ATSDR (2004) Guidance manual for the assessment of joint toxic action of chemical mixtures (final). Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services, Atlanta, GA. Available at www.atsdr.cdc.gov/interactionprofiles/ipga.html.

2 US Environmental Protection Agency (2000) Supplementary guidance for conducting health risk assessment of chemical mixtures. Risk Assessment Forum. Office of Research and Development. Washington, D.C. EPA/630/R-00/002. Available at http://www.epa.gov/ncea/raf/pdfs/chem_mix/chem_mix_08_2001.pdf.

3 NRCEE (1991) *Environmental Epidemiology, Environmental Epidemiology, vol. 1, Public Health and Hazardous Wastes*, National Academy Press, Washington, DC.

4 Feron, V.J., Cassee, F.R., Groten, J.P., van Vliet, P.W., Job, A., and van Zorge, J.A. (2002) International issues on human health effects of exposure to chemical mixtures. *Environ. Health Perspect.*, **110** (Suppl. 6), 893–899.

5 World Health Organization (2009) Assessment of Combined Exposures to Multiple Chemicals: Report of a WHO/IPCS International Workshop. Available at http://www.who.int/ipcs/methods/hormonization/areas/aggregate/en/index.html.

6 Shermer, M. (2005) Rumsfeld's wisdom. *Sci. Amer.*, **293**, 38.

7 CDC (2009) Fourth National Report on Human Exposure to Environmental Chemicals. Available at http://www.cdc.gov/exposurereport/.

8 ATSDR (2004) Interaction profile for persistent chemicals found in fish (chlorinated dibenzo-*p*-dioxins, hexachlorobenzene, *p,p′*-DDE, methylmercury, and polychlorinated biphenyls). Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services, Atlanta, GA. Available at www.atsdr.cdc.gov/interactionprofiles.

9 Lindsay, D.G. (2005) Nutrition, hermetic stress and health. *Nut. Res. Rev.*, **18**, 249–258.

10 Calabrese, E.J. and Baldwin, L.A. (2003) The hormetic dose–response model is more common than the threshold model in toxicology. *Toxicol. Sci.*, **71**, 246–250.

11 Cook, R. and Calabrese, E.J. (2007) The importance of hormesis to public health. *Cein. Saude Colet.*, **12**, 955.

12 Calabrese, E.J. (2008) Another California milestone: the first application of hormesis in litigation and regulation. *Int. J. Toxicol.*, **27**, 31–33.

13 Grandjean, P. (2005) Implications of the precautionary principle for public heath practice and research. *Human Ecol. Risk Assess.*, **11**, 3–15.

14 Landrigan, P. (2005) Opening remarks: Collegium Ramazzini. *Human Ecol. Risk Assess.*, **11**, 7–8.

15 Johnson, B.J. (2005) Editorial *Human Ecol. Risk Assessment*, **11**, 1–2.

16 Bucher, J. and Lucier, G.E. (1998) Current approaches toward chemical mixtures studies at the National Institute of Environmental Health Sciences and the U.S. National Toxicology Program. *Environ. Health Perspect.*, **106** (Suppl. 6), 1295–1298.

17 Yang, R.S.H., El-Masri, H.A., Thomas, R.S., Dobrev, I., Dennison, J.E. Jr., Bae, D.S., Campain, J.A., Liao, K.H., Reisfeld, B., Andersen, M.E., and Mumtaz, M.M. (2004) Chemical mixture toxicology: from descriptive to mechanistic, and going on to *in silico* toxicology. *Environ. Toxicol. Pharmacol.*, **18**, 65–81.

18 ACGIH (2006) TLV/BEI Resources. American Conference of Governmental and Industrial Hygienists. Available at www.acgih.org/TLV.

19 Mumtaz, M.M., Sipes, I.G., Clewell, H.J., and Yang, R.S.H. (1993) Risk assessment of chemical mixtures: biological and toxicologic issues. *Fundam. Appl. Toxicol.*, **21**, 258–269.

20 Combination Toxicology: Proceedings of a European Conference (1996) *Food Chem. Toxicol.*, **34**, 1025–1185.

21 Mason, A.M., Borgert, C.J., Bus, J.S., Mumtaz, M.M., Simmons, J.E., and Sipes, I.G. (2007) Improving the scientific foundation for mixtures joint toxicity and risk assessment: contributions from the SOT mixtures project – introduction. *Toxicol. Appl. Pharmacol.*, **223**, 99–103.

22 Groten, J.P., Heijne, W.H.M., Stierum, R.H., Freidig, A.P., and Feron, V.J. (2004) Toxicology of chemical mixtures: a challenging quest along empirical sciences. *Environ. Toxicol. Pharmacol.*, **18**, 185–192.

23 Mumtaz, M.M., De Rosa, C.T., Cibulas, W., and Falk, H. (2004) Seeking solutions to chemical mixtures challenges in public health. *Environ. Toxicol. Pharmacol.*, **18**, 55–63.

24 Andersen, M.E. and Dennison, J.E. (2004) Mechanistic approaches for mixtures risk assessments: present capabilities with simple mixtures and future directions. *Environ. Toxicol. Pharmacol.*, **16**, 1–11.

25 Boobis, A., Budinsky, R., Collie, S., Crofton, K., Embry, M., Felter, S., Hertzberg, R., Kopp, D., Mihlan, G., Mumtaz, M., Price, P., Solomon, K., Teuschler, L., Yang, R., and Zaleski, R. Critical analysis of literature on low dose synergy for use in screening chemical mixtures for risk assessment. *Critical Rev. Toxicol.*, in press.

26 Teuschler, L.K., Hertzberg, R.C., Rice, G.E., and Simmons, J.E. (2004) EPA project-level research strategies for chemical mixtures: targeted research for meaningful results. *Environ. Toxicol. Pharmacol.*, **18** (3), 193–199.

27 SRP (2010) National Institutes of Environmental Health Sciences, RTP, NC, USA. Available at http://www.niehs.nih.gov/research/supported/srp/about/index.cfm (accessed 2 March, 2010).

28 Suk, W.A., Anderson, B.E., Thompson, C.L., Bennett, D.A., and VanderMeer, D.C. (1999) Creating multidisciplinary research opportunities: a unifying framework model helps researchers to address the complexities of environmental problems. *Environ. Sci. Technol.*, **33** (11), 241A–244A.

29 Wilson, S.H. and Suk, W.A. (2005) Framework for environmental exposure research: the disease-first approach. *Mol. Interv.*, **5** (5), 262–267.

30 Suk, W.A., Olden, K., and Yang, R.S.H. (2002) Chemical mixtures research: significance and future perspectives. *Environ. Health Perspect.*, **110** (Suppl. 6), 891–892.

31 Suk, W.A. and Wilson, S.H. (2002) Overview and future of molecular biomarkers of exposure and early disease in environmental health, in *Biomarkers of Environmentally Associated Disease* (eds S.H. Wilson and W.A. Suk), CRC Press LLC/Lewis Publishers, Boca Raton, FL, pp. 3–15.

32 Yang, R.S., Thomas, R.S., and Gustafson, D.L. (1998) Approaches to developing alternative and predictive toxicology based on PBPK/PD and QSAR modeling. *Environ. Health Perspect.*, **106** (Suppl. 6), 1285–1293.

33 Suk, W.A. and Olden, K. (2004) Multidisciplinary research: strategies for assessing chemical mixtures to reduce risk of exposure and disease. *Euro. J. Oncol.*, **2**, 1–10.

34 Lazarou, J., Pomeranz, B.H., and Corey, P.N. (1998) Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA*, **279**, 1200–1205.

35 Moore, T.J., Cohen, M.R., and Furberg, C.D. (2007) Serious adverse drug events reported to the Food and Drug Administration, 1998–2005. *Arch. Intern. Med.*, **167** (16), 1752–1759.

36 Reynolds, E.S., Brown, B.R., Jr., and Vandam, L.D. (1972) Massive hepatic necrosis after fluroxene anesthesia: a case of drug interaction? *New Engl. J. Med.*, **286**, 530–531.

37 Harrison, G.G. and Smith, J.S. (1973) Massive lethal hepatic necrosis in rats anesthetized with fluroxene, after microsomal enzyme induction. *Anesthesiology*, **39** (6), 619–625.

38 Jevtovic-Todorovic, V., Hartman, R.E., Izumi, Y., Benshoff, N.D., Dikranian, K., Zorumski, C.F., Olney, J.W., and Wozniak,

D.F. (2003) Early exposure to common anesthetic agents causes widespread, neurodegeneration in the developing rat brain and persistent learning deficits. *J. Neurosci.*, **23**, 876–882.

39 Brater, D.C. (1990) *Toxic Interactions* (eds R.S. Goldstein, W.R., Hewitt, and J.B. Hook), Academic Press, San Diego, pp. 149–173.

40 Hardman, R. (2006) A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environ. Health Perspect.*, **114** (2), 165–172.

41 Royal Commission on Environmental Pollution (2008) Novel Materials in the Environment: The Case of Nanotechnology, 27th Report, p. 147.

42 Maynard, A.D., Aitken, R.J., Butz, T., Colvin, V., Donaldson, K., Oberdorster, G., Philbert, M.A., Ryan, J., Seaton, A., Stone, V., Tinkle, S.S., Tran, L., Walker, N.J., and Warheit, D.B. (2006) Safe handling of nanotechnology. *Nature*, **444**, 267.

43 Yang, R.S.H., Chang, L.W., Yang, C.S., and Lin, P. (2010) Pharmacokinetics and physiologically-based pharmacokinetic modeling of nanoparticles. *J. Nanosci. Nanotech.*, **10**, 1–8.

44 Nel, A., Xia, T., Madler, L., and Li, N. (2006) Toxic potential of materials at the nanolevel. *Science*, **311** (5761), 622–627.

45 Yang, R.S., Chang, L.W., Wu, J.P., Tsai, M.H., Wang, H.J., Kuo, Y.C., Yeh, T.K., Yang, C.S., and Lin, P. (2007) Persistent tissue kinetics and redistribution of nanoparticles, Quantum Dot 705, in mice: ICP-MS quantitative assessment. *Environ. Health Perspect.*, **115** (9), 1339–1343.

46 Lin, P.P., Chen, J.W., Chang, L.W., Wu, J.P., Redding, L., Chang, H., Yeh, T.K., Yang, C.S., Tsai, M.H., Wang, H.J., Kuo, Y.C., and Yang, R.S.H. (2008) Computational and ultrastructural toxicology of a nanoparticle, Quantum Dot 705, in mice. *Environ. Sci. Technol.*, **42**, 6264–6270.

47 De Rosa, C.T., Hansen, H., Wilbur, S., Pohl, H.R., El-Masri, H.A., and Mumtaz, M.M. (2001) Interactions, in *Patty's Toxicology*, 5th edn, vol. 1 (eds E. Bingham, B. Cohrssen, and C.H. Powell), John Wiley & Sons, Inc., pp. 233–284.

48 NRC (2008) *Science and Decisions: Advancing Risk Assessment, National Research Council*, The National Academy Press, Washington, DC.

49 NRC (1983 *Risk Assessment In The Federal Government: Managing The Process*, Committee on the Institutional Means for Assessment of Risks to Public Health, Commission on Life Sciences, National Research Council, National Academy Press, Washington, DC.

50 Mumtaz, M., Ruiz, P., and De Rosa, C. (2007) Toxicity assessment of unintentional exposures to multiple chemicals. *Toxicol. Appl. Pharmacol.*, **223**, 104–113.

51 Mumtaz, M.M., De Rosa, C.T., Groten, J., Feron, V.J., Hansen, H., and Durkin, P.R. (1998) Estimation of toxicity of chemical mixtures through modeling of chemical interactions. *Environ. Health. Perspect.*, **106** (Suppl. 6), 1353–1360.

52 Calabrese, E.J. (1991) *Multiple Chemical Interactions*, Lewis Publishers, Chelsea, MI.

53 Yang, R.S.H. (2010) Toxicologic interactions of chemical mixtures, in *Comprehensive Toxicology. Vol. 1. General Principles* (ed. J. Bond), Elsevier Ltd., Oxford, in press.

54 Yang, R.S.H. and Andersen, M.E. (2005) Physiologically based pharmacokinetic modeling of chemical mixtures, in *Physiologically Based Pharmacokinetics: Science and Applications* (eds M.B. Reddy, R.S.H. Yang, H.J. ClewellIII, and M.E. Andersen), John Wiley & Sons, Inc., New York, pp. 349–373.

55 Yang, R.S.H. and Lu, Y. (2007) The application of physiologically based pharmacokinetic (PBPK) modeling to risk assessment, in *Risk Assessment for Environmental Health* (eds M.G. Robson and W.A. Toscano), John Wiley & Sons, Inc., Hoboken, NJ, pp. 85–120.

56 Mumtaz, M.M., Poirier, K.A., and Coleman, J.T. (1997) Risk assessment for chemical mixtures: fine-tuning the hazard index approach. *J. Clean Technol. Environ. Toxicol. Occup. Med.*, **6**, 189–204.

57 Mumtaz, M.M. and Durkin, P.R. (1992) A weight-of-evidence scheme for assessing interactions in chemical mixtures. *Toxicol. Ind. Health*, **8**, 377–406.

58 Mumtaz, M.M., El-Masri, H., Chen, D., and Pounds, J. (2000) Joint toxicity of inorganic chemical mixtures: the role of dose ratios, in *Metal Ions in Biology and Medicine*, vol. 6 (eds J.A. Centeno, P.H. Collery, G. Vernet, R.B. Finkelman, H. Gibb and J.C. Etienne), John Libbey Eurotext, Paris, pp. 297–299.

59 El-Masri, H.A., Mumtaz, M.M., and Yushak, M.L. (2004) Application of physiologically based pharmacokinetic modeling to investigate the toxicological interaction between chlorpyrifos and parathion in the rat. *Environ. Toxicol. Pharmacol.*, **16**, 57–71.

60 Ray, M., Ritger, S.E., Mumtaz, M., Ruiz, P., Welsh, C., Fowler, D.A., Keys, D., and Fisher, J. (2009) Addressing public exposures to priority solvents using human PBPK models. *Toxicol. Sciences*, **108** (1), 471.

61 Haddad, S., Pelekis, M.L., and Krishnan, K. (1996) A methodology for solving physiologically-based pharmacokinetic models without the use of simulation software. *Toxicol. Letters*, **85**, 113–126.

62 Jonker, D., Jones, M.A., van Bladeren, P.J., Woutersen, R.A., Til, H.P., and Feron, V.J. (1993) Acute (24 hr) toxicity of a combination of four nephrotoxicants in rats compared with the toxicity of the individual compounds. *Food Chem. Toxicol.*, **31**, 45–52.

63 Jonker, D., Woutersen, R.A., and Feron, V.J. (1996) Toxicity of mixtures of nephrotoxicants with similar or dissimilar mode of action. *Food Chem. Toxicol.*, **34**, 1075–1082.

64 Heijne, W.H., Jonker, D., Stierum, R.H., van Ommen, O.B., and Groten, J.P. (2005) Toxicogenomic analysis of gene expression changes in rat liver after a 28-day oral benzene exposure. *Mutat. Res.*, **575** (1–2), 85–101.

65 Kienhuis, A.S., Wortelboer, H.M., Hoflack, J.C., Moonen, E.J., Kleinjans, J.C., van, O.B., van Delft, J.H., and Stierum, R.H. (2006) Comparison of coumarin-induced toxicity between sandwich-cultured primary rat hepatocytes and rats *in vivo*: a toxicogenomics approach. *Drug Metab. Dispos.*, **34** (12), 2083–2090.

66 McMillian, M., Nie, A.Y., Parker, J.B., Leone, A., Bryant, S., Kemmerer, M., Herlich, J., Liu, Y., Yieh, L., Bittner, A., Liu, X., Wan, J., and Johnson, M.D. (2004) A gene expression signature for oxidant stress/reactive metabolites in rat liver. *Biochem. Pharmacol.*, **68** (11), 2249–2261.

67 McMillian, M., Nie, A.Y., Parker, J.B., Leone, A., Kemmerer, M., Bryant, S., Herlich, J., Yieh, L., Bittner, A., Liu, X., Wan, J., and Johnson, M.D. (2004) Inverse gene expression patterns for macrophage activating hepatotoxicants and peroxisome proliferators in rat liver. *Biochem. Pharmacol.*, **67** (11), 2141–2165.

68 Natsoulis, G., El, G.L., Lanckriet, G.R., Tolley, A.M., Leroy, F., Dunlea, S., Eynon, B.P., Pearson, C.I., Tugendreich, S., and Jarnagin, K. (2005) Classification of a large microarray data set: algorithm comparison and analysis of drug signatures. *Genome Res.*, **15** (5), 724–736.

69 van Delft, J.H., van Agen, A.E., van Breda, S.G., Herwijnen, M.H., Staal, Y.C., and Kleinjans, J.C. (2005) Comparison of supervised clustering methods to discriminate genotoxic from non-genotoxic carcinogens by gene expression profiling. *Mutat. Res.*, **575** (1–2), 17–33.

70 Thomas, R.S., O'Connell, T.M., Pluta, L., Wolfinger, R.D., Yang, L., and Page, T.J. (2007) A comparison of transcriptomic and metabonomic technologies for identifying biomarkers predictive of two-year rodent cancer bioassays. *Toxicol. Sci.*, **96**, 40–46.

71 Thomas, R.S., Allen, B., Nong, A., Yang, L., Bermudez, E., Clewell, H.J. III, and Andersen, M.E. (2007) A method to integrate benchmark dose estimates with genomic data to assess the functional effects of chemical exposure. *Toxicol. Sci.*, **98**, 240–248.

72 Hendricksen, P., Freidig, A.P., Jonker, D., Thissen, U., Bogaards, J.J.P., Mumtaz, M.M., Groten, J.P., and Stierum, R.H.

(2007) Transcriptomics analysis of interactive effects of benzene, trichloroethylene, and methyl mercury within binary and ternary mixtures on the liver and kidney following subchronic exposure in the rat. *Toxicol. Appl. Pharmacol.*, **225**, 171–188.

73 Callahan, M.A. and Sexton, K. (2007) If cumulative risk assessment is the answer, what is the question? *Environ. Health Perspect.*, **115** (5), 799–806.

74 ICCVAM (2010) National Institutes of Environmental Health Sciences, RTP, NC, USA. Available at http://iccvam.niehs.nih.gov/ (accessed 2 March, 2010).

75 UN (2003) Skin corrosion/irritation. UN Globally Harmonized System of Classification and Labelling of Chemicals. ST/SG/AC.10/30, United Nations, New York, Geneva, pp. 123–135.

76 Houck, K.A. and Kavlock, R.J. (2008) Understanding mechanisms of toxicity: insights from drug discovery research. *Toxicol. Appl. Pharmacol.*, **277**, 163–178.

77 Dix, D.J., Houck, K.A., Martin, M.T., Richard, A.M., Setzer, R.W., and Kavlock, R.J. (2007) The ToxCast program for prioritizing toxicity testing of environmental chemicals. *Toxicol. Sci.*, **95**, 5–12.

78 Judson, R., Richard, A., Dix, D., Houch, K., Elloumi, F., Martin, M., Cathey, T., Transue, T., Spencer, R., and Wolf, M. (2008) ACToR: aggregated computational toxicology resource. *Toxicol. Appl. Pharmacol.*, **233**, 7–13.

79 Mattingly, C.J., Colby, G.T., Forrest, J.N., and Boyer, J.L. (2003) The comparative toxicogenomics database (CTD). *Environ. Health Perspect.*, **111**, 793–795.

80 NRC (2007) *Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment*, The National Academy Press, Washington, DC.

81 NAS (2007) *Toxicity Testing in the 21st Century: A Vision and a Strategy*, The National Academy Press, Washington, DC.

82 Bredel, M. and Jacoby, E. (2004) Chemogenomics: an emerging strategy for rapid target and drug discovery. *Nat. Rev. Genet.*, **5** (4), 262–275.

83 US Environmental Protection Agency (2004) Potential Implications of Genomics for Regulatory and Risk Assessment Applications at EPA. Science Policy Council, U.S. Environmental Protection Agency, Washington, D.C. 20460.

84 NAS (2007) *Green Healthcare Institutions: Health, Environment, and Economics: Workshop Summary*, The National Academies Press, Washington, DC.

85 Mokadad, A.H., Marks, J.S., Stroup, D.F., and Gerberding, J. (2004) Actual causes of death in the United States, 2000. *JAMA*, **291** (10), 1238–1245.

86 Mumtaz, M.M., Ruiz, P., Whittaker, M., Dennison, J., Fowler, B.A., and De Rosa, C.T. (2006) Chemical mixtures risk assessment and technological advances, in *Biological Concepts and Techniques in Toxicology: An Integrated Approach* (ed. J.E. Riviere), Taylor & Francis, New York, pp. 177–204.

87 Gorell, J.M., Johnson, C.C., Rybicki, B.A., Peterson, E.L., Kortsha, G.X., Brown, G.G., and Richardson, R.J. (1997) Occupational exposure to metals as risk factors for Parkinson's disease. *Neurology*, **48**, 650–658.

88 Gorell, J.M., Johnson, C.C., Rybicki, B.A., Peterson, E.L., and Richardson, R.J. (1998) The risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. *Neurology*, **50**, 1346–1350.

89 Pimentel, D., Tort, M., D'Anna, L., Krawic, A., Gerger, J., Rossman, J., Mugo, F., Doon, N., Shriberg, M., and Howard, E. (1995) Ecology of increasing disease: population growth and environmental degradation. *Bioscience*, **48**, 817–826.